Biol Res 28: 73-80 (1995)



# Elementary forms of synaptic plasticity in the visual cortex

ALFREDO KIRKWOOD and MARK F BEAR

Department of Neuroscience and Institute for Brain and Neural Systems, Brown University, Providence, Rhode Island, USA

*The neocortex is an important site of memory storage, and memories are believed to be formed in the cortex by the activity-dependent modification of synaptic connections. However, in contrast to the hippocampus where there has been an increasingly sophisticated analysis of synaptic plasticity, relatively little is known about the mechanisms of synaptic modification in neocortex. Here we summarize the results of a series of experiments conducted on slices of visual cortex* ***in vitro****, aimed at elucidating the elementary mechanisms of synaptic plasticity in the superficial layers of neocortex. We show that long-term potentiation (LTP) and depression (LTD) result from high- and low-frequency conditioning stimulation, respectively, of the middle layers of cortex. Both forms of synaptic plasticity are input-specific and dependent on activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors. The critical variable in determining the sign of the synaptic modification appears to be the level of postsynaptic depolarization during conditioning stimulation. The data support a model in which the state of correlation of pre- and post-synaptic activity is converted by the voltage-dependent NMDA receptor channel into a graded postsynaptic $Ca^{2+}$ signal. LTD is triggered by a modest but sustained elevation in postsynaptic $Ca^{2+}$, while LTP is elicited by larger changes in $Ca^{2+}$. An important variable that regulates synaptic plasticity in the neocortex is intracortical inhibition, which constrains the patterns of activity that can reach the modifiable synapses in layer III.*

***Key terms****: long-term depression, long-term potentiation, memory, synaptic plasticity, visual cortex.*

## INTRODUCTION

Neural network models have shown that learning and memory can result if mechanisms exist to strengthen synapses whose activity coincides with target depolarization beyond some threshold level (called "Hebbian" modification), and conversely, to weaken synapses whose activity consistently fails to correlate with postsynaptic activation (Frégnac, 1991). Activity-dependent synaptic strengthening has been demonstrated directly in slices of hippocampus; for example, brief high frequency activation of the Schaffer collateral (SC) pathway yields a long-term potentiation (LTP) of the stimulated synapses in CA1. Importantly, LTP can also be evoked using low-frequency stimulation when it is paired with strong intracellular depolarization (Kelso *et al*, 1986; Malinov and Miller, 1986; Sastry *et al*, 1986; Wigstrom *et al*, 1986), a condition that fulfills the requirements of a Hebbian modification. The biophysical basis for this form of LTP in hippocampus has now been worked out in considerable detail. Activation of *N*-methyl-D-aspartate (NMDA) receptors by presynaptic stimulation leads to a postsynaptic $Ca^{2}$+ flux

**Correspondence to**: Dr Alfredo Kirkwood, Department of Neuroscience and Institute for Brain and Neural Systems, Brown University, Providence, Rhode Island 02912, USA. Fax: (1-401) 863-1074.

when $Mg^{2+}$ is displaced from the NMDA channel by strong postsynaptic depolarization (Gustafsson *et al*, 1987); an elevation of postsynaptic $Ca^{2+}$ is essential for triggering the biochemical mechanisms that lead to lasting synaptic potentiation (Malenka *et al*, 1988).

A mechanism for weakening synapses has also been demonstrated in CA1. It was recently found that prolonged repetitive activation of the Schaffer collaterals at low frequencies causes a long-term depression (LTD) of CA1 synaptic responses (Dudek and Bear, 1992). This forms of LTD is also dependent upon activation of NMDA receptors and requires for induction an elevation of postsynaptic $Ca^{2+}$ during the conditioning stimulation (Dudek and Bear, 1992, 1993; Mulkey and Malenka, 1992). Taken together, the results obtained in CA1 support a model in which the state of correlation of pre- and post-synaptic activity is converted by the voltage-dependent NMDA receptor channel into a graded postsynaptic $Ca^{2+}$ signal that triggers LTP when it exceeds some critical value (the "modification threshold") and triggers LTD when it falls below this level.

The successful elucidation of a Hebbian mechanism in hippocampus inspired a number of laboratories to use slices of neocortex to assess whether plasticity here is governed by similar mechanisms. However, the results using this approach have yielded quite varied results (for review, see Bear and Kirkwood, 1993). Perhaps this was to be expected since even in the hippocampus different mechanisms can be elicited under some conditions without participation of NMDA receptors (Grover and Teyler, 1990). Indeed, it is entirely plausible that different mechanisms might operate in different cytoarchitectural areas as well as in different layers of neocortex.

We set out to investigate the issue NMDA-dependent plasticity in the superficial layers of primary visual cortex using a novel stimulation-recording configuration (Kirkwood and Bear, 1994a,b; Kirkwood *et al*, 1993). We have shown that NMDA-receptor dependent LTP and LTD can be evoked reliably in visual cortical slices of adult rats and developing kittens. These forms of plasticity are input specific, and are evoked in neocortex using precisely the same types of conditioning stimulation that are effective in CA1. As in CA1, a crucial variable in determining the sign and magnitude of synaptic plasticity appears to be the amount of post-synaptic activity.

## LTP AND LTD ARE EVOKED IN VISUAL CORTEX BY LAYER IV STIMULATION

Experiments were performed using slices of visual cortex prepared from adult (≥ 150 g) rats or kittens aged 5-7 weeks as described previously (Kirkwood and Bear, 1994a; Kirkwood *et al*, 1993). Stimulation typically was applied at a site in the middle of the cortical thickness (600-800 μm from the pia) that corresponds mainly to layer IV, and responses were recorded in layer III. Stimulation at this site activates direct projections of layer IV cells to layer III, and ascending intracortical and cortico-cortical axons that pass through layer IV *en route* to layer III. The response to layer IV stimulation typically was monitored using the negative field potential which reflects the amplitude of an excitatory synaptic current sink.

In order to make possible a direct comparison of layer III and CA1 plasticity, we adopted the same conditioning protocols that have been used successfully in CA1. Thus, to induce LTP, we delivered 2-5 episodes of high frequency stimulation (HFS), modeled after the "theta-burst" protocol (Larson *et al*, 1986). Theta burst stimulation consists of 10-13 stimulus trains delivered at 5-7 Hz with each train consisting of 4 pulses at 100 Hz. To induce LTD we used low frequency stimulation (LFS) consisting of 900 pulses delivered at 1 Hz (Dudek and Bear, 1992). The effects of these stimulation paradigms on the layer III field potential amplitude are shown in Fig 1. Following HFS, the amplitude of the synaptic response in layer III gradually increased until it reached a stable value 10 minutes after the conditioning stimulation (Fig 1A). LFS, on the other hand, caused a decrease in response magnitude that was immediately apparent upon cessation of the conditioning (Fig 1B). In the experiments shown in Fig 1 the changes were monitored for up to 1 hour without observing signs of recovery.

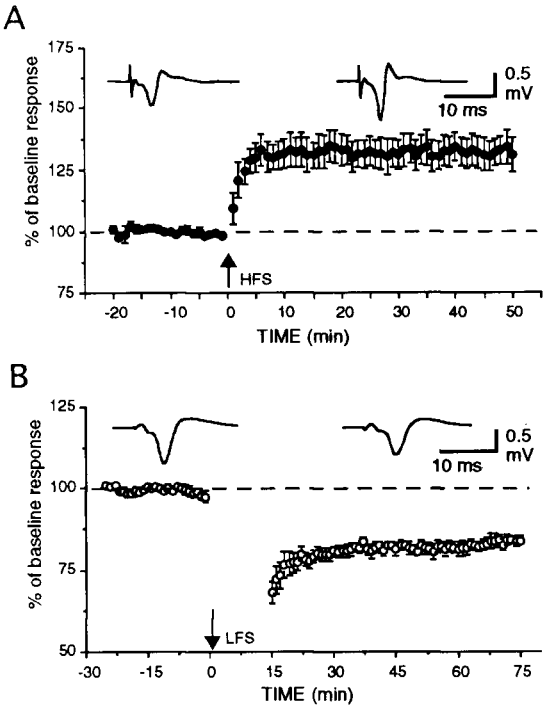


**Fig 1.** Conditioning stimulation of layer IV induces long-term changes in the synaptic responses recorded in layer III. Time course of effects of HFS (A) and LFS (B) on amplitude of layer III field potentials evoked by layer IV stimulation. Responses at 1 every 15 s and averaged over 1 min. Each point, mean (± SEM) of 5 (A) or 4 (B) experiments. Insets, field potentials from representative experiments, averaged from 4 consecutive responses recorded 1 min before (left) and 50 min after (right) conditioning stimulation.

To confirm that the changes in the layer III field potential reflects, at least in part, a change in the EPSP's of layer III neurons, we recorded simultaneously intracellular and extracellular potentials in layer III of rat visual cortex. Figure 2 illustrates the results of 5 experiments in which field potentials were recorded in layer III and EPSP's were recorded simultaneously from a nearby layer III neuron. After a suitable baseline period, induction of LTP was attempted using HFS, and subsequently, LTD was attempted in the same preparation by delivering LFS. As a result, both the field potential and EPSP changed with similar time courses. Although the amplitude of the EPSP's are plotted in Fig 2, it should be noted that the changes in the peak of the negative field potential also were always correlated with changes in the initial slope of the EPSP. This is evident in the traces in Fig 2B.

## SYNAPTIC PLASTICITY IN HIPPOCAMPUS AND VISUAL CORTEX HAVE SIMILAR PROPERTIES

### *Input specificity*

One of the key properties of synaptic modification in CA1 is that only those synapses that have undergone conditioning stimulation are modified. Inputs onto the same postsynaptic neuron that are inactive during conditioning stimulation fail to show any change. To isolate separate inputs to layer III, we made a radial cut in the slice that extended from the white matter through layer IV. Then stimulation was applied to layer IV on either side of the cut and the layer III field potential was monitored (Fig 3A). Field potential responses to stimulation of the two sites showed summation that is expected from independent inputs. Using this approach, we found that HFS of layer IV on one side of the cut produced potentiation of test stimuli only on that side of the cut (Fig 3B). Similarly, when LFS was applied only the responses to test stimuli on the conditioned side were depressed (Fig 3C).

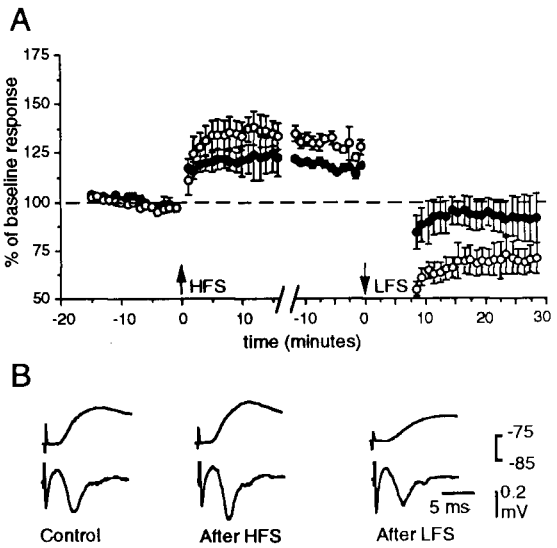


**Fig 2.** Intracellular correlates of LTP and LTD in layer III. **A.** Effects of HFS and LFS on amplitude of simultaneously recorded EPSP's (filled circles) and field potentials (open circles). Each point, mean (± SEM) of 4 experiments. LFS (900 pulses at 2 Hz) applied between 25 to 40 min after HFS. **B.** Records from a representative case, taken 1 min before HFS (left), 20 min after HFS (center) and 20 min after LFS (right). Traces averaged from 4 consecutive responses (EPSP's, upper traces; field potentials, lower traces).

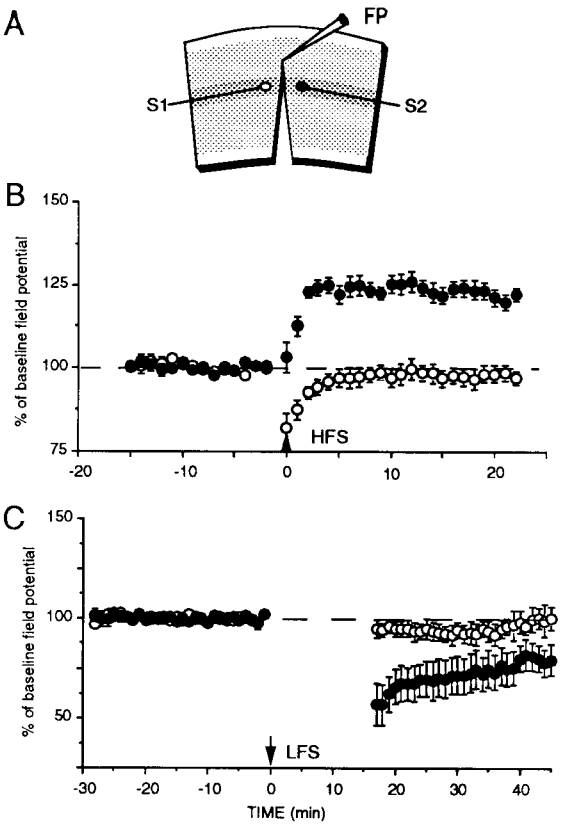


**Fig 3.** LTP and LTD are input-specific. Schema of stimulus-recording configurations used to assess input specificity (**A**). Layer IV stimulated on either side of a radial cut that extended from the white matter through layer IV. Conditioning, either HFS (n = 4) or LFS (n = 5), was delivered on one side of the cut. HFS (**B**) and LFS (**C**) affected the size of responses of the conditioned pathways (filled circles), but not the unconditioned pathways (open circles).

*Role of NMDA receptors*

In hippocampus, the induction of homosynaptic LTP and LTD is prevented by application of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5) (Collingridge *et al*, 1983; Dudek and Bear, 1992; Mulkey and Malenka, 1992). In visual cortex, in contrast, the situation has been controversial (reviewed by Bear and Kirkwood, 1993). Therefore, it was of interest to assess whether LTP and LTD in our neocortical preparation were sensitive to NMDA receptor blockade. Figure 4A and B illustrates the effects of bath applied 100 μM D,L-AP5 on the induction of LTP by HFS in rat visual cortex (Fig 4A) and in kitten visual cortex (Fig 4B). In no case were we able to induce LTP in AP5; however, in all cases LTP could be elicited following wash-out of the drug. Similarly, LFS did not result in LTD when delivered in the presence of AP5, but it did cause LTD when it was delivered after the drug was removed (Fig 4C).

*LTP is Hebbian*

If LTP in visual cortex were triggered by NMDA receptor activation, as in the hippocampus, we expected to be able to induce it by pairing low frequency stimulation of layer IV with strong intracellular depolarization of the neurons in layer III. After a suitable baseline period, during which both the field potential and intracellular responses were monitored, layer III cells were depolarized by direct current injection while 90 stimulus pulses were delivered to layer IV at 1 Hz (Fig 5). After this conditioning we observed a marked potentiation in the intracellular responses, but no change in the extracellular field potentials (LTD of the field potential did not result because of the small number of pulses). Depolarization alone, or 100 pulses at 1 Hz alone, never caused a lasting increase in the evoked EPSP confirming that the LTP was a result of the pairing.

*LTD depends on stimulation frequency*

In hippocampus the same number of conditioning pulses delivered at different frequencies yielded different effects, ranging from LTD to LTP (Dudek and Bear, 1992). Therefore, it was of interest to determine whether synaptic plasticity in visual cortex similarly varies as a function of stimulation frequency. We found that in comparison with the robust LTD caused by 1 Hz stimulation, stimulation at 2 and 4 Hz caused only a slight depression of the field potential amplitude, both in rat (Fig 6A) and in kitten (not shown). Unlike the situation in CA1, however, the same number of pulses at higher frequencies did not yield potentiation. This may be attributed to possible deleterious effects of prolonged high frequency stimulation in the neocortical preparations, which tend to be more fragile than hippocampal slices. Therefore, we had to modify our conditioning protocol. The new conditioning protocol, which we called "delta burst", consisted of brief bursts of

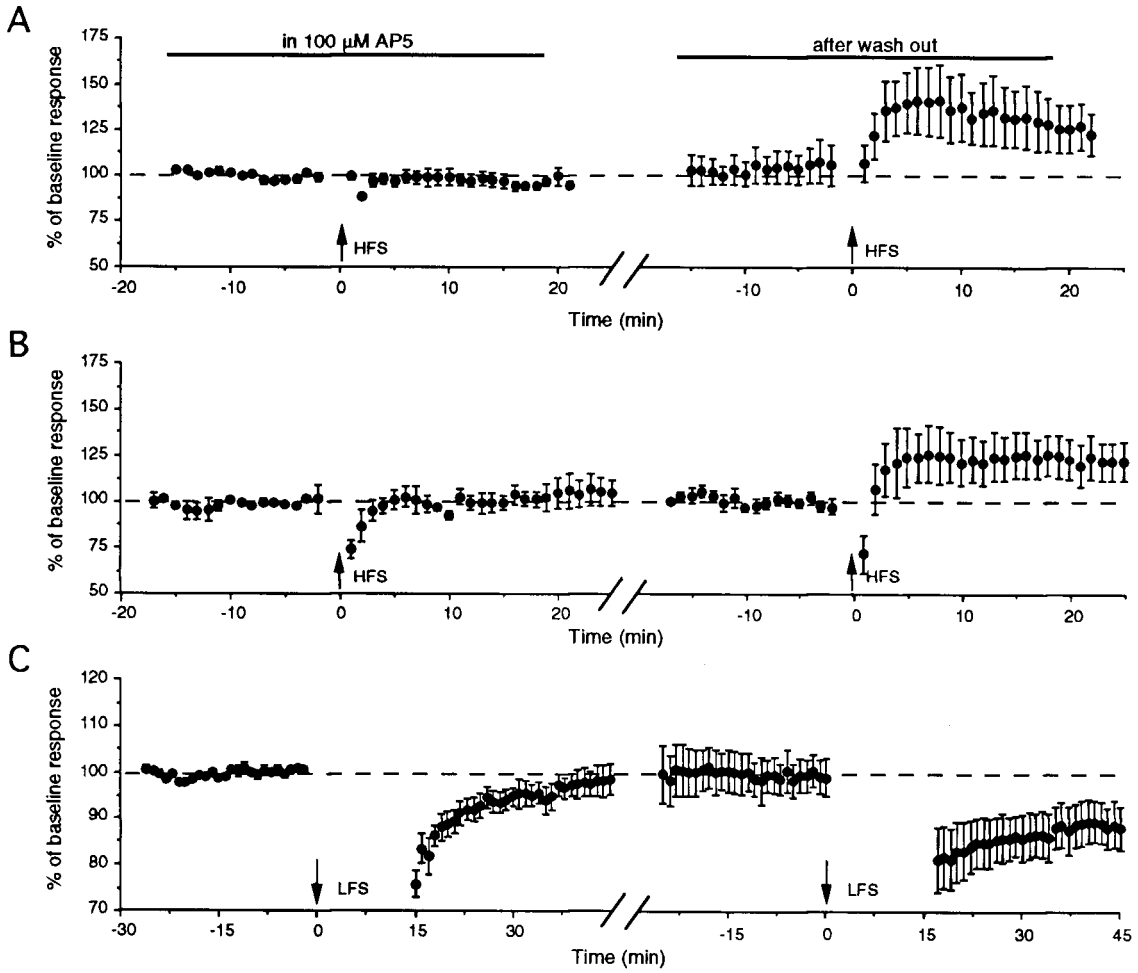


Fig 4. NMDA-receptor antagonist AP5 reversibly blocks the induction of LTP and LTD. Bath application of 100 μM AP5 prevented the induction of LTP by HFS in rat (**A**, n = 4) and kitten (**B**, n = 4) slices, and the induction of LTD by LFS in rat slices (**C**, n = 4). Following wash-out of the drug, however, LTP and LTD were reliably produced. Time elapsed from last data point in left panels (with the drug) and first data point in right panels (after wash-out) varied, but was not greater than 30 min.

stimuli at 100 Hz that were repeated at 1 Hz (the delta frequency). Although the changes in field potential amplitude that resulted from this type of delta burst stimulation were of small amplitude, presumably due to the small number of pulses, they did vary systematically from depression to potentiation as the number of stimuli in each burst increased (Fig 6B).

## LAYER IV MAY ACT AS A GATE THAT LIMITS SYNAPTIC PLASTICITY IN LAYER III

The traditional approach to study neocortical plasticity *in vitro* has been to stimulate the white matter and record in layer III. However, using this approach most investigators have been unable to elicit LTP in adult slices without the use of drugs that reduce inhibition (reviewed by Bear and Kirkwood, 1993). Therefore, it was of interest to directly compare the effects of high- and low-frequency conditioning stimulation to layer IV and the white matter. We found that HFS applied to the white matter did not result in significant LTP, whereas layer IV stimulation did result in LTP (Fig 7A). This difference in plasticity does not apply to the effects of LFS: LTD of similar magnitude resulted regardless of whether the stimulation was applied to the white matter or to layer IV (Fig 7B).

The failure of HFS, but not LFS, to induce plasticity when applied to the white matter suggests that HFS of layer IV may evoke different responses in layer III compared to

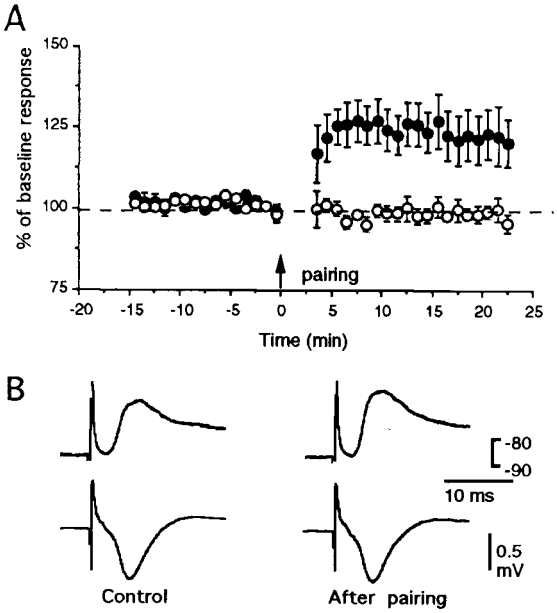


**Fig 5.** Induction of LTP by pairing layer IV stimulation with postsynaptic depolarization. **A**. Average of 7 experiments in which LFS of layer IV (90 pulses at 1 Hz) was paired with intracellular depolarization (0.7 to 1.5 nA DC current to bring membrane potential 15 to 5 mV below EPSP's reversal potential). Baseline responses collected at 0.07 Hz for both intracellular EPSP's (filled circles) and extracellular field potentials (open circles). Pairing delivered at time indicated by arrow and resulted in marked potentiation of intracellular response, but not of field potential. **B**. Intra- and extracellular traces from a representative experiment collected 2 min before (left) and 20 min after pairing (right).

that evoked by HFS of white matter. To address this possibility, the responses of layer III neurons were recorded intracellularly during HFS of layer IV or of the white matter. Representative examples of the intracellular responses to a single 2-s episode of HFS under the different conditions are shown in Fig 7C. HFS of layer IV consistently resulted in a rapid development of a stable depolarization of 10-30 mV on which the individual burst responses would ride. This pattern of depolarization was not observed when white matter was stimulated. To quantify this impression, the area of the depolarization during the tetanus was measured in each case and these values were compared across the two experimental groups and in relation to the development of LTP (Fig 7D). This analysis confirmed that the experimental group in which layer IV was stimulated, which also showed LTP after HFS, was characterized by a significantly greater response during the conditioning.

As we mentioned, a number of groups have reported that application of bicuculline methiodide (BMI) to neocortical slices will allow for the generation of LTP in layer III after white matter stimulation (Artola and Singer, 1987; Kimura *et al*, 1989; Bear *et al*, 1992). We therefore extended our analysis to include HFS of the white matter in the presence of BMI. Under these conditions, the tetanus evoked a response that resembled that evoked during layer IV stimulation without BMI, and it also produced LTP.

Taken together, the analysis suggests that inhibitory circuitry within or deep to layer IV may act to filter high-frequency patterns of activity evoked from white matter, and therefore limit LTP. LTD in unaffected because it is induced using low frequency stimulation. We have called this idea that intracortical circuitry contains the patterns of activity that reach modifiable synapses in layer III the "plasticity gate hypothesis" (Kirkwood and Bear, 1994a). This "gate" might be a site where modulatory inputs to neocortex converge to exert effects on synaptic plasticity (cf Bear and Singer, 1986).

## POSSIBLE SIGNIFICANCE OF NEOCORTEX AS A PREPARATION TO STUDY LTP AND LTD

The evidence summarized in this article is consistent with the idea that very similar mechanisms govern synaptic plasticity in the superficial layers of the visual cortex and in the CA1 region of the hippocampus. Our data do not speak directly to the issue of whether **all** neocortical areas exhibit the same forms of plasticity. However, the fact that the basic rules appear so similar in hippocampus and visual cortex of two widely divergent mammalian species tempts us to speculate that these might represent universal mechanisms for synaptic plasticity in all cortical regions that express NMDA receptors.

Regardless of whether the LTP and LTD described here are "universal" or not, our findings do suggest that many of the properties and mechanisms of synaptic plasticity that have been so extensively studied in CA1 are applicable to visual cortex. The visual cortex offers an important -perhaps unique- advantage over CA1 for the eventual understanding of how LTP and LTD contribute

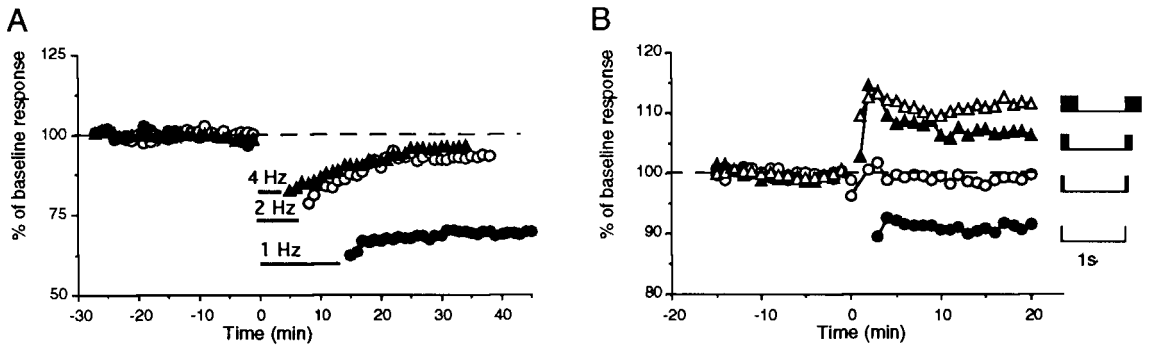


**Fig 6.** LTD is dependent on frequency of conditioning stimulation. **A.** Averaged effects of 900 pulses at 1 Hz (solid circles; n=7), 2 Hz (open circles; n=8) and 4 Hz (filled triangles; n=6) on field potential response evoked in layer III by stimulation of layer IV. **B.** Effects of "delta burst" stimulation on synaptic responses in layer III. A total of 200 conditioning stimuli were delivered to layer IV in different patterns, illustrated on right side of figure. Patterns consisted of bursts of 1 to 8 stimulus pulses repeated every second. Burst frequency was 100 Hz. Left side of figure, average change in synaptic response in layer III following these different types of conditioning stimulation: 1 pulse, filled circles (n =10); 2 pulses, open circles (n = 6); 4 pulses filled triangles (n=18); and 8 pulses, open triangles (n = 9). Error bars omitted in A and B for clarity.

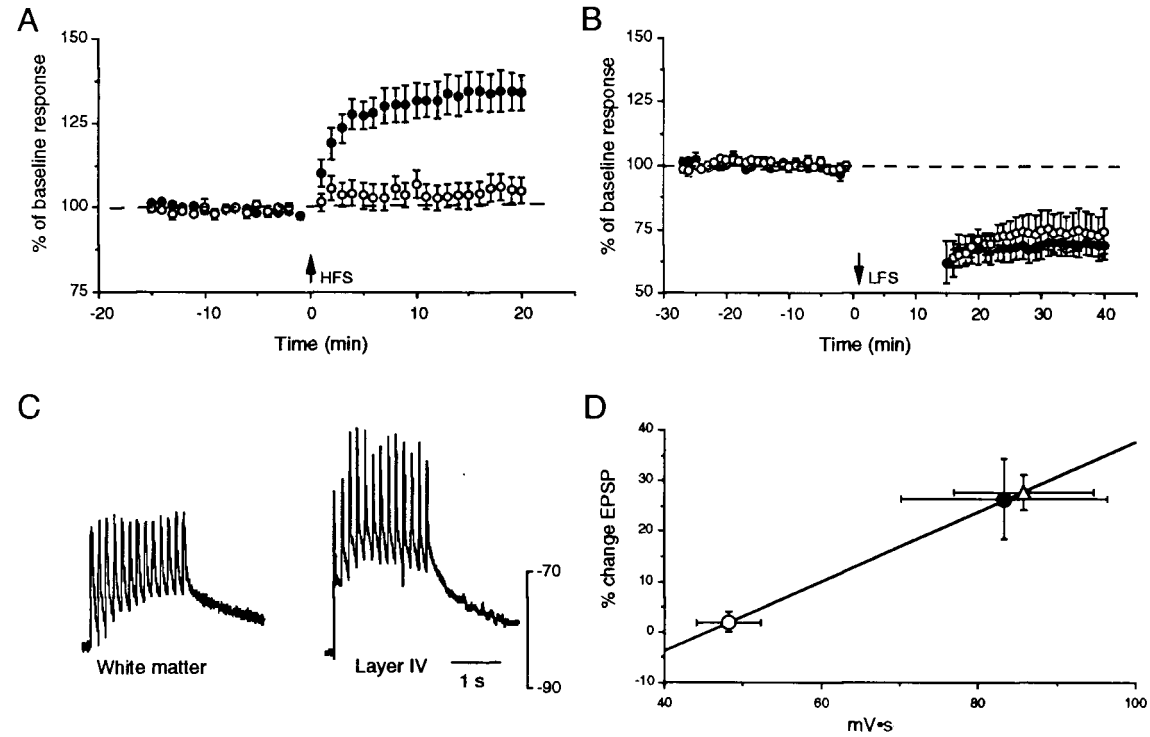


**Fig 7.** Comparison of layer IV and white matter stimulation. **A.** Effects of HFS on field potential responses evoked from white matter (open circles, n = 25) and from layer IV (filled circles, n = 44). **B.** Effects of LFS on field potential responses evoked from white matter (open circles, n = 5) and from layer IV (filled circles, n = 7). **C,D.** Intracellular responses of layer III neurons to theta-burst stimulation of white matter and layer IV. **C.** Representative responses during HFS delivered under indicated conditions. In each case, stimulus amplitude adjusted to yield an EPSP that was 2/3 of action potential threshold. In case of layer IV stimulation, there was marked summation of the four EPSP's within each burst, and a longer-lasting depolarizing potential that bridged the interval between bursts. **D.** Magnitude of change in EPSP amplitude 20 min after HFS plotted as a function of the area of depolarization (mV • s; mean ± SEM) during the HFS. Open circle: white matter stimulation (n = 9); filled circle: layer IV stimulation (n = 6); open triangle: white matter stimulation with BMI applied topically to superficial layers (n = 6).

to the functioning of the brain. This advantage is that the visual cortex lies much closer to the interface between electrophysiology and behavior than does the hippocampus. While the exact significance of the hippocampus to "memory" continues to be debated, no one would question the importance of area 17 to vision. Visual capabilities can be

modified by experience, particularly in young animals, but also in adults. Do these modifications employ the mechanisms of LTP and LTD? We do not know the answer yet, but it is noteworthy that experience-dependent synaptic modifications in the kitten visual cortex, as well as LTP and LTD, are disrupted by the blockade of cortical NMDA receptors (Bear *et al*, 1990).

ACKNOWLEDGMENTS

We thank HK Lee for reading and help editing the manuscript.

REFERENCES

ARTOLA A, SINGER W (1987) Long-term potentiation and NMDA receptors in rat visual cortex. Nature 330: 649-652

BEAR MF, KIRKWOOD A (1993) Neocortical long-term potentiation. Curr Opinion Neurobiol 3: 197-202

BEAR MF, KLEINSCHMIDT A, GU Q, SINGER W (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J Neurosci 10: 909-925

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

BEAR MF, SINGER W (1986) Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 320: 172-176

COLLINGRIDGE GL, KEHL SL, MCLENNAN H (1993) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol, London 334: 33-46

DUDEK SM, BEAR MF (1992) 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 (1993) Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J Neurosci 13: 2910-2918

FRÉGNAC Y (1991) Computational approaches to network processing and plasticity. In: BAUDRY M, DAVIS JL (eds) Long-term Potentiation: A Debate of Current Issues. Cambridge: MIT Press. pp 425-435

GROVER LM, TEYLER TJ (1990) Two components of long-term potentiation induced by different patterns of afferent activation. Nature 347: 477-479

GUSTAFSSON B, WIGSTROM H, ABRAHAM WC, HUANG YY (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. J Neurosci 7: 774-780

KELSO SR, GANONG AH, BROWN T (1986) Hebbian synapses in the hippocampus. Proc Natl Acad Sci USA 83: 5326-5330

KIMURA F, NISHIGORI A, SHIROKAWA T, TSUMOTO T (1989) Long-term potentiation and NMDA receptors in the visual cortex of young rats. J Physiol, London 414: 125-144

KIRKWOOD A, BEAR MF (1994a) Hebbian synapses in visual cortex. J Neurosci 14: 1634-1645

KIRKWOOD A, BEAR MF (1994b) Homosynaptic long-term depression in visual cortex. J Neurosci 14: 3404-3412

KIRKWOOD A, DUDEK SD, GOLD JT, AIZENMAN CD, BEAR MF (1993) Common forms of synaptic plasticity in hippocampus and neocortex *in vitro*. Science 260: 1518-1521

LARSON J, WONG D, LYNCH G (1986) Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res 368: 347-350

MALENKA RC, KAUER JA, ZUCKER RS, NICOLL RA (1988) Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242: 81-84

MALINOW R, MILLER JP (1986) Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. Nature 320: 529-530

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

SASTRY RB, GOH JW, AUYEUNG A (1986) Associative induction of posttetanic and long-term potentiation in CA1 neurons of rat hippocampus. Science 232: 988

WIGSTROM H, GUSTAFSSON B, HUANG Y-Y, ABRAHAM WC (1986) Hippocampal long-term potentiation is induced by pairing simple afferent volleys with intracellularly injected depolarizing pulses. Acta Physiol Scand 126: 317-319

ZALUTSKY RA, NICOLL RA (1990) Comparison of two forms of long-term potentiation in single hippocampal neurons. Science 248: 1619-1624.