Bidirectional Control of Quantal Size by Synaptic Activity in the Hippocampus

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Analysis of strontium-induced asynchronous release of quanta from stimulated synapses revealed that long-term potentiation and long-term depression in the CA1 region of the mammalian hippocampus are associated with an increase and a decrease, respectively, in quantal size. At a single set of synapses, the increase in quantal size seen with long-term potentiation was completely reversed by depotentiating stimuli. Long-term potentiation and depression are also associated with an increase and decrease, respectively, in the frequency of quantal events, consistent with an all-or-none regulation (up or down) of clusters of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, a change in the release of transmitter, or both.

Long-term potentiation (LTP) and long-term depression (LTD) in the CA1 region of the hippocampus are thought to depend on the activation of postsynaptic *N*-methyl-D-aspartate receptors (NMDARs) and a rise in intracellular calcium (Ca^{2+}). The site at which LTP and LTD are expressed, however, has not been unequivocally determined (*1*). Initially, quantal analysis suggested that increases in both quantal content, a presynaptic variable, and quantal size, a postsynaptic measure, occurred during LTP (*2*). However, more recent reports in which presumptive single synaptic sites were studied did not observe a change in quantal size during LTP (*3*, *4*) or LTD (*3*) but found a significant change in synaptic failures that could indicate a change in the probability of transmitter release.

One approach to identify the site responsible for a change in synaptic strength has been to examine the size and frequency of spontaneous quantal excitatory postsynaptic currents (EPSCs) (*5*). A change in the size of these events is thought to reflect a postsynaptic change in the response to the neurotransmitter. In contrast, a change in frequency is thought to represent a change in neurotransmitter release. Because NMDAR-dependent LTP and LTD are limited to the stimulated pathway, only a small subset of the synapses on the recorded cell express the plasticity. Thus, analysis of spontaneous EPSCs, which originate from all of the synapses contacting the cell, is unlikely to detect any changes at the modified syn-

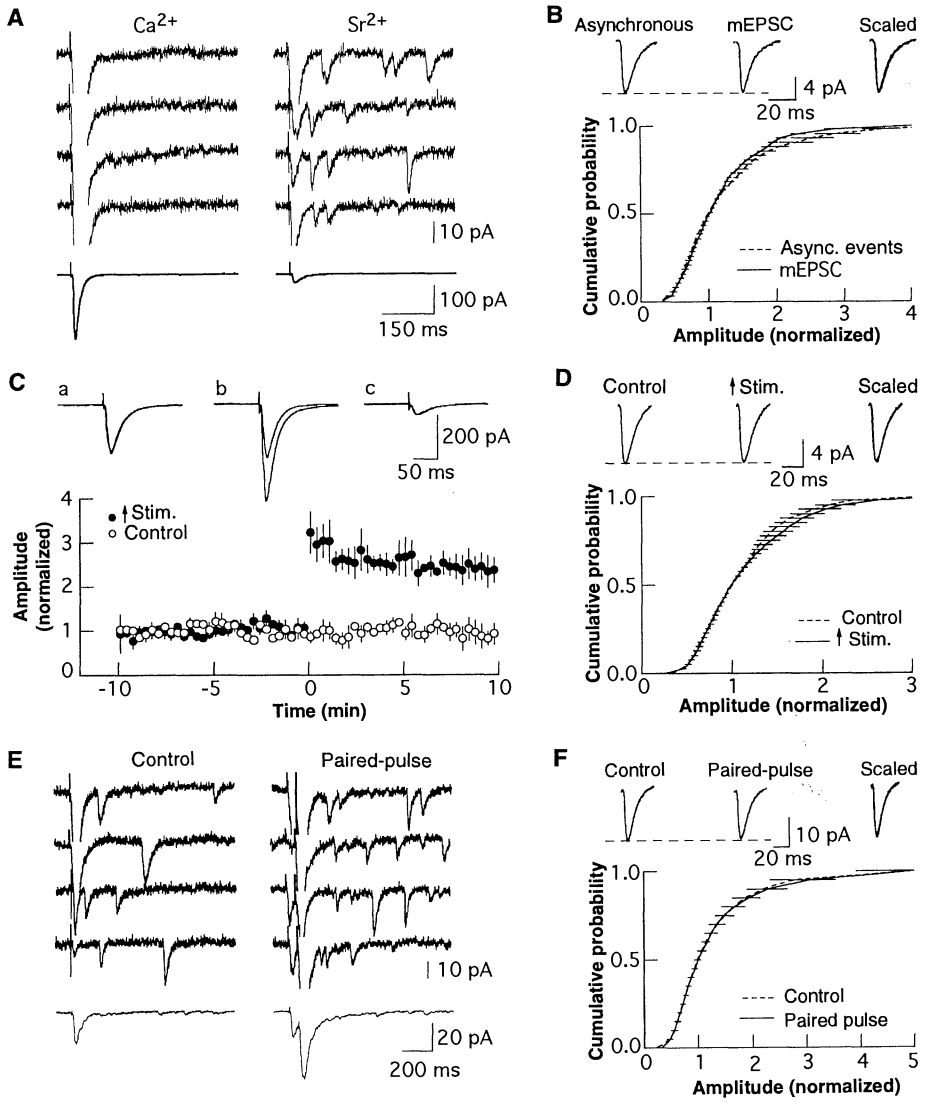

Fig. 1. Substitution of Sr^{2+} for Ca^{2+} induces asynchronous quantal release. (**A**) Sample traces of evoked EPSCs obtained in a guinea pig CA1 neuron in the presence of Ca^{2+} or Sr^{2+}. The bottom two traces are averages of 30 EPSCs shown at a different scale. (**B**) Cumulative amplitude distributions comparing events associated with Sr^{2+}-induced asynchronous release (dotted line) with miniature EPSCs (mEPSCs) recorded in TTX (solid line). Data from six different CA1 neurons were averaged. No statistical difference in quantal amplitude was found between the two distributions ($P > 0.07$). Averages of 100 of these quantal events obtained in a single cell are displayed above the plot. (**C**) Summary graph of four cells where the stimulus strength was increased in one pathway (filled symbols) before Sr^{2+} replaced Ca^{2+} in the extracellular solution. The traces are superimposed averages of six successive sweeps taken from a typical experiment before (a) and after (b) modification of the synaptic response in the test pathway, as well as in the presence of Sr^{2+} (c). (**D**) Cumulative amplitude distributions obtained from the cells illustrated in (C) comparing the asynchronous events in the control pathway (dotted line) with those from the pathway where stimulus strength was increased (solid line). No statistical difference was found between the two distributions ($P > 0.25$). Averages of 100 of these events obtained in a single cell are shown above the plot. (**E**) Sample traces of evoked EPSCs originating from two independent pathways in the presence of Sr^{2+}. On one pathway, paired-pulse stimulation was given. The lower traces are averages of 15 EPSCs shown at a different scale. (**F**) Cumulative amplitude distributions comparing asynchronous events obtained from control (dotted line) and paired-pulse (solid line) pathways. Data from four different neurons were averaged. No difference in quantal amplitude was found between the two distributions ($P > 0.1$). Averages of 100 of these quantal events obtained in a single cell are displayed above the plot.

apses. Because of this complication, alternative approaches, such as application of glutamate agonists (*6*, *7*) or activation of voltage-sensitive Ca^{2+} channels (*8*, *9*), have been devised to permit modification of all of the synapses on a cell. These approaches produce changes in both quantal size and frequency. However, the relevance of the synaptic plasticity elicited by these manipulations to standard NMDAR-dependent LTP and LTD that are evoked by synaptic stimulation has been questioned (*10*, *11*). Thus, we set out to study, in isolation, the quantal EPSCs that originate specifically from the subset of synapses that are expressing standard LTP and LTD.

When strontium (Sr^{2+}) is substituted for Ca^{2+}, the stimulation-evoked synchronous release of transmitter is reduced, but asynchronous release of quanta is markedly and selectively enhanced (*12*, *13*). This permits detailed analysis of quantal events from the set of stimulated synapses that have undergone the plasticity. Using standard electrophysiological techniques (*14*), we first examined the effect of substituting Sr^{2+} for Ca^{2+} on excitatory synaptic transmission in the hippocampal slice (Fig. 1A). Stimulation of excitatory fibers in stratum radiatum in a solution containing 4 mM Ca^{2+} evoked a fast synchronous EPSC with no associated quantal events after each of several consecutive stimuli. Substituting the Ca^{2+} with Sr^{2+} (4 mM) quickly led to a decrease in the size of the synchronous EPSC and to the appearance of numerous asynchronous quantal events, which lasted for a few hundred milliseconds after the stimulus. In the absence of synaptic stimulation, the frequency of spontaneous events did not increase in the presence of Sr^{2+} ($n = 6$). The amplitude distribution of quanta for the asynchronous events evoked in Sr^{2+} was not different from that obtained in the same cell after action potentials were blocked by tetrodotoxin (TTX) (miniature EPSC) ($P > 0.07$) (Fig. 1B). Thus, the asynchronous events observed after stimulation were, in fact, quantal (*13*, *15*).

Because the induction of LTP washes out quickly (~15 min) with whole-cell recording (*16*), sequential application of Sr^{2+} before and after LTP induction was not feasible. Thus, we tested the possibility of comparing, at the same time, quantal events in a control pathway and a modified pathway. We first determined if the sizes of the quantal responses evoked in the presence of Sr^{2+} were the same in two independent pathways, even when the sizes of the evoked responses in the two pathways differed (Fig. 1C). Comparison of the quantal size distribution in experiments in which the stimulus to one of the pathways was increased and then Sr^{2+} was applied (Fig. 1D) revealed no difference ($P > 0.25$) in the asynchronous quantal EPSCs evoked from these two pathways, even though the number of synapses being activated and contributing to the generation of quantal EPSCs was considerably different in the two inputs. As expected, the pathway with the larger input was also associated with an increase in the frequency of events. We also examined whether changing the probability of transmitter release in one of the two inputs could affect the size of the quantal EPSCs. Applying paired stimuli (interstimulus interval of 30 ms) to one input (Fig. 1E), which facilitates transmitter release, increased the frequency of quantal EPSCs (by a factor of 2.04 ± 0.23 compared to control inputs, $n = 4$) but had no effect on mean quantal amplitude (+0.5 ± 2.9%) or the cumulative amplitude distribution ($P > 0.1$) (Fig. 1F).

Having established the validity of this technique, we examined whether LTP had any effect on the size distribution of the quanta evoked in Sr^{2+} (Fig. 2A). After obtaining baseline evoked responses, LTP was induced by pairing synaptic stimulation with depolarization in one of the pathways. (The stimulus to the other pathway was turned off during the pairing procedure.) After pairing, there was a selective potentiation in the paired pathway that remained stable for 40 min. Then, Sr^{2+} was applied, and the quantal EPSCs evoked by the stimulation of one pathway and then the other pathway were collected [see (*14*) for details]. The size distribution of quantal responses collected from the potentiated pathway was shifted to the right when compared with the control quantal responses (Fig. 2B), and the mean size increased by 33%, consistent with previous results (*2*, *6*). With scaling, the averaged traces from the two pathways were superimposable. The shift in the cumulative distribution of quantal EPSC size was highly sig-

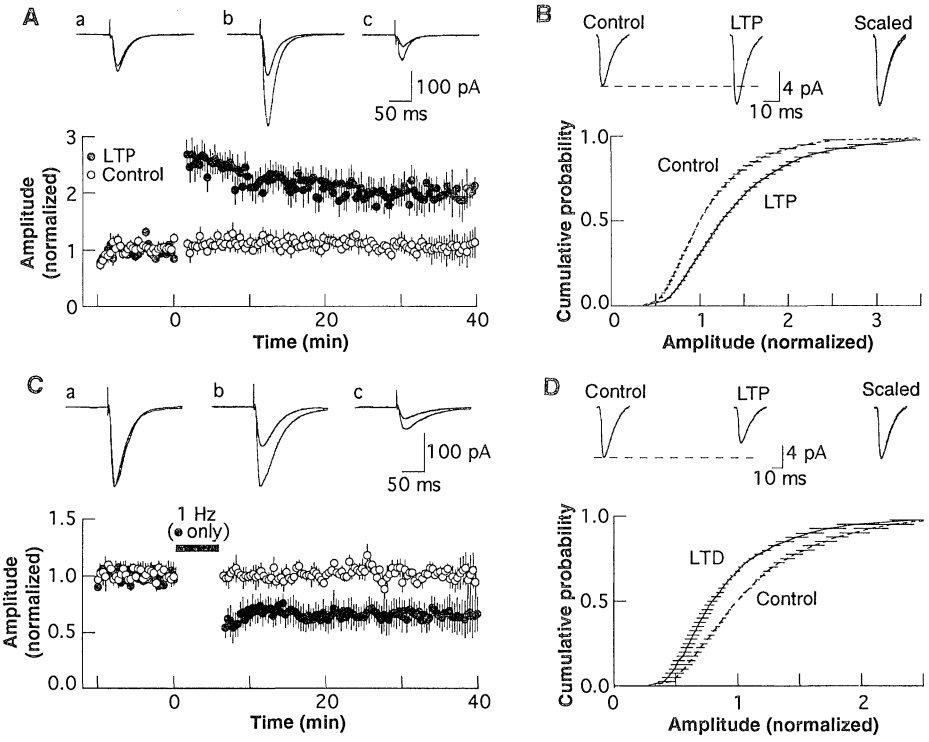

Fig. 2. LTP and LTD are associated with changes of quantal size. (**A**) Summary of six whole-cell recordings obtained from guinea pig neurons where synaptic stimulation was paired with membrane depolarization in one pathway (filled symbols). The sample records are superimposed averages of six successive sweeps taken as in Fig. 1C. (**B**) Corresponding cumulative amplitude distributions of the events associated with Sr^{2+}-induced asynchronous release obtained from the paired (solid line) and unpaired (dotted line) pathways. The distributions are statistically different ($P < 0.0001$). Averages of 100 of these events obtained in a single cell are shown above the plot. (**C**) Summary graph of seven whole-cell recordings obtained from rat neurons where depression of one pathway (filled symbols) was induced by low-frequency stimulation (1 Hz, 6 min; thick bar). The sample records are superimposed averages of six successive sweeps taken as in Fig. 1C. (**D**) Corresponding cumulative amplitude distributions of the events associated with Sr^{2+}-induced asynchronous release, obtained from the depressed (solid line) and control (dotted line) pathways. The two distributions are statistically different ($P < 0.0001$). Averages of 100 of these events obtained in a single cell are shown above the plot.

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nificant in all six experiments in which quantal responses were collected 50 to 60 min after pairing ($P < 0.0001$) (Fig. 2B). These experiments were repeated in the presence of the NMDAR antagonist D(−)-2-amino-5-phosphonovalerate (APV) (100 μM), which blocked both LTP and the increase in quantal size ($P > 0.07$, $n = 5$).

To examine if quantal size decreases during LTD, we induced LTD by low-frequency stimulation (LFS) (1 Hz for 6 min) at the resting membrane potential in current clamp. This stimulation caused a large stable depression in the experimental pathway (Fig. 2C). When quantal events were collected in Sr^{2+} 55 min after LFS, quantal size was depressed (mean quantal size decreased by 25%). The summary for all experiments in which LTD was generated (Fig. 2D) shows a shift to the left in the quantal size distribution, and for each experiment, the shift was highly significant ($P < 0.004$, $n = 7$). In the presence of APV, LFS did not induce LTD, and no change in quantal size was detected ($P > 0.08$, $n = 4$).

In a final set of experiments, we determined if, in the same set of synapses, the increase in quantal size that accompanies LTP could be reversed. A tetanus was used to induce LTP so that we could compare the results with those obtained by pairing (Fig. 3). Strontium was applied shortly after the induction of LTP, and the quantal EPSCs evoked in both the control and potentiated pathways were compared (Fig. 3A). We then returned to the control, Ca^{2+}-containing medium, and after establishing that the LTP had remained stable, we repeatedly applied LFS to obtain maximal depotentiation. This procedure routinely resulted in evoked responses smaller than those obtained before LTP (Fig. 3A). Then Sr^{2+} was reapplied, and the quantal EPSCs evoked from each of the pathways were examined. The increase in quantal size associated with LTP (mean = 24%) was completely reversed (−31%) and converted into a clear depression (−8%) (Fig. 3B). A summary of five experiments shows the reliability of this bidirectional control of quantal size at the same set of synapses (Fig. 3, C and D).

A summary of the changes in mean quantal size for the various manipulations is shown in Fig. 4A. There was no difference in the size of quantal events evoked in the presence of Sr^{2+} and the size of miniature EPSCs evoked in the presence of TTX, indicating that the former are not composed of multiple quantal events. A similar highly significant increase in quantal size was observed for LTP induced by pairing and by tetanic stimulation. In contrast, LTD induced by LFS gave a clear decrease in quantal size. When the decrease in quantal size produced by depotentiation was compared to the control pathway, the effect did not reach significance. However, most importantly, when quantal size after depotentiation was compared with quantal size recorded in the same pathway during LTP, the decrease was highly significant. The observed changes in quantal size likely indicate that during LTP, the sensitivity or the number of AMPA receptors (or both) on a postsynaptic spine increases, and that during LTD, the opposite occurs (*17*). An alternative and less likely explanation that cannot be excluded by the present data is that LTP is associated with a presynaptic mechanism, whereby a cooperative synchronous release of multiple quanta occurs (*18*).

Like standard LTP and LTD, the changes in quantal size were entirely blocked by

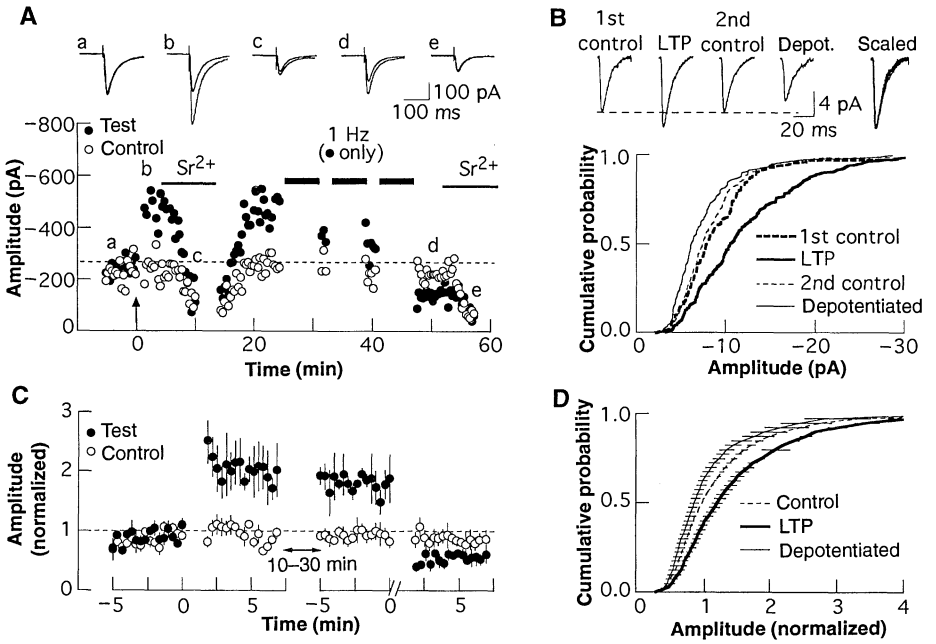

Fig. 3. Quantal size can be sequentially increased and decreased at a single set of synapses. (**A**) Example of a recording from a rat neuron where a test pathway (filled symbols) was first tetanized (arrow) and then depotentiated by successive application of periods of low-frequency stimulations (thick bars). After the tetanus, Sr^{2+} was washed in and out quickly and then reapplied once depotentiation had occurred. The sample records are superimposed traces (averages of six successive sweeps) taken at the indicated times in both the control and test pathways. (**B**) Corresponding cumulative amplitude distributions of the asynchronous events associated with potentiation (thick solid line), depotentiation (thin solid line), and their respective controls (dotted lines). The increase and decrease in quantal size associated with LTP and depotentiation, respectively, are statistically significant ($P < 0.0001$). The first control was collected after LTP, and the second control, after depotentiation. Averages of 100 of these events are shown above the plot. (**C** and **D**) Summary graphs for five experiments. The time necessary to apply and wash out Sr^{2+} varied from 10 to 30 min. To obtain maximal depotentiation, we applied LFS one to six times [break in (C)]. The control distributions were pooled together in the averaged cumulative amplitude plot because they were not statistically different.

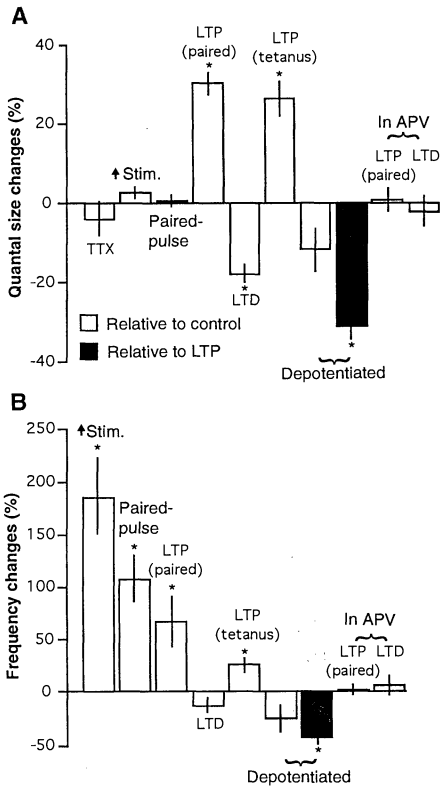

Fig. 4. Summary of the effects of LTP, LTD, and depotentiation on the size and frequency of Sr^{2+}-induced quantal events. (**A**) Differences in mean quantal size observed under different conditions. (**B**) Changes in the frequency of quantal events observed under different conditions. Data are expressed as percent changes relative to control (open) or potentiated (solid) pathways. Asterisks indicate significant difference ($P < 0.05$).

APV. In addition to the changes in quantal size, changes in quantal frequency were also observed (Fig. 4B). The frequency was increased during both pairing-induced and tetanus-induced LTP. During LTD, the decrease in frequency was not significant. However, depotentiation caused a decrease that was significant when compared with the frequency of quantal responses during LTP in the same pathway.

Important control experiments were to examine the effects of increasing stimulus strength or applying paired stimuli, pure presynaptic manipulations, on the size and frequency of quantal responses. When the increase in EPSC size recorded for LTP was matched by increasing the stimulus strength (Fig. 1C), the increase in frequency was larger than with LTP. Furthermore, the increase in frequency caused by applying paired stimuli was also much larger than that accompanying LTP. Thus, whatever the origin of the increase in quantal frequency, it cannot by itself account for the increase in the evoked EPSC size. The change in quantal size presumably accounts for this discrepancy. In contrast, neither increasing the number of activated synapses nor increasing the probability of transmitter release caused a change in quantal size. Thus, a pure increase in frequency had no effect on our measurements of quantal size.

A change in frequency of quantal events is usually interpreted as reflecting a change in the release of transmitter, although such an interpretation is difficult to reconcile with MK-801 experiments in which no change in release probability during LTP was observed (*19*). Recent studies (*20*) have suggested that a portion of synapses in the CA1 region may contain functional NMDARs but no functional AMPA receptors, that is, they are silent at negative membrane potentials. After LTP, some of these silent synapses become functional. Such a mechanism provides an alternative explanation for a change in the frequency of quantal events because after LTP there would be an increase in the number of functional synapses. The present results, in which changes in both quantal size and frequency were observed, are therefore consistent with the proposal that the function or number of AMPA receptors (or both) at individual synapses can be up- or down-regulated depending on the pattern of synaptic activation of NMDARs. However, it should be emphasized that our results do not exclude a presynaptic contribution to the plasticity we have studied.

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14. Hippocampal slices were prepared from 3- to 5-week-old Hartley guinea pigs and from 2- to 4-week-old Sprague-Dawley rats as described previously (*6, 8*). Slices were superfused with standard extracellular solution containing 4 mM $CaCl_2$ and 4 mM $MgCl_2$. Whole-cell patch-clamp recordings were made from CA1 neurons with the "blind" recording technique [M. G. Blanton, J. J. Lo Turco, A. R. Kriegstein, *J. Neurosci. Methods* **30**, 203 (1989)]. Patch electrodes were filled with an internal solution containing 122.5 mM Cs-gluconate, 10 mM CsCl, 8 mM NaCl, 10 mM Hepes, 0.2 mM EGTA, 2 mM Mg–adenosine triphosphate, 0.1 mM cyclic adenosine monophosphate, 0.3 mM Na_3–guanosine triphosphate, 5 mM QX-314, and 10 mM glucose. For LTD experiments, Cs was replaced by K, and QX-314 was omitted. Series resistance was monitored throughout the experiments. Two bipolar, stainless steel stimulating electrodes placed in the stratum radiatum equidistant from the pyramidal cell layer on each side of the recording site were used to alternatively evoke two independent EPSCs at a frequency of 0.05 Hz. Data were collected with an axopatch-1D, filtered at 1 kHz, sampled at 2 kHz, and analyzed on-line as previously described [R. M. Mulkey and R. C. Malenka, *Neuron* **9**, 967 (1992)]. Once the EPSC amplitude reached a steady level in the presence of Sr^{2+} (5 to 15 min), each pathway was stimulated alternatively (every 30 s) at 2 Hz for 10 s for a period of 7 to 15 min. Asynchronous events were measured during the 400-ms period after the end of the synchronous response and were analyzed off-line, as previously described (*6, 8*). Entries in the cumulative histograms ranged from 163 to 946. Data were compared statistically either by the nonparametric Kolmogorov-Smirnov test or by the paired Student's *t* test. Averaged cumulative amplitude histograms were obtained by normalizing each distribution to the corresponding median value obtained in the control pathway. In the TTX experiments, the criterion used for including cells in the analysis was a lack of difference between the rise-time distributions of the asynchronous quantal events and the miniature EPSCs. Pairing-induced LTP was obtained by pairing membrane depolarization (0 mV) with synaptic stimulation at 2 Hz for 30 to 60 s. Tetanus-induced LTP was obtained by using a 100-Hz stimulus for 1 s in current clamp. This procedure was performed four times at 20-s intervals.
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17. Although the changes in quantal size evoked by LTP and LTD were highly significant, there was still a discrepancy, especially for LTP, between the magnitude of the change recorded on the evoked EPSC compared with the change in quantal size. If all of the recorded quantal responses come from the synapses expressing the plasticity, one possible explanation is that events that were below the detection threshold before LTP become detectable but are smaller than the basal mean quantal size. These events would add to the enhancement of the evoked response but would actually counteract the increase in mean size of the quantal events.
18. Another possibility is that a change in quantal size is caused by a relative change in the frequency of large quantal events. In considering this possibility, one must consider what effect such a selective change will have on the overall frequency and the shape of the distribution. We have examined this issue for all of the cells by attempting to reproduce the observed change in distribution and mean quantal size by changing the relative frequency of subsets of quantal events larger than the mean. In all cases where the shape of the distribution was not significantly different from the observed distribution, the overall increase in frequency would have had to have been several times (2- to 20-fold) greater than that observed experimentally for a selective change in the frequency of large events to account for the observed change in mean quantal size.
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