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LTP of AMPA and NMDA Receptor–Mediated Signals: Evidence for Presynaptic Expression and Extrasynaptic Glutamate Spill-Over

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Summary

We have addressed the expression of long-term potentiation (LTP) in hippocampal CA1 by comparing AMPA and NMDA receptor–(AMPAR- and NMDAR-) mediated postsynaptic signals. We find that potentiation of NMDAR-mediated signals accompanies LTP of AMPAR-mediated signals, and is associated with a change in variability implying an increase in quantal content. Further, tetanic LTP of NMDAR-mediated signals can be elicited when LTP of AMPAR-mediated signals is prevented. We propose that LTP is mainly expressed presynaptically, and that, while AMPARs respond only to glutamate from immediately apposed terminals, NMDARs also sense glutamate released from terminals presynaptic to neighboring cells. We also find that tetanic LTP increases the rate of depression of NMDAR-mediated signals by the use-dependent blocker MK-801, implying an increase in the glutamate release probability. These findings argue for a presynaptic contribution to LTP and for extrasynaptic spill-over of glutamate onto NMDARs.

Introduction

Long-term potentiation (LTP) is an important example of synaptic plasticity, which may underlie some forms of learning, development, and neuronal injury (Bliss and Collingridge, 1993). LTP in the CA1 region has been found to be accompanied by an increase in quantal content, which has conventionally been interpreted as reflecting a presynaptic increase in release probability (Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Kullmann and Nicoll, 1992; Larkman et al., 1992; Liao et al., 1992; Voronin, 1993; but see Foster and McNaughton, 1991). An alternative explanation, however, is that postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are normally nonfunctional or absent at a proportion of synapses, but can be uncovered with LTP (Kullmann, 1994; Isaac et al., 1995; Liao et al., 1995). This proposal accounts for the observation that, under baseline conditions, the quantal content sensed by AMPARs on a given cell is normally less than that sensed by N-methyl-D-aspartate receptors (NMDARs). It also explains why LTP has been reported to be preferentially, if not selectively, expressed by AMPARs, with little or no change in the NMDAR-mediated component of the synaptic signal (reviewed by Kullmann and Siegelbaum, 1995). A similar process may underlie developmental changes in synaptic transmission (Durand et al., 1996).

There are, nevertheless, some observations that are difficult to reconcile with the "latent AMPAR cluster" hypothesis (Kullmann and Siegelbaum, 1995). First, in two recent studies of trial-to-trial fluctuations of unitary postsynaptic signals, LTP was accompanied by a decrease in the probability of transmission failures, with no change in the average amplitude of the excitatory postsynaptic currents (EPSCs) (Stevens and Wang, 1994; Bolshakov and Siegelbaum, 1995). This is difficult to reconcile with the appearance of new clusters of AMPARs, because synchronous activation of several clusters should give rise to occasional larger EPSCs. Second, a phenomenon akin to LTP in cultured cells is accompanied by increased presynaptic vesicle cycling, implying that the increase in quantal content has a presynaptic origin (Malgaroli et al., 1995). And third, although several groups have reported little or no potentiation of the NMDAR-mediated component with LTP (Kauer et al., 1988; Muller and Lynch, 1988; Muller et al., 1988; Perkel and Nicoll, 1993; Kullmann, 1994), others have shown that isolated NMDAR-mediated signals can undergo potentiation after tetanic stimulation (Asztely et al., 1992; Bashir et al., 1991; Beretta et al., 1991; Tsien and Malinow, 1990; Xie et al., 1992). LTP of AMPAR-mediated signals has, moreover, been reported to be accompanied by a potentiation of the NMDAR-mediated component under some conditions (Aniksztejn and Ben-Ari, 1995; Asztely et al., 1992; Clark and Collingridge, 1995). This potentiation of the NMDAR-mediated component calls for a revision of the latent AMPAR cluster hypothesis.

One possible factor that may shed some light on this disagreement is the fact that different procedures have been applied to elicit LTP. The two conventional induction methods are tetanic stimulation of a large number of presynaptic afferents on the one hand, and low frequency pairing of presynaptic stimulation and postsynaptic depolarization on the other. While LTP of the NMDAR-mediated component has been observed with tetanic stimulation (Bashir et al., 1991; Beretta et al., 1991; Tsien and Malinow, 1990; Asztély et al., 1992; Aniksztejn and Ben-Ari, 1995; Clark and Collingridge, 1995; Xie et al., 1992), it has not previously been reported with pairing (Perkel and Nicoll, 1993; Kullmann, 1994; Selig et al., 1995). Here, we show that pairing-induced LTP of AMPAR-mediated signals is accompanied by a consistent, albeit small, potentiation of NMDAR-mediated signals, and that this potentiation is associated with a correspondingly small increase in quantal content. We also show that tetanic LTP NMDAR-mediated signals can be obtained under conditions when LTP of AMPAR-mediated signals is prevented. We propose a novel explanation to account both for these results and for the discrepancy in quantal contents sensed by AMPARs and NMDARs, which places the major locus of expression of LTP in the presynaptic terminal and requires that NMDARs of one cell sense glutamate released from terminals that are presynaptic to neighboring cells. Finally, we provide supportive evidence for a presynaptic

contribution to tetanic LTP by examining the rate at which the use-dependent NMDAR blocker MK-801 attenuates postsynaptic NMDAR-mediated signals.

Results

Pairing-Induced LTP of AMPAR- and NMDAR-Mediated Signals

In the first series of experiments, we addressed the issue of whether pairing-induced LTP of AMPAR-mediated excitatory postsynaptic potentials (EPSPs) is associated with a potentiation of NMDAR-mediated signals. To keep the induction protocol and recording conditions as close as possible to previous studies, we initially recorded the AMPAR-mediated component of the synaptic signal and verified that LTP of this signal was induced, before recording the NMDAR-mediated component elicited by the same presynaptic stimuli.

We recorded extracellular field potentials in CA1, with three stimulating electrodes positioned in stratum radiatum to activate separate groups of afferents converging on the same dendritic region. One pathway was repeatedly tetanized, while a second pathway was stimulated with single pulses timed to coincide with the start of each tetanus (Gustafsson and Wigström, 1986). This caused a large potentiation in both pathways (Figure 1A). The potentiation in the first pathway (50% ± 5%) reflects tetanic LTP, and that in the second pathway (27% ± 3%), pairing-induced LTP, since the single pulses coincide with the depolarization generated by the tetani. AMPARs were subsequently blocked with 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM), and the extracellular Mg^{2+} concentration was decreased to 0.1 mM to relieve the block of NMDARs. This allowed the NMDAR-mediated component of the EPSPs to be assessed with no change to the stimulus intensity or frequency.

To determine whether there was a potentiation of the NMDAR-mediated signal, we normalized the initial slope of the NMDAR-mediated EPSPs in each pathway by the average initial slope of the AMPAR-mediated EPSPs prior to LTP induction. This relies on the assumption that the ratio of the two components of the synaptic signal is the same in the different pathways studied in each slice. This has been validated by Selig et al. (1995) for field EPSPs, and we have confirmed that this also holds true for EPSCs (see Experimental Procedures). The normalized initial slopes of the NMDAR-mediated EPSPs were then compared with those of the third and control pathway, which was not stimulated during the induction procedure. This revealed a potentiation of the NMDAR-mediated EPSPs of both the tetanized and the paired pathways (Figure 1A). When this measurement was repeated in 22 cells and a paired *t* test applied, a significant potentiation of the NMDAR-mediated signal was seen in both the tetanized (23% ± 5%, $p < 10^{-7}$) and the paired (8% ± 3%, $p = 0.014$) pathways (Figure 1B).

A consistent finding was that LTP of the NMDAR-mediated EPSPs was smaller than that of the AMPAR-mediated EPSPs (Figure 1C). Expressed as a fraction of the AMPAR-mediated EPSP potentiation, the potentiation of the NMDAR-mediated component measured 46% and 30% in the tetanized and paired pathways, respectively. It is unlikely that this reflected a continued slow decline of LTP with time, since in separate experiments where we followed the AMPAR-mediated component, the potentiation at 40 min remained within ±15% of that at 25 min (data not shown). An alternative possible explanation for the smaller potentiation of the NMDAR-mediated component is that measurement of NMDAR-mediated EPSPs *per se* induced a potentiation: since Ca^{2+} ions must flow into the cell via the NMDARs when the Mg^{2+} concentration is low, a necessary condition for induction of LTP is satisfied. If this inadvertently induced potentiation were occluded by the prior conditioning, the magnitude of LTP of the NMDAR-mediated EPSPs in the test pathways could have been underestimated. To avoid this, we took advantage of the finding that, with the whole-cell technique, LTP can only be induced by pairing within the first 20 min of recording (Malinow and Tsien, 1990; Kato et al., 1993; Kullmann, 1994). If the measurement of the NMDAR-mediated component is delayed beyond this time, there should be no further potentiation, assuming that the induction requirements for LTP of AMPAR- and NMDAR-mediated signals are similar.

In 42 cells, we paired low frequency stimulation with depolarization to 0 mV within 12 min of entering whole-cell mode. After a further 20 min, AMPARs were blocked with DNQX, and the postsynaptic cell was held at a positive potential to record the NMDAR-mediated EPSCs (Figure 2). The experimental protocol was identical to that used by Kullmann (1994), and the data include 23 cells reported in that study. Only a small potentiation of the NMDAR-mediated component was seen when the average EPSCs in the test and control pathways were compared, and the standard error bars overlapped, suggesting no significant difference between the pathways. There was, however, considerable variability among different cells, both in the relative amplitude of the two components, and in the amount of LTP, which could have concealed a statistically significant potentiation. We therefore applied the same normalization procedure as in Figure 1: the amplitude of the NMDAR-mediated EPSCs in each pathway was normalized by the mean amplitude of the AMPAR-mediated EPSCs before the pairing. The normalized NMDAR-mediated EPSCs in the test and control pathways were then compared with a paired *t* test. This revealed a small but highly significant potentiation of the NMDAR-mediated component (14% ± 4%, $p < 0.001$). In Figure $2B_2$, the control pathway was rescaled to 100%, and the same scaling factor was applied to the test pathway, in order to display the difference between the pathways.

The potentiation of the NMDAR-mediated component was again smaller than the potentiation of the AMPAR-mediated EPSCs (62% ± 8%; potentiation of the two components different at $p < 10^{-9}$). We thus conclude that LTP of AMPAR-mediated EPSP/Cs, either tetanic or pairing-induced, is associated with a small but consistent potentiation of NMDAR-mediated EPSP/Cs.

Variance Analysis of Pairing-Induced Potentiation of NMDAR-Mediated EPSCs

We next addressed the synaptic locus of pairing-induced LTP of the NMDAR-mediated component, by

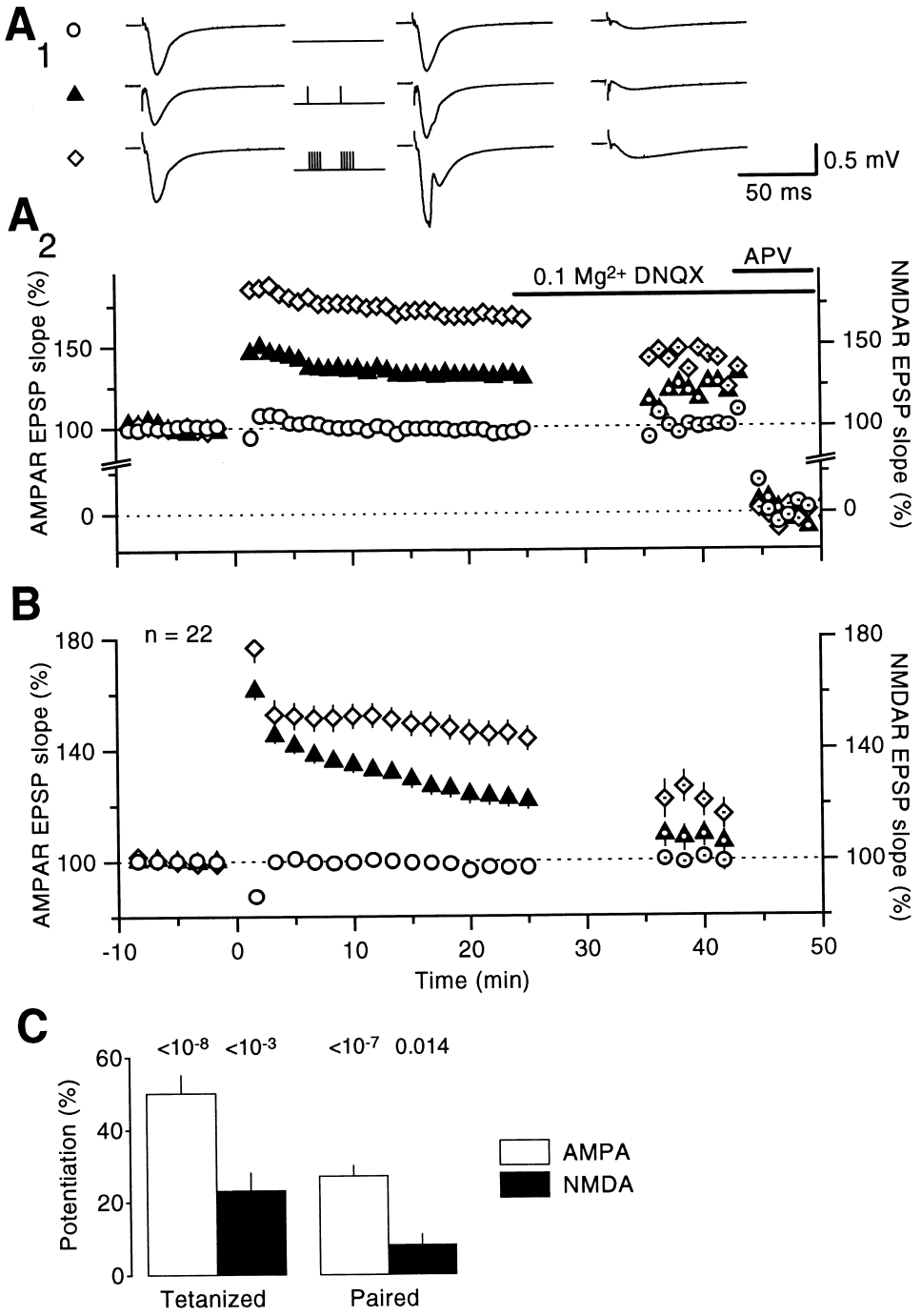

Figure 1. LTP Is Associated with a Potentiation of NMDAR-Mediated EPSPs

(A_1) Traces from one experiment before (left) and after (middle) induction of LTP, and after switching to 0.1 mM Mg^{2+} and 10 μM DNQX (right). Top to bottom: control, paired, tetanized pathways.

(A_2) EPSP initial slope plotted against time (averages of five successive stimuli). At t = 0, one pathway (open diamonds) was repeatedly tetanized (five pulses at 100 Hz), with single stimuli delivered to the second pathway (filled triangles), synchronous with the first pulse of each tetanus. (Open circles: control pathway.) DNQX and low Mg^{2+} were later added to record NMDAR-mediated EPSPs, and then 100 μM D, L-aminophosphonovaleric acid (APV) to confirm that AMPARs were blocked. The average NMDAR-mediated EPSP slopes in the control pathway were rescaled to the average baseline AMPAR-mediated EPSP slope, and the same scaling factor was applied to the test pathways (symbols with dots).

(B) Results from 22 slices (averages of ten successive stimuli ± SEM), showing that NMDAR-mediated EPSPs were potentiated, but less than AMPAR-mediated EPSPs.

(C) Histogram of the potentiation of AMPAR- and NMDAR-mediated EPSPs (averaged over 5 min), with p values for paired *t* tests. LTP of NMDAR-mediated EPSPs was smaller than LTP of AMPAR-mediated EPSPs with both induction methods ($p < 0.001$).

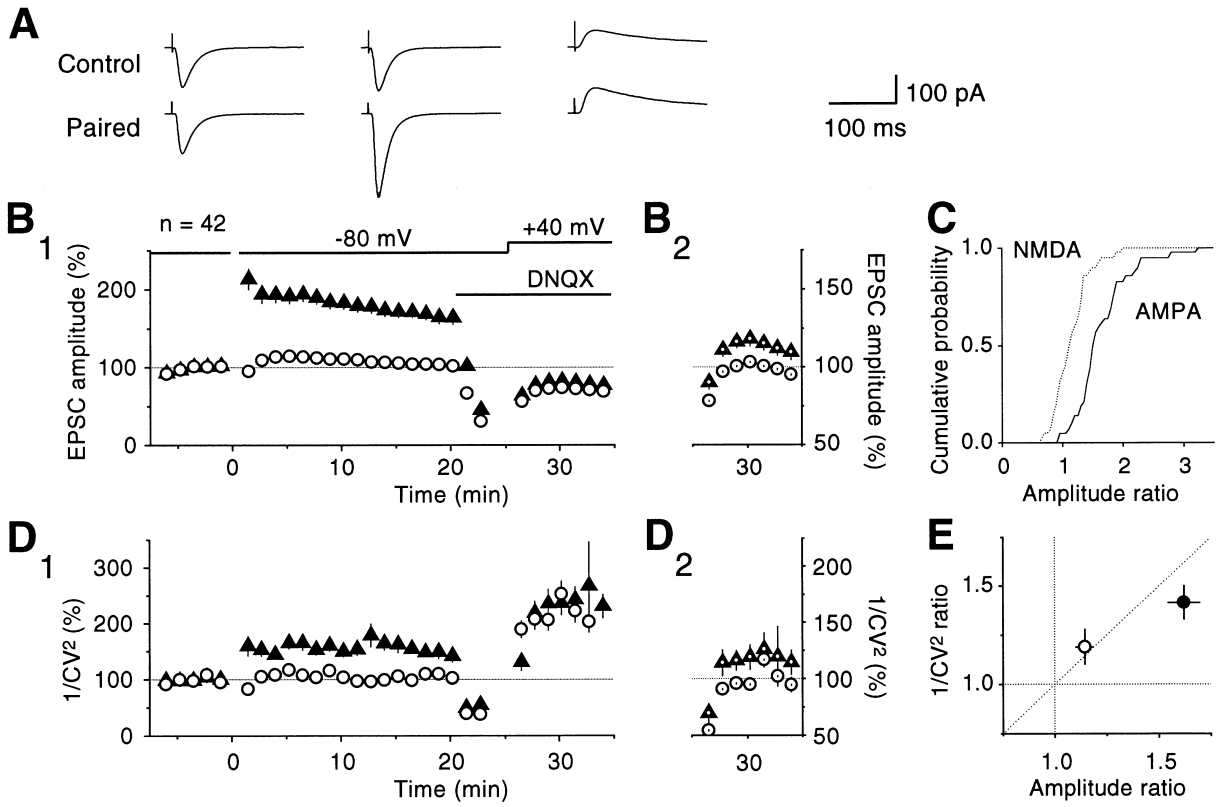

Figure 2. Pairing-Induced LTP of NMDAR-Mediated EPSCs Is Associated with an Increase in $1/CV^2$, Implying an Increase in Quantal Content

(A) Averaged traces from one cell before (left) and after (middle) induction of LTP by pairing, and after switching to a positive holding potential in DNQX (right).

(B_1) EPSC amplitude plotted against time in 42 cells (averages of 25 trials). At t = 0, stimulation of one pathway (2 Hz, 120 pulses; filled triangles) was paired with depolarization to 0 mV. DNQX was later added, and the membrane potential switched to +40 mV, to record the NMDAR component. Open circles show the control pathway EPSCs.

(B_2) NMDAR-mediated EPSCs, rescaled to set the average control EPSCs = 100%, showing a small potentiation in the paired pathway (triangles with dots).

(C) Cumulative histogram showing the potentiation of the AMPAR- and NMDAR-mediated components. The amplitude ratio for each cell was calculated by dividing the average EPSC amplitude in the test pathway by the average amplitude in the control pathway (after normalizing each pathway by the baseline amplitude of the AMPAR-mediated component).

(D_1) Time course of $1/CV^2$, corrected for the background noise variance, showing an increase with LTP and a further increase when switching to +40 mV in DNQX.

(D_2) $1/CV^2$ for the NMDAR component, rescaled as in B_2, showing an increase in the paired pathway.

(E) Fractional increase in $1/CV^2$ plotted against fractional increase in amplitude for AMPAR- and NMDAR-mediated components (filled and open symbols, respectively). The increases are larger for the AMPAR- than for the NMDAR-mediated components.

examining the trial-to-trial amplitude fluctuations of the EPSCs with the statistic $1/CV^2$ (inverse of the coefficient of variation squared). $1/CV^2$ varies with quantal content but is independent of the mean quantal amplitude, and has been shown to increase with LTP of the AMPAR-mediated component (Malinow and Tsien, 1990; Bekkers and Stevens, 1990). We confirmed this result in the same 42 cells: $1/CV^2$ for the AMPAR-mediated EPSCs increased by 42% ± 9% after LTP induction, which is consistent with a large increase in quantal content (also see Larkman et al., 1992; Manabe et al., 1993) (Figure 2D). $1/CV^2$ was also much larger for the NMDAR- than for the AMPAR-mediated components of the EPSCs, as expected if the quantal content sampled by NMDARs was larger than that sampled by AMPARs (Kullmann, 1994; Isaac et al., 1995; Liao et al., 1995; Selig et al., 1995): for the control pathway, the ratio of $1/CV^2$ for the NMDAR- and AMPAR-mediated components was 2.1 ± 0.1.

To determine whether the potentiation of the NMDAR-mediated component was also associated with an increase in quantal content, we applied the same normalization procedure as for the mean amplitude change: $1/CV^2$ for the NMDAR-mediated component of both pathways was divided by $1/CV^2$ of the AMPAR-mediated component prior to pairing. A comparison of the paired and control pathways revealed a difference of 19% ± 9% ($p = 0.044$) (Figure $2D_2$). Although this is an indirect measurement, and $1/CV^2$ can also be affected by variability in quantal amplitude or release probability (Faber and Korn, 1991), these results are most simply explained by postulating that potentiation of the NMDAR-mediated component is indeed associated with an increase in quantal content.

LTP of both components is thus associated with an increase in $1/CV^2$, although this is smaller for the NMDAR- than for the AMPAR-mediated EPSCs. Figure 2E shows the fractional increase in $1/CV^2$ plotted against the fractional increase in amplitude for the two components. Assuming a simple binomial or Poisson model of transmitter release, an increase in quantal content should cause the point to fall on or above the line of identity. This was the case for the NMDAR-mediated component. For the AMPAR-mediated component, in contrast, the fractional increase in $1/CV^2$ was less than the fractional increase in amplitude (Larkman et al., 1992; Manabe et al., 1993). This is compatible with an increase in both quantal amplitude and release probability, as has been argued by several groups who examined the clustering of successive EPSCs at different amplitudes (Kullmann and Nicoll, 1992; Liao et al., 1992; Larkman et al., 1992; Stricker et al., 1996; but see Stevens and Wang, 1994; Bolshakov and Siegelbaum, 1995).

Although the *fractional* increase in $1/CV^2$ for the NMDAR-mediated EPSCs was smaller than for the AMPAR-mediated EPSCs, this was because the denominator was larger for the NMDAR-mediated component: multiplying the fractional increase in $1/CV^2$ by the ratio of $1/CV^2$ for the NMDAR- and AMPAR-mediated components in the control pathway gives $19\% \times 2.1 = 40\%$, which is similar to the fractional increase in $1/CV^2$ for the AMPAR-mediated EPSCs (42%). A possible interpretation for this coincidence is that, while the *baseline* quantal content was larger for the NMDAR- than for the AMPAR-mediated EPSCs, the *absolute increase* in quantal content with LTP was identical for both components. This led to the following conjecture: First, synaptic AMPARs only sense glutamate released from presynaptic terminals directly apposed to the recorded cell. This is compatible with their relatively low affinity for glutamate and with the estimated concentration of the transmitter transiently reached within the synaptic cleft, which may briefly exceed 1 mM (Clements et al., 1992). Second, NMDARs, in contrast, as a result of their much higher affinity for glutamate (Patneau and Mayer, 1990), respond not only to glutamate released from immediately apposed terminals, but also to glutamate spilling over from nearby terminals that are presynaptic to other cells. Third, LTP is expressed, in large part, by a presynaptic increase in release probability.

The difference in the baseline quantal content for the two components is thus explained by the hypothesis that NMDARs sense a larger number of release sites, without invoking a nonuniform distribution of the two receptor types (Kullmann and Siegelbaum, 1995). We propose that pairing-induced LTP is expressed, in large part, through an increase in release probability from presynaptic terminals. Terminals presynaptic to neighboring cells would not sense the putative retrograde factor, because of their relative remoteness. Since glutamate released from these terminals is unaffected by pairing, the *fractional* increase in the amplitude and $1/CV^2$ for the NMDAR-mediated component should be considerably smaller than for the AMPAR-mediated component, in agreement with the results illustrated in Figure 2. The *absolute* increase in quantal content is, however, the same for both components, as is also suggested by the present results.

Selective Potentiation of NMDAR-Mediated EPSCs

So far, the evidence for the spill-over hypothesis is indirect, and changes in the statistic $1/CV^2$ are open to alternative interpretations (Faber and Korn, 1991). We therefore tested an important prediction from this model. Since (we propose) NMDARs sense glutamate released from terminals presynaptic to neighboring cells, preventing the induction of tetanic LTP of AMPAR-mediated EPSCs in a single cell should not prevent potentiation of NMDAR-mediated EPSCs: if LTP is induced at some of the synapses on neighboring cells, then there should be a higher probability of glutamate spill-over from those terminals. We tested this prediction in two different ways.

In the first series of experiments, we simultaneously recorded EPSCs in one cell with a whole-cell pipette, and the population field EPSPs with an extracellular electrode positioned nearby. LTP of the field EPSPs in one pathway was elicited with tetanic stimulation. We prevented conventional LTP induction in the voltage-clamped cell by holding its membrane potential at -80 mV during the tetanization (hyperpolarization of a single cell has previously been shown to prevent tetanic LTP of AMPAR-mediated EPSPs in that cell, but not in neighboring cells; Malinow and Miller, 1986). We then added DNQX and recorded the NMDAR-mediated component of the EPSCs.

Figure 3 shows the results of this experiment in 17 cells: while LTP of the AMPAR-mediated EPSCs was prevented (potentiation $= 1\% \pm 6\%$, $p = 0.870$), the extracellular AMPAR-mediated EPSPs still underwent a large increase ($54\% \pm 5\%$, $p < 10^{-6}$). In contrast with the AMPAR-mediated component, the NMDAR-mediated component of the EPSCs in the tetanized pathway still exhibited a small but significant potentiation: normalizing by the amplitude of the AMPAR-mediated EPSCs before tetanization revealed an increase of $14\% \pm 6\%$ ($p = 0.036$). This argues that a potentiation of NMDAR-mediated signals can still be obtained when induction of tetanic LTP of the AMPAR-mediated component is prevented.

Thus far, all the experiments have relied on normalizing NMDAR-mediated signals by the AMPAR-mediated component prior to the induction procedure. In the second series of experiments, we avoided this requirement by recording the isolated NMDAR-mediated component of the synaptic signal in the continued presence of DNQX. We again asked whether tetanization could elicit LTP of NMDAR-mediated EPSCs, when conventional LTP was prevented by voltage clamping the cell at -80 mV during the tetani. Since we were not able to monitor whether this would indeed have prevented LTP of the AMPAR-mediated component, we applied two further measures to block conventional LTP: first, we waited for 45 min before tetanizing, and second, we included 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA, 5 mM) in the pipette solution to chelate Ca^{2+} ions. Figure 4 shows that in 25 cells, high frequency tetani (2×50 pulses, 100 Hz) again elicited a small potentiation of isolated NMDAR-mediated EPSCs (potentiation at 25 min following tetanus: $11\% \pm 5\%$ relative to control pathway; $p = 0.033$).

The results illustrated in Figures 3 and 4 confirm that

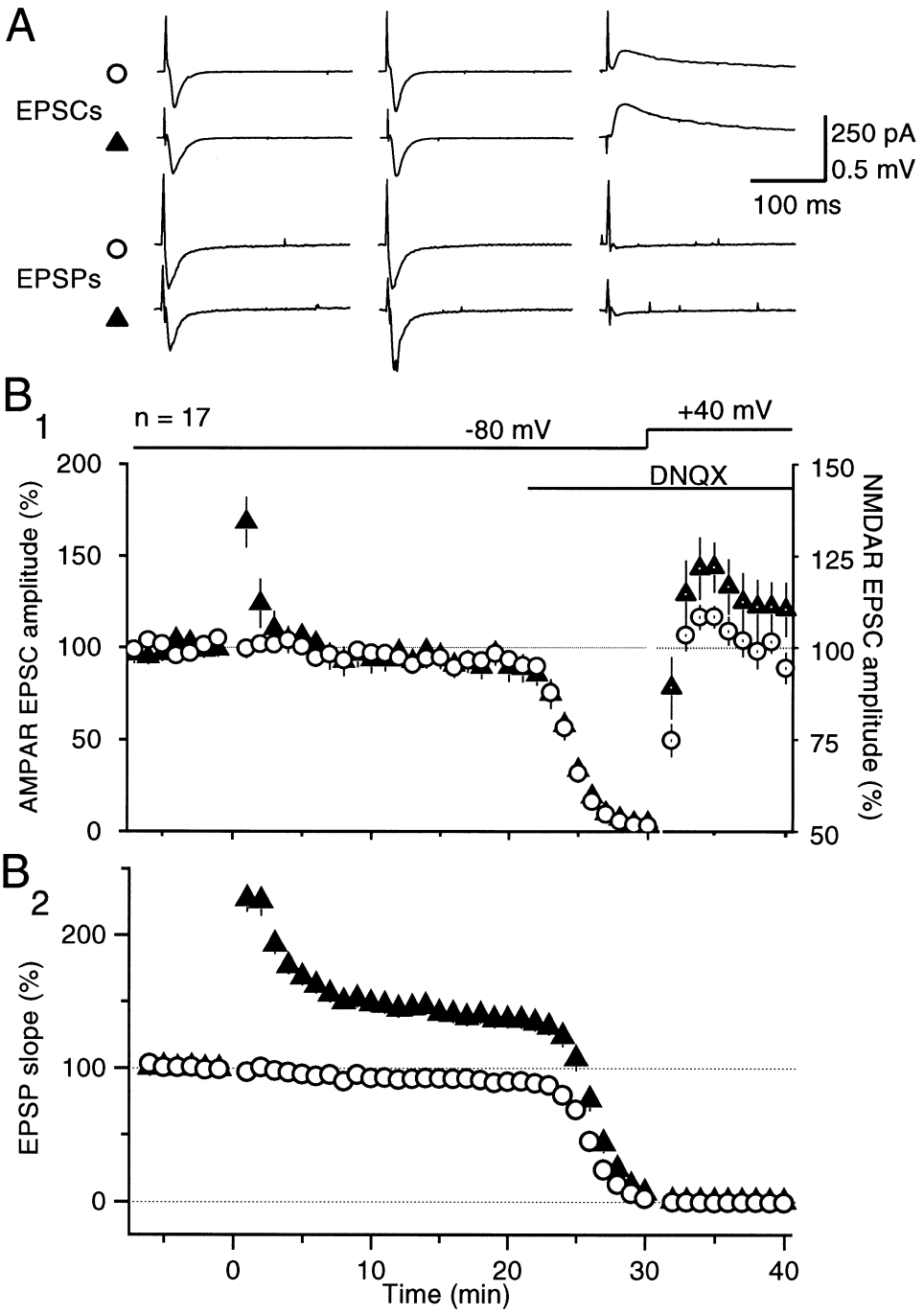

Figure 3. Tetanic LTP of NMDAR-Mediated EPSCs Can Be Elicited when LTP of AMPAR-Mediated EPSCs Is Prevented

(A) Averaged EPSCs (top) and extracellular field EPSPs (bottom) taken from one experiment where the postsynaptic cell was held at −80 mV during high frequency stimulation of the test pathway. The traces were taken from the baseline period (left), after stable LTP had been obtained in the field EPSP (middle) and in the presence of DNQX, while holding the cell at a positive potential (right). Holding the cell at −80 mV during the tetanus prevented LTP of the AMPAR-mediated EPSCs, but not of the field EPSPs. Nor did it prevent a small potentiation of the NMDAR-mediated EPSCs, indicating that LTP of the two components can be dissociated.

(B_1) EPSC amplitudes recorded from 17 cells, which were held at −80 mV during high frequency stimulation at time = 0 (averages of ten successive trials). Filled triangles show the paired pathway EPSCs, and open circles show the control pathway. Symbols with dots show the NMDAR-mediated EPSCs after rescaling to set the control pathway = 100%.

(B_2) Extracellular field EPSPs recorded simultaneously.

While the field EPSPs showed robust LTP, the whole-cell EPSC response B_1 underwent only post-tetanic potentiation. DNQX was added and the holding potential changed at the times indicated. In contrast with the AMPAR-mediated EPSCs, the NMDAR-mediated EPSCs of the tetanized pathway were potentiated ($p = 0.036$).

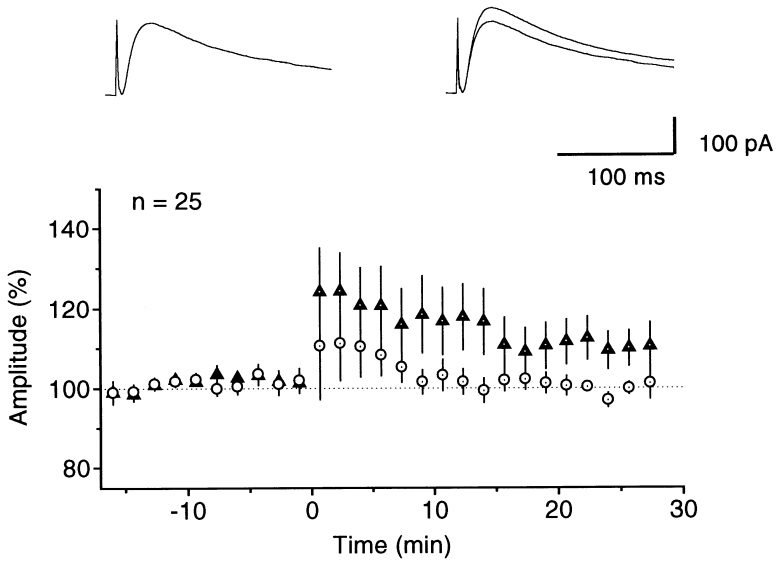

Figure 4. Tetanic LTP of Isolated NMDAR-Mediated EPSCs under Conditions Designed to Prevent LTP of AMPAR-Mediated EPSCs

Average NMDAR-mediated EPSC amplitudes from 25 cells, where tetanic stimulation was given at time 0, under conditions designed to preclude conventional LTP of AMPAR-mediated responses. The cells were held at a positive potential in the presence of DNQX and dialyzed with a BAPTA-containing solution. At time 0 (45 min after entering whole-cell mode), one pathway (filled triangles) was tetanized while holding the cell at −80 mV. A small but significant potentiation was seen ($p = 0.033$). The averaged traces are taken from one experiment before (left) and 25 min after (right) the tetanus.

tetanic LTP of the NMDAR-mediated component can still be induced when LTP of the AMPAR-mediated component is prevented. They argue in favor of the hypothesis that NMDARs of one cell, where LTP is blocked, can sense glutamate release from terminals presynaptic to other cells, at which LTP is elicited.

Enhanced Glutamate Release Revealed by Faster Decay of NMDAR–EPSCs in MK-801

Although our model of LTP expression accounts for the greater potentiation of AMPAR- than NMDAR-mediated signals, there is a major source of evidence against a presynaptic increase in glutamate release with LTP: Manabe and Nicoll (1994) examined the rate at which the use-dependent blocker MK-801 attenuated successive NMDAR-mediated EPSCs and found that prior induction of LTP by pairing had no detectable effect on the half decay time when compared with a control pathway. Since an increased glutamate release probability should cause NMDAR-linked ionophores to open more frequently and therefore be more susceptible to open-channel block, this argues against a presynaptic locus for pairing-induced LTP. One possible explanation for this result, which is still compatible with a presynaptic contribution to LTP, is that the increase in the rate of decay was too small to have been detected. This could have been the case if a large fraction of the NMDAR-mediated signal arose through spill-over of glutamate from neighboring synapses that were not potentiated.

In our hands, low frequency pairing induces only a modest potentiation of the NMDAR-mediated signal (see Figure 2), but it should be possible to increase this by tetanizing presynaptic afferents during the depolarization. This induction procedure, which we term "tetanic pairing," should elicit LTP not only at synapses on the postsynaptic cell, but also at synapses on neighboring cells. We tested two predictions. First, tetanic pairing should give rise to a relatively larger degree of potentiation of the NMDAR-mediated component than either low frequency pairing (see Figure 2) or tetanization while hyperpolarizing the postsynaptic cell (see Figures 3 and 4). This is because NMDARs should sense enhanced glutamate release both from immediately presynaptic terminals and from terminals presynaptic to neighboring cells. Second, tetanic pairing should be accompanied by an increase in the rate of decay of successive NMDAR-mediated EPSCs in the presence of MK-801.

We induced tetanic pairing LTP in 25 cells, by delivering two 100 Hz tetani while holding the cells at 0 mV (Figure 5), within 12 min of entering whole-cell mode. DNQX was subsequently added, and the cells depolarized, in the same way as in Figures 2 and 4, to assess the degree of potentiation of the NMDAR-mediated component. Stimulation was then interrupted while MK-801 (40 μM) was washed in and allowed to equilibrate. After 10 min, stimulation was restarted and the amplitude of successive NMDAR-mediated EPSCs was followed as the NMDAR-linked ionophores were progressively blocked.

The magnitude of the potentiation of the NMDAR-mediated component, prior to the addition of MK-801, was larger than that measured with the alternative induction methods: 21% ± 9% (Figure 5D), compared with 14% ± 4% with low frequency pairing (see Figure 2) and 14% ± 6% with tetanization at −80 mV (see Figure 3). This difference did not, however, reach significance ($p > 0.1$). When expressed as a fraction of the potentiation of the AMPAR-mediated component, the increase in the NMDAR-mediated component was also larger with tetanic pairing than with low frequency pairing (30% and 23%, respectively), but this again was not significant. This aspect of the results thus lends only relatively weak support for the spill-over hypothesis.

MK-801 did, however, cause a significantly faster decay of the NMDAR–EPSCs in the tetanized pathway than in the control pathway (Figure 5B). The test and control pathways in each cell were compared in two independent ways. First, the successive EPSC amplitudes in the control and test pathways, normalized by the first pair of responses in MK-801, were plotted against one another (Manabe and Nicoll, 1994). (The EPSCs in each cell and

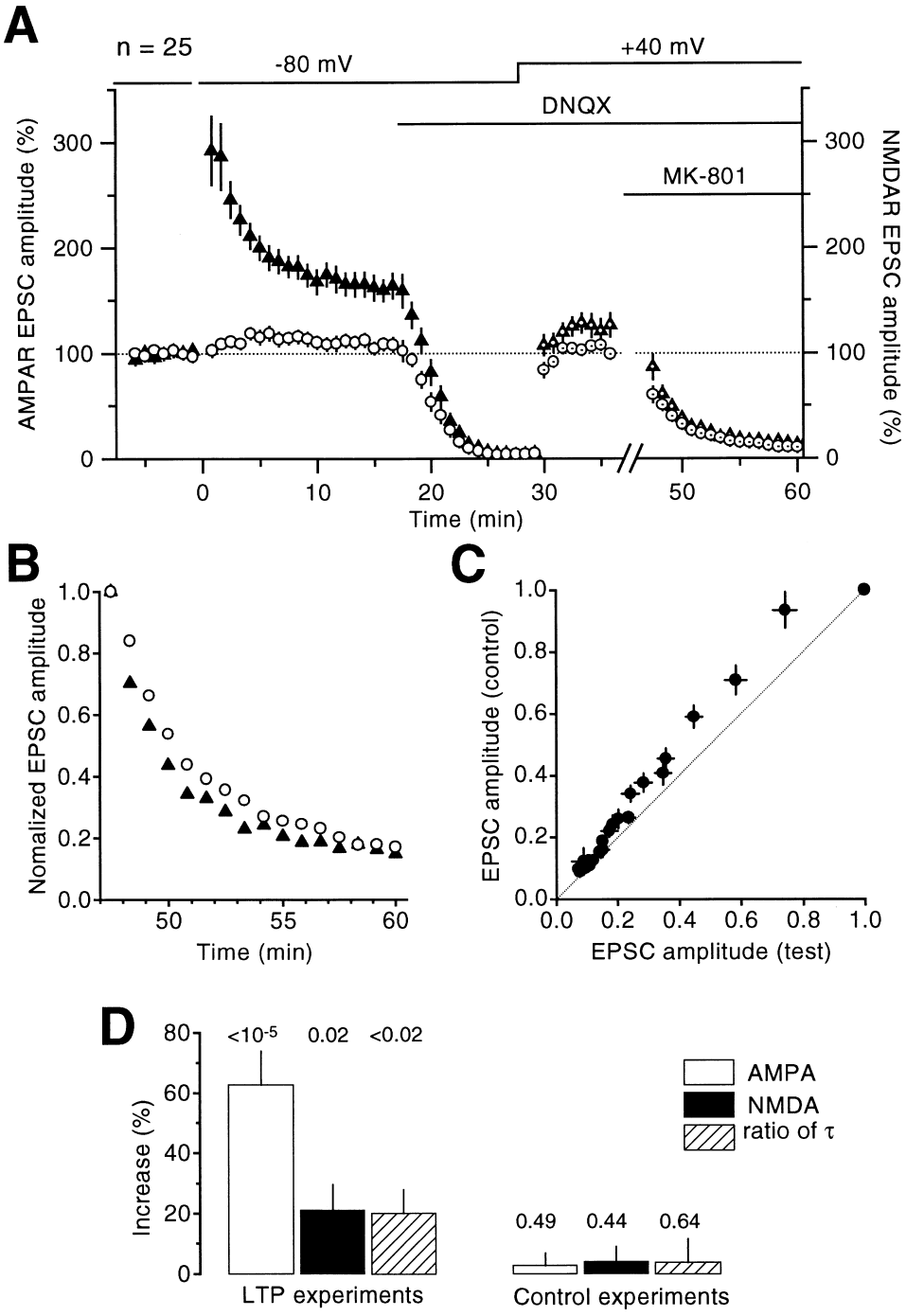

Figure 5. Enhancement of Glutamate Release with LTP Induced by Tetanic Pairing

(A) Average amplitudes of EPSCs in 25 cells (averages of five stimuli). At time = 0, one pathway (filled triangles) was tetanized while the cell was held at 0 mV. Both AMPAR- and NMDAR-mediated EPSCs were potentiated. Stimulation was interrupted for 10 min while MK-801 (40 μM) was washed in.

(B) The mean amplitudes of successive NMDAR–EPSCs recorded in the presence of MK-801 were averaged across all cells and normalized by the first response (averages of five successive stimuli). The decay rate of the tetanized pathway was greater than that of the control pathway. Error bars are not displayed, because decay time constants varied considerably across cells, although they were positively correlated between the two pathways.

(C) The figure plots the amplitude of each average of five successive EPSCs in the control pathway against the corresponding amplitude in the test pathway (±SEM), both normalized by the first point recorded in MK-801. The points lie above the line of identity, indicating that the MK-801-induced decay was more rapid in the test than in the control pathway and implying that individual NMDARs were exposed to glutamate more frequently after LTP than in the control pathway.

(D) Histogram showing the amount of LTP of the AMPAR- and NMDAR-mediated EPSCs, and the difference in time constants fitted to successive NMDAR-mediated EPSCs ([control-test]/test), together with p values for paired *t* tests. At right is a histogram showing results

in each pathway were averaged in groups of five successive trials before the normalization, in order to minimize the error in the estimate of the denominator.) Figure 5C shows that the points lay above the line of identity, indicating a more rapid decay in the test pathway. Second, single exponential decay time constants were fitted to the successive amplitudes measured in MK-801 and compared within each cell (Figure 5D). (The EPSC amplitudes in this case were not averaged into groups of successive trials before fitting.) The decay was faster in the test than in the control pathway ($p < 0.02$, paired *t* test), lending further support to the conclusion that NMDARs open more frequently after LTP induction. An unexpected feature of these results is that most of the difference in the decay rates between the test and control pathways was in the first few trials after restarting stimulation (Figure 5C). We performed a simple numerical simulation of the effect of MK-801 on a population of synapses to investigate this finding. By eliminating synapses whenever they had released transmitter, we found that a trajectory similar to that seen in Figure 5C could be obtained if, first, there was a wide range of initial release probabilities, and second, synapses with low and high initial release probabilities underwent similar fractional increases in probability. In contrast, a selective increase in probability at sites with an initially low probability gave rise to a larger difference later in the sequence of trials.

Both methods of comparison of the decay rate (Figures 5C and 5D) are independent of amplitude scaling, so they do not rely on normalization by the AMPAR-mediated components of the EPSCs. We nevertheless verified in 21 cells that there was no consistent tendency for the amplitude of the NMDAR-mediated component, or the rate of decay in MK-801, to differ between two pathways when no conditioning stimulation was given (Figure 5D). We therefore conclude that the rate of decay of NMDAR-mediated EPSCs in MK-801 was indeed increased by tetanic pairing, implying that glutamate release was potentiated and lending support to a presynaptic contribution to LTP expression.

Discussion

The major findings in the present study are as follows. First, potentiation of NMDAR-mediated signals consistently accompanies both tetanic and pairing-induced LTP of AMPAR-mediated signals, although it is much smaller. Second, the increase in the NMDAR-mediated signal with pairing-induced LTP is accompanied by an increase in $1/CV^2$ suggestive of a small fractional increase in quantal content. Correcting for the larger quantal content of NMDAR- than AMPAR-mediated signals in the baseline, the estimated absolute quantal content increase with LTP is remarkably similar for both components. Third, tetanization potentiates the NMDAR-mediated signal even when conventional LTP or the AMPAR-mediated signal is blocked in an individual cell. Fourth, tetanic LTP is associated with an increase in the rate of decay of the NMDAR-mediated signal in the presence of MK-801, implying an increase in glutamate release. On the basis of these results, we propose that LTP is expressed, at least in part, by an increase in glutamate release probability, and that NMDARs normally sense glutamate release not only from immediately apposed presynaptic release sites, but also from terminals that are presynaptic to neighboring cells (Figure 6).

Although we interpret the increase in $1/CV^2$ for the NMDAR-mediated component as an increase in quantal content, the difference between the test and control pathways was small and required normalization by the baseline $1/CV^2$ for the AMPAR-mediated component to reach significance at $p < 0.05$. $1/CV^2$ is also affected by variability in release probability and quantal amplitude (Faber and Korn, 1991), so on its own the change in $1/CV^2$ cannot be taken as compelling evidence for an increase in transmitter release. For the AMPAR-mediated component of transmission, moreover, the synaptic mechanism underlying the increase in quantal content has undergone some revision, with the proposal that latent clusters of AMPARs become activated (Kullmann, 1994; Liao et al., 1995; Isaac et al., 1995). Could a similar postsynaptic activation of latent clusters of NMDARs explain the present results? It is difficult to see how this could explain the faster decay of NMDAR-mediated EPSCs in MK-801. An alternative explanation might be that the kinetics of NMDARs were altered after the induction of LTP: if their open probability was increased, they would be more susceptible to the blocking action of MK-801. Although a uniform increase in the open probability of NMDARs would predict no change in $1/CV^2$, it is still possible to accommodate an increase in $1/CV^2$: if, under baseline conditions, the open probability at some active sites was lower than at other sites and the NMDAR-mediated quanta consequently variable, then a selective increase in open probability at sites with a low probability could reduce the site-to-site variability in quantal amplitude. Since the overall variance of the synaptic signal is a function both of quantal variability and of stochastic transmitter release, $1/CV^2$ could increase. Clearly, however, this proposal cannot account for the observation that NMDAR-mediated signals can be potentiated by tetani when LTP of AMPAR-mediated signals is blocked, unless LTP of NMDAR-mediated signals is mediated by completely different induction and expression mechanisms than LTP of AMPAR-mediated signals. If potentiation of the two components does share mechanisms, therefore, an exclusively postsynaptic site of expression is very difficult to reconcile with the present results.

The spill-over model provides a novel explanation for the observation that NMDAR-mediated EPSCs can be recorded with minimal stimuli when no AMPAR-mediated EPSCs are seen (Liao et al, 1995; Isaac et al., 1995;

from 21 cells where no conditioning stimulation was given, but AMPAR- and NMDAR-mediated EPSCs and the effect of MK-801 were otherwise measured in an identical fashion: there was no significant difference in either the amplitude of the two components or in the decay time constants.

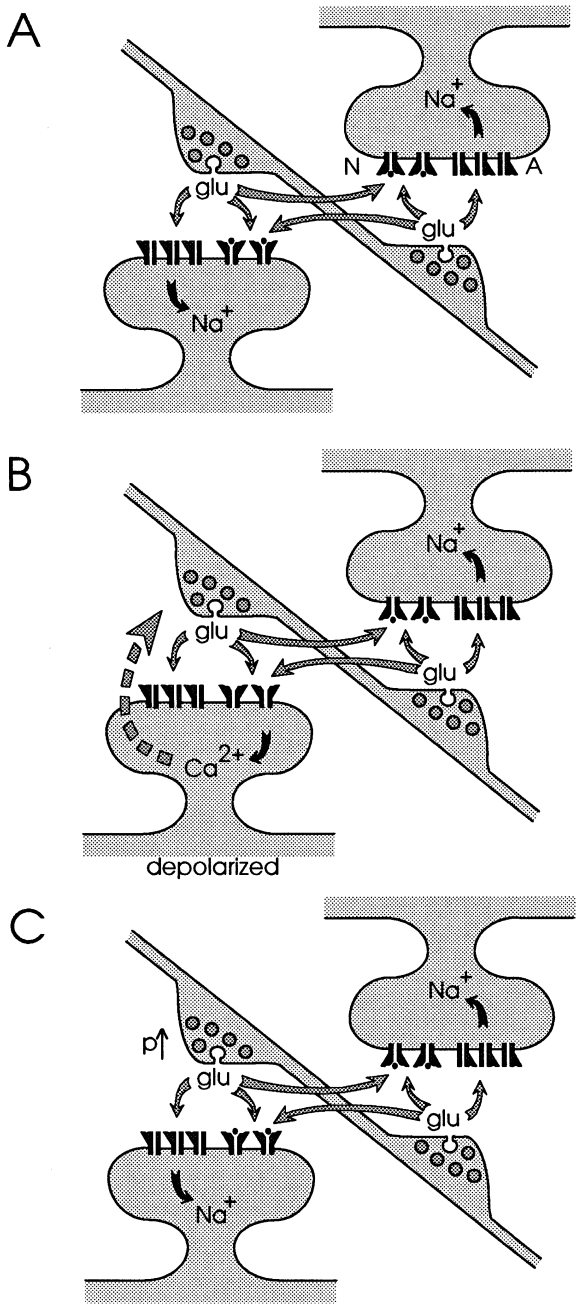

Figure 6. The Glutamate Spill-Over Hypothesis and Presynaptic Expression of LTP

(A) Two dendritic spines on neighboring pyramidal cells are illustrated, supplied by terminals from the same afferent fiber (the synapses could equally be supplied by different afferents). The extracellular concentration of glutamate, stochastically released from each terminal, transiently reaches $\sim 10^{-3}$ M in the synaptic cleft, activating both postsynaptic AMPARs (A) and NMDARs (N) on the postsynaptic cell. Outside the synaptic cleft, the transmitter may reach 10^{-5} M. This is sufficient to activate NMDARs, but not AMPARs, of a neighboring synapse made on a different pyramidal cell. Stochastic release from a population of terminals, only some of which are presynaptic to a given cell, will give rise to EPSCs in that cell with a larger quantal content for the NMDAR- than the AMPAR-mediated component. This explains the discrepancy between $1/CV^2$ for the two components. If there are no active release sites on the recorded cell, spill-over of glutamate from neighboring synapses will be sensed as pure NMDAR-mediated EPSCs.

(B) LTP is induced when activation of NMDARs coincides with postsynaptic depolarization. This generates a retrograde factor (broken arrow), which only reaches the immediately presynaptic terminal, not more distant synapses.

(C) LTP is expressed by an increase in release probability (there may be an additional postsynaptic change in AMPAR properties, which is not illustrated here). LTP can be generated at a terminal whose initial release probability is 0, if the postsynaptic NMDARs are liganded by glutamate released from a terminal presynaptic to a neighboring cell, and if this coincides with postsynaptic depolarization. This could explain the apparent activation of functional AMPAR clusters at "silent synapses".

Durand et al., 1996). Rather than reflecting absence of AMPARs at some synapses, this arises when glutamate is released from a sparse population of active terminals, none of which is in direct synaptic contact with the recorded cell. Glutamate spill-over generates NMDAR–EPSCs, while AMPARs fail to sense the transmitter as a result of their lower affinity. If this explanation is correct, it further implies that activation of NMDARs of neighboring cells by spill-over can occur for individual quantal release events and is not an artifact of synchronous activation of many presynaptic terminals with multifiber stimuli, causing overlapping "domains" of glutamate to reach a sufficient concentration to activate NMDARs.

Both Liao et al. (1995) and Isaac et al. (1995) showed that pairing postsynaptic depolarization with presynaptic stimuli that initially give rise only to NMDAR-mediated EPSCs can cause AMPAR-mediated EPSCs to appear. This was taken as strong evidence in favor of the latent AMPAR cluster hypothesis, but could the same observation be explained by glutamate spill-over and a presynaptic locus of expression? LTP could be induced at a synapse where the initial release probability is 0 (a "presynaptically silent" synapse) if the NMDARs postsynaptic to the 0 probability terminal are liganded by glutamate spilling over from a nearby terminal, which is presynaptic to a neighboring cell. If this is paired with postsynaptic depolarization, Ca^{2+} influx through the NMDARs will occur, and a necessary condition for LTP induction will therefore be satisfied. This will allow the putative retrograde messenger, be it a diffusible messenger or a more direct mechanical linkage, to trigger an increase in presynaptic release probability. For this explanation to hold, there are some additional requirements. First, the distance between the "donor" terminal (from which glutamate is released) and the "target" synapse must be small (see below). And second, in order to preserve the specificity of LTP, the presynaptic increase in release probability must only occur at target terminals that belong to the paired pathway, since otherwise there will simply be a diffuse potentiation of all the synapses on the postsynaptic cell that are in the vicinity of the donor terminal. If there is a sufficiently high density of terminals with 0 release probability, then the first requirement could be satisfied, since this will allow a silent synapse to occur in the vicinity of a donor terminal in a substantial proportion of cases. As for the maintenance of specificity, this would be assured if the putative

retrograde messenger only triggers an increase in release probability if there has been an action potential in the presynaptic terminal. There is conflicting evidence on whether presynaptic activity is an essential requirement for the induction of LTP: Cormier et al. (1993), Kamiya et al. (1993) and Neveu and Zucker (1996) have demonstrated long-term plasticity with postsynaptic manipulations, which apparently shares mechanisms with conventional LTP. Kullmann et al. (1992), Zhuo et al. (1993), and Arancio et al. (1995), on the other hand, have argued that presynaptic activity is necessary.

An alternative proposal could obviate the need for any additional requirements to reconcile LTP at a presynaptically silent synapse with the specificity of LTP: multiple release sites can occur in very close proximity on large boutons that make synaptic contacts on different postsynaptic cells (Sorra and Harris, 1993). Spill-over from one release site onto NMDARs postsynaptic to another site could allow the release probability at the latter site to increase from 0, as long as activity of the bouton is paired with depolarization postsynaptic to the silent site.

There are several other observations that are explained by the spill-over hypothesis. First, both Asztely et al. (1992) and Isaac et al. (1995) described simultaneous measurements of the early and late parts of dual-component postsynaptic signals in CA1, designed to estimate the relative sizes of the AMPAR- and NMDAR-mediated components. Both groups showed that the two components initially scale linearly as the stimulus strength is increased, but at high stimulus intensities the late part of the synaptic signal increases less than the early part, implying that the NMDAR-mediated component scales sublinearly with the AMPAR-mediated component. This may reflect mutual occlusion of domains of glutamate released from neighboring active terminals, saturating the NMDARs in their vicinity and preventing them from sensing the recruitment of further release sites. This does not occur for the AMPAR-mediated signal because of the lower affinity of these receptors.

Second, Hestrin et al. (1990) reported that the noncompetitive glutamate uptake blocker dihydrokainate could selectively increase the NMDAR-mediated component of the postsynaptic signal, with no effect on AMPAR-mediated EPSCs. This is compatible with an extrasynaptic action of glutamate at NMDARs, at least in the presence of dihydrokainate: if glutamate only acted on synaptic receptors, reducing its clearance would be expected to have a greater effect on the AMPAR- than the NMDAR-mediated component of the EPSCs, since AMPARs have a lower affinity for the transmitter. It also argues for a critical role of glutamate uptake in limiting the extent of glutamate spill-over. Inconsistent results have been obtained with the competitive uptake blocker L-trans-pyrrolidine-2,4-dicarboxylate (Sarantis et al., 1993; Isaacson and Nicoll, 1993). This may be because a competitive uptake blocker, in contrast with a nonsaturating concentration of a noncompetitive uptake blocker, may shift the background extracellular glutamate concentration to a higher level, giving rise to a greater degree of steady-state desensitization of glutamate receptors.

Third, taken together with the proposal that LTP is expressed in part presynaptically, the spill-over hypothesis reconciles the relatively greater potentiation of AMPAR- than NMDAR-mediated signals with the evidence for an increase in quantal content (Kullmann and Siegelbaum, 1995). Although the change in $1/CV^2$ that we observed suggested an additional increase in quantal amplitude (Figure 2E), Stevens and Wang (1994) and Bolshakov and Siegelbaum (1995) saw no change in the amplitude of EPSCs with LTP, and only observed a decrease in transmission failure rate. As was mentioned in the Introduction, this is difficult to explain on the basis of activation of latent clusters of AMPARs, since larger events should have been observed on occasion. A genuine increase in transmitter release probability, on the other hand, explains these observations. Why Stevens and Wang (1994) and Bolshakov and Siegelbaum (1995) saw no increase in quantal amplitude is not clear, since this has been reported by several groups who applied similar or complementary techniques (or both) (Manabe et al., 1992; Foster and McNaughton, 1991; Kullmann and Nicoll, 1992; Larkman et al., 1992; Liao et al., 1992; Oliet et al., 1996; Stricker et al., 1996; also see Voronin, 1993).

Fourth, the present model is also compatible with the observation that synaptic glutamate release can be sensed with NMDARs in an outside-out membrane patch positioned in a hippocampal slice and that this is potentiated by LTP induction (O'Connor et al., 1995; but see Isaacson and Nicoll, 1993).

Although a larger potentiation of the NMDAR-mediated component was seen when tetanic stimulation was combined with postsynaptic depolarization (tetanic pairing), there was still a large discrepancy between the increase in the AMPAR- and NMDAR-mediated signals: the potentiation of the NMDAR-mediated component was only 30% as large as that of the AMPAR-mediated component. A plausible explanation for this difference is that tetanic LTP is not elicited at all terminals from which glutamate release is sensed by the NMDARs on the recorded cell. Some of the active synapses, for instance, may be on neighboring cells that are not sufficiently depolarized during the tetanus for LTP to be generated. As a result, the potentiation of the NMDAR-mediated component is generally smaller than that of the AMPAR-mediated component. This could account for the fact that it was not reported in several studies (Kauer et al., 1988; Muller and Lynch, 1988; Muller et al., 1988), and is consistent with the observation by Anikszteyn and Ben-Ari (1995) that potentiation of the NMDAR-mediated component can be seen with strong, but not weak, tetani.

Other studies have reported tetanic LTP of the NMDAR-mediated component, which, relative to the potentiation of the AMPAR-mediated component, was similar to that seen in the present study (Asztély et al., 1992) or even larger (Clark and Collingridge, 1995). A possible explanation for these inconsistencies is that the degree of synaptic cross-talk witnessed by NMDARs varies between preparations. A suggestion that this is so comes from a comparison of the present results with those of Selig et al. (1995): although tetanic LTP was associated with a small potentiation of the NMDAR-mediated component, Selig et al. reported no change with pairing-induced LTP. The ratio of $1/CV^2$ for the NMDAR- and

AMPAR-mediated components was, however, over 3-fold, implying that there may have been a larger degree of extrasynaptic spill-over, on average, in the experiments of Selig et al. (1995) than in the present study, where the ratio was only 2.1. With a greater degree of spill-over, we predict that the fractional increase in the NMDAR-mediated component with pairing should be smaller, possibly explaining why it was not observed by Selig et al. (1995).

This explanation may also provide the clue to the observation by Manabe and Nicoll (1994) that pairing-induced LTP was not associated with a detectable increase in the rate of decay of NMDAR-mediated EPSCs in MK-801. Although we have not repeated the experiments with pairing, we predict that there should be a small difference in the decay rates, since some of the NMDAR-mediated signal did increase with this manipulation in the present study. Manabe and Nicoll (1994) did not, however, state whether LTP in their experiments was associated with a significant change in the NMDAR-mediated component.

The ratio in $1/CV^2$ for the two components in the present study implies a similar ratio of quantal contents sampled by NMDARs and AMPARs as does a comparison of transmission failure rates with minimal stimulation (Liao et al., 1995): since the simple binomial model of transmission predicts that $1/CV^2$ varies linearly with the number of release sites, we estimate that NMDARs sample just over twice as many release sites as do AMPARs. This is in rough agreement with the estimate by Liao et al. (1995) that roughly 60% of release events are sampled exclusively by NMDARs. How does this estimate compare with what is known of the diffusional limits of glutamate in the hippocampus? Neighboring excitatory synapses with different pre- and postsynaptic elements are frequently found within $\sim$1 μm of one another in rodent CA1 (for example, see Figure 6 in Sorra and Harris, 1993). This is within the distance that glutamate released from a single vesicle could diffuse and activate NMDARs, but not AMPARs, given some assumptions about vesicle contents, and ignoring obstacles to diffusion and glutamate uptake (Wahl et al., 1995). The spill-over model is thus compatible with current knowledge of hippocampal ultrastructure and extracellular diffusion of glutamate, although there are many critical parameters that are not known with sufficient precision to test the hypothesis quantitatively: among these are the extracellular effective diffusion coefficient of glutamate, the actual contents of a single vesicle, and the density and turn-over rate of glutamate uptake pumps. In this rough analysis, moreover, we have assumed that only synaptic NMDARs respond to glutamate spill-over from neighboring synapses, whereas extrasynaptic receptors may also play a critical role.

The proposal that NMDARs sense glutamate released from distant synapses calls for a novel view of glutamate as acting both locally at AMPARs and nonlocally at NMDARs. It also has the possibly wide-ranging consequence that a cell samples the activity of a larger number of terminals when depolarized than at its resting potential. This could have extensive repercussions for information processing if it occurs in vivo.

Experimental Procedures

Hippocampal slices 450 μm thick were prepared from 4–5-week-old guinea pigs and stored in a solution containing the following: 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, and 11 mM glucose (bubbled with 95% O_2/5% CO_2). All the recordings were made in a submerged slice chamber perfused with this solution, with added picrotoxin (100 μM), at 20°C–24°C. CA3 was cut away to prevent epileptiform bursting from spreading to CA1. Stratum radiatum fibers were stimulated with bipolar stainless steel electrodes positioned on either side of the recording pipette, and both of these were located at approximately the same distance from stratum pyramidale. Extracellular field EPSPs were recorded via a glass pipette containing 3 M NaCl. Whole-cell recording pipettes were filled with the following: 117.5 mM Cs gluconate, 17.5 mM CsCl, 10 mM HEPES, 0.2 mM EGTA, 8 mM NaCl, 2 mM MgATP, 0.3 mM GTP, and 5 mM QX-314 Br (pH 7.2) (295 mOsm). The series resistance was continuously monitored by delivering a voltage step command, was less than 16 MΩ, and changed by less than 20% in the cells that were accepted for analysis. Junctional potentials were not corrected. Recordings were made with Axopatch 1D amplifiers (Axon Instruments, Foster City, CA), filtered at 1 kHz, and sampled at 2 or 4 kHz. The trials were stored on computer disk for off-line analysis. The initial slope of the EPSPs was measured over a 2 ms period for AMPAR-mediated component and a 4 ms period for the NMDAR-mediated component. EPSC amplitudes were measured over a 7–10 ms period coinciding with the peak of the average time course. This was usually about 10 ms later for the NMDAR- than the AMPAR-mediated component. For the measurement of trial-to-trial EPSC amplitude variance (Var_{EPSC}), identical measurements were also made from interleaved traces where no stimuli were delivered, in order to estimate the variance of the background noise (Var_{noise}). $1/CV^2$ was estimated for successive epochs of 25 trials as $(\text{mean EPSC})^2/(Var_{EPSC} - Var_{noise})$.

In the field potential experiments of Figure 1, we took care to avoid overlap between the three pathways by verifying that there was no cross-facilitation. The tetanized and control pathway electrodes were usually positioned to one side of the recording electrode, and the paired pathway on the other side. The stimulus intensity in the paired and control pathways were adjusted to make baseline EPSP slopes as similar as possible. The baseline EPSP slope in the tetanized pathway was, however, up to 75% larger, in order to provide sufficient depolarization during the tetanus to induce LTP in the paired pathway. In the rest of the study, the stimulus intensities were adjusted to give baseline AMPAR-mediated EPSC amplitudes, which were similar in the test and control pathways. The test stimulus frequencies in the different experiments were 0.2 Hz (Figure 1), 0.33 Hz (Figure 2), 0.1667 Hz (Figures 4 and 5), and 0.1 Hz (Figure 6). LTP was elicited in the experiments illustrated in Figure 1 by pairing brief tetani (five impulses at 100 Hz, repeated 50 times at 0.5 Hz, at control intensity) in one pathway, with single pulses in another pathway. The single pulses coincided with the start of each tetanus. In Figure 2, LTP was elicited by pairing 120 pulses at 2 Hz with depolarization to 0 mV, and was carried out within 12 min of breaking into whole-cell mode. In Figure 4, the conditioning consisted of tetanization (100 Hz, 1 s, repeated once after 20 s), while holding the cells at -80 mV. In Figure 5, the tetani were briefer (0.5 s) and were delivered only after a minimum of 45 min had elapsed from breaking into whole-cell mode. In Figure 6, tetanic pairing consisted of two 100 Hz, 1 s tetani while holding the cell at 0 mV. This was again delivered within 12 min of breaking in.

Throughout the study, all experiments were included in the analysis, whether LTP was obtained or not. To display the effects of conditioning stimuli, NMDAR-mediated EPSP/Cs after conditioning (E_{NMDA}) were normalized by dividing by the baseline amplitude of the AMPAR-mediated component in the same pathway, averaged over at least 5 min (BL_{AMPA}): $E^*_{NMDA} = E_{NMDA}/BL_{AMPA} \cdot E^*_{NMDA}$ in the control pathway (c) was then rescaled to 100%, and this scaling factor was applied to the test pathway(s) (t) to estimate the potentiation of the NMDAR-mediated signal. This is equivalent to $(E^*_{NMDA(t)}/E^*_{NMDA(c)} - 1) \times 100\%$. To control for minor drifts in recording conditions, the same procedure was applied to the AMPAR-mediated component after the conditioning procedure (E_{AMPA}): $E^*_{AMPA} = E_{AMPA}/BL_{AMPA}$; potentiation of the AMPAR-mediated component was given by the following: $(E^*_{AMPA(t)}/E^*_{AMPA(c)} - 1) \times 100\%$.

The normalization procedure relies on the assumption that the ratio of NMDAR- to AMPAR-mediated components is the same in different pathways impinging on a given cell (Selig et al., 1995). We tested this in 20 cells by comparing the NMDAR/AMPAR ratio in two pathways, neither of which was conditioned. The ratio in the second pathway was 99% ± 4% of that in the first pathway.

To estimate the rate of decay of NMDAR-mediated EPSCs in the presence of MK-801 (Figure 6), a single exponential time constant with zero asymptote was fitted to all the EPSCs recorded in the presence of the blocker (Marquardt-Levenburg algorithm). This was less sensitive to sampling error than estimating decay half-times (Manabe and Nicoll, 1994). Although a double exponential gave a better fit (Rosenmund et al., 1993; Hessler et al., 1993), the additional parameters prevented a simple comparison of the test and control pathways.

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