Mechanisms Shaping Fast Excitatory Postsynaptic Currents in the Central Nervous System

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How different factors contribute to determine the time course of the basic element of fast glutamate-mediated excitatory postsynaptic currents (mEPSCs) in the central nervous system has been a focus of interest of neurobiologists for some years. In spite of intensive investigations, these mechanisms are not well understood. In this review, basic hypotheses are summarized, and a new hypothesis is proposed, which holds that desensitization of AMPA receptors plays a major role in shaping the time course of fast mEPSCs. According to the new hypothesis, desensitization shortens the time course of mEPSCs largely by reducing the buffering of glutamate molecules by AMPA receptors. The hypothesis accounts for numerous findings on fast mEPSCs and is expected to be equally fruitful as a framework for further experimental and theoretical investigations.

1 Factors Shaping the Kinetics of Fast mEPSCs: Historical Background

Fusion of synaptic vesicles with presynaptic membrane of excitatory synapses in the central nervous system leads to a release of glutamate. Glutamate is believed to reach high concentrations before decaying rapidly (Clements, 1996). The kinetics of glutamate release, diffusion, binding by the receptors, and uptake by the transporters, as well as synaptic geometry, are all expected to influence its spatiotemporal concentration profile in the synaptic cleft, which together with the receptor properties (kinetics, density, and spatial distribution) determine the amplitude and the time course of the basic element of synaptic transmission: miniature excitatory postsynaptic current (mEPSC). The fast component of the excitatory postsynaptic current (EPSC) in neurons of the central nervous system results from activation of AMPA ($\alpha$-amino-3-hydroxy-methyl-isoxazole)-type glutamate receptors. How different factors contribute to shaping the time course of mEPSCs has been a focus of interest of neurobiologists for some years. In spite of intensive investigations, these mechanisms are not well understood, though determining them is critical to issues of synaptic specificity and the induction of synaptic plasticity. In this review, basic hypotheses of
mechanisms shaping the time course of fast glutamate-mediated excitatory postsynaptic currents in the central nervous system are summarized and a new hypothesis is proposed.

Three hypotheses have been traditionally put forward to explain the rate of decay of the synaptic current (Jonas & Spruston, 1994). The first follows the line of argument used to explain the time course of postsynaptic current at the neuromuscular junction (Magleby & Stevens, 1972). The decay of glutamate concentration in the synaptic cleft is assumed to be very rapid, the decay time of the postsynaptic current being therefore determined by channel closure, similar to deactivation (Hestrin, Sah, & Nicoll, 1990; Tang, Shi, Katchman, & Lynch, 1991).

The second hypothesis argues that the decay of glutamate concentration is slow and that the postsynaptic current is terminated by desensitization of AMPA receptor channels (Trussell, Thio, Zorumski, & Fischbach, 1988; Trussell & Fischbach, 1989; Isaacson & Nicoll, 1991; Vyklicky, Patneau, & Mayer, 1991; Hestrin, 1992; Trussell, Zhang, & Raman, 1993; Livsey, Costa, & Vicini, 1993; Raman & Trussell, 1995). This is plausible since AMPA receptor channels desensitize very rapidly (they are the fastest desensitizing ligand-gated ion channels).

According to the third hypothesis, the postsynaptic current is determined in a complex manner by the time course of deactivation and desensitization, as well as glutamate concentration (its decay has been argued to be between the two extremes postulated in the first and second hypothesis; Clements, Lester, Tong, Jahr, & Westbrook, 1992; Barbour, Keller, Llano, & Marty, 1994; Tong & Jahr, 1994a).

2 Deactivation and Desensitization of Currents Evoked by Glutamate, Released Either Synaptically or by Local Application

Deactivation is by convention defined as the decrease of current following the end of a very brief pulse of glutamate applied to excised outside-out membrane patches. Since it reflects the random closure of AMPA channels in the absence of glutamate, deactivation should determine the rate of decay of mEPSCs if the glutamate transient in the synaptic cleft is very fast. Intrinsically desensitization is defined as the decrease in current observed during a prolonged application of glutamate. The time constants of deactivation and desensitization of AMPA receptors have been studied in membrane patches from a variety of central neurons to evaluate these hypotheses.

In inhibitory interneurons of neocortex (Jonas, Racca, Sakmann, Seeburg, & Monyer, 1994) and hippocampus (Livsey et al., 1993) and in cochlear nucleus neurons (Raman & Trussell, 1992; Trussell et al., 1993), the gating of AMPA channels is rapid; it is slower in hippocampal granule cells and pyramidal neurons and neocortical pyramidal neurons (Colquhoun, Jonas, & Sakmann, 1992; Hestrin, 1992, 1993; Jonas et al., 1994). In all these cells,
the time course of postsynaptic currents is well correlated with the time course of currents induced by short pulses of glutamate (Raman & Trussell, 1992; Silver, Traynelis, & Cull-Candy, 1992; Stern, Edwards, & Sakmann, 1992; Jonas, Major, & Sakmann, 1993). Because intrinsic desensitization is typically two- to four-fold slower than the decay of well-clamped fast EPSCs, many authors have argued that at these synapses, desensitization is too slow to affect the time course of mEPSCs or even EPSCs (Colquhoun et al., 1992; Hestrin, 1992; Stern et al., 1992; Trussell et al., 1993; Jonas et al., 1993; Partin, Patneau, Winters, Mayer, & Buonanno, 1993; Jonas et al., 1994; Jonas & Spruston, 1994; Raman & Trussell, 1995; Edmonds, Gibb, & Colquhoun, 1995; Hausser & Roth, 1997).

3 Need for Reevaluation

For several reasons, these notions need to be reevaluated. First, the apparent time course of intrinsic desensitization is not necessarily a good indicator of the entry into desensitized states, which may develop much faster; thus, a glutamate pulse much shorter than the time course of desensitization leads to a diminished response to a subsequent short test pulse of glutamate (Raman & Trussell, 1995). Second, the decay time of mEPSCs becomes markedly amplitude dependent when AMPA receptor desensitization is blocked by aniracetam or cyclothiazide (Ghamari-Langroudi & Glavinovic, 1998; Atassi & Glavinovic, 1999). This finding suggests that (1) desensitization of synaptic currents depends on the number of glutamate molecules released and therefore is concentration dependent (unlike intrinsic desensitization), and (2) in the absence of desensitization, another process renders the decay phase of mEPSCs concentration dependent.

Alternative explanations may be given for the amplitude dependence of mEPSCs in the absence of desensitization. Larger mEPSCs may be of multivesicular origin, and the positive correlation may arise at least partly from asynchronous vesicular release. The recent report that spontaneous release becomes less multivesicular with maturation (Wall & Usowicz, 1998) argues against such an explanation (both studies were done in hippocampal slices from adult rats; Ghamari-Langroudi & Glavinovic, 1998; Atassi & Glavinovic, 1999). Another explanation would be that the deactivation rate is concentration dependent. High glutamate concentrations may preferentially activate large-conductance channels (Rosenmund, Stern-Bach, & Stevens, 1998) that remain open for longer times (Stern-Bach, Russo, Newman, & Rosenmund, 1998). Though plausible, it seems an unlikely explanation for dentate granule cells or CA1 pyramidal cells of hippocampus; in excised patches, decay times of currents produced by short (1 ms) pulses of glutamate are essentially concentration independent (over the range 0.2–1.0 mM). In CA3 pyramidal cells of hippocampus, however, such a mechanism may be more important (Colquhoun et al., 1992).
Current Observations

Our recordings in hippocampal slices showed that the removal of desensitization by specific pharmacological agents prolonged mEPSCs and greatly enhanced the amplitude dependence of their decay times. To investigate this phenomenon further, we made a detailed simulation of the underlying mechanisms using well-established Monte Carlo methods to examine glutamate release into the synaptic cleft, and its interaction with AMPA receptors, and the interaction of short and long pulses of glutamate with AMPA receptors in excised patches. More specifically, we aimed to clarify what factors shape the amplitude and time course of mEPSCs and what determines the relationship among the time course of intrinsic desensitization, deactivation, mEPSCs, and the time course of the occupancy of desensitized states (for synaptic and patch currents; Glavinović & Rabie, 1998).

The Monte Carlo method follows individual glutamate molecules as they diffuse randomly within the synaptic cleft and interact with the postsynaptic receptors (see Figure 1A; Bartol, Land, Salpeter, & Salpeter, 1991; Wahl, Pouzat, & Stratford, 1996; Kruk, Horn, & Faber, 1997; Glavinović & Rabie, 1998; Glavinovic, 1999). Our model assumes a 200 x 200 nm synaptic contact area and 15 nm wide cleft, bounded by a three-dimensional infinite space. Further assumptions are that the movement of a glutamate molecule depends only on its present position (and not on its history) and that the probability of each receptor’s remaining in its present state or changing to another state depends only on its present state. Both the movement and the gating kinetics are thus Markovian processes. In the kinetic scheme for the receptors (see Figure 1B; Jonas et al., 1993), two glutamate molecules must bind to the receptor before the channel can open. AMPA receptor can therefore be unbound (U), singly bound (SB), doubly bound (DB), open (O), or in one of the three desensitized states (D1, D2, or D3). The interaction between glutamate molecules and receptors is defined by the three rates in the kinetic scheme (K+1, K+2, and K+3) and by associating with each receptor state a surface area and a probability of binding, given that a glutamate molecule hits this receptor surface.

There is no agreement concerning the level of occupancy of AMPA receptors at the peak of the current. Although AMPA receptors may saturate at some synapses, several lines of experimental evidence indicate that this is unlikely to occur in the cerebellum and hippocampus: noise analysis at single-site synapses in the cerebellum (Silver, Colquhoun, Cull-Candy, & Edmonds, 1996), the high coefficient of variation for single-site mEPSCs in hippocampal culture (Forti, Bossi, Bergamaschi, Villa, & Malgaroli, 1997), and the effects of local agonist applications at single-site synapses in hippocampal cultures (Liu, Choi, & Tsien, 1999). We therefore simulated mEPSCs generated by the release of glutamate molecules, varying in number over a very wide range (150–20,000) and covering all degrees of saturation.
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Figure 1:

(A) Diagram (approximately to scale) showing how glutamate molecules, after release from an instantaneous point source, diffuse in synaptic cleft and interact with postsynaptic receptors. (B) Kinetic scheme of gating of AMPA receptor channels by glutamate, used for the Monte Carlo simulations. U, SB, DB, and O indicate the unbound, singly bound, doubly bound, and open state respectively. D₁, D₂, and D₃ are three desensitized states. The rate constants, taken from Jonas et al. (1993) were adjusted for the temperature of simulations (37°C) and assuming a Q₁₀ of 4.0 (Glavinović & Rabie, 1998). They are:

- for glutamate binding:
  - \( K_{C1} \) = \( 3.67 \times 10^7 \text{M}^{-1} \) \( \alpha \), \( K_{C2} \) = \( 2.61 \times 10^7 \text{M}^{-1} \) \( \beta_1 \), \( K_{C3} \) = \( 1.02 \times 10^7 \text{M}^{-1} \) \( \beta_2 \), \( K_{C4} \) = \( 366 \text{s}^{-1} \) \( \beta_3 \), and \( K_{C5} \) = \( 1.02 \times 10^7 \text{M}^{-1} \) \( \alpha_2 \) for glutamate binding;
  - for channel opening:
    - \( \alpha_1 \) = \( 3.39 \times 10^4 \text{s}^{-1} \), \( \beta_1 \) = \( 7200 \text{s}^{-1} \), for channel opening; and \( \alpha_1 \) = \( 2.31 \times 10^4 \text{s}^{-1} \), \( \beta_1 \) = \( 314 \text{s}^{-1} \), \( \alpha_2 \) = \( 1376 \text{s}^{-1} \), \( \beta_2 \) = \( 5.82 \text{s}^{-1} \), \( \alpha_3 \) = \( 142 \text{s}^{-1} \), \( \beta_3 \) = \( 32 \text{s}^{-1} \), \( \alpha_4 \) = \( 134.4 \text{s}^{-1} \), \( \beta_4 \) = \( 1523 \text{s}^{-1} \) for the desensitization pathway.

There was good agreement between the predictions of the simulations and experimental observations:

- Both rise and decay times of mEPSCs are prolonged when desensitization is reduced or abolished, but the amplitude changes only marginally (Isaacson & Walmsley, 1996; Ghamari-Langroudi & Glavinović, 1998; Atassi & Glavinović, 1999).
- The decay phase of mEPSCs, which is not (or is only modestly) amplitude dependent in the presence of desensitization becomes markedly so when desensitization is absent (see Figure 2A; Isaacson & Walms-
Figure 2: Under control conditions, the decay times of fast unitary excitatory postsynaptic current (mEPSC) are only marginally amplitude dependent, but they become markedly so when desensitization is suppressed. (A) Amplitude dependence of the decay time of mEPSCs, recorded from CA1 pyramidal cells in rat hippocampal slices, in control conditions and after suppression of AMPA receptor desensitization by 100 μM cyclothiazide (cyclo; from Atassi & Glavinović, 1999). (B) Amplitude dependence of the decay time of simulated fast mEPSCs, when desensitization is either present (control) or absent (No Des), with either single (Single Bind) or repeated (Repeat Bind) glutamate binding to receptors permitted. The amplitude was normalized to 1.0, when all AMPA channels are open. Over a wide range, the amplitude dependence of simulated mEPSCs is evident only if there is repeated binding; this indicates that desensitization shortens mEPSCs and markedly reduces the amplitude dependence of their decay times by eliminating glutamate repeated binding (“buffering”) by AMPA receptors. Slower decay of observed mEPSCs (A versus B) can be ascribed to the lower temperature of recordings (32° versus 37° for the simulations), without excluding likely different kinetics in CA1 versus CA3 neurons (rate constants listed in Figure 1B were obtained from CA3 neurons; Jonas et al., 1993).
Intrinsic desensitization is concentration independent (Colquhoun et al., 1992) and two to three times slower than the decay of "perfectly clamped" mEPSCs (see Figures 2B and 3A; Colquhoun et al., 1992; Hestrin, 1992; Stern et al., 1992; Trussell et al., 1993; Jonas et al., 1993, 1994; Partin et al., 1993; Jonas & Spruston, 1994; Raman & Trussell, 1995; Edmonds et al., 1995; Hausser & Roth, 1997; Glavinovic & Rabie, 1998).

Deactivation and perfectly clamped mEPSCs have very similar decay times (see Figures 2B and 3A; Raman & Trussell, 1992; Silver et al., 1992; Stern et al., 1992; Jonas et al., 1993; Glavinovic & Rabie, 1998).

5 Revised Desensitization Hypothesis

Although it is clear that desensitization plays a major role in shaping the time course of post-synaptic currents, the picture that emerges from these simulations differs considerably from the classical desensitization hypothesis (the second hypothesis).

According to our revised hypothesis, desensitization shapes the time course of mEPSCs largely by limiting the repeated binding of glutamate molecules to AMPA receptors (i.e., by reducing glutamate buffering by AMPA receptors) and, to a lesser extent, through the termination of burst openings of AMPA channels. This conclusion is indicated by two sets of observations on simulated mEPSCs. First, if only single binding of glutamate is permitted, their decay times are very similar (brief and virtually amplitude independent) in both the presence and absence of desensitization (see Figure 2B); second, if multiple glutamate binding is permitted, the decay times are long and strongly amplitude dependent in the presence of desensitization. The short duration and the similar time course of mEPSCs and deactivation in the presence of desensitization can thus be explained by the absence of repeated binding of individual glutamate molecules. In excised patches, this happens because only a brief pulse of glutamate is applied; during mEPSCs, repeated binding is curtailed by desensitization. The decay times are thus very similar not due to the lack of desensitization but because of its importance.

The occupancy of the single bound activatable state is especially relevant if one wishes to understand what determines the time course of mEPSCs. As the simulations indicate, suppression of desensitization leads to a higher occupancy of the single bound state (see Figure 4A), as glutamate molecules both unbind from it and subsequently rebind more frequently. The high rate of glutamate binding to the singly bound state (it is 6.2 times greater than binding to the unbound state; see Figure 1B; see also Jonas et al., 1993; Wahl et al., 1996; Glavinovic & Rabie, 1998) further
Figure 3: Although decay of intrinsic desensitization is slower than decay of deactivation, the desensitization of AMPA receptors during an mEPSC is very pronounced and depends strongly on how many molecules of glutamate are released (Monte Carlo simulations). (A) Decay time due to intrinsic desensitization (defined as a decrease in responsiveness to a constant prolonged pulse of glutamate) is two to three times longer than the decay time by deactivation, although most AMPA receptors enter into desensitized states during an mEPSC. (B) Fraction of the AMPA channels in the unbound state (filled and empty circles) is higher in the absence of desensitization. Desensitization of AMPA receptors is very pronounced and strongly depends on the number of molecules released (all desensitized states are taken together; filled squares). The occupancy of all states (unbound and desensitized) was estimated 1 ms after instantaneous release (from Glavinović & Rabie, 1998).
Figure 4: In the absence of desensitization, both the fraction of bound receptors and late, but not early, phase of cleft concentration of glutamate are higher regardless of how many glutamate molecules are released (Monte Carlo simulations). The fraction of single- (circles) and double- (triangles) bound AMPA receptors (A) and the synaptic cleft concentration (C) as a function of the number of molecules of glutamate molecules released, in the presence and absence of desensitization. The occupancies of all receptor states and the concentration of glutamate were estimated 1 ms after instantaneous release (from Glavinović & Rabie, 1998). (B) The fast constant of the cleft concentration is, however, independent of the number of molecules released, the presence of AMPA receptors, and their ability to desensitize. Smooth lines are the best multiexponential fits to the concentration traces.

enhances the frequency of repeated binding. Its proximity to the open state (unlike that of the unbound or desensitized state D1; see Figure 2A) results in prolongation of mEPSCs and greater synaptic efficacy (i.e., a higher
probability of opening of AMPA channels). Higher occupancy of the single bound state raises the occupancy of the double-bound state (DB; see Figure 1B) and thus enhances the likelihood of repeated opening of AMPA channels.

Simulations also reveal that during and following an mEPSC, the occupancies of all states, including desensitized states, depend on the number of molecules released (i.e., they are concentration dependent; see Figures 3 and 4). This is not surprising given that three out of seven kinetic rates are concentration dependent (see Figure 1B). The postulated concentration dependence of desensitization is not in contradiction experimentally (Jonas et al., 1993) or theoretically (Glavinović & Rabie, 1998) with the well-documented concentration independence of desensitization in excised patches (intrinsic desensitization; see Figure 3A). Desensitization that develops during a single mEPSC is concentration dependent mainly because it abolishes glutamate buffering—a concentration-dependent process that is not present during desensitization in excised patches.

Finally, our simulations remove one of the main objections to the idea that desensitization plays an important role in shaping the time course of mEPSCs: the supposed requirement that the time course of glutamate concentration in the synaptic cleft be very slow (Edmonds et al., 1995). The decay of glutamate concentration is actually predicted to be faster in the presence of desensitization of AMPA channels because of reduced buffering of glutamate by AMPA receptors (see Figure 4B and Figure 5).

A word of caution is, however, necessary. The comparative importance of desensitization as an inactivation pathway as opposed to its role in diminishing glutamate buffering may vary from synapse to synapse and may depend on external conditions. Desensitization is more likely to act as an inactivation pathway if the synaptic cleft is wide and the synaptic contact area small. The kinetics of gating of AMPA channels and their density are also significant factors. The theoretical simulations (Wahl et al., 1996; Glavinović & Rabie, 1998; Glavinović, 1999) assumed that all rates have the same $Q_{10}$, but that is not necessarily the case. If the association rates have lower $Q_{10}$ (Jones, Sahara, Dzubay, & Westbrook, 1998), desensitization would have a greater importance as an inactivation pathway at higher temperatures than we evaluated.

In the simulations, all glutamate molecules that reached the edge of the synapse diffused freely into the surrounding infinite space (Glavinović & Rabie, 1998; Glavinović, 1999). If, as seems likely, there are cellular barriers to such free diffusion (e.g., neuroglia), they would slow the clearance of glutamate from the synaptic cleft and enhance the role of desensitization in diminishing glutamate buffering by AMPA receptors—bearing in mind that significant removal of glutamate by cellular uptake probably accelerates the clearance of glutamate (see below); this needs to be included explicitly in further simulations. In any case, the experimental findings strongly support the idea that repeated binding is important in shaping the time course of
Figure 5: Diagram compares the time course of glutamate concentration in synaptic cleft and unitary excitatory postsynaptic current (below), in the presence and absence of desensitization. (Top) Glutamate concentration appears as a large but short pulse that decays instantaneously and is followed by a small, prolonged tail. According to our model, the effect of glutamate is not only more evanescent in the presence of AMPA receptor desensitization, but its concentration in the synaptic cleft decays faster. Both effects are closely linked to a higher occupancy of the single bound receptor state that produces the greater and more prolonged postsynaptic effect (downward arrows) but also elevates its concentration in the synaptic cleft, owing to greater buffering by the AMPA receptors (curved upward arrows).

mEPSCs at low (22–27°C) and especially at high (32°C) temperatures (Atassi & Glavinovic, 1999).

6 Fast-Application Protocol on Excised Membrane Patch as Surrogate Synapse

Since it is difficult to examine the steps that are rate limiting at an intact synapse, fast glutamate applications to excised outside-out membrane patches are often used as a surrogate synapse (Colquhoun et al., 1992; Raman & Trussell, 1992; Hestrin, 1992, 1993; Trussell et al., 1993; Livsey et al., 1993; Jonas et al., 1994). This protocol is attractive because it permits the investigator to control not only postsynaptic but also presynaptic (i.e., release) param-
eters. One can thus systematically examine how the glutamate concentration and its pulse length influence the time course of the postsynaptic currents.

For at least three reasons, the surrogate synapse often gives a misleading picture of synaptic events (Glavinović & Rabie, 1998). First, the time course of transmitter in the synaptic cleft is never a square pulse: it has a more complicated shape, and the concentration is spatially nonuniform (Ogston, 1955; Holmes, 1995; Wahl et al., 1996; Kleinle et al., 1996; Glavinović & Rabie, 1998; Glavinovic, 1999). Second, in such a system, the binding and unbinding of transmitter from receptors do not affect the concentration of transmitter that activates the responses. In the synaptic cleft, accumulations (and depletions) of transmitter can occur because of such binding and unbinding, and they can exert a profound influence on the time course of the postsynaptic current (Mennerick & Zorumski, 1994, 1995; Glavinovic & Rabie, 1998). They are likely to be especially relevant if the occupancy of the AMPA receptors in the single bound state is more than negligible. Third, the rate of onset of intrinsic desensitization differs from that of entry into the desensitized states. Unlike the time course of intrinsic desensitization, the occupancy of the desensitized states during a synaptic current clearly is concentration dependent.

7 Pharmacological Inhibition of Desensitization of AMPA Receptor Channels

A variety of agents (such as aniracetam or cyclothiazide) are known to inhibit the desensitization of AMPA receptor channels (Ito, Tanabe, Kohda, & Sugiyama, 1990; Yamada & Tang, 1993). They produce a slower decay of the spontaneous and evoked postsynaptic potentials (Larson, Le, Hall, & Lynch, 1994) and currents (Isaacson & Nicoll, 1991; Tang et al., 1991; Trussell et al., 1993; Partin et al., 1993; Ghamari-Langroudi & Glavinovic, 1998; Atassi & Glavinovic, 1999), which is often taken as an argument that desensitization plays an important role in shaping the time course of postsynaptic currents. This interpretation is not universally accepted on the grounds that these agents also make the kinetics of deactivation slower (Jonas & Spruston, 1994; Edmonds et al., 1995). Such criticisms may not be valid because deactivation should be slower when desensitization is suppressed, though there is no slowing of the rate of closing of AMPA channels (Glavinovic & Rabie, 1998). A strong correlation between the time constants of deactivation and of desensitization, observed in a variety of central synapses (Partin, Fleck, & Mayer, 1996), suggests that deactivation is slower largely (and probably entirely) owing to reduced desensitization.

8 Time Course of Glutamate Concentration in the Synaptic Cleft

Albeit of considerable interest, the time course of glutamate concentration is not known with precision. Given that diffusion is fundamentally a mul-
tiexponential process (Carslaw & Jaeger, 1959), the time course of transmitter concentration in the synaptic cleft should decay multiexponentially (Ogston, 1955; Eccles & Jaeger, 1958; Wathey, Nass, & Lester, 1979; Silver et al., 1996; Clements, 1996). At least two exponentials are expected even when considering a two-dimensional extracellular space (Destexhe & Sejnowski, 1995). The time constant of the fast initial concentration decay ($\tau_f$) is expected to be governed by lateral diffusion and determined primarily by the diffusion coefficient ($D$) of the transmitter and by the cleft radius ($r$; $\tau_f = r^2/4D$; Bartol & Sejnowski, 1993; Destexhe & Sejnowski, 1995; Clements, 1996; Silver et al., 1996). This has been confirmed by recent Monte Carlo simulations (Bartol et al., 1991; Wahl et al., 1996; Glavinovic & Rabie, 1998). Fitting four exponentials to the time course of the concentration of cleft glutamate simulated assuming an instantaneous point source of glutamate (see Figure 4B) yields a very fast initial time constant (2–6 $\mu$s) that is independent of the number of glutamate molecules released (300–10,000), the presence or absence of 196 receptors on the 200 $\times$ 200 nm postsynaptic membrane, or their ability to desensitize. Though this value is clearly smaller than values reported before (50–200 $\mu$s; Eccles & Jaeger, 1958; Burger et al., 1989; Faber, Young, Legendre, & Korn, 1992; Bartol & Sejnowski, 1993), the agreement between them is good when the differences of the cleft radius are taken into account (0.25–0.5 $\mu$m as opposed to 0.1 $\mu$m). The cleft diameters of excitatory synaptic terminals on CA1 pyramidal neurones are variable ranging from 0.1 to 1.0 $\mu$m (Palay & Chan-Palay, 1974; Bekkers, Richerson, & Stevens, 1990).

Within 50 $\mu$s, the transmitter concentration is essentially spatially uniform throughout the synapse (Wahl et al., 1996; Clements, 1996; Glavinovic, 1999). The amplitudes and the time constants of the slower components, however, depend not only on the diffusion constant and cleft geometry but also on receptor density and its kinetics (Wahl et al., 1996; Glavinovic & Rabie, 1998; see Figure 4B). The longest time constant ranges from 30 $\mu$s to 1.2 ms, values comparable to earlier estimates for the slow component (Clements, 1996). Its amplitude is 1.5 to 15.0% of that for the fastest component. The effective diffusion constant of glutamate in the synaptic cleft influences all components of the time course of glutamate concentration: if the rate of diffusion is lower than in simple aqueous solutions (Longsworth, 1953), the glutamate concentration will change more slowly (Vogt, Luscher, & Streit, 1995; Kleinle et al., 1996). In contrast to the neuromuscular junction, a high-turnover enzyme for transmitter degradation is absent from the synaptic cleft of glutamatergic synapses. This would tend to make the time course of glutamate in the cleft relatively long. However, a significant compensating factor is the presence of transmitter-selective transporters (not found at cholinergic synapses), which affects the time course of glutamate (Tong & Jahr, 1994b; Takahashi, Sarantis, & Attwell, 1996), especially if, as evidence suggests, the transporters are at a high density, surpassing that of AMPA receptor channels by

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more than an order of magnitude (Arriza et al., 1994; Otis, Kavanaugh, & Jahr, 1997; Diamond & Jahr, 1997). The rate of binding of glutamate to the transporters appears to be at least as rapid as binding to the AMPA receptors; the rate of glutamate unbinding is low, and its turnover rate is very low. Therefore, on the timescale of the fast excitatory postsynaptic current, the binding of glutamate to the transporters is essentially irreversible, and it will speed up the clearance of glutamate from the cleft.

The time course of release of the vesicular content will also influence the time course of glutamate concentration in the synaptic cleft. The short rise time of well-clamped, fast, spontaneous EPSCs (Stern et al., 1992; Trussell et al., 1993; Jonas et al., 1993; Spruston, Jonas, & Sakmann, 1995) would argue for a rapid onset of the release of the vesicular content and a rapid subsequent rise of glutamate concentration in the synaptic cleft. However, the time course of release of vesicular content appears to be highly variable, judging by the marked variability in rate of rise of mEPSCs, even when all are equally well clamped (Bekkers & Stevens, 1996; Ghamari-Langroudi & Glavinovic, 1998; Atassi & Glavinovic, 1999). This is not surprising because the intravesicular concentrations of glutamate and vesicular sizes differ considerably. The estimates of the intravesicular glutamate concentration range from 60 to 210 mM (Burger et al., 1989; Riveros, Fiedler, Lagos, Muñoz, & Orrego, 1986; Shupliakov, Brodin, Cullheim, Ottersen, & Storm-Mathisen, 1992) and those of the vesicle diameters from 25 to 45 nm (Palay & Chan-Palay, 1974; Bekkers et al., 1990). Assuming identical fusion pore geometry and time course of opening, larger vesicles are expected to release their contents more slowly than smaller vesicles (Glavinovic, 1999). The amplitude dependence of rise times of mEPSCs recorded in the presence and especially the absence of desensitization (Ghamari-Langroudi & Glavinovic, 1998; Atassi & Glavinovic, 1999) supports such a conclusion. Any variability of the geometry of the fusion pore and its time course of opening would provide another important modulating influence on the rate of release of vesicular content. Finally, according to several recent reports on neuroendocrine secretory cells, agents are not released by simple diffusion, but first have to dissociate from the gel matrix onto which they are stored at high concentration (Helle, 1990; Yoo & Lewis, 1993; Schroeder, Jankowski, Senyszyn, Holz, & Wightman, 1994; Walker, Glavinovic & Trifaro, 1996). By slowing the release process, similar mechanism of storing glutamate in vesicles would provide an additional variable in the process of release of vesicular content.

9 Conclusion

According to the revised hypothesis presented in this review, desensitization of AMPA receptors plays a major role in shaping the time course of fast excitatory postsynaptic currents. This hypothesis differs from the classical desensitization hypothesis in several important respects and proposes that: (1) the glutamate concentration decays faster in the presence of desensiti-
zation of AMPA channels (see Figure 5), (2) desensitization shapes the time course of mEPSCs to a large extent through its effect on buffering of glutamate molecules by AMPA receptors, (3) the occupancy of the single bound activatable state is an important determinant of both the level of glutamate buffering and the efficacy of glutamate in opening AMPA channels, (4) during an mEPSC, the occupancy of all desensitized states (taken individually or all together) and of all other states, as well as the extent of glutamate buffering, are concentration dependent, and (5) desensitization is concentration dependent largely because it abolishes the glutamate buffering (a concentration-dependent process). Thus, the revised hypothesis accounts for numerous findings on fast mEPSCs. It is expected to be equally fruitful as a framework for further experimental and theoretical investigations.

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References


