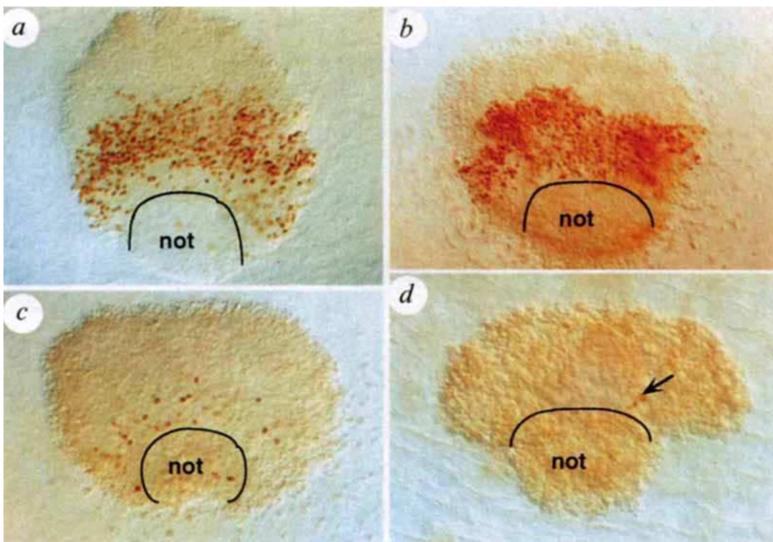

FIG. 4 Antibody-mediated blocking of motor-neuron induction by notochord. *a*, *b*, Examples of Islet-1 induction in neural plate explants cultured in contact with control notochords. Notochord in (*a*) was pre-incubated in Ringer's solution. Notochord in (*b*) was pre-incubated in pre-immune serum and cultured in the presence of pre-immune serum. *c*, *d*, Examples of Islet-1 induction in explants cultured in contact with notochords, in the presence of an affinity-purified rabbit polyclonal antiserum specific to the 19K N-terminal Shh peptide (Ab 80)[2]; arrow points to 2 Islet-1 expressing cells.
METHODS. Notochords from stage 6 to 7 chick embryos[21] were incubated for 3 h at 37 °C in a 5% CO_2 atmosphere in Ringer's solution, or Ringer's supplemented with either 50 $\mu g\ ml^{-1}$ of pre-immune serum or an affinity-purified antibody raised against the amino peptide of Shh (Ab 80)[2]. Pre-immune serum (from the rabbit used to generate the Ab 80 antibody) was purified on a protein A–Sepharose CL-4B column (Pharmacia), eluted in 150 mM glycine (pH 2.5), neutralized, then dialysed against PBS. Affinity purification of Ab 80 is described elsewhere[2]. Tissue recombinants were prepared and cultured as described in Fig. 1. Culture media for the experimental tissue recombinants was supplemented with 5 $\mu g\ ml^{-1}$ of Ab 80 and for the control notochords with 5 $\mu g\ ml^{-1}$ of pre-immune serum.

In summary, our results demonstrate that the active signalling component of Shh is the amino 19K peptide. A similar conclusion has recently been reached with respect to its *Drosophila* counterpart Hh[20,27]. This is both necessary and sufficient for the induction of distinct ventral cell types *in vitro*. Thus the 19K peptide appears to act as a morphogen. Whether the different responses depend on the actual concentration of the factor itself or the method of presentation to the receiving cell is not yet clear. The demonstration that Shh can induce uncommitted neural progenitors to form motor neurons may have clinical significance. Moreover, the widespread expression of Shh in prechordal plate mesoderm, notochord and the ventral midline of the CNS along the entire rostral–caudal length of the CNS[30] raises the possibility that Shh may induce other ventral cell types at different axial levels. For example, it has recently been demonstrated that dopaminergic neurons, which arise in the ventral midbrain, are induced in response to a signal derived from floor plate[28]. In addition, Shh has been implicated in patterning structures outside the CNS, most notably along the anterior–posterior axis of the limb[18]. It is not yet known whether recombinant Shh peptide is able to mirror the dose-dependent effects of ZPA signalling. Finally, there are obvious similarities with the role of *Drosophila* Hh. However, *Drosophila*, Hh appears to act indirectly, by regulating the expression of a second signal encoded by the *TGF-β* superfamily member *decapentaplegic*[1,11,14,15,17]. We have no evidence for a second signal regulated by Shh in early neural tissue, however, suggesting that Shh may act directly to pattern the vertebrate CNS. □

Received 23 February; accepted 2 May 1995.

1. Smith, J. C. *Curr. Biol.* **3,** 582–585 (1993).
2. Bumcrot, D. A., Takada, R. & McMahon, A. P. *Molec. cell. Biol.* **15,** 2294–2303 (1995).
3. Echelard, Y. *et al. Cell* **75,** 1417–1430 (1993).
4. Krauss, S., Concordet, J.-P. & Ingham, P. W. *Cell* **75,** 1431–1444 (1993).
5. Roelink, H. *et al. Cell* **76,** 761–775 (1994).
6. Fietz, M. J. *et al. Development* (Suppl.) **120,** 43–51 (1994).
7. Mohler, J. *Genetics* **120,** 1061–1072 (1988).
8. Lee, J. L., von Kessler, D. P., Parks, S. & Beachy, P. *Cell* **71,** 33–50 (1992).
9. Ingham, P. W. *Nature* **366,** 560–562 (1993).
10. Heberlein, U., Wolff, T. & Rubin, G. M. *Cell* **75,** 913–926 (1993).
11. Ma, C., Zhou, Y., Beachy, P. A. & Moses, K. *Cell* **75,** 927–938 (1993).
12. Tabata, T. & Kornberg, T. B. *Cell* **76,** 89–102 (1994).
13. Heemskerk, J. & DiNardo, S. *Cell* **76,** 449–460 (1994).
14. Basler, K. & Struhl, G. *Nature* **368,** 208–214 (1994).
15. Díaz-Benjumea, F. J., Cohen, B. & Cohen, S. M. *Nature* **372,** 175–179 (1994).
16. Lee, J. J. *et al. Science* **266,** 1528–1537 (1994).
17. Ingham, P. W. & Fietz, M. J. *Curr. Biol.* **5(4),** 432–441 (1995).
18. Riddle, R. D., Johnson, R. L., Laufer, E. & Tabin, C. *Cell* **75,** 1401–1416 (1993).
19. Chang, D. T. *et al. Development* **120,** 3339–3353 (1994).
20. Porter, J. A. *et al. Nature* **374,** 363–366 (1995).
21. Hamburger, V. & Hamilton, H. L. *J. Morph.* **88,** 49–92 (1951).
22. Tsuchida, T. *et al. Cell* **79,** 957–970 (1995).
23. Tanaka, H. & Obata, K. *Devl Biol.* **106,** 26–37 (1984).
24. Yamada, T., Placzek, M., Tanaka, H., Dodd, J. & Jessell, T. M. *Cell* **64,** 635–647 (1991).
25. Thor, S., Ericson, J., Brännström, T. & Edlund, T. *Neuron* **7,** 881–889 (1991).
26. Yamada, T., Pfaff, S. L., Edlund, T. & Jessell, T. M. *Cell* **73,** 673–686 (1993).
27. Fietz, M. J., Jacinto, A., Taylor, A. M., Alexandre, C. & Ingham, P. W. *Curr. Biol.* (in the press).
28. Hynes, M., Poulsen, K., Tessier-Lavigne, M. & Rosenthal, A. *Cell* **80,** 95–101 (1995).
29. Dubendorff, J. W. & Studier, F. W. *J. molec. Biol.* **219,** 45–59 (1991).
30. Martí, E., Takada, R., Bumcrot, D. A., Sasaki, H. & McMahon, A. P. *Development* (in the press).

ACKNOWLEDGEMENTS. We thank C. Tabin for the chick Shh complementary DNA probe; T. Jessell for the chick Islet-2 cDNA probe; T. Edlund for the Islet-1 antiserum; the Developmental Hybridoma Bank for the SC-1/BEN antiserum; and T. Yamada, M. Tessier-Lavigne and M. Dickinson for advice on explant cultures. This work was supported by a grant from the NIH to A.P.M., and postdoctoral fellowships from the NIH to E.M. and the Muscular Dystrophy Association to D.A.B.

A critical period for long-term potentiation at thalamocortical synapses

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In mammalian development, the refinement of topographical projections from the thalamus to the cortex is thought to arise through an activity-dependent process in which thalamic axons compete for cortical targets[1,2]. In support of this view, if activity is altered during a critical period in early development, normal connectivity is disrupted[1,2]. It has been proposed that synaptic connections are strengthened during development by correlated pre- and postsynaptic activity[3,4], and a likely mechanism for this process would be *N*-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP)[5,6]. However, the evidence that LTP is involved in normal development remains inconclusive. We have examined LTP in the thalamocortical synapses that form whisker barrels in rat somatosensory cortex (S1). We report here that the period during which LTP can be induced matches closely the critical period during which the barrels can be modified by sensory perturbations. Moreover, the loss of susceptibility to LTP with age is accompanied by a decrease in NMDA receptor-mediated synaptic currents. These findings provide compelling evidence that LTP is important for the development of cortical circuitry.

The barrels of S1 cortex are a somatotopic representation of sensory vibrissae on the rodent's muzzle which are formed by aggregates of ventrobasal thalamic (VB) afferents that innervate discrete clusters of layer IV cells[7,8]. Although the development of the gross morphology of barrels does not appear to require

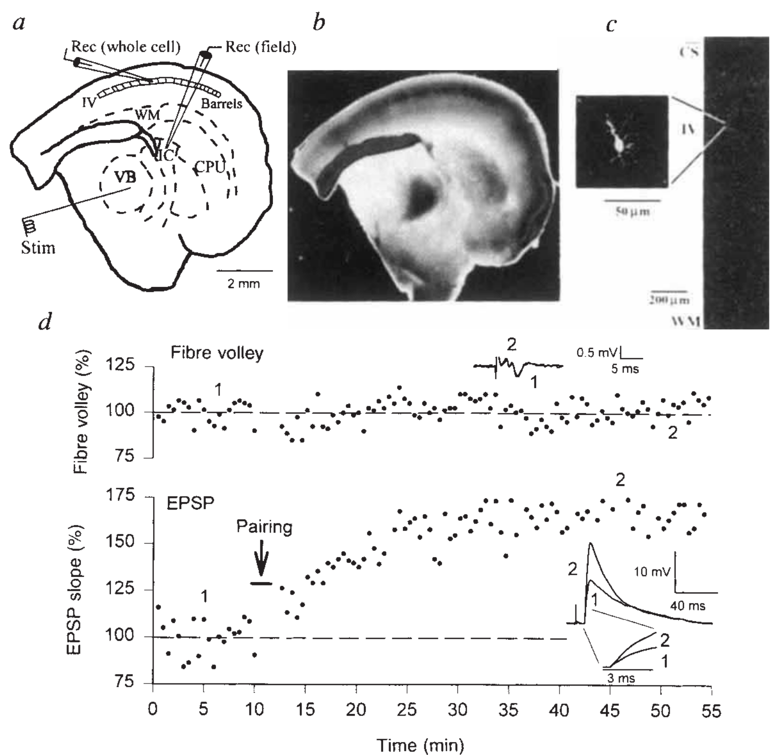

FIG. 1 Long-term potentiation (LTP) in layer IV cells of a thalamocortical slice preparation. *a*, Diagram of the preparation. Stimulating electrodes (Stim) are placed directly in the ventrobasal nucleus of the thalamus (VB) and monosynaptic responses are recorded from layer IV cells (Rec, whole cell). CPU, caudate/putamen; IC, internal capsule; WM, white matter. *b*, Slice preparation (P9) in the recording chamber, illustrating that transillumination reveals identifiable layer IV barrels and VB. *c*, An example of a fluorescent Nissl-stained section (P5) with a Lucifer yellow filled layer IV cell (CS, cortical surface). *d*, Example of LTP from P3 slice showing the slow growth of the potentiation in the absence of any change in the simultaneously recorded fibre volley from the internal capsule. Insets show example data traces taken at the times indicated by the numbers in the graph. Note that expansion of the traces demonstrates a clear increase in the initial slope of the e.p.s.ps.

METHODS. Slices from pups of timed-pregnant Sprague-Dawley rats (P0 is defined as the first 24 h after birth) were prepared as described[12]. Recording techniques, data acquisition and data analysis were as described[29]. Afferent stimulation was maintained at 0.033 Hz. The composition of the extracellular perfusing solution was 119 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$ and 11 mM glucose. The whole-cell recording pipette solution contained 117.5 mM caesium gluconate, 17.5 mM CsCl, 8 mM NaCl, 10 mM HEPES, 0.2 mM EGTA, 4 mM Mg-ATP and 0.3 mM GTP (pH = 7.2, 280–290 mosmol). Cells were studied only if upon break-in they generated overshooting action potentials, had input resistances >300 MΩ, and synaptic responses were stable with no signs of drift for >10 min before LTP induction. Only one cell per slice was studied. For voltage-clamp experiments the fast peak of the excitatory postsynaptic current (e.p.s.c.) was measured. In all experiments, a fibre volley was simultaneously recorded from the internal capsule and the experiment was discarded if its amplitude increased. Data in the text and in the summary graphs are presented as mean ± s.e.m. except where noted. The mean percentage change in e.p.s.p./e.p.s.c. magnitude was calculated by averaging over a 10 min window taken 30–40 min after the LTP induction protocol and comparing this value to the 10 min baseline period. Slices with Lucifer yellow CH (1%)-filled cells were counterstained with the fluorescent Nissl stain bisbenzamide (2.5 μg ml^{-1}) to identify laminae.

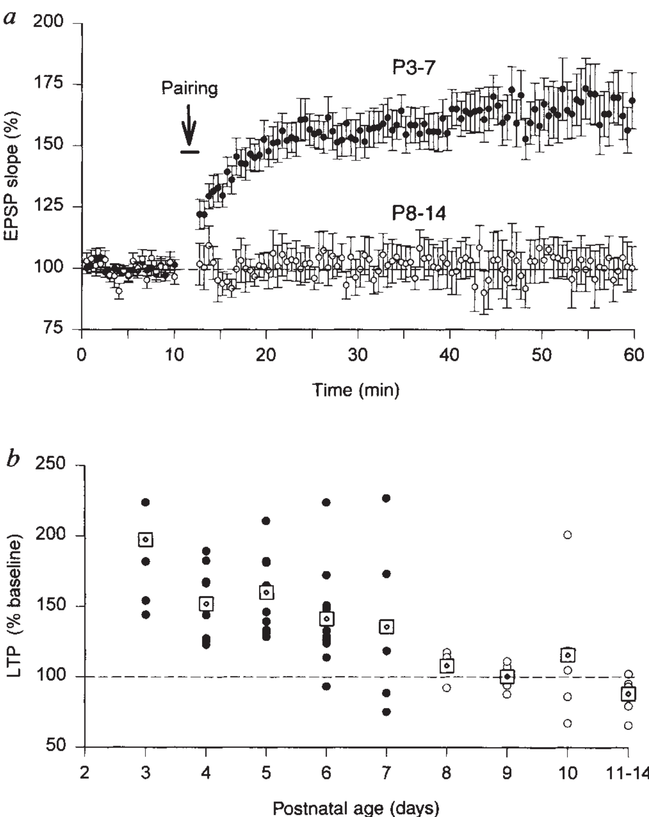

FIG. 2 LTP is limited to the critical period. *a*, Summary graphs of the effects on synaptic transmission of applying afferent stimulation (100 stimuli at 1 Hz) while the cell is depolarized (−10 to 0 mV) (pairing) in P3–7 slices ($n = 43$) and P8–14 slices ($n = 20$). *b*, A scatter diagram showing that the magnitude of LTP gradually decreases during the critical period. Each point represents a single cell which contributed to the graphs in *a*. For clarity, one P3 cell is not displayed (LTP magnitude = 282%). The squares show the mean LTP magnitude for that age.

postnatal cortical activity[9], manipulations of the sensory periphery during the critical period cause a profound rearrangement of the normal somatotopic patterning of VB afferents[10], an effect that is activity dependent[11]. Using a novel thalamocortical slice preparation[12] (Fig. 1*a*), we have examined the occurrence and properties of LTP at the synapse between VB afferents and layer IV cells. Transillumination of the slice (Fig. 1*b*) reveals cytoarchitectonic structures that clearly identify layer IV and VB, allowing for placement of microelectrodes under visual control. To confirm the location of layer IV in each slice, the characteristic profile of field excitatory postsynaptic potentials (e.p.s.ps) across lamina were recorded[12]. In addition, several cells ($n = 6$) were stained with Lucifer yellow, and all were found to be in layer IV (Fig. 1*c*). To ensure that monosynaptic e.p.s.ps were examined, recordings were rejected if there was any change in the latency of the e.p.s.p. when adjusting stimulation strength from threshold to saturation or during higher frequency (1 Hz) stimulation. Moreover, synaptic strength was assayed by measuring the very early initial slope of e.p.s.ps over the first 0.3–1.5 ms of the response.

Initially, we prepared slices from animals within one week of birth (postnatal day (P) 3–7) and attempted to elicit LTP by applying presynaptic stimulation (100 stimuli at 1 Hz to VB)

during direct postsynaptic depolarization of layer IV cells (to between −10 mV and 0 mV), a protocol (termed 'pairing' in Figs 1–3) which ensures that the essential ingredients required for generation of LTP are provided. In P3–7 slices, this protocol elicited robust LTP of the monosynaptic e.p.s.p. in the absence of any change in the afferent fibre volley (Figs 1*d* and 2). Like other forms of cortical LTP[13], the increase in synaptic strength developed slowly over 5–15 min before stabilizing for the duration of the recording. Consistent with the path length of axons from VB to S1, the latency of the e.p.s.ps was long (10.3 ± 0.6 ms, $n = 14$) and did not change following LTP (Fig. 1*d*).

After the first postnatal week, even severe sensory manipulations do not disrupt layer IV barrel field topography[10,11]. To determine whether LTP at layer IV thalamocortical synapses is limited to the critical period, we examined the ability to generate LTP in slices prepared from older animals (P8–14). Although robust LTP could be generated in slices up to P7 (158 ± 6%, $n = 43$), thereafter it was extremely difficult to elicit any LTP (102 ± 6%, $n = 20$) (Fig. 2*a*). From P3 to P7, the magnitude of LTP gradually decreased (Fig. 2*b*) such that, by P8, LTP (greater than 120%) was observed in only 1 of 20 cells. The input resistance of layer IV cells also decreased with development (P3–7, 844 ± 68 MΩ, $n = 43$; P8–14, 420 ± 23 MΩ, $n = 20$, $P < 0.01$). However, this cannot account for the inability to elicit LTP in older slices because all cells were held near the e.p.s.p. reversal potential during the LTP induction protocol.

To understand the mechanisms responsible for the loss of LTP it was first necessary to define its basic properties in young slices (P3–6). We initially tested whether postsynaptic depolarization was in fact required for eliciting LTP, and found that voltage clamping cells at −95 mV prevented LTP (96 ± 9%, $n = 5$) (Fig. 3*a*). Depolarizing cells in the absence of afferent stimulation caused a small increase in synaptic strength (Fig. 3*b*) (116 ± 6%, $n = 18$) which was clearly much less ($P < 0.001$) than that elicited by the standard LTP induction protocol. To determine whether LTP requires a rise in postsynaptic calcium concentration, cells were loaded with the calcium chelator BAPTA (10 mM), which completely blocked the LTP normally elicited by stimulating afferents during postsynaptic depolarization (Fig. 3*c*) (97 ± 8%, $n = 10$). The requirements for afferent stimulation, postsynaptic depolarization and a rise in postsynaptic calcium suggested that synaptic activation of NMDA receptors is critically important for generating LTP. Consistent with this proposal, D-APV (50 μM) strongly inhibited LTP (Fig. 3*d*) (116 ± 5%, $n = 25$).

Previous work has suggested that the properties of synaptic NMDA receptors change early during development[14,15], a change that in visual cortex[15] occurs during a comparable period of development to that examined here. To determine if similar changes occur in layer IV thalamocortical synapses in S1, we examined pharmacologically isolated NMDA receptor-mediated synaptic currents. There was a profound decrease in the relative contribution of NMDA receptors to synaptic currents as a function of age (Fig. 4*a*, *b*), such that the ratio of currents mediated by NMDA receptors to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors decreased by over fivefold from 1.62 ± 0.9 (mean ± s.d.) for P3–7 slices ($n = 14$) to 0.30 ± 0.2 for P8–14 slices ($n = 9$; $P < 0.0001$). This change could be due either to a relative decrease in synaptic NMDA receptors or a relative increase in synaptic AMPA receptors. However, the time course of NMDA receptor synaptic currents also decreased dramatically with age (Fig. 4*c*, *d*). Their estimated time constant of decay decreased from 248 ± 62 ms (mean ± s.d.) for P3–7 cells ($n = 14$) to 113 ± 26 ms for P8–14 cells ($n = 9$, $P < 0.0001$). Thus,

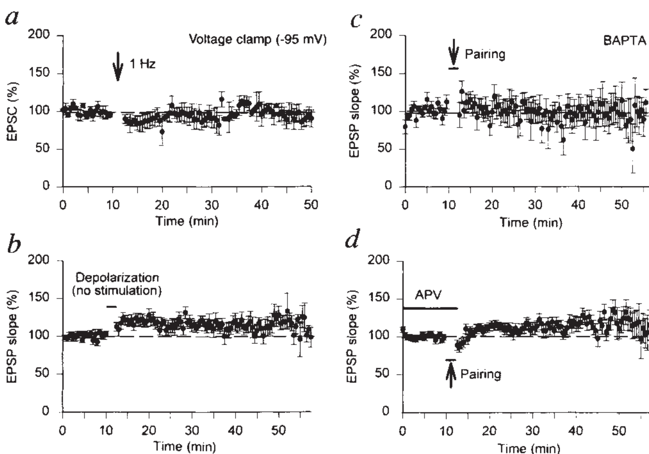

FIG. 3 Properties of LTP induction in P3–6 slices. *a*, Voltage clamping cells at −95 mV ($n = 5$) during 1 Hz stimulation (100 s) prevented the generation of LTP. *b*, Depolarizing cells ($n = 18$) (−10 to 0 mV for 100 s) in the absence of afferent stimulation did not elicit LTP. *c*, Loading cells ($n = 10$) with BAPTA (10 mM in the pipette solution) prevented the LTP normally elicited by combining afferent stimulation with postsynaptic depolarization ('pairing'). *d*, Bath applying the NMDA receptor antagonist D-APV (50 μM; $n = 25$) prevented LTP. LTP is defined as an increase in synaptic strength >120%. The small potentiation elicited by depolarization alone (*b*) or in the presence of APV (*d*) may be due to activation of voltage-dependent Ca^{2+} channels as it was blocked by hyperpolarization (*a*) or BAPTA (*c*).

METHODS. For all experiments, control cells (included in Fig. 2) were interleaved with experimental cells. BAPTA (10 mM) replaced caesium gluconate in the pipette solution to maintain osmolality.

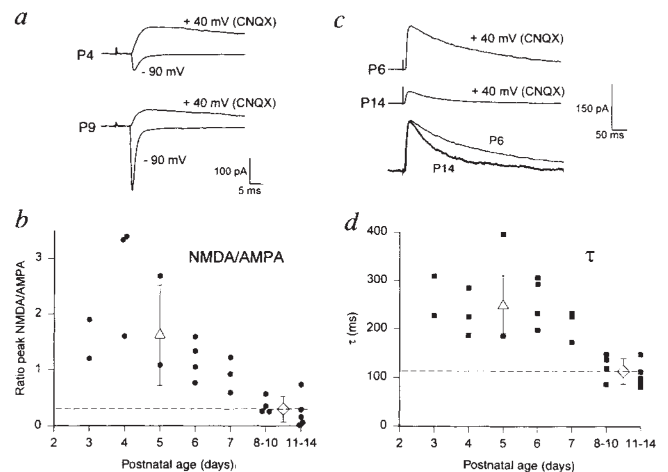

FIG. 4 NMDA receptor-mediated synaptic currents decrease with age. *a*, Sample e.p.s.cs from P4 and P9 slices recorded at −90 mV to measure AMPA receptor-mediated currents and at +40 mV in the presence of the non-NMDA receptor antagonist CNQX (10 μM) and picrotoxin (100 μM) to measure NMDA receptor-mediated currents. *b*, The ratio of peak NMDA receptor-mediated synaptic currents to AMPA receptor-mediated synaptic currents as a function of age. The open symbols indicate the mean ± s.d. for P3–7 and P8–14 cells. Note that all P3–7 cells had a ratio larger than the mean (broken line) for P8–14 cells. *c*, Sample NMDA receptor-mediated e.p.s.cs from P6 and P14 cells. Below, the peak of the P14 e.p.s.c. was aligned with that of the P6 e.p.s.c. to illustrate the difference in the decays. *d*, The time constant of decay of NMDA receptor e.p.s.cs as a function of age. Open symbols are as in *b*. Note that τ (time constant of decay; see Methods) for all P3–7 cells was larger than the mean (broken line) for P8–14 cells.

METHODS. E.p.s.cs were recorded at −90 mV for at least 10 min and then, during continued afferent stimulation, CNQX (10 μM) and picrotoxin (100 μM) were bath applied and the cell depolarized to +40 mV. The peak AMPA receptor- or NMDA receptor-mediated synaptic current was obtained by averaging together 10–20 consecutive e.p.s.cs at either holding potential. τ was calculated as the time it took the current to decay to 1/e of the peak current. In 24 of 25 cells, an NMDA receptor-mediated e.p.s.c. remained after application of CNQX, providing further evidence that monosynaptic responses were being recorded.

independent of any change in the relative proportion of NMDA and AMPA receptors at S1 thalamocortical synapses, the properties of synaptic NMDA receptors have changed in a manner which would make it more difficult to elicit LTP.

Theoretical work[3,4,16,17] suggests that hebbian synaptic modifications are critical for the precise patterning of neuronal connectivity during development. However, experimental evidence in support of this hypothesis has been limited because it is difficult to find manipulations that block such modifications (e.g. LTP) without affecting normal neural activity (see ref. 18). We report here that, at the thalamocortical synapses which form the barrels of rodent S1, LTP is limited to the first postnatal week and essentially cannot be generated thereafter, a time period which closely matches the critical period for the topographic reorganization of VB afferents by sensory experience. Although this strong correlation does not prove causality, it provides compelling evidence that NMDA receptor-dependent LTP is critical for the rearrangement of cortical maps induced by sensory experience. Consistent with this hypothesis is earlier work which demonstrated developmental changes in LTP at unidentified synapses in visual cortex[19] and the occurrence of LTP at developing retinogeniculate synapses[20]. Unlike layer IV cells that are directly activated by thalamic afferents, the peripherally evoked responses of cells in infra- and supragranular layers can be modified by sensory perturbations after the critical period[21,22]. This may be attributable to the LTP which can be generated at intracortical connections in older animals[13].

During the course of development, the molecular composition of NMDA receptors can change[23,24] and thereby influence NMDA receptor inactivation kinetics[25,26]. Because LTP is thought to require an increase in intracellular calcium concentration beyond some critical threshold[27], changes in NMDA receptors that decrease the time course and relative contribution of NMDA receptor-mediated synaptic currents provide a mechanism for the difficulty in eliciting LTP at thalamocortical synapses in S1 after the critical period. Changes in local inhibitory circuits may also influence evoked NMDA receptor currents[28], although these cannot account for our results because inhibition was blocked when recording NMDA receptor-mediated synaptic currents. In visual cortex[15] and superior colliculus[14], the change in NMDA receptor properties appears to occur later in development than in S1, suggesting that the exact timing of critical periods in different brain areas may be dependent on the local molecular regulation of NMDA receptors. □

Received 22 February; accepted 6 April 1995.

1. Goodman, C. S. & Shatz, C. J. *Cell* **72,** 77–98 (1993).
2. Shatz, C. J. *Neuron* **5,** 745–756 (1990).
3. Hebb, D. O. *The Organization of Behaviour* (Wiley, New York, 1949).
4. Stent, G. S. *Proc. natn. Acad. Sci. U.S.A.* **70,** 997–1001 (1973).
5. Collingridge, G. L. & Singer, W. *Trends Neurosci.* **11,** 290–296 (1990).
6. Kandel, E. R. & O'Dell, T. J. *Science* **258,** 243–245 (1992).
7. Woolsey, T. A. & Van der Loos, H. *Brain Res.* **1,** 205–242 (1970).
8. Killackey, H. P. & Leshin, S. *Brain Res.* **86,** 469–472 (1975).
9. Chiaia, N. L., Fish, S. E., Bauer, W. R., Bennett-Clarke, C. A. & Rhoades, R. W. *Devl Brain Res.* **66,** 244–250 (1992).
10. Woolsey, T. A. & Wann, J. R. *J. comp. Neurol.* **170,** 53–66 (1976).
11. Schlaggar, B. L., Fox, K. & O'Leary, D. D. M. *Nature* **364,** 623–626 (1993).
12. Agmon, A. & Connors, B. W. *Neuroscience* **41,** 365–379 (1991).
13. Bear, M. F. & Kirkwood, A. *Curr. Opin. Neurobiol.* **3,** 197–202 (1993).
14. Hestrin, S. *Nature* **357,** 686–689 (1992).
15. Carmignoto, G. & Vicini, S. *Science* **258,** 1007–1011 (1992).
16. Bienenstock, E. L., Cooper, L. N. & Munro, P. W. *J. Neurosci.* **2,** 32–48 (1982).
17. Miller, K. D., Keller, J. B. & Stryker, M. P. *Science* **245,** 605–615 (1989).
18. Fox, K. & Daw, N. W. *Trends Neurosci.* **16,** 116–122 (1993).
19. Komatsu, Y., Fujii, K., Maeda, J., Sakaguchi, H. & Toyama, K. *J. Neurophysiol.* **59,** 124–141 (1988).
20. Mooney, R., Madison, D. V. & Shatz, C. J. *Neuron* **10,** 815–825 (1993).
21. Fox, K. *J. Neurosci.* **12,** 1826–1838 (1992).
22. Diamond, M. E., Huang, W. & Ebner, F. F. *Science* **265,** 1885–1888 (1994).
23. Watanabe, M., Inoue, Y., Sakimura, K. & Mishina, M. *Neuroreports* **3,** 1138–1140 (1992).
24. Williams, K., Russel, S. L., Shen, Y. M. & Molinoff, P. B. *Neuron* **10,** 267–278 (1993).
25. Monyer, H. *et al. Science* **256,** 1217–1221 (1992).
26. Kutsuwada, T. *et al. Nature* **358,** 36–41 (1992).
27. Malenka, R. C. & Nicoll, R. A. *Trends Neurosci.* **16,** 521–527 (1993).
28. Agmon, A. & O'Dowd, D. K. *J. Neurophysiol.* **68,** 345–349 (1992).
29. Mulkey, R. M. & Malenka, R. C. *Neuron* **9,** 967–975 (1992).

ACKNOWLEDGEMENTS. This work was supported by the NIH, the McKnight Endowment Fund for Neuroscience, and the National Association for Research on Schizophrenia and Depression. We thank R. Nicoll and M. Stryker for helpful comments on the manuscript, and M. Silver for help with confocal microscopy.

Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience

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Long-term potentiation (LTP) is a lasting enhancement of excitatory synaptic transmission that follows specific patterns of electrical stimulation[1]. Although the mechanism of LTP has been intensively studied, particularly in the hippocampus, its significance for normal brain function remains unproven. It has been proposed that LTP-like mechanisms may contribute to naturally occurring, experience-dependent synaptic modifications in the visual cortex[2–8]. The formation of normal binocular connections within the visual cortex requires simultaneous input from both eyes during a postnatal critical period[9–12] that can be delayed by rearing animals in complete darkness[13,14]. To explore the role of LTP in this experience-dependent maturation process, we induced LTP in visual cortical slices taken at different ages from light-reared and dark-reared rats. Susceptibility to LTP coincides with the critical period and, like the critical period, can be prolonged by rearing animals in darkness. These findings support the hypothesis that LTP reflects a normal mechanism of experience-dependent synaptic modification in the developing mammalian brain.

For many years the visual cortex has been used as a model to study experience-dependent synaptic plasticity. Modifications can be elicited *in vivo* with simple manipulations of visual experience, such as monocular deprivation, and these modifications have clear behavioural consequences, such as blindness of the deprived eye[15]. Although carnivores and primates have traditionally been used for studies of experience-dependent visual cortical development, the same principles apply to rodents[12]. In rat visual cortex, for example, visual response properties mature rapidly from postnatal day (P) 18, when the optics clear, to P45. As in other species, dark-rearing from birth postpones this maturation. Moreover, response properties in the small binocular region of rat visual cortex can be altered by monocular deprivation during a critical period. Susceptibility of binocular connections to monocular deprivation is greatest when deprivation is begun at approximately three weeks of age but declines rapidly, such that deprivation initiated at 5 weeks of age has little effect[12,16].

Various forms of activity-dependent modifications have been described by studying synaptic plasticity in the visual cortex *in vitro*[2–6,17]. The common approach has been to record intracellular or population excitatory postsynaptic potentials in layer III that are evoked by electrical stimulation at the border of the white matter and layer VI. In the absence of treatments to reduce inhibition, conditioning stimulation of the white matter fails to produce robust LTP in layer III in slices of visual cortex prepared from normal adult (⩾P35) rats[3,7,17]. However, it has been reported that LTP may be elicited using this stimulation-