Estimating the Distribution of Synaptic Reliabilities

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Huang, Emily P. and Charles F. Stevens. Estimating the distribution of synaptic reliabilities. J. Neurophysiol. 78: 2870–2880, 1997. Using whole cell recording from CA1 hippocampal pyramidal neurons in slices, we examined the progressive decrease of N-methyl-D-aspartate receptor-mediated synaptic responses in the presence of the open-channel blocker MK-801. Previous studies analyzing this decrease have proposed that hippocampal synapses fall into two distinct classes of release probabilities, whereas studies based on other methods indicate a broad distribution of synaptic reliabilities exists. Here we derive the theoretical relationship between the MK-801–mediated decrease in excitatory postsynaptic current amplitudes and the underlying distribution of synaptic reliabilities. We find that the MK-801 data are consistent with a continuous distribution of synaptic reliabilities, in agreement with studies examining individual synapses. In addition, changes in the MK-801–mediated decrease in response size as a consequence of altering release probability are consistent with this continuous distribution of synaptic reliabilities.

INTRODUCTION

Synapses release neurotransmitter probabilistically (Katz 1969). Release probability—the probability that at least one exocytotic event will occur when a nerve impulse invades a presynaptic terminal—is regulated by the Ca$^{2+}$-dependent processes governing synaptic vesicle exocytosis and is generally much less than one in central synapses (Allen and Stevens 1994; Hessler et al. 1993; Jack et al. 1981; Korn et al. 1986; Raastad 1995; Raastad et al. 1992; Rosenmund et al. 1993; Walmsley et al. 1988).

Synaptic release probability ($P_r$) plays an important role in determining the efficacy, or strength, of a given synapse: the average size of the postsynaptic response is proportional to $P_r$. Thus the strength of a synapse can be altered by modification of its release probability (Bekkers and Stevens 1990; Bolshakov and Siegelbaum 1995; Magleby 1987; Malinow and Tsien 1990; Manabe et al. 1993; Stevens and Wang 1994; Zucker 1989). Because synaptic strength is so important for the function of neuronal circuits, a significant question is how release probabilities are distributed in central synapses. In other words, we want to know the density function that describes the occurrence frequency of synapses with a given $P_r$.

Two studies (Hessler et al. 1993; Rosenmund et al. 1993) addressed this question in large populations of hippocampal synapses in culture and in brain slices. These studies analyzed the progressive decrease of N-methyl-D-aspartate (NMDA) receptor-mediated synaptic current amplitudes in the presence of the open-channel NMDA receptor blocker MK-801. MK-801 blocks receptors only when they are opened, and because receptors open only when transmitter is released, the progressive block of receptors in MK-801 provides an indirect measure of the transmitter release probability.

The conclusion from this analysis was that populations of hippocampal synapses are nonuniform in release probability. More specifically, it was proposed that hippocampal synapses fall into two classes with about sixfold difference in release probability; we call these classes unreliable and very unreliable. In this scenario, more than half of the synapses belong to the very unreliable class. This conclusion, however, is in conflict with the results of studies using other, more direct methods to examine $P_r$ of synapses (Allen and Stevens 1994; Murthy et al. 1997); these suggest a wide range of release probabilities exists. Indeed, Rosenmund et al. (1993) note the possibility that more than two classes of synaptic $P_r$ are present, although they do not pursue this analytically.

Murthy et al. (1997) measured the endocytotic uptake of the styryl dye FM-143 at stimulated synaptic terminals in culture. The amount of dye taken up at each synapse is directly proportional to its release probability (a synapse with high $P_r$ releases synaptic vesicles more often in response to stimulation and therefore undergoes more vesicle endocytosis). Murthy et al. thus could calculate the $P_r$ for each of a large number of synapses. When a frequency histogram of synaptic $P_r$ was plotted, the distribution appeared to be a continuous function skewed heavily toward low $P_r$ values; in contrast to the conclusions of the MK-801 studies, this directly observed $P_r$ distribution cannot be fitted as a bimodal function. These results are corroborated by data from minimal stimulation experiments (Allen and Stevens 1994), which indicate synapses have $P_r$ ranging continuously from <0.1 to 1.0.

In the following analysis, we demonstrate that the progressive block of NMDA-mediated synaptic responses in the presence of MK-801 is in fact consistent with a continuous release probability distribution. First, we analyze the MK-801 method and derive the mathematical relationship between the progressive block of synaptic responses and the underlying release probability distribution. Then we show that a continuous distribution fits the data as well as the bimodal (two-class) distribution postulated in previous studies. Finally, we demonstrate that the same continuous distribution fits robustly the progressive block of excitatory postsynaptic current (EPSC) amplitudes in MK-801 under two conditions that alter the synaptic release probability: paired pulse facilitation and changing the external Ca$^{2+}$ concentration.

METHODS

Slice preparation and physiology

Transverse hippocampal slices (350-μm thick) were prepared from 2- to 3-wk-old Long-Evans rats using standard procedures.
Our goal is to determine the relationship between this stimulation pattern and superfused with external solution identical to the storage solution described above with the addition of 50 μM picrotoxin (a cut was made between area CA3 and CA1 in the slices to prevent epileptiform activity). Flow rate was 2 ml/min. Whole cell recordings were obtained in CA1 pyramidal cells using standard procedures as described by Stevens and Wang 1993. Recording pipettes were 3–4 MΩ and were filled with internal solution containing (in mM) 130 Cs gluconate, 5 CsCl, 5 NaCl, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 0.5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid, 1 MgCl₂, 2 Mg-ATP, and 0.2 Li-guanosine 5’-triphosphate.

**MK-801 experiments**

Whole cell recordings were obtained as above in standard recording solution containing 5 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX). NMDA-mediated EPSCs were evoked by stimulating Schaffer collateral fibers with a bipolar tungsten electrode while clamping the recorded cell at −40 mV; the stimulus repetition interval was 8 s. Recording solution containing MK-801 was added as specified in the text. DNQX and MK-801 were obtained from Research Biochemicals.

Two-pathway experiments were performed as follows: two bipolar tungsten electrodes were placed in the Schaffer collaterals, each ~200 μm to either side of the recorded cell. The electrodes were stimulated alternately, evoking two independent populations of synapses on the cell. Pathway independence was ensured by confirming there was no paired pulse facilitation when one pathway was stimulated shortly after the other (at a 50-ms interval). In two-pathway paired pulse facilitation (PPF) experiments, the PPF interval was 50 ms; the stimulation patterns (repetition = 8 s) were arranged so that the same number of pulses were delivered to PPF and control pathways in the presence of MK-801, as follows:

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Control  |   |   |   |   |   |   |   |
PPF      |   |   |   |   |   |   |   |
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In Ca²⁺ experiments, the external Ca²⁺ concentration was established by perfusing slices for ≥15 min at the specified [Ca²⁺] before beginning the experiment.

**Minimal stimulation**

Minimal stimulation experiments were performed using previously described methods (Dobrunz and Stevens 1997; Raastad et al. 1992; Stevens and Wang 1995). Whole cell recordings were obtained in standard recording solution, and EPSCs were evoked by stimulating the Schaffer collaterals with a tungsten bipolar electrode while clamping the cell at −70 mV. Stimulation rate was 0.1 Hz. Stimulation intensity was lowered until a significant number of failures were seen among the responses and the presence of a putative single synapse was confirmed as described in Dobrunz and Stevens 1997. The criteria for single synapse selection can be summarized as follows: the stimulus intensity is just high enough to elicit responses; small changes in stimulus intensity (+5%) do not alter failure probability or average response amplitude; there is no variation in epsc shape or latency; and the response amplitude histogram does not change when the synapse is paired-pulse facilitated. Failure rate for the synapse was calculated as the fraction of responses that are failures; success rate (or P₀) was one minus the failure rate.

PPF at minimal stimulation was evoked by stimulating in pairs with interstimulus intervals of 19–25 ms (pairs were presented at a rate of 0.1 Hz). The PFF ratio was calculated as the success rate on the second pulse divided by success rate on the first pulse, whereas initial P₀ was just the success rate on the first pulse.

**Analysis**

Signals were filtered at 2 kHz, digitized at 5 kHz, and stored for analysis. Analysis of epscs was performed using programs written in AXOBASIC; relative EPSC size was measured by integrating the current in a 40-ms window around the peak. For minimal stimulation experiments, responses were inspected visually to determine failures.

All fits of data with mathematical models were carried out in Mathcad by least-square fitting procedures. Differential equations describing the NMDA-receptor kinetic scheme also were solved in Mathcad.
the block of open channels by MK-801 (Hestrin et al. 1990; Huettner and Bean 1988). EPSCs were fitted with a simple four-state kinetic model (Fig. 2B), in which channels make the indicated transitions between the unbound, closed, open, or blocked states. An impulse of transmitter is assumed. For any given experiment, control EPSCs were first fitted with the blocking rate set to zero; EPSCs in the presence of MK-801 then were fitted using the same rate constants as control and allowing the blocking rate to vary. In general, an “unblocking” rate was unnecessary to fit the EPSCs except at low concentrations of MK-801 (Jahr 1992). When unblocking was used, the unblocking rate was at least an order of magnitude smaller than the blocking rate.

The estimated blocking rate increased as the concentration of MK-801 was varied from 10 to 40 μM, indicating a dose dependence. To determine channel block fraction (the fraction of open channels that get blocked by MK-801 during a single synaptic response), the differential equations for the kinetic scheme (Fig. 2B) were solved with the fitted rate constants to calculate the time dependence of the blocked state; block fraction was calculated as the fraction of opened channels that get blocked by MK-801 during a stimulus. This value is plotted against MK-801 concentration in Fig. 2C, and the data are fitted by an effective dose-response curve with \( k_{1/2} = 28.63 \) μM MK-801. We emphasize that this constant \( k_{1/2} \) is not the equilibrium dissociation constant of MK-801 binding to the NMDA receptor. Rather, it is the concentration of MK-801 at which half of the receptors that open during a stimulus get blocked. This value depends on the opening and closing kinetics of the receptor as well as the specific experimental conditions. In all subsequent experiments, a concentration of 40 μM MK-801 is used, with an estimated average channel block fraction of 0.61 ± 0.068 (mean ± SE; \( n = 5 \)).

One might expect that block fraction, as determined above, would influence the rate at which NMDA receptor channels are blocked with successive stimuli. That is, an increase in the efficacy of MK-801 block would be expected to yield experimental blocking functions that decrease faster as a function of stimulus number. To compare the rate of decrease of two different blocking functions, we plotted the amplitude points of one as a function of the amplitude points of the other for each stimulus number. For instance, when we plotted the amplitudes of one 40 μM MK-801 blocking function against the amplitudes of another for each stimulus number (Fig. 3A), the points fell along a straight line of slope 1, indicating the two functions have an identical rate of decrease. When we compared a 10 μM and a 40 μM MK-801 experiment in the same way (Fig. 3B), however, the points deviated greatly from the slope = 1 line, highlighting the slower decrease of EPSC amplitudes in 10 μM MK-801. Plots for decreases in 20 μM against those in 40 μM MK-801 fell in between (plots not shown).

As the value of block fraction does clearly affect the blocking function, we must be sure the block fraction does not change during the course of the experiment. This point can be settled by scaling and comparing EPSCs after different number of stimuli in the presence of MK-801. Figure 3C shows typical results from an experiment in 40 μM MK-801; EPSCs at later stimulation numbers scale perfectly to EPSCs at early numbers. Thus block fraction does not change with either time or stimulus number in the course of our experiments.

**Decrease of EPSC amplitudes in MK-801 depends on block efficacy and synaptic release probability**

Now we can derive the quantitative relationship between synaptic release probability and the decrease of NMDA-mediated EPSC amplitudes in the presence of MK-801, that is, between the synaptic reliability distribution and the blocking function. First, let us outline the assumptions used in our derivation. We assume we repetitively stimulated a fixed number of synapses (with a fixed number of postsynaptic NMDA receptors) the release probabilities of which remained constant throughout the experiment [Allen and Stevens (1994) found this to be the case for the synapses in their sample]. With each stimulus, a fraction of the available postsynaptic NMDA receptors are blocked by MK-801. This fraction (a) depends on three factors: \( P_r \), the release probability of the stimulated synapses; \( \theta \), the fraction of receptors participating in the current given a release event; and \( m \), the fraction of these participating receptors that get blocked by MK-801 (block fraction). If \( P_r \) varies across synapses [Allen and Stevens (1994); Hessler et al. (1993); Murthy et al. (1997); Rosenstein et al. (1993)], a also will depend on \( \rho (P_r) \), the
function that describes the relative occurrence frequency of synapses with a given $P_r$.

With these initial assumptions, we write and solve a difference equation describing the fraction of synaptic receptors remaining unblocked at $n$th stimulus, $\hat{F}_n$ (see APPENDIX for full derivation). Our experimental blocking functions measure synaptic current, however, not receptor fraction, so we then calculate from $F$, the predicted synaptic current on the $n$th stimulus, $S_n$. When this is done (see APPENDIX), we get

$$S_n = \int_0^\infty dP_r P_r e^{-\alpha n}$$

$S_n$ is normalized so that $S_0 = 1$

From Eq. 1, we see that the predicted decrease of synaptic amplitudes in the presence of MK-801 on $m\theta$, the fraction of NMDA receptors blocked by MK-801 when a synapse releases transmitter, as well as $\rho(P_r)$, the $P_r$ weighting distribution. We now are prepared to determine what release probability distributions fit our MK-801 data.

Decrease of EPSC amplitudes in MK-801 is consistent with a continuous release probability distribution

Previous studies analyzing the decrease of EPSC amplitudes in MK-801 (Hessler et al. 1993; Rosenmund et al. 1993) have suggested that synapses are distributed between two classes of release probabilities (termed high and low). As noted earlier, the conclusion that there is a bimodal distribution of release probabilities, however, contradicts more direct estimates of the $P_r$ weighting function from studies using other methods (Allen and Stevens 1994; Murthy et al. 1997), in which release probabilities seem to follow a continuous distribution.

To resolve this conflict, we next examined whether the MK-801 blocking function could be explained as well by a continuous distribution of release probabilities as by the two-class (bimodal) $P_r$ distribution. An ideal bimodal weighting function, as used by Hessler et al. and Rosenmund et al., is represented mathematically as

$$\rho_b(P_r) = A\delta(P_r - P_{r1}) + (1 - A)\delta(P_r - P_{r2})$$
characterized as a function with a negative exponential dependence on $P_r$ (see APPENDIX), such as

$$\rho_n(P_r) = \frac{1}{1/(1 + n/r)} e^{-nr},$$

where $\lambda$ is a constant; $1/\lambda$ is the “characteristic $P_r$” of the distribution.

When Eq. 1 is evaluated with the above bimodal and continuous distributions, we get two predictions for $S_n$, the decrease in EPSC amplitudes in the presence of MK-801

$$S_n = Ae^{-b_1} + (1 - A)e^{-b_2},$$

where $b_1 = P_1 m \theta$, $b_2 = P_2 m \theta$, $0 \leq A \leq 1$, and

$$S_n = 1/(1 + n/r)$$

where $r = \lambda/m \theta$. We see that $S_n$ for the bimodal distribution is the sum of two exponentials (the form used by Hessler et al. 1993; Rosenmund et al. 1993), whereas $S_n$ for the continuous distribution is the sum of an infinite number of exponentials. Note that the parameter $r$ is inversely proportional to the characteristic release probability $1/\lambda$.

Experimentally obtained blocking functions in the presence of 10, 20, and 40 $\mu$M MK-801 were fitted with the predicted $S_n$ functions incorporating the bimodal and continuous distributions by a least-squares-fitting procedure (Fig. 4). For all cells ($n = 15$), the amplitude decreases were fitted by both models nearly indistinguishably; for 40 $\mu$M MK-801 ($n = 6$), mean values of $A$, $b_1$, $b_2$, and $r$ were $0.677 \pm 0.012$, $0.116 \pm 0.007$, $0.014 \pm 0.0006$, and $9.78 \pm 0.502$, respectively. We conclude that the data from MK-801 experiments are perfectly consistent with at least one type of continuous distribution of synaptic release probabilities as well as with a bimodal $P_r$ distribution.

To obtain a quantitative description of the above $P_r$ distributions, we must calculate $P_1 (=b_1/m \theta)$ and $P_2 (=b_2/m \theta)$, the $P_r$ values of the two synaptic classes in the bimodal distribution, and $1/\lambda (\lambda = r m \theta)$, the characteristic $P_r$ for the continuous distribution. Calculation of these values requires an estimate of $m \theta$, the fraction of receptors blocked

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Block fraction affects rate of decrease of EPSC amplitudes in MK-801. A: normalized amplitudes of one 40 $\mu$M MK-801 blocking function are plotted against the normalized amplitudes of another for each stimulus number. Data fall on a straight line with slope = 1.0, indicating an identical rate of decrease. B: amplitudes of a 10 $\mu$M MK-801 blocking function plotted against the amplitudes of a 40 $\mu$M MK-801 blocking function for each stimulus number. Points deviate considerably from a line of slope = 1, indicating a far slower rate of amplitude decrease in 10 $\mu$M MK-801. C: representative EPSCs at early and late stimulus numbers in the presence of 40 $\mu$M MK-801. i: average of the 1st 5 responses and average of 6 responses beginning at stimulus number 50 in the presence of MK-801. In ii, the EPSCs are scaled so that their initial peaks match. There is no significant change in the decay course of the EPSCs, indicating that block fraction remains unchanged.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{MK-801 data are fitted by a continuous release probability distribution. Representative blocking function in 40 $\mu$M MK-801 fitted by $S_n$ incorporating a bimodal distribution ($\cdots$) and a continuous distribution ($\cdots$). To reduce variability and for the purposes of quantitative fitting, the EPSC amplitudes are normalized to the $y$ intercept of a line fitted to the 1st 10 points in the decrease. \cdots, fit of the data by a $S_n$ incorporating a distribution derived from minimal stimulation experiments, as described in the DISCUSSION.}
\end{figure}
per stimulus. \( m \) is the fraction of participating receptors on a given trial that get blocked and was estimated earlier as 0.611 for 40 \( \mu \)M MK-801. \( \theta \) is the fraction of receptors that participate in the current when transmitter is released; we take \( \theta \) at the previously estimated value 0.5 (Rosenmund et al. 1995) and at 1.0. When \( \theta \) is 0.5, \( P_1 \), \( P_2 \), and \( \lambda \) are 0.38, 0.05, and 2.99, respectively; when \( \theta \) is 1, \( P_1 \), \( P_2 \), and \( \lambda \) are 0.19, 0.025, and 5.98, respectively. Thus for the bimodal distribution, the "high" synaptic \( P \), may range from 0.19 to 0.38 and the low \( P \), from 0.025 to 0.05. For the continuous distribution, the characteristic release probability \( 1/\lambda \) ranges between 0.17 and 0.33.

**PPF of a continuous \( P \), distribution**

PPF is an increase in presynaptic release probability that occurs when a synapse is stimulated to release within a few hundred milliseconds of a previous stimulation event; the increased \( P \), is thought to reflect residual \( Ca^{2+} \) in the axon terminal (see Zucker 1989). To examine the effect of PPF on the blocking function, we performed experiments in which two separate and independent pathways onto the same recorded CA1 cell were stimulated alternately. This was accomplished by stimulating the Schaffer collaterals on either side of the cell. When such pathways were alternately given single stimuli in the presence of MK-801, the EPSC amplitudes of one pathway could be plotted against the amplitudes of the other (for each stimulus number) as a straight line (Fig. 5A), with a slope of 1.

When one of the pathways was paired pulse facilitated, however, a plot of the first pulse of each pair in the PPF pathway against every other pulse in the control pathway (with stimulus number as the plot parameter) deviated considerably from a line of slope equal to one (Fig. 5B). This deviation reflects a more rapid decrease of the blocking function for the paired pulse pathway, presumably due to an increase in release probability. To see whether we could relate directly the amount of PPF enhancement to the change in amplitude decrease, we modified our continuous distribution model (see APPENDIX) to accommodate an increase in \( P \), for every second pulse. This increase in \( P \), on the second pulse is given by the function \( \phi(P_r) \), defined as the amount of PPF (or PPF ratio) as a function of initial release probability. The result of this simple modification is

\[
S_n = \int_0^\infty dP_r e^{-\lambda P_r} e^{-\theta(1 - \phi(P_r))} dP_r \; \text{m}^0
\tag{2}
\]

All the parameters of this equation, except for \( \phi(P_r) \), are fixed by values derived from fits of data from the control pathway (\( \lambda, m, \theta \)). The problem, of course, is to determine \( \phi(P_r) \) because the MK-801 experiments do not provide a direct measure of the changes in release probabilities due to PPF. To estimate \( \phi(P_r) \), we turned to the technique of minimal stimulation, which provides a means of activating putative single synapses on a neuron recorded in slices (Dobrunz and Stevens 1997; Raastad 1995; Stevens and Wang 1995). Synapses stimulated in this manner exhibit frequent response failures that are due to the probabilistic nature of transmitter release (Allen and Stevens 1994). In minimal stimulation, the \( P \), of a given synapse is calculated as one minus the failure rate of responses to the minimal stimulus (Allen and Stevens 1994; Raastad et al. 1992; Stevens and Wang 1994). When closely spaced pairs of the minimal stimuli are given, the \( P \), of the second pulse is increased relative to the \( P \), of the first pulse, as expected with PPF. Thus we were able to measure PPF ratio and initial \( P \), for a number of synapses (\( n = 25 \), see METHODS). In these experiments, we found synapses with \( P \),s ranging continuously from 0.1 to 1.0; there was no evidence of a bimodal distribution of synaptic \( P \), (see Fig. 6).

When the PPF ratio is plotted as a function of initial \( P \), (Fig. 6), we see a decrease in PPF as \( P \), goes from zero to one. We thus can approximate \( \phi(P_r) \) as a straight line

\[
\phi(P_r) = \text{PPF}_{\max} - (\text{PPF}_{\max} - 1)P_r \tag{3}
\]

\( \text{PPF}_{\max} \) is the PPF ratio at very low \( P \), values; \( \phi(P_r) \) goes to one at \( P_r = 1.0 \). We assessed \( \text{PPF}_{\max} \) in our MK-801 experiments by calculating average PPF ratio at late stimulus numbers; then, for ease of calculation, we further approximated \( \phi(P_r) \) as a staircase function decreasing regularly
from PPF$_{\text{max}}$ to one in 10 steps. When this $\phi(P_r)$ was incorporated into Eq. 2, we obtained an expression describing $S_n$ for PPF synapses that had no free parameters. For four experiments, this expression fitted the MK-801 mediated decrease of EPSC amplitudes in the PPF pathway very well (Fig. 7, A and B). The model incorporating the continuous distribution $\rho(P_r) = e^{-rP}$ is therefore robust when the release probabilities are modified, such as with PPF.

We also calculated the $S_n$ for PPF synapses assuming a bimodal $P_r$ distribution

$$S_n = \int_0^{P_{\text{max}}} dP_r [A\delta(P_r - P_1) + (1 - A)\delta(P_r - P_2)] e^{-r(1 + \phi(P_r) - 1)\theta} \, dP_r$$

(4)

Again, the parameters $A$, $P_1$, $P_2$, $m$, and $\theta$ are taken from fits of data from the control pathway, and $\phi(P_r)$ is taken from the minimal stimulation data by selecting the values of Eq. 3 at $P_1$ and $P_2$. The PPF blocking functions are fitted acceptably with these calculated $S_n$, so we cannot exclude a bimodal $P_r$ distribution from consideration on the basis of fitting the PPF data. As we noted before, however, that the $\phi(P_r)$ obtained from minimal stimulation data are itself a continuous function; in Eq. 4, it is treated artificially as a bimodal function because only two of its points are selected in the calculation of $S_n$. Ignoring this inherent contradiction yields reasonable fits but indicates a bimodal $P_r$ distribution is a less appropriate choice than a continuous one.

$Ca^{2+}$ alters the $P_r$ distribution according to the Dodge-Rahamimoff equation

Transmitter release is a $Ca^{2+}$-dependent process, and changes in external $Ca^{2+}$ profoundly affect synaptic release probability (Dodge and Rahamimoff 1967). We examined the MK-801 blocking function at four different external $Ca^{2+}$ concentrations: 1.0, 1.75, 2.5 (standard), and 6.0 mM. Figure 8A shows blocking functions under these four conditions.

Blocking functions for all $Ca^{2+}$ concentrations were well fitted by our model incorporating a continuous $P_r$ distribution. The average best-fit $r$ for external $[Ca^{2+}]$ of 1.0 mM ($n = 2$), 1.75 mM ($n = 5$), 2.5 mM ($n = 6$), and 6.0 mM ($n = 4$) were $22.51 \pm 4.5$, $12.67 \pm 0.868$, $9.78 \pm 0.502$, and $8.81 \pm 0.607$, respectively.

The change in $r$ reflects changes in the $P_r$ distribution at different $[Ca^{2+}]$ concentrations. $r$, however, is also affected by MK-801 block efficacy (recall that $r = \lambda/m\theta$); because $Ca^{2+}$ ions may interact with the MK-801 block site (Reynolds and Miller 1988), we examined the EPSCs from each condition to determine whether block fraction had been significantly changed from standard conditions (2.5 mM $[Ca^{2+}]$). Analysis of the EPSCs revealed that MK-801 block fraction was not significantly affected by changing the external $Ca^{2+}$ concentration except at 6.0 mM $[Ca^{2+}]$, where the average block fraction was reduced from $0.611 \pm 0.068$ to $0.330 \pm 0.029$. Because $r$ is inversely proportional to block fraction, the fitted $r$ for the condition of 6.0 mM $[Ca^{2+}]$ was scaled by a factor of $0.330/0.611 = 0.54$ for the purposes of quantitative comparison.

Figure 8B shows normalized $r^{-1}$ plotted against external $[Ca^{2+}]$; the data are fitted by the Dodge-Rahamimoff equation (Dodge and Rahamimoff 1967). Best fit by a least-
In the work presented here, we analyzed the relationship between synaptic release probability and the decrease of NMDA-mediated synaptic current amplitudes in the presence of MK-801. Previous studies exploiting this method (Hessler et al. 1993; Rosenmund et al. 1993) have argued that the simplest fit of the MK-801 blocking function points to two classes of synapses with a sixfold difference in release probability (although Rosenmund et al. did not exclude the possibility of multiple $P_r$ classes). The proposal of two synaptic $P_r$ classes, however, is inconsistent with the results of studies in which synaptic $P_r$ is directly measured (Allen and Stevens 1994; Murthy et al. 1997) (see also Fig. 6); such studies indicate synapses range over all possible release probabilities. Furthermore, studies have shown that individual synapses are capable of both increasing and decreasing their $P_r$ by varying amounts under conditions such as long-term potentiation and long-term depression (Bolshakov and Siegelbaum 1995; Stevens and Wang 1994), implying that two $P_r$ classes may be inadequate to describe the available $P_r$ landscape.

We have demonstrated that the data from MK-801 can be described with a continuous $P_r$ distribution. One attractive feature of this distribution is that it describes the MK-801 blocking function with a single parameter ($r$). In contrast, three parameters are needed with the bimodal distribution ($A, b_1, b_2$). Our distribution thus provides the simplest fit of the MK-801 data. Furthermore, this distribution fits the MK-801 blocking function robustly under conditions of altered release probability, such as PPF and changes in external $[\text{Ca}^{2+}]$. In our PPF experiments, we can account for the change in MK-801 blocking function in terms of our distribution and the amount of observed PPF alone. In our $\text{Ca}^{2+}$ experiments, the predicted change in our distribution at different $\text{Ca}^{2+}$ concentrations follows the Dodge-Rahamimoff equation. In contrast, we cannot identify a consistent relationship between the parameters of the bimodal distribution and the $[\text{Ca}^{2+}]$.

The equation we use to describe the continuous distribution, $\rho(P_r) = e^{-k_r r}$, is, of course, a mathematical idealization. For instance, we actually expect the distribution to go to zero at $P_r = 0$, as described in Murthy et al. (1997). Our idealization is acceptable because very low values of $P_r$ are not detectable by the MK-801 method, although this method is more accurate at low $P_r$ than minimal stimulation. We estimate the MK-801 method cannot detect synaptic $P_r < 0.05$; with minimal stimulation, synapses begin to be heavily underrepresented at $P_r$ of 0.1–0.2. The FM1–43 method used by Murthy et al. (1997) is also inaccurate for low probability synapses and underrepresents synapses with release probabilities less than ~0.05–0.1.

Instead of idealizing the minimal stimulation-derived distribution as a negative exponential, we can express it exactly as a histogram (Allen and Stevens 1994). This “real” distribution is problematic in that it assuredly underestimates the relative number of synapses at the lower end of the $P_r$ range (<0.2, as discussed earlier), so it is of limited use for analysis. Nonetheless, to see whether this distribution is consistent with the MK-801 data, we calculated its $S_n$ and fitted it to the experimental blocking function. The calculated $S_n$ had
only two free parameters, which were varied for best fit: \( \theta \), the number of receptors that participate in the current, and \( y \), a small constant added to the fitting equation to compensate for the underrepresentation of low \( P_r \) synapses. When these parameters were adjusted, the data were fitted quite well (Fig. 4, \( \cdots \cdots \) ); the best fit yielded \( \theta = 0.54 \). Thus a real, albeit incomplete, distribution derived from minimal stimulation data are consistent with the MK-801 blocking function.

Although we have confirmed here that the MK-801 blocking functions reflect the distribution of synaptic reliabilities, the limitations of this method for estimating synaptic reliabilities must be stressed. We consider these limitations in the following text.

We have used a particular continuous weighting function of synaptic reliabilities \( [\rho(P_r) = e^{-\lambda P_r}] \) to describe our data—this distribution is the lowest order approximation to a more general function—but we must emphasize that the MK-801 method alone cannot be used to determine the exact shape of the function that describes the synaptic reliability distribution. The problem is that the MK-801 blocking function can be fitted with sums of anywhere between two (bimodal) and an infinite (continuous) number of exponential functions. Distinguishing whether a function with even a small amount of noise is the sum of 2, 10, or more exponentials is difficult because exponential functions with similar rate constants are very similar in shape. On the basis of our analysis, the reliability distribution \( \rho(P_r) \) could be a continuous exponential function, a sum of two delta functions, or any function in between (such as sums of multiple delta or Gaussian functions); with correctly chosen parameters, the predicted \( S_n \) are nearly indistinguishable. We can determine the range of \( \rho(P_r) \) that is consistent with the observed blocking function, but within that range, cannot decide whether one \( \rho(P_r) \) is better based on fit. To make that decision, one must use other criteria, as we have done in the preceding text.

Whatever the precise problems with the MK-801 method for determining the distribution of synaptic reliabilities, we have resolved the conflict between the observed continuous distribution of release probabilities and the original interpretation of the MK-801 blocking data as indicating the presence of two or a few classes of synapses. A firm conclusion of the present work is that synapses are generally quite unreliable and that a broad distribution of reliabilities that is weighted to the less reliable synapses is present in slices. A measure of how much the distribution is weighted to low \( P_r \) is provided by the characteristic release probability \( 1/\lambda \) of our fitted continuous distribution: two-thirds of the synapses have a \( P_r \) value less than or equal to the characteristic \( P_r \). Our estimation of \( 1/\lambda \) is 0.17–0.33. We believe that any theory of brain function will have to take account of this broad distribution of synaptic reliabilities.

**APPENDIX**

**MK-801 blocking equation**

Here we derive the relationship between synaptic \( P_r \) and the decrease of EPSC amplitudes in the presence of MK-801. Let us start with the simplest case where a hypothetical population of synapses all have the same release probability. In that case, the fraction \( F_n \) of receptors remaining unblocked after \( n \) stimulation trials in the presence of MK-801 can be calculated by solving the difference equation

\[
F_{n+1} = (1 - \alpha)F_n
\]

where \( \alpha \), the fraction of receptors blocked for any given stimulus, is the product of three factors: \( P_r \), synaptic release probability; \( \theta \), the fraction of receptors participating in the current; and \( m \), the fraction of these participating receptors that get blocked by MK-801 (block fraction). In the text, we found \( m = 0.611 \) for an MK-801 concentration of 40 \( \mu \)M.

Solving the above difference equation yields

\[
F_n = (1 - \alpha)^n
\]

For small \( \alpha \), such as when release probability \( P_r \) is small, this equation becomes

\[
F_n \approx e^{-\alpha n}
\]

Thus if release probability is uniform across a population of synapses and \( m \) and \( \theta \) are constants, the number of unblocked receptors at trial \( n \) will decline as a single exponential. Actual EPSC amplitude at trial \( n \), \( S_n \), will be proportional to the product of the fraction of unblocked receptors \( (F_n) \), the probability of release \( (P_r) \), and the average current per receptor \( (z) \)

\[
S_n \sim zP_r F_n
\]

The actual value of \( S_n \) can be obtained by scaling this quantity by the total number of receptors. From the preceding text, we see that the assumption of uniform \( P_r \) yields a measured quantity \( S_n \) that declines exponentially; so for this hypothetical case, the blocking function is an exponential. However, as has been noted previously (Hessler et al. 1993; Rosenmund et al. 1993), the decline of synaptic current amplitudes in the presence of MK-801 cannot be fitted with a single exponential, indicating that release probability is not constant across the population of synapses responsible for the measured synaptic currents.

Because release probability is not constant, there must be a probability density function \( \omega(P_r) \) that describes the relative distribution of synaptic release probabilities in the population; thus \( F_n \) can be weighted accordingly

\[
F_n = \int_0^1 \omega(P_r)e^{-\alpha P_r}dP_r = \int_0^1 \omega(P_r)e^{-\alpha P_r}dP_r
\]

The upper limit of the integral can be taken as infinity if \( \omega(P_r) \) approaches zero rapidly as \( P_r \) approaches one, so that higher values of \( P_r \) do not contribute; in practical terms, this means there are many more low \( P_r \) synapses than high \( P_r \) synapses. Several studies (Allen and Stevens 1994; Hessler et al. 1993; Rosenmund et al. 1993) have shown this to be the case.

Again, synaptic current amplitude for trial \( n \) will be proportional to the product of \( F_n \), \( P_r \), and the size of the average current as a function of \( P_r \), \( z(P_r) \)

\[
S_n = \int_0^1 dP_rz(P_r)\omega(P_r)P_r e^{-\alpha P_r} = \int_0^1 dP_r\rho(P_r)z(P_r)e^{-\alpha P_r}
\]

where \( \rho(P_r) = \omega(P_r)z(P_r)P_r \).

The actual magnitude of \( S_n \) depends on the total number of receptors, but this factor is unimportant in our analysis because we will normalize \( S_n \) to its initial value.

**Motivation for \( \rho(P_r) \) as a negative exponential function**

The choice of an exponential weighting function can be motivated as follows. First, recall
\[ S_n = \int_0^\infty dP \rho(P) e^{-\alpha P}, \]

which also can be written as

\[ S_n = \int_0^\infty dP \rho(P) e^{-\alpha P}, \]

Expanding the function \( \ln \rho(P) \) to first order about \( \bar{P} \), we find the equation

\[ S_n = \int_0^\infty dP \rho(P) e^{-\alpha \bar{P}}, \]

where \( k \) is a constant.

Generally, \( \rho(P) \) decreases with \( P \), so \( k \) is negative. Taking the first order approximation, we get

\[ S_n \sim \int_0^\infty dP \rho(P) e^{-\alpha P}. \]

Thus the continuous distribution \( e^{-\lambda P} \) can be thought of as the lower order approximation to the general function \( \rho(P) \). This distribution reasonably describes the dominant tail portion of \( P \), histograms derived from the study of individual synapses (Allen and Stevens 1994; Murthy et al. 1997).

**MK-801 blocking equation for PPF synapses**

A modified \( S_n \), for paired pulse facilitated synapses is derived next. The assumptions and analysis are similar to those used above to derive \( S_n \) for normal synapses (see Eq. 1). We write the difference equation that describes the fraction of unblocked receptors at stimulus trial \( k \) for the case in which every second pulse is facilitated

\[ F_{k+1} = F_k - r_1 F_k, \]

where \( r_1 = a \) if \( k \) is even (first pulse) and \( r_1 = \alpha \) if \( k \) is odd (second pulse).

Take \( n = k/2 \), so that \( n \) counts the first stimulus of each pair. The fraction of unblocked receptors on the first pulse of the \( n \)th stimulus pair is

\[ F_n = (1 - a)^n (1 - \alpha)^n = e^{-(a+\alpha)n}. \]

Using the same reasoning as in the text, the actual synaptic response size for the first stimulus of the \( n \)th pair is given by

\[ S_n \sim \int_0^\infty dP \rho(P) e^{-(a+\alpha)n}. \]

Remember that the fraction blocked \( a \) on the first pulse equals \( P_{middle} \). For the second (facilitated) pulse, the fraction blocked \( a \) is related to \( a \) by

\[ \alpha = \phi(P) a, \]

where \( \phi(P) \) is the PPF ratio at a given \( P \). Thus

\[ S_n = \int_0^\infty dP \rho(P) e^{-\alpha(1+\phi(P)) P}, \]

With a continuous distribution \( \rho(P) = e^{-\lambda P}, \)

\[ S_n = \int_0^\infty dP e^{-\lambda P} e^{-\alpha(1+\phi(P)) P}, \]

With a bimodal distribution \( \rho(P) = A\delta(P - P_1) + (1 - A)\delta(P - P_2) \)

\[ S_n = \int_0^\infty dP A\delta(P - P_1) + (1 - A)\delta(P - P_2) e^{-\alpha(1+\phi(P)) P}, \]

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