Analytical Description of the Activation of Multi-State Receptors by Continuous Neurotransmitter Signals at Brain Synapses

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ABSTRACT Chemical synaptic transmission is a fundamental component of interneuronal communications in the central nervous system (CNS). Discharge of a presynaptic vesicle containing a few thousand molecules (a quantum) of neurotransmitter into the synaptic cleft generates a transmitter concentration signal that drives postsynaptic ion-channel receptors. These receptors exhibit multiple states, with state transition kinetics dependent on neurotransmitter concentration. Here, a novel and simple analytical approach for describing gating of multi-state receptors by signals with complex continuous time courses is used to describe the generation of glutamate-mediated quantal postsynaptic responses at brain synapses. The neurotransmitter signal, experienced by multi-state *N*-methyl-D-aspartate (NMDA)- and L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-type glutamate receptors at specific points in a synaptic cleft, is approximated by a series of step functions of different intensity and duration and used to drive a Markovian, multi-state kinetic scheme that describes receptor gating. Occupancy vectors at any point in time can be computed iteratively from the occupancy vectors at the times of steps in transmitter concentration. Multi-state kinetic schemes for both the low-affinity AMPA subtype of glutamate receptor and for the high-affinity NMDA subtype are considered, and expected NMDA and AMPA components of synaptic currents are calculated. The amplitude of quantal responses mediated by postsynaptic receptor clusters having specific spatial distributions relative to foci of quantal neurotransmitter release is then calculated and related to the displacement between the center of the postsynaptic receptor cluster and the focus of synaptic vesicle discharge. Using this approach we show that the spatial relation between the focus of release and the center of the postsynaptic receptor cluster affects synaptic efficacy. We also show how variation in this relation contributes to variation in synaptic current amplitudes.

INTRODUCTION

Receptor-mediated signal transduction is a key component of many physiological processes. Biological receptors typically can exist in any one of a number of distinct kinetic states with some or all of the transition rate constants between these multiple states modulated by the signal intensity. In the case of neurotransmitter receptor-ion-channels, the signal is the neurotransmitter concentration. The channel component can either be conducting or nonconducting, and the receptor component can either be resting, activated, or desensitized with receptor binding sites for neurotransmitter either occupied or free. Although the mechanics of signal transduction mediated by many types of receptors are becoming well understood, a challenge remains to predict how given receptors will respond to complex signal waveforms such as occur in real biological systems.

Receptor-mediated gating of ion-channels generally can be discussed in terms of Markovian kinetics (e.g., for a given receptor-channel in a given kinetic state, distribution of the probabilities of transitions to other states of the kinetic scheme does not depend on the receptor-channel prehistory). Analytical formalisms that describe and predict responses of multi-state kinetic schemes to step changes in signal intensity are commonly used to define the pharmacodynamics of receptor-channel systems (for review, see Colquhoun and Hawkes, 1995). These formalisms allow the results of a variety of experimental protocols, designed to accentuate specific state transitions, to be used to select a kinetic scheme that best describes the pharmacodynamics of a particular system. This approach is an important feature of the current biophysical exploration of ion-channel dynamics (see Sakmann and Neher, 1995).

Here we take that approach one step further by developing a strategy for estimating how continuous physiological signals of any shape, as opposed to a single-step function, will drive multi-state receptors in the generation of physiological responses. Our strategy has been to expand and generalize an analytical formalism introduced previously to describe interactions between a three-state kinetic receptor system and phasic activation (Uteshev and Pennefather, 1996a). The actual time course of a complex physiological signal is approximated by a sequence of step functions with adjustable intensities and durations. Occupancy vectors at any point in time can be computed iteratively from the occupancy vectors at the times of steps in transmitter concentration. Therefore, the response of the receptor system to the entire continuous signal can be approximated by a sequence of responses of the system to a defined series of step functions.

We illustrate the approach by analyzing the generation of postsynaptic quantal responses at synapses between neurons in the brain, and explore how synaptic structure might influence synaptic efficacy. A number of previous studies have used various types of numerical integrations and sim-

Received for publication 3 September 1996 and in final form 10 December 1996.

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0006-3495/97/03/1127/08 $2.00

ulations strategies to model how quantal synaptic responses are generated following the discharge of a transmitter-containing vesicle (Wathey et al., 1979; Land et al., 1981; Bartol et al., 1991; Khanin et al., 1994; Van der Kloot, 1995; Holmes, 1995; Stiles et al., 1996). Although these numerical approaches delivered a satisfying precision to the results, they do not directly establish relationships between synaptic structure and different subprocesses contributing to synaptic transmission such as vesicular release, diffusion in the synaptic cleft, uptake, postsynaptic receptor binding and activation; nor do they permit a formal generalization of principles and further analytical development. In contrast, these goals can be achieved once an analytical formulation is established.

Our present approach is novel in that we have developed a generalized analytical formalism for producing a mathematical description of the dynamics of synaptic transmission including all its major steps. Using classical diffusion theory, we first define a continuous signal that represents the time course of neurotransmitter concentration in a synaptic cleft of a specific geometry, as it is seen by receptors at different locations on the postsynaptic membrane relative to focus of the discharge of a single quantum of neurotransmitter into the synaptic cleft (see Appendix, Note 3; Uteshev and Pennefather, 1996b). We then replace the expected continuous signal by a sequence of step functions of variable duration, where the concentration is set at a constant level corresponding to the actual concentration at the midpoint of each step. Hence, for each jth step we can unambiguously determine occupancy vectors, $\mathbf{p}_j(t)$, representing probabilities of occupancies of each of the multiple kinetic states of the receptor during that step. Time t runs within each step between 0 and the duration of the step. Once the relation between $\mathbf{p}_j(t)$ and receptor displacement is known, then the mean occupancy vector for receptors in a postsynaptic receptor cluster of specified dimensions and distance from the focus of release can be calculated.

Using this approach we show that the spatial relation between the focus of release and the center of the postsynaptic receptor cluster affects synaptic efficacy. We also show how variation in this relation contributes to variation in synaptic current amplitudes. Multi-state kinetic schemes for both the low-affinity L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtype of glutamate receptor (see Jonas et al., 1993), and for the high-affinity N-methyl-D-aspartate (NMDA) subtype (see Lester and Jahr, 1992) are considered, and expected NMDA and AMPA components of synaptic currents are calculated. We show that for the AMPA component, "off-center" release has a significant effect on peak amplitude despite the small displacements expected within the dimensions of a CNS synapse. The NMDA component of synaptic currents is shown to be less sensitive to displacement than the AMPA component. The approach is also extended to predicting the response of postsynaptic receptors to trains of quantal release events where events within the train can exhibit different displacements from spatially localized receptors. We show that AMPA receptor desensitization can lead to interactions between quantal events occurring as far away as 0.4 μm, a distance large enough to represent an adjacent synapse.

MATHEMATICAL FORMALISM

Interaction between continuous signals and multi-state receptors

Consider a complex signal that may in fact consist of several identical subsignals following each other in a train, such as in the case during repeated synaptic stimulation. In our formulation we determine a continuous time course of the complex signal as a sequence of several, for example J, step functions of different durations. Therefore, the status of the receptor at any particular time of stimulation is fully determined by the occupancy vector $\mathbf{p}_j(t)$, where the subscript j refers to the jth step function. At the transitions between the adjacent $(j-1)$th and jth step functions, we define $\mathbf{p}_{j-1}(t_{j-1}) = \mathbf{p}_j(0)$, where t_{j-1} is the duration of the $(j-1)$th step. The occupancy vectors for the jth step can be determined in terms of a matrix-exponential function

$$\mathbf{p}_j(t) = \mathbf{p}_j(0)e^{\mathbf{Q}_j t} = \mathbf{p}_{j-1}(t_{j-1})e^{\mathbf{Q}_j t} \tag{1}$$

where $\mathbf{p}_j(0)$, $\mathbf{p}_j(t_j)$, and $\mathbf{p}_j(t)$ are occupancy vectors at the beginning, at the end, and at arbitrary time t ($t \in [0, t_j]$) during the jth step, respectively; $\mathbf{Q}_j$ is a matrix introduced previously by Colquhoun and Hawkes, (1977) (see Appendix, Note 1).

We have used this approach to determine how the distribution of multi-state receptors on the postsynaptic membrane relative to the point of release of the contents of a synaptic vesicle affects the time course of miniature or unitary evoked postsynaptic currents (uePSCs). We first discretize the concentration function and then determine the probability of finding a receptor in a particular state at a particular time as a function of displacement from the point of vesicular discharge into the cleft. Repeated application of Eq. 1 gives the jth vector occupancy as a function of time within the jth step ($t \in [0, t_j]$)

$$\mathbf{p}_j(t) = \mathbf{p}_1(0)\mathbf{Б}_{j-1}e^{\mathbf{Q}_j t} \tag{2}$$

where

$$\mathbf{Б}_{j-1} = \left(\prod_{m=1}^{j-1} e^{\mathbf{Q}_m t_m}\right) \tag{2'}$$

Here we have specified the following definition: $\mathbf{Б}_0 = (\prod_{m=1}^{0} e^{\mathbf{Q}_m t_m}) \overset{\text{def}}{=} \mathbf{E}$; where $\mathbf{E}$ is either a 7×7 or 5×5 identity matrix for AMPA and NMDA kinetic schemes, respectively (Fig. 1 B). It can be shown (see, for example, Colquhoun and Hawkes, 1995) that the steady-state vector occupancy can be calculated as

$$\mathbf{p}_{ss} = \mathbf{e}(\mathbf{S}\mathbf{S}^{\mathrm{T}})^{-1} \tag{3}$$

where $(\ldots)^{-1}$ is an inverse matrix, $\mathbf{S}$ is a defined modification of the $\mathbf{Q}_{SS}$ matrix, $\mathbf{S}^{\mathrm{T}}$ is a matrix transposed to the $\mathbf{S}$ matrix, and $\mathbf{e} = (1, 1, \ldots, 1)$ is a unity vector-row (see

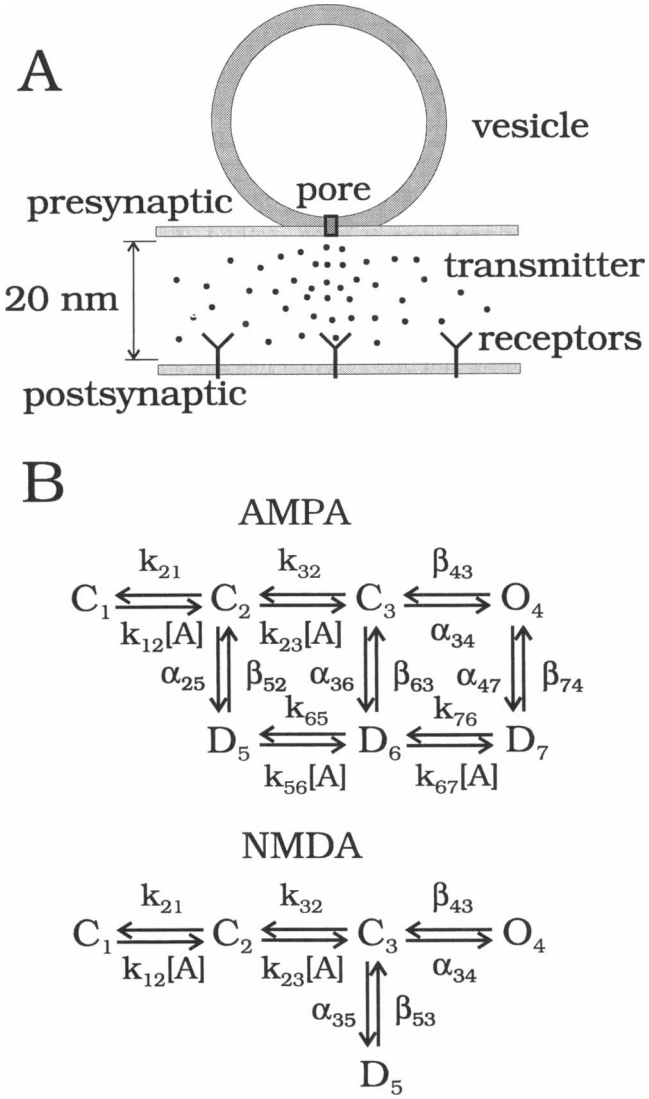

FIGURE 1 Models of synaptic transmission and postsynaptic gating. (*A*) A schematic representation of a CNS synapse considered in this description. A presynaptic vesicle (~17 nm in radius) will release neurotransmitter molecules into the synaptic cleft (~20 nm in width) by diffusion through a fusion pore (~1 nm in diameter) that spans the synaptic vesicle and presynaptic terminal membrane bilayers (10 nm in length). The overall postsynaptic receptor response will reflect the interaction between a continuous intrasynaptic signal (concentration) and postsynaptic receptors of a particular kinetic scheme [see (*B*)]. (*B*) Two kinetic schemes for AMPA and NMDA receptors that were considered in the present study. The validity of the kinetic schemes was tested experimentally elsewhere [see Jonas et al., 1993 (AMPA), and Lester and Jahr, 1992 (NMDA)]. Kinetic constants for interstate transitions were as follows: AMPA receptors: $k_{12} = 4.59 \cdot 10^3\ mM^{-1}\ ms^{-1}$; $k_{21} = 4.26\ ms^{-1}$; $k_{23} = 2.84 \cdot 10^4\ mM^{-1}\ ms^{-1}$; $k_{32} = 3.26\ ms^{-1}$; $\alpha_{25} = 2.89\ ms^{-1}$; $\beta_{52} = 3.92 \cdot 10^{-2}\ ms^{-1}$; $\alpha_{34} = 4.25\ ms^{-1}$; $\beta_{43} = 0.9\ ms^{-1}$; $\alpha_{36} = 0.17\ ms^{-1}$; $\beta_{63} = 7.27 \cdot 10^{-4}\ ms^{-1}$; $\alpha_{47} = 1.77 \cdot 10^{-2}\ ms^{-1}$; $\beta_{74} = 4 \cdot 10^{-3}\ ms^{-1}$; $k_{56} = 1.27 \cdot 10^3\ mM^{-1}\ ms^{-1}$; $k_{65} = 4.57 \cdot 10^{-2}\ ms^{-1}$; $k_{67} = 1.68 \cdot 10^{-2}\ ms^{-1}$; $k_{76} = 0.19\ ms^{-1}$; NMDA receptors—$k_{12} = 10^4\ mM^{-1}\ ms^{-1}$; $k_{21} = 4.7 \cdot 10^{-3}\ ms^{-1}$; $k_{23} = 0.5 \cdot 10^4\ mM^{-1}\ ms^{-1}$; $k_{32} = 9.4 \cdot 10^{-3}\ ms^{-1}$; $\alpha_{34} = 8.4 \cdot 10^{-3}\ ms^{-1}$; $\beta_{43} = 1.8 \cdot 10^{-3}\ ms^{-1}$; $\alpha_{35} = 46.5 \cdot 10^{-3}\ ms^{-1}$; $\beta_{53} = 91.6 \cdot 10^{-3}\ ms^{-1}$.

Appendix, Note 1). If we assume that stimulation began from steady-state conditions, and therefore $\mathbf{p}_1(0) = \mathbf{p}_{SS}$, then combining Eq. 3 with Eqs. 2–2′ we obtain the final result for the time dependence of the vector occupancy within the jth step

$$\mathbf{p}_j(t) = \mathbf{e}(\mathbf{S}\mathbf{S}^T)^{-1}\mathbf{Б}_{j-1}e^{Q_j t} \tag{4}$$

where time $t \in [0, t_j]$. The entire vector-curve, therefore, can be built by joining the end of the response to the jth step function to the beginning of the $(j + 1)$th response, for all $j \in [1, J]$.

Synaptic parameters

The processes and structures involved in synaptic transmission are well defined. In response to a presynaptic signal (typically an action potential-induced influx of Ca^{2+} ions) at a nerve terminal active zone, a small packet or quantum of neurotransmitter molecules (typically 1000–5000 molecules) contained in a synaptic vesicle is discharged into a synaptic cleft. Loss of neurotransmitter, due to diffusion within and out of the cleft and to uptake, begins simultaneously with its appearance in the cleft so that receptors eventually return to their resting state. In our study we consider and compare responses of fast (AMPA) and slow (NMDA) glutamate receptors. Both kinds of postsynaptic receptors have been described previously using kinetic schemes containing seven states for low-affinity AMPA receptors (Jonas et al., 1993), and five states for high-affinity NMDA (Lester and Jahr, 1992) (Fig. 1 *B*).

Fig. 1 *A* shows a schematic picture of synaptic transmission. A detailed justification of all parameters and assumptions used here is presented elsewhere (Uteshev and Pennefather, 1996b). The postsynaptic membrane is characterized in electron micrographs by a halo of electron-dense material referred to as the postsynaptic density (PSD). This region is roughly circular, with a diameter of ~0.4 μm. There is evidence that postsynaptic receptors are organized into clusters (PRCs) restricted to the PSD region (see Uteshev and Pennefather, 1996b, for discussion). The opposing presynaptic membrane is also characterized by a halo of electron-dense material that is thought to delimit the active zone region capable of supporting evoked release of neurotransmitter. There is a high degree of overlap of the dimensions of the PSD and the active zone. For our calculations we have defined an idealized synapse as a circular region of close apposition between pre and postsynaptic neurons. The diameter of this region is set at 0.4 μm, and the synaptic cleft separating the two neurons is given a width of 20 nm. After initiation of synaptic transmission, a synaptic vesicle (radius 17 nm) forms a 1 nm-wide fusion pore with the presynaptic membrane through which the vesicular contents of ~5000 molecules are discharged into the synaptic cleft over a period of a few hundred microseconds. This process of release can be approximated by an exponentially declining point source with a time constant of 0.2 ms. The transmitter is allowed to diffuse with a diffusion coefficient of

0.3 μm^2/ms in a two-dimensional plane with a thickness of 20 nm. For simplicity, uptake is considered to be homogeneous and nonsaturable with a time constant of 1 ms^{-1}. This is equivalent to assigning a finite and exponentially distributed lifetime to each transmitter molecule that enters the synaptic cleft. Because of an excess of neurotransmitter molecules in the synaptic cleft (~5000) over available postsynaptic receptors (~100) we assume that depletion of neurotransmitter concentration in the cleft because of receptor binding is absent. These parameters and assumptions allow us to calculate the time course of glutamate concentration in the synaptic cleft at any distance from the point of release. Our analytical discretization approach then allows us to calculate the response of AMPA and NMDA multistate receptors to this signal at any point in time or space within the synaptic cleft.

RESULTS

Responses to the release of a single quantum of neurotransmitter

Fig. 2 shows results of our analysis for a single receptor positioned either 0.04 μm (*top row*, Fig. 2) or 0.2 μm (*bottom row*, Fig. 2) from the foci of transmitter release. The expected transmitter signal was calculated as described in the Appendix (Note 2; see also Uteshev and Pennefather, 1996b) and found to be different at these two points, rising more slowly and reaching a smaller maximum value at the larger displacement (*bottom row*, Fig. 2). Using the kinetic schemes described in Fig. 1 *B* for AMPA and NMDA receptors and our discretization method, we find that efficacy of activation of the AMPA receptor is reduced with increasing displacement distance from foci of transmitter release. This arises because there is a gradient of transmitter concentration within the cleft that has a peak at the focus of release (see Uteshev and Pennefather, 1996b). This gradient, however, makes little difference for activation of the NMDA receptor.

Both NMDA and AMPA receptors appear to be clustered within the region of the postsynaptic density (for discussion, see Uteshev and Pennefather, 1996b) and the overall response of a PRC will be the sum of responses of all functional receptors of the PRC. Once a shape and size of the PRC is assumed, the overall response can be calculated from the function relating state probability of a single receptor to the displacement of that receptor from the focus of

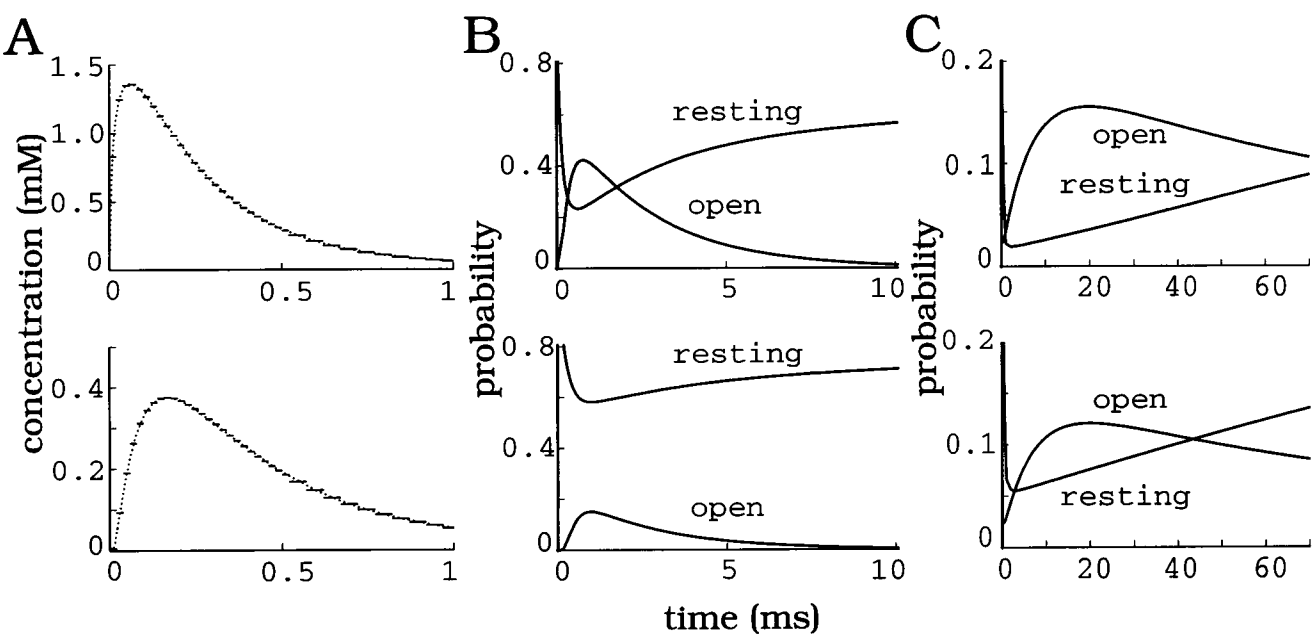

FIGURE 2 Responses of a single receptor placed at different locations on the postsynaptic membrane relative to the focus of presynaptic release. The top row (*A–C*) corresponds to a single receptor located at 0.04 μm from the point of projection of the focus of release onto the postsynaptic membrane. The bottom row corresponds to a single receptor located at 0.2 μm from the point of projection. (*A*) Concentration time course experienced by postsynaptic receptor at different distances from the focus of release. A continuous intrasynaptic signal that is the time course of neurotransmitter concentration in the synaptic cleft is approximated by a series of step functions with different durations and intensities (see text). Calculation of the intrasynaptic concentration time course has been described previously (Uteshev and Pennefather, 1996b, and as is outlined in Note 2). Duration of the steps are: 0.02 ms during the first 0.52 ms of the signal and 0.05 ms during the remaining interval. Within each step concentration of neurotransmitter in the vicinity of the receptor is constant. (*B*) Responses of a single AMPA receptor to a continuous intrasynaptic signal. Two examples of responses of a single AMPA receptor described by the scheme shown in Fig. 1 *B*. The time dependence of occupancy of the state C_1 is indicated by "resting"; time dependence of occupancy of the state O_4 is marked by "open." With a 0.2-μm displacement between the receptor and the point of projection of the transmitter onto postsynaptic membrane discharge occupancy of open state reaches ~15% at the peak. If, however, the release occurs within 0.04 μm of the receptor, the peak open state probability is ~40%. (*C*) Responses of a single NMDA receptor to the continuous intrasynaptic signal. A paradigm similar to that used in (*B*) is employed for a single NMDA receptor, whose affinity is ~20× higher than for the AMPA receptor. The kinetic scheme shown in Fig. 1 *B* is used with "resting" and "open" time courses corresponding to the probability that the receptor is in states C_1 and O_4, respectively.

release. For our analysis we assume a circular shape for the PRC and that the density of receptors in PRC is not a function of the radius of the PRC. For a PRC that matches the dimensions of the active zone (e.g., concentric and with a 0.4 μm diameter), our calculations predict that even if a presynaptic vesicle is discharged over the exact center of the PRC (and in this case also at the center of the active zone) just under 30% of AMPA receptors will be activated at the peak of the uePSC (Fig. 3, *A* and *B*). Displacement of the release site by 0.2 μm to the edge of the active zone reduces efficacy to about one-half this value (Fig. 3, *A* and *B*). However, if the center of the PRC is located more than 0.4 μm from the release site (i.e., a distance that could represent an adjacent synapse) there is little signal produced by this release event. Thus, as we have pointed out elsewhere (Uteshev and Pennefather, 1996b), the noninstantaneous release and low affinity of AMPA receptors combine to focus the response of the PRC associated with a particular active zone to the quantum of transmitter released from that active zone. With PRCs made up of NMDA receptors, which have a higher affinity for glutamate than AMPA receptors, this focusing effect is much less pronounced, and responses as much as one-third the optimal are expected with displacements of 1 μm (the largest displacement considered in Fig. 3). Hence, more NMDA receptor-mediated cross talk between adjacent synapses is expected than AMPA receptor-mediated response cross talk.

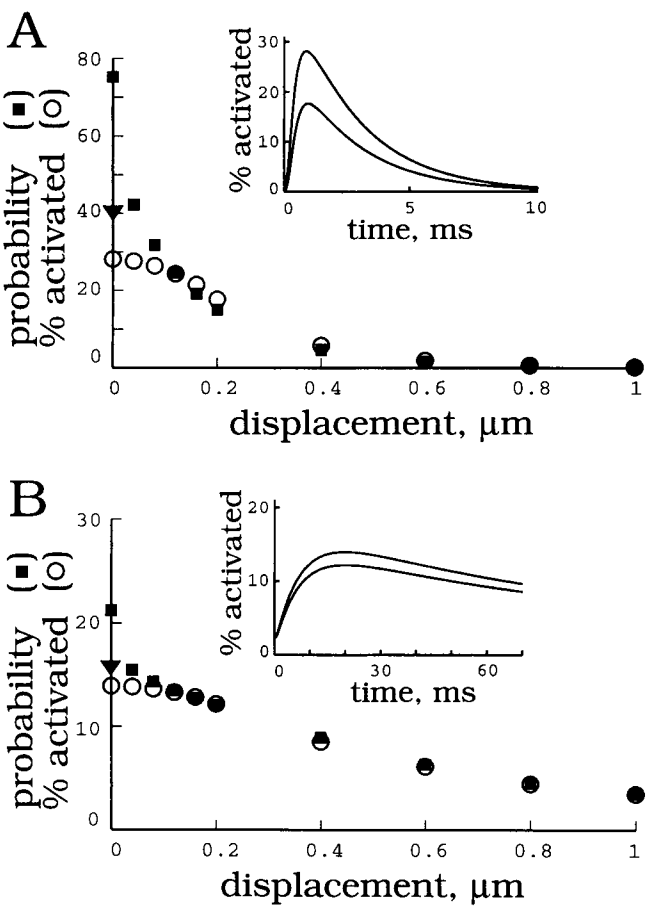

FIGURE 3 Responses from a postsynaptic receptor cluster and a single receptor: comparison. Response-displacement functions for responses from the AMPA (*A*) and NMDA (*B*) PRCs, 0.4 μm in diameter (*open circles*) and single receptors (*closed squares*). The triangle indicates the peak response expected for a circular PRC with a smaller diameter of 0.24 μm. Responses of the PRCs are calculated in terms of percent activated receptors, while responses of single receptors are calculated in terms of the probability of opening. The insets show examples of simulated responses from AMPA (*A*) and NMDA (*B*) circular PRCs (0.4 μm in diameter) displaced by 0.04 μm (the largest trace in each figure) or 0.2 μm (the smallest trace in each figure) from the point of projection of the focus of release onto postsynaptic membrane. Displacement is calculated from the center of PRC. NMDA responses demonstrate less sensitivity to displacement between the focus of release and the center of the PRC than did AMPA receptors.

Fig. 3 also shows that reducing the size of the PRC increases the sensitivity of the response to displacement of the center of the receptor cluster from the focus of release. Central synapses should contain ~100 AMPA receptors, and if these were tightly packed at a density of 2200/μm^2, they would form a cluster with a diameter of 0.24 μm (see Uteshev and Pennefather, 1996b). The response expected for a PRC of this size and a centered release is indicated by a triangle in Fig. 3, *A* and *B*. The response is intermediate between that expected for a single receptor and a larger PRC. Hence, our description suggests that efficacy and variability of unitary responses mediated by AMPA receptors will be dependent on the relative sizes and disposition of the active zone and the PRC. These considerations are less important for NMDA-mediated responses. Uteshev and Pennefather (1996b) have argued that part of the large coefficient of variation of AMPA receptor mediated uePSCs is due to variation in the point of release of quanta within the active zone, resulting in variation in the displacement between the center of the PRC and the point of release. Thus, our analysis makes the prediction that the NMDA receptor-mediated component of uePSCs recorded at a unitary synapse should be less variable in amplitude than the AMPA-mediated component.

The case of a train of quantal events

We have also used our description to consider the case where there is a train of quantal discharges into a synaptic cleft. The question we wished to consider was the degree to which the resulting series of postsynaptic responses are independent of one another. We analyzed the response of a single postsynaptic AMPA receptor to the neurotransmitter signal that it should experience following a train of quantal discharges. A specific train consisting of three synaptic discharges displaced by either 0.04 μm, 0.2 μm, or 0.4 μm from the receptor, and a fourth release event occurring at 0.04 μm from the focus of release, was considered (see Appendix, Note 3). Although this particular sequence of events is unlikely to occur given our expectation that release is equally likely from any position in the active zone, it nevertheless illustrates how timing and spatial dispersion of the successive release events influence the interaction between synaptic responses in the train.

When all release events occur near the postsynaptic AMPA receptor, desensitization leads to a pronounced and progressive attenuation of successive responses with >70% depression of the fourth response (Fig. 4 *A*). When the conditioning release events occur at a more distant point (0.2 μm in Fig. 4 *B*), and the test release occurs at a point close to the receptor (0.04 μm), the responses to this fourth release are still reduced by 50% rather than 70%. Thus, there may be an advantage for quantal release events to occur randomly within an active zone, since this will lead to less accumulation of desensitization with repeated activation than if the vesicles were always discharged at the same point. When the receptor is displaced 0.4 μm from the point of discharge of the conditioning release events, a distance which could conceivably correspond to an adjacent synaptic unit, the response to a proximal release event is still reduced by 34% (Fig. 4 *C*) due to AMPA receptor desensitization. This is despite the expected lack of direct responsiveness to quantal events occurring at 0.4 μm away from receptors (Fig. 3 *A*). Thus, our calculations suggest the possibility of *desensitization cross talk* between synapses during trains of synaptic activity, but only if these synapses are very close to each other and if there are no diffusion barriers separating those adjacent synapses.

DISCUSSION

The method of analytical discretization of continuous signals

A system of differential equations that describes the kinetics of postsynaptic multi-state ion receptor-channels during presynaptic release can be written as follows

$$\frac{d\mathbf{p}(t)}{dt} = \mathbf{p}(t)Q[C(t)] \tag{5}$$

with initial conditions $\mathbf{p}(t = 0) = \mathbf{p}_{SS}$. Here, the $\mathbf{Q}$ matrix exhibits dependence of the transmitter concentration in the synaptic cleft (C) which is, in turn, time-dependent [$C = C(t)$]. In general, systems like Eq. 5 do not have analytical solutions. Numerical methods exist, though, that can approximate Eq. 5 by a system of algebraic equations $\mathbf{p}(t + \Delta t) - \mathbf{p}(t) = \mathbf{p}(t)\mathbf{Q}(t)\Delta t$, and the system can be solved (see Wathey et al., 1979; Land et al., 1981; Ortega and Poole, 1981; Holmes, 1995). Various methods of numerical simulations such as the Monte Carlo method are also available that permit numerical modeling of the processes occurring during synaptic transmission, and predict the outcome (Rubinstein, 1981; Bartol et al., 1991; Van der Kloot, 1995;

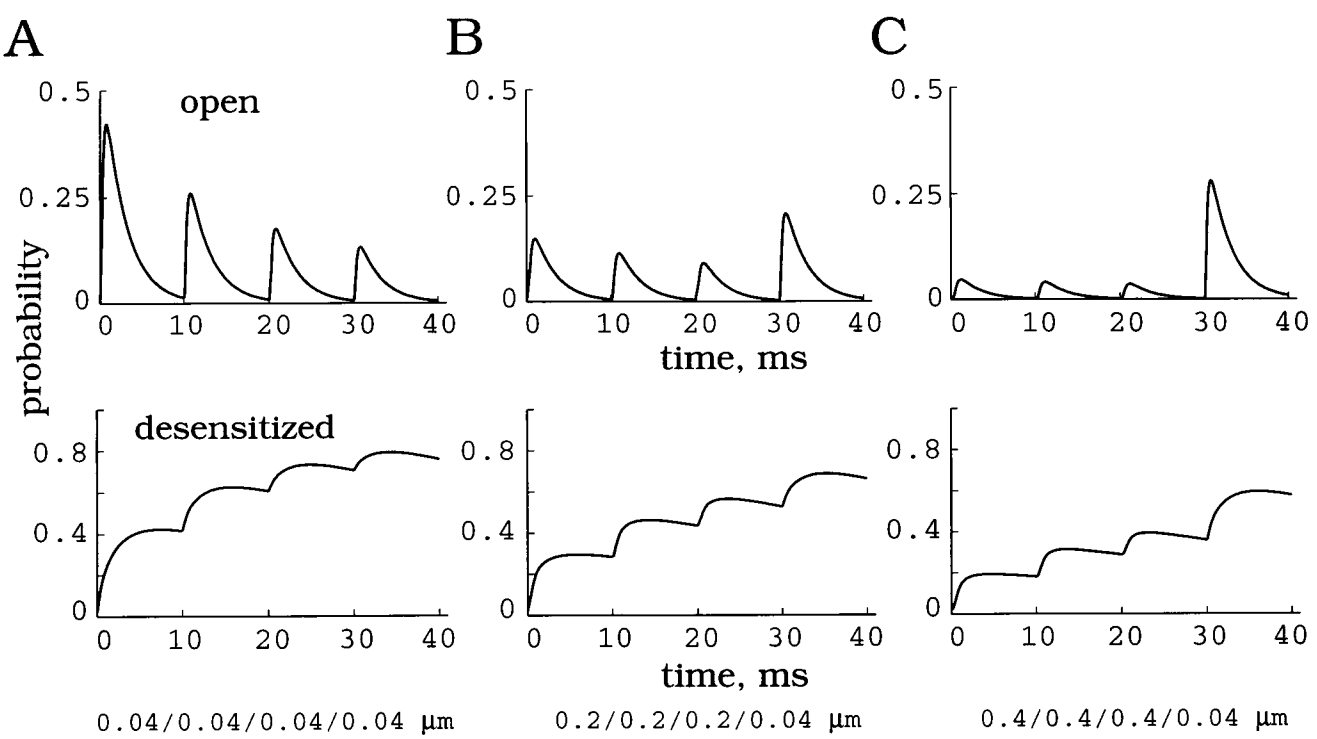

FIGURE 4 Responses of a single AMPA receptor to a continuous signal containing a train of release events at different distances relative to the receptor. Three different paradigms are considered: (*A*) All release events occur at the same focus proximal to the receptor (within 0.04 μm); (*B*) Three release events occur at a distant point (0.2 μm away) that could be within the same synaptic region, while the fourth release occurs at the proximal point (0.04 μm away); (*C*) Three release events occur at a distant point (0.4 μm), which is far enough away to originate in the active zone of a neighboring synapse, while the fourth release occurs at the proximal point (0.04 μm away). Open (state O_4, *top row*) and desensitized (a sum of states $D_5 + D_6 + D_7$, *bottom row*) states are considered (see notations in Fig. 1 *B*). Comparing the amplitudes of the first response in (*A*) with the amplitude of the fourth response in (*C*), one can see how distant release events, possibly from a neighboring synapse, can modify the synaptic efficacy of a receptor response to a release event in its immediate vicinity.

Khanin et al., 1994; Stiles et al., 1996). However, these numerical integrations and simulations techniques require specific knowledge of numerical mathematics and skills in computer programming. Without such knowledge and skills, these techniques cannot be accepted as straightforward. In contrast, we present here a simple analytical method of approximating a system of differential equations containing time-dependent **Q** matrices (Eq. 5) by a series of systems of differential equations with constant but different **Q** matrices. Our straightforward method uses discretization of continuous complex signals as a tool, reducing, therefore, the problem of interactions between multi-state systems and continuous signals to the well-characterized problem of interactions between these systems and specific step functions. Our method of discretization allows a general analytical description of this problem in standard terms of vector occupancy and **Q** matrices. Therefore, in addition to useful specific applications, this method permits further development of theoretical formalisms describing the behavior of multi-state kinetic systems at a greater level of detail. Moreover, our formalism is readily interpreted by high-level computer languages, such as *Mathematica*, making it possible to solve any particular problem regarding interactions between multi-state receptors and continuous signals without any specialized knowledge of numerical mathematics or extensive computer programing experience.

Implications of results

An important goal of any mathematical description of a physiological response is to assist in exploration of the physiological significance of details of proposed dynamic schemes describing subprocesses involved in the generation of that response. By comparing observed and theoretical responses, we can infer how properties of the subprocesses are reflected in the overall response, and begin to test hypotheses of how those subprocesses might be shaped and modulated. In the case discussed here we have examined how the properties of neurotransmitter discharge into and diffusion within the synaptic cleft, as well as details of kinetic schemes describing gating of multi-state ionotropic glutamate receptors, influence the generation of an integrated physiological response, namely a unitary quantal synaptic current. Diffusion out of the synaptic vesicle and within the cleft can be inferred from fundamental principles and from observed structural dimensions of the cleft and of synaptic vesicles (Khanin et al., 1994; Van der Kloot, 1995; Holmes, 1995). Kinetic gating schemes can be inferred from a variety of studies of the response of glutamate receptors to rapid application of known concentrations of glutamate (Lester and Jahr, 1992; Jonas et al., 1993). However, it is not obvious how these processes come together to generate a synaptic current, and a precise description is required. Although numerical approaches have been used to look at this problem in the past, we believe that our analytical approach provides more insight into the origin and accuracy of the results of the analysis.

The generalized description presented here confirms our earlier conclusion, based on a simplified two-state receptor gating scheme, that "off-center" release will increase the variability of the AMPA component of uePSCs, and that the low affinity of AMPA receptors minimizes response cross talk between adjacent synapses (see Uteshev and Pennefather, 1996b). The NMDA component, however, was found to be relatively insensitive to the "off-center" release effect, and PRCs as far away as 1 μm from the focus of a vesicular discharge could give a respectable response. This may account in part for the observation made on multi-quantal ePSCs that the AMPA component was more variable than the NMDA component (Kullmann, 1994). Our results support the suggestion by Kullmann et al. (1996) that extrasynaptic glutamate spill-over can explain the observation that under certain conditions evoked excitatory PSCs will have only an NMDA component before inducing long-term potentiation (LTP), but exhibit both AMPA and NMDA components after LTP has been induced. Our description suggests that glutamate diffusing from nearby synapses on dendrites of adjacent neurons could activate NMDA receptors at synapses on the recorded neuron without activating AMPA receptors at those same synapses. After an LTP induced enhancement of release probability at previously silent synapses, both NMDA and AMPA responses will now be observed. Our results also predict that diffusion between synapses leading to either a spill-over NMDA response or to AMPA receptor desensitization cross talk may account in part for observed cooperative effects in synaptic plasticity.

APPENDIX

Note 1

The $\mathbf{Q}_j$ matrix for the n-state kinetic scheme corresponding to the jth step function can be written as follows

$$Q_j = \begin{pmatrix} -\sum_{h=1,(h\neq 1)}^{n} k^j_{1h} & k^j_{12} & \cdots & k^j_{1m} & \cdots & k^j_{1n} \\ k^j_{21} & -\sum_{h=1,(h\neq 2)}^{n} k^j_{2h} & \cdots & k^j_{2m} & \cdots & k^j_{2n} \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ k^j_{m1} & k^j_{m2} & \cdots & -\sum_{h=1,(h\neq m)}^{n} k^j_{mh} & \cdots & k^j_{mn} \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ k^j_{n1} & k^j_{n2} & \cdots & k^j_{nm} & \cdots & -\sum_{h=1,(h\neq n)}^{n} k^j_{nh} \end{pmatrix} \quad \text{(A1)}$$

The elements on the main diagonal (r_{mm}) of the $\mathbf{Q}_j$ matrix are negative sums of rate constants characterizing transitions from the mth state to all other states h, i.e., $r_{mm} = -\Sigma^{n}_{h=1,(h\neq m)} k^j_{mh}$; every remaining element (r_{mh}) standing not on the main diagonal is a rate constant characterizing transition from the state m to the state h for the jth step, i.e., $r_{mh} = k^j_{mh}$. In the case when the interval between neighboring stimuli in a train is large compared to time constants of the slowest transitions between kinetic states, the $\mathbf{Q}_j$ matrices corresponding to subsequent stimuli might not differ significantly. In general, however, this will not be the case because of the buildup of neurotransmitter concentration in the cleft during repetitive stimulation. Note that the determinant of the $\mathbf{Q}_j$ matrix is always zero, reflecting the fact that new states with zero kinetic rate constants can be

always incorporated into any kinetic scheme without distortion. Therefore, a straightforward method of finding steady-state solutions $\mathbf{p}_{ss}$ ($d\mathbf{p}_{ss}/dt = \vec{0} = \mathbf{p}_{ss}Q_{ss}$, where $\mathbf{Q}_{SS}$ is a $\mathbf{Q}$ matrix corresponding to the steady-state concentration of neurotransmitter in the cleft) does not lead anywhere. However, one can show that the steady-state solutions can be presented in a form of Eq. 3 (see, for example, Colquhoun and Hawkes, 1995).

Note 2

Here we present calculations of the time course of neurotransmitter concentration in the synaptic cleft during a train of continuous neurotransmitter releases occurring at different moments at different points of the presynaptic active zone (see also Uteshev and Pennefather, 1996b). The following assumptions have been made: 1) noninstantaneous (exponential) source of release; 2) two-dimensional diffusion in the cleft; 3) homogeneous nonsaturable uptake; and 4) binding of neurotransmitter molecules to postsynaptic receptors does not affect neurotransmitter concentration in the cleft. With a continuous source of neurotransmitters that represents a train of signals, concentration of molecules in the cleft at different times can be found as a convolution of the source $\Phi_\Sigma(t)=\Sigma_{i=1}^{I}\Phi_i(t)=\Sigma_{i=1}^{I}q_i[\Theta(t-t_i)-\Theta(t-t_{i+1})]e^{-\phi_i(t-t_i)}$ with the fundamental solution of the diffusion equation (i.e., the solution for instantaneous sources, each ith of which ($i \in [1, I]$) is displaced by a radius-vector $\mathbf{r}_i$), $\mathcal{E}(\mathbf{r}, \mathbf{r}_i, t) = (4\pi Dht)^{-1}e^{-(\mathbf{r}-\mathbf{r}_i)^2/4Dt}$. Therefore, the time course of concentration from the entire source (all I continuous sources) can be found explicitly as a sum

$$C(\mathbf{r}, t) = \sum_{i=1}^{I}(\mathcal{E}(\mathbf{r}, \mathbf{r}_i, t)*\Phi_i(t))$$

$$= (4\pi Dh)^{-1}\sum_{i=1}^{I}\left(q_i e^{\phi_i t_i}\int_0^t\left\{[\Theta(\tau - t_i) - \Theta(\tau - t_{i+1})]e^{-((\mathbf{r}-\mathbf{r}_i)^2/4D(t-\tau)+\phi_i\tau)}\right\}\frac{d\tau}{(t-\tau)}\right) \quad \text{(A2)}$$

where $\Theta(t)$ is a Heaviside function; $h = 0.02$ μm is the width of the cleft; $D = 0.3$ μm^2/ms is the diffusion coefficient; q_i and ϕ_i (in ms^{-1}) are amplitude and time constants of the exponential release source (see for values Uteshev and Pennefather, 1996b); t (in ms) is time counted from the beginning of transmission, t_i (in ms) is time from the beginning of stimulation to the beginning of the ith stimulus ($t_1 = 0$); τ is a variable of integration.

Note 3

Responses from the entire PRC have been calculated in two steps. We have first calculated the time course of transmitter concentration on the postsynaptic membrane at different distances (between 0 μm and 1 μm with the step of 0.04 μm) from the focus of release. Then, a PRC of diameter 0.4 μm was placed at different displacements from the focus of release: 0 μm, 0.04 μm, . . . , 1 μm, and responses from different areas of PRC were approximated as described previously (see Uteshev and Pennefather, 1996b, Appendix B).

This work was supported by the Medical Research Council and Neuroscience Network (NCE) of Canada.

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