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A Mathematical Description of Miniature Postsynaptic Current Generation at Central Nervous System Synapses

Vladimir V. Uteshev and Peter S. Pennefather

Department of Physiology and Faculty of Pharmacy, MRC Nerve Cell and Synapse Group, University of Toronto, Toronto, Ontario M5S 2S2 Canada

ABSTRACT Variation in the amplitude of miniature postsynaptic currents (mPSCs) generated by individual quanta of neurotransmitter is a major contributor to the variance of evoked synaptic responses. Here we explore the possible origins of this variability by developing a mathematical description of mPSC generation and consider the contribution of "off-center" release to this variability. By "off-center" release we mean variation in the distance between the position where a presynaptic vesicle discharges its content of neurotransmitter into the synaptic cleft and the center of a cluster of postsynaptic receptors (PRCs) that responds to those transmitter molecules by generating an mPSC. We show that when the time course of quantal discharge through a fusion pore (noninstantaneous release) is considered, elementary analytical descriptions of the subsequent diffusion of transmitter within the synaptic cleft (with or without uptake) predict the development of significant gradients of transmitter concentration during the rising phase of mPSCs. This description of diffusion is combined with a description of the pharmacodynamics of receptors in the PRC and of the time dependence of the gradient of transmitter concentration over the area of the PRC to reconstruct the time course and amplitude of an mPSC for a synapse of a given geometry. Within the constraints of known dimensions of presynaptic active zones and postsynaptic receptor clusters at CNS synapses, our analysis suggests that "off-center" release, produced by allowing release to occur anywhere within an anatomically defined presynaptic active zone, can be an important contributor to mPSC variability. Indeed, modulation of the influence of "off-center" release may be a novel way of controlling synaptic efficacy. We also show how noninstantaneous release can serve to focus the action of neurotransmitter within a given synapse and thereby reduce cross-talk between synapses.

INTRODUCTION

Synaptic neurotransmission in the central nervous system (CNS) is the product of a chain of rapidly (<1 ms) developing processes occurring within each synapse. These include vesicular release of neurotransmitter, diffusion of neurotransmitter in the synaptic cleft, collision with and activation of postsynaptic receptors by neurotransmitter, and uptake and clearance of neurotransmitter from the cleft. Each of these processes is modulated by the structure and geometry of the synapse. To fully understand the mechanism of neurotransmission at CNS synapses, it is important to establish relationships between all of the processes involved. Experimental observations place constraints on the values of physical and structural parameters of synapses under different experimental conditions. On the other hand, theoretical considerations, made explicit through mathematical formulation, predict consequences of various combinations of the synaptic parameters and therefore establish testable rules of how the underlying mechanisms could interact in the expression of synaptic transmission at particular synapses. Here we develop a mathematical description of synaptic transmission from first principles with the aim of exploring those rules.

A fairly accurate picture of the structure of central synapses has developed over the last few decades. These are characterized by a cluster of about 100 small electronlucent synaptic vesicles, 40 nm in diameter, localized to axonal varicosities or bouton-like swellings at the ends of axonal branches. A small area of the varicosity or bouton surface membrane forms a flattened region of close apposition with the shaft of a dendrite or the head of a dendritic spine, creating a synaptic cleft 15–20 nm in width (Fig. 1 *A*). This region of presynaptic membrane is often associated with electron-dense material that is thought to delimit an area known as the active zone (AZ), where evoked transmitter release is thought to occur. Typically at central synapses the AZ has an area of about 0.07 μm^2 (Rosahl et al., 1995; Sur et al., 1995), and assuming circular geometry, this predicts a mean diffusional distance of about 0.15 μm from the center to the edge of such a synapse.

At many synapses there is also a region of electron-dense material referred to as the postsynaptic receptor density (PSD) with dimensions closely corresponding to those of the AZ. Both histological (Craig et al., 1993; Nusser et al., 1995; Weinberg et al., 1995) and physiological (Jones and Baughman, 1991; Vogt et al., 1995) experiments indicate that the postsynaptic membrane opposite the presynaptic AZ, and the associated clusters of vesicles, contain a much higher density of neurotransmitter receptors than is found in the surrounding dendritic region. Although there is less information about the exact distribution of receptors within the postsynaptic region, it is clear that at many types of synapses between CNS neurons the average amplitude of

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Address reprint requests to Dr. P. Pennefather, Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, ON M5S 2S2 Canada. Tel.: 416-978-6609; Fax: 416-978-8511; E-mail: uteshev@phm.utoronto.ca; pspen@phm.utoronto.ca.

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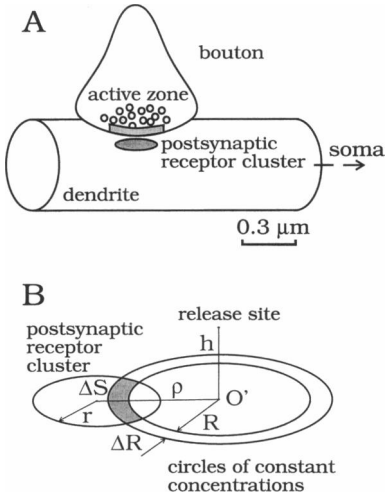

FIGURE 1 Geometry of the PRC. (*A*) A schematic representation of a typical punctate synapse. In the CNS, many synapses releasing glutamate, GABA, or glycine are made up of a terminal bouton formed at the end of an axon branch that abuts onto a dendritic shaft or a dendritic spine. The bouton contains small, clear vesicles localized over a region of electron-dense material that is thought to delimit the region where evoked and spontaneous quantal release can occur (AZ). A postsynaptic receptor cluster (PRC) appears to be localized below the presynaptic AZ and separated from the presynaptic terminal by a synaptic cleft about 20 nm wide. (*B*) Calculation of regions of constant concentration. If we assume that the disk on the left (of radius r) is a PRC and that the pair of concentric circles define regions of essentially equal concentration between distances R and $R + \Delta R$ from the focus of release (O', the point of projection of the release site onto the postsynaptic membrane), then the area of the PRC (ΔS) exposed to an approximately constant concentration at any moment after release can be calculated, and numbers of receptors in that region can be determined if receptors are assumed to be evenly distributed throughout the PRC.

fast mPSCs reflects the simultaneous activation of around 20 receptor channels (Traynelis et al., 1993; Legendre and Korn, 1994; Abdul-Ghani et al., 1996). Because the largest mPSCs can be three to five times greater than the average value, the maximum number of channels activated by a quantum of transmitter at these synapses will be 60–100. If receptor saturation is approached at the peak of these largest responses, a typical PRC would contain 100 receptors. Distributed over a typical PSD region (0.07 μm^2), these 100 receptors would have a density of 1400/μm^2 (higher densities would be indicated if receptors were not saturated during the largest mPSCs). Although this density may be an underestimate, it is nevertheless an order of magnitude lower than that reported for structurally similar nicotinic receptor found at the neuromuscular junction, where receptors are known to be tightly packed (see Land et al., 1981). Therefore, for the purpose of generality, we introduce the concept of a postsynaptic receptor cluster (PRC) to represent a functional unit of receptors distributed over an area smaller than the PSD and in which receptors are tightly packed (e.g., if 100 receptors were packed at a density of 10,000/μm^2 they would occupy a circular PRC with a diameter of 0.113 μm). In this paper we show how the size and position of the PRC relative to that of the AZ will influence the efficacy of mPSC generation resulting from the discharge of transmitter molecules found in a single synaptic vesicle.

Within the constraints of known information regarding synaptic structure, we show how two opposing processes, 1) the discharge of neurotransmitter from a synaptic vesicle into the synaptic cleft and 2) the subsequent clearance of those transmitter molecules from the synaptic cleft by diffusion and uptake, determine the time course of the concentration of neurotransmitter in the vicinity of receptors in PRCs. When combined with receptor pharmacodynamics, this concentration time course determines the amplitude and shape of mPSCs.

Special attention is given to the approximation of instantaneous vesicular release, which is widely used in models of mPSC generation (Land et al., 1981; Faber et al., 1992; Holmes, 1995; Wahl et al., 1996). We show that although the release of neurotransmitter into the synaptic cleft is likely to be fast, the approximation of instantaneous release may be misleading. In particular, we find that the distribution of transmitter within the synaptic cleft is very sensitive to the duration and rate of efflux of transmitter from the synaptic vesicle into the synaptic cleft. By modeling noninstantaneous release as being mediated by a stable fusion pore (see Spruce et al., 1990; Khanin et al., 1994; Van der Kloot, 1995), we describe vesicular release as equivalent to an exponentially declining source with a time constant that is directly related to the conductance of the fusion pore. We demonstrate that despite the small dimensions of a CNS synapse, significant gradients of neurotransmitter concentration can exist within the cleft after release. Noninstantaneous release leads to a stabilization of these gradients such that they persist during the rising phase of the mPSC. Moreover, we will show that noninstantaneous release results in less cross-talk between adjacent synapses.

The presence of gradients of neurotransmitter in the synaptic cleft during the rising phase of the mPSC will determine the consequences of "off-center" release. By "off-center" release we mean release that occurs at a significant distance from the center of the PRC. This might arise if release can occur at any point within the AZ. Our analysis suggests that "off-center" release may cause a greater contribution to mPSC amplitude variation than has previously been suggested by models employing the assumption of instantaneous release (see Faber et al., 1992; Wahl et al., 1996). Indeed, modulation of the influence of "off-center" release may be a novel way of controlling synaptic efficacy.

MATERIALS AND METHODS

The neurotransmitter concentration transient experienced by postsynaptic receptors during synaptic transmission depends on a synthesis of two opposing processes: delivery of neurotransmitter to and clearance from the synaptic cleft, which together with the pharmacodynamic properties of those receptors determine the amplitude and time course of synaptic currents. Two subprocesses are principally responsible for the clearance of neurotransmitter from the cleft: diffusion away from the focus of release

and uptake (loss), both of which depend on the concentration of neurotransmitter in the cleft (Eccles and Jaeger, 1958; Mennerick and Zorumski, 1995) and therefore will depend on the time course of neurotransmitter release into the cleft. To estimate the sensitivity of the process of mPSC generation to the rate and effective duration of the delivery of neurotransmitter to the cleft we have considered and compared two hypothetical types of quantal release: instantaneous and noninstantaneous (exponentially declining source).

Three general assumptions have been made to simplify our mathematical description: 1) At a unitary punctate synapse with one AZ, only one quantum of neurotransmitter can be released at a given time. 2) Neurotransmitter moves from the synaptic vesicle into the synaptic cleft by first adding to a line source perpendicular to the pre- and postsynaptic membranes and spanning the width of the cleft; hence after release, neurotransmitter will diffuse in two dimensions away from this line source at the focus of release. 3) Neurotransmitter uptake is homogeneous, irreversible, and nonsaturable (see Appendix A). In addition, to simplify the mathematical description, we considered synapses of particular geometry and ion channels of particular kinetics; a circular PRC is considered to contain evenly distributed two-state (closed-open) receptors (Fig. 1).

Detailed development of the mathematical formulation of the problem is presented in the appendices. Appendix A describes the process of diffusion in two dimensions, with the contribution of an exponential source and uptake factored into the diffusion equation. Appendix B describes how the PRC can be divided into regions experiencing roughly constant concentrations of neurotransmitter at any point in time. Appendix C describes how the pharmacodynamics of the channel receptor is collapsed into a two-state formalism and considered. In general, other geometries of the PRCs as well as more complicated kinetic schemes could have been incorporated into the description. But this model is sufficient to demonstrate a number of general concepts. Appendix D describes the method used to build an expected amplitude density function on the basis of an arbitrary function that provided successful fits to the results obtained for mPSC amplitudes expected with different displacements between the point of release and the center of the PRC (Figs. 4 and 5). In all of these calculations we have deliberately not considered the influence of receptor desensitization (Raman and Trussell, 1992), stochastic variance (Faber et al., 1992), or variation in the amount of glutamate released with each quantal event (Bekkers et al., 1990). These additional sources of variance will add to the particular source of variance considered here. All descriptions were programmed and explored using the symbolic calculation program Mathematica, run on a Pentium personal computer or a Digital Equipment Corporation α 3000 workstation.

Synaptic parameters

Synaptic geometry

Several reports suggest that for punctate synapses found in the CNS the width of the synaptic cleft is 0.02 μm (Peters et al., 1976; Suedhof, 1995) and the area of the presynaptic AZ is around 0.07 μm^2 (Rosahl et al., 1995; Sur et al., 1995). Here we consider a circular AZ with a diameter of 0.3 μm (area 0.071 μm^2) and a circular PRC, concentric with the AZ, with a diameter of either 0.125 μm or 0.3 μm. These PRC diameters lie within the observed range of sizes of PSDs that are thought to encompass the PRC at glutamate synapses (Suedhof, 1995). The density of receptors in the PRC was set to be 8150/μm^2 or 1408/μm^2 (i.e., 100 receptors per PRC). These density values are somewhat arbitrary because the outcome of all calculations was obtained in terms of the percentage of PRC receptors activated, but are chosen to ensure that a calculated "average" mPSC generally reflects the activation of about 20 channels (see Traynelis et al., 1993; Legendre and Korn, 1994; Abdul-Ghani et al., 1996). Because the number of receptors within the PRC is expected to be small, we did not consider possible depletion of neurotransmitter molecules in the cleft due to receptor binding.

Diffusion

Diffusion of neurotransmitter in the synaptic cleft was considered to be two-dimensional. Free diffusion of molecules the size of glutamate, GABA, or glycine in water is characterized by a diffusion coefficient of around 1 μm^2/ms (Herz et al., 1969). In a restricted space such as a CNS synaptic cleft, where many different types of organic molecules are simultaneously present, in addition to structural obstruction (see Ichimura and Hashimoto, 1988), a smaller value for a diffusion coefficient can be expected (see Holmes, 1995). In our calculations we use a value of 0.3 μm^2/ms. This value is similar to that used by others to define the diffusion of small neurotransmitter molecules in narrow synaptic clefts (Land et al., 1981; Faber et al., 1992; Holmes, 1995).

Vesicular contents and release

In our calculations we assume either 5000 or 2500 molecules per synaptic vesicle (N). These values are consistent with the contents of small clear synaptic vesicles estimated from a variety of sources (ACh-containing vesicles at frog neuromuscular junction [Kuffler and Yoshikami, 1975]; glutamate containing vesicles isolated from brain synaptosomes [Burger et al., 1989; Orrego and Villanueva, 1993]). When a noninstantaneous source was used, it was described by an exponential function of time and given by the formula $\Phi(t) = N\phi e^{-\Phi t}$, where $\Phi(t)$ is the intensity of the source (number of molecules released per unit of time at time t). As described by Khanin et al. (1994) and Van der Kloot (1995), an exponentially declining source would be expected if release of the vesicular neurotransmitter contents occurred through a small fusion pore spanning the synaptic vesicle and the presynaptic terminal membrane. The decay rate ϕ (ms^{-1}) would then be equal to $(\pi r^2 D/VL)$, where r is the radius of the pore, L is the length of the pore, V is the volume of the vesicle, and D is the diffusion coefficient of neurotransmitter in the pore. For small molecules like glutamate or glycine with a Stokes-Einstein radius of around 0.5 nm, drag in a pore of radius 1 nm will reduce D to 0.3 μm^2/ms or about 30% the diffusion coefficient of the neurotransmitter in free solution (Deen, 1987; Khanin et al., 1994).

Uptake

To simplify our calculation, uptake was assumed to be homogeneous, irreversible, and not saturable. This type of uptake implies that the lifetime of molecules in the synaptic cleft after being discharged from a synaptic vesicle is exponentially distributed such that the total number of neurotransmitter molecules in the cleft will decline exponentially. A number of different studies have suggested time constants of neurotransmitter clearance in the cleft ranging from 100 to 300 μs (Eccles and Jaeger, 1958; Tong and Jahr, 1994) to 1.2–1.5 ms (Clements et al., 1992). Accordingly, we introduced a removal process with rate constants of 1 ms^{-1} or 3 ms^{-1} and determined the influence of this simulated uptake on the predicted mPSC amplitude.

Channel pharmacodynamics

Receptors of the PRC were considered within a two-state kinetic model,

$$C \underset{k_2}{\overset{k_1}{\rightleftarrows}} O,$$

where k_1 and k_2 are derived from a more complicated kinetic model:

$$C + nA \underset{\beta}{\overset{k_D}{\rightleftarrows}} A_nC \overset{\alpha}{\rightleftarrows} A_nO,$$

where $k_2 = \beta$ and $k_1 = \alpha A_nC = \alpha(A/(A + K_D))^n$; concentration (in mM) is calculated for each time and each position on the postsynaptic membrane, using Eq. A3, which describes diffusion and loss of neurotransmitter in the synaptic cleft (Appendix A). In this model, k_2 will correspond to the mean open time of the channel, K_D is a dissociation constant defining the affinity of equivalent binding sites for neurotransmitter on closed receptor channels, and n will reflect the number of those sites per synaptic receptor that must be occupied for the associated channel to open with a rate α.

A model of glutamate and glycine synapses

For our analysis we will focus primarily on excitatory glutamate synapses. We believe, however, that the principles are also applicable to other types of synapses, because excitatory and inhibitory synapses in the CNS can exhibit similar dimensions (Suedhof, 1995 (glutamate); Nusser et al., 1995 (GABA); Sur et al., 1995 (glycine)). We assume that activation of GluR receptors responsible for mPSCs is adequately described by our two-state model (see above) and the following parameters: $K_D = 600$ μM, $n = 2$, $\alpha = 4.2$ ms^{-1}, $\beta = k_2 = 0.3$ ms^{-1}. These parameters predict responses to brief applications of glutamate similar to those predicted by a more elaborate model presented by Jonas et al., (1993) to describe mPSCs recorded in pyramidal neurons of the hippocampal CA_3 region (data not shown). At our hypothetical excitatory synapse we have set the rate of glutamate release from the vesicle at $\phi = 5$ ms^{-1}; this is close to what is predicted for a glutamate vesicle with a radius of 17 nm ($V = 2.06 \times 10^{-5}$ μm^3; Bekkers et al., 1990) and a fusion pore of radius 1 nm and length 10 nm (Spruce et al., 1990). These parameters are similar to those used by Khanin et al. (1994) and Van der Kloot (1995) to estimate the rate of diffusion of ACh out of small, electronlucent, synaptic vesicles undergoing exocytosis at the frog neuromuscular junction. Although glycine synapses exhibit dimensions similar to those of glutamate synapses, the affinity of glycine receptors is higher. In some calculations, therefore, we consider receptors with $K_D = 20$ μM and $n = 1.7$. Despite leaving the parameters α and β the same as for glutamate, our two-state formalism predicts responses similar to those predicted by the more elaborate model of glycine receptor pharmacodynamics used by Faber et al. (1992) (data not shown).

RESULTS

Dependence of the pre-open state (A_nC) occupancy of a single receptor on its position relative to the focus of release

On the basis of a 2D diffusion model and the kinetic scheme described above we were able to calculate explicitly the pre-open state (A_nC) occupancy of individual receptors on the postsynaptic membrane as a function of the distance between that receptor and the point of projection of the focus of release onto the postsynaptic membrane. This calculation ignores the subsequent stabilization of the pre-open state that occurs after isomerization to the open state, but provides a first approximation of receptor occupancies at early times after the initiation of release during the rising phase of the mPSC.

Fig. 2 shows how this occupancy changes as the displacement between the focus of release and the position of an individual receptor increases. Each line describes this function at different times after release. Glutamate receptor pharmacodynamics are assumed, and both noninstantaneous and instantaneous release models are considered. Fig. 2 *A* shows what is predicted for release associated with an exponential source of transmitter. A pronounced dependence of the pre-open state upon displacement over spatial dimensions similar to those of a synaptic cleft (e.g., 0–0.2 μm) and over a time period during which the mPSC is generated (e.g., 0–500 μs) reflects a significant gradient of neurotransmitter concentration within the cleft during mPSC generation. This gradient in turn can generate a range of postsynaptic responses depending on the position of the

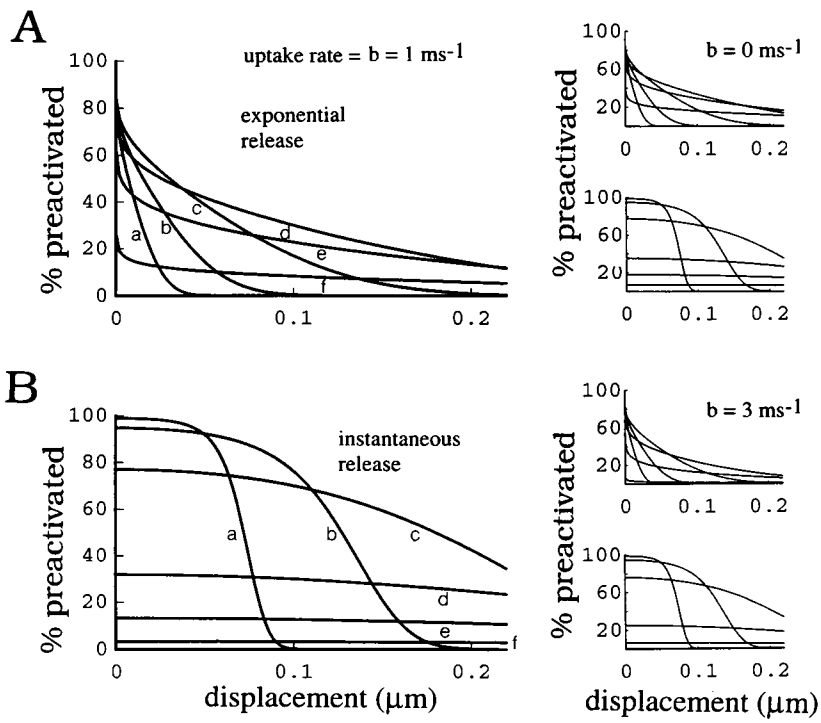

FIGURE 2 Dependence of the occupancy of the pre-open state (A_nC) of an individual receptor on its position relative to the focus of release. (*A*) An exponential source of neurotransmitter releasing 5000 molecules with a time constant of 0.2 ms. Displacement indicates a distance between a single receptor located on the postsynaptic membrane and a source of neurotransmitter within the model of synapse discussed in the text. Parameters appropriate for a glutamatergic synapse are assumed. Letters below each curve indicate that the curves refer to the following times after release: a, 1 μs; b, 5 μs; c, 25 μs; d, 125 μs; e, 250 μs; f, 500 μs. (*B*) A synaptic model similar to that considered in *A*, but with an instantaneous source. Letters correspond to the same time points as in *A*. An instantaneous source does not provide strong gradients of concentrations in the cleft within 100 μs after release. In both *A* and *B*, the uptake rate (b) is set at 1 ms^{-1}. The inset on the right shows plots similar to those in *A* and *B*, but for b set at 0 ms^{-1} (*top*) and 3 ms^{-1} (*bottom*).

center of the PRC relative to the focus of release events responsible for particular mPSCs.

This gradient originates as the result of an interaction between delivery of transmitter into the cleft at a specific point and diffusion of transmitter molecules from that point conditioned by uptake. With the diffusion coefficient and the synaptic dimensions used here, instantaneous release leads to relatively uniform distributions of the preactivated receptors shortly after release (within 25 μs; Fig. 2 *B*), reflecting a rapidly developing equilibration of transmitter concentration within the cleft (see also Clements, 1996). However, if diffusion is made unreasonably slow, gradients during the rising phase of the mPSC are seen, even with instantaneous release (not shown). The simple form of uptake used here will reduce transmitter concentration homogeneously in space and exponentially in time (Appendix A) and thus not affect the nature of the gradient. This is evident from the results shown in the inset to Fig. 2, which shows analogous results only with uptake rate, *b*, set at either 0 (*top pair*) or 3 ms^{-1} (*bottom pair*). Hence, with uptake rates ranging from 0 to 3 ms^{-1} and reasonable diffusion coefficients and synaptic dimensions, the position of the PRC relative to the release point is less important in determining the amplitude of the resulting mPSCs with instantaneous release than with exponential release.

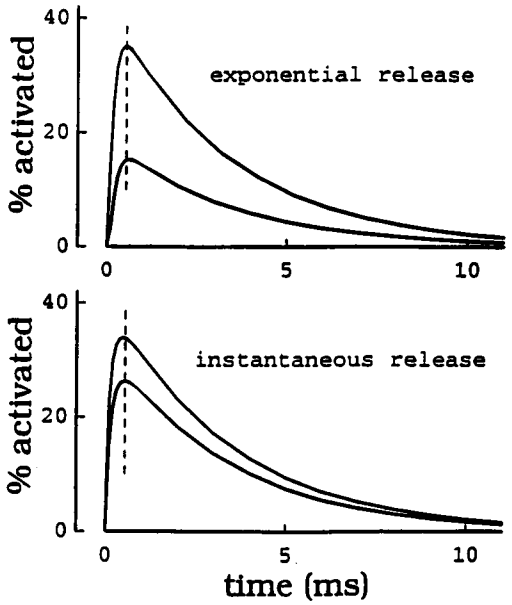

FIGURE 3 The time course of the simulated miniature synaptic currents with instantaneous and noninstantaneous release. Synaptic parameters were similar to those used in Fig. 2 (i.e., glutamate receptor pharmacodynamics; uptake rate, 1 ms^{-1}; circular AZ with diameter 0.3 μm and concentric PRC with diameter 0.125 μm). (*Top traces*) Two mPSCs generated by an exponential source of neurotransmitter. The larger current corresponds to the case where the center of the small PRC is displaced only 0.02 μm from the center of the AZ and the smaller current is that predicted for a displacement of 0.15 μm. (*Bottom traces*) Two mPSCs obtained for the same displacements and within the same model of synapse as in the top traces, but for an instantaneous source of neurotransmitter. All currents reach their maximum within the interval 0.6 to 0.8 ms after the release has occurred. The dashed line indicates the time of occurrence of the peak of the larger current at the top of the inset.

Influence of synaptic parameters on the effect of "off-center" release

Using Eq. C4 of Appendix C, we have built mPSCs by integrating the receptor occupancy predicted for receptors distributed over the area of the PRC and plotting these values as a function of time. This was done for various distances between the center of the PRC (here, 0.125 μm in diameter) and the focus of release (0.02 μm and 0.15 μm in Fig. 3), when either an exponential source (Fig. 3, *top traces*) or an instantaneous source (Fig. 3, *bottom traces*) was assumed. The type of source did not have a significant influence on the rising phase of simulated mPSCs (*dashed lines*), but it did influence how amplitudes of mPSCs depended on this displacement; the difference between amplitudes of mPSCs generated by release foci with different displacements from the PRC is greater with an exponential source (Fig. 3, *top traces*) than with an instantaneous source (Fig. 3, *bottom traces*).

Fig. 4 shows the influence of "off-center" release for different sets of synaptic parameters when a PRC with a diameter equal to and concentric with the AZ (0.3 μm) is considered. The peak amplitudes for mPSCs expected for seven different displacements were calculated by numerical integration, and then a line was fit to the points using the arbitrary function (see Appendix D). In Fig. 4 *A*, glutamate pharmacodynamics are used; the uptake rate, *b*, was set at 1 ms^{-1}; and vesicle efflux rate, ϕ, was set at 5 ms^{-1} (*open circles*) or made instantaneous (*closed circles*). Instantaneous release leads to slightly greater synaptic efficacy with centered release but is associated with much less attenuation of the mPSC peak with displacement between the release focus and the center of the PRC than is observed with exponential release.

It is physically possible for a 0.4-μm displacement to represent the influence of release at an adjacent synapse. With instantaneous release the mPSC amplitude expected with this displacement is still almost one-third the amplitude produced by centered release. However, the amplitude observed with exponential release and this displacement is much smaller. Thus, exponential or noninstantaneous release will serve to minimize cross-talk between synapses. Synaptic uptake or loss is also important in minimizing cross-talk. Fig. 4 *B* shows that eliminating uptake reduces the decrement in amplitude associated with displacement and increases the synaptic efficacy. Increasing the uptake rate to 3 ms^{-1} has the opposite effect. It is apparent, however, that relying only on the simple form of uptake described here to minimize cross-talk will compromise synaptic efficacy. There may thus be a biological advantage to minimizing cross-talk by slowing influx into the cleft rather than by enhancing efflux out of the cleft; with noninstantaneous release a smaller number of components (e.g., receptors and transmitter molecules) are used more efficiently.

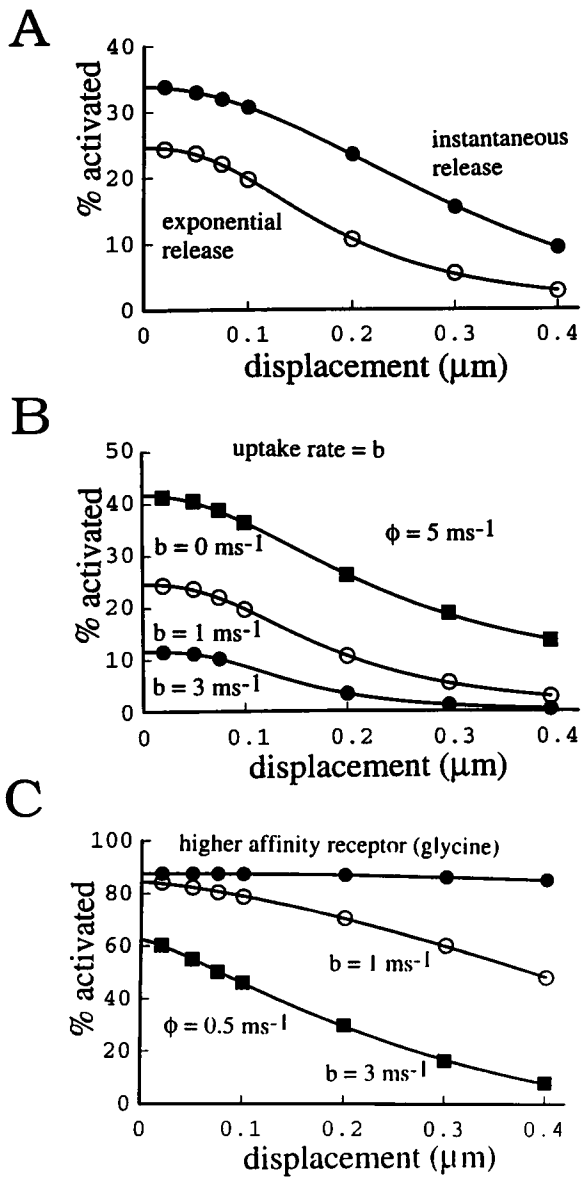

FIGURE 4 Influence of synaptic parameters on the relation between mPSC amplitudes and the position of the PRC relative to the focus of release. Displacement refers to the distance between centers of the PRC and the focus of release. In all cases, the diffusion coefficient was set at 0.3 $\mu m^2/ms$. Kinetic parameters governing binding and activation for *A* and *B* were as described in text for glutamate receptors, and for *C* they were as described for glycine receptors. Both PRC and AZ were concentric, with a diameter of 0.3 μm. For each set of parameters, the amplitude for seven displacement distances was determined. These points were than fit by the function $I/\varsigma = a + b/(1 + (\varsigma/c)^d)$, where ς is a displacement (solid line). (*A*) Instantaneous vesus exponential release. ●, Instantaneous source (N = 5000); ○, exponential source (ϕ = 5 ms^{-1}; N = 5000). (*B*) Effect of uptake rate with an exponential source (ϕ = 5 ms^{-1}; N = 5000). The uptake rate is indicated below each curve. Increasing uptake rate reduces the efficiency of capture of transmitter and increases the decrement in efficiency caused by "off-center release." (*C*) Effect of increased receptor affinity (a glycine synapse). Receptors at this synapse have a 30-fold higher affinity for neurotransmitter. Three conditions are considered: an instantaneous source (N = 5000) (●); an exponential source (ϕ = 0.5 ms^{-1}; N = 5000) (○); the same exponential source but with an uptake rate of 3 ms^{-1} instead of 1 ms^{-1} (■). Note that little variability in mPSC amplitudes is predicted if the release of transmitter is instantaneous (●).

Fig. 4 *C* shows the effect of increasing receptor affinity by decreasing the K_D concentration for generation of the pre-open state by 30-fold (from 600 to 20 μM). This will create receptor pharmacodynamics similar to those expected at glycine or GABA synapses. As reported by Faber et al. (1992), there is no effect of displacement with instantaneous release. However, an effect of displacement can be observed if vesicle efflux rate is reduced to 0.5 ms^{-1}, and this effect is accentuated if uptake rate is increased from 1 to 3 ms^{-1}. With these parameters, synaptic efficacy is still greater than 50% for centered release. The efflux rate might be reduced by increasing the drag on the transmitter molecules exiting the vesicle through the fusion pore or by rendering the fusion pore unstable, so that it flickers open and closed. A number of combinations of uptake rate, efflux rate, and receptor affinity will create a situation where synaptic efficiency is reasonable (i.e., >25%) with centered release and yet falls off with displacement in a way that reduces cross-talk between synapses. This observation also emphasizes the point that synaptic efficacy and synaptic focusing (e.g., enhancement of the influence of displacement) are complex functions of neurotransmitter influx into the cleft, the pharmacodynamics of postsynaptic receptors, and the clearance of transmitter from the cleft.

Fig. 5 shows plots similar to those in Fig. 4, only now obtained from a model, similar to that used in Fig. 3, where the PRC is still concentric with the AZ of diameter 0.3 μm but has a diameter of 0.125 μm. With other parameters equal, this change in geometry enhances synaptic efficacy and synaptic focusing (compare open symbols in Fig. 5 *A* to open circles in Fig. 4, *A* and *B*). Indeed, with centered release the mPSC is larger with the more tightly packed PRC reflecting the fact that the same number of receptors will be on average closer to a centered focus of release. Fig. 5 also describes the effect of reducing vesicle efflux rate. This causes a proportional decrease in the amplitude of mPSCs resulting from centered release. In Fig. 5 *B* efflux rate has been reduced by reducing the number of transmitter molecules in the vesicle. The dashed line has the same shape as that describing the effect of displacement, with all points having been multiplied by 2. The fact that this scaled curve overlaps that obtained with N = 5000 suggests that vesicular content has little effect on the synaptic focusing effect and that the mPSC amplitude will be proportional to vesicular content. Thus, variability in vesicular contents, such as might arise from variation in vesicle volume (see Bekkers et al., 1990), or contents released, such as might occur if the fusion pore closes before discharge is complete, should accentuate synaptic variability, but in a manner that is independent of synaptic geometry.

Once the relation between displacement and amplitude has been defined, it becomes a simple matter to predict the influence of "off-center" release on amplitude distribution functions. All that is needed is to make an assumption regarding the distribution of release points in the AZ. If it is assumed that release can occur anywhere within the AZ with equal probability, then the chance of centered release

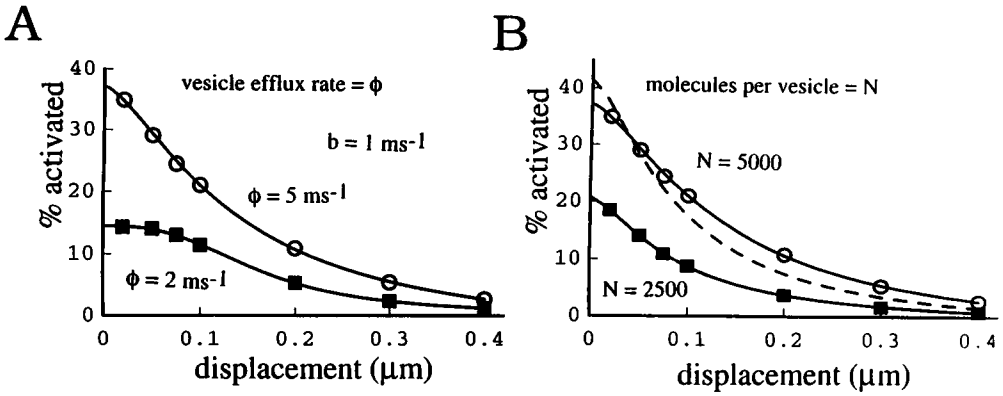

FIGURE 5 Influence of displacement on mPSC amplitude when the PRC is smaller (diameter 0.125 μm) than the AZ (diameter 0.3 μm). (*A*) Effect of vesicle efflux rate (ϕ). The data presented are similar to those in Fig. 4, except that the PRC diameter is smaller (0.125 μm). The points described by the open circles were generated using the same parameters as the open circles in *A*, where the PRC has the same dimensions as the AZ. Note that reducing the size of the PRC increases the efficiency of centered release and increases the decrement with displacement. Reducing ϕ reduces the synaptic efficiency, but has a greater effect on "centered" release than on "off-center" release. (*B*) Effect of the number of transmitter molecules (N) released per vesicle. The PRC dimensions are the same as in *A*, but similar results are obtained with the larger PRC. The dashed line has the same shape as the solid line through the closed squares (N = 2500), but is multiplied by 2. The overlap between this dashed line and the line through the open circles (N = 5000) indicates that the influence of N is roughly linear, even when the PRC is displaced.

will be much less than for "off-center" release. Fig. 6 shows amplitude distributions predicted for three distinct geometries, assuming equal release probability anywhere within a circular AZ, using glutamate receptor pharmacodynamics, a diffusion coefficient of 0.3 μm^2/ms, setting uptake rate at 1 ms^{-1}, and efflux rate at 5 ms^{-1}. The synaptic geometries are represented schematically on the right of each section. In Fig. 6 *A* equal and concentric PRC and AZ areas are assumed. Here centering the release enhances the mPSC amplitude by only 66% relative to mPSC produced by maximally displaced release within the AZ. The peak on the right of the distribution is artifactual because the derivative of the arbitrary function used to fit the displacement data approaches zero at small displacements. Therefore the density function becomes ill-defined for the small displacements associated with the largest mPSCs (see Eq. D2, Appendix D). Fig. 6 *B* shows that if the PRC is made smaller than the AZ, many release sites will be outside the region facing the PRC and the amplitude distribution will be skewed, with a preponderance of small mPSCs. When the radius of the PRC is half that of the AZ, "off-center" release can result in more than a twofold spread in amplitudes. Fig. 6 *C* shows that other geometries can cause an even greater spread of mPSC amplitudes.

DISCUSSION

We have described analytically three main components of synaptic transmission: 1) diffusion of neurotransmitter in synaptic cleft with either an instantaneous or exponential source of neurotransmitter release; 2) homogeneous, irreversible, and nonsaturable uptake of neurotransmitter in the cleft; 3) ion channel activation defined by a two-state kinetic model. A particular synaptic structure has also been considered. Two types of ion channel kinetics have been considered that correspond to high-affinity (glycine) and low-affinity (glutamate) receptors. We have found that gradients of neurotransmitter concentration in the synaptic cleft, which together with ion channel pharmacokinetics determines the overall synaptic response, strongly depend on the relationship between the processes of neurotransmitter release, diffusion, and uptake. As a result, the relative position of sites of release (determined by the geometry of the AZ) and the center of the PRC (determined by the geometry of the PSD) is an important determinant of mPSC amplitude. We have shown here that for diffusion coefficients in CNS synaptic clefts expected for neurotransmitter amino acids, instantaneous sources will not generate much variability as the result of "off-center" release. This explains why Faber et al. (1992) concluded that off-centered release introduced little additional variability to the amplitude distribution; an instantaneous release source was assumed in that study. Nevertheless, because release mediated through a fusion pore is expected to occur over hundreds of microseconds (see above), conclusions based on previous models assuming instantaneous release should be reexamined.

Wahl et al. (1996) have described a Monte Carlo simulation of glutamate-mediated fast synaptic transmission at CNS synapses. Like Faber et al. (1992), they concluded that "off-center" release has little effect on mPSC amplitude. Many of their assumptions are similar to those used here, but are nevertheless slightly different. For example, they settle on a diffusion coefficient of 0.76 μm^2/ms and a cleft height of 15 nm compared to 0.3 μm^2/ms and 20 nm used here. Furthermore, to predict responses at 37°C, they arbitrarily increased all of the kinetic rate constants, derived by Jonas et al., (1993) to describe responses observed at 22°C, by three- to fourfold. More importantly, they also assumed instantaneous release in the simulation in which the effect of "off-center" release was examined. Although they com-

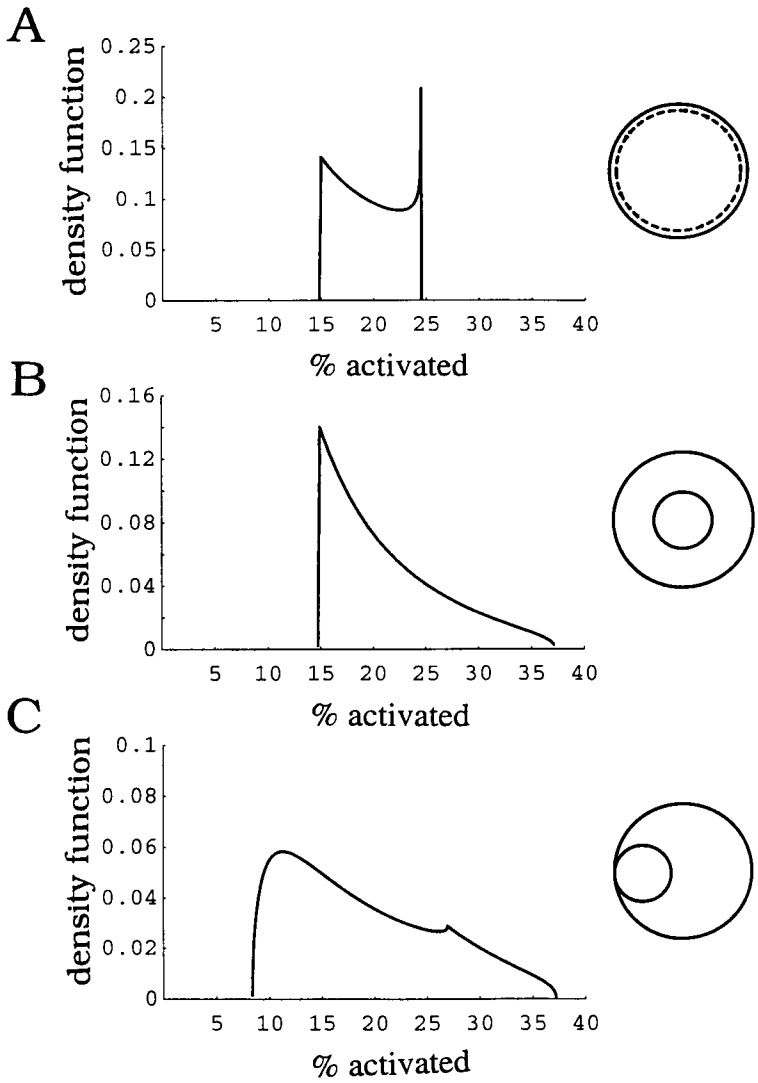

FIGURE 6 Amplitude distributions predicted for different PRC dimensions. The functions described in Fig. 4 relating mPSC amplitude to the displacement of the focus of release from the center of the PRC were used to predict the amplitude distributions expected for different relative positions of the AZ and PRC (represented schematically on the right of each section). These distributions assume that vesicle fusion can occur anywhere within the AZ area with equal probability. (*A*) Equal and concentric AZ and PRC areas (diameter 0.3 mm). This distribution was generated using the line through the open circles in Fig. 4 *A*. The maximum displacement of a release point is 0.15 μm. The flattening of that curve with small displacements leads to the peak in this distribution at the largest amplitude (see text). (*B*) Concentric AZ and PRC with smaller PRC (diameter 0.125 mm). Note the skewed distribution. The large number of small mPSC reflects the large area of the AZ that is outside of the region facing the PRC. (*C*) Smaller PRC offset from the center of the AZ. This asymmetric geometry produces a rising phase to the distribution (as opposed to the discrete transition seen in *A* and *B*) and increases the range of amplitudes expected.

mented on the influence of a fusion pore on mPSC generation, they did not report on the interaction between noninstantaneous release and displacement between the release site and the PRC. Using parameters similar to theirs, we obtain mPSCs with almost identical time courses and amplitudes. This confirms the accuracy of their calculations but not their assumptions.

Another numerical simulation of glutamate-mediated synaptic transmission at CNS synapses has recently been described by Holmes (1995). However, again the results must be interpreted with caution because of the method used to define the influx of glutamate into the synaptic cleft. Holmes's (1995) model essentially proposed that release resulted from an instantaneous expansion of a fusion pore to the width of a synaptic vesicle (i.e., to 0.05 μm), which should allow an efflux rate of at least 125 ms^{-1}, a rate indistinguishable from instantaneous release (see Wahl et al., 1996). Even if the fusion pore did expand, the consequences for the rate of delivery of transmitter into the cleft would be slight, provided fusion expanded linearly with time over a 1–2-ms period (see Spruce et al., 1990); with an initial discharge rate of 5 ms^{-1} most of the transmitter will have left the vesicle within the first 200 μs, during which the fusion pore will have a conductance close to its initial value.

On the basis of our analysis it becomes clear that there are ranges of parameters where instantaneous and exponential sources of release give rise to average synaptic currents of similar shape and amplitude. For example, near the focus of the release amplitudes of currents from the two sources are similar, and rise times of these currents lie within the same range of about 0.6–0.8 ms (Fig. 3). However, the similarities in amplitude break down when "off-center" release is considered. Because isomerization is rate-limiting for channel opening with the Jonas et al. (1993) pharmacodynamic parameters, rise time is not very sensitive to "off-center" release.

We assumed that desensitization did not influence the amplitudes of synaptic currents. It is notable, however, that Tang et al. (1994) reported that a benzothiadiazide, cyclothiazide, which has been shown to destabilize the desensitized state, reduces the variability of the non-NMDA com-

ponents of excitatory postsynaptic currents (EPSCs). This agent also increases the affinity of glutamate receptors for neurotransmitter and may increase the saturation of PRC receptors (Tang et al., 1994). As we showed in Fig. 4 *C*, our model predicts that increasing receptor affinity and receptor saturation at the peak of the mPSC will reduce the variability of mPSCs.

Tong and Jahr (1994) have shown that a block of glutamate uptake by D-L-theo-b-hydroxyaspartic acid (THA) influences mPSC amplitude at 34°C but does not influence it at 24°C. The variability of mPSC amplitudes in both cases remained large (8–20-fold). The authors concluded that a complex of processes with different temperature dependencies was involved in the governing of neurotransmission. It is notable that a pronounced mPSC variation (~8-fold) was still present at 24°C, when THA did not affect the mPSC amplitude distribution. Thus, the origins of amplitude variation, whatever they may be, are affected but not eliminated by increasing temperature. This is consistent with our conclusion that the origin of variability of mPSCs has in fact a complex nature with a number of elementary origins, each of which contributes to the final variability but does not solely determine it.

The amplitude of mPSCs recorded at individual synaptic contacts between CNS neurons exhibits considerable variation (C.V. ~0.5). This appears to be true of synapses between CNS cells found in dissociated cell culture (see Bekkers and Stevens, 1989; Abdul-Ghani et al., 1996) and in brain slices (Raastad et al., 1992). This variance of mPSC amplitudes is likely to be important for information transfer at CNS synapses (see Otmakhov et al., 1994) and thus of biological significance. Rather than being a limitation of the ingenuity of nature (i.e., simply due to variance in the size and hence volume of synaptic vesicles; Bekkers et al., 1990), this variance in response amplitude might be a design feature of synapses that can be regulated. Indeed, despite the amplitude variability observed at individual synapses, the mean mPSC amplitude is remarkably similar for all synapses (regardless of origin) on a given neuron but varies between cells (Liu and Tsien, 1995). Thus, neurons appear to be able to regulate the size of the quantal response but also appreciate quantal diversity.

We have not ruled out the possibility that release only occurs at one point in the active zone, and all of the variance of mPSC amplitudes is due differences in the amount of transmitter released into the cleft, due either to variation in the transmitter content of the vesicle or to the fusion pore duration. Nor, however, can the contribution of "off-center" release to this variability be ruled out. For example, Frerking et al. (1995) have argued that correlation between the amplitudes of GABA-mediated mPSCs recorded simultaneously in two amacrine cells linked by a dynapse supports the variation in transmitter concentration model. However, our model is equally consistent with that data, provided the PRCs on the two sides of the dynapse have a similar spatial relation to that of the release points where a particular mPSC was generated. The standardized mPSC distribution that they observe at the dynapses formed by amacrine cells may simply reflect common features of the fusion pore behavior and a match-up of AZ and PRC dimensions on either side of the dynapse. Indeed, mPSC amplitude variability may have multiple independent sources that complement each other. But our structural hypothesis suggests a simple way by which this variability can be tuned, namely, by changing the shape and position of the PRC relative to the AZ (see Fig. 6).

The principles described here may be of use in explaining how mPSC amplitude can change as the result of induction of forms of synaptic plasticity such as long-term potentiation (LTP), depression (LTD), and kindling. Amplitude histograms of mPSCs are often asymmetrical, with both median amplitudes and shape of the distributions subject to short- and long-term modulation (see Malgaroli et al., 1995). Our analysis shows how this modulation could result from changes in synaptic geometry and the position of foci of release relative to clusters of postsynaptic receptors (see also Edwards, 1995) or small changes in parameters of neurotransmitter release, diffusion, and uptake in the synaptic cleft. Our analysis may provide a starting point for developing a description of how examples of synaptic plasticity are related to concomitant changes in synaptic morphology and biophysical parameters describing the function of synaptic components.

APPENDIX A: 2D DIFFUSIONS IN SYNAPTIC CLEFT (A NONINSTANTANEOUS SOURCE)

In the case of instantaneous release when each quantum contains a constant number of molecules (N), the concentration of neurotransmitter in the cleft can be obtained from the diffusion equation as a function of time and distance within a 2D model of diffusion (a fundamental solution):

$$C(R, t) = \frac{N}{4\pi htD} e^{-R^2/4tD}, \tag{A1}$$

where h is a width of the synaptic cleft; R is a distance between the point of projection of the release site on the postsynaptic membrane and a point of the postsynaptic membrane where the concentration of neurotransmitter is being calculated; and D is a diffusion coefficient.

When a noninstantaneous source $\Phi(t) = N\phi e^{-\phi t}$ is considered, the concentration of neurotransmitter in the cleft can be obtained as a convolution of the fundamental solution (Eq. A1) with the source function $\Phi(t)$ (see Vladimirov, 1971). Thus

$$A(R, t) = \Phi(t) * C(R, t) \tag{A2}$$

If a homogeneous, irreversible, nonsaturable uptake and loss in the cleft also is considered, the final equation for the concentration on the postsynaptic membrane will be

$$A(R, t) = \frac{N\phi e^{-\mu t}}{4\pi hD} \int_0^t e^{-\left(\phi\tau + \frac{R^2}{4D(t-\tau)}\right)} \frac{d\tau}{t-\tau}. \tag{A3}$$

Note: In the case where diffusion of neurotransmitter in the cleft occurs in the presence of uptake (irreversible chemical reaction),

$$(\text{free} \overset{\mu}{\Rightarrow} \text{bound}),$$

the diffusion equation,

$$\left\{\frac{\partial}{\partial t} - D\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}\right) + \mu\right\}A = 0$$

can be reduced to the ordinary diffusion equation

$$\left\{\frac{\partial}{\partial t} - D\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}\right)\right\}A^* = 0$$

by a substitution: $A(\vec{R},t) = A^*(\vec{R},t)e^{-\mu t}$ (Carslaw and Jaeger, 1959).

APPENDIX B: GEOMETRY OF THE PRC

A circular PRC provides a geometric simplification that facilitates the calculations of the fraction of receptors in the PRC exposed to given concentration of neurotransmitter at any given time. On the basis of geometric considerations it can be shown that the area of cross section of two circles of radii r and R whose centers are separated by the distance ρ is

$$S(R, r) = \frac{1}{2}[R^2(\varphi_R - \sin\varphi_R) + r^2(\varphi_r - \sin\varphi_r)], \quad \text{(B1)}$$

where

$$\varphi_r = 2 \arccos\left(\frac{\gamma^2 + \delta^2 - 1}{2\gamma\delta}\right) \quad \text{(B2)}$$

$$\varphi_R = 2 \arccos\left(\frac{\gamma^2 - \delta^2 + 1}{2\gamma}\right)$$

$$\delta = \frac{r}{R} \qquad \gamma = \frac{\rho}{R}. \quad \text{(B3)}$$

If we assume that one of these circles (of radius r) is a receptor PRC and the second one describes a line of equal concentration at a distance R from the focus of release, then the area of the PRC that is exposed to a certain, almost constant, concentration of neurotransmitter will be $\Delta S \approx dS = \partial S/\partial R\, dR$ (Fig. 1 *B*). Thus, the number of channels in the PRC separated from the point O' by distance R (Fig. 1 *B*) that are exposed to a certain, almost constant concentration at any moment t will be

$$dN = \sigma(R)\frac{\partial S}{\partial R}dR, \quad \text{(B4)}$$

where $\sigma(R)$ is a density of receptors over the PRC.

APPENDIX C: PHARMACODYNAMICS OF ION CHANNELS

Within a two-state kinetic scheme the solution for the percentage of receptors that are still closed at time t will be given by the following equation (for any particular radius R) (see Synaptic Parameters):

$$\frac{d}{dt}\zeta(R, t) + \left(k_2 + \alpha\left(\frac{A(R, t)}{A(R, t) + K_D}\right)^n\right)\zeta(R, t) = k_2. \quad \text{(C1)}$$

Solving this equation (it is referred to as a Ricatti equation), one obtains

$$\zeta(R, t) = e^{-\vartheta(R,t)}\left(1 + k_2\int_0^t e^{\vartheta(R,t')}dt'\right)d, \quad \text{(C2)}$$

where

$$\vartheta(R, t) = k_2 t + \alpha\int_0^t\left(1 + \frac{K_D}{A(R, \tau)}\right)^{-n}d\tau, \quad \text{(C3)}$$

where t' and τ are variables of integration and concentration is taken from Eq. A3. Therefore, the proportion of receptors that are open at a time t will be $1 - \zeta(R,t)$. This number of receptors will determine the contribution of the particular cross section of receptors of width dR, that is located at a distance of R from the center O' (Fig. 1 *B*) and is exposed to a given concentration of agonist (curves of equal concentrations).

To obtain a current from the entire PRC one should integrate over the whole area of the PRC. The density of receptors within the PRC can be taken as constant, thus $\sigma(R) \equiv \sigma_0$. Therefore, the proportion of activated receptors over the entire PRC will be

$$\eta(t) = \frac{n(t)}{\pi\sigma_0 r^2} = \frac{1}{\pi r^2}\iint_{(PRC)}[1 - \zeta(R, t)]\,dS(R). \quad \text{(C4)}$$

APPENDIX D: AN AMPLITUDE DISTRIBUTION OF MINIS

We assume that release can occur with equal probability at any point on the AZ. If AZ is a circle, equations similar to Eqs. B1–B3 can be used to determine a spatial distribution of releases that locate within the AZ at the same distance ς from the point of projection of the center of PRC to the presynaptic membrane and therefore will produce synaptic currents of the same amplitude. The distribution function $f(\varsigma)$ will be determined by a derivative of the area of cross section (see Eqs. B1–B3) of the AZ and a circle of radius ς, with the center located at the point of projection of the center of PRC to the presynaptic membrane. On the other hand, the relationships between the peak amplitude of mPSC and the distance ς shown in Figs. 4 and 5 can be well fit by the function

$$I(\varsigma) = a + \frac{b}{1 + (\varsigma/c)^d}. \quad \text{(D1)}$$

Parameters of the function D1 were found to be different for different types of synaptic parameters and conditions of the release (not shown). Therefore, it is possible to establish analytically the amplitude distribution of synaptic currents $g(I)$ in the case of a circular AZ and a circular PRC, the main axes of which are separated by a distance ρ. The following sequence of expressions gives the result

$$dG = f[\varsigma(I)]d\varsigma = f[\varsigma(I)]\left|\frac{\partial\varsigma(I)}{\partial I}\right|dI = g(I)dI, \quad \text{(D2)}$$

where dG is the probability that release occurred within the AZ at a given distance from the projected center of the PRC. Therefore, it is also the probability of occurrence of a synaptic current with an amplitude lying between I and $I + dI$. $\varsigma(I)$ is an inverse function to $I(\varsigma)$ and $f[\varsigma] = 1/S_{AZ}\, d/d\varsigma\,\{S(\varsigma,r_{AZ})\}$ is a normalized spatial distribution of presynaptic releases as seen from the center of the PRC, where S_{AZ} is the area of AZ and the function $S(\varsigma,r_{AZ})$ is taken from Eq. B1 with the following substitutions of parameters: $\varsigma \to R$ and $r_{AZ} \to r$. The derivative of the function $S(\varsigma,r_{AZ})$ is somewhat complex and is not presented explicitly here. Therefore, $g(I)$ will be

$$g(I) = \frac{f[\varsigma(I)]}{(I - a)^2}\left|\frac{bc}{d}\left(\frac{b}{I - a} - 1\right)^{1/d-1}\right|. \quad \text{(D3)}$$

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