require further study in intact systems and the testing of NMDA antagonist drugs in combination with drugs designed to block such other mechanisms.

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12. Oxygen-glucose deprivation was initiated by medium exchange to a deoxygenated solution containing (in mM) NaCl (116 for pH 7.4, 129.5 for pH 6.4), KCl (5.4), $MgSO_4$ (0.8), NaH_2PO_4 (1), $CaCl_2$ (1.8), Pipes (10), $NaHCO_3$ (15 for pH 7.4, 1.5 for pH 6.4), and phenol red (10 mg/liter). Exposure was terminated by medium exchange with oxygenated Eagle's minimum essential media (MEM, Earle's salts) at pH 7.4, supplemented with glutamine and glucose (final concentrations, 2 mM and 5.5 mM, respectively).
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29. We thank K. Rose for technical assistance. D.A.K. was supported by the National Stroke Association. R.G.G. is an Anesthesiology Young Investigator Award recipient from the Foundation for Anesthesia Education and Research. Work was supported by Clinical Investigator Development Award grant NS 01425 (R.G.G.) and NIH grant NS 26907 (D.W.C.).

27 January 1993; accepted 29 March 1993

Common Forms of Synaptic Plasticity in the Hippocampus and Neocortex in Vitro

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Activity-dependent synaptic plasticity in the superficial layers of juvenile cat and adult rat visual neocortex was compared with that in adult rat hippocampal field CA1. Stimulation of neocortical layer IV reliably induced synaptic long-term potentiation (LTP) and long-term depression (LTD) in layer III with precisely the same types of stimulation protocols that were effective in CA1. Neocortical LTP and LTD were specific to the conditioned pathway and, as in the hippocampus, were dependent on activation of *N*-methyl-D-aspartate receptors. These results provide strong support for the view that common principles may govern experience-dependent synaptic plasticity in CA1 and throughout the superficial layers of the mammalian neocortex.

Activity-dependent synaptic plasticity in the mammalian brain is best understood in the CA1 region of the adult hippocampus, where conditioning stimulation of the Schaffer collateral pathway in vitro can induce *N*-methyl-D-aspartate (NMDA) receptor-dependent LTP (*1, 2*) and LTD (*3, 4*). An important question is whether what has been learned about the hippocampus can be applied generally to synaptic plasticity in the cerebral cortex. Although LTP has been demonstrated in the visual cortex (*5*), in the mature neocortex in vitro it has been reported to occur with low probability (*6–8*) and to require for induction pharmacological treatments to reduce inhibition (*9–11*) and stimulation patterns that vary substantially from those that are effective in the hippocampus (*6, 7, 12–14*). Similarly, the conditions for evoking LTD in the hippocampus may be very different from those in the neocortex. For example, in the hippocampus low-frequency stimulation evokes NMDA-dependent LTD (*3, 4*), whereas the same type of stimulation in the neocortex can yield LTP (*6*). Furthermore, strong stimulation in the presence of NMDA receptor antagonists causes LTD in the neocortex (*15, 16*) but no change in the CA1 region (*17*). In order to reconcile these disparate results, we directly compared the plasticity of synaptic responses evoked in adult rat hippocampal field CA1 with those evoked in adult rat and immature cat visual cortical layer III. To more closely approximate the stimulation-recording arrangement in the hippocampus (stimulating the Schaffer collaterals and recording in CA1), in neocortical preparations we stimulated the direct input to layer III from layer IV rather than using the traditional approach of stimulating the white matter (*18*).

In CA1, brief high-frequency (100 Hz) bursts of stimulation delivered to the Schaffer collaterals at the theta rhythm (5 to 7

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Hz) consistently lead to induction of LTP that is specific to the conditioned pathway and is sensitive to the pharmacological blockade of NMDA receptors (*19*) (Fig. 1A). Consistent with previous observations (*20*), we observed that the potentiated response often decayed over the 20 to 40 min immediately after the tetanus until a stable value was reached, which in our experiments was 143 ± 7% of the base line control (n = 4). LTD was produced by 900 pulses delivered at 1 to 3 Hz, either from a potentiated (Fig. 1A, row 2) or a naïve (Fig. 1A, row 5) state. Like LTP, this synaptic depression persisted for many hours in vitro, was input-specific, and depended on NMDA receptor activation for its induction (*3, 4*). From the naïve state, 900 pulses at 1 Hz reduced the response to 80 ± 4% of the base line control, measured 45 min after the cessation of conditioning stimulation (n = 5).

Despite the fact that the superficial layers of the sensory neocortex are rich in NMDA receptors (*21*), attempts to evoke plasticity in layer III with protocols identical to those in hippocampal experiments have generally met with limited success (*14*). The standard approach has been to stimulate ascending corticipetal fibers at the white matter–layer VI border and to record either intracellularly or extracellularly in layer III (*5–14*). However, this arrangement may not be optimal because much of the activity evoked in layer III by white matter stimulation is relayed by layer IV neurons (*22*). Therefore, to better approximate the situation in CA1, we attempted to evoke synaptic plasticity in visual neocortex by stimulating the more direct path from layer IV to layer III.

Stimulation of layer IV in slices of adult rat visual cortex evoked a large negative field potential in layer III with a peak latency of ~4 ms (Fig. 1B, row 3) (*23*). As with the hippocampus, theta-burst stimulation (TBS) potentiated the layer III response to test stimulation, and low-frequency stimulation (LFS) caused LTD from either a potentiated or a naïve state (Fig. 1B). In contrast to the hippocampus, there was no evidence for a decaying form of potentiation in rat visual cortex immediately after TBS (Fig. 1B, row 4); rather, the response increased in the first 15 min and reached a plateau value of 132 ± 4% of the base line control (n = 43). However, just like hippocampus (*3*), LFS depressed the naïve synaptic response in a frequency-dependent manner; 900 pulses at 1 Hz reduced the response to 71 ± 7% of the base line control (n = 5; Fig. 1B, row 5) but at 4 Hz had no lasting effect (98 ± 10%, n = 4). These forms of plasticity are not peculiar to the rat cortex; layer III responses in visual cortex prepared from 5- to 7-week-old kittens also exhibited LTP and LTD after TBS and LFS, respectively, of layer IV (Fig. 1C). To confirm that changes in the field potential truly reflect modifications of synaptic potentials, we simultaneously recorded intracellular excitatory postsynaptic potentials (EPSPs) and extracellular field potentials in rat visual cortical layer III (n = 5). In all cases, a change in the field potential amplitude correlated with a change in the EPSP (Fig. 2, A and B).

In the hippocampus, both LTP and LTD are input-specific; only the conditioned inputs demonstrate the plasticity (*1, 3*). We addressed this issue in our neocortical preparation by monitoring the response to a second input originating in layer III just lateral to the recording site (Fig. 2C). If the plasticity induced by layer IV stimulation was not input-specific, the LTP and LTD would have been reflected in the response to layer III stimulation. However, conditioning stimulation of layer IV caused a change only in the base line response evoked from layer IV; responses evoked from adjacent layer III were unaffected (Fig. 2D).

Previous work in CA1 has shown that induction of LTD by low-frequency stimulation can be blocked by antagonists of NMDA receptors (*3*). In contrast, a form of LTD has been reported for the neocortex, whose induction by patterned high-frequency stimulation (400 to 500 pulses delivered in 50- to 100-Hz bursts of 1 to 2 s in duration) is actually promoted by NMDA receptor blockade (*15, 16*). This difference could be accounted for by the different stimulation protocols or, alternatively, by differences in the two types of cortex. To address this question, we applied the NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid (AP5; 100 μM) to slices of rat visual cortex and attempted to induce LTD with 1-Hz stimulation. Blockade of NMDA receptors in the visual cortex inhibited the induction of LTD by LFS, as

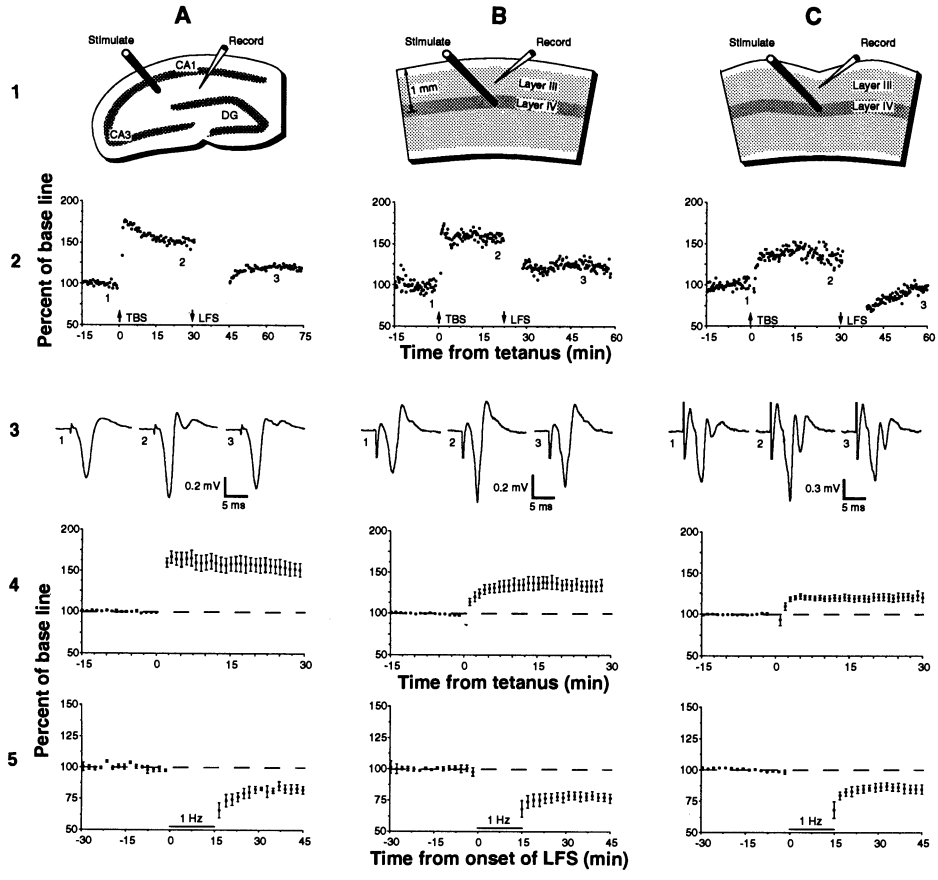

Fig. 1. Similar forms of synaptic plasticity in slices of adult rat hippocampus (**A**), adult rat visual cortex (**B**), and immature cat visual cortex (**C**). Row 1 shows the stimulation-recording configurations (DG, dentate gyrus). Row 2 shows changes in the extracellular field potential induced by TBS and by LFS [900 pulses were delivered at 1 Hz in (A) and (C) and at 3 Hz in (B)]. Response magnitude was measured as the change in the initial slope of the negative field potential in (A) and as the peak negativity in (B) and (C). Row 3 shows averages of four consecutive field potentials taken in each preparation before conditioning stimulation, after TBS, and after LFS for the experiments in row 2. Row 4 shows the average change in response magnitude after TBS [n = 4 for (A); n = 19 for (B); and n = 9 for (C)]. Row 5 shows the average change in response after LFS (900 pulses at 1 Hz), starting from an unpotentiated state [n = 5 for (A); n = 5 for (B); and n = 6 for (C)].

in the hippocampus (Table 1). Also, induction of LTP by TBS in both rat and kitten visual cortex was blocked by AP5, as has been reported for CA1 (*24*) (Table 1).

The plasticity we report here for the neocortex occurs with high probability and does not require the use of drugs such as bicuculline. Our success may be attributable to the fact that we stimulated layer IV rather than using the traditional approach of stimulating the fibers at the layer VI–white matter border. Indeed, a direct comparison of the effects of TBS delivered to layers VI and IV (without the use of bicuculline) confirms that these two configurations have different effects on the layer III evoked response (Fig. 3A). The average change in response magnitude 20 min after conditioning stimulation of layer VI was only 108 ± 4% of the base line control, which was less than that obtained after layer IV stimulation ($P < .001$; t test). Although the half-maximal responses collected for the base line are greater when layer IV is stimulated (683 ± 62 μV) than when layer VI is stimulated (318 ± 59 μV), this does not appear to be sufficient to account for the difference in LTP (Fig. 3B). We believe that the key difference lies in the distinct patterns of cortical activation that result from stimulation of the two sites (*25*).

Our work demonstrates that when similar preparations of neocortex and hippocampus are studied in vitro, they can yield similar forms of synaptic plasticity. Although some differences remain in the details (*26*), our data suggest the existence of a common substrate, across both phylogeny and postnatal age, for activity-dependent synaptic plasticity in CA1 and in the superficial layers of the neocortex. The substrate we describe here involves the modification of excitatory synaptic effectiveness according to the pattern or amount of NMDA receptor activation. One implication of this finding is that pharmacological manipulations of NMDA receptors in vivo are likely to interfere equally with mechanisms of synaptic enhancement and depression. Recently, NMDA receptor–independent forms of synaptic plasticity have been reported for both visual cortex (*15, 16, 27, 28*) and CA1 (*29*). If these similarly prove to be governed according to common principles, it may be possible to construct a

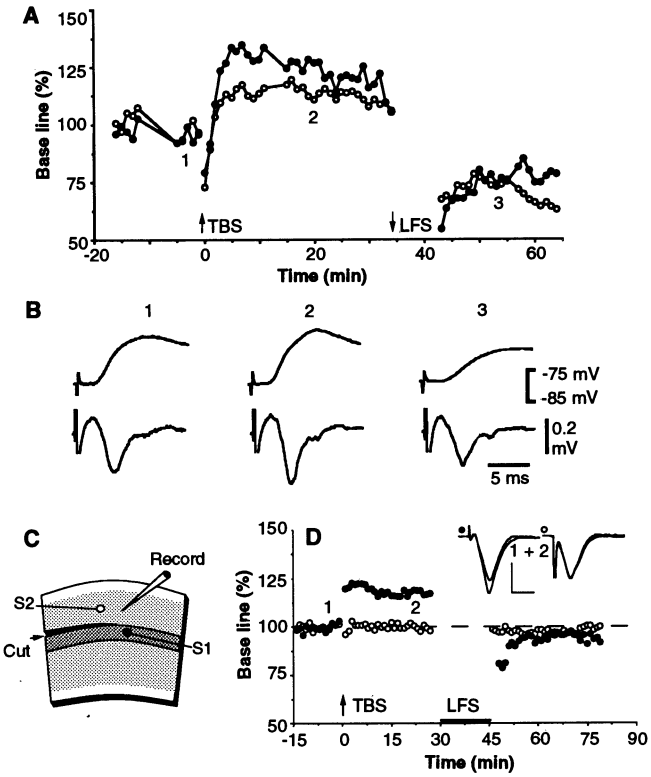

Fig. 2. Intracellular correlates of plasticity of the layer III field potential. (**A**) Record of an experiment in which intracellular recordings were made from a layer III neuron as nearby layer III field potentials were monitored. Open circles are the amplitude of the EPSPs, and filled circles are the negative peak of the field potentials. (**B**) Average (n = 4) EPSPs (upper row) and field potentials (lower row) taken from the experiment in (A) at the indicated times. (**C**) Stimulation-recording configuration to assess the input specificity of LTP and LTD in visual cortex. Field potentials were evoked by stimulation of either layer IV (S1) or layer III (S2). We isolated the layer III stimulation site from layer IV by making a partial transection of the slice as shown. The field potentials evoked by S1 and S2 were matched for amplitude and were additive at all stimulus intensities, which suggests that the responses were generated by independent inputs converging on the same target population of postsynaptic neurons. (**D**) Input-specific LTP and LTD with the stimulation-recording arrangement in (C). TBS stimulation of S1 produced an enhancement of the response to base line stimulation of S1 only (filled circles); LFS of S1 produced a depression of the response to base line stimulation of S1 only. Each data point is the average from four experiments; error bars were omitted for clarity. Inset shows average of four consecutive field potentials evoked from layer IV (filled circle) or from layer III (open circle) before and after TBS in one of these cases at the indicated times. Scale bars of inset: 5 ms, 0.25 mV.

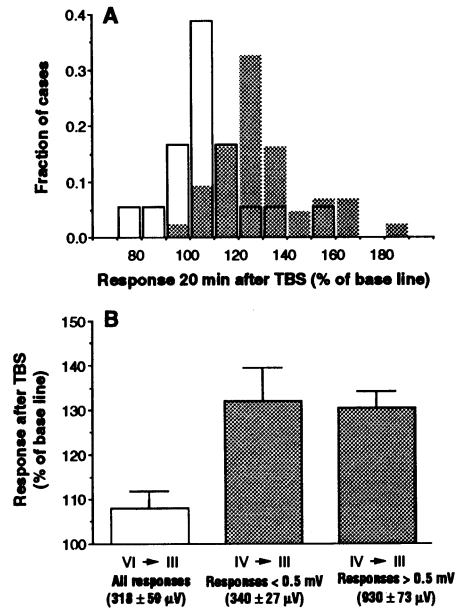

Fig. 3. Differential effects of TBS on the layer III field potential evoked by stimulation of layers IV and VI. (**A**) The fraction of cases in which TBS induced a change in the field amplitude (percent of base line) when layer VI was stimulated (open bars, n = 18) and when layer IV was stimulated (hatched bars, n = 43). These distributions were significantly different ($P < 0.001$, t test). (**B**) Differences in LTP are not accounted for by the differences in field amplitude. The layer IV cases were divided according to whether the half-maximal field amplitude was greater or less than 0.5 mV. The low-amplitude layer IV group, with a mean response size comparable to the layer VI group, still yielded significantly greater LTP ($P < 0.01$, t test).

Table 1. Effects of conditioning stimulation on synaptic responses (mean ± SEM) in the visual cortex in the presence or absence of 100 μM AP5. The AP5 condition was different than the wash group in all cases ($P \leq 0.01$, t test).

Group	Change in synaptic responses					
	Theta burst in rat		Theta burst in cat		1 Hz in rat	
	% of base line control	n	% of base line control	n	% of base line control	n
Controls	132 ± 4	43	122 ± 4	14	71 ± 7	5
AP5	100 ± 5	4	96 ± 3	4	99 ± 4	4
Wash	128 ± 13	4	128 ± 13	4	87 ± 8	4

general theory for experience-dependent synaptic modification in the mammalian cerebral cortex.

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18. Brain slices (400 μm) were prepared and maintained in an interface chamber (Medical Systems, Greenvale, NY) as described (*3, 11*). Microelectrodes were filled with 1 M NaCl (1 to 2 megohms) for extracellular recording or 3 M potassium acetate (80 to 120 megohms) for intracellular recording. Synaptic responses were evoked with 0.02-ms pulses (amplitude, 10 to 200 μA) delivered with a bipolar concentric stimulating electrode (outside diameter, 200 μm). In every experiment, a full input-output curve was generated, and base line responses were obtained at 0.07 to 0.03 Hz with a stimulation intensity that yielded a half-maximal response. To induce LTP, we delivered two to five episodes of TBS at 0.1 Hz. TBS consisted of 10 to 13 stimulus trains delivered at 5 to 7 Hz; each train consisted of four pulses at 100 Hz. To induce LTD, we delivered 900 pulses at 1 to 3 Hz. All rats used were adults (>40 days old).
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22. Anatomical experiments indicate that fibers ascending through layer VI terminate densely in layer IV but relatively sparsely in layer III (*30*), and current-source density analysis shows that the prominent current sink in layer III that is evoked by stimulation of the white matter is mostly di- and polysynaptic and always follows activation of layer IV synapses (*6, 31*). Accordingly, the EPSPs evoked by white matter stimulation fail to exhibit a monosynaptic component in half the layer III neurons recorded (*6, 27*). Electrical stimulation of layer IV, besides activating the projection from layer IV neurons, also recruits any direct inputs to layer III that ascend from the white matter.
23. This negative peak was maximal at a depth of ~300 μm from the pia, and current-source density analysis indicates that it reflects a prominent layer III synaptic current sink; therefore, the amplitude of this field potential was used routinely as a measure of synaptic effectiveness.
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25. We caution, however, that these consequences of layer IV stimulation need not be accounted for solely by activation of the projection of layer IV neurons onto the dendrites in layer III. Clearly, the effects of intracortical stimulation can be quite complex and, in principle, could include the antidromic activation of some layer III neurons and orthodromic activation of intracortical and corticocortical fibers passing through layer IV [cells in the infragranular layers are not part of the essential circuit as these can be cut away and LTP is left intact (131 ± 6%; n = 7)]. However, these complexities are not unique to experiments where layer IV is stimulated. Indeed, stimulation of the cortical white matter may be even more complex considering that in addition to activation of corticipetal fibers and synaptic activation of layer IV neurons, the cells in layers VI, V, and III could all be activated antidromically. The effects of white matter stimulation are further complicated by the requisite use of bicuculline, which, at the concentrations usually used, often promotes widespread epileptiform activation of cortical slices (*32*). Thus, despite some potential complications, layer IV stimulation appears to be an advantageous preparation for the investigation of synaptic plasticity in the superficial layers of the neocortex.
26. One of these residual differences is an early time-course of the responses immediately after the TBS. In the hippocampus, a potentiated response typically is observed immediately after the tetanus and declines to a stable value in about 30 min. The decaying form of potentiation in the hippocampus has been termed short-term potentiation (STP) and may be mechanistically distinct from LTP (*33*). STP typically is not observed in the neocortex.
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34. Supported by grants from the National Eye Institute and the U.S. Office of Naval Research. A.K. was supported in part by a grant from the Human Frontiers Science Program. We thank J. Lisman, L. Cooper, and B. Connors for critically reading an earlier version of the manuscript and J. P. Donoghue for helpful discussions.

4 December 1992; accepted 25 February 1993

Presence of Mitochondrial Large Ribosomal RNA Outside Mitochondria in Germ Plasm of *Drosophila melanogaster*

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Mitochondrial large ribosomal RNA (mtlrRNA) has been identified as a cytoplasmic factor that induces pole cell formation in embryos whose ability to form a germ line has been abolished by treatment with ultraviolet light. In situ hybridization analyses reveal that mtlrRNA is enriched in germ plasm and is tightly associated with polar granules, the distinctive organelles of germ plasm, which supports the idea that mtlrRNA functions in pole cell formation. This suggests that a product from the mitochondrial genome, along with nuclear products, participates in a key event in embryonic development: determination of the germ line.

The segregation of the germ and somatic line in animal embryos represents one of the basic events in cell and developmental biology. In many animal groups, the factor required for germ-line establishment has been postulated to be localized in a histologically remarkable region in egg cytoplasm, the germ plasm (*1*). In *Drosophila*, germ plasm is referred to as polar plasm because it is localized in the posterior pole region of oocytes and cleavage embryos (*2, 3*). The polar plasm contains factors for germ-line and abdomen formation (*4–6*). The factor for abdomen formation has been identified as the product of the *nanos* (*nos*) gene (*7, 8*). The factors for germ-line formation, however, have remained elusive. Recent genetic analyses have identified seven maternally acting genes called posterior group genes [*cappuccino* (*capu*), *spire* (*spir*), *staufen* (*stau*), *oskar* (*osk*), *vasa* (*vas*), *valois* (*vls*), and *tudor* (*tud*)] whose functions are required for the localization of factors for the germ line as well as of the *nos* product in the polar plasm (*4, 9, 10*). Embryos from these mutants fail to form a germ line and abdomen. In addition, they lack polar granules, the distinctive organelles of polar plasm, which suggests that polar granules are essential for both germ-line and abdomen formation (*9*).

After fertilization, nine nuclear divisions take place without cytokinesis in the central region of embryos (the cleavage stage). The nuclei then migrate to the periphery (the syncytial blastoderm stage). As the nuclei enter the posterior polar plasm, each of them is included in a cytoplasmic protrusion that contains polar

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