Rapid decay of averaged single-channel NMDA receptor activations recorded at low agonist concentration

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SUMMARY

The NMDA class of glutamate receptors have the unique property of binding some agonists, including glutamate, for a very long period of time. One manifestation of this is that brief (1 ms) application of glutamate (1 mM) produces a slowly decaying current, the major component of which has a time constant of approximately 200 ms. Application of glutamate at low concentrations allows identification of groups ('superclusters') of openings in the data record that probably correspond to a single period during which one or more molecules of glutamate are bound to the receptor, i.e. a single activation of the channel. The length of such superclusters is long on average (74 ms); the longest component of the distribution has a duration of approximately 300 ms, and comprises about 25% of the area. However, aligning many superclusters to obtain an average current reveals that the decay is mainly fast; the major component has a time constant of around only 5 ms. It is shown that incorporation of a distribution of first latencies (from the time of the jump to the first opening) can explain at least part of this discrepancy.

1. INTRODUCTION

Release of glutamate from synaptic terminals in the mammalian central nervous system results in the activation of at least two classes of glutamate receptors. One class, selectively activated by AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) or kainate, underlies the fast component of the synaptic current, whereas a second class, activated by NMDA (N-methyl-D-aspartate), gives rise to a slower component which lasts a few hundred milliseconds (Forster & Westbrook 1988; Collingridge et al. 1988; Bekkers & Stevens 1989; Silver et al. 1992). It has been shown recently that the slow decay arises not from the prolonged presence of glutamate in the synaptic cleft, but from the fact that the agonist remains bound to the receptor, and produces openings, for a remarkably long time (Lester et al. 1990; Gibb & Colquhoun 1991, 1992). We show here, for example, that brief (1 ms duration) applications of glutamate to outside-out membrane patches yield slowly decaying currents similar to those recorded at glutamatergic synapses (see also Lester et al. 1990; Lester & Jahr 1992).

For applications of glutamate that are sufficiently short, the NMDA receptor is not likely to become occupied by agonist more than once, and the agonist concentration will be zero throughout almost the entire timecourse of the recorded response (i.e. except for the first millisecond of a 1000–1500 ms response). Under these conditions the observed decay of an ensemble current should reflect the average behaviour of NMDA channels during a single period throughout which one or more molecules of agonist is bound to the receptor.

It is therefore of interest to see the extent to which the timecourse of the response can be predicted by the single channel 'activations' recorded at very low agonist concentrations. In this study, as in Gibb & Colquhoun (1991), an 'activation' is defined as the observed duration of a single period during which at least one agonist molecule is bound to the receptor, i.e. the time elapsed from the beginning of the first opening to the end of the last opening of such a period; it does not, therefore, include either the latency to first opening or the latency to dissociation after the last closing. A very low agonist concentration was used to ensure that groups of openings that correspond to individual channel activations (identified as superclusters) could be identified. In this way, each group of openings resembles a brief concentration jump or synaptic current, in that agonist rebinding does not contribute to the decay kinetics (Lester et al. 1990).

The low-concentration studies show that glutamate produces superclusters of openings that often last for over 200 ms (see also Gibb & Colquhoun 1991, 1992). It was suggested by Gibb & Colquhoun (1991, 1992) that the long superclusters, which probably correspond to single activations of the receptor, underlie the slow macroscopic currents. We have now investigated this question more quantitatively. For example, although one component (24%) of the distribution of supercluster lengths had a length (mean ≈ 300 ms) comparable with that of the slow decay of the current observed in jump experiments, simply aligning the first opening of several superclusters yields a rapidly decaying average current. As a result of this finding, we have begun to investigate other properties of the
NMDA channel currents with this pipette solution was of NaOH solution (diluted by 10% before use) contained in millimoles pulled without fire polishing to a final resistance of prepared from thick-walled borosilicate glass (Clark Electroimmersion objective (Zeiss), and outside-out patches were made from granule cells in dentate gyrus. Patch pipettes were approximately +15 mV. All recordings were made at room temperature (22— 26 °C).

Slices of a theta glass partition (pulled to an external diameter of about 200 pm). The theta glass (Clark Electromedical GCI50F-7.5) and coated with Sylgard resin (Dow Corning 184) to within 100 μm of the tip. Pipettes were pulled without fire polishing to a final resistance of 10—20 MΩ (for concentration jump experiments) or 20—30 MΩ (for low-concentration experiments). The pipette solution (diluted by 10% before use) contained (in millimoles per litre): 140 NaCl, 10 NaCl, 10 HEPES, 10 EGTA and 6.5 CaCl₂. Internal pH was adjusted to 7.4 by addition of NaOH (ca. 2.2 ml of 4 M). The reversal potential of NMDA channel currents with this pipette solution was approximately +15 mV. All recordings were made at room temperature (22—26 °C).

2. METHODS

(a) Preparation

Hippocampal slices (250 μm thick) were prepared from 11—14 day-old rats using standard procedures (Edwards et al. 1989). Transverse slices were cut with a Vibroslice (Camden Instruments) and then incubated at 32 °C for 15—30 min in standard Ringer before use. Ringer was made from high-purity chemicals to minimize contamination by Mg²⁺ ions, and comprised (in millimoles per litre): 125 NaCl, 2.5 KCl, 1.0 CaCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, 25 glucose and 1 MgCl₂. Solutions were bubbled continuously with 95% O₂ and 5% CO₂ to maintain a pH of approximately 7.2. Slices were viewed under Nomarski optics using a 40 x water immersion objective (Zeiss), and outside-out patches were made from granule cells in dentate gyrus. Patch pipettes were prepared from thick-walled borosilicate glass (Clark Electromedical GCI50F-7.5) and coated with Sylgard resin (Dow Corning 184) to within 100 μm of the tip. Pipettes were pulled without fire polishing to a final resistance of 10—20 MΩ (for concentration jump experiments) or 20—30 MΩ (for low-concentration experiments). The pipette solution (diluted by 10% before use) contained (in millimoles per litre): 140 NaCl, 10 NaCl, 10 HEPES, 10 EGTA and 6.5 CaCl₂. Internal pH was adjusted to 7.4 by addition of NaOH (ca. 2.2 ml of 4 M). The reversal potential of NMDA channel currents with this pipette solution was approximately +15 mV. All recordings were made at room temperature (22—26 °C).

(b) Concentration jump experiments

Fast application of agonist was accomplished by rapid movement across the tip of the patch pipette of the interface of two solutions (drug and control) flowing from either side of a theta glass partition (pulled to an external diameter of about 200 μm). The theta glass (Clark Electromedical TGC150-10) was moved with a 100 V piezotraper (Physik Instrumente, Model P-810.10). The method is a modification of that of Franke et al. (1987), as used by Colquhoun et al. (1992). Concentration jumps of 1 ms duration were made every 8 s from a control solution (same as Ringer defined above) which lacked Mg²⁺ but contained 5 μM glycine and 5 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), to the same solution plus 1 mM glutamate.

Currents were recorded with a L/M EPC-7 (List Electronic, F.R.G.) at a holding potential of —60 mV and filtered at a final cutoff frequency of 1 kHz (—3dB, 8-pole Bessel). The timing of cycles, the DAC output that controlled the piezotraper and the sampling of the response (at 10 kHz) were all done by interrupt-driven instructions to a CED 1401 plus interface (Cambridge Electronic Design), from a protected mode FORTRAN program.

Ensemble currents were obtained by averaging of individual sweeps off-line. The decay phases of these ensembles were fitted by using the method of least squares with the sum of two exponential components.

(c) Low-concentration experiments

A low concentration of glutamate (30 nM) was applied, together with a low concentration of glycine (100 nM), by positioning the patch pipette in front of the appropriate barrel of the theta glass application system. The glycine concentration was lowered to reduce channel activity to avoid lowering glutamate to a level comparable to that of contaminating amino acids in solution. Use of a low concentration of glycine is unlikely to alter the characteristics of channel activations (Gibb & Edwards 1991; see also Discussion). Channel currents were recorded on FM tape (Racal Store 4DS, DC—8kHz. —3dB, Bessel).

(d) Analysis of low-concentration experiments

Data were played back from tape, amplified, filtered at a final calculated cutoff frequency of 2 kHz (—3dB, 8-pole Bessel) and sampled at 20 kHz onto computer (PPD-11/73 via a CED 502 interface (Cambridge Electronic Design). The durations of open and closed times were determined by the method of timecourse fitting (see Colquhoun & Sigworth 1983) which in these experiments allowed the detection of openings and shuttings as brief as 70—80 ps and 60—70 ps, respectively. Thus, a high-resolution idealized record was constructed which included sublevels of the channel.

Supercluster and closed time distributions are shown as histograms with the log of the duration on the abscissa and the square root of the frequency on the ordinate (Sigworth & Sine 1987). With this method of display, the peaks of the fitted distribution correspond to the means of the exponential components. Each distribution was fitted with the sum of exponential components by the method of maximum likelihood (Colquhoun & Sigworth 1983).

(e) Definition of bursts, clusters and superclusters in steady-state records

Groups of openings were classified as belonging to bursts, clusters or superclusters according to a critical duration (t₉₅%) defined for each by the closed time distribution. In most cases, t₉₅ was calculated so as to equalize the percentage of gaps belonging to a long gap distribution misclassified as short and the percentage of gaps belonging to the short distribution misclassified as long (Colquhoun & Sakmann 1985). Thus, bursts were defined as groups of openings separated by a gap longer than the t₉₅ between the second and third components of the shut time distribution (Howe et al. 1991; Traynelis & Cull-Candy 1991; Gibb & Colquhoun 1991, 1992). Similarly, clusters contained the first three gap components, and superclusters contained all but the longest (fifth) gap component.

(f) Missed events

Although the fitted time constants suggest that there were substantial numbers of events too brief to be detected, there is no practicable way to make corrections for their omission in records of this complexity. This will become possible only when we postulate a formal mechanism for the receptor (see Hawkes et al. 1992).

(g) Timecourse of activations aligned from low-concentration experiments

Superclusters were identified in the current record according to the specified value of t₉₅%. Sections of data of fixed length (300—600 ms) were marked for subsequent averaging. Before saving events to disk, each marked section was checked to make sure that it contained only one supercluster; if a gap longer than t₉₅ occurred after the initial opening then all openings after this gap were changed to a mean baseline current value.

After 100–400 superclusters had been collected, all events were averaged with the first opening of each supercluster occurring simultaneously. Ensemble decays were then fitted with the sum of two exponential components.

3. RESULTS

(a) Concentration jumps

The time taken for solution exchange (10—90% risetime) was approximately 140 μs as estimated from shifts in liquid junction potentials at the tip of a bare patch pipette (figure 1a, inset). An average timecourse (figure 1a) of the NMDA component of current activated by a 1 ms application of glutamate is shown in figure 1, together with records produced by a single application of glutamate (figure 1b).

The decay phase of each ensemble current was fitted with two exponential components. The results were \( \tau_1 = 34.6 \pm 4.3 \text{ ms (relative amplitude } 37 \pm 10\% ), \), \( \tau_2 = 279 \pm 70 \text{ ms (relative amplitude } 63 \pm 10\% ) \) (n = 4). The faster of the two time constants was shorter, and possessed a smaller relative amplitude, than was reported previously for cultured hippocampal cells (Lester et al. 1990; Lester & Jahr 1992). Part of this discrepancy may be related to the shorter concentration jumps used in this study (unpublished observations), but part may be explained by the difference in the age of animal used (Hestrin 1992).

(b) Single-channel currents with very low agonist concentrations

Previous studies using continuous application of a low concentration of glutamate showed the presence of unusually long shut times (mean ≈ 10 ms) within an individual activation, and it was quite likely that a longer gap component, with mean ≈ 100 ms, also occurred (Gibb & Colquhoun 1992). The use of a lower glycine concentration in this work made it easier to distinguish one activation from the next. The slowest component of the shut time distribution, which we interpret as the time between individual activations, ranged from 0.84 s to 7.7 s.

Distributions of apparent open and closed times (figure 2a) were fitted with the sum of two and five exponential components, given as time constants (in milliseconds) with relative areas, with the following values: (i) 0.047, 47%; (ii) 0.51, 17%; (iii) 12.9, 9.6%; (iv) 314, 4.1%; and (v) 6148, 22%. (b) A distribution of supercluster lengths is shown for a different patch; fitted with the sum of three exponential components (same format as in (a)): (i) 0.07, 31%; (ii) 7.5, 39%; and (iii) 300, 30%.

Figure 1. Brief concentration jumps of glutamate produce slowly decaying currents. (a) A 1 ms application of 1 mM glutamate yields an ensemble (average of 30 sweeps) current shown together with fitted exponentials (\( \tau_1 = 27 \text{ ms, relative amplitude } 42\% )\). The brief deflection at the left-hand side of the top trace shows the time of application of glutamate. Inset: the timecourse of a junction potential shift across the tip of a bare patch pipette (resistance ≈ 20 MΩ) using Ringer and 20% Ringer. Top trace indicates the time of switching of the piezotranslator. Note that the lag between the time of switching and the time of concentration change at the patch pipette is typical, and generally ranged from 1–1.3 ms. (b) Two individual sweeps are shown for the ensemble current obtained from the patch shown in (a). Calibrations are the same as those shown in (a).

Figure 2. Long shut periods and long superclusters of openings can be detected in the presence of a low concentration of glutamate. (a) An example of a distribution of shut times obtained with 30 nM glutamate and 100 nM glycine. The distribution is shown fitted with the sum of five exponential components, given as time constants (in milliseconds) with relative areas, with the following values: (i) 0.047, 47%; (ii) 0.51, 17%; (iii) 12.9, 9.6%; (iv) 314, 4.1%; and (v) 6148, 22%. (b) A distribution of supercluster lengths is shown for a different patch; fitted with the sum of three exponential components (same format as in (a)): (i) 0.07, 31%; (ii) 7.5, 39%; and (iii) 300, 30%.
Table 1. Single channel distributions with 30 nM glutamate and 100 nM glycine

(Values for tfcrlt used to identify bursts, clusters and superclusters were 2.2 ms, 37 ms and 642 ms, respectively.)

<table>
<thead>
<tr>
<th>Component</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shut times mean/ms</td>
<td>0.06 (0.01)</td>
<td>0.71 (0.09)</td>
<td>12.5 (1.0)</td>
<td>360 (36)</td>
<td>4213 (1551)</td>
</tr>
<tr>
<td>Area</td>
<td>0.42 (0.02)</td>
<td>0.22 (0.02)</td>
<td>0.09 (0.00)</td>
<td>0.05 (0.02)</td>
<td>0.23 (0.01)</td>
</tr>
<tr>
<td>Open times mean/ms</td>
<td>0.22 (0.06)</td>
<td>3.0 (0.2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Area</td>
<td>0.30 (0.05)</td>
<td>0.70 (0.05)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Burst duration mean/ms</td>
<td>0.05 (0.01)</td>
<td>2.3 (0.7)</td>
<td>14.6 (5.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Area</td>
<td>0.49 (0.04)</td>
<td>0.19 (0.07)</td>
<td>0.32 (0.09)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cluster duration mean/ms</td>
<td>0.05 (0.01)</td>
<td>5.0 (0.4)</td>
<td>38.0 (2.8)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Area</td>
<td>0.50 (0.06)</td>
<td>0.36 (0.04)</td>
<td>0.14 (0.02)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Supercluster duration mean/ms</td>
<td>0.05 (0.01)</td>
<td>6.6 (0.04)</td>
<td>329 (35)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Area</td>
<td>0.45 (0.01)</td>
<td>0.32 (0.05)</td>
<td>0.24 (0.07)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* All values given with standard errors in parentheses.

Figure 3. Aligned activations from low-concentration experiments decay rapidly. (a) The average decay timecourse is shown for 297 aligned superclusters along with the fitted curve ($\tau_1 = 4.6$ ms, relative amplitude = 82% ; $\tau_2 = 38$ ms). (b) Six individual superclusters used to construct the average in (a) are shown digitally filtered to a final calculated half-power frequency (Gaussian) of 1 kHz.

For $t_{crlt}$ used to identify bursts, clusters and superclusters were 2.2 ms, 37 ms and 642 ms, respectively. The three briefest components of the shut time distribution (table 1) were similar in duration to values reported previously for CA1 pyramidal cells (Gibb & Colquhoun 1992). The fourth component had a mean value of 360 ± 36 ms and appears to be the longest occurring within a single activation of the receptor. It was somewhat longer (and smaller in area) than the value, 137 ± 26 ms, reported by Gibb & Colquhoun (1992) for CA1 cells, possibly because the present results are from dentate granule cells, or because the extremely long duration of the slowest (fifth) component in this study allowed clearer resolution of the fourth.

Groups of channel openings can be subdivided into bursts, clusters and superclusters. Distributions of supercluster lengths were fitted with the sum of three exponential components, and both the longest component of the distribution as well as the overall mean of the distribution were long on average (329 ms and 74 ms, respectively; table 1). An example of this distribution is shown in figure 2b, and examples of the superclusters on which it is based are shown in figure 3b. The slow component of the cluster length distribution ($\tau = 38$ ms), and of the supercluster length distribution ($\tau = 329$ ms), were comparable with the time constants for the decay of macroscopic current seen in concentration jump experiments or in synaptic currents.

Alignment of low-concentration superclusters

To determine whether the low-concentration protocol produced NMDA receptor activations (identified here with superclusters) which could account for the decay of current in jump experiments, we aligned several superclusters identified in low-concentration experiments to approximate the lining up of activations which occurs with a short pulse of agonist. Ensemble averages, aligned with the start of the first opening of the activation, were constructed for three experiments in which activations were most clearly distinguishable (figure 3a). Examples of the superclusters that were averaged are shown in figure 3b. Average aligned activations were fitted with two exponential components; the results were $\tau_1 = 5.3 ± 0.7$ ms with relative amplitude = 83 ± 4 %, and $\tau_2 = 36.9 ± 10.5$ ms with relative amplitude = 17 ± 4 %. Decays of the ensemble currents were thus considerably faster than the decay recorded with brief jumps of saturating agonist. The distribution of supercluster length clearly implies the presence of a slow (≈ 300 ms) component in this ensemble average; however, the amplitude of this component was too small to be detected. For example, the late openings in the first and fifth traces of figure 3b.
contribute little to the overall average current shown in figure 3a.

(d) First latency simulations

The discrepancy between the decay rates of the brief jumps and the aligned activations must arise, in part, because the latency between agonist binding and the first opening of the channel is not taken into account when activations are aligned as above. To assess the effect on the decay kinetics of the fact that activations do not start simultaneously after an agonist pulse, each supercluster was offset by a first latency before averaging. The first latencies were random values from the distribution of first latencies predicted by the kinetic model for the NMDA receptor that was described recently by Lester & Jahr (1992). Their microscopic association rate constant was 4.7 s⁻¹, for both steps, and the other rates (s⁻¹) were as shown:

\[
\begin{align*}
A & \rightarrow AR \rightarrow A_{1}R \rightarrow A_{1}R^* \\
& \rightarrow \frac{4.7}{0.6} 1.8 \frac{1}{s} \times 8.4 \\
& \rightarrow A_{2}D
\end{align*}
\]

This model describes quite well the timecourse of the response to brief concentration jumps; however, it does not correctly describe, for example, open or shut time distributions or correlations between shut times (see, for example, Gibb & Colquhoun 1992), so only qualitative results can be expected. Using a short (0.2 ms) pulse of 1 μM Glu, the first latency distribution, calculated from the model as described by Colquhoun & Hawkes (1987), consisted of the sum of two exponential components, \( \tau_1 = 15.5 \text{ ms (area 86%) } \) and \( \tau_2 = 641 \text{ ms (area 14%) } \), with an overall mean of 102 ms (similar values were obtained with a 1 ms pulse). After assigning latencies, the decay of the resulting ensemble average could be fitted with a single exponential with a mean time constant of \( 32 \pm 1.6 \text{ ms } (n = 3) \) (figure 4a). This value is similar to the faster component of decay found in the jump experiments. However, allowing for the first latency distribution, to the extent that the model used is adequate, still does not account for the slow component of macroscopic decay, with a time constant in the 200–300 ms range.

As these simulation results do not adequately describe the macroscopic jump current, we have calculated the first latency distribution that would have to be postulated to account for the macroscopic current after a brief jump, if it were true that individual channel activations had the same structure in the low-concentration experiments as they have following a brief concentration jump.

Derivation of the convolution. The expected macroscopic current, \( I(t) \), at time \( t \) after a brief concentration jump is given by

\[ I(t) = iNP(t), \quad \text{(1)} \]

where \( P(t) \) is the probability of a channel being open at time \( t \), \( N \) is the number of channels in the patch (which is not generally known), and \( i \) is the (mean) single-channel current. If individual channel activations from a low-concentration record are aligned on their first openings before averaging, the expected average current at time \( t \) can be denoted

\[ I_{\text{avg}}(t) = \int_{0}^{t} P_{\text{avg}}(t - t' \delta(t')) \, dt', \quad \text{(2)} \]

where

\[ P_{\text{avg}}(t) = \text{prob} [ \text{open at } t \mid \text{first opening starts at time zero}, \quad \text{(3)} \]

\[ P_{\text{avg}}(t) = \text{prob} [ \text{open at } t_0 + t \mid \text{first opening starts at } t_0]. \quad \text{(3)} \]

(Note that each activation originates from one channel only.) A channel will open at a specified time \( t \) following a jump if both: (i) the latency to the first opening is \( t'_0 \); and (ii) the channel is open at \( t = t_0 + t' \), given that it first opened at \( t_0 \). Thus \( t' \), the time from first opening to \( t \), is given by \( t - t_0 \), so the probability of the latter event is, from (3), \( P_{\text{avg}}(t - t_0) \). Denoting the probability density function for the duration, \( t_0 \), of the first latency as \( f(t_0) \), and integrating over all possible values of \( t_0 \) (from 0 to \( t \)), we obtain

\[ P(t) = \int_{t_0=0}^{t} f(t_0) P_{\text{avg}}(t - t_0) \, dt_0. \quad \text{(4)} \]

This is the convolution of \( f \) with \( P_{\text{avg}} \), and can be solved for the first latency distribution, \( f(t) \), by deconvolution. The solution can be found most conveniently by using Laplace or Fourier transforms. Thus

\[ f(t) = \mathcal{F}^{-1} \left[ \mathcal{F} \left[ P(t) \right] \right], \quad \text{(5)} \]

where \( \mathcal{F} \) and \( \mathcal{F}^{-1} \) denote Fourier and inverse Fourier transforms. Apart from an unknown constant, the observed \( I(t) \) and \( I_{\text{avg}}(t) \) can be substituted for \( P(t) \) and \( P_{\text{avg}}(t) \), respectively.

Application of the result. Equation (5) was evaluated with the Fourier transforms supplied with Mathcad (V3.1, MathSoft). A representative macroscopic current (shown in figure 1a) was used as \( I(t) \), and the timecourse of aligned activations, \( I_{\text{avg}}(t) \), was supplied in the form of values calculated from the equation fitted to the raw data (use of the actual data would result in a noisier output). The output was then exported to a FORTRAN program in which it was fitted with a sum of two exponentials.

Figure 4b shows an example of a deconvolved first latency distribution obtained using an experiment for which the fitted decay of aligned activations (\( \tau_1 = 4.6 \text{ ms, amplitude 82% } \); \( \tau_2 = 38.4 \text{ ms, amplitude 18% } \)) was representative of the mean values. For three sets of activation timecourses the average fitted first latency distribution was \( \tau_1 = 14.9 \pm 1.1 \text{ ms (area = 10 \pm 2%) } \); \( \tau_2 = 174 \pm 1.7 \text{ ms (area = 90 \pm 2%) } \). In terms of area (but not amplitude) this is dominated by the slow component, which has a time constant that is virtually the same as the slow time constant found with concentration jumps. The time constants for the first
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(a) Jahr (1992), but the slower component has a far larger from a distribution of first latencies (see Results). A single with a first latency, each of which was assigned randomly An ensemble current is shown for superclusters convolved with the fitted exponential curve (rx = 13 ms, relative frequency = 2 kHz. amplitude = 63%; r2 = 176 ms). This latency density was the current. Results are from the same patch as in figure 3. activations following a concentration jump are similar to those observed at low agonist concentration. Sampling (a , b) Deconvolved first latency probability density is shown and the timecourse of decay of aligned superclusters shown in figure 3a, i.e. it is based on the premise that activations following a concentration jump are similar to those observed at low agonist concentration. Sampling frequency = 2 kHz.

The fact that ‘24% of superclusters are long’ (mean 329 ms; see table 1) might lead one to expect a substantial slow component when superclusters are aligned. However, even though the slow component may carry something of the order of half the charge that is passed, the amplitude of the slow component in the averaged current will be small because the amplitude of a component is proportional to (area)/(time constant) (e.g. using the time constants for the two slower components of supercluster length from table 1, a relative amplitude for the slowest component of roughly 0.5 × (6.6/329), i.e. around 1%, might be expected). There are two obvious reasons for the failure of the averaged aligned activations to predict the macroscopic decay. (i) The first latency from the time of the jump to the first opening is often far from negligible; preliminary results from patches with few (possibly one) channels show that the first-latency distribution was a prominent slow component with a time constant of hundreds of milliseconds. And (ii) activations elicited by a brief pulse of high agonist concentration may differ from those elicited by very low agonist concentrations. Experimental measurements of these quantities are in progress, but are very difficult to obtain because there is no simple way to control or determine the number of channels in the patch. In the meantime, our results allow something to be said about each of these possibilities.

(b) Activations in jump and steady-state experiments

4. DISCUSSION

Our results show that aligned activations obtained at very low glutamate concentrations decay far too rapidly to account for the macroscopic jump current. The fact that ‘24% of superclusters are long’ (mean 329 ms; see table 1) might lead one to expect a substantial slow component when superclusters are aligned. However, even though the slow component may carry something of the order of half the charge that is passed, the amplitude of the slow component in the averaged current will be small because the amplitude of a component is proportional to (area)/(time constant) (e.g. using the time constants for the two slower components of supercluster length from table 1, a relative amplitude for the slowest component of roughly 0.5 × (6.6/329), i.e. around 1%, might be expected).

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experiments, but is hard to determine for the jump protocol.) This would not be surprising in principle, despite the fact that the agonist concentration is zero, or very small, for most of the time in both sorts of experiment. The evolution of the single-channel behaviour will depend on the initial occupancies of the various states at \( t = 0 \) (the vector \( 0(0) \) in Colquhoun & Hawkes (1987)). These occupancies may well be different in the low-concentration recordings (for which the equilibrium initial vector, described in Colquhoun & Hawkes (1982), will apply), and in the jump experiments in which the important values are the occupancies at the end of the brief pulse. It is possible, for example, that activations observed at low concentrations reflect primarily the activity of a monoliganded channel, although even brief application of a high concentration of glutamate may produce a substantial number of multiply liganded receptors.

A less likely possibility for the rapid decay of aligned activations is that unbinding of glycine, which is required for channel opening (Kleckner & Dingledine 1988; Lerma et al. 1990; Henderson et al. 1990; Vytkicky et al. 1990) terminates the activation prematurely in the low-concentration experiments. The reported rate constant for glycine unbinding varies between \( 1 \text{ s}^{-1} \) (Johnson & Ascher 1992) and \( 3.1 \text{ s}^{-1} \) (Benveniste et al. 1990). Assuming an unbinding rate of \( 3 \text{ s}^{-1} \), the mean duration of occupancy of the receptor by glycine is about nine times longer than the slow component of 37 ms for the decay of the aligned activations. Moreover, the mean supercluster length determined in these experiments using low glycine is nearly the same as the supercluster length found previously with nearly saturating (1 \( \mu \text{M} \)) glycine in CA1 pyramidal cells (Gibb & Colquhoun 1992). Thus, it appears unlikely that glycine unbinding is increasing the rate of decay of aligned activations in these experiments.

In conclusion, it seems likely that a long first latency distribution, and a higher \( P_{\text{open}} \) in activations elicited by a short jump, both contribute to the slow component of the macroscopic decay. To take this investigation further it is clearly essential to have experimental measurements of both the distribution of first latencies, and of the characteristics of the activations that are elicited by brief concentration jumps. Such experiments are in progress, but they are greatly complicated by the need to know the number of channels that are in the membrane patch before their results can be interpreted.

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