Bidirectional Changes in Spatial Dendritic Integration Accompanying Long-Term Synaptic Modifications

Zhiru Wang,[1] Ning-long Xu,[1] Chien-ping Wu,[1] Shumin Duan,[1,*] and Mu-ming Poo[1,2,*]
[1]Institute of Neuroscience
Shanghai Institutes of Biological Sciences
Chinese Academy of Sciences
Shanghai
China
[2]Division of Neurobiology
Department of Molecular and Cell Biology
University of California
Berkeley, California 94720

Summary

Information processing in the neuron requires spatial summation of synaptic inputs at the dendrite. In CA1 pyramidal neurons of the hippocampus, a brief period of correlated pre- and postsynaptic activity, which induces long-term potentiation (LTP) or long-term depression (LTD), results in a persistent increase or decrease in the linearity of spatial summation, respectively. Such bidirectional modification of the summation property is specific to the modified input and reflects localized dendritic changes involving I_h channels and NMDA receptors. Thus, correlated pre- and postsynaptic activity alters not only the strength of the activated input but also its dendritic integration with other inputs.

Introduction

Information received by the neuron via synaptic inputs is integrated by spatial and temporal summation of excitatory and inhibitory synaptic potentials at the dendrite, a process dependent on both passive cable properties and the distribution of voltage-dependent ion channels along the dendrite (Rall, 1964; Magee, 1999, 2000; Spruston et al., 2001; Cash and Yuste, 1998, 1999). The neural circuits are susceptible to short- and long-term modification by neuronal activity. The best understood form of activity-dependent neuronal plasticity is the persistent increase or decrease in synaptic efficacy, commonly referred to as LTP or LTD, respectively (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). In addition to synaptic modifications, activity may also induce changes in the morphology of dendritic spines (Maletic-Savatic et al., 1999; Engert and Bonhoeffer, 1999; Toni et al., 1999; Yuste and Bonhoeffer, 2001) and in the intrinsic neuronal excitability of the pre- or postsynaptic neurons (Turrigiano et al., 1994; Desai et al., 1999; Ganguly et al., 2000; Aizenman and Linden, 2000; Armano et al., 2000). For example, tetanic stimulation that induces LTP also increases the postsynaptic excitability, known as EPSP-to-spike (E-S) potentiation (Bliss and Lømo, 1973; Andersen et al., 1980; Pugliese

*Correspondence: shumin@ion.ac.cn (S.D.), mpoo@uclink.berkeley. edu (M.-m.P.)

et al., 1994; Chavez-Noriega et al., 1990). Such changes in neuronal excitability have been attributed to a reduction of inhibitory inputs (Lu et al., 2000; Abraham et al., 1987; Tomasulo and Ramirez, 1993; Chavez-Noriega et al., 1989) and local modulation of active conductances at the potentiated synapse (Hess and Gustafsson, 1990; Asztely and Gustafsson, 1994; Jester et al., 1995). In principle, local morphological changes of dendrites and alteration in the property or distribution of ion channels are all likely to modify dendritic summation of synaptic potentials, an effect separate from the activity-induced modifications of synaptic strength. In the present study, we have examined systematically the consequence of correlated synaptic activity on dendritic integration of synaptic inputs in hippocampal pyramidal neurons by measuring changes in the property of spatial summation of excitatory postsynaptic potentials (EPSPs). We found that following the induction of LTP/LTD there were corresponding bidirectional changes in the efficacy of dendritic integration between the activated synapses with other dendritic inputs. Further studies of underlying mechanisms revealed that local dendritic modifications of I_h channels and NMDA subtype of the glutamate receptors, which are known to influence dendritic summation of EPSPs (Magee, 1998, 1999; Cash and Yuste, 1999), may account for the observed changes in the linearity of summation. Thus, correlated synaptic activity can induce persistent changes in the integrative properties of the postsynaptic dendrite, in addition to modifications of synaptic efficacy.

Results

Increase in the Linearity of Spatial Summation Accompanying LTP

Perforated whole-cell recording was made from CA1 pyramidal cells of rat hippocampal slices to monitor monosynaptic EPSPs elicited by extracellular stimulation of Schaffer collateral/commissural fibers. Spatial summation of EPSPs at two inputs (S_p and S_c) elicited by stimulating electrodes placed in the *s. radiatum* (Figure 1A) was examined. In general, we found that the amplitude of the EPSP elicited by synchronous costimulation of the two inputs (termed "measured EPSP") is lower than the arithmetic sum ("expected EPSP") of the two EPSPs obtained by stimulating each input separately (Figure 1A), consistent with that reported previously (Cash and Yuste, 1999). To induce LTP, S_p was repetitively stimulated (20 Hz for 0.5 s, ten times at 4 s intervals) to evoke subthreshold EPSPs, each paired with a postsynaptic action potential elicited by current injection (2 ms, 1.6 nA) 5 ms after the onset of the EPSP. The potentiation of input S_p after correlated stimulation was shown as a persistent increase in the EPSP amplitude, without any change in the EPSP amplitude of the control input S_c elicited by a constant test stimulus (Figure 1B). The potentiation of the stimulated input was also reflected by a leftward shift in the stimulus-response relationship (Figure 1C; see Experimental Procedures).
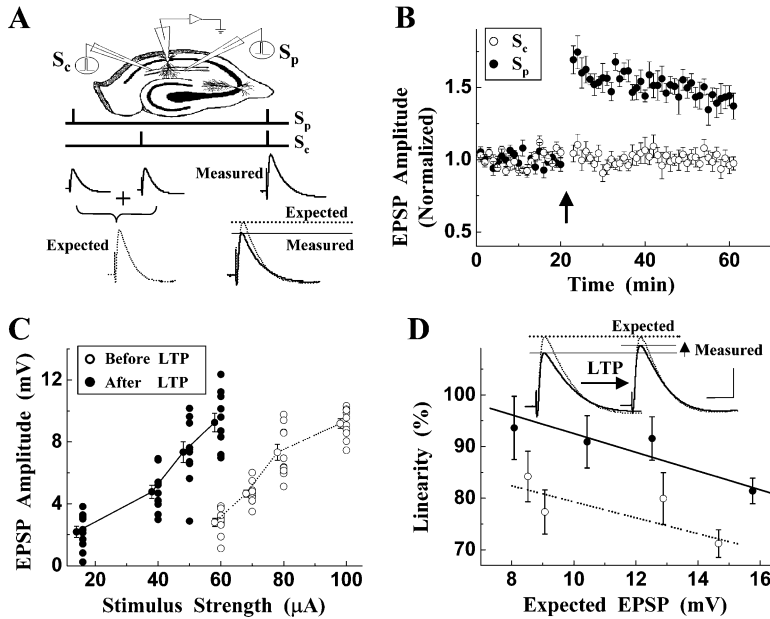
Figure 1. The Increase in the Linearity of Spatial Summation Following LTP Induction

(A) Spatial summation at the apical dendrite is sublinear. The schematic drawing depicts the recorded pyramidal cell in the CA1 region of the hippocampal slice, with two stimulating electrodes placed in the *s. radiatum*. The spatial summation of EPSPs at the two inputs (S_p and S_c) was examined by comparing the measured EPSPs (solid line), obtained by synchronous stimulation of the two inputs, with the expected EPSPs (dotted line), derived from the sum of the EPSPs evoked separately.

(B) Summary of the induction of LTP. Repetitive stimulation of S_p (20 Hz for 0.5 s, ten times at 4 s intervals, at the time marked by the arrow), each paired with a postsynaptic spike, initiated at 5 ms after the onset of each EPSP by injection of a brief depolarizing current (2 ms, 1.6 nA). The EPSP amplitudes were normalized by the mean amplitude observed during the control period (t = 0–20 min). Data depict averages (±SEM) from nine experiments.

(C and D) Results from one example experiment, showing the induction of LTP, reflected as a leftward shift in the stimulus-response curve in (C) and an increased linearity of summation in (D). Dendritic summation between the potentiated input (S_p) and the control input (S_c) was tested by applying a range of stimulus currents to S_p (ten repetitions at each stimulus strength at 0.04 Hz) and a fixed stimulus to S_c before and after the induction of LTP. The linearity is defined as the ratio (in percentage) of the amplitude of the measured EPSP (solid line) to that of the expected EPSPs (dotted line). Lines in (C) connect the average values (±SEM) of the responses at various stimulus strengths. Each point in (D) represents the average of ten measurements (±SEM) at one stimulus intensity. Lines in (D) depict the best linear fits of the data. Inset: average traces of all EPSPs in this experiment, showing that the measured EPSP (solid) was increased after LTP induction, for the same expected EPSPs (dotted). Scales: 4mV, 50 ms.

To measure dendritic summation of EPSPs elicited at S_p and S_c, a range of stimulus currents were applied to S_p before and after LTP induction, either alone to elicit a series of subthreshold EPSPs (range 2–10mV) or synchronously with stimulation of S_c at a constant strength that evoked EPSPs in the range of 4–7mV. After LTP induction, the range of stimulus strength applied to S_p was reduced in order to test the spatial summation with the control input over the same range of EPSP amplitudes. We found that the linearity of summation, as defined by the ratio of the measured EPSP to the expected EPSP, was markedly increased over the same range of amplitude tested (Figure 1D).

Changes in the Linearity of Summation Is Input Specific

To further determine whether the increase in the linearity of spatial summation of EPSPs accompanying the induction of LTP reflects a local dendritic change specific to the potentiated input, we have also monitored the spatial summation of the control, unpotentiated inputs. As shown in Figures 2A and 2B, the increase in linearity was observed for summation of the potentiated input (S_p) with a control input (S_{c1}) following LTP induction at S_p, but not for summation of the two control inputs (S_{c1} and S_{c2}) onto the same postsynaptic dendrite. To summarize the results from all experiments, the data from each cell were normalized by the linearity value observed at 10mV of the expected EPSP before LTP induction (Figures 2C and 2D), as estimated from the best-fit line (indicated by the arrow in Figure 2A). After LTP induction, a clear overall increase in the linearity was

observed for $S_p + S_{c1}$, but not for $S_{c1} + S_{c2}$. In a different format, we plotted the linearity determined at 10mV of the expected EPSP for each cell before and after LTP induction. It is clear that significant increase in the linearity was restricted only to the summation with the potentiated input (Figure 2E).

To determine whether the activity-induced changes in dendritic summation depend on the localization of the input on the apical dendrite, we further compared changes in the spatial summation between the potentiated input (S_p) with either a proximal (S_{c1}) or a distal (S_{c2}) control input, which was located at a distance of 50 ± 10 μm from S_p, as estimated from the position of the stimulating electrode relative to the pyramidal cell body layer. Results from all six experiments are shown in Figure 2F. We found that the linearity of summation was similarly increased for the summation of the potentiated input with either the proximal or the distal input. In another set of experiments, we measured the summation of a potentiated input at the distal dendrite with a control input on the basal dendrite. We observed no significant increase in the average linearity of summation (n = 7, data not shown).

Decrease in the Linearity of Summation Accompanying LTD

Beside LTP, repetitive correlated activity at Schaffer collateral-CA1 pyramidal cell synapses can also induce LTD (Markram et al., 1997; Bi and Poo, 1998; Nishiyama, et al., 2000). As shown in Figure 3A, significant reduction in the EPSP amplitude was induced at the input S_d by repetitive presynaptic stimulation (2 Hz, 50 s), each
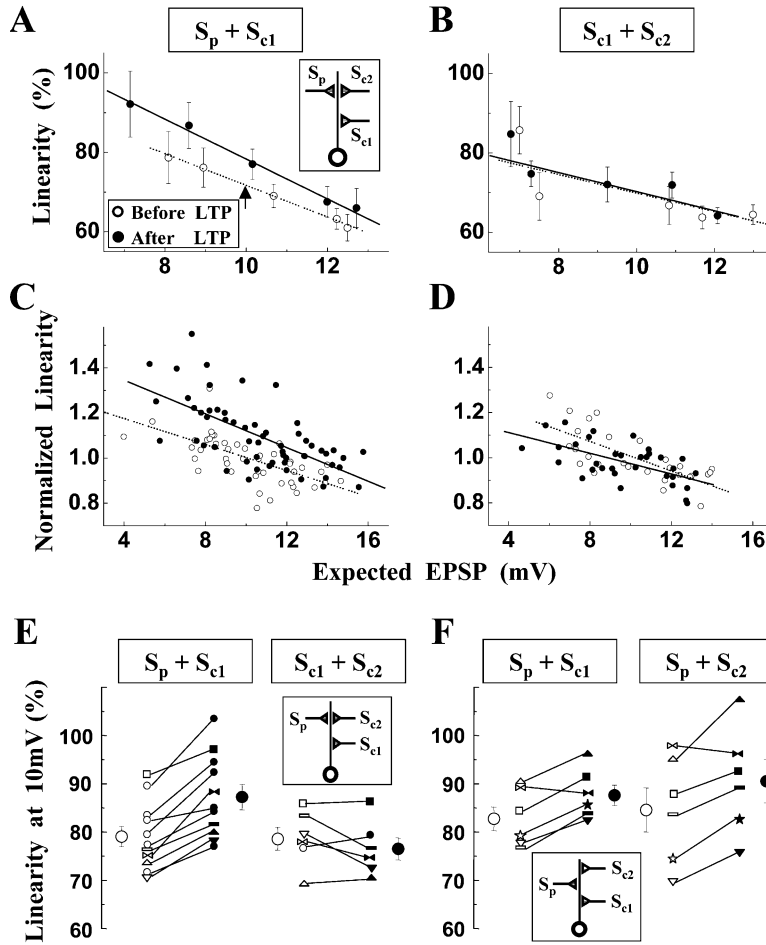
Figure 2. Position Dependence of the Increase in the Linearity of Summation

(A and B) An example of recording showing that after LTP induction an increase in linearity was observed for the summation of the potentiated input (S_p) with a control input (S_{c1}), but not for the summation of the two control inputs (S_{c1} and S_{c2}) to the same postsynaptic cell.

(C and D) Summary of all the experiments (n = 11 for S_p + S_{c1}, n = 6 for S_{c1} + S_{c2}) similar to that shown in (A) and (B). The data from each cell were normalized by the linearity at the expected EPSP of 10mV (marked by the arrow in [A]), with the error bars omitted for clarity. Dotted and solid lines represent the best linear fit of all data points before (open circles) and after (close circles) LTP induction, respectively.

(E) The linearity observed at 10mV expected EPSP before (open symbols) and after (closed symbols) LTP induction. Data from the same cell are connected by the lines and depicted by the same symbols, except circles, for which S_p + S_{c1} and S_{c1} + S_{c2} were tested in independent experiments. Data with error bars (±SEM) are averages. The difference was significant for S_p + S_{c1} (p < 0.001, paired t test), but not for S_{c1} + S_{c2}. The same data set as that shown in (C) and (D).

(F) Similar increase in the linearity was observed for the summation of a potentiated input (S_p) located 200 ± 10 μm from soma with a more proximal (S_{c1}, 150 ± 10 μm) or with a more distal (S_{c2}, 250 ± 10 μm) control input, respectively. Changes in the linearity of summation at 10mV after LTP induction were plotted. Data from the same neuron are depicted by the same symbols. Data with error bars (±SEM) are averages. There was significant increase in the linearity after LTP induction for both sets of data (p < 0.03, paired t test).

paired with a postsynaptic spike elicited 20 ms before the onset of the EPSP. The EPSP amplitude of the control input (S_c) elicited by a constant test stimulus remained unchanged. The stimulus-response curve of the stimulated input (S_d) also showed a rightward shift following the induction of LTD (Figure 3B). Using the same procedure as that described above, we found that the linearity of spatial summation of the stimulated (S_d) and control (S_c) inputs was reduced following the induction of LTD (Figures 3C–3E).

Induction of LTP/LTD of the excitatory inputs to these CA1 pyramidal neurons may involve changes in the density of AMPA subtype of glutamate receptors in the postsynaptic membrane (Lüscher et al., 1999; Carroll et al., 1999). Following the induction of LTP/LTD in our experiments, the strength of presynaptic stimulation was readjusted in order to measure spatial summation over the same range of EPSP amplitudes. This should alter the number of fibers activated, which in turn might cause the change in the linearity of summation. To test this possibility, we partially blocked AMPA receptors in these pyramidal neurons with a low concentration (0.2 μM) of receptor antagonist DNQX. Within 10 min after bath application of DNQX, the EPSP amplitude was

reduced to and remained stable at a level similar to that resulted from LTD. The test stimuli were then increased to evoke EPSPs with amplitudes similar to those during the control period. We found no significant change in the linearity of summation (−0.8% ± 1.3%, SEM; Figure 4C). Thus, the decrease in the linearity of summation after LTD induction was not due to a reduction of AMPA receptor density or to the change in the strength of test stimuli.

Postsynaptic Induction Mechanisms
Similar to the induction of LTP/LTD in the CA1 region of the hippocampus (Bliss and Collingridge, 1993; Malenka, 1994; Malenka and Nicoll, 1999), we found that changes in dendritic integration induced by correlated activity required activation of NMDA receptors. When the hippocampal slice was exposed to the NMDA receptor antagonist AP-5 (50 μM), correlated pre- and postsynaptic spiking failed to induce any enhancement or reduction of synaptic efficacy, and no change in the linearity of summation was observed (Figure 4A). In addition, we found that treatment of the slices with nimodipine (25 μM), an L-type calcium channel antagonist, abolished both the induction of LTD (Christie et al., 1997)
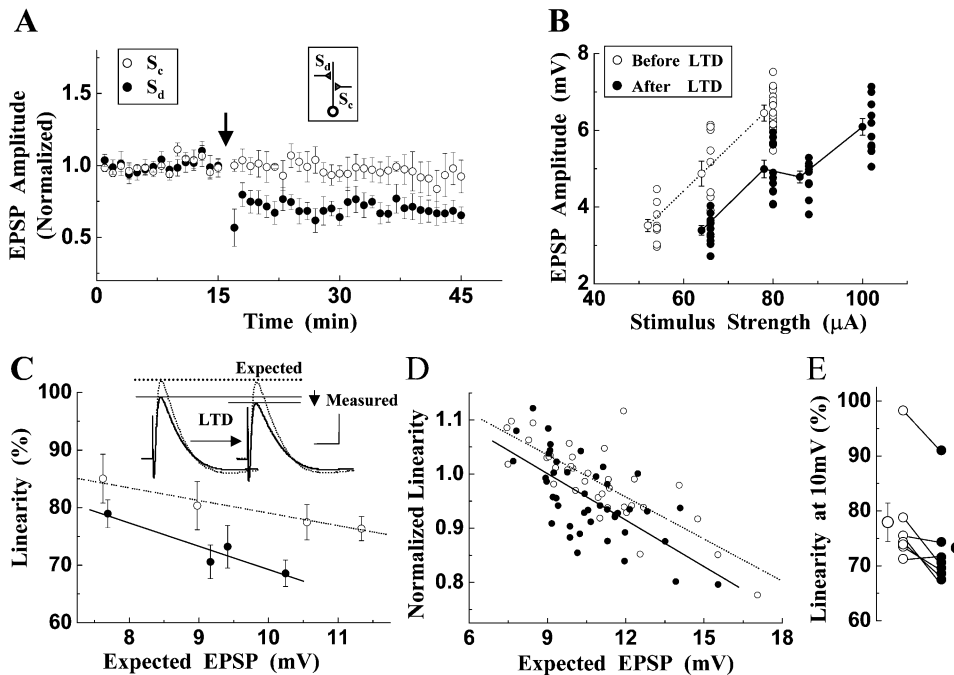
Figure 3. Decrease in the Linearity of Summation Following LTD Induction

(A) LTD was induced at one input (S_d) to the CA1 pyramidal neuron by repetitive correlated spiking similar to that used for inducing LTP (Figure 1B), except that the stimulus frequency was 2 Hz, and EPSPs were elicited 20 ms after postsynaptic spiking. A persistent reduction in the EPSP amplitude (normalized as in Figure 1B) was observed at the paired input (S_d), but not at the control input (S_c). Data depict averages (± SEM) from four experiments.

(B and C) Results from one example experiment, showing a rightward shift of the stimulus-response curve after LTD induction (B) and a reduction in the linearity of summation (C). Lines in (B) connect the average values (± SEM) of the responses at various stimulus strengths. Lines in (C) depict the best linear fits of the data. Inset: sample traces (averages of 40 events) depicting that, for the same individual EPSP amplitude of S_d and S_c (same expected EPSPs, dotted line), measured EPSPs elicited by synchronous stimulation of S_d and S_c (solid lines) were reduced after LTD induction. Scales: 3mV, 50 ms.

(D) Summary of all experiments (n = 7) similar to that shown in (B) and (C). The data were normalized as in Figure 2C, with the error bars omitted for clarity. Dotted and solid lines represent the best linear fits of the data before (open circles) and after LTD (close circles) induction, respectively.

(E) Changes in the linearity of summation after LTD induction were represented only for the linearity observed at the expected sum of 10mV. Data before (open circles) and after (close circles) LTD induction were connected by a line. Significant reduction in the linearity was found (p < 0.02, paired t test). Data with error bars (±SEM) are averages.

and the reduction in the linearity of summation (Figure 4A). Thus, changes in spatial summation and the induction of LTP/LTD appear to require activation of the same membrane receptors and channels. However, two lines of evidence suggest a divergence of mechanisms downstream from Ca^{2+} influx through NMDA receptors or L-type Ca^{2+} channels. First, we found that the degree of changes in the linearity of summation exhibited no significant correlation with the extent of LTP and a clear negative correlation with the extent of LTD (Figure 4B). Second, the increase in the linearity of summation induced by correlated activity was completely abolished when the slices were preincubated (for 1 hr) with a low concentration of an inhibitor of protein kinase C, GF109203X (50 nM) (Toullec et al., 1991), or an inhibitor of calcium/calmodulin kinase II (CaMKII), KN-93 (10 μM) (Tessier et al., 1999) (Figure 4D). On the other hand, these treatments only resulted in a small reduction in the extent of LTP (Figure 4D), consistent with the known dependence of LTP on the activity of these two enzymes (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). Thus, changes in the linearity of summation appear to depend on cytoplasmic processes that are more sensi-

tive to the inhibitor of PKC or CaMKII than those associated with the induction of LTP.

The Role of I_A K^+ and I_h Channels

It has been shown that E-S potentiation associated with the induction of LTP may result from either an activity-induced reduction of inhibitory inputs (Lu et al., 2000; Abraham et al., 1987; Tomasulo and Ramirez, 1993; Chavez-Noriega et al., 1989) or a local modulation of voltage-dependent ion channels near the potentiated synapses (Hess and Gustafsson, 1990; Asztely and Gustafsson, 1994; Jester et al., 1995). In the present study, the summation properties were examined in the presence of picrotoxin; thus changes in inhibitory inputs are unlikely to be involved. We have further examined whether changes in the ion channels are responsible for the alteration in dendritic summation. Of particular interest is the I_A potassium channel, which is known to exert significant effect on the sublinearity of summations in the apical dendrite of CA1 pyramidal neuron (Cash and Yuste, 1999; Hoffman et al., 1997) and to be downregulated by dendritic activation of PKA and PKC (Hoffman and Johnston, 1998). As shown in Figure 5A, we found a
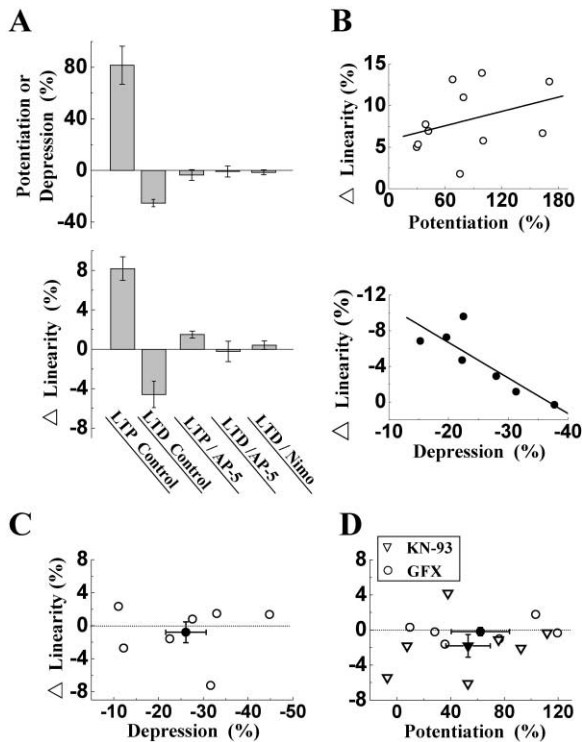
Figure 4. Divergence of Mechanisms for LTP/LTD and for Changes in Spatial Summation

(A) Results from the same set of experiments show similar dependence on the induction mechanisms. The extent of synaptic potentiation or depression is represented by the mean percentage increase or decrease (±SEM) in the EPSP amplitude at 20–30 min after correlated stimulation (top). Changes in summation are shown by the mean change in linearity (±SEM) at the expected EPSP amplitude of 10mV (bottom). LTP control: normal induction of LTP (n = 11). LTD control: normal induction of LTD (n = 7). LTP/AP-5: blocking LTP by preincubation with AP-5 (50 μM) (n = 4). LTD/AP-5: blocking LTD by preincubation with AP-5 (50 μM) (n = 5). LTD/Nimo: blocking LTD by preincubation with nimodipine (25 μM) (n = 4). All drugs were bath applied for at least 1 hr prior to the recording.

(B) Change in the linearity of summation plotted against the extent of synaptic potentiation/depression (percent change in the EPSP amplitude 20–30 min after correlated stimulation). No significant correlation (correlation coefficient: 0.36) was found for LTP (top), whereas significant negative correlation was found (coefficient: −0.85) for LTD (bottom).

(C) Change in the linearity of summation for each neuron plotted against the extent of synaptic depression 10 min after bath application of DNQX (0.2 μM). The DNQX treatment depressed the EPSPs to an extent similar to that associated with LTD, as shown in (A), but did not change the linearity of summation. Closed circle represents the mean values of the changes in the linearity and of the extent of synaptic depression (±SEM, n = 7).

(D) Preincubation of the slices (for 1 hr) with a low concentration of GFX (50 nM) or KN-93 (10 μM) abolished the increase in linearity, but only had a small effect on LTP. The degree of synaptic potentiation and the change in linearity are plotted for each neuron. The closed circle and triangle represent the mean values (±SEM) of the extent of synaptic potentiation and of the change in the linearity from the experiments using bath application of GFX (n = 6) or KN-93 (n = 7).

marked increase in the linearity of summation in the presence of 4-AP (6 mM), which is known to block most of I_A K$^+$ channels in these neurons (Hoffman et al., 1997).

Following the 4-AP treatment, we found that LTP was not inducible by correlated spiking at dendritic inputs

at about 200 μm from the cell body layer (Figure 1), but induction was observed for a more distal site of 280 ± 20 μm. By examining spatial summation of the potentiated input with a control input (200 ± 10 μm from the cell body layer), we found that, despite the increased linearity of summation during the control period at these distal sites, correlated spiking remained effective in further increasing both the linearity of summation and the synaptic efficacy, to the same extent as that in untreated slices (Figures 5A and 5B). Thus, changes in dendritic I_A K$^+$ channel activity are unlikely to account for the observed changes in spatial summation.

We further investigated the role of the hyperpolarization-activated (I_h) channel, which is known to be distributed at a high density on the apical dendrite (Magee, 1998; Stuart and Spruston, 1998) and to have a major influence on temporal summation of EPSPs (Magee, 1998, 1999). Interestingly, treatment with ZD 7288 (20 μM), a specific blocker of I_h channels, increased the linearity of spatial summation to a similar extent to that with 4-AP treatment (Figure 5A). Thus, I_h and I_A K$^+$ channels have similar influence on spatial summation by reducing the linearity. However, unlike the 4-AP treatment that blocks the I_A K$^+$ channels, we found that ZD 7288 treatment significantly reduced the magnitude of the increase in the linearity of summation accompanying the induction of LTP (Figures 5A and 5B). On the other hand, the extent of LTP in treated slices was similar to that in the untreated slices at the typical dendritic site of 200 μm (Figure 5B). These results suggest that a modification of the I_h channel activity may account for a major part of the increase in spatial summation after LTP induction.

It is known that dendritic I_h channels exert a significant effect on the duration of EPSPs (Magee, 1998). In the present study, we also found that blocking I_h channel prolonged the rising and decaying phases of the EPSPs (Figure 5C). In the presence of ZD 7288 (20 μM), the rise time (10%–90% of the peak amplitude) and the decay time (90%–10%) of the EPSPs were 9.7 ± 0.4 ms and 204.1 ± 12.8 ms (SEM, n = 7), respectively, which were significantly longer than those found in the untreated control (rise time = 5.9 ± 0.3 ms, decay time = 43.0 ± 2.8 ms, n = 18, p < 0.001, t test). Furthermore, we found that LTP induction, which presumably modified local dendritic I_h currents during EPSPs, significantly increased the rise time of EPSPs (Figure 5D). On the other hand, LTD induction resulted in a significant decrease in the rise time. In addition, the increase in the rise time of the EPSPs following LTP was largely blocked by ZD 7288 (Figure 5D), consistent with an alteration of the I_h current accompanying LTP induction. Treatment with 4-AP, which also broadened the EPSPs (rise time = 12.1 ± 1.0 ms, decay = 68.6 ± 14.5 ms, n = 7, p < 0.001, t test), had no effect on the increase in the rise time of EPSPs accompanying LTP induction (Figure 5D). Although changes in the linearity of summation and the rise time of EPSPs share the common locus of expression (the change in I_h channel activity), there was no clear correlation between their magnitudes after LTP/LTD induction (Figure 5E). This suggests that the two events are not causally linked. We found that there was no significant change in the decay time after LTP/LTD induction. This could be due to a large variation in the
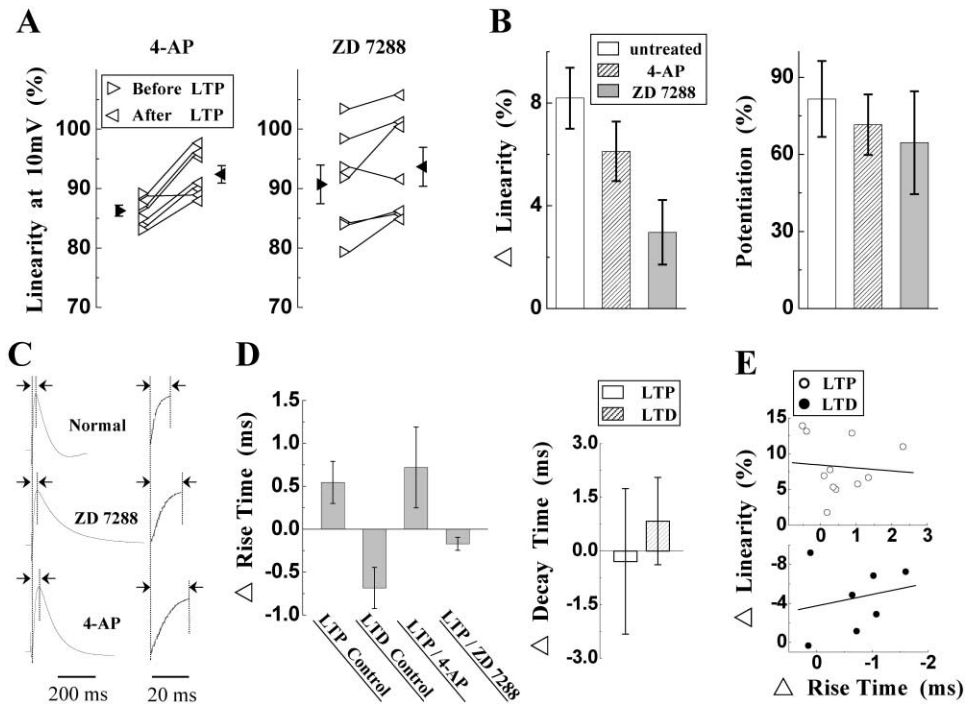
Figure 5. Involvement of I_A K^+ and I_h Channels in the Changes of Linearity

(A) Changes in the linearity at the expected sum of 10mV following the induction of LTP in the presence of 4-AP (6 mM) or ZD 7288 (20 μM). Note that both 4-AP and ZD 7288 treatments resulted in an increased linearity prior to the induction protocol (p < 0.01, t test, see Figures 2E and 3E). Values obtained before and after LTP induction from the same cell are connected by a line. Data with error bars (±SEM) are average values.

(B) Summary of all experiments using 4-AP (n = 7) or ZD 7288 (n = 7) treatment and those performed in untreated slices (n = 11, also shown in Figure 4A). Unlike 4-AP, the treatment of ZD 7288 significantly attenuated the increase in the linearity of summation (p < 0.01, t test), without significant effects on the LTP induction.

(C) Samples of typical EPSPs in the control, untreated slices and those treated with ZD 7288 (20 μM) or 4-AP (6 mM). The rising phase (between arrows) was also shown at a higher temporal resolution. Both the rise time and the decay time were significantly increased by bath application of ZD 7288 or 4-AP (p < 0.001, t test).

(D) Changes in the rise time and decay time of EPSPs of the same amplitudes before and after induction of LTP or LTD. LTP control: normal induction of LTP (n = 11). LTD control: normal induction of LTD (n = 7). LTP/4-AP: induction of LTP in the presence of 4-AP (6 mM) (n = 7). LTP/ZD 7288: induction of LTP in the presence of ZD 7288 (20 μM) (n = 7). Significant increase or decrease in the rise time, but not in the decay time, was found after the induction of LTP or LTD in the absence of the drug treatment (p < 0.05, paired t test), and the increase in the rise time after LTP induction was blocked by ZD 7288 (p < 0.05, t test), but not by 4-AP. All drugs were applied 1 hr before the onset of recording. All columns represent the mean ± SEM.

(E) No significant correlation was found between changes in the rise time and changes in the linearity of summation after the induction of LTP (open circles) or LTD (close circles). Data set are the same as that shown in Figures 2E and 3E.
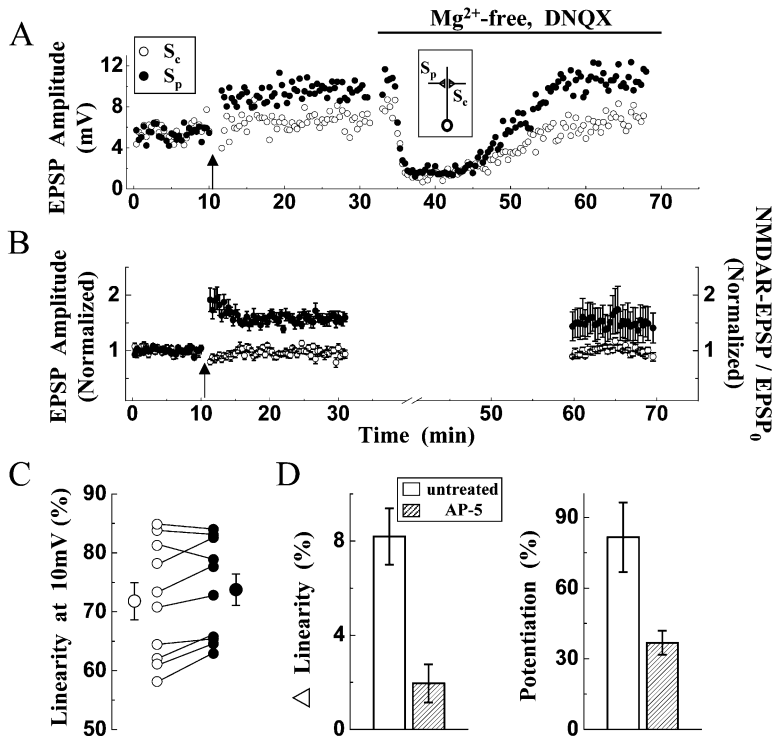
recorded decay time, which may mask small effects caused by local modification of I_h channel activity.

The Role of NMDA Receptors

Besides I_h channels, other conductances may also be involved in altering spatial summation, since in the presence of ZD 7288 there was still a residual increase in the linearity of summation associated with LTP induction. It is known that NMDA receptors can increase the linearity of spatial summation (Cash and Yuste, 1999), due to the cooperativity in the voltage-dependent removal of magnesium block. Following the induction of CA1 hippocampal LTP by tetanic stimulation or by pairing presynaptic stimulation with postsynaptic depolarization, there is an increase in the NMDA receptor-mediated EPSP (NMDAR-EPSP) (Kullmann et al., 1996). Such an upregulation of NMDA receptors may further facilitate the summation of EPSPs after LTP induction.

We first examined whether LTP induced by correlated pre- and postsynaptic spiking can also result in an increase in the NMDAR-EPSP. As shown in Figures 6A and 6B, two pathways at the same distal level, S_p (potentiated) and S_c (control), were examined. After LTP was induced at S_p, the extracellular solution was replaced with Mg^{2+}-free solution containing 10 μM DNQX, and the total NMDAR-EPSP was recorded. We found that, after LTP induction, the ratio of the amplitude of the total NMDAR-EPSP to the pre-LTP amplitude of the normal EPSP ($EPSP_0$) was significantly increased at S_p, as compared to that at S_c (Figures 6A and 6B), indicating that there was an increase in NMDAR-EPSP after correlated spiking (Kullmann et al., 1996).

Further experiments were performed to examine whether blocking NMDA receptors affects the changes in the linearity of summation associated with LTP induction. Summation was first measured in the presence of AP-5 (50 μM), followed by 20 min of washing to remove
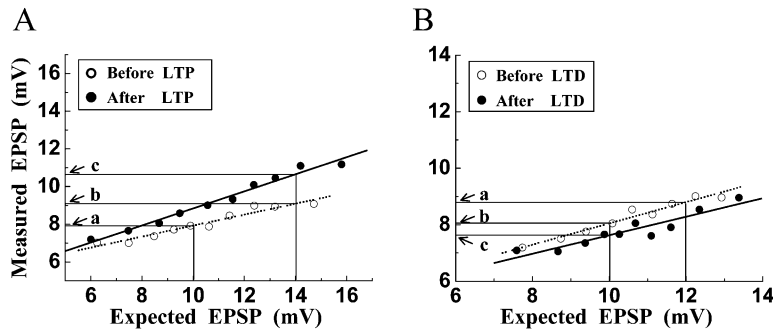
Figure 6. NMDA Receptors Contribute to the Increase in the Linearity of Summation

(A) An example showing increased NMDA receptor-mediated EPSP after LTP induction. LTP was induced at S_p located at 200 μM distal dendrites at the time depicted by the arrow. Another input (S_c) was at the same distal level as S_p and was not stimulated during induction. After the LTP induction protocol, the extracellular solution was replaced with a Mg^{2+}-free solution containing DNQX (10 μM). The response first diminished to a level near zero and then slowly increased as the Mg^{2+} block was washed out from the NMDA receptors. After the responses increased to a stable level, we found a larger NMDAR-EPSP at S_p (close circles) than at S_c (open circles).

(B) Summary of all results from experiments similar to that shown in (A) (mean ± SEM, n = 9). Data from S_p (close circles) and S_c (open circles) were normalized by the average amplitude of the EPSPs at each input before LTP induction ($EPSP_0$). The data after the drug treatment (Mg^{2+} free and DNQX) were further normalized by setting the initial normalized value ($NMDAR-EPSP/EPSP_0$) at S_c to 100% (see Kullmann et al., 1996). During the drug treatment, the responses before the stabilization of NMDAR-EPSPs were omitted, because different cells show different rise times to a stable response. Before LTP induction, the stimulus strength at S_p was adjusted to evoke EPSPs with similar sizes (95% ± 2.5%, SEM) to those at S_c. The arrow marks the time of LTP induction.

(C) Changes in the linearity of summation recorded in the presence of AP-5 (50 μM) before (open circles) and after (close circles) LTP induction at the expected sum of 10mV. Data from the same cell were connected by a line. After a control test on summation in the presence of AP-5, LTP was induced after 20 min washing of AP-5, followed by a second measurement of the linearity of summation after reapplication of AP-5. Note that the treatment of AP-5 reduced the pre-LTP linearity (p < 0.05, t test, see Figures 2E and 3E). Data with error bars (± SEM) are mean values.

(D) Summary of all the results with AP-5 treatment shown in (C) (n = 10) and those from untreated slices (also shown in Figure 4A). Under such AP-5 treatment, there was marked reduction in both the increase in the linearity of summation (p < 0.001, t test) and synaptic potentiation (p < 0.02, t test), although a significant increase in the linearity remained (p < 0.03, paired t test). Each column represents mean ± SEM.

AP-5, subsequent induction of LTP, and then a second measurement of the linearity of summation after reapplication of AP-5. Under this procedure, we found that both the increase in the linearity and the extent of LTP were significantly reduced (Figures 6C and 6D). The reduced extent of LTP is likely to result from incomplete washing of AP-5. We also found that the pre-LTP summation was less linear than that found in the untreated slices (Figure 6C), consistent with the effect of NMDAR on the summation. Thus the increase in the NMDAR-EPSP after LTP induction may contribute to the increased linearity. Significantly, there was still a residual increase in the linearity under the AP-5 treatment (Figures 6C and 6D), suggesting involvement of other mechanisms, such as changes in the I_h currents described above.

Discussion

Integration of synaptic inputs and initiation of action potentials are essential for signal processing in the neuron. Spatial and temporal summation of excitatory synaptic potentials at the dendrite, which determines the spiking activity of the postsynaptic neuron, is known to be nonlinear in the CA1 pyramidal neurons of the hippocampus (Rall, 1964; Magee, 1999, 2000; Cash and Yuste, 1999; Nettleton and Spain, 2000, Wessel et al., 1999). In the present study, we showed an activity-dependent plasticity in the property of spatial summation of EPSPs between converging inputs at the dendrite of these pyramidal cells. Repetitive correlated pre- and postsynaptic spiking, which is effective in inducing LTP or LTD of synaptic transmission, results in an increase or decrease in the linearity of summation, respectively. This bidirectional modification is specific to the summation of the modified input with other coincident inputs on the same postsynaptic dendrite. The changes in the linearity, averaged +8.2% and −4.6%, respectively, were persistent for at least 30 min after a brief period of correlated spiking. The immediate consequence of such changes in the summation property is an enhancement or decrement in the weight of the modified synapse in initiating firing of the postsynaptic pyramidal neuron. In terms of its role in signal processing, this effect is independent of, but synergistic with, the activity-dependent modification of synaptic efficacy.

A small change in the linearity of spatial summation can exert a significant effect on the information relay at the dendrite. Because dendritic summation is sublinear, and the sublinearity increases with the EPSP amplitude (Figure 1D), a synaptic input is represented less effectively in the dendrite when it is summed together with

Figure 7. Consequences of the Changes in Linearity in Information Relay at the Dendrite

(A) Correlation between the amplitudes of measured and expected EPSPs (for summation of two inputs) before (open circles, dotted line) and after (close circles, solid line) LTP induction at one input. Each dot represents the average value of the measured and expected EPSPs from the summation tests from 11 experiments with the expected EPSPs within the same amplitude bin. The lines depict the best linear fit of the data. Arrows indicate the amplitudes of the measured EPSPs at the expected amplitude of 10 mV before LTP induction (a), and of 14 mV before (b) and after (c) LTP induction, respectively.

(B) Correlation between the amplitudes of the measured and the expected EPSPs before (open circles, dotted line) and after (close circles, solid line) LTD induction. Each dot represents the average value of the measured and expected EPSPs from seven experiments. Arrows indicate the amplitude of measured EPSPs at the expected amplitude of 12mV before LTD induction (a), and of 10mV before (b) and after (c) LTD induction, respectively.

other inputs. This effect is best discerned by examining Figure 7, which plots the correlation between measured and expected EPSPs before and after LTP/LTD induction for all the data (without drug treatments) obtained in the present study. Considering the summation of two 5mV inputs at a pyramidal neuron, the measured sum is about 8mV (Figure 7A). Prior to LTP induction, a 4mV increment at one input results in only a 1–1.5mV increment in the measured summed EPSP (Figure 7A, arrows a to b). Following LTP induction, however, a 4mV increment at the potentiated input will result in an additional 1–1.5mV (or doubling) increment in the summed EPSP (Figure 7A, arrows b to c) because of the increase in linearity. Similarly, a 2mV decrease at one input prior to LTD induction causes 0.5–1mV decrement in the measured sum (Figure 7B, arrows a to b), and an additional decrement of 0.5mV in the sum occurs after LTD induction (Figure 7B, arrows b to c) because of the reduced linearity of summation. Thus, changes in the linearity of summation associated with LTP/LTD, an effect caused by local modifications of the active conductances beyond the strengthening/weakening of the synaptic connection, can increase the fidelity of information relay at the potentiated/depressed input.

It is known that postsynaptic spiking induced by synaptic inputs is elevated after LTP induction, a phenomenon known as E-S potentiation (Bliss and Lømo, 1973; Andersen et al., 1980; Pugliese et al., 1994; Chavez-Noriega et al., 1990). There is evidence that E-S potentiation is caused by depressed inhibitory inputs (Lu et al., 2000; Abraham et al., 1987; Tomasulo and Ramirez, 1993; Chavez-Noriega et al., 1989). However, other reports have shown the persistence of E-S potentiation in the absence of inhibitory inputs (Hess and Gustafsson, 1990; Asztely and Gustafsson, 1994; Jester et al., 1995) and have suggested that local changes in intrinsic excitability of the postsynaptic neurons underlie E-S potentiation. In hippocampal cultures, induction of LTP is accompanied by an increased intrinsic excitability of the presynaptic neuron, as a result of changes in the Na^+ channel kinetics (Ganguly et al., 2000). This presynaptic modulation appears to be a global retrograde effect that requires postsynaptic activation of NMDA receptors and Ca^{2+} influx. The present results further provide evidences that induction of LTP/LTD is accompanied by

local changes of at least two types of active conductances, I_h channels and NMDA receptors, that modify the properties of summation between the modified input and other converging inputs. Although most of the increase in the linearity appeared to be eliminated when the summation was examined in the presence of AP-5 (Figures 6C and 6D), a large part of this effect may be attributed to the fact that the extent of LTP was significantly lower than that in the untreated slices, due to the difficulty in complete clearing of AP-5. The actual contribution of NMDA receptors to the increase in linearity is thus likely to be much smaller than the apparent effect shown in Figures 6C and 6D.

It was reported that the presynaptic I_h currents play an important role in the expression of hippocampal mossy fiber LTP (Mellor et al., 2002). Our results indicate that induction of CA1 LTP causes a downregulation of the effect of postsynaptic I_h channel activity on the summation of EPSPs, resulting in a significant increase in the linearity of dendritic summation. This conclusion is supported by the observation that the increase in the linearity associated with LTP induction was significantly eliminated in the presence of I_h channel blocker ZD 7288. Furthermore, the increase in the rise time of EPSP following LTP induction was also blocked by ZD 7288. The voltage-dependent activation curve for I_h channels is known to be sensitive to the intracellular adenylate cyclase or cAMP activity (Ingram and Williams, 1996; Pape, 1996). Calcium entry via NMDA receptors induced by the correlated activity may modify the I_h channel activity via activation of postsynaptic Ca^{2+}-dependent adenylate cyclase. This in turn could result in both a more linear summation and a longer rise time of EPSPs, by downregulating the effect of I_h currents on these events.

In the present study, we found that the extent of LTP did not correlate with the magnitude of the increase in linearity of summation. Surprisingly, we observed a strong negative correlation between the reduction in linearity and the magnitude of LTD. This finding is reminiscent of the negative correlation between the stimulation frequency (or the level of postsynaptic Ca^{2+}) and the magnitude of LTD during the transition between LTD to LTP, as described by the BCM rule (Bienenstock et al., 1982; Dudek and Bear, 1992). Furthermore, when LTP was induced under a partial inhibition of PKC or

CaMKII by GFX or KN-93, no increase in the linearity of summation was observed. Thus, modifications of synaptic efficacy and dendritic summation are not causally linked and require activation of different cytoplasmic signaling pathways. Different patterns of electrical activity or neurotrophic modulation may trigger differential modifications of these two separate aspects of neuronal signal processing.

Experimental Procedures

Slice Preparation
Hippocampal slices were prepared as described previously (Kato et al., 1993). Sprague-Dawley rats (~14–22 days old) were anaesthetized with sodium pentobarbital. After decapitation, hippocampal formation was dissected rapidly and placed in ice-cold oxygenated solution containing 119 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl$_2$, 1.3 mM MgSO$_4$, 1 mM NaH$_2$PO$_4$, 26.2 mM NaHCO$_3$, and 11 mM glucose, pH 7.4. Transverse slices (400 μm thick) were cut with a tissue chopper (MalLWAIN, Mickle Lab) and maintained in an incubation chamber for at least 2 hr at room temperature before recording. During the experiments, individual slices were transferred to a submersion recording chamber and, where they were continuously perfused with the above extracellular solution (~4.0–5.0 ml/min) at 28°C–29°C. Slices were visualized with an Olympus microscope (DX50WI), using infrared video microscopy and differential interference contrast optics.

Electrophysiology
All experiments were carried out in the presence of a GABA$_A$ antagonist, picrotoxin (100 μM). Perforated whole-cell recordings with amphotericin B (Sigma, 0.5 mg/ml) were made from pyramidal neurons in the cell body layer of CA1, using a patch-clamp amplifier (Axopatch 200B, Axon Instruments). Patch pipettes were pulled from 1.2 mm outside diameter borosilicate glass and had a resistance of ~3.5–4.5 MΩ. Pipettes were routinely filled with a solution containing 136.5 mM K-Gluconate, 17.5 mM KCl, 9.0 mM NaCl, 1.0 mM MgCl$_2$, 10.0 mM HEPES, and 0.2 mM EGTA, with pH adjusted to 7.3 using KOH. The resting membrane potential was about −65mV, except those in the presence of ZD 7288, which was about −75mV. The membrane potential was maintained at a level of about −75mV by injection of a constant current (<100 pA). The membrane potential for most neurons was stable throughout the recording. The input resistance was 100–200 MΩ and was checked repeatedly in some neurons by measuring the voltage response to a step-hyperpolarizing current pulse (30 pA, 150 ms) throughout the experiments. In all cases for which input resistance was monitored, we found no change in the input resistance during control recording and after LTP/LTD induction (n = 11). To test whether break-in occurred during our perforated-patch recording, we performed experiments in which 5 mM TEA was included in the intracellular solution throughout the experiment. For all neurons recorded (n = 8), the same membrane potential as that observed in the absence of TEA was found. Glass pipettes (tip diameter 2–3 μM) filled with extracellular solution were used as extracellular-stimulating electrodes and were connected to stimulators (S88K, Grass Instruments) and isolators (SIU7, Grass Instruments). Monosynaptic EPSPs were elicited with the latency between stimulus and EPSP onset less than 3 ms. In a typical experiment, two stimulating electrodes were placed in the *s. radiatum* at distances of about 150 (S$_c$) and 200 μm (S$_p$), respectively, distal from the cell body layer, with the distance of at least 300 μm between the two electrodes. Paired-pulse test (with a 50 ms interval) was used to examine the independence of two different inputs. In all tests using the stimulating pipettes described above, we have never detected any overlap in the stimulated fibers. This absence of a significant overlap was also supported by the finding that LTP/LTD observed was always specific to the stimulated input, even when the control input was at the same distal level as the potentiated/depressed input. GF109203X and KN-93 were purchased from Calbiochem, and ZD 7288 was from Tocris. All other chemicals were from Sigma.

Data Collection and Analysis
Data were recorded at least 20 min after pipette seal on the soma, and filtered at 1 kHz, digitized, and stored by Axoscope 8.01 and a 1200a A/D board (Axon Instruments). In a typical measurement of the linearity of summation, an initial strength of stimulus was chosen to evoke the individual EPSPs with amplitude in the range of 4–7mV at S$_p$ (input to be potentiated) or S$_d$ (input to be depressed), followed by stimuli at other strengths (ten repetitions at 0.04 Hz for each measurement of EPSP amplitude). Stimulation with the initial stimulus strength was repeated prior to the induction of LTP or LTD. The extent of LTP/LTD is estimated by the percentage change in the EPSP amplitude evoked by the initial stimulus strength at 20–30 min after the correlated spiking as compared to that observed during the control period prior to the induction. The linearity of summation was defined as the ratio (in percentage) of the measured peak amplitude of EPSPs evoked by simultaneous activation of the two inputs (measured sum) to the arithmetic summation of two individual EPSPs elicited by stimulating two inputs separately (expected sum). For the EPSPs of measured and expected summation, we also measured the linearity by comparing the amplitudes at a fixed time, i.e., the peak time of EPSPs evoked at the control input. Comparing these two approaches of the measurements of linearity, there was slight difference in the value (< 5%), which occurred with similar magnitude to both the summation before and after LTP/LTD induction, thus did not affect the estimated changes in the linearity after LTP/LTD induction.

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