The Mechanism of α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionate Receptor Desensitization after Removal of Glutamate

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ABSTRACT We have examined responses of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors in the chick nucleus magnocellularis to pairs of pulses of glutamate and determined the extent of desensitization and the rate of recovery. Receptors recovered from desensitization with a time constant of 16 ms, regardless of the concentration or duration of the conditioning pulse. Even with very brief conditioning pulses, evoking submaximal currents, desensitization occurred at a consistent rate after the removal of free ligand. A quantitative kinetic model based on these data shows that receptors must desensitize from a closed state. The results provide evidence that very brief exposure to glutamate, on the time scale of uniquantal synaptic transmission, will result in a significant reduction in sensitivity of postsynaptic receptors.

INTRODUCTION

In neurons of the central nervous system, glutamate receptors of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) subclass share the characteristic of fast desensitization. Receptors usually desensitize within several milliseconds, although the dominant decay time constants vary with cell type and range from 0.75–1 ms for chick nucleus magnocellularis (Raman and Trussell, 1992a; Raman et al., 1994) to 10–12 ms for rat hippocampus (Colquhoun et al., 1992). In terms of channel kinetics, current desensitization is interpreted as the accumulation of receptors in a bound, unopenable state. In response to prolonged exposure to rapidly applied glutamate, currents in outside-out patches generally desensitize by well over 90% (e.g., Trussell and Fischbach, 1989; Tang et al., 1991; Smith et al., 1991; Raman and Trussell, 1992a; Colquhoun et al., 1992; Hestrin, 1992, 1993; Livsey et al., 1993; Raman et al., 1994). Thus, in these cell types, the desensitized state is presumably a thermodynamically stable or high affinity state.

Upon removal of glutamate, receptors exit from one or more liganded, desensitized states and ultimately lose glutamate and become available for reactivation. The time course of recovery from desensitization can be measured by using rapid flow techniques to apply ligands. Recovery from desensitization has been described for a variety of central nervous system cell types, including neurons of the cochlear nucleus, hippocampus, spinal cord, and visual cortex (Tang et al., 1989; Trussell and Fischbach, 1989; Smith et al., 1991; Colquhoun et al., 1992; Hestrin, 1992; Trussell et al., 1993). These types of measurements can provide information about the kinetic states of receptors and, furthermore, may allow inferences to be made about the activity of synaptic receptors under physiological conditions. For example, at some synapses, release of transmitter leads to desensitization of receptors, in which case the kinetics of recovery from desensitization contribute to the reversal of synaptic depression (Trussell et al., 1993).

A surprising finding has been that desensitization can occur even after ligand applications that are significantly briefer than the intrinsic time course of desensitization (Trussell and Fischbach, 1989; Colquhoun et al., 1992; Patneau et al., 1992; Hestrin, 1992, 1993). To explore the basis for this effect, we have examined responses of AMPA receptors in the nucleus magnocellularis (nMAG), one of the avian cochlear nuclei (Raman and Trussell, 1992a; Zhou and Parks, 1992; Raman et al., 1994). AMPA receptors recovered at a uniform rate, regardless of the duration of agonist application. However, recovery occurred after a delay, during which receptors continued to desensitize, despite the removal of free glutamate. The results raise the possibility that, even at those synapses in which the time course of transmitter is brief compared with the desensitization time constant of the synaptic receptors, receptors may still enter a desensitized state. Desensitization after transmitter removal may therefore modify synaptic strength during repetitive firing.

MATERIALS AND METHODS

Preparation of cells

Neurons from the nMAG of late-stage embryonic chicks (E18 to E21) were isolated as described in Raman and Trussell (1992a). Briefly, 300-μm-thick brainstem slices were cut and incubated at 25°C in an Eagle's minimal essential medium-based solution (GIBCO BRL, Gaithersburg, MD) containing 40 units/ml papain (Worthington, Freehold, NJ). After 20 min, the slices were washed in enzyme inhibitor (ovomucoid, Worthington), and the nMAG was microdissected and triturated to release individual cells. Neurons were allowed to settle in the recording chamber onto a poly-D-lysine-coated plastic coverslip.

Recording conditions

The extracellular solution (bathing solution) was composed of 140 mM NaCl, 5 mM KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 20 mM

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glucose, and 1 mM pyruvate, buffered to pH 7.3 with NaOH. In addition, 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Neuramin, Bristol, U.K.) was added to the bath to reduce an apparently excitotoxic effect on the neurons from accumulation of glutamate and/or kainate in the bath, despite bath perfusion. After recordings were made from each patch, the bath was replaced with fresh bathing solution and CNQX. Electrodes were pulled from borosilicate glass (2–5 MΩ) and heat polished. Electrode filling solution consisted of 70 mM Cs_2SO_4, 85 mM sucrose, 4 mM NaCl, 1 mM $MgCl_2$, 10 mM HEPES, and 5 mM BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetracetic acid), buffered to pH 7.3 with CsOH. Agonists L-glutamic acid (Sigma Chemical Co., St. Louis, MO) and kainic acid (Cambridge Research Biochemicals, Wilmington, DE) were dissolved in bathing solution without pyruvate or CNQX. Recordings were made with an Axopatch-1D patch clamp amplifier (Axon Instruments, Foster City, CA) with a filter cut-off frequency of 5 kHz and analyzed with pCLAMP software (Axon Instruments), except as noted. All recordings were made from outside-out patches that were voltage clamped at −70 mV (corrected for a 10-mV junction potential).

Drug application

A piece of theta glass (WPI, New Haven, CT) pulled to a 150-μm-diameter tip was mounted on a piezoelectric manipulator (Physik Instrumente, Waldbronn, Germany) as described in Raman et al. (1994). One barrel of the theta glass contained control solution, which was bathing solution without pyruvate or CNQX (except as noted), and the other contained a drug solution, which was ligand dissolved into control solution that had been diluted by 2–4% with water (Lester and Jahr, 1992; Raman et al., 1994). Flow of solutions was maintained by a syringe pump (Harvard Apparatus, South Natick, MA). After recordings had been made from each patch, the electrode tip was cleared by application of positive pressure. Solution exchange was repeated and changes in junction current were recorded with the open-tipped electrode. In this way the precise duration and rate of solution application could be measured for each patch (See also Results, Fig. 1*A*).

Nonstationary variance (NSV) analysis

Applications of glutamate (30 ms) were given repetitively to patches, with a 2-s interval between applications to ensure recovery from desensitization. Data were used for NSV analysis only if the responses showed no systematic decline in amplitude with successive applications of glutamate. Data were digitized at 32 μs per point. The mean current at each point, $I(t)$ in pA, and the variance at each point, $\sigma^2(t)$ in pA^2, were calculated from 9 to 64 records (average, 32 records) per patch. Analysis was similar to Sigworth (1980), Hestrin (1992), and Jonas et al., (1993). Data were binned into 5-pA bins, or occasionally 0.5- or 1-pA bins for very small currents, and the average $\sigma^2(t)$ was calculated for each bin (MicroCal Origin, Northampton, MA). The $\sigma^2(t)$ was plotted against $I(t)$. When possible, the data were fitted with a parabola of the form $\sigma^2(t) = iI(t) - I^2(t)/N + \sigma_n^2$, where $\sigma^2(t)$ and $I(t)$ are as defined above, i is the single channel current, N is the number of receptors in the patch, and σ_n^2 is the variance of the patch noise in the absence of glutamate. For data that did not approximate a parabola, the points were fit by linear regression with a straight line of the form $\sigma^2(t) = iI(t) + \sigma_n^2$.

Simulations based on kinetic models were run on a personal computer with SCOP software (Simulation Resources, Berrien Springs, MI). Data are reported as mean ± SD. In some traces, capacitative artifacts associated with charging the piezoelectric device were omitted for clarity.

RESULTS

We ascertained that the solution exchange times estimated from junction current measurements were comparable with exchange at the outside-out patches by recording kainate-activated currents upon a change in driving force. Patches were stepped from a solution containing 40 mM NaCl, 100 mM sucrose, and 1 mM kainate into a test solution containing 140 mM NaCl and 1 mM kainate. The change in driving force produced an increase in kainate-evoked current, as shown in Fig. 1*A*, top panel. The mean 10–90% rise/fall time of these currents was 175 ± 37 μs ($N = 4$). A junction current measured with an open-tipped electrode and the same exchange parameters are shown in Fig. 1*A*, bottom panel. The exchange times and duration are nearly identical to those measured in the patch (see figure legend), indicating that the junction current measurement accurately reflects the time course of exchange at the receptors.

As described previously, a prolonged application of 1–10 mM glutamate to a patch held at −70 mV produced a rapidly activating inward current, with a 10–90% rise time ranging from 0.13 to 0.18 ms, which was probably limited by the time course of solution exchange (Raman et al., 1994). The response to glutamate in patches from enzymatically isolated nMAG neurons is due entirely to activation of AMPA or non-NMDA (*N*-methyl-D-aspartate) receptors (Raman and Trussell, 1992a). As shown by the thin trace in Fig. 1*B*, the onset of desensitization began immediately and could be described with one or two exponential time constants, as described by Raman and Trussell (1992a). When glutamate was removed before desensitization was complete, the current fell more abruptly as receptors deactivated, as illustrated by the thick trace in Fig. 1*B*. Thus, the onset of deactivation is evident as a point of inflection in the trace when compared

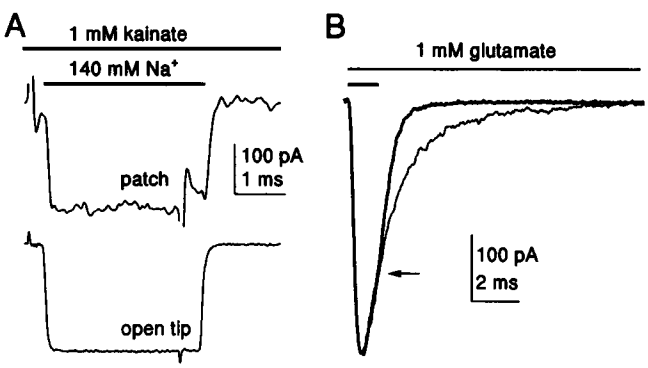


FIGURE 1 Precise applications of ligand can be used to measure kinetic parameters. (*A*) Top panel: in the chronic presence of 1 mM kainate, an outside-out patch was stepped from a solution containing 40 mM NaCl and 100 mM sucrose to a solution containing 140 mM NaCl, for the duration indicated by the bars. The change in driving force was accompanied by an increase in inward current, which reflects the rate and time course of solution exchange at the receptors. The 10–90% rise and fall times of this current were 160 and 210 μs, respectively, and the duration was 3.18 ms. Mean of seven replications. Lower panel: solution exchange measured as the change in junction current at an open tipped electrode, with the same exchange parameters as in the top panel. The 10–90% rise and fall times are 100 and 150 μs, respectively, and the duration is the same as for the patch. Vertical scale bar applies only to the top panel. (*B*) A long pulse of 1 mM glutamate (thin bar and trace) applied to an outside-out patch produces a rapidly activating current that desensitizes by >99% in ~10 ms. A brief (1.16-ms) pulse (thick bar and trace) evokes a current that activates and begins to desensitize. Upon removal of glutamate (arrow), the current deactivates as channels close. Deactivation occurs more quickly than desensitization. Mean of two records.

with the response to a long pulse of glutamate, indicated by the arrow in Fig. 1*B*.

The time course of recovery from desensitization was assessed by exposing patches to paired pulses of glutamate. By comparing responses to short and long conditioning pulses, we investigated whether the rate of recovery from desensitization is dependent on the duration of exposure to ligand, as shown by Boyd (1987) and Trussell and Fischbach (1989). The interval between the first, conditioning pulse and the second, test pulse ranged from 0.5 to 200 ms. An index of recovery was obtained by measuring the ratio of the peak amplitude of the test response to the amplitude of the conditioning response. Examples of paired responses with a 20-ms conditioning pulse of 1 mM glutamate are shown in Fig. 2*A*. In Fig. 2*B*, the index of recovery is plotted against interpulse interval for a 20- and 40-ms conditioning pulse, both of which induced >99% desensitization. Recovery time was indistinguishable between these two conditions, suggesting that the longer, 40-ms exposure did not drive receptors to a more profoundly desensitized state (i.e., with a slower rate of recovery). A single-exponential fit to the averaged data gave a time constant of recovery (τ_{rec}) of 15.8 ms. The best fit was obtained only when a 2- to 3-ms delay before recovery was included in the exponential function (see Fig. 2*B* legend). A time constant of ~16 ms is consistent with the time course of recovery measured with applications of 3 mM glutamate and a 5-ms conditioning pulse (Trussell et al., 1993). Recovery in nMAG is considerably faster than in hippocampus (e.g., Colquhoun et al., 1992), consistent with the more rapid kinetics of AMPA receptors in the auditory pathway (Raman et al., 1994).

Incomplete desensitization can be achieved with briefer conditioning pulses (Fig. 1*B*). For example, at 1 ms after the onset of application of 1 mM glutamate, the response has fallen to ~80% of the peak. Fig. 3*A* shows examples of paired responses to a 0.96-ms conditioning pulse. With a conditioning pulse of 1 mM glutamate for 0.96 ms, the time course of recovery was 16.0 ms, as plotted in Fig. 3*B*. To reveal any effect of concentration, we measured recovery from brief, 1.4-ms pulses of 10 mM glutamate. Under these conditions, the time constant of recovery was 16.7 ms, plotted in Fig. 3*C*. Based on a previous study, 1 mM and 10 mM are, respectively, 0.5 and 5 times the EC_{50} for this receptor (Raman and Trussell, 1992a). The similarity of the recovery times, regardless of the duration of the conditioning pulse (0.96 or 1.4 ms vs 20 or 40 ms) or concentration of agonist (1 vs 10 mM), indicates a common route for recovery from a desensitized state.

The earliest phase of recovery could be examined by measuring the amplitude of test responses at times immediately after brief pulses of 300 μM glutamate. With this concentration, activation and desensitization were slow enough to

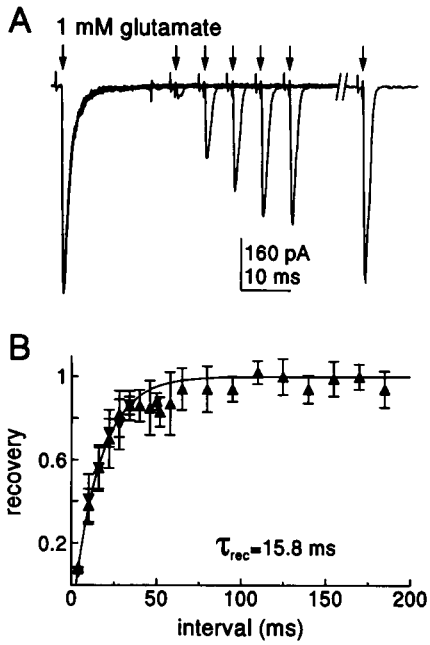


FIGURE 2 Recovery from desensitization to long pulses of glutamate. (*A*) Pairs of responses from a single patch. The conditioning pulse was 20 ms and the test pulse was 6.4 ms. Intervals were 4, 10, 16, 22, 28, and 125 ms. Arrows indicate the onset of each application of glutamate. (*B*) Plot of recovery, measured as the ratio of the peak amplitude of the test response to the peak amplitude of the conditioning response, versus interpulse interval. Upward triangles (▲) are data from a conditioning pulse of 20 ms and downward triangles (▼) are from a conditioning pulse of 40 ms. Data from five patches. The time course of recovery could be fit with a single exponential of the form, recovery = $1 - [\exp(t_0 - t/\tau_{rec})]$, where t is the interval between pulses, t_0 represents the delay before recovery and is estimated at 2.6 ms, and τ_{rec} is the time constant of recovery, estimated at 15.8 ms.

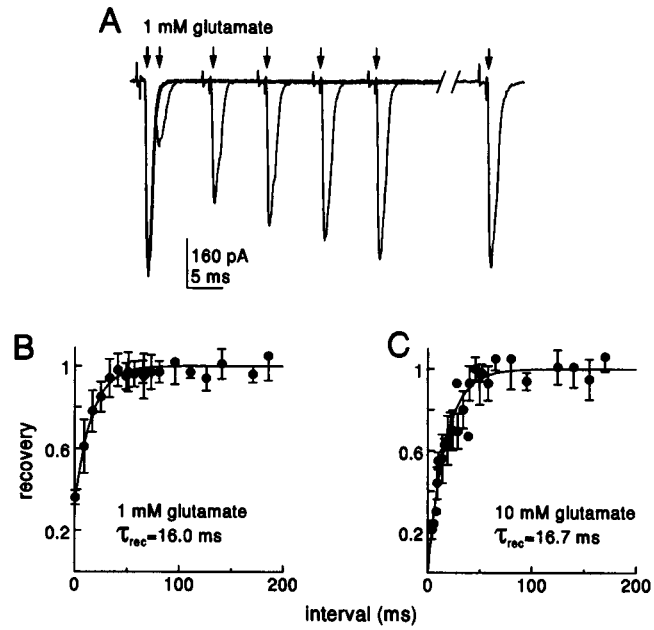


FIGURE 3 Recovery from desensitization to short pulses of glutamate. (*A*) Pairs of responses from the same patch as in Fig. 2*A*. The conditioning pulse was 0.96 ms and the test pulse was 6.4 ms. Intervals were 0.64, 9, 17, 25, 33, and 65 ms. Arrows indicate the onset of each application of glutamate. (*B*) Plot of recovery to a 0.96-ms pulse of 1 mM glutamate, measured as in Fig. 2*B*, against interpulse interval. The τ_{rec} from a single-exponential fit was 16.0 ms. Data from seven patches. (*C*) Plot of recovery to a 1.4-ms pulse of 10 mM glutamate, measured as in Fig. 2*B*. The τ_{rec} was 16.7 ms. Data from one to eight patches at each point.

permit pulses to be delivered with intervals that were short compared with the time constant of desensitization. Fig. 4*A* shows representative traces of 0.42-ms conditioning and 0.42-ms test responses to 300 μM glutamate. Interestingly, the test response first declined and then began to increase with larger intervals, as though desensitization had continued to develop after the end of the conditioning pulse.

To examine more closely the relationship between the extent of desensitization and the onset of recovery, we varied the duration of the conditioning pulse of 1 mM glutamate from 1 to 10 ms, while keeping the interval between the pulses constant at 2 ms. We then compared the peak amplitude of the test response ($P2_{peak}$) with the amplitude of the conditioning response at the end of the conditioning pulse ($P1_{end}$). Thus, a ratio of $P2_{peak}$ to $P1_{end}$ equal to 1 would indicate that all the channels that were open at the end of the conditioning pulse were available for activation 2 ms later, but no recovery occurred during the interval. Fig. 4*B* displays pairs of responses to test pulses ranging in duration from 1 to 10 ms. The ratio of $P2_{peak}/P1_{end}$ varied depending on the length of the conditioning pulse, plotted as triangles in Fig. 4*C*. For conditioning pulses longer than 5 ms, the ratio was greater than 1, suggesting that some recovery did occur within 2 ms. However, the current amplitudes were not more than 2–4% of the maximal amplitude of the conditioning response, i.e., a few picoamps, making the final data points somewhat imprecise. By contrast, for pulses briefer than 5 ms, the ratio was less than 1 and declined as the conditioning pulse was shortened to ~1 ms. In other words, for brief conditioning pulses, desensitization proceeded after the pulse, thus delaying the onset of recovery.

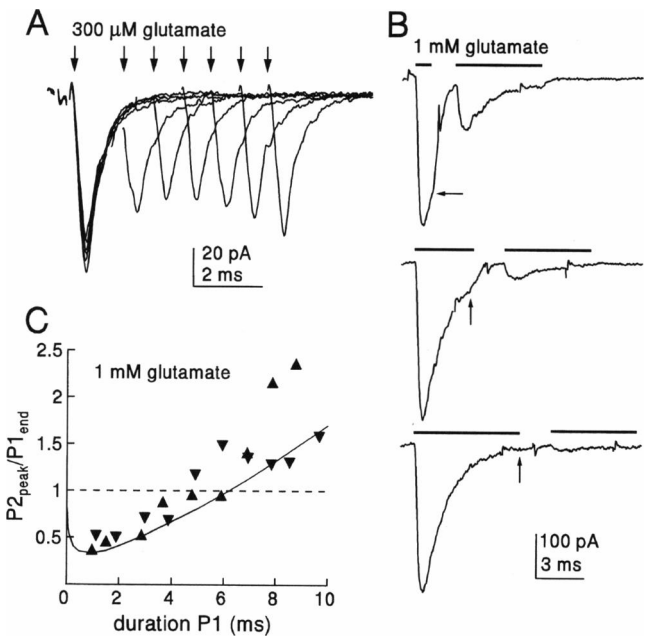


FIGURE 4 Relation between the extent of desensitization and the onset of recovery. (*A*) Paired responses to 0.42-ms applications of 300 μM glutamate with interpulse intervals of 1.00, 1.86, 2.70, 3.48, 4.30, and 5.12 ms, illustrating the delay in recovery due to continued desensitization. Arrows indicate the onset of each glutamate application. (*B*) Paired responses to 1 mM glutamate with increasing duration of the conditioning pulse (1.1, 3.9, and 9.7 ms) and a constant interpulse interval (2 ms). Bars indicate the duration of the pulses. Arrows point out the amplitude of the conditioning response upon removal of glutamate ($P1_{end}$). (*C*) Plot of the ratio of the peak amplitude of the test response ($P2_{peak}$) to $P1_{end}$ versus duration of conditioning pulse. The different symbols indicate data from two different patches. The dashed line at a ratio of 1 indicates the expected ratio if no recovery occurs during the 2-ms interval but all open channels at the end of the conditioning pulse are available for reactivation upon application of the test pulse. The continuous line is the ratio predicted by the model presented in the Discussion.

Quantification of the amount of continuing desensitization during brief intervals revealed an interesting property of the receptors. During a 0.96-ms pulse of 1 mM glutamate, the current only desensitized to 79 ± 8% of the peak amplitude (N = 8 patches). If no recovery occurred during the brief (0.64-ms) interval, but all of the undesensitized receptors were available for reactivation, the test response should be at least equal to the amplitude of the current at the end of the conditioning response. Instead, the second response was 36 ± 3.5% (N = 7 patches) of the conditioning response, consistent with the continuation of desensitization described above. The extent of desensitization is remarkably similar to the degree of desensitization expected from a constant pulse of glutamate; with an exponential time constant of desensitization of 1.40 ms for 1 mM glutamate (Raman et al., 1994), if glutamate had been applied continuously for 2.0 ms (0.96 ms pulse + 0.64 ms interval + 0.4 ms total rise time of test response), the current amplitude would have decayed to 32% of the peak of the conditioning response. To illustrate this effect, Fig. 5*A* (thick trace) shows an expansion of the first response of Fig. 3*A* with the double pulse, superimposed on a fully desensitizing response (thin trace) to a long pulse of glutamate measured in the same patch. The peak of the test response matches the amplitude of the response to the long pulse of glutamate. This enveloping of the test response by a more fully desensitizing response, first documented in visual cortical neurons by Hestrin (1992), indicates that entry into the desensitized state is not predicated upon the time spent in the open state.

Briefer applications of glutamate could produce this enveloping effect, as demonstrated by shortening the conditioning pulse so that it evoked a submaximal response. A response of a patch to a conditioning pulse of 1 mM glutamate for 0.26 ms and a subsequent pulse after 0.72 ms is shown in Fig. 5*B*. Superimposed is a current evoked by a long (30-ms) pulse of 1 mM glutamate. Although the initial response is submaximal, the test response follows exactly the amplitude and time course of the fully desensitizing current. This result suggests that receptors bound agonist, and even those that did not open, did desensitize.

This phenomenon was examined more closely with a lower concentration of glutamate, in experiments like those of Fig. 4, *B* and *C*, but with briefer test pulses and a shorter interval. Responses to conditioning pulses of 300 μM glutamate with a 0.84-ms interval, followed by a longer test pulse are shown in Fig. 5*C*. The horizontal dashed line indicates the level reached by a long, unconditioned response.

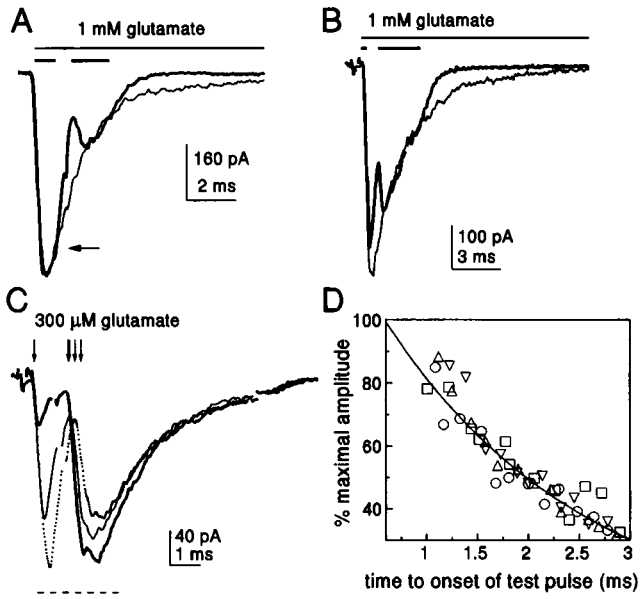


FIGURE 5 Enveloping of test responses to long, desensitizing responses to glutamate. (*A*) The thick line is the trace from Fig. 3*A* with the 0.64-ms interval, shown on an expanded time scale. Thick bars indicate the duration of glutamate application. Superimposed (thin trace) is a desensitizing response in the same patch, obtained by a long pulse of glutamate (thin bar). During the presence of glutamate, the test response from the paired protocol follows the envelope defined by the desensitizing response. (*B*) Thick trace shows the responses to a submaximally activating, 0.26-ms conditioning pulse and a 3.2-ms test pulse (thick bars). Superimposed (thin trace) is the desensitizing response in the same patch to a long pulse of glutamate (thin bar). Enveloping persists, even though the maximal number of channels were not opened by the very brief conditioning pulse. (*C*) Paired responses to 300 μM glutamate. The duration of the conditioning pulse was varied (0.24, 0.42, or 0.62 ms) to give a range of submaximally conditioning responses. The interval and test pulse duration were constant, at 0.84 ms and 6.4 ms, respectively. Arrows indicate the onset of glutamate applications. The thick line shows the responses to the briefest (smallest) conditioning response; the dotted line shows responses to the longest (largest) conditioning response. Dashed line indicates peak amplitude achieved by a long pulse of 300 μM glutamate in the same patch. (*D*) Plot of percent maximal amplitude, measured as the ratio of the peak amplitude of the test pulse to the amplitude of a long (30-ms) pulse of 300 μM glutamate, versus time from the onset of the conditioning pulse to the onset of the test response. Data from four patches, each represented by a different symbol. The solid line is an exponential decay that has a value of 100% at 0.6 ms, the rise time of a maximal response to 300 μM glutamate, and that has a time constant of 2 ms, equal to the time constant of desensitization to a long pulse of 300 μM glutamate.

Even with the briefest conditioning pulse, which produced a tiny response (thick line), desensitization was evident in the test response. Indeed, in this trace, almost as many receptors desensitized as were initially activated.

In Fig. 5*D*, we quantify the extent of enveloping after a test pulse of 300 μM glutamate. The plot shows the percent maximal amplitude achieved by the test response, plotted against the time from the onset of the conditioning pulse to the onset of the test response. The duration of the conditioning pulse ranged from 0.19 to 2.27 ms in ~0.16-ms increments (precise intervals were measured for each patch; see Materials and Methods), and the interval was fixed at 0.84 ms. Note that the longer the time after the onset of the conditioning pulse, the smaller the amplitude of the test response. Moreover, the reduction in the amplitude of the test response with longer conditioning pulses describes an exponential decay. The time constant of desensitization for 300 μM glutamate is 2.01 ± 0.12 ms and the 10–90% rise time is 0.59 ± 0.21 ms (N = 4). To illustrate the extent to which the amplitude of the test response is enveloped by the fully desensitizing response, an idealized trace with a decay of 2 ms is superimposed on the data.

The preceding data were used to develop a quantitative model of binding, opening, desensitization, and recovery at this AMPA receptor (see Discussion). To improve the accuracy of our estimates of certain transition rates, we obtained a measure of the open probability at the peak of the response (P_o), as well as the single channel conductance (γ), by performing NSV analysis on the desensitizing receptors. The mean current, $I(t)$, and variance, $\sigma^2(t)$, of responses of patches to repeated 30-ms applications of 1 mM glutamate were measured. Plots of the variance against the mean current amplitude gave fairly linear relationships that rarely rolled over to approximate a parabola. These data suggest that the P_o for 1 mM glutamate is well under 0.5. Linear regression over the data points provided an estimate of γ (i/driving force, assuming a reversal of 0 mV; Raman and Trussell, 1992b) of 15.0 ± 1.63 pS (N = 4). For 10 mM glutamate, in six of seven patches, the variance-versus-mean plot rolled over sufficiently to be fit with a parabola as described in Materials and Methods. The seventh patch studied with 10 mM glutamate gave a linear variance-mean plot, indicative of a P_o considerably less than 0.5 for this patch. From the parabolic fits, the P_o for 10 mM glutamate was calculated as $P_o = I_{peak}/iN$, where I_{peak} is the maximal value of the mean current, and was equal to 0.54 ± 0.10 (N = 6). An example of a variance-mean plot with a parabolic fit is shown in Fig. 6*A*. This patch had a P_o of 0.69, the highest measured for any of the patches, and a γ, (i/driving force, as above), of 24.7 pS. The mean γ for 10 mM glutamate was 19.3 ± 4.7 pS (N = 7) and was not significantly different from the γ for 1 mM (α = 0.05). The conductance estimate obtained from compiling the data from the 1 mM and the 10 mM patches was 17.7 ± 4.4 pS (N = 11). These values are within the range described for single channel responses with steady-state applications of 10–30 μM glutamate (Raman et al., 1994).

To confirm the measurements of P_o and γ, we examined the coefficient of variation (CV = σ_{peak}/I_{peak}, where σ_{peak} is the standard deviation of the peak current) as a function of the peak macroscopic conductance, G (Jonas et al., 1993), for responses to both 10 and 1 mM glutamate (N = 7 and N = 6, respectively; 16 to 55 records per patch). The data are plotted in Fig. 6*B*. CV varies as a function of N according to the equation $CV(N) = \sqrt{[(1/P_o)-1]}/\sqrt{N}$. As $G = N\gamma P_o$, the relation between CV and G can be written as $CV(G) = \sqrt{(\gamma-\gamma P_o)}/\sqrt{G}$. Substituting 18 pS for γ and 0.54 for P_o gives the solid line in Fig. 6*C*, which appears to describe the data points for 10 mM glutamate quite well. The P_o for 1 mM

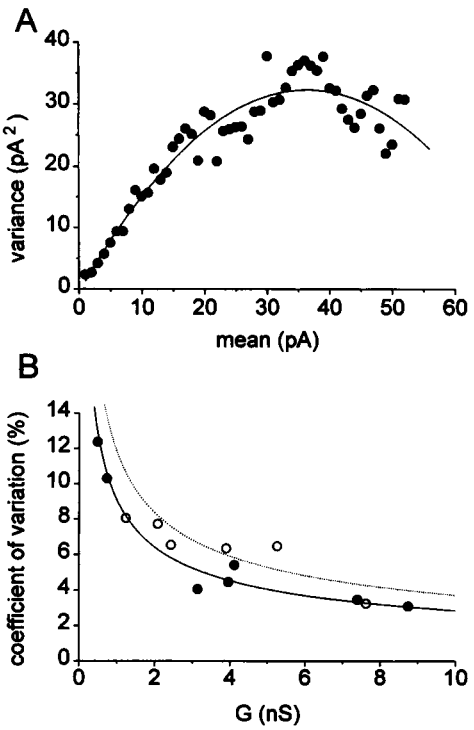


FIGURE 6 Estimates of open probability, P_o, and single-channel conductance, γ. (*A*) Variance, $\sigma^2(t)$, versus mean current, $I(t)$, obtained by NSV analysis of currents evoked by 10 mM glutamate. The data were fit by a parabola (see text). The parameters i and N were estimated to be 1.80 pA and 40 channels, respectively, corresponding to a γ of 24.7 pS and a P_o of 0.69. (*B*) Plot of the coefficient of variation, CV, measured as σ_{peak}/I_{peak}, versus the peak macroscopic conductance, G, for responses to 10 mM glutamate (●) and 1 mM glutamate (○). The lines are functions described in the text, with γ set at 18 pS, and P_o = 0.54 (——) and P_o = 0.22 (·····) for 10 mM and 1 mM glutamate, respectively.

glutamate was estimated from the dose-response curve for glutamate in these neurons (Raman and Trussell, 1992a), which indicates that the P_o for 1 mM glutamate is 1/2.44 times that for 10 mM glutamate. Thus, the dotted line in Fig. 6*B* is derived from setting P_o = 0.22. Although the data points for 1 mM glutamate are more scattered, they are generally consistent with the predicted curve.

DISCUSSION

In recent years, several common properties of AMPA receptors have been identified. For example, two to three molecules of glutamate must bind to gate the channel (Trussell and Fischbach, 1989; Patneau and Mayer, 1990; Raman and Trussell, 1992a; Hestrin, 1992; Colquhoun et al., 1992). With NSV analysis, the mean channel conductance is ~18 pS in brainstem and cerebellum (Traynelis et al., 1993; see above), ~8 pS in visual cortex and hippocampus (Hestrin, 1992; 1993; Jonas et al., 1993), and as high as 27 pS in nonpyramidal visual cortical neurons (Hestrin, 1993). Although NSV technique cannot distinguish among subconductance states or detect mixtures of channels with different conductances, the weighted mean value for conductance we obtained is consistent with single channel measurements in the chick (Zorumski and Yang, 1988; Trussell and Fischbach, 1989; Raman et al., 1994). After binding, activation of the channel occurs very quickly, as glutamate- and kainate-evoked responses, as well as somatic synaptic currents, peak well within 1 ms (Colquhoun et al., 1992; Raman and Trussell, 1992a; Schneggenburger et al., 1992; Zhang and Trussell, 1994).

Other aspects of the responses of AMPA receptors differ quantitatively in different brain regions. For example, desensitization rates measured with essentially identical techniques may vary over 10-fold (Colquhoun et al., 1992; Hestrin, 1992; 1993; Livsey et al., 1993; Raman et al., 1994), depending on the cell types studied. Also, recovery time constants range from 16 ms in nMAG to a few tens or hundreds of ms in hippocampal cells and rat spinal neurons (Colquhoun et al., 1992; Smith et al., 1991). In some regions, such as the nMAG and hippocampal CA1 and CA3 pyramidal cells, recovery occurs in a single exponential phase, whereas in dentate gyrus and cultured spinal neurons, two phases of recovery have been described (Trussell and Fischbach, 1989; Colquhoun et al., 1992). Finally, the value for peak open probability, P_o, is lower in nMAG cells (0.54) for 10 mM glutamate than that observed for 3 mM glutamate in CA3 pyramidal cells (0.71; Jonas et al., 1993) or 5 mM glutamate in neurons of the visual cortex (0.7; Hestrin, 1992). This difference in open probability may reflect, in part, a lower apparent affinity of the nMAG AMPA receptor for glutamate and a shorter channel open time (Raman and Trussell, 1992a).

At some synapses that have AMPA receptors with very rapid desensitization rates, the free transmitter is present in the synaptic cleft long enough to desensitize receptors and terminate the excitatory postsynaptic current (EPSC; Trussell et al., 1993). At other synapses, the transmitter is cleared more quickly than the intrinsic desensitization rate, so that channel burst or open time, and not desensitization, limits the EPSC duration (Clements et al., 1992; Colquhoun et al., 1992; Hestrin, 1992). The present work shows that desensitization proceeds after glutamate removal. As discussed below, this property of AMPA receptors may lead to desensitization upon exposures to transmitter as brief as tens of microseconds.

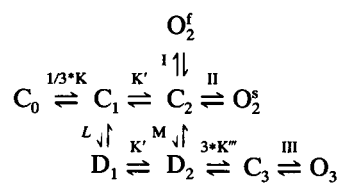
Before discussing the implication of this work for synaptic transmission, it is helpful to describe a plausible kinetic scheme for activation and desensitization of the AMPA receptor. Because the behavior of the receptor is complicated, we will first discuss some properties of simpler kinetic schemes to justify more sophisticated models. A general sequential, two-site model will be explored, with any one of three possible routes for desensitization, from singly liganded closed (C_1), doubly liganded closed (C_2), or open (O_2) receptors. Subscripts indicate the number of ligand molecules bound to a receptor.

$$\begin{array}{ccccccc} C_0 & \leftrightharpoons & C_1 & \leftrightharpoons & C_2 & \leftrightharpoons & O_2 \\ & & \{\downarrow\uparrow & & \downarrow\uparrow & & \downarrow\uparrow\} \\ & & D_1 & & D_2 & & D_2' \end{array}$$

Scheme 1

Forward binding rates were set to 10^7 M^{-1} s^{-1}, unbinding rates at 10^5/s, the opening rate at 6×10^5/s and the closing rate at 2.5×10^3/s. The model was tested by setting the desensitization rates to zero for two of the three routes to desensitization. Forward rates of desensitization were adjusted to give macroscopic desensitization times of between 1 and 10 ms, and the backward rate from desensitization was made low enough to produce 90–99% steady-state desensitization after the peak. A primary goal was to determine in each scheme how much desensitization can occur after very brief pulses of ligand at a high concentration. With desensitization from either O_2 or C_2 and applications just long enough to generate a maximal response (~200 μs), a second, equivalent pulse of agonist 1 ms later always produced a smaller response. Thus, some desensitization always occurred, as expected with relatively fast desensitization and slow recovery. However, neither of these schemes produced the kind of enveloping responses observed in this study in which, after the conditioning pulse was over, receptors desensitized at the same rate and to the same extent as seen with a long conditioning pulse. By contrast, desensitization from C_1 could account for enveloping behavior but only at certain concentrations. At high doses, desensitization to long pulses became quite slow because the receptors were only infrequently in C_1. In this case, brief pulses actually produced greater desensitization than long pulses. By including a route to a desensitized state from both C_1 and either C_2 or O_2, it was possible to maintain enveloping behavior as well as rapid rates of desensitization to high concentrations. Thus, it is most likely that there are multiple desensitized states, including transitions to desensitized states from closed, monoliganded receptors. This conclusion is consistent with the observation that very low doses of glutamate can desensitize a large proportion of receptors, apparently without opening (Kiskin et al, 1986; Trussell and Fischbach, 1989; Smith et al., 1991; Raman and Trussell, 1992a; Colquhoun et al., 1992).

As desensitization to high concentrations of glutamate is nearly complete, the backward rates from the doubly liganded desensitized state must be very slow. Therefore, to account for the relatively quick recovery that we measured, it is necessary provide an alternative route for recovery by linking the singly and doubly liganded states. This approach has been used previously in models of ligand-gated channels (Katz and Thesleff, 1957; Patneau and Mayer, 1991; Raman and Trussell, 1992a; Jonas et al., 1993). In this work, we will consider the model presented in Raman and Trussell (1992a):



Scheme 2

Superscripts f and s denote states with fast and slow closing rates, respectively. Several parameters have been refined to account for new observations, including the deactivation and recovery rates, as well as the enveloping phenomena described above. As discussed previously, C_3 and O_3 are needed to account for a biphasic (rising and falling) dose-response curve for steady-state responses to glutamate (Geoffroy et al., 1991; Raman and Trussell, 1992a). In this scheme, as before, C_1 and C_2 have very different affinities (3000-fold), to account for desensitization by low doses and activation by high doses. To reproduce these results with more equivalent binding sites, the rate of desensitization from C_1 must be high, resulting in a marked slowing of the desensitization rate with increasing agonist concentration, in contrast to the actual behavior of the receptor (Smith et al., 1991; Raman and Trussell, 1992a; Jonas and Sakmann, 1992).

The rates in Scheme 2 (Table 1) have been set to account for the dose-response relations and rates described in Raman and Trussell (1992a) and Raman et al. (1994). The model (with data for the nMAG receptor in parentheses) exhibits an EC_{50} of 2 mM (2.1 mM), an IC_{50} for desensitization by low doses of glutamate of 4 μM (5.2 μM), and desensitization time constants of 1.0 and 3.2 ms (0.96 and 3.50 ms) with the fast component contributing 79% (75%) of the current in response to 10 mM glutamate. The P_o is 0.53 (0.54) for 10 mM and 0.22 (0.22) for 1 mM glutamate, a single deactivation time constant of 0.48 ms (0.50 ms), and a single recovery time constant of 16 ms (15.8 ms). Fig. 7*A* shows desensitizing and deactivating responses to ligand applied for different durations. (Note that, in Fig. 7*A*, the probabilities of all open states have been summed). Fig. 7*B* illustrates recovery after a 10-ms pulse of 10 mM agonist, comparable with Fig. 2. Fig. 7, *C* and *D*, illustrate the model's responses to protocols like those in Fig. 5 and show that the model accounts for the observed enveloping behavior. In these panels, the response to a long pulse of agonist is shown as a dotted line. Note that, in Fig. 7*C*, the peak of the test response to 300 μM ligand, after a 0.1-ms conditioning pulse, exceeds the envelope defined by the long pulse (asterisk); however, peaks of subsequent responses match the dotted trace quite well. In the experiment shown in Fig. 5*D*, a similar deviation was observed for the briefest conditioning pulses. With 10 mM ligand (Fig. 7*D*), enveloping is perfect, and the dotted trace is completely obscured by the test responses.

Fig. 8, *A* and *B*, show the probabilities of the closed, unbound state (C_o), the summed open states, and the singly

TABLE 1 Parameters for scheme 2

Equilibrium constant =	Backward rate (s^{-1}) ÷	Forward rate (s^{-1})
K	300	10^7/M
K′	3×10^5	10^7/M
K″	519	10^7/M
K‴	220	10^7/M
I	3000	6×10^4
II	350	3000
III	2000	6
L	300	1000
M	14	2.7×10^4

Forward rates are to the right and down in scheme 2, except O_2^f.

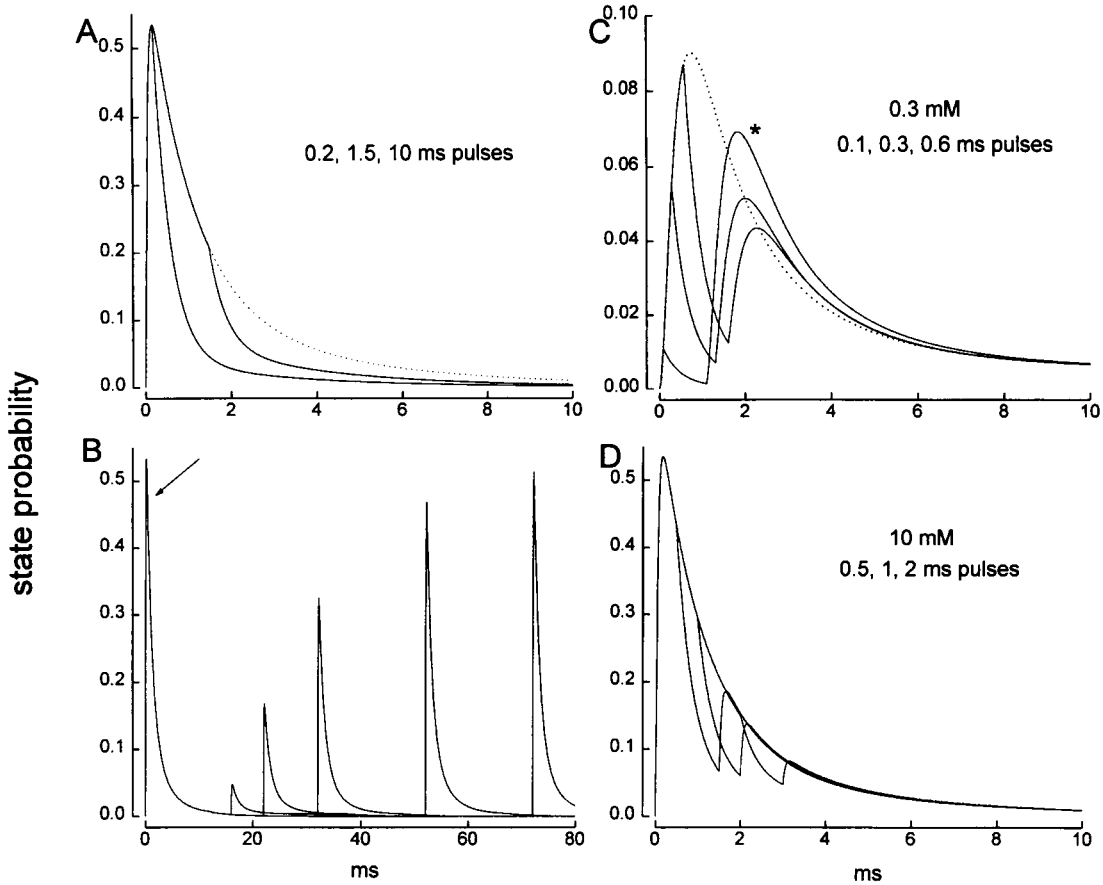


FIGURE 7 Simulations of glutamate-activated currents by Scheme II. (*A*) Total open probability in response to pulses of 10 mM agonist that begin at t = 0 and last for 0.2, 1.5 (——), or 10 ms (·····). Note faster deactivation rate, as observed in Fig. 1 for experimentally observed responses to glutamate. (*B*) Recovery after the complete desensitization to 10 mM agonist. The conditioning pulse (marked by arrow) begins at t = 0 and lasts 10 ms. Test applications of 10 mM agonist lasted 5 ms and were delivered at various intervals after the end of the conditioning pulse. (*C*) A 10-ms test pulse of 300 μM agonist, given 1 ms after conditioning pulses lasting 0.1, 0.3, or 0.6 ms. A long pulse of agonist beginning at t = 0 is shown (·····) to illustrate the maximal possible extent of desensitization. * The test response after the 0.1-ms conditioning pulse. (*D*) A 10-ms test pulse of 10 mM agonist 1 ms after conditioning pulses lasting 0.5, 1, and 2 ms. A long pulse of agonist was also applied at t = 0, as in *C*, but the response is obscured by the overlying test responses.

bound desensitized state (D_1) for a 0.2- and 10-ms pulse of ligand. Of particular interest is the evolution of D_1. With either the short or long pulse protocol, the probability of the D_1 state increases dramatically after the end of the pulse. The gradual accumulation of receptors in this state may explain a variety of experimental observations described above, including the continued desensitization of receptors after removal of free glutamate and the apparent delay in the onset of recovery from desensitization.

After release of a single vesicle, the lifetime of the transmitter in the synaptic cleft could be as brief as 100 μs, as suggested by the brevity of miniature EPSCs (in nMAG, the rise time is 91 μs and decay time is 180 μs at 29–32°C; Zhang and Trussell, 1994). Because the piezoelectric device could not reliably produce pulses shorter than 250 μs, we have used Scheme 2 to predict the extent of desensitization of the receptors after extremely brief pulses of ligand. Fig. 8*C* simulates the responses to pairs of 80-μs pulses of 2 mM ligand (the EC_{50}) delivered at varying intervals. Even after such a brief pulse, which activates only a small fraction of receptors, the amplitude of subsequent responses is markedly reduced. Additionally, in the simulation, recovery from desensitization began after a delay and proceeded with the time course characteristic of recovery after longer pulses (not shown). This effect was more completely examined by simulating the experiment shown in Fig. 4, *B* and *C*. The results are plotted in Fig. 4*C* as a continuous line, which shows that the amount of recovery after 2 ms is dependent on the duration of the conditioning pulse. For conditioning pulses as short as 50 μs, the model predicts depression of the test response; with concentrations greater than 1 mM, even shorter pulses produce depression. Because a significant degree of desensitization is expected to occur after the briefest applications of ligand, receptors may desensitize even upon release of a single vesicle of transmitter.

In some systems, the time course of glutamate in the synaptic cleft has been estimated to be longer, with a time constant of 1.2 ms (Clements et al., 1992); thus assuming the receptor has qualitatively the same features as those described above, desensitization could limit receptor availability even at noncalyceal synapses. Indeed, the kinetic model of Jonas et al. (1993), which describes hippocampal AMPA

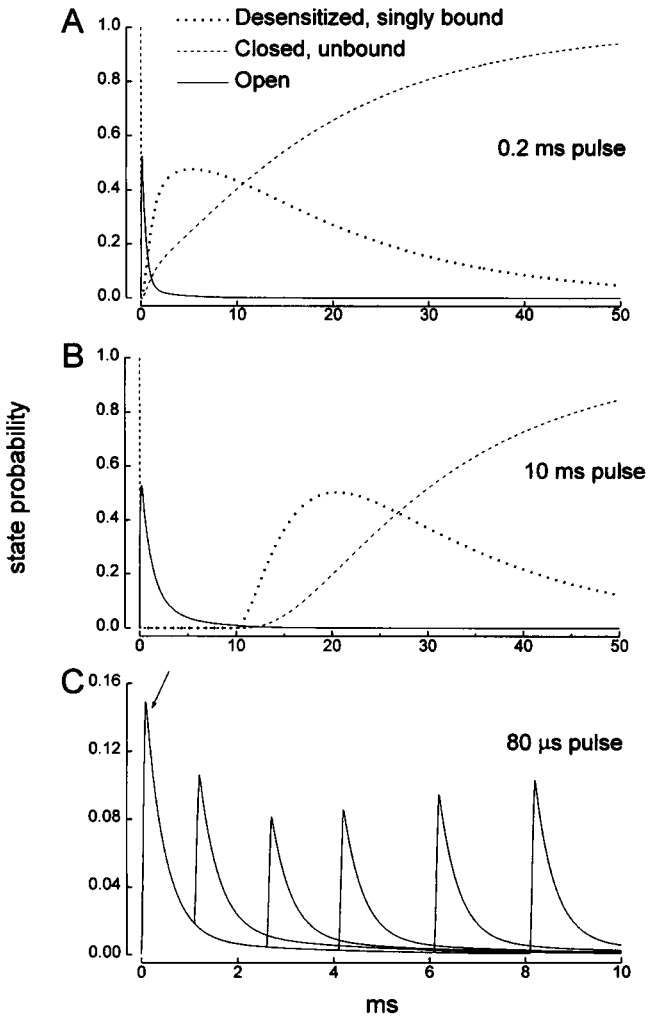


FIGURE 8 Predictions made from Scheme 2 for different states. (*A* and *B*) Probability of the desensitized, singly bound state (·····; D_1 in the model) and of the closed, unbound state (– – –; C_0) and the summed probability of all the open states (——) during and after a 0.2-ms (*A*) and 10-ms (*B*) pulse of 10 mM ligand. Note that, in both cases, D_1 gradually increases and declines during the recovery process. (*C*) An 80-μs conditioning pulse of 2 mM agonist (arrow) followed by 80-μs, 2 mM pulses at various intervals.

receptors with slower kinetics, also predicts substantial depression with paired brief pulses of glutamate. Testing this hypothesis further may prove difficult because, with sequential stimuli, a given active zone may not release transmitter each time, and thus postsynaptic receptors would not be challenged at the stimulus rate.

It is important to distinguish the effect of desensitization described in this study from that described by Trussell and Fischbach (1989) and Trussell et al. (1993). In those studies, desensitization appeared to determine the decay rate of the synaptic conductance. As noted above, this is not the case at all glutamatergic synapses (e.g., Colquhoun et al., 1992; Hestrin, 1992), and, as demonstrated in the nMAG, desensitization contributes less to the EPSC decay time when quantal content is reduced (Trussell et al., 1993). Accordingly, in Fig. 8*C*, the decay of the conductance is faster than the desensitization rate and is determined by the burst duration of the channels. Notably, in the model, deactivation is independent of desensitization, as the deactivation rate stays constant even after the desensitization rates are set to zero. Nevertheless, desensitization does regulate the amplitude of the second response and, therefore, may contribute to synaptic depression. In this context, the rate of recovery from desensitization at a variety of synapses could determine the maximal rate of firing before postsynaptic depression limits synaptic strength (Colquhoun et al., 1992; Hestrin, 1993).

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