dictions must await the crystallization and structural resolution of these membrane proteins. □

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1. Schofield, P. R. *et al. Nature* **328,** 221–227 (1987).
2. Khrestchatisky, M. *et al. Neuron* **3,** 745–753 (1989).
3. Lolait, S. J. *et al. FEBS Lett.* **246,** 145–148 (1989).
4. Shivers, B. D. *et al. Neuron* **3,** 327–337 (1989).
5. Frieda, S., Pearce, A., Preston-HurlBurt, P. & Hawrot, E. *Proc. R. Soc.* B**241,** 207–213 (1990).
6. Galzi, J. L. *et al. FEBS Lett.* **294,** 198–202 (1991).
7. O'Leary, M. E. & White, M. M. *J. biol. Chem.* **267,** 8360–8365 (1992).
8. Dennis, M. *et al. Biochemistry* **27,** 2346–2357 (1988).
9. Tomaselli, G. F., McLaughlin, J. T., Juman, M. E., Hawrot, E. & Yellen, G. *Biophys. J.* **60,** 721–727 (1991).
10. Schmieden, V., Kuhse, J. & Betz, H. *EMBO J.* **11,** 2025–2032 (1992).
11. Vandenberg, R. J., Handford, C. A. & Schofield, P. R. *Neuron* **9,** 491–496 (1992).
12. Del Castillo, J & Katz, B. *Proc. R. Soc.* B**146,** 369–381 (1957).
13. Weiss, D. S. & Magleby, K. L. *J. Neurosci.* **9,** 1314–1324 (1989).
14. Twyman, R. E., Rogers, C. J. & Macdonald, R. L. *J. Physiol., Lond.* **423,** 193–220 (1990).
15. Casalotti, S. O., Stephenson, A. & Barnard, E. *J. biol. Chem.* **261,** 15013–15016 (1986).
16. Deng, L., Ransom, R. W. & Olsen, R. W. *Biochem. biophys. Res. Commun.* **138,** 1308–1314 (1986).
17. Sigel, E., Baur, R., Trube, G., Mohler, H. & Malherbe, P. *Neuron* **5,** 703–711 (1990).
18. Sigel, E., Baur, R., Kellenberger, S. & Malherbe, P. *EMBO J.* **11,** 2017–2023 (1992).
19. Nicoll, R. A., Eccles, J. C., Oshima, T. & Rubia, F. *Nature* **258,** 625–627 (1975).
20. Barker, J. L. & Ransom, B. R. *J. Physiol., Lond.* **280,** 355–372 (1978).
21. Nicoll, R. A. & Wojtowicz, J. M. *Brain Res.* **191,** 225–237 (1980).
22. Schulz, D. W. & MacDonald, R. L. *Brain Res.* **209,** 177–188 (1981).
23. Branden, C & Tooze, J. *Introduction to Protein Structure* (Garland, New York, 1991).
24. Galzi, J.-L., Revah, F., Bessis, A. & Changeux, J.-P *A. Rev. Pharmac.* **31,** 37–72 (1991).
25. Crawford, J. L., Lipscomb, W. N. & Schellman, C. G. *Proc. natn. Acad. Sci. U.S.A.* **70,** 538–542 (1973).
26. Levitan, E. S., Blair, L. A. C., Dionne, V. E. & Barnard, E. A. *Neuron* **1,** 773–781 (1988).
27. Verdoorn, T. A., Draguhn, A., Ymer, S., Seeburg, P. H. & Sakmann, B. *Neuron* **4,** 919–928 (1990).
28. Pritchett, D. B. *et al. Nature* **338,** 582–585 (1989).
29. Saiki, R. K. *et al. Science* **239,** 487–491 (1988).
30. Colquhoun, D. & Hawkes, A. G. *Proc. R. Soc.* B**211,** 205–235 (1981).
31. Colquhoun, D. & Ogden, D. C. *J. Physiol., Lond.* **395,** 131–159 (1988).
32. Colquhoun, D., Ogden, D. C. & Cachelin, A. B. *Ion Channels in Neural Membranes* (Plenum, New York, 1986).
33. Sakmann, B., Hamill, O. P. & Bormann, J. *J. Neural Transm.* **18**(suppl.), 83–95 (1983).
34. Newland, C. F., Colquhoun, D. & Cull-Candy, S. G. *J. Physiol., Lond.* **432,** 203–233 (1991).

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The probability of transmitter release at a mammalian central synapse

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When an action potential reaches a synaptic terminal, fusion of a transmitter-containing vesicle with the presynaptic membrane occurs with a probability (p_r) of less than one[1]. Despite the fundamental importance of this parameter, p_r has not been directly measured in the central nervous system. Here we describe a novel approach to determine p_r, monitoring the decrement of NMDA (*N*-methyl-D-aspartate)-receptor mediated synaptic currents in the presence of the use-dependent channel blocker MK-801 (ref. 2). On a single postsynaptic CA1 hippocampal slice neuron, two classes of synapses with a sixfold difference in p_r are resolved. Synapses with low p_r contribute to over half of transmission and are more sensitive to drugs enhancing transmitter release. Switching between these two classes of synapses provides the potential for large changes in synaptic efficacy and could underlie forms of activity-dependent plasticity.

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To determine the probability of release (p_r) at a central glutamatergic synapse, we monitored the decrement of elicited NMDA-mediated transmission as a function of repeated trials in the presence of MK-801. Because MK-801 is an irreversible NMDA-channel open channel blocker[3], only those receptors on synapses that release transmitter can be blocked, preventing their participation in subsequent transmission. Intuitively, if p_r is high, a large fraction of available synapses will be eliminated with each trial, and transmission will decay quickly. If p_r is low, then a small fraction of synapses will be eliminated with each trial, and transmission will decay slowly.

The relation between the amplitude of NMDA-mediated e.p.s.cs for consecutive trials in the presence of MK-801 is given by

$$R(t+1) = R(t) \times (1-F) \quad (1)$$

where $R(t)$ is the synaptic response for the tth trial; and F, the fraction of NMDA channels blocked by MK-801 in one trial out of all available NMDA channels, is calculated from

$$F = p_f \times p_r \times FB \quad (2)$$

where, FB = fraction of NMDA channels blocked by MK-801 at a synapse that releases transmitter; p_r = probability that a terminal receiving an action potential will release transmitter; p_f = the probability that a fibre stimulated with an extracellular electrode fires an action potential that is conducted to a synaptic terminal.

In MK-801, the predicted decrement in transmission is given by solving difference equation (1):

$$R(t) = R_0 \exp(-t/\tau)$$

where $\tau = -1/ln(1-F)$ and R_0 is the initial response in MK-801. Thus, from equation (2)

$$p_r = \frac{1-\exp(-1/\tau)}{p_f \times FB} \approx \frac{1}{\tau \times p_f \times FB} \text{ if } \tau \text{ is } > 5 \quad (3)$$

Therefore if τ, p_f and FB are measured, then the probability of transmitter release, p_r, can be determined.

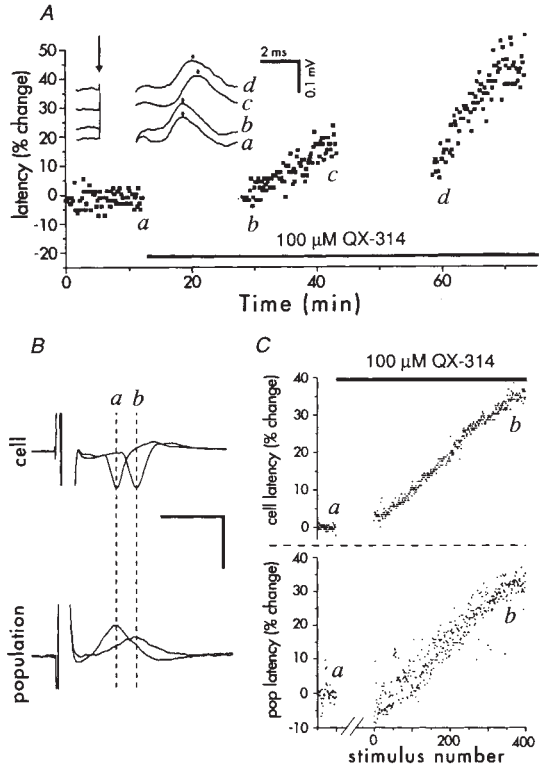
Stable NMDA-mediated synaptic responses between Schaffer collateral/commissural fibres and CA1 neurons were recorded in hippocampal slices in the presence of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Fig. 1). In MK-801, individual NMDA-mediated e.p.s.cs decayed more rapidly with little effect on the initial slope of the response (Fig. 1*B*; ref. 4), confirming the open-channel blocking nature of the drug. With continued stimulation, the peak amplitude progressively diminished (Fig. 1*A*) without changes in response kinetics (Fig. 1*C*, *b*, *c*). This argues against an increase in MK-801 concentration at the synapse after the initial response in the drug and against poor voltage control of NMDA-mediated currents at the subsynaptic membrane. Halting stimulation for 10–30 min after partial or total inhibition of transmission by MK-801 yielded no recovery of the response ($n=3$; data not shown), supporting the irreversibility of channel block.

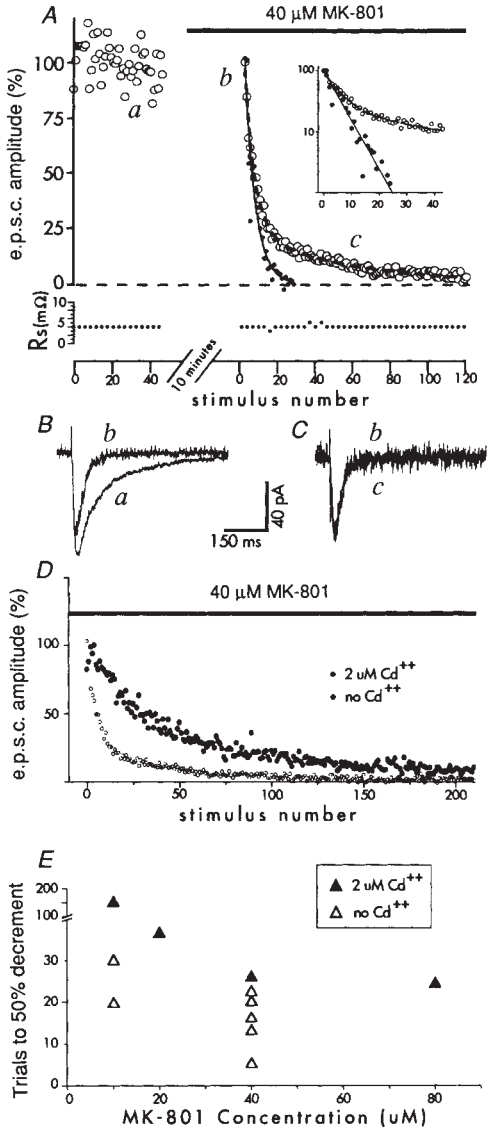
Equation (3) predicts that p_r is inversely related to τ. We tested this directly by examining the effect of extracellular Cd^{2+} (which blocks presynaptic calcium channels) on the decrement of transmission produced by MK-801 (Fig. 1*D*). In MK-801, transmission decayed more slowly in the presence of Cd^{2+} ($n=4$) than in its absence ($n=9$; Fig. 1*D*, *E*)

To estimate p_f, we used the voltage-dependent Na^+ channel open-channel blocker QX-314 (ref. 5). Extracellular application of this drug produced a use-dependent slowing of action potential conduction ($n=5$, Fig. 2*A*). To estimate p_f of a population of fibres, antidromic responses were recorded simultaneously from a single CA3 cell and from the surrounding population (Fig. 2*B*). With repeated stimuli, the same fractional change in conduction velocity was noted in the population as in the individual cell that fired every trial (Fig. 2*B*, *C*; $n=3$). This indicates

FIG. 1 Decay of NMDA-mediated responses in the presence of the open-channel blocker MK-801. *A*, top, Normalized peak amplitude of synaptic NMDA currents (empty circles) measured at −10 mV plotted versus stimulus trials. MK-801 (40 μM) was applied where indicated followed by interruption of stimuli for 10 min. In parallel experiments without MK-801, the stability of transmission was affected little by a 10 min interruption of afferent stimulation ($N=7$). Least-square fit (dashed line) of the decrement of transmission in MK-801 to the sum of 2 exponentials gives time constants $\tau_1=5.3$ trials (79%) and $\tau_2=59$ trials (21%). Normalized peak amplitude of responses (filled circles) to brief (2 ms) application of glutamate (1 mM) in the presence of MK-801 (40 μM) and CNQX (4 μM) from a different cell overlaid for comparison. Response decrement was fitted well by a single exponential (solid line, $\tau=5.0$ trials). Inset, Expanded view of response decrement plotted on a logarithmic scale. Bottom, Series resistance plotted versus stimulus number. *B*, Ensemble averages of 30 (*a*) and 2 (*b*) consecutive e.p.s.cs recorded before and after MK-801 application (epochs indicated in *A*). *C*, Ensemble averages of 2 (*b*) and 30 (*c*) consecutive e.p.s.cs obtained where indicated in *A* scaled to match peak amplitude. Note close kinetic agreement of normalized traces. Experiments in which the later e.p.s.cs decayed faster than early e.p.s.cs were not further analysed. *D*, Peak amplitude of synaptic NMDA currents plotted versus stimulus trial for an experiment with no Cd^{2+} (empty circles) and 2 μM Cd^{2+} (filled circles) included in superfusate. Cd^{2+} inhibited transmission by 66% (not shown). 40 μM MK-801 produced a slower decrement of transmission in experiment with Cd^{2+}, with greater effect on the initial fast decrement. *E*, Plot of trials required for peak amplitude of NMDA mediated e.p.s.c. to decay to 50% of initial value in MK-801 versus concentration of MK-801 for experiments in 2 μM cadmium (filled triangles) and no cadmium (empty triangles).

METHODS. Transverse hippocampal slices were prepared from 11–19-day-old rats and whole-cell recordings were obtained as previously described[12]. NMDA currents were isolated by addition of 3–10 μM CNQX (RBI) and 100 μM picrotoxin (Sigma) to the bath. Transmission was elicited (0.1 Hz) with a stimulating electrode placed in the CA1 stratum radiatum. Responses to agonist were elicited by injecting (Picosprizter, General Valve; 2 ms, 20 p.s.i.) glutamate (1 mM) through a patch pipette placed in stratum radiatum. Input (100–300 MΩ) and series (3.5–10 MΩ) resistances were monitored throughout an experiment (*A*). If series resistance deviated more than 30% during an experiment, the experiment was removed from analysis. The decrement of transmission as a function of trials (t) after MK-801 (RBI) application was fit (least-squares, MicroCal Origin) to the function $y(t)=A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)+y_0$ allowing A_1, A_2, τ_1 and τ_2 to vary.





that fibres contributing to the population response also fired with $p_f\sim 1$.

To obtain a simple and direct measurement of *FB*, we monitored the decrement of NMDA-mediated responses to pressure-applied glutamate in the presence of MK-801 (Fig. 1*A*). In 40 μM MK-801 the decrement was fitted well by a monoexponential function with time constant $\tau=4.0\pm0.8$ ($n=6$), which

FIG. 2 Fibres excited with an extracellular stimulating electrode fire with high probability. *A*, Plot of change in conduction time versus time for an antidromically activated population of CA3 neurons. QX-314 (RBI) is applied to bath where indicated. Note no increase in conduction time in the absence of stimuli demonstrating a use-dependent block. Inset, Average of 5 sweeps obtained at times indicated on plot. Arrow indicates stimulus artefact; asterisks indicate peak of antidromic response. *B*, Antidromic responses from a single CA3 cell (cell-attached patch, top) and a nearby population of CA3 cells (field electrode, bottom) obtained at time indicated on graph in *C*. Calibration bars: top 4 ms, 20 pA; bottom 4.75 ms, 1.25 mV. Note similar change in relative conduction time in the individual cell as in the population of cells. *C*, Plot of conduction time measured in a single fibre (top) and in the population of cells (bottom). Drug is applied where indicated. The change in conduction time in the single fibre versus that of the population correlate (least squares linear regression) with slope of 1 indicating that the population of cells is affected by QX-314 as much as a single fibre that fires every time. Perfusate contained 10 μM CNQX, 80 μM MK-801 and 10 μM 2-chloro-adenosine (Sigma) to block synaptic transmission.

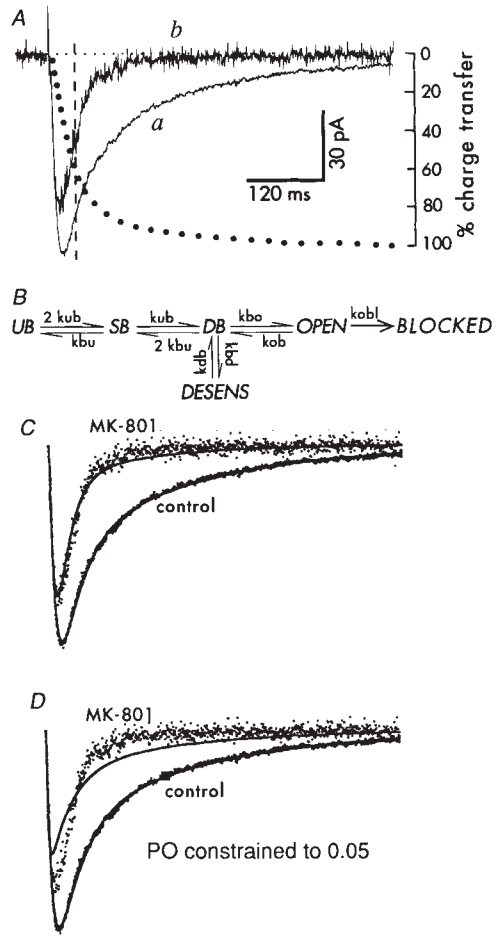


FIG. 3 Fraction of NMDA receptors blocked by MK-801 during an e.p.s.c. (*FB*) calculated with two methods. *A*, e.p.s.cs recorded before (*a*, average of 30 traces) and during (*b*, average of the first 2 traces) application of 40 μM MK-801. In the absence of MK-801, e.p.s.cs (10%–90% rise time = 12.4 ± 1.6 ms; biexponential decay with τ_1 = 55.7 ± 3.2 and τ_2 = 225 ± 16; N = 13) resembled currents resulting from application of saturating concentrations of glutamate to membrane patches[6,13,14]. Percentage of total charge (integrated current) transferred during synaptic response in MK-801 (filled circles). Time for 60% of charge to be transferred ($t_{60\%}$ = 41 ms) indicated by dashed line. In membrane patch studies[3] a similar $t_{60\%}$ is observed if [MK-801] = 3–10 μM and 40–65% of patch channels are blocked. The lipophilicity of MK-801 may explain the apparent 4–10-fold difference between drug concentration in the bath and that inferred to be at the synapse. Records are from same experiment as in Fig. 1*B*. *B*, Reaction scheme used to model opening and blocking of synaptic NMDA receptors[10] *C*, e.p.s.c. averages (dots) in the absence and presence of MK-801, with least-squared fits (solid line) obtained using reaction scheme in *B*. *D*, e.p.s.c. averages (dots) overlaid with least-square fits (solid line) with $P_{open} = k_{bo}/(k_{ob} + k_{bo})$ constrained to 0.05. This value for P_{open}, suggested by other studies[2,15], is lower than the value from our unconstrained simulations (P_{open} = 0.31 ± .05; N = 13). Note the poor fit to e.p.s.c. in MK-801 with such constraints. In addition a 300% increase in receptor number was required (experiment N19T02). A heterogeneity in *FB* sufficient to account for τ_2/τ_1 (see text) is inconsistent with several lines of evidence. First, the time course of synaptic responses (as can be measured by $t_{60\%}$) was similar for all trials after application of MK-801 (Fig. 1*C*). For different concentrations of MK-801, $t_{60\%}$ = 1.6 × *FB* + intercept (n = 13). Thus, a sixfold difference in blocking rate (necessary to explain τ_2/τ_1) would show up as a 10-fold increase in $t_{60\%}$ which is not seen. Second, differential binding of agonist (such as non-homogeneous exposure of agonist to channels or homogenous exposure to channels with different agonist affinities) is not consistent with the data obtained with Cd^{2+}. Addition of extracellular Cd^{2+} primarily slowed the faster decrement, τ_1 (τ_2/τ_1 = 2.7 ± 1.1 in Cd^{2+}, n = 4; τ_2/τ_1 = 5.8 ± 0.9 in no Cd^{2+}, n = 9; $P < 0.05$; Fig. 1*D*). If τ_2 corresponds to the population of receptors with lower binding efficacy one would predict a similar or greater effect of Cd^{2+} on this component of transmission. And finally, the monoexponential decrement of responses to exogenously applied glutamate argues against heterogeneity of receptor binding, opening or blocking rates, although admittedly, the temporal and spatial agonist concentration profile or receptors recruited with such application may not faithfully reflect synaptic transmission. We note that if a single vesicle does not saturate all NMDA receptors, our estimate of p_r may be low.

METHODS. In simulations, a brief pulse of transmitter was delivered and model parameters were adjusted to fit optimally synaptic currents recorded with no MK-801 using a least-squares fitting procedure in the SCoPfit program (Simulation Resources). With these parameters fixed, blocking rate and receptor number were allowed to vary to fit optimally e.p.s.c. in MK-801. With such a procedure, changes in receptor number (reflecting variations in transmission during the 10 min interruption) were minimal (−7 ± 5%; N = 13) Glutamate binding rate was constrained to a previously estimated value (5 $\mu M^{-1} s^{-1}$ ref. 14), and blocking rate, k_{obl}, was set to 0 in fits to currents recorded in no MK-801. State and rate constant abbreviations along with values obtained in no MK-801 (N = 13): UB, unbound; SB, single bound; DB, double bound; DESENS, desensitized; k_{ub}, binding rate; k_{bu}, unbinding rate (9.5 ± 0.6); k_{bo}, opening rate (25 ± 1.5); k_{ob}, closing rate (59 ± 5); k_{bd}, desensitization rate (16 ± 1.7); k_{db}, resensitization rate (13 ± 1.2); k_{obl}, block rate. Units of k_{ub} are $\mu M^{-1} s^{-1}$ and of all other rates are s^{-1}.

implies (from equation (3)) *FB* = 25 ± 6%. But spatial–temporal agonist concentration profiles or the receptor pools activated may differ for synaptic and exogenously applied transmitter. Thus, two other independent methods were used to determine *FB*. One method uses the observation that the fraction of NMDA channels on isolated membrane patches blocked by MK-801 during a single brief application of glutamate is related to the time required for 60% of the charge to be transferred, $t_{60\%}$ (ref. 3; Fig. 3 legend). In hippocampal slices with 40 μM MK-801, $t_{60\%}$ = 65 ± 8 ms (n = 6, Fig. 3*A*) implying (from ref. 3) that about 40–50% of channels at synapses that release transmitter are blocked (Fig. 3 legend). For a third independent estimate of *FB*, we used a kinetic model that accounts for the macroscopic behaviour of NMDA currents from isolated patches and from synapses of cultured neurons (Fig. 3*B*, refs 6–8). Least-squares fits of this model to experimental e.p.s.cs in the absence and presence of 40 μM MK-801 (Fig. 3*C*) yielded a value of *FB* = 30 ± 8% (n = 6), generally in agreement with the values for *FB* obtained above.

To calculate τ, the decrement of peak e.p.s.c. amplitude produced by MK-801 was fitted to exponential functions (Fig. 1 legend). In general (8 of 9 experiments), the decrement over trials (t) could be best fitted by the sum of two exponentials, $A_1 \exp(t/\tau_1) + A_2 \exp(t/\tau_2)$ with $A_2/(A_1 + A_2)$ = 0.54 ± 0.05 and τ_2/τ_1 = 6.4 ± 0.7 (N = 8; Fig. 1*A*). This biexponential decrement indicates heterogeneity in p_f, *FB* and/or p_r. To account for the observed $A_2/(A_1 + A_2)$ and τ_2/τ_1 with multiple values of p_f, approximately 85% of axons must have a $p_f < 0.15$, which is inconsistent with the results in Fig 2. A heterogeneity of *FB* would require receptors that are differentially blocked by MK-801, those with greater *FB* being removed from transmission earlier. Such heterogeneity is not supported by (1) the similar response kinetics throughout the block (Fig. 1*C* and Fig. 3 legend); (2) the observation that Cd^{2+} differentially affects the two components of the biexponential decrement (Fig. 1*D* and Fig. 3 legend); or (3) the monoexponential decrement of responses to brief exogenous application of glutamate in the presence of MK-801. Thus our data argue against heterogeneity in p_f or *FB*, suggesting that there are two populations of synapses with high and low p_r. For each of the eight experiments showing a biexponential decrement of transmission in MK-801, τ_1 and τ_2 were computed together with *FB* (using the kinetic model) allowing calculation of high p_r = 0.37 ± 0.04 and low p_r = 0.06 ± 0.01.

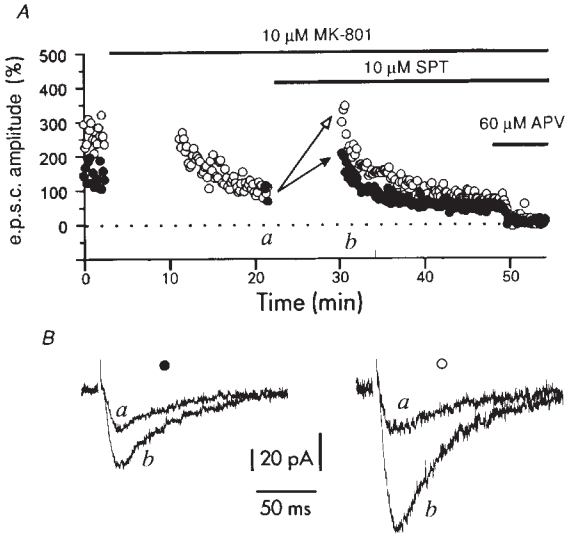


FIG. 4 Synapses with low probability of release are more sensitive to drugs enhancing release. *A*, Peak NMDA synaptic currents in 4 μM CNQX plotted versus time for two inputs onto a single neuron, normalized to current amplitude at time *a*. After addition of MK-801, one pathway (empty circles) was stimulated 60 times (0.1 Hz), the other pathway (filled circles) was stimulated 3 times (0.1 Hz). Addition of SPT (10 μM, RBI) to disinhibit transmission by blocking presynaptic adenosine receptors, produces a larger enhancement in the pathway retaining primarily low p_r synapses (empty circles). *B*, e.p.s.c. averages obtained where indicated on graph from two inputs (empty and filled circles).

To test further for synapses with differing p_r, we designed an experiment to isolate these two populations and examined the effect of drugs that act presynaptically. We reasoned that synapses with a lower p_r should be affected more by manipulations that increase release[9–11]. Synapses with a low p_r were partially isolated by stimulating afferent inputs repeatedly (more than 40 times at 0.1 Hz) in the presence of MK-801 (Fig. 4*A*). This would be expected to remove predominantly synapses with a higher p_r. Another input to the cell, stimulated for only a few trials in MK-801, would be expected to retain both high and low p_r synapses. Bath application of agents acting presynaptically produced a greater enhancement in the pathway that had been repeatedly stimulated in MK-801 (Fig. 4*A*, *B*). The enhancement was $37 \pm 4\%$ greater with 8-(*p*-sulphophenyl)theophylline (10–20 μM, $n = 4$, $P < 0.05$, paired *t*-test), and $90 \pm 10\%$ greater with 4-amino-pyridine (60 μM, $n = 3$, $P < 0.05$ paired *t*-test). These results provide additional support to the view that there are separable populations of synapses with high and low probability of release[16–19].

We have found evidence for two classes of central excitatory synapses with a sixfold difference in the probability of release. Because they contribute about equally to transmission, if the quantal size of the two classes is similar then 85% of these synapses have a low p_r. Although it is surprising that most synapses appear to be virtually silent, such synapses may transmit primarily in physiological conditions increasing release such as during bursts of presynaptic activity (as occurs with sensory stimuli) or after local secretion of modulators. Conversion of synapses from low to high p_r (as possibly by induction of long-term potentiation) may render these synapses capable of recreating sensory traces in the absence of externally driven bursts.

Note added in proof: A preliminary report[15] published after this study was initiated and a more complete report[20] published since its completion used similar techniques to investigate cultured hippocampal autapses and reached conclusions generally in agreement with those presented here. □

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1. Katz, B. *The Release of Neural Transmitter Substances* (Thomas, Springfield, Illinois, 1969).
2. Huettner, J. E. & Bean, B. P. *Proc. natn. Acad. Sci. U.S.A.* **85,** 1307–1311 (1988).
3. Jahr, C. E. *Science* **255,** 470–472 (1992).
4. Hestrin, S., Sah, P. & Nicoll, R. A. *Neuron* **5,** 247–253 (1990).
5. Strichartz, G. *J. gen. Physiol.* **62,** 37–57 (1973).
6. Edmonds, B. & Colquhoun, D. *Proc. R. Soc.* B**250,** 279–286 (1992).
7. Lester, R. A. J. & Jahr, C. E. *J. Neurosci.* **12,** 635–643 (1992).
8. Clements, J. D., Lester, R. A. J., Tong, G., Jahr, C. E. & Westbrook, G. L. *Science* **258,** 1498–1501 (1992).
9. Rahamimoff, R. *J. Physiol., Lond.* **195,** 471–480 (1968).
10. Jack, J. J. B., Redman, S. J. & Wong, K. *J. Physiol., Lond.* **321,** 111–126 (1981).
11. Lin, J-W. & Faber, D. S. *J. Neurosci.* **8,** 1313–1325 (1988).
12. Liao, D., Jones, A. & Malinow, R. *Neuron* **9,** 1089–1097 (1992).
13. Lester, R. A. J., Clements, J. D., Westbrook, G. L. & Jahr, C. E. *Nature* **346,** 565 (1990).
14. Clements, J. D. & Westbrook, G. L. *Neuron* **7,** 605–613 (1991).
15. Rosenmund, C., Clements, J. D. & Westbrook, G. L. *Biophys. Soc. Abstr.* A327 (1993).
16. Atwood, H. L. *Nature* **215,** 57–58 (1967).
17. Redman, S. & Walmsley, B. *J. Physiol. Lond.* **343,** 135–145 (1983).
18. Walmsley, B., Edwards, F. R. & Tracey, D. J. *J. Neurophys.* **60,** 889–908 (1988).
19. Clamann, H. P., Mathis, J. & Luscher, H.-R. *J. Neurophys.* **61,** 403–416 (1989).
20. Rosenmund, C., Clements, J. D. & Westbrook, G. L. *Science* **262,** 754–757 (1993).

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Phosphatidylinositol transfer protein required for ATP-dependent priming of Ca^{2+}-activated secretion

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ELUCIDATION of the reactions responsible for the calcium-regulated fusion of secretory granules with the plasma membrane in secretory cells would be facilitated by the identification of participant proteins having known biochemical activities. The successful characterization of cytosolic[1–3] and vesicle[4,5] proteins that may function in calcium-regulated secretion has not yet revealed the molecular events underlying this process. Regulated secretion consists of sequential priming and triggering steps which depend on ATP and Ca^{2+}, respectively, and require distinct cytosolic proteins[6]. Characterization of priming-specific factors (PEP proteins) should enable the ATP-requiring reactions to be identified. Here we show that one of the mammalian priming factors (PEP3) is identical to phosphatidylinositol transfer protein (PITP)[7]. The physiological role of PITP was previously unknown. We also find that SEC14p, the yeast phosphatidylinositol transfer protein which is essential for constitutive secretion[8–10], can substitute for PEP3/PITP in priming. Our results indicate that a role for phospholipid transfer proteins is conserved in the constitutive and regulated secretory pathways.

The ATP-dependent priming of Ca^{2+}-activated noradrenaline secretion from permeable PC12 cells was previously found to require proteins present in rat brain cytosol[6]. Gel filtration of rat brain cytosol revealed three size classes of factors with priming activity (ref. 6, and Fig. 1*b*). These PEP factors (for priming in exocytosis proteins) had apparent molecular masses of ~500,000 (500K) (PEP1), ~120K (PEP2), and ~20K (PEP3). In cytosol from rat liver, only the smallest factor, PEP3, was detectable (not shown); we used this cytosol to purify PEP3 as a 32K protein (Fig. 2*b* and *d*). The amino-terminal sequence of the purified PEP3 was VLLKEYRVILPVSVDEYQVGQLYSVA, which is identical to residues 2–27 of the 32K protein predicted from a rat brain complementary DNA encoding PITP[11]. PITP was originally identified by its ability to transfer

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