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Bidirectional Long-Term Modification of Synaptic Effectiveness in the Adult and Immature Hippocampus

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Previously we showed that delivering 900 pulses to the Schaffer collateral–CA1 pathway at 1–3 Hz causes a lasting depression of synaptic effectiveness that is input specific and dependent on NMDA receptor activation (Dudek and Bear, 1992a). Here we describe experiments aimed at further characterizing this homosynaptic long-term depression (LTD) and comparing it with long-term potentiation (LTP). To address the question of whether depressed synapses can still be potentiated and vice versa, LTP was saturated with repeated high-frequency tetani, and then LTD was induced with low-frequency stimulation (LFS). A second strong tetanus then restored the potentiation, indicating that the same synapses whose transmission had been depressed by LFS were capable of subsequently supporting potentiation. In a complementary experiment, LTD was induced first and then a strong high-frequency tetanus was delivered. We found that the resulting LTP achieved the same absolute magnitude as that observed in control slices that had received the high-frequency stimulation alone. Next, the postnatal development of LTD was investigated in slices prepared from rats at 6–35 d of age. The consequences of LFS were far more pronounced in slices from young rats. LTD following 900 pulses at 1 Hz measured −45 ± 4% in CA1 of rats less than 2 weeks old as compared with −20 ± 4 in animals at 5 weeks postnatal. It was also found that LTD precedes the developmental onset of LTP in CA1. Finally, we addressed the question of whether LTD could be saturated by repeated episodes of LFS in slices prepared from 3-week-old rats. It was observed that a floor to the LTD effect is reached after ~1800 pulses and measures approximately −50%. From these data, we estimate that the dynamic range over which the population of Schaffer collateral synapses can be modified from a naive state under our experimental conditions is roughly ±50%.

[Key words: synaptic plasticity, development, long-term potentiation, long-term depression, hippocampus, NMDA receptors]

The phenomenon of long-term potentiation (LTP), first described by Bliss and Lømo (1973), has attracted the intense interest of experimentalists and theoreticians alike. The experimental appeal stems partly from the fact that this form of synaptic plasticity can be evoked in brain slices under rigorously controlled conditions. This approach has led to a significant elucidation of the mechanisms of LTP induction, particularly in the Schaffer collateral synapses onto CA1 pyramidal cells in the hippocampus (reviewed by Nicoll et al., 1989; Gustafsson and Wigström, 1990). The theoretical appeal of LTP is that it fulfills the requirements of "Hebbian" synaptic modification, which has been shown to be a powerful means to store associative memories in neural networks.

A key property of Hebbian synaptic plasticity and LTP is "input specificity," which is to say that only those synapses that have undergone conditioning stimulation are potentiated (Bliss and Lømo, 1973). Inputs onto the same postsynaptic neuron that are inactive during conditioning stimulation generally fail to show LTP. Indeed, under some stimulation conditions, other converging inputs show a modest but significant depression of synaptic effectiveness (Lynch et al., 1977; Levy and Steward, 1979; Abraham and Goddard, 1983; Bradler and Barrionuevo, 1990). This effect has been termed "heterosynaptic depression" because it depends on a burst of activity generated at synapses other than the ones modified. Heterosynaptic depression appears to be triggered by strong postsynaptic depolarization (Pockett et al., 1990), is promoted by NMDA receptor activation (Abraham and Wickens, 1991; Desmond et al., 1991; but see Bradler and Barrionuevo, 1990), and may be mediated by calcium entry via voltage-sensitive channels (Wickens and Abraham, 1991).

For theoretical reasons (i.e., Sejnowski, 1977; Cooper et al., 1979; Bienenstock et al., 1982; Willshaw and Dayan, 1990), a second form of long-term depression (LTD) that shares with LTP the property of input specificity has also been sought. The first report of such a "homosynaptic" LTD came in 1989 when Stanton and Sejnowski presented evidence that concurrent input activity and postsynaptic hyperpolarization caused a lasting reduction in the effectiveness of the stimulated pathway in CA1. Unfortunately, this effect proved to be difficult to replicate (Stevens, 1990), possibly because it depends on additional factors that are not yet fully understood (cf. Yang and Faber, 1991; Christie and Abraham, 1992). Artola et al. (1990) and Hirsch and Crepel (1990) subsequently presented data from neocortical slices suggesting that homosynaptic LTD requires (1) that input activity coincide with postsynaptic *depolarization* and (2) that

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NMDA receptors *not* be recruited by the stimulation. Very recent data suggest that this effect may also be triggered by voltage-gated Ca^{2+} entry (Bröcher et al., 1992). These results imply that strong stimulation in the presence of an NMDA receptor antagonist should be a sufficient condition to induce homosynaptic LTD. However, a direct test of this prediction in CA1 by Goldman et al. (1990) yielded negative results. Thus, the questions of whether homosynaptic LTD can be evoked reliably in the cerebral cortex and, if so, whether it has similar properties in hippocampus and neocortex have remained unsettled.

Work in CA1 had shown that prior LTP could be "depotentiated" by sustained periods of low-frequency stimulation (LFS; Barrionuevo et al., 1980; Staubli and Lynch, 1990; Fujii et al., 1991). We adopted this protocol for experiments in virgin (unpotentiated) hippocampal slices, and found that it produces a modest (~20%) but reliable depression of Schaffer collateral synaptic transmission in CA1 (Bear et al., 1991; Dudek and Bear, 1992a). Like LTP, this synaptic depression persists for many hours *in vitro*, is input specific, and depends on NMDA receptor activation for its induction (Dudek and Bear, 1992a). This homosynaptic LTD is also reproducible; it has been replicated very recently in CA1 by Mulkey and Malenka (1992a) and in visual cortex by Kirkwood et al. (1992).

Here we describe experiments in CA1 aimed at further characterizing this form of LTD. We address three specific questions. First, to what extent is there an interaction between the processes responsible for LTD and LTP, and does one form of plasticity reverse the other at the same synapses? Second, what is the developmental time course of LTD, and how does this compare with that reported for LTP? Third, can LTD like LTP be saturated? Our data suggest (1) that depressed synapses can still be potentiated and vice versa, (2) that LTD is more prominent early in postnatal development and precedes the onset of LTP, and (3) that LTD can be saturated. Our results support a model in which high- and low-frequency stimulation modify synaptic effectiveness at a common site.

These data were presented at the 1992 Society for Neuroscience meeting (Dudek and Bear, 1992b).

Materials and Methods

The experiments described in this article were performed on transverse slices prepared from the hippocampi of adult (≥150 gm) or immature albino rats of both sexes. Each animal was given an overdose of sodium pentobarbital (~75 mg/kg, i.p.) and was decapitated soon after the disappearance of any corneal reflexes. The brain was rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl, 124; KCl, 5; NaH_2PO_4, 1.25; $MgCl_2$, 1.5; $CaCl_2$, 2.5; $NaHCO_3$, 26; and dextrose, 10. The hippocampus was dissected free and sectioned into 0.4-mm-thick slices using a vibrating microtome. These slices were collected in ice-cold ACSF and gently transferred to an interface slice chamber. Here, the slices were maintained in an atmosphere of humidified 95% O_2, 5% CO_2, and superfused at a rate of 1 ml/min with 35°C ACSF saturated with 95% O_2, 5% CO_2.

After at least 1 hr of equilibration in the slice chamber, a concentric bipolar stimulating electrode was placed in the trajectory of the Schaffer collateral fibers projecting to the stratum radiatum of area CA1. In some experiments a second stimulating electrode was placed on the opposite (subicular) side of the recording location in order to activate a second converging input. The recording pipette was filled with 1 M NaCl and was placed in the apical dendritic layer of CA1. Population EPSPs were evoked using 10–30 μA stimuli of 0.2 msec duration. These responses were digitized at 20 kHz and stored on a computer. The initial slope of the population EPSP was extracted as a measure of the magnitude of the response.

At the start of each experiment, a full input–output curve was constructed. A stimulation intensity was selected for baseline measurements that yielded between one-half and two-thirds of the maximal response (these population EPSPs were not accompanied by population spikes). In general, slices were studied only if the maximal population EPSP amplitude was ≥2 mV and, as a rule, slices were studied for no longer than 6 hr *in vitro*. Baseline measurements were collected using single shocks every 15–30 sec. The conditioning stimulation for LTD consisted of 900 pulses delivered at 1 Hz, which we showed previously to be effective in producing LTD in virgin preparations (Dudek and Bear, 1992a). The conditioning stimulation for LTP consisted of 3–4 epochs of theta burst stimulation (TBS) delivered at 0.1 Hz. TBS consists of 10 stimulus trains delivered at 5 Hz; each train consists of four pulses at 100 Hz. When needed, an F test was used to confirm that any change in the response after conditioning could not be explained by a linearly drifting baseline (Bear et al., 1992).

Results

Potentiation of previously depressed responses

An example of stable LTD following 900 pulses of 1 Hz stimulation is illustrated in Figure 1. This experiment was performed using a virgin hippocampal slice prepared from an adult rat. Previously, we offered several lines of evidence that this effect was indeed a manifestation of synaptic plasticity rather than fatigue of or damage to the stimulated synapses (Dudek and Bear, 1992a). For example, the depression is frequency dependent; no LTD is produced by the same number of pulses at 10 Hz. This coupled to the fact that the LTD is prevented by application of antagonists of postsynaptic NMDA receptors indicated that the decrease in synaptic effectiveness was not caused by presynaptic fatigue or neurotransmitter depletion. Nonetheless, the possibility remained that the conditioning stimulation caused some irreversible postsynaptic change, perhaps damage to the dendritic spines due to prolonged NMDA receptor activation. Arguing against this possibility was the observation, also illustrated in Figure 1, that pathways with depressed synapses can still support robust LTP following TBS. However, because the measured responses reflect the activity of a large and heterogeneous population of synapses, the demonstration of LTP after LTD cannot be taken as evidence that the same synapses that underwent LTD can subsequently be potentiated.

In order to address this issue further, we took advantage of the fact that LTP can be saturated (Bliss and Lømo, 1973), this is to say, that the synaptic strength of a tetanized input has a certain "ceiling" that cannot be exceeded once the physical limit has been reached in the synaptic parameter(s) that is modified to produce the potentiation. If LTD reflects the adjustment of the same synaptic parameter(s), but in the opposite direction as LTP, then it should be possible to "unsaturate" LTP with 1 Hz conditioning stimulation. And then, by necessity, any potentiation caused by subsequent high-frequency stimulation must be occurring at the same synapses that had previously undergone depression (depotentiation).

This reasoning led us to perform the experiments illustrated in Figure 2. Figure 2*A* shows an example of the effects of two distinct episodes of TBS separated by a 2 hr interval. The first TBS led to LTP that stabilized at a value of approximately 140% of the preconditioning baseline response. In contrast, the second TBS caused little additional potentiation. The long interval between tetani makes it unlikely that this effect is explained by the desensitization of LTP induction mechanisms that has recently been demonstrated (Huang et al., 1992); rather, it appears that the first TBS was sufficient to saturate the LTP. Figure 2*B* illustrates an experiment that was identical to that in Figure 2*A*, with the only difference being that LFS (i.e., 900 pulses at 1 Hz) was given 10 min after the first TBS. This example illustrates

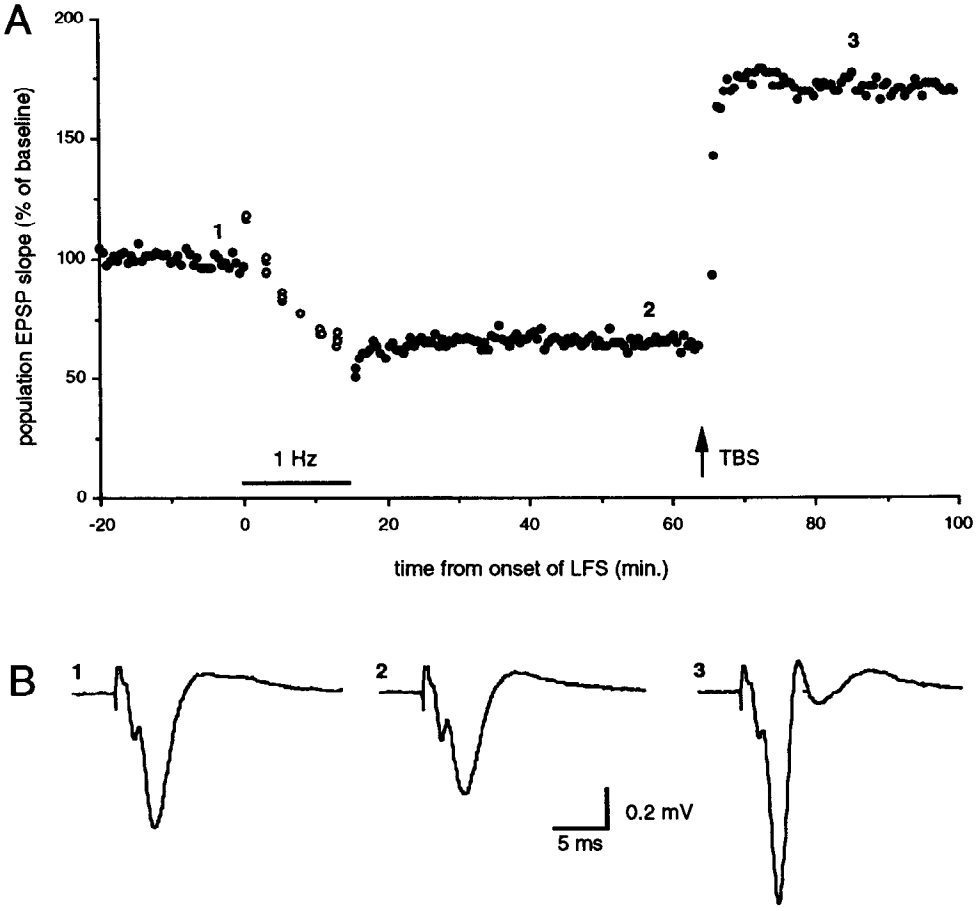

Figure 1. Example of LTD evoked in the Schaffer collateral–CA1 pathway. *A*, Plot of population EPSP initial slope measured every 30 sec. At time zero, 1 Hz stimulation was applied for 15 min (900 pulses). *Open circles* show selected responses measured during the conditioning stimulation. At the completion of the 1 Hz conditioning, baseline measurements were resumed at 0.03 Hz. Note the persistent depression of the response to baseline stimulation. At the *arrow*, "theta burst" stimulation (TBS) was applied, and LTP resulted. *B*, Field potentials (averages of 10 consecutive sweeps) taken before 1 Hz stimulation (*1*), after 1 Hz stimulation (*2*), and after the TBS (*3*). The times at which the illustrated sweeps were taken are indicated in *A*.

the reduction of potentiated responses by LFS, as had been reported previously (Staubli and Lynch, 1990; Fujii et al., 1991). However, also illustrated here is that the reduction of the potentiated response after LFS permits further potentiation by a second TBS. The group data are illustrated in Figure 2*C*, where the magnitude of the potentiation measured 30 min after each TBS was calculated relative to the baseline period just prior to each TBS. In control experiments ($n = 4$) we found that the first TBS produced a potentiation of 43 ± 7% above baseline while the second TBS produced an additional increase of only 5 ± 1%. In contrast, the second TBS that followed LFS ($n = 5$) caused a potentiation that measured 22 ± 5%. We interpret these data to mean that the LFS unsaturated the LTP established by the first TBS and, further, that the subsequent potentiation caused by the second TBS occurred at the same synapses that had undergone depression.

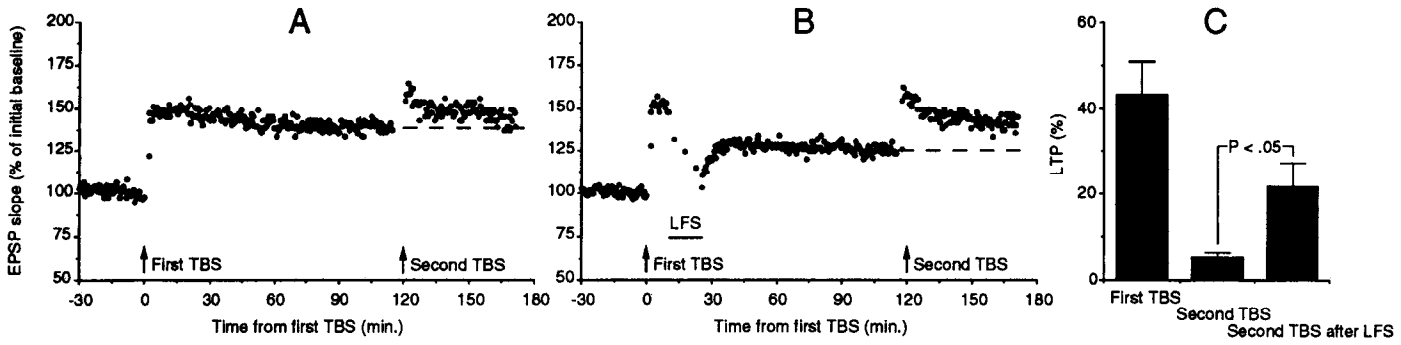

Figure 2. Evidence that depressed synapses can be potentiated. *A*, Plot of EPSP slope versus time in one representative experiment in which TBS was applied twice with a 2 hr interval. Note that the first TBS nearly saturates the LTP; little additional potentiation is caused by a second tetanus. *B*, Plot of another experiment identical to that shown in *A* with the exception that 900 pulses at 1 Hz were delivered 10 min after the first TBS. In this case, subsequent TBS 2 hr later was able to reverse the depression caused by the LFS. *C*, Summary data where the magnitude of the potentiation measured 30 min after each TBS was calculated relative to the baseline period just prior to each TBS. In control experiments, such as that illustrated in *A* ($n = 4$), we found that the first TBS produced a potentiation of 43 ± 7% above baseline while the second TBS produced an additional increase of only 5 ± 1%. In experiments such as that illustrated in *B*, where the second TBS followed LFS ($n = 5$), it caused a potentiation that measured 22 ± 5%.

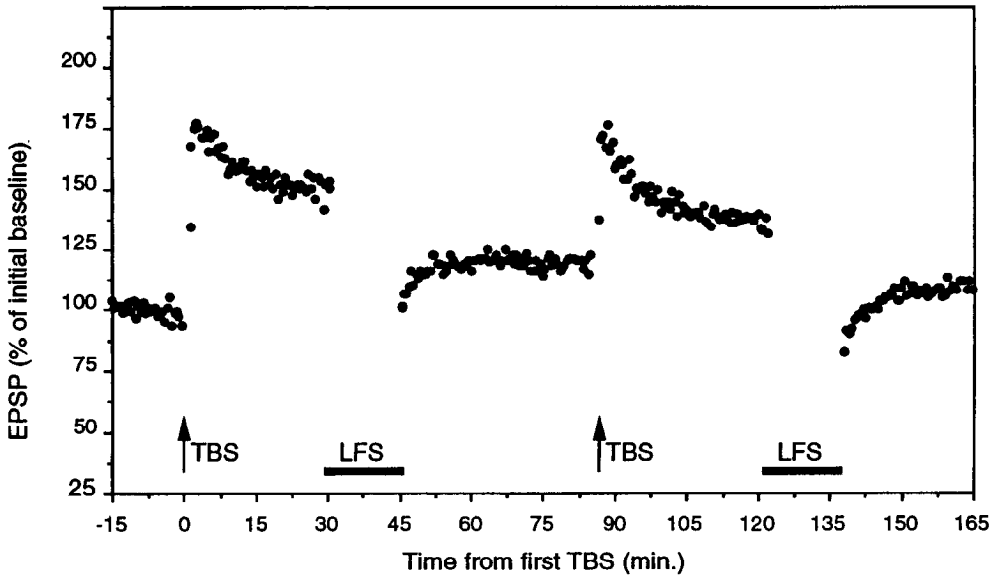

Figure 3. Record of one experiment in which LFS unsaturated LTP repeatedly, even when applied 30 min after the TBS.

The argument could be made, however, that the effect of LFS only 10 min after establishment of potentiation could be a deconsolidation of LTP rather than the LTD that we have documented in the virgin preparations. Against this explanation is evidence, illustrated in Figure 3, that LFS can unsaturate LTP even 30 min after TBS. However, we felt that a better way to address this potential confound would be first to induce a stable LTD in virgin slices, and then to assess whether it is possible to achieve the same LTP "ceiling" as in control slices. To do this, we turned to a preparation in which the effects of TBS and LFS are amplified: hippocampal slices prepared from younger animals. The development of LTD will be described in more detail below; for now, let it suffice to say that both LTP and LTD are of greater relative magnitude in slices from 3-week-old rats. Using this preparation, we were able to compare the magnitude of LTP in virgin slices ($n = 4$) with that observed after establishment of stable LTD ($n = 4$). As Figure 4 illustrates, even though prior LFS caused a depression of the EPSP slope to less than 75% of the initial baseline, subsequent TBS was able to achieve virtually the same absolute amount of potentiation as in control slices.

Postnatal development of LTD

LTP-like and LTD-like mechanisms have previously been invoked to account for aspects of activity-dependent cortical development (e.g., Bear et al., 1987); therefore, we set out to investigate the development of homosynaptic LTD in CA1. Slices of hippocampus were prepared from animals ranging in age from postnatal day 6 (P6) through adulthood, and the magnitude of the LTD caused by 900 pulses at 1 Hz was assessed 30–40 min

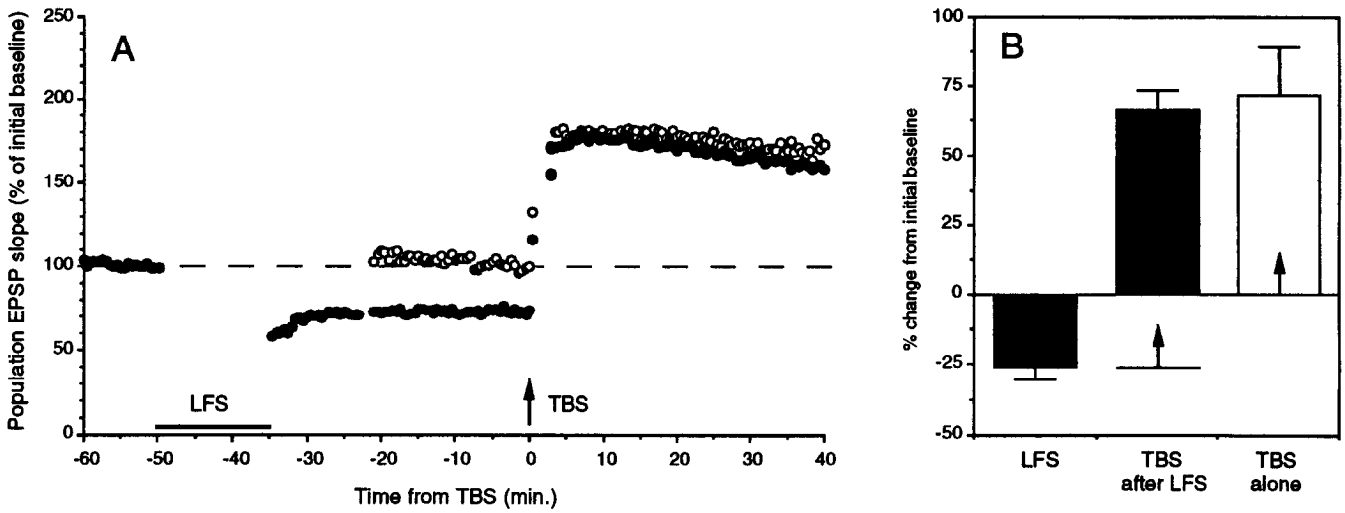

Figure 4. Evidence that LTD does not lower the ceiling for maximal LTP. *A*, Comparison of LTP magnitude following induction of LTD (*solid circles*) with that observed in control slices (*open circles*). Each data point represents the average of four different experiments performed on slices from 3-week-old rats. *B*, Summary of the mean (±SEM) effects of LFS, TBS after LFS, and TBS alone. Mean effects were calculated as follows. Ten consecutive sweeps were averaged for each case 30 min after TBS, and these were expressed as a percentage change from the average of 10 sweeps taken during the initial baseline period. These values for each experiment were then averaged and expressed as the mean ± SEM. The magnitude of the LTD was calculated in a similar fashion. Note that the absolute magnitude of the LTP following TBS, measured as a percentage change from the initial baseline, was not reduced by the prior LTD.

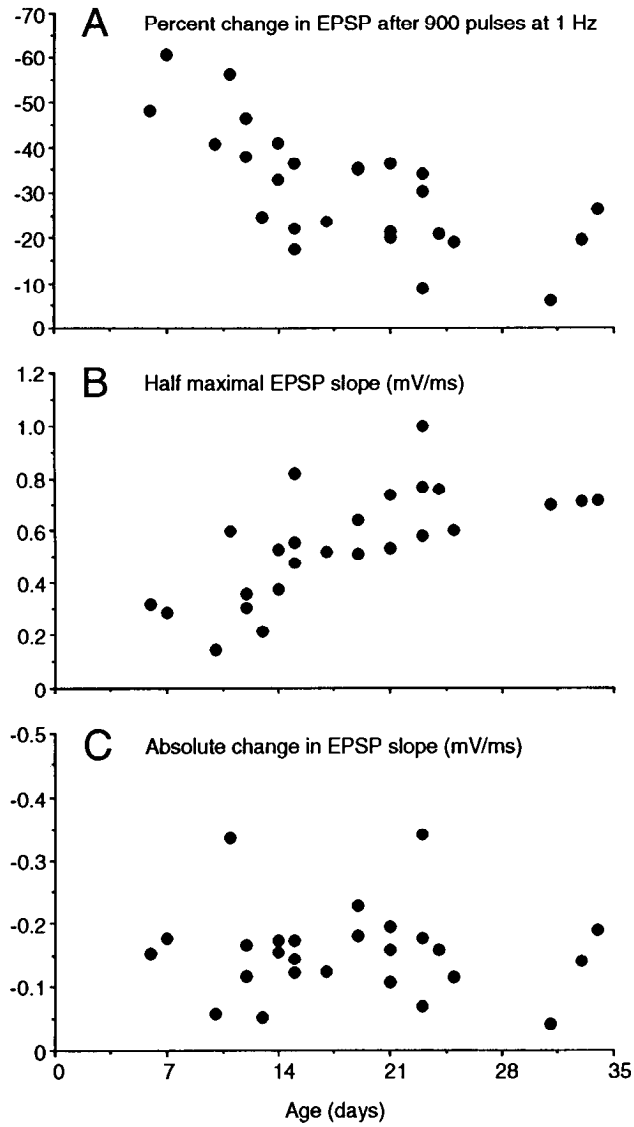

Figure 5. Postnatal changes in LTD and population EPSP slopes. *A*, Scatter plot of LTD measurements made 30 min after 1 Hz conditioning stimulation in experiments performed on hippocampal slices at different ages. Each point represents a single experiment. The decline in LTD magnitude as a function of age is significant using ANOVA at $P < 0.003$. *B*, Plot of the half-maximal EPSP slopes used for baseline measurements in the same experiments as shown in *A*. Note that there is a clear increase in the magnitude of these responses that is also significant using ANOVA at $P < 0.001$. *C*, Absolute difference in EPSP slopes before and 30 min after LFS in the same experiments plotted in *A* and *B*. Using this measure of LTD, there is no significant age effect ($P > 0.9$).

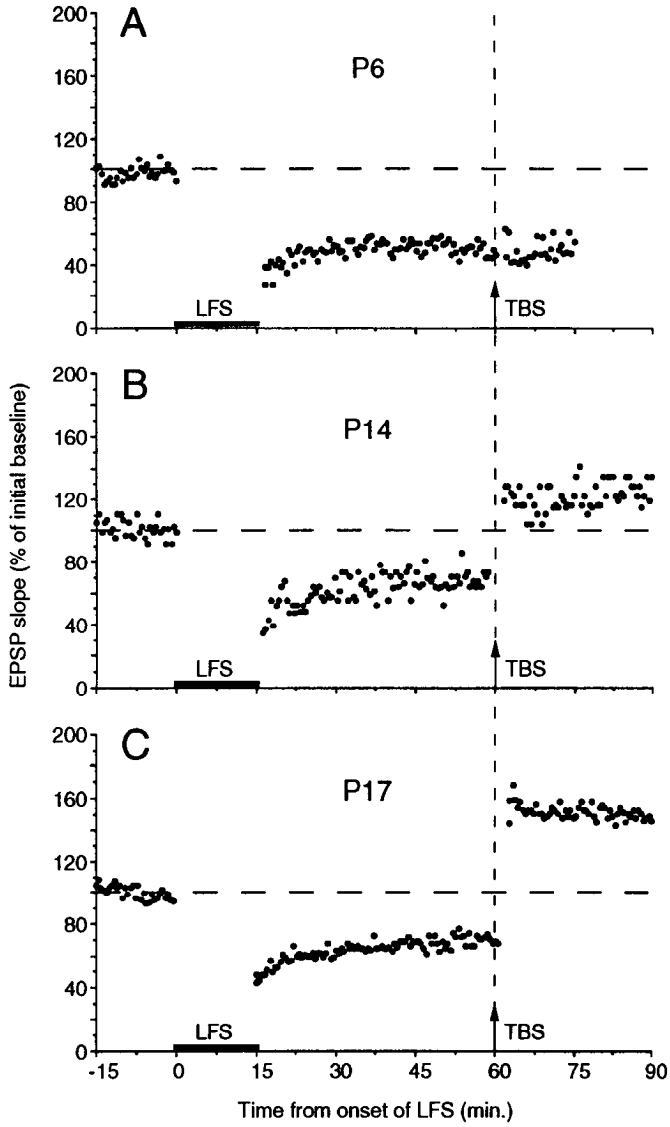

Figure 6. Examples of experiments on slices taken from rats at different ages in which LTD was induced by LFS and then, 45 min later, TBS was applied in an attempt to induce LTP. During the first postnatal week, LFS produces robust LTD but no LTP was observed following TBS ($n = 2$). A representative example from P6 is illustrated in *A*. By the second postnatal week, TBS is effective in reversing LTD (five of five cases) and causing an additional small potentiation (four of five cases). An example from P14 is illustrated in *B*. By the third postnatal week, TBS yields robust and reliable LTP regardless of whether it follows LTD or not. An example from P17 is shown in *C*.

after cessation of conditioning stimulation. Figure 5*A* plots the change in EPSP slope expressed as a percentage of the initial baseline versus the age of the rat at the time of the experiment. The data expressed in this way show a very clear age dependence for LTD. The average change (±SEM) in slices from animals aged less than 2 weeks (P6–P13, $n = 7$) was $-45 \pm 4\%$. The same conditioning during the third postnatal week (P14–P20, $n = 8$) resulted in a change of $-30 \pm 3\%$, and during the fourth postnatal week (P21–P27, $n = 8$) LFS resulted in LTD measuring $-24 \pm 3\%$. By the fifth postnatal week, the magnitude of the LTD had clearly stabilized at the adult value, which is $-20 \pm 4\%$ ($n = 5$). This age-dependent decline in the magnitude of LTD is significant at $P < 0.003$ (ANOVA).

The data in Figure 5*A* show that the consequences of conditioning LFS are relatively more pronounced in the hippocampus from rats in the first 3 postnatal weeks. This postnatal decline in LTD magnitude could be explained by a developmental downregulation of the mechanisms that trigger this form of synaptic plasticity (e.g., Dudek and Bear, 1989). However, as illustrated in Figure 5*B*, there is also a clear developmental increase in the baseline (half-maximal) response magnitude during this same period, from an initial EPSP slope of 318 ± 54 μV/msec in the slices prepared from animals less than 2 weeks

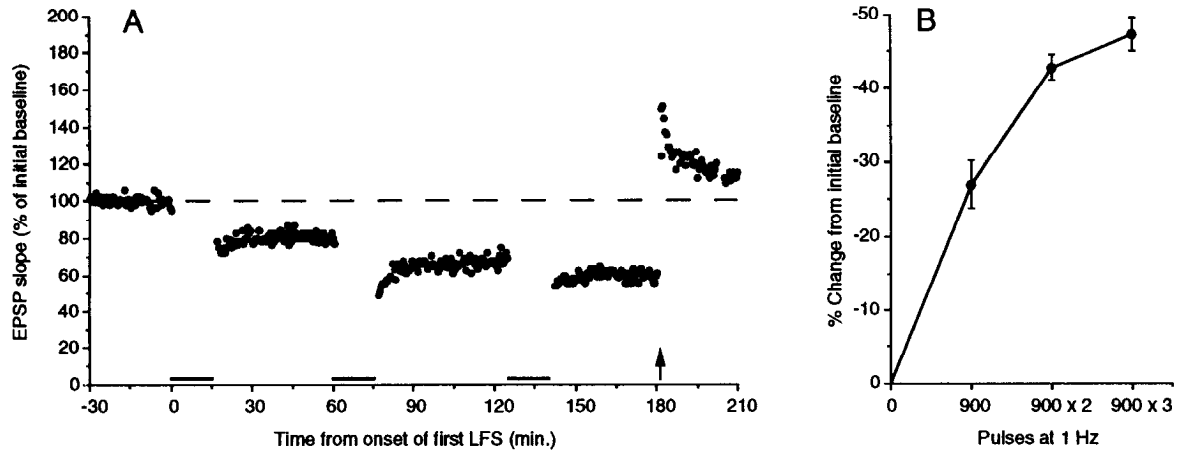

Figure 7. Saturation of LTD by repeated episodes of LFS in slices from 3-week-old rats. *A*, Record of one experiment in which three episodes of 1 Hz stimulation were applied (900 pulses each) with a spacing of ~45 min. It is apparent that the depression approaches an asymptote at a value of about 60% of baseline control. However, despite prolonged conditioning stimulation, TBS continues to yield a potentiation. *B*, Summary data from five experiments similar to that shown in *A*. Plotted is the average (±SEM) cumulative effect measured 30 min after each episode of LFS. The cumulative LTD following 2700 pulses is −47 ± 2%.

of age to 709 ± 5 μV/msec in the slices prepared from animals >4 weeks old. Thus, the absolute effectiveness of LFS in depressing synaptic transmission might actually remain constant during this period; the increase in baseline response magnitude alone could account for the apparent decline in relative LTD magnitude. To address this possibility, in Figure 5*C* we plot the absolute difference in EPSP slope before and after LFS versus age. This analysis reveals that the absolute decrease in EPSP slope caused by LFS remains relatively constant during early postnatal development; there is no significant effect of age on this measure of LTD.

NMDA receptors appear to be fully functional in CA1 at birth (Muller et al., 1989; Kleckner and Dingledine, 1991), which could explain why LTD was observed at the earliest ages studied (P6). However, previous work has shown that LTP develops postnatally and is not fully expressed before about 2 weeks of age (Harris and Teyler, 1984; Muller et al., 1989). These data then predict that these two forms of NMDA receptor–dependent plasticity are dissociable during the early postnatal period. This prediction was tested in a series of experiments in which TBS was given after establishment of LTD in slices from very young animals. In two experiments on slices from animals less than 1 week old, tetanic stimulation failed to cause any potentiation, despite the establishment of robust LTD. An example from a P6 slice is shown in Figure 6*A*. In contrast, by the third postnatal week TBS not only reversed the LTD, but also caused a robust LTP (Fig. 6*C*, P17). Coincident with the reported onset of LTP in virgin slices during the second postnatal week (Harris and Teyler, 1984; Muller et al., 1989), we find that TBS at these ages ($n = 5$) is also sufficient to reverse LTD and typically causes an additional small potentiation (Fig. 6*B*, P14).

Saturation of LTD

We originally settled on 900 pulses at 1 Hz because it yields a reliable LTD, albeit one of modest amplitude (Dudek and Bear, 1992a). Left unanswered, however, has been the question of whether this type of conditioning is sufficient to achieve a maximal effect and, if not, whether LTD could be "saturated" by additional LFS. Therefore, experiments were carried out using slices from ~3-week-old animals to investigate this issue. The design was to give 15 min episodes of 1 Hz stimulation (900 pulses per episode) repeated every 45 min. An example of such an experiment is shown in Figure 7*A*, where it can be seen that a second application of LFS practically doubled the magnitude of the LTD, but that a third episode of conditioning stimulation had relatively little additional effect. It can also be noted from this figure that despite the length of the experiment and these repeated episodes of conditioning stimulation, TBS still resulted in potentiation.

Summary data from five identical experiments are presented in Figure 7*B*. These data show that the magnitude of the synaptic depression appears to be linearly related to the number of conditioning pulses up to, but not exceeding, 1800. After that, additional stimulation has little additional effect, suggesting a "floor" to population synaptic strength changes evoked by LFS of about −50% in ~3-week-old rat CA1.

Discussion

Here we have followed up on our previous study that showed that delivering 900 pulses to the Schaffer collateral–CA1 pathway at 1–3 Hz causes a lasting depression of synaptic effectiveness that is input specific and dependent on NMDA receptor activation (Dudek and Bear, 1992a). The major additional findings of the present study are, first, that the same synapses that are depressed by LFS can be potentiated by patterned high-frequency stimulation; second, that the consequences of LFS are more pronounced early in postnatal development and precede the development of LTP; and third, that the absolute magnitude of LTD can be substantial, but has a physical limit.

Saturation of LTD

A characteristic of LTP in CA1 is that it can be saturated with repeated bursts of high-frequency tetanic stimulation (cf. Bliss and Lømo, 1973). Synaptic strength has certain physical limits imposed by the number of release sites, probability of transmitter release, and the quantal size (Korn et al., 1986); presumably saturation of LTP occurs once a limit has been reached in one or more of these potentially modifiable parameters. For LTP, this limit appears to be reached after three or four episodes of TBS, and in our experiments on virgin slices this stimulation

rendered a maximal change in the population EPSP after 30 min of between +40% and +50% (Fig. 2). Thus, it is of interest that the limit for LTD appears to be of approximately the same magnitude, between −40% and −50% (Fig. 7). And, just like TBS, the synchronous activation of hippocampal neurons at 1 Hz for prolonged periods may closely resemble natural patterns of activation in some behavioral states (Green et al., 1990). Thus, without entering into the debate here about the possible significance of electrically evoked synaptic plasticity for understanding normal hippocampal function, these observations suggest that LTD may be as important as LTP for gaining insights into the mnemonic function of the hippocampus.

Development of LTD

Activity-dependent decreases in synaptic effectiveness are an important feature of normal cortical development. It is this process that is responsible for the refinement of topographic maps and the establishment of neuronal stimulus selectivity (cf. Wolpaw et al., 1991). A classic example of synaptic LTD in the visual cortex is the loss of responsiveness to stimulation of one eye after it has been deprived of normal vision for several days (reviewed by Bear et al., 1987). This type of plasticity appears to be far more pronounced during a critical period of postnatal development, and the proposal has been made that this might be explained by a developmental overexpression of the mechanism(s) responsible for LTD (Dudek and Bear, 1989). Thus, it was of interest to examine the development of LTD in hippocampus as a model cortical system.

Our data confirm that the consequences of conditioning LFS are far more pronounced during early development. The magnitude, relative to baseline, of LTD in 1-week-old rats was double that observed after 3 weeks of age (Fig. 5). However, further analysis showed that the consequences of LFS during the first 3 weeks are exaggerated by virtue of the fact that the baseline responses are generally smaller during this period. This explanation does not diminish the functional significance of LTD during early development; a baseline synaptic response in neonates can be nearly halved by stimulation that in adults only alters the response by one-fifth. Nonetheless, this analysis suggests that there need not be a developmental overexpression of a specific mechanism linked to LTD to account for the data.

Interaction of LTD and LTP

We have presented two lines of evidence that the same synapses that have undergone depression can still be potentiated by high-frequency stimulation. In the first experiment we saturated LTP and then showed that the same inputs could be potentiated again by high-frequency stimulation that followed induction of LTD (Figs. 2, 3). Regardless of whether the effects of the second high-frequency tetanus are to be viewed as "LTP" or "de-depression," this experiment shows that the same synapses that had been depressed could subsequently be increased in effectiveness. This conclusion is supported by the additional finding that the absolute magnitude (measured from the initial baseline) of LTP produced by a saturating TBS is equivalent regardless of whether or not the synaptic pathway had been depressed previously (Fig. 4). Taken together, the data argue against the hypothesis that the homosynaptic LTD that follows LFS is caused by some irreversible damage to the stimulated synapses.

These same experiments also address the question of whether LTD and LTP affect synaptic strength at a common site. Consider, for example, a model in which LTP is caused by a change in postsynaptic receptor number while LTD is caused by a decreased probability of neurotransmitter release. According to this hypothesis, saturation of LTP (maximal increase in receptors) could not be reversed by induction of LTD (decreased transmitter release), and prior LTD would lower the maximal synaptic strength attained by saturating LTP. However, in a model in which LTP and LTD affect the same variable, LTD would "unsaturate" LTP and leave the ceiling for maximal synaptic strength unaffected. Our data are consistent with this latter hypothesis.

We cannot rule out the possibility that LFS-induced "depotentiation" following LTP is qualitatively different from LFS-induced LTD in virgin preparations, nor can we rule out the possibility that TBS-induced "de-depression" following LTD is mechanistically distinct from TBS-induced LTP in naive slices. Indeed, work on synaptic plasticity in the invertebrate *Aplysia* has shown that two seemingly similar forms of synaptic enhancement, sensitization and dishabituation, differ in terms of both mechanism (Ghirardi et al., 1992) and developmental time of onset (Carew, 1989). On the other hand, we have no data to suggest such a dichotomy; in our experiments "de-depression" appears in development at the same ages that have been reported for LTP, suggesting that they are one and the same process.

Relationship to previous studies

Our finding of LTD after LFS in virgin slices appears to be somewhat at odds with several previous studies. For example, Barrionuevo et al. (1980) and Staubli and Lynch (1990) found that 1 Hz stimulation of the Schaffer collateral projections in the rat hippocampus *in vivo* would cause a reduction of synaptic effectiveness in pathways that had been previously potentiated, but not in control (unpotentiated) pathways. The inability of these investigators to observe LTD in the naive preparations is likely explained by the relatively brief stimulus trains that were used, ranging from 100 pulses at 1 Hz to 250 pulses at 5 Hz. If the magnitude of the depression is linearly related to the number of stimulus pulses (before LTD saturation), then from our data we would predict that 100 pulses at 1 Hz would yield an effect of less than 3%. More difficult to explain, however, are the results of Fujii et al. (1991), who were also unable to induce depression by as many as 1000 pulses at 1 Hz in virgin hippocampal slices. We do not know the reason for this discrepancy, but we note that their experiments were performed on guinea pig slices at 31°C whereas ours were on rat slices at 35°C. In any case, Fujii et al. were able to produce substantial depression of previously potentiated responses, even 100 min after LTP induction. As Yang and Faber (1991) have recently shown in the goldfish Mauthner cell, the synaptic modification one gets with a given stimulation paradigm can vary depending on where the synaptic strengths are within their available dynamic range at the time of the conditioning. In our hands, the synaptic efficacy of the rat Schaffer collateral–CA1 pathway in freshly prepared slices can be adjusted ±50%.

From the dependence of LTD on NMDA receptors we presume that an elevation of postsynaptic Ca^{2+} is involved in the induction process (Dudek and Bear, 1992a), and preliminary work using Ca^{2+} chelators supports this hypothesis (Mulkey and Malenka, 1992a). Thus, it is possible that the LTD we describe here bears some commonality with that described in neocortex by Artola et al. (1990) and by Hirsch and Crepel (1990), which has also been shown to be Ca^{2+} dependent (Bröcher et al., 1992). These authors report that strong stimulation [i.e., 500 pulses in

50 Hz bursts using a stimulation intensity that was 3–7× that required to elicit spikes in the postsynaptic neurons (Bröcher et al., 1992)] will induce LTD as long as NMDA receptors are *not* recruited by the stimulation. This differential involvement of NMDA receptors in LTD is not accounted for by a distinction between archi- and neocortex; we have recently found that the same LFS protocol used here yields NMDA receptor–dependent homosynaptic LTD in slices of both adult rat and immature cat visual cortex (Kirkwood et al., 1992). Thus, the difference appears to be best accounted for by the different types of stimulation. If LTD depends on an intermediate rise in intracellular Ca^{2+} that is below the threshold for inducing LTP (Bear et al., 1987; Lisman, 1989), it would be reasonable to expect that this condition could be achieved in different ways (i.e., low-frequency NMDA receptor activation, voltage-gated Ca^{2+} entry, release of intracellular Ca^{2+} stores), although different degrees of "input specificity" might also be expected of these different routes of calcium entry. Indeed, it is possible that "homosynaptic" and "heterosynaptic" LTD might differ only in terms of the spatial extent of the Ca^{2+} signals evoked by stimulation (cf. White et al., 1990). However, this speculative model must also be considered in light of evidence from CA1 that brief elevations in intracellular Ca^{2+} by way of NMDA receptor activation always produce an enhancement of synaptic strength, even when the Ca^{2+} signals are of small amplitude (Malenka, 1991).

In any case, now that a reliable model of homosynaptic LTD has been established for rat CA1 *in vitro*, we can look forward to rapid progress in unraveling the mechanisms of induction and expression of this potentially important form of synaptic plasticity.

Note added in proof

Since submission of this article, Mulkey and Malenka (1992b) published a complete account of their experiments on the mechanisms of homosynaptic LTD in CA1 of young (P12–P22) rats. They confirm our original observations that LTD is input specific and blocked by NMDA receptor antagonists. In addition, they have provided several new lines of evidence that LTD is triggered, at least in part, by Ca^{2+} entering through the NMDA receptor. Specifically, they find that LTD induction is sensitive to extracellular [Ca] and is blocked by both intracellular hyperpolarization and injection of a Ca^{2+} chelator.

References

Abraham WC, Goddard GV (1983) Asymmetric relations between homosynaptic long-term potentiation and heterosynaptic long-term depression. Nature 305:717–719.

Abraham WC, Wickens JR (1991) Heterosynaptic long-term depression is facilitated by blockade of inhibition in area CA1 of the hippocampus. Brain Res 546:336–340.

Artola A, Brocher S, Singer W (1990) Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. Nature 347:69–72.

Barrionuevo G, Schottler F, Lynch G (1980) The effects of low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus. Life Sci 27:2385–2391.

Bear MF, Cooper LN, Ebner FF (1987) A physiological basis for a theory of synaptic modification. Science 237:42–48.

Bear MF, Dudek SM, Gold JT (1991) Homosynaptic long-term depression in CA1 of rat hippocampus *in vitro*. Soc Neurosci Abstr 17: 533.11.

Bear MF, Press WA, Connors BW (1992) Long-term potentiation in slices of kitten visual cortex and the effects of NMDA receptor blockade. J Neurophysiol 67:1–11.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 2:32–48.

Bliss TVP, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:331–356.

Bradler JE, Barrioneuvo G (1990) Heterosynaptic correlates of long-term potentiation induction in hippocampal CA3 neurons. Neurosci 35:265–271.

Bröcher S, Artola A, Singer W (1992) Intracellular injection of Ca^{++} chelators blocks induction of long-term depression in rat visual cortex. Proc Natl Acad Sci USA 89:123–127.

Carew TJ (1989) Developmental assembly of learning in *Aplysia*. Trends Neurosci 12:389–394.

Christie BR, Abraham WC (1992) Priming of associative long-term depression in the dentate gyrus by q frequency synaptic activity. Neuron 9:79–84.

Cooper LN, Liberman F, Oja E (1979) A theory for the acquisition and loss of neuron specificity in visual cortex. Biol Cybern 33:9–28.

Desmond NL, Colbert CM, Zhang DX, Levy WB (1991) NMDA receptor antagonists block the induction of long-term depression in the hippocampal dentate gyrus of the anesthetized rat. Brain Res 552: 93–98.

Dudek SM, Bear MF (1989) A biochemical correlate of the critical period for synaptic modification in the visual cortex. Science 246: 673–675.

Dudek SM, Bear MF (1992a) Homosynaptic long-term depression in area CA1 of hippocampus and the effects of NMDA receptor blockade. Proc Natl Acad Sci USA 89:4363–4367.

Dudek SM, Bear MF (1992b) Homosynaptic long-term depression in CA1 *in vitro*: development, mechanism, and interaction with LTP. Soc Neurosci Abstr 18:1499.

Dudek SM, Bowen WD, Bear MF (1989) Postnatal changes in glutamate stimulated phosphoinositide turnover in rat neocortical synaptoneurosomes. Dev Brain Res 47:123–128.

Fujii S, Saito K, Miyakawa H, Ito K-i, 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.

Ghirardi M, Braha O, Hochner B, Montarolo PG, Kandel ER (1992) Roles of PKA and PKC in facilitation of evoked and spontaneous transmitter release at depressed and nondepressed synapses in *Aplysia* sensory neurons. Neuron 9:479–489.

Goldman RS, Chavez-Noriega LE, Stevens CF (1990) Failure to reverse long-term potentiation by coupling sustained presynaptic activity and *N*-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci USA 87:7165–7169.

Green EJ, McNaughton BL, Barnes CA (1990) Exploration-dependent modulation of evoked responses in fascia dentata: dissociation of motor, EEG, and sensory factors and evidence for a synaptic efficacy change. J Neurosci 10:1455–1471.

Gustafsson B, Wigström H (1990) Long-term potentiation in the hippocampal CA1 region: its induction and early temporal development. Prog Brain Res 83:223–232.

Harris KM, Teyler TJ (1984) Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. J Physiol (Lond) 346: 27–48.

Hirsch JC, Crepel F (1991) Blockade of NMDA receptors unmasks a long-term depression in synaptic efficacy in rat prefrontal neurons *in vitro*. Exp Brain Res 85:621–624.

Huang Y-Y, Colino A, Selig DK, Malenka RC (1992) The influence of prior synaptic activity on the induction of long-term potentiation. Science 255:730–733.

Kirkwood A, Aizenman CD, Bear MF (1992) Common forms of plasticity in hippocampus and visual cortex *in vitro*. Soc Neurosci Abstr 18:1499.

Kleckner N, Dingledine R (1991) Regulation of hippocampal NMDA receptors by magnesium and glycine during development. Mol Brain Res 11:151–159.

Korn H, Faber DS, Burnod Y, Triller A (1984) Regulation of efficacy at central synapses. J Neurosci 4:125–130.

Levy WB, Steward O (1979) Synapses as associative memory elements in the hippocampal formation. Brain Res 175:233–245.

Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. Proc Natl Acad Sci USA 86: 9574–9578.

Lynch GS, Dunwiddie T, Gribkoff V (1977) Heterosynaptic depression: a postsynaptic correlate of long term potentiation. Nature 266: 737–739.

Malenka RC (1991) Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. Neuron 6:53–60.

Mulkey RM, Malenka RC (1992a) Homosynaptic NMDA receptor-dependent long-term depression in rat hippocampal slices. Soc Neurosci Abstr 18:1498.

Mulkey RM, Malenka RC (1992b) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Muller D, Oliver M, Lynch G (1989) Developmental changes in synaptic properties in hippocampus of neonatal rats. Dev Brain Res 49: 105–114.

Nicoll RA, Kauer JA, Malenka JA (1989) The current excitement in long-term potentiation. Neuron 1:97–103.

Pockett S, Brookes NH, Bindman LJ (1990) Long-term depression at synapses in slices of rat hippocampus can be induced by bursts of postsynaptic activity. Exp Brain Res 80:196–200.

Sejnowski TJ (1977) Storing covariance with nonlinearly interacting neurons. J Math Biol 69:385–389.

Stanton PK, Sejnowski TJ (1989) Associative long-term depression in the hippocampus induced by Hebbian covariance. Nature 229: 215–218.

Staubli U, Lynch G (1990) Stable depression of potentiated synaptic responses in the hippocampus with 1–5 Hz stimulation. Brain Res 513:113–118.

Stevens CF (1990) A depression long awaited. Nature 347:16.

White G, Levy WB, Steward O (1990) Spatial overlap between populations of synapses determines the extent of their associative interaction during the induction of long-term potentiation and depression. J Neurophysiol 64:1186–1198.

Wickens JR, Abraham WC (1991) The involvement of L-type calcium channels in heterosynaptic long-term depression in the hippocampus. Neurosci Lett 130:128–132.

Willshaw D, Dayan P (1990) Optimal plasticity from matrix memories: what goes up must come down. Neural Comp 2:85–93.

Wolpaw JR, Schmidt JT, Vaughn TM, eds (1991) Activity-driven CNS changes in learning and development. Ann NY Acad Sci 627.

Yang X-D, Faber DS (1991) Initial synaptic efficacy influences induction and expression of long-term changes in transmission. Proc Natl Acad Sci USA 88:4299–4303.