Desensitization of *N*-methyl-D-aspartate receptors: A problem of interpretation

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ABSTRACT **The phenomenon of desensitization is universal, but its mechanism is still ill-understood and controversial. A recently published study [Lin, F. & Stevens, C. F. (1994) *J. Neurosci,* 14, 2153–2160] attempted to cast light on the mechanism of desensitization of *N*-methyl-D-aspartate (NMDA) receptors, in particular the vexed question of whether the channel must open before it can desensitize. During the desensitizing preexposure to agonist in those experiments, more desensitization was produced when channel openings were observed than when no openings were observed. The conclusion that "desensitization occurs more rapidly from the open state" unfortunately was based on a stochastic fallacy, and we present here a theoretical treatment and illustration showing that the observed behavior is predicted by a simple mechanism in which desensitization can occur only from a shut state.**

The phenomenon of desensitization is universal, but little is known about its mechanism. It may be argued, in the case of muscle nicotinic receptors, that it is a purely experimental phenomenon with little physiological importance. However, in the case of *N*-methyl-D-aspartate (NMDA) receptors, it is likely that long-lived fully-liganded shut states, which may reasonably be termed "desensitized states," are important for determining the slow time course of synaptic currents mediated via NMDA receptors (1–3). Thus, the mechanism of desensitization of NMDA receptors is of direct physiological relevance. In particular, the question of whether ion channels must open before they can desensitize has given rise to much discussion (but few unambigous answers) over many years. Lin and Stevens (4) published some ingenious experiments that were designed to cast light on the difficult problem of whether or not NMDA receptors can desensitize from one or more open states, shut states, or both. They found that more desensitization was produced in cases where channel openings were observed during the desensitizing preexposure to agonist, compared with the smaller extent of desensitization that was found when no openings were observed during the preexposure. They concluded that "desensitization occurs more rapidly from the open states." Unfortunately this conclusion is based on a fallacy, and behavior of the sort that was observed is predicted by a simple mechanism in which desensitization can occur only from a shut state.

In the experiments of Lin and Stevens (4), a prepulse of NMDA (e.g., 2.5 μM for 600 ms) was applied to outside-out patches from cultured hippocampal neurones. The amount of desensitization produced by the prepulse was assessed by measuring the peak response to a test pulse of NMDA (e.g., 100 μM for 1 s), which was applied at the end of the prepulse. Each such episode (prepulse plus test pulse) will be referred to here as a "sweep." Sweeps for which one or more channel

FIG. 1. The mechanism proposed by Lester and Jahr (2). The open channel is denoted R*, and the desensitized channel is denoted D. The values for the rate constants used for the calculations were those proposed by Lester and Jahr for glutamate, viz. $k_{+1} = 5 \times 10^6 \, \text{M}^{-1} \cdot \text{s}^{-1}$, $k_{-1} = 4.7 \, \text{s}^{-1}$, $k_{+D} = 8.4 \, \text{s}^{-1}$, $k_{-D} = 1.8 \, \text{s}^{-1}$, $\alpha = 91.6 \, \text{s}^{-1}$, and $\beta = 46.5 \, \text{s}^{-1}$. In this diagram, k_{+1} is the association rate constant for agonist binding, k_{-1} is the dissociation rate constant for agonist unbinding, k_{+D} and k_{-D} are the rate constants for entry into and exit from the desensitized state (state 2), and α and β are the rate constants for the shutting and opening of the fully liganded channel. The states are numbered as shown for identification purposes in the text.

openings were detected during the prepulse were separated from sweeps for which no openings were detected during the prepulse. When these two categories were averaged separately, it was found that substantially less desensitization occurred in the latter case (no prepulse openings) than in the former. However, it cannot be concluded from these observations that desensitization occurs faster (or occurs at all) from the open state(s).

The relevant theory for this problem is given in the *Appendix*, and it will be illustrated here by the simple mechanism proposed for the NMDA receptor by Lester and Jahr (2). This mechanism is shown in Fig. 1, together with the values for the transition rates that were suggested by Lester and Jahr (2) for glutamate. It has four shut states, one open state, and no correlations. It is, therefore, too simple to account for most single-channel results, but it can describe the time course of macroscopic currents quite well. Shut state 2 is quite long-lived and may be considered (somewhat arbitrarily) to be the desensitized state. The important thing about this mechanism for the present purposes is that desensitization can occur only from a shut state (state 3).

The behavior predicted by this mechanism, for conditions that resemble those of Lin and Stevens (4), can be calculated from the general results given in the *Appendix* and are illustrated in Fig. 2. It can be seen that it is predicted that much more desensitization will occur following prepulses that contain one or more channel openings, relative to the amount of desensitization that results following prepulses with no openings.

The reason for this result is made clear by the values given in Table 1. With the particular values used for this calculation, it is predicted (from *Appendix*, Eq. 5) that 87% of sweeps will have no openings during the prepulse and 13% will have one

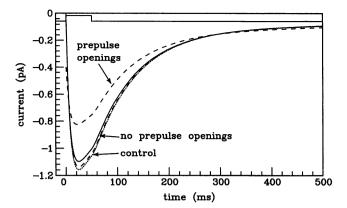Abbreviation: NMDA, *N*-methyl-D-aspartate.

FIG. 2. Results of calculations using the mechanism and rate constants specified in Fig. 1. The solid line shows the response to a test pulse of agonist (1000 μM for 50 ms), following a desensitizing prepulse (0.25 μM for 600 ms) (peak, −1.10 pA). The concentrations used for this calculation differ from those used by Lin and Stevens (4) because the rate constants proposed by Lester and Jahr (2) are for glutamate rather than NMDA. The upper dashed curve shows the calculated response for only those sweeps that have one or more openings during the prepulse (peak, −0.82 pA), and the lower dashed curve is the response for sweeps that have no prepulse openings (peak, −1.14 pA). The latter differs little from the control (no prepulse) response, which is shown as a dotted curve (peak, −1.16 pA). The calculations are for one 50-ps channel at −80 mV, so the maximum possible current is 4 pA.

or more openings during the prepulse. The response to the test pulse depends on the fraction of channels that are in each state at the end of the prepulse (i.e., the initial condition for the test pulse). On average, in 5.5% of sweeps, the channel will be in state 2 ("desensitized") at the end of the prepulse; however, the channel will be desensitized in 32% of sweeps that have one or more openings during the prepulse, compared with only 1.7% for sweeps that have no openings during the prepulse (we may notice, incidentally, that this ratio, 1.7/32, is considerably underestimated by the ratio of the peak responses to the test pulse). Similarly, if we consider shut state 3, which is the only state from which desensitization can occur, we see that the channel is in state 3 at the end of the prepulse in 2.5% of sweeps. However, when there are no prepulse openings, only 0.6% of channels are in state 3, whereas for sweeps with one or more openings during the prepulse, 16% of channels are in state 3. Differences in the opposite direction occur for shut states 4 and 5.

One way of looking at the reason for this behavior is to note that many channels will never reach state 3 (the only state from which desensitization can occur) during the prepulse. The mean lifetime of a sojourn in the compound state (4, 5), given that the sojourn starts in state 5, is 2704 ms, which is quite long compared with the 600-ms length of the prepulse. Thus, many (though not all) of the "no prepulse opening" sweeps will

Table 1. Occupancy of each state at the end of the prepulse (i.e., the initial condition for the test pulse)

State no.	p(0)	p₀(0)	p₁(0)
1	0.013	0.0	0.101
2	0.055	0.017	0.318
3	0.025	0.006	0.155
4	0.297	0.305	0.240
5	0.610	0.671	0.186

Fig. 1 identifies each state. The column headed $p(0)$ gives the overall values for all sweeps (*Appendix*, Eq. **4**). The column headed $p_0(0)$ gives the values for those sweeps that have no prepulse openings (*Appendix*, Eq. **6**), and the column headed $p_1(0)$ gives the values for those sweeps that have one or more prepulse openings (*Appendix*, Eq. **8**).

spend their entire time in states 4 and 5. On the other hand, it is obvious from inspection of Fig. 1 that any channel that has opened (and shut again) must have experienced at least two sojourns in state 3, during which desensitization may occur. These numbers would be different, of course, with a different agonist (e.g., NMDA rather than glutamate) or with a more realistic model, but the main qualitative conclusions of this paper would be unchanged.

It is clear from this illustration that the phenomenon occurs because the channel will have, on average, a different distribution among the various shut states at the end of the prepulse for sweeps that have prepulse openings compared with sweeps that have no prepulse openings. The fact that this behavior (which at first sight suggests that the channel has "remembered" that it opened) can be generated by a "memoryless" Markovian mechanism can thus be rationalized in much the same way as the existence of correlations in such mechanisms (5, 6). Although the observations could be generated by mechanisms in which desensitization occurs from open state(s), there is clearly no necessity for such a conclusion.

APPENDIX

The notation used here is that of Colquhoun and Hawkes (7). Explanations of this approach may be found in refs. 6 and 8. The mechanism is specified in terms of a matrix, **Q**, of the transition rates between the k states in which the receptor can exist ($k = 5$ in Fig. 1). The **Q** matrix is partitioned into k_{sd} open states and $k_{\mathscr{F}}$ shut states. For the particular mechanism in Fig. 1 we have

$$\mathbf{Q}(x) =$$

$$\begin{bmatrix} -\alpha & 0 & \alpha & 0 & 0 \\ 0 & -k_{-D} & k_{-D} & 0 & 0 \\ \beta & k_{+D} & -(\beta + k_{+D} + 2k_{-1}) & 2k_{-1} & 0 \\ 0 & 0 & k_{+1}x & -(k_{-1} + k_{+1}x) & k_{-1} \\ 0 & 0 & 0 & 2k_{+1}x & -2k_{+1}x \end{bmatrix},$$

[1]

where **Q** has been expressed as a function of the agonist concentration, x, and $k_{sd} = 1$ and $k_{\mathscr{F}} = 4$. Thus, we can define, in our example, $\mathbf{Q}_p = \mathbf{Q}(0.25 \times 10^{-6})$ as the transition rate matrix during the prepulse, and similarly we have $\mathbf{Q}_t = \mathbf{Q}(0.001)$ during the test pulse and $\mathbf{Q}_0 = \mathbf{Q}(0)$ after the test pulse. If the test pulse starts at $t = 0$ and has duration T, then the response during the test pulse is given by

$$\mathbf{p}(t) = \mathbf{p}(0)\exp(\mathbf{Q}_t t), \qquad t \le T, \qquad [2]$$

where $\mathbf{p}(t)$ is a vector containing the occupancies of each of the k states at time t from the start of the test pulse, and $\mathbf{p}(0)$ contains the occupancies at the start of the test pulse (i.e., at the end of the prepulse). The occupancies so found at time T form the initial occupancies for the jump to zero concentration at the end of the test pulse, so after the end of the pulse we have

$$\mathbf{p}(t) = \mathbf{p}(0)\exp(\mathbf{Q}_t T)\exp[\mathbf{Q}_0(t - T)], \qquad t > T. \qquad [3]$$

In this case only state 1 is open, so the current plotted in Fig. 2 is $ip_1(t)$ where $i = 4$ pA is the current while the channel is open. The exponential parts of these expressions are always the same regardless of the prepulse; differences in the response to the test pulse depend only on differences in the initial vector, $\mathbf{p}(0)$. Therefore, the problem is to find the initial vector $\mathbf{p}(0)$, for (*i*) all sweeps, (*ii*) sweeps with no prepulse openings, and (*iii*) sweeps with one or more prepulse openings.

The Overall Initial Vector. If the duration of the prepulse is t_p then the prepulse starts at $t = -t_p$. The overall initial vector can be found exactly as in Eq. 2 and is

Neurobiology: Colquhoun and Hawkes

Proc. Natl. Acad. Sci. USA 92 (1995)   10329

$$p(0) = p(-t_p)\exp(Q_p t_p), \qquad [4]$$

where Q_p is the Q matrix at the concentration used for the prepulse and $p(-t_p)$ is the vector of occupancies at the start of the prepulse; thus, for the example in Fig. 1, $p(-t_p) = (0\ 0\ 0\ 0\ 1)$.

The Probability That No Openings Occur During the Prepulse. There will be no openings during a prepulse of length t_p, for a one-channel patch, if the latency to the first opening is t_p or greater. Integration of the first latency distribution (see refs. 5, 6, and 8) gives the probability that no openings occur during the prepulse as

$$P_0 = p_{\mathscr{F}}(-t_p)\exp(Q_{\mathscr{F}\mathscr{F}}t_p)u_{\mathscr{F}}, \qquad [5]$$

where $Q_{\mathscr{F}\mathscr{F}}$ is the $k_{\mathscr{F}} \times k_{\mathscr{F}}$ section of the Q_p matrix (the part that corresponds to the shut states—i.e., the bottom right 4×4 elements in Eq. 1 in our case), $p_{\mathscr{F}}(-t_p)$ is the occupancy of the shut states at the start of the prepulse [i.e., $(0\ 0\ 0\ 1)$ in our example], and $u_{\mathscr{F}}$ is a column vector with unit elements.

The Initial Vector Conditional on There Being No Prepulse Openings. The matrix $\exp(Q_{\mathscr{F}\mathscr{F}}t_p)$ has elements that give (see ref. 7) the probabilities that the channel stays within the shut states throughout the prepulse and is in shut state j at time 0, given that the channel was in shut state i at $t = -t_p$. The initial conditions for the test pulse, the occupancies of the states at time 0 conditional on no openings occurring, will be denoted by $p_0(0)$ and are given by

$$p_0(0) = [0 \quad p_{\mathscr{F}}(-t_p)\exp(Q_{\mathscr{F}\mathscr{F}}t_p)/P_0]. \qquad [6]$$

From Eq. 5, these probabilities must sum to 1. The conditional probabilities of occupying the open states at time 0 are obviously zero; the conditional probabilities of occupying the various shut states are given by the expression in the right hand partition of $p_0(0)$.

The Initial Vector Conditional on the Occurrence of One or More Prepulse Openings. This vector, the occupancies at time 0 when one or more openings occur during the prepulse, will be denoted $p_1(0)$. It can be found from the fact that the overall initial vector, from Eq. 4, must be a combination of $p_0(0)$ and $p_1(0)$, in proportions P_0 and $(1 - P_0)$, respectively. Thus,

$$p(0) = P_0 p_0(0) + (1 - P_0)p_1(0). \qquad [7]$$

Hence, we can find the final quantity that we need as

$$p_1(0) = [p(0) - P_0 p_0(0)]/(1 - P_0). \qquad [8]$$

These results are general for any Markovian mechanism with constant transition probabilities. The three initial vectors, calculated from Eqs. 4, 6, and 8, are given in Table 1 for the example in Fig. 1. Substitution of each of them for $p(0)$ in Eq. 2 allows calculation of the response to the test pulse, as illustrated in Fig. 2. The control (no prepulse) result is given by taking $p(0) = (0 \quad 0 \quad 0 \quad 0 \quad 1)$.

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