Neuron, Vol. 15, 427–434, August, 1995, Copyright © 1995 by Cell Press

Evidence for Silent Synapses: Implications for the Expression of LTP

John T. R. Isaac,* Roger A. Nicoll,†‡ and Robert C. Malenka*†
*Department of Psychiatry
Center for Neurobiology and Psychiatry
†Department of Physiology
‡Department of Cellular and Molecular Pharmacology
University of California, San Francisco
San Francisco, California 94143

Summary

Recent work has suggested that some proportion of excitatory synapses on hippocampal CA1 pyramidal cells that express NMDA receptors (NMDARs) may not express functional AMPA receptors (AMPARs), thus making these synapses silent at the resting membrane potential. In agreement with this hypothesis, we demonstrate here that it is possible to stimulate synapses that yield no detectable excitatory postsynaptic currents (EPSCs) when the cell is held at −60 mV; yet at positive holding potentials (+30 to +60 mV), EPSCs can be elicited that are completely blocked by the NMDAR antagonist, D-APV. When these functionally silent synapses are subjected to an LTP induction protocol, EPSCs mediated by AMPARs appear and remain for the duration of the experiment. This conversion of silent synapses to functional synapses is blocked by D-APV. These results suggest that LTP may involve modification of AMPARs that, prior to LTP, were either not present in the postsynaptic membrane or electrophysiologically silent. This mechanism may account for several experimental results previously attributed to presynaptic changes in quantal content.

Introduction

Whether the site of expression for long-term potentiation (LTP) at the Schaffer collateral–CA1 pyramidal cell synapse is primarily pre- or postsynaptic has been the subject of intense investigation and much debate. Many different experimental approaches have been taken to address this question, each with its own advantages, limitations, and assumptions. These include (but are not limited to): measurement of glutamate concentrations in perfusate from control and potentiated tissue (Bliss et al., 1986; Bliss and Collingridge, 1993); comparison of the changes in synaptic responses mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropimate (AMPA) receptors (AMPARs) with those mediated by N-methyl-D-aspartate (NMDA) receptors (NMDARs; Kauer et al., 1988; Muller and Lynch, 1988; Asztely et al., 1992; Perkel and Nicoll, 1993; Clark and Collingridge, 1995; O'Connor et al., 1995); calculation of changes in the coefficient of variation (CV) of synaptic responses during LTP (Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Kamiya et al., 1991; Manabe et al., 1993; Kullmann, 1994); formal quantal analysis of LTP in which various statistical methods were used to define integral "peaks" in amplitude distributions of synaptic responses (Malinow and Tsien, 1990; Foster and McNaughton, 1991; Kullmann and Nicoll, 1992; Larkman et al., 1992; Liao et al., 1992); examination of LTP-induced changes in the amplitude and frequency of miniature synaptic currents (Malgaroli and Tsien, 1992; Manabe et al., 1992); measurement of responses to exogenous application of AMPA to monitor changes in postsynaptic receptor sensitivity (Davies et al., 1989; Wyllie et al., 1994); and monitoring of so-called failures of synaptic transmission in response to minimal stimulation of single or small numbers of synapses (Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Kullmann and Nicoll, 1992; Stevens and Wang, 1994).

The reasons for the lack of resolution to this ongoing debate despite this major effort can generally be attributed to two factors. First, for some experiments there is a lack of consensus about the results themselves. This problem is exemplified by the disagreement concerning the apparently simple question of whether NMDAR- and AMPAR-mediated synaptic responses increase in parallel during LTP, or whether LTP occurs primarily on AMPAR responses (the second approach above). Second, even when results are agreed upon, different conclusions are reached by different groups, perhaps because assumptions concerning the basic processes of synaptic transmission in the hippocampus remain untested.

One important result that appears to be reliable from laboratory to laboratory is the ability to identify failures of synaptic transmission using minimal stimulation, and that LTP is accompanied by a decrease in the relative proportion of these failures. Failures have classically been attributed entirely to the probabilistic nature of transmitter release, resulting in a failure of transmitter release when an action potential invades the presynaptic terminal. Thus, the change in the percentage of failures that occurs with LTP has been most easily explained by a net increase in the probability of neurotransmitter release during LTP. However, several investigators have pointed out that, formally, this result could be explained if, prior to LTP, there existed so-called silent synapses that do not contain functional or electrophysiologically detectable AMPARs, and thus cannot yield a response when transmitter release does occur. The induction of LTP could then conceivably cause the uncovering or insertion of clusters of AMPARs at these functionally silent sites, and this would appear as a change in the frequency of failures (Edwards, 1991; Kullmann and Nicoll, 1992; Liao et al., 1992; Lisman and Harris, 1993; Manabe et al., 1993). Accepting that LTP is normally synapse specific, this hypothesis would also require the existence of synapses that contain only functional NMDARs.

Recently, experiments examining the CV, or the related statistical measure $1/CV^2$, of AMPAR- and NMDAR-

mediated EPSCs (AMPAR EPSCs and NMDAR EPSCs, respectively) have provided indirect evidence in support of this idea (Kullmann, 1994; Selig et al., 1995 [this issue of *Neuron*). It was found that the basal CV of AMPAR EPSCs was consistently larger than that of NMDAR EPSCs (Kullmann, 1994), a result suggesting that more synapses contain NMDARs than AMPARs. Moreover, following LTP (Kullmann, 1994; Selig et al., 1995) or long-term depression (LTD; Selig et al., 1995), there was a significant change in the CV or $1/CV^2$ of AMPAR EPSCs, but no change in the CV or $1/CV^2$ of NMDAR EPSCs. These results are difficult to reconcile with a presynaptic locus of expression for LTP (or LTD) and provided strong impetus for designing experiments that would permit more direct tests of the existence of functionally silent synapses and their conversion to "responsive" synapses following LTP.

In the present study, we provide evidence that a proportion of synapses on CA1 pyramidal cells contain only NMDARs and no detectable AMPARs. Furthermore, induction of LTP at these synapses results in the rapid appearance of AMPAR EPSCs. These results suggest that LTP is caused, at least in part, by the modification of AMPARs at synapses that previously were functionally silent and appeared to contain only NMDARs. This postsynaptic mechanism for changing the number of functional synapses during LTP can unify many of the seemingly disparate results in the field.

Results

In an initial set of experiments, we used an experimental protocol that allowed us to search for synapses that contained NMDARs but no detectable AMPARs. We reasoned that, if such synapses existed as some reasonable proportion of the total number of synapses, it should be possible on occasion to stimulate them selectively using very low stimulation strengths. If successful, our prediction was that we should record no evoked synaptic currents while holding the cell at negative membrane potentials, because of the strong voltage dependence of the NMDAR; but when the cell was depolarized to positive potentials, some evoked synaptic currents should be observed, and these should be completely blocked by the NMDAR antagonist D-aminophosphonovalerate (D-APV).

Our standard experimental protocol was to stimulate afferents initially at an intensity sufficient to evoke a clear AMPAR EPSC (at −60 mV), and then to decrease stimulation strength to a point at which a minimum of 100 consecutive stimuli elicited no detectable EPSC (Figure 1). To avoid any subjective judgments or assumptions with regard to distinguishing individual failures from responses in each trace, especially when examining NMDAR EPSCs, we averaged these consecutive traces and both measured and visually inspected the average. We examined only cells (n = 42) in which the averaged trace exhibited no deflection different from that seen when 10 μM 6-cyano-7-dinitroquinoxaline-2,3-dione (CNQX) was present in the perfusate, confirming that the stimuli failed to evoke a response. To confirm further that stimulation elicited no detectable EPSCs, at the end of each experiment in which NMDAR EPSCs were detected, the baseline data were represented as a histogram, and cells were included only if the number of negative values did not exceed the number of positive values (see Experimental Procedures).

At this point in the experiment, two possibilities existed: the stimulus strength had been decreased to such an extent that no synapses were being activated (assuming that probability of release at activated synapses was at least >0.01); or we were activating synapses with only NMDARs,

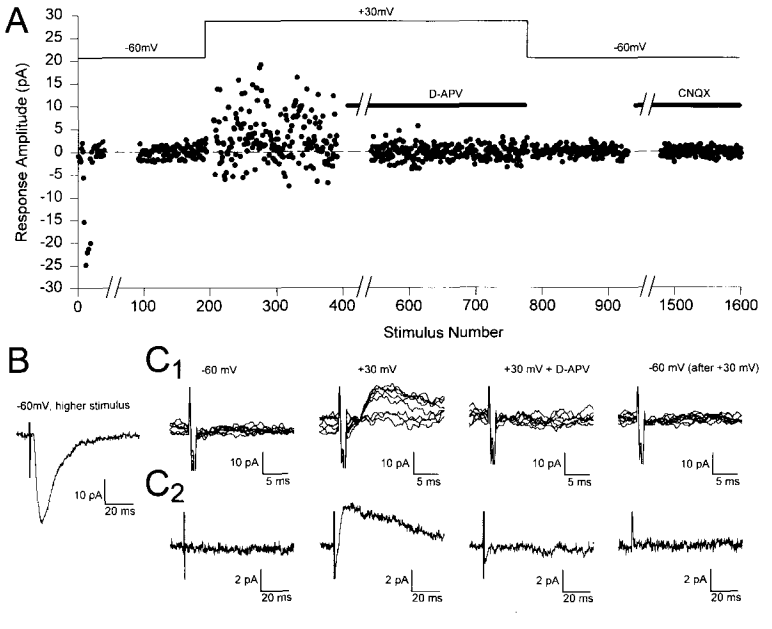

Figure 1. Example of an Experiment Demonstrating the Existence of Silent Synapses

(A) Graph of an experiment to illustrate the standard experimental protocol. The cell was held at −60 mV, and after obtaining a small EPSC, stimulus intensity was decreased so that no EPSCs were detected for 100 consecutive stimuli. The cell was then depolarized to +30 mV, and stimulation now evoked responses that were completely blocked by the application of D-APV (25 μM), indicating that they were mediated by NMDARs. The cell was then returned to −60 mV, where again no EPSCs could be detected, as evidenced by the lack of effect of CNQX (10 μM), which was applied at the end of this experiment.

(B) Sample of the EPSC (average of 10 responses) recorded at the beginning of the experiment.

(C) Examples of 8 consecutive traces (C_1) or the average of 100 consecutive responses (C_2; including the traces in C_1) taken at the indicated times during the course of the experiment. For all experiments in this study, once stimulation was commenced, it was maintained at the same rate throughout the entire experiment.

so the released glutamate failed to activate AMPARs. To test this possibility, we checked for the occurrence of NMDAR EPSCs by depolarizing the cell to positive potentials (+30 to +60 mV), while continuing afferent stimulation at the same rate. The likelihood of inducing LTP during the depolarization was minimized because cells were dialyzed by maintaining the whole-cell recording configuration for an average of 20 min before the depolarization (Malinow and Tsien, 1990). Furthermore, holding cells at positive membrane potentials reduces the driving force for Ca^{2+} entry, which also decreases the probability of inducing LTP (Malenka et al., 1988; Perkel et al., 1993). In ~60% of cells (25 of 42), we still could not detect any responses in either the individual traces or averages of 100 consecutive sweeps. However, in ~40% of cells (17 of 42), EPSCs could clearly be distinguished in some of the individual traces and always in the averaged trace (Figure 1C). These responses exhibited the slow time course characteristic of NMDAR EPSCs and were completely blocked by D-APV (Figures 1A and 1C).

Figure 2 shows a summary of the experiments in which NMDAR EPSCs were evoked by afferent stimulation that elicited no detectable AMPAR EPSCs. In this graph, the mean amplitude of each group of 25 consecutive sweeps is plotted as a function of time during the course of the experiment. Two observations make it unlikely that during the course of this experiment, while the cell was being held at positive membrane potentials, the stimulation intensity or position of the electrode had drifted, such that additional synapses containing detectable AMPARs were being activated. First, D-APV completely blocked the response, leaving no trace of an EPSC at the positive holding potential. Second, after applying D-APV we returned the holding potential to −60 mV (see Figure 1) and failed to detect AMPAR EPSCs in individual or averaged traces (n = 10). Furthermore, in 2 additional cells, after confirming the existence of NMDAR EPSCs, we held the cell at −90 mV to increase the driving force for AMPAR EPSCs and again were unable to detect any currents. Finally, CNQX (10 μM) was applied at the end of experiments (n = 7 of the 10 experiments), and we did not observe any significant difference in the individual or averaged traces before and after depolarizing the cells, except for the disappearance of some spontaneous EPSCs. Thus, we were able to confirm at the end of this experiment that the synapses responsible for the observed NMDAR EPSCs at positive holding potentials did not contain detectable AMPARs. (In an additional 7 cells, the same results were obtained, except after blocking the NMDAR EPSCs at positive potentials with D-APV, the cells were not returned to −60 mV. Thus, these cells are not included in Figure 2.)

In the previous experiments, ~60% of cells exhibited no detectable responses at positive holding potentials. If, as suggested by the previous data, a proportion of synapses contain only NMDARs and no functional AMPARs, in some cases it would be expected that small increases in stimulus strength would preferentially recruit these synapses. Thus, the relative contribution of NMDARs and AMPARs to evoked EPSCs would change with increasing

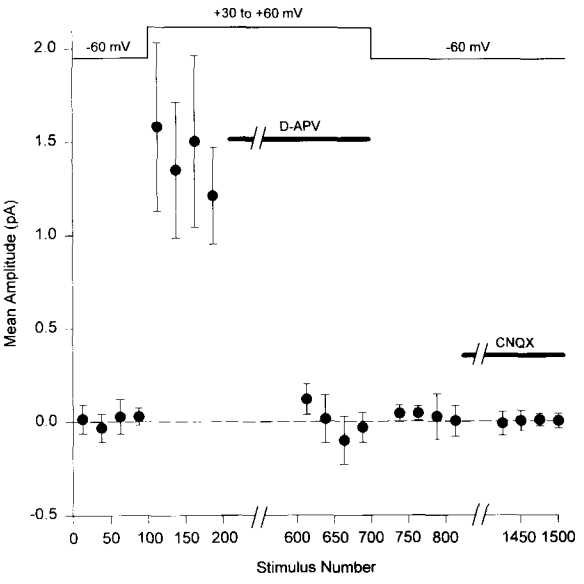

Figure 2. Summary of Experiments like That Illustrated in Figure 1

Each point represents the mean of 25 consecutive traces (n = 10 experiments). At −60 mV, there were no detectable responses. When the cell was depolarized to +30 to +60 mV, clear EPSCs could be detected, and these were completely blocked by D-APV (25 μM). At the end of these experiments, the cell was returned to −60 mV (n = 10), and after collecting 100 additional responses, CNQX (10 μM) was applied (n = 7). It can be seen that the mean values for the data collected in CNQX were not different from those recorded in its absence at the beginning of the experiment.

stimulus strength. To test this prediction, we again held cells at −60 mV and reduced stimulation strength to a level at which no EPSCs were observed for at least 100 consecutive stimuli. Cells were then depolarized to +30 mV, another 50 stimuli were given, and then stimulation strength was gradually increased in small increments. EPSC amplitudes were measured at early ($EPSC_E$) and late ($EPSC_L$) time points that predominantly reflect AMPAR and NMDAR EPSCs, respectively (see Experimental Procedures). Two examples of this experiment are shown in Figure 3. Initially, increasing stimulus strength had no effect on $EPSC_E$, even though $EPSC_L$ clearly increased. At higher stimulus strengths, both components of the EPSC increased. The differential effects of stimulation strength on $EPSC_E$ and $EPSC_L$ are clearly evident in Figures $3A_3$ and $3B_3$, in which $EPSC_L$ is plotted as a function of $EPSC_E$. This presumably reflects the fact that, in these cells at low stimulus strengths, synapses with only NMDARs and no detectable AMPARS were activated, and higher stimulus strengths were required to activate synapses with both NMDARs and AMPARs. Similar results to those in Figure 3 were obtained in 7 of 14 cells. In the other 7 cells, the relative contribution of AMPARs and NMDARs to the evoked EPSC remained constant as stimulus strength was increased, suggesting that in these cells the proportion of stimulated synapses containing only NMDARs did not change with stimulus strength.

Thus far we have presented data consistent with the

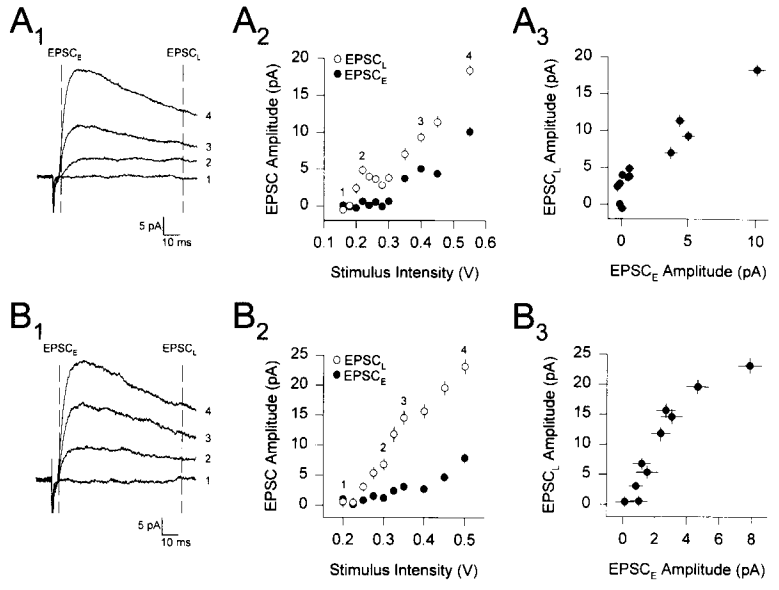

Figure 3. Differential Effects of Increasing Stimulus Strength on Early and Late Components of Evoked EPSCs

(A_1) EPSCs (average of 50 consecutive responses) recorded at the stimulus intensities indicated by the numbers on the graph in (A_2). Dashed lines show the early ($EPSC_E$) and late ($EPSC_L$) time points at which amplitudes were calculated.

(A_2) Graph of $EPSC_E$ and $EPSC_L$ amplitudes as a function of stimulus intensity. Each point represents the mean ± SEM of 50 consecutive traces.

(A_3) $EPSC_L$ amplitude plotted as a function of $EPSC_E$ amplitude. Data are the same as those illustrated in (A_2).

(B_1–B_3) Results from another cell.

proposal that there are a significant proportion of synapses on CA1 cells that contain NMDARs but no detectable AMPARs. An obvious and important question is whether these synapses can be modified during LTP or remain functionally "silent." To address this question, we used a protocol similar to that used in our initial experiments. The cell was held at −60 mV, and stimulus intensity was reduced to a level at which no AMPAR EPSCs were observed for a minimum of 100 consecutive stimuli (Figure 4). At this point, the cell was depolarized to −10 mV while the stimulation rate was maintained for 100 stimuli. Following this "pairing" protocol, which provides the essential requirements for the induction of LTP, the cell was returned to −60 mV, still maintaining a constant stimulation rate. To minimize the effects of dialysis on LTP, the cell was depolarized within ~8 min of break-in. As can be seen in Figure 4, pairing in this cell resulted in the appearance of AMPAR EPSCs that were readily distinguished from failures in individual traces (Figure $4B_1$). The ability of an LTP induction protocol to generate responses where none existed previously was also apparent when averaged responses of 100 consecutive traces were examined (Figure $4B_2$). Figure 5 shows a summary graph of these experiments. Cells (n = 14) were included in this graph if they fit either of two criteria: following the pairing protocol, AMPAR EPSCs were observed (n = 10), or in the cases

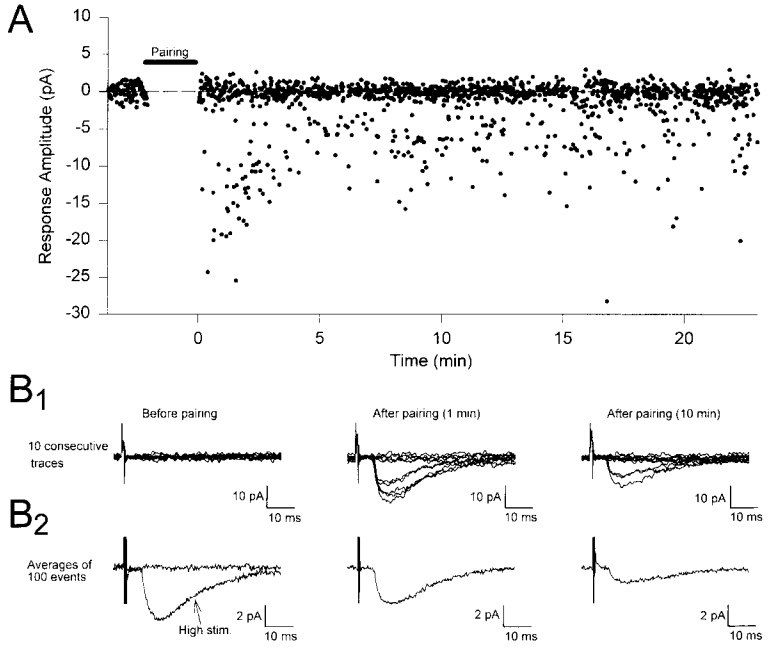

Figure 4. A Silent Synapse Can Exhibit LTP

(A) Graph of an experiment in which stimulus intensity was reduced so that no EPSCs were elicited at −60 mV. While continuing afferent stimulation, the cell was depolarized to −10 mV (Pairing; bar). When the cell was returned to −60 mV, clear EPSCs could now be elicited, and these remained for the duration of the recording.

(B) Examples of 10 consecutive traces (B_1) or the average of 100 consecutive traces (B_2; including traces in B_1) taken at the indicated times during the course of the experiment.

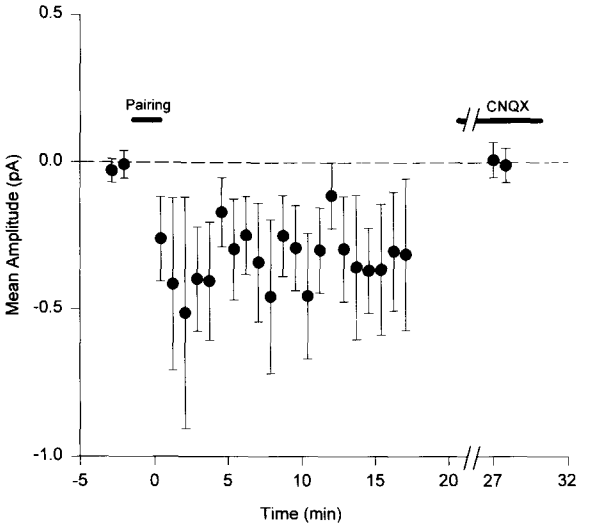

Figure 5. Summary of the LTP Experiments

Each point represents the mean of 50 consecutive traces (n = 14 experiments). At the beginning of the experiment, stimulation did not elicit EPSCs. After pairing to induce LTP, clear EPSCs could now be detected. Application of CNQX (10 μM) at the end of the experiment in the majority of cells (n = 9) confirmed that, initially, stimulation did not evoke any EPSCs mediated by AMPA receptors. These data include 4 experiments in which no EPSCs were elicited for 2 min after the pairing protocol. These cells were then depolarized, and APV-sensitive EPSCs were observed.

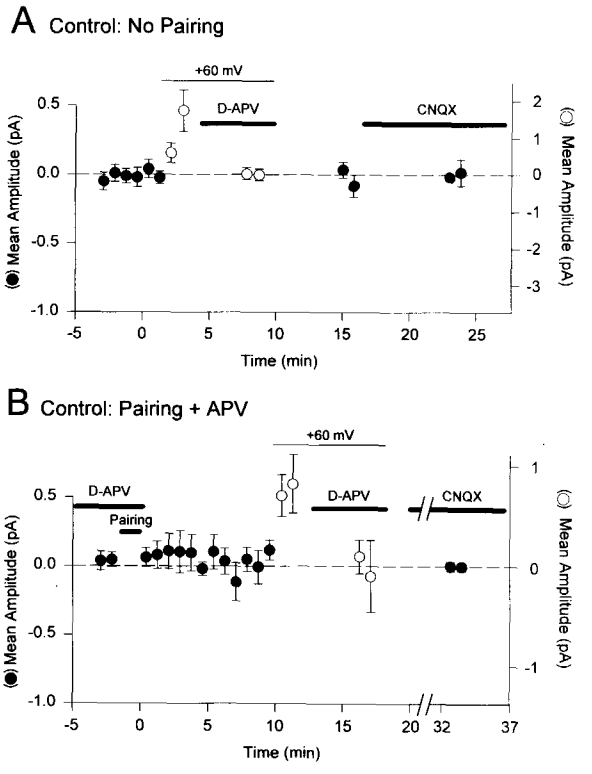

Figure 6. Summary of LTP Control Experiments

(A) Summary of experiments (n = 6) in which afferent stimulation was continued for a time period exceeding that during which pairing occurred in the experiments summarized in Figure 5. The cells were then depolarized to +60 mV, a membrane potential at which EPSCs could be elicited, and these were completely blocked by D-APV (25 μM). When the cells were returned to −60 mV, at ~20 min after the start of the experiment, again EPSCs were not elicited, as confirmed by the lack of effect of CNQX (n = 4).

(B) Summary of experiments (n = 7) performed exactly like those in Figure 5, except D-APV (25 μM) was present during the pairing protocol. After the pairing, D-APV was washed out, and traces were monitored for 8–10 min, at which point cells were depolarized to +60 mV, a membrane potential at which D-APV-sensitive EPSCs were elicited. Application of CNQX at the end of the experiment (n = 4) again confirmed that no EPSCs were elicited at the start of the experiment. Both sets of control experiments (A and B) were interleaved with the experiments summarized in Figure 5.

where no AMPAR EPSCs occurred following the pairing protocol, NMDAR EPSCs could be evoked when the cell was depolarized to +60 mV (n = 4). Thus, in 10 of 14 cells, a standard LTP induction protocol caused the appearance of AMPAR EPSCs where none were detectable previously, and these AMPAR EPSCs remained stable for the duration of the recording.

We also performed several controls, interleaved with the experiments illustrated in Figure 5. To ensure that AMPAR EPSCS did not simply appear spontaneously during the course of the experiment because of some change in the stimulation conditions, one set of cells (n = 6; Figure 6A) was kept at −60 mV for the 100 stimuli (when the pairing would have taken place), and then for a further 100 stimuli following that (when AMPAR EPSCs started to appear in the cells in which LTP was induced by pairing). After these stimuli, which did not elicit detectable AMPAR EPSCs, cells were depolarized to +60 mV (average time to depolarization following break-in was 17 min) and included in the data set if NMDAR EPSCs were observed. The cells were then returned to −60 mV, where again no EPSCs were elicited. Perhaps the most important control experiment is shown in Figure 6B. In these cells (n = 7), the protocol was identical to that shown in Figure 4 and Figure 5, except D-APV (25 μM) was present during the pairing protocol. Again, cells were included in this data set only if the occurrence of NMDAR EPSCs was confirmed after D-APV had been washed out. From these experiments, it is clear that blocking NMDA receptors, and thus the induction of LTP, prevented the appearance of AMPAR EPSCs following pairing. Together, these control experiments make it very unlikely that the positive results summarized in Figure 5 are due to some nonspecific experimental drift, and instead indicate that the appearance of AMPAR EPSCs was indeed due to the induction of LTP by the pairing protocol.

Discussion

We have presented direct experimental evidence that a proportion of excitatory synapses on CA1 cells contain NMDARs but no detectable AMPARs. Thus, at the normal resting potential, they are functionally silent in that they do not generate a detectable synaptic response when neurotransmitter is released. However, when these synapses are subject to a typical LTP induction protocol during which

the NMDARs are activated repetitively, AMPAR EPSCs appear and remain for the duration of the experiment. This result is consistent with the proposal that a significant proportion of synapses on CA1 cells do not contain detectable AMPARs and that LTP involves the uncovering or insertion of clusters of latent AMPARs at these synapses (Edwards, 1991; Kullmann and Nicoll, 1992; Liao et al., 1992; Lisman and Harris, 1993; Manabe et al., 1993).

The present results and conclusion are also consistent with previous work showing that the basal CV of NMDAR EPSCs is smaller than that of AMPAR EPSCs, and does not change during LTP even though the CV of AMPAR EPSCs changes significantly (Kullmann, 1994). In the accompanying paper (Selig et al., 1995), we have confirmed these results and extended them by demonstrating that the CVs of NMDAR and AMPAR EPSCs are also differentially affected during LTD. Together, these results strongly support the existence of functionally silent synapses containing only or predominantly NMDARs. The existence of synapses containing only NMDARs has also been proposed based on the examination of miniature EPSCs in cultured hippocampal neurons (Bekkers and Stevens, 1989). It is conceivable that dendritic filtering may preferentially mask AMPAR EPSCs because of their fast time course relative to NMDAR EPSCs. However, unless LTP causes a dramatic change in dendritic filtering, such filtering cannot account for the appearance of AMPAR EPSCs following LTP induction. Our results do not allow us to distinguish between synapses that do not contain functional AMPARs and those that contain a very low number of AMPARs or that contain AMPARs with an extremely low probability of opening or a very low single channel conductance. However, considered in the context of previous work on the site of expression of LTP, all such synapses are equivalent since all are electrophysiologically silent when recording from the soma.

Formally, there are alternative interpretations to explain the changes seen following the induction of LTP. Activation of NMDARs at functionally silent synapses could release a retrograde messenger that "turns on" adjacent presynaptic boutons that, prior to the LTP induction protocol, did not release transmitter when stimulated. This hypothesis requires that these presynaptic boutons always occur at postsynaptic sites that contain functional AMPARs, and that the postsynaptically silent synapses containing no detectable AMPARs be involved in the triggering of LTP yet not in its expression. Alternatively, at some synapses prior to LTP, the amount of glutamate released into the synaptic cleft may be sufficient to activate NMDARs but too low to activate AMPARs. LTP could then result by increasing the amount of glutamate released into the cleft to a level that would now activate AMPARs.

The evidence presented here that LTP results in the appearance of detectable AMPARs at previously silent synapses has significant consequences for the interpretation of previous results. LTP is associated with changes in the CV of AMPAR EPSCs, changes in the percentage of failures in response to minimal stimulation, and changes in the frequency of miniature EPSCs. All of these results, which previously have been attributed to presynaptic changes in quantal content, can be explained by the conversion of silent synapses to functional synapses. It is recognized that the current results do not rule out increases in transmitter release accompanying LTP. However, considered in the context of the lack of change in several independent electrophysiological measures of transmitter release, including paired-pulse facilitation (McNaughton, 1982; Gustafsson et al., 1988; Muller and Lynch, 1989; Zalutsky and Nicoll, 1990; Manabe et al., 1993) and the MK801-induced decay of NMDAR EPSCs (Manabe and Nicoll, 1994), we think that the current results make the hypothesis that presynaptic changes contribute significantly to LTP less attractive.

Determining the exact mechanisms by which synapses devoid of detectable AMPARs become responsive during LTP will be challenging. Possible mechanisms include: the "uncovering" of AMPARs already present in the dendritic spine membrane, perhaps via modifications in the cytoskeleton (Lynch and Baudry, 1984); the insertion of AMPARs into the membrane, perhaps via a mechanism analogous to the quantal release of neurotransmitter; and dramatic changes in the affinity or biophysical properties of AMPARs that, prior to LTP, would have to have existed in a functionally quiescent state. Although the conversion of silent synapses to functional synapses during LTP can explain many previous results, this mechanism is unlikely to explain the increase in mean quantal size observed during LTP using quantal analysis (Kullmann and Nicoll, 1992; Larkman et al., 1992; Liao et al., 1992) or examination of miniature EPSCs (Manabe et al., 1992). Thus, independent but possibly related mechanisms must be invoked, such as the direct modification of preexisting functional AMPARs or the addition of clusters of AMPARs at synapses already containing some number of functional AMPARs (Edwards, 1991).

An important question that we have not addressed is whether homosynaptic LTD in CA1 cells, which shares many properties with LTP (Malenka, 1994), is at least in part due to a mechanism opposite to that proposed here, specifically the removal, turning off, or covering up of clusters of functional AMPARs. Such a mechanism would explain the results in the accompanying paper (Selig et al., 1995) as well as the ability of certain patterns of synaptic activity to reverse LTP (Fujii et al., 1991; Mulkey and Malenka, 1992; Dudek and Bear, 1993; O'Dell and Kandel, 1994).

A recent report (Liao et al., 1995) published after review of this paper reached conclusions similar to some of those presented in this article.

Experimental Procedures

Transverse hippocampal slices (400 μm) were prepared from 12- to 20-day-old Sprague–Dawley rats and allowed to recover for 2–4 hr before being transferred to a recording chamber, where they were submerged beneath a continuously superfusing solution at room temperature (23°C–25°C) saturated with 95% O_2, 5% CO_2. The standard extracellular solution contained 119 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 30 mM sucrose, 11 mM glucose, and 0.1 mM picrotoxin (pH 7.4; 320–330

mOsm). A cut was made between the CA3 and CA1 regions to prevent the propagation of epileptiform activity.

Somatic whole-cell voltage-clamp recordings (Blanton et al., 1989) were made from CA1 pyramidal cells using 2–5 MΩ electrodes. The whole-cell solution contained 107.5 mM Cs–gluconate, 20 mM HEPES, 0.2 mM EGTA, 5 mM QX314-Br, 8 mM NaCl, 10 mM TEA–Cl, 4 mM Mg–ATP, and 0.3 mM GTP (pH 7.2 with CsOH; osmolarity adjusted to 295–297 mOsm with sucrose or distilled water). Cells were held at −60 mV during recordings unless indicated otherwise. Input resistances of cells were 350–500 MΩ at −60 mV. Series resistance was estimated by measuring the whole-cell fast capacitance transient in response to a 20 mV step using a filter frequency of 20 kHz and sampled at >50 kHz (electrode fast capacitance transient was compensated for when cell-attached), and was monitored throughout each recording (mean series resistance = 18.9 ± 1.1 MΩ; n = 87; range = 4–50 MΩ). A glass monopolar stimulating electrode placed in stratum radiatum was used to evoke EPSCs at a stimulation frequency of 1 Hz. To avoid any changes in synaptic response associated with changes in stimulus frequency, once stimulation was commenced at 1 Hz, it was maintained at that frequency throughout the duration of the recording. LTD was not elicited by this stimulation (n = 4), presumably because cells were voltage clamped at −60 mV.

Data were collected using an Axopatch-1D amplifier in voltage-clamp mode, filtered on-line at 2 kHz (80 dB/decade low-pass Bessel filter), sampled at 5 kHz, and analyzed on-line as previously described (Huang et al., 1992; Mulkey and Malenka, 1992). EPSC amplitudes were measured using a window at the peak of the event (1–4 ms for AMPAR EPSCs and 5–10 ms for NMDAR EPSCs), relative to the baseline taken immediately before the stimulus artifact. To control for possible contamination of measurements by the stimulus artifact, for the majority of recordings at −60 mV, CNQX was applied at the end of the experiment while cells were held at −60 mV. The mean value in CNQX was then subtracted from the experimental values. Similarly, for recordings at +30 to +60 mV, the mean value in D-APV was subtracted from the previous experimental values. When recording at −60 mV, traces containing spontaneous EPSCs not temporally correlated with the stimulus and that interfered with the measurement of the baseline or peak were not included in the subsequent analysis.

To test that our evaluation of failures during the baseline period was accurate, histograms were created from the data sets of the 100 stimuli immediately preceding depolarization of the cells to positive holding potentials. These were then fit with a single Gaussian, and a χ^2 test was used to determine if the fit was good. The data were well fit by a single Gaussian ($\chi^2 > 0.05$). In some cells (n = 9), the data had small symmetrical tails in the distributions that made them non-Gaussian and were likely due to small spontaneous events not removed from the analysis. Histograms skewed in the negative direction were never observed, confirming our initial impression during the experiment that the stimulus was not evoking detectable AMPAR EPSCs. For the experiments in Figure 3, $EPSC_E$ amplitudes were measured using a 0.5–1.0 ms window on the rising phase of the EPSC, at a time point at which the EPSC was dominated by AMPAR current, as determined by the subsequent blockade by >80% of $EPSC_E$ by CNQX (5–10 μM). $EPSC_L$ amplitudes were measured using a 5 ms window at 70 ms after the stimulus artifact, a time point at which >98% of $EPSC_L$ was blocked by D-APV (25 μM).

Acknowledgments

We thank Shaul Hestrin and members of our laboratories for many helpful discussions. This work was supported by grants from the National Insitute of Mental Health (R. A. N. and R. C. M.), the National Institute on Aging (R. C. M.), and the McKnight Endowment Fund for Neuroscience (R. C. M.). J. T. R. I. was supported by a Wellcome Trust Travelling Research Fellowship. R. A. N. is a member of the Silvio Conte Center for Neuroscience Research and the Keck Center for Integrative Neuroscience.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 USC Section 1734 solely to indicate this fact.

Received May 9, 1995; revised June 15, 1995.

References

Asztely, F., Wigstrom, H., and Gustafsson, B. (1992). The relative contribution of NMDA receptor channels in the expression of long-term potentiation in the hippocampal CA1 region. Eur. J. Neurosci. *4*, 681–690.

Bekkers, J. M., and Stevens, C. F. (1989). NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus. Nature *341*, 230–233.

Bekkers, J. M., and Stevens, C. F. (1990). Presynaptic mechanism for long-term potentiation in the hippocampus. Nature *346*, 724–729.

Blanton, M., LoTurco, J., and Kriegstein, A. (1989). Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. J. Neurosci. Meth. *30*, 203–210.

Bliss, T. V. P., and Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31–39.

Bliss, T. V. P., Douglas, R. M., Errington, M. L., and Lynch, M. A. (1986). Correlation between long-term potentiation and release of endogenous amino acids from dentate gyrus of anaesthetized rats. J. Physiol. *377*, 391–408.

Clark, K. A., and Collingridge, G. L. (1995). Synaptic potentiation of dual-component excitatory postsynaptic currents in the rat hippocampus. J. Physiol. *482*, 39–52.

Davies, S. N., Lester, R. A. J., Reymann, K. G., and Collingridge, G. L. (1989). Temporally distinct pre- and postsynaptic mechanisms maintain long-term potentiation. Nature *338*, 500–503.

Dudek, S. M., and Bear, M. F. (1993). Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J. Neurosci. *13*, 2910–2918.

Edwards, F. A. (1991). LTP is a long-term problem. Nature *350*, 271–272.

Foster, T., and McNaughton, B. (1991). Long-term enhancement of CA1 synaptic transmission is due to increased quantal size, not quantal content. Hippocampus *1*, 79–91.

Fujii, S., Saito, K., Miyakawa, H., Ito, K., and Kato, H. (1991). Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. Brain Res. *555*, 112–122.

Gustafsson, B., Huang, Y. Y., and Wigstrom, H. (1988). Phorbol ester-induced synaptic potentiation differs from long-term potentiation in guinea pig hippocampus *in vitro*. Neurosci. Lett. *85*, 77–81.

Huang, Y. Y., Colino, A., Selig, D. K., and Malenka, R. C. (1992). The influence of prior synaptic activity on the induction of long-term potentiation. Science *255*, 730–733.

Kamiya, H., Sawada, S., and Yamamoto, C. (1991). Persistent enhancement of transmitter release accompanying long-term potentiation in the guinea pig hippocampus. Neurosci. Lett. *130*, 259–262.

Kauer, J. A., Malenka, R. C., and Nicoll, R. A. (1988). A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. Neuron *1*, 911–917.

Kullmann, D. M. (1994). Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. Neuron *12*, 1111–1120.

Kullmann, D. M., and Nicoll, R. A. (1992). Long-term potentiation is associated with increases in quantal content and quantal amplitude. Nature *357*, 240–244.

Larkman, A., Hannay, T., Stratford, K., and Jack, J. (1992). Presynaptic release probability influences the locus of long-term potentiation. Nature *360*, 70–73.

Liao, D., Jones, A., and Malinow, R. (1992). Direct measurements of quantal changes underlying long-term potentiation in CA1 hippocampus. Neuron *9*, 1089–1097.

Liao, D., Hessler, N. A., and Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of

hippocampal slice. Nature *375*, 400–404.

Lisman, J. E., and Harris, K. M. (1993). Quantal analysis and synaptic anatomy—integrating two views of hippocampal plasticity. Trends Neurosci. *16*, 141–147.

Lynch, G., and Baudry, M. (1984). The biochemistry of memory: a new and specific hypothesis. Science *224*, 1057–1063.

Malenka, R. C. (1994). Synaptic plasticity in the hippocampus: LTP and LTD. Cell *78*, 535–538.

Malenka, R. C., Kauer, J. A., Zucker, R. S., and Nicoll, R. A. (1988). Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science *242*, 81–84.

Malgaroli, A., and Tsien, R. W. (1992). Glutamate-induced long-term potentiation of the frequency of miniature synaptic currents in cultured neurons. Nature *357*, 134–139.

Malinow, R., and Tsien, R. W. (1990). Presynaptic enhancement shown by whole-cell recording of long-term potentiation in hippocampal slices. Nature *346*, 177–180.

Manabe, T., and Nicoll, R. A. (1994). Long-term potentiation: evidence against an increase in transmitter release probability in the CA1 region of the hippocampus. Science *265*, 1888–1892.

Manabe, T., Renner, P., and Nicoll, R. A. (1992). Postsynaptic contribution to long-term potentiation revealed by analysis of miniature synaptic currents. Nature *355*, 50–55.

Manabe, T., Wyllie, D. J. A., Perkel, D. J., and Nicoll, R. A. (1993). Modulation of synaptic transmission and long-term potentiation: effects of paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. J. Neurophysiol. *70*, 1451–1459.

McNaughton, B. L. (1982). Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. J. Physiol. *324*, 249–262.

Mulkey, R. M., and Malenka, R. C. (1992). Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of hippocampus. Neuron *9*, 967–975.

Muller, D., and Lynch, G. (1988). Long-term potentiation differentially affects two components of synaptic responses in hippocampus. Proc. Natl. Acad. Sci. USA *85*, 9346–9350.

Muller, D., and Lynch, G. (1989). Evidence that changes in presynaptic calcium are not responsible for long-term potentiation in hippocampus. Brain Res. *479*, 209–299.

O'Connor, J. J., Rowan, M. J., and Anwyl, R. (1995). Tetanically induced LTP involves a similar increase in the AMPA and NMDA receptor components of the excitatory postsynaptic current: investigations of the involvement of mGlu receptors. J. Neurosci. *15*, 2013–2020.

O'Dell, T. J., and Kandel, E. R. (1994). Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of phophatases. Learn. Mem. *1*, 129–139.

Perkel, D. J., and Nicoll, R. A. (1993). Evidence for all-or-none regulation of neurotransmitter release: implications for long-term potentiation. J. Physiol. *471*, 481–500.

Perkel, D. J., Petrozzino, J. J., Nicoll, R. A., and Connor, J. A. (1993). The role of Ca^{2+} entry via synaptically activated NMDA receptors in the induction of long-term potentiation. Neuron *11*, 817–823.

Selig, D. K., Hjelmstad, G., Herron, C., Nicoll, R. A., and Malenka, R. C. (1995). Independent mechanisms for long-term depression of AMPA and NMDA responses. Neuron, this issue.

Stevens, C. F., and Wang, Y. (1994). Changes in reliability of synaptic function as a mechanism for plasticity. Nature *371*, 704–707.

Wyllie, D. J. A., Manabe, T., and Nicoll, R. A. (1994). A rise in postsynaptic Ca^{2+} potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. Neuron *12*, 127–138.

Zalutsky, R. A., and Nicoll, R. A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. Science *248*, 1619–1624.