results obtained with ammonium chloride and leupeptin indicate that alternative processing in the endosomal-lysosomal pathway generates a complex set of COOH-terminal derivatives that includes potentially amyloidogenic forms (Fig. 3). We cannot, however, exclude the possibility that the potentially amyloidogenic forms are produced to some extent by an atypical nonlysosomal protease inhibited by both ammonium chloride and leupeptin.

Our finding that the βAPP is processed by the endosomal-lysosomal system is supported by studies showing punctate intracellular labeling by antibodies to βAPP consistent with localization of βAPP to lysosomes (*5*), and reports by Cole and co-workers (*6*) demonstrating that inhibitors of endosomal-lysosomal processing increase the steady-state level of βAPP and βAPP derivatives. Moreover, the βAPP contains a cytoplasmic sequence known to target membrane-associated glycoproteins for endocytosis (7).

The specific proteases that produce amyloid are potential therapeutic targets in Alzheimer's disease (AD). Our data showing that endosomal-lysosomal processing produces potentially amyloidogenic βAP-bearing COOH-terminal derivatives focus the search for these proteases on the endosomal-lysosomal system. βAP-bearing COOH-terminal derivatives are probably further processed in this system to produce small fragments similar or identical to the βAP. These fragments could be quite stable because they would tend to aggregate into a β-pleated sheet structure, but fragments like this have not yet been detected in normal cells.

The apparent localization of the βAPP to lysosomes (*5*) and the immunocytochemical detection of lysosomal proteases in senile plaques (*8*) have independently led several groups to propose that aberrant lysosomal processing may play a role in AD. Our data indicate that normal endosomal-lysosomal processing produces potentially amyloidogenic βAPP fragments, and they support the hypothesis that dysfunctional endosomal-lysosomal processing may play a critical role in the pathogenesis of AD. There is evidence that amyloid deposition arises through mutations in the βAPP gene in familial AD (*9*), but it is not yet known whether these mutations alter endosomal-lysosomal processing in a way that is amyloidogenic.

REFERENCES AND NOTES

1. F. S. Esch *et al.*, *Science* **248**, 1122 (1990).
2. S. Estus *et al.*, *ibid.* **255**, 726 (1992).
3. S. S. Sisodia *et al.*, *ibid.* **248**, 492 (1990).
4. P. O. Seglen, *Methods Enzymol.* **96**, 737 (1983). Ammonium chloride is a weak base amine that is concentrated in acidic compartments where it inhibits its endosomal-lysosomal proteolysis by raising the pH of these compartments. It is generally considered the most specific inhibitor of endosomal-lysosomal proteolysis. Leupeptin is a dipeptidyl protease inhibitor. It effectively inhibits endosomal-lysosomal proteolysis by inhibiting serine and cysteine proteases. Because protease inhibitors like leupeptin only affect a specific class of proteases, they are usually not as effective as the weak base amines. However, because lysosomal enzymes work sequentially, as much as 80% of the amine-sensitive degradation can be inhibited with agents like leupeptin.
5. G. M. Cole *et al.*, *Neurochem. Res.* **14**, 933 (1989); L. I. Benowitz *et al.*, *Exp. Neurol.* **106**, 237 (1989); G. M. Cole *et al.*, *Neurobiol. Aging* 12, **85** (1991); M. Kawai *et al.*, *Am. J. Pathol.*, in press.
6. G. M. Cole *et al.*, in *Molecular Biology and Genetics of Alzheimer's Disease*, T. Miyatake *et al.*, Eds. (Elsevier, Amsterdam, 1990), pp. 113–122.
7. W.-J. Chen *et al.*, *J. Biol. Chem.* **265**, 3116 (1990).
8. A. M. Cataldo *et al.*, *Brain Res.* **513**, 181 (1990); A. M. Cataldo and R. A. Nixon, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3861 (1990).
9. A. Goate *et al.*, *Nature* **349**, 704 (1991); G. Lucotte *et al.*, *ibid.* **351**, 530 (1991); J. Murrell *et al.*, *Science* **254**, 97 (1991); M.-C. Chartier-Harlin, *Nature* **353**, 844 (1991).
10. The forward oligonucleotides used to generate the deletion mutants are βAPP410 (5′-GTATCTCGAGATCTCTGAAGTGAAGATGG-3′); βAPP419 (5′-GTATCTCGAGGGGTTGACAAATATCAAGA-3′); βAPP423 (5′-GTATCTCGAGCGACCAGGTTCTGGGTTGA-3′); and βAPP438 (5′-GTATCTCGAGGAGCCTGTTGATGCCCGCC-3′). These primers correspond to nucleotides 1771–1789, 1744–1762, 1732–1750, and 1687–1705, respectively, of the βAPP_{695} sequence [J. Kang *et al.*, *Nature* **325**, 733 (1987)] and contain a common 5′ sequence, GTATCTCGAG, that allows cleavage with Xho 1 and in-frame fusion of the amplified DNAs encoding the various COOH-terminal regions with the DNA encoding the 304 residues at the NH_2-terminal end of the deletion mutants. The reverse primer used for each mutant was βAPP2121 (5′-GACTCGAGTCGACGGATCCGTGTCCAACTTCAGAGGCTGC-3′), which contains a 5′ adaptor sequence of 20 base pairs followed by nucleotides 2121–2101 of βAPP_{695} (*11*).
11. We thank G. Perry for antibody to βAPP_{45-62}.

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The Influence of Prior Synaptic Activity on the Induction of Long-Term Potentiation

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Long-term potentiation (LTP) is an extensively studied model of synaptic plasticity, in part because it is a plausible biological correlate for the Hebbian synaptic modification that forms the basis for theoretical models of neural development, learning, and memory. Although these models must incorporate algorithms that constrain synaptic weight changes, physiological evidence for such mechanisms is limited. Examination of LTP in area CA1 of the hippocampus revealed that the threshold for LTP induction was not fixed but could be strongly influenced by the recent history of synaptic activity. This effect was transient, synapse-specific, and dependent on postsynaptic *N*-methyl-D-aspartate (NMDA) receptor activation. These results suggest that the threshold for LTP induction may be continually adjusted according to the recent history of NMDA receptor activation and provide a physiological mechanism by which LTP can be transiently inhibited.

ACTIVITY-DEPENDENT MODIFICATIONS in synaptic strength are critical for the development of neural networks and for certain forms of learning and memory. Most attention has focused on one form of LTP that is dependent on activation of postsynaptic NMDA receptors, because its induction requires coincident pre- and postsynaptic activity, thus fulfilling the criteria for a synaptic plasticity mechanism originally postulated by Hebb (*1*). Although by incorporating a Hebbian scheme of synaptic modification, artificial neural network models successfully reproduce many aspects of neural development, learning, and memory, they also require a means for limiting changes in synaptic weights (*2*). One theoretical mechanism, which has been incorporated into some neural network models, permits an adjustment of the threshold for synaptic modification according to the recent history of postsynaptic cell activity (*3*). We have tested whether the history of synaptic activation influences the induction of LTP in the CA1 region of the hippocampus.

In all experiments we simultaneously recorded excitatory postsynaptic potentials (EPSPs) in response to two independent inputs synapsing on the same cell or population of cells (*4*). When a weak tetanus (30 Hz; 0.1 to 0.2 s) that elicits decremental, NMDA receptor–dependent short-term potentiation (STP) (*5, 6*) was given repetitively (four to six times) at 2-min intervals, a subsequent stronger tetanus (100 Hz; 0.5 s) capable of eliciting LTP in a control pathway did not elicit LTP in the test pathway

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(Fig. 1A) (five of eight experiments). LTP induction was also inhibited when the stimulus intensity was increased and single strong shocks (7) rather than weak tetani were applied at 2-min intervals to the test pathway (five of seven experiments). A summary of these experiments (Fig. 1B) (n = 15) indicates that the inhibition of LTP was synapse-specific; LTP in the test, but not the control, pathway was inhibited.

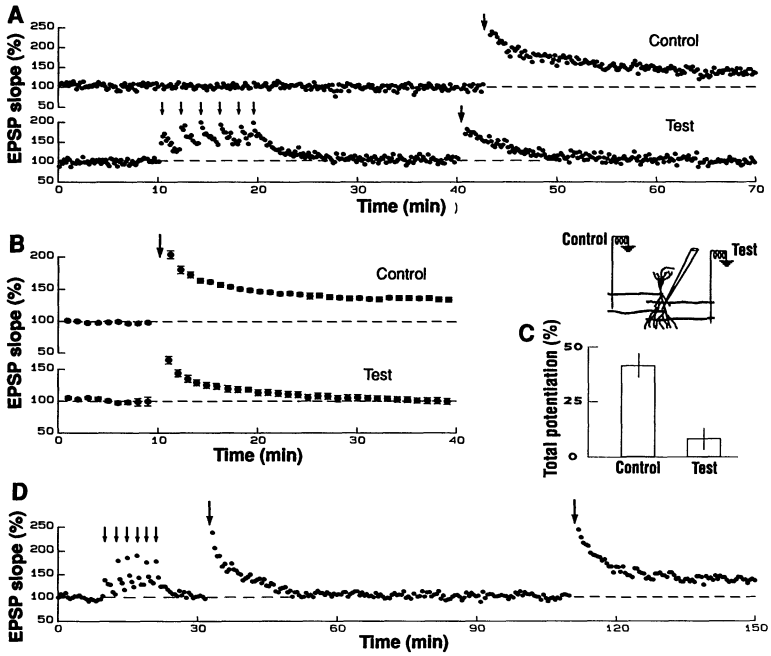

Fig. 1. Inhibition of LTP induction by prior synaptic activity. (**A**) Plot of a typical experiment in which field EPSPs were recorded from a single site (inset) in response to two independent inputs (control and test). Small arrows, weak tetanus (30 Hz, 0.15 s); large arrows, strong tetanus (100 Hz, 0.5 s). (**B**) Summary of experiments in which either weak tetani (n = 8) or single strong shocks (n = 7) were given to the test pathway before induction of LTP (arrow, tetanus, 100 Hz, 0.5 s; points, mean ± SEM). (**C**) The total potentiation (mean ± SEM) (*4*) evoked in control and test pathways from same experiments in (B) (control, 41 ± 5%; test, 7 ± 4%; $P < 0.01$; n = 15). (**D**) Example of an experiment demonstrating that the inhibition of LTP induction is transient. Small arrows, weak tetanus (30 Hz, 0.15 s); large arrows, strong tetanus (100 Hz, 0.5 s).

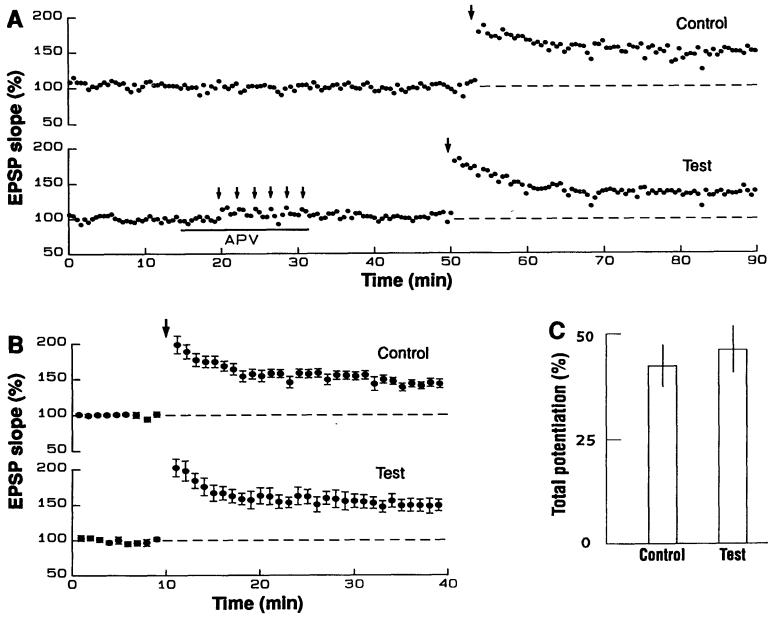

Fig. 2. The NMDA receptor antagonist D-APV prevents the inhibition of LTP induction by prior synaptic activity. (**A**) Plot of a typical experiment in which D-APV (25 to 50 μM) was applied before application of the weak tetani (small arrows; 30 Hz, 0.15 s). After washout of D-APV, a stronger tetanus (large arrows; 100 Hz, 0.5 s) was given, and LTP was induced in both pathways. (**B** and **C**) Summary (n = 11) of the time course and magnitude of the potentiation (control, 42 ± 5%; test, 46 ± 5%; $P > 0.10$; n = 11).

The responses after the weak tetani or strong shocks routinely decayed back to initial baseline values before any LTP-inducing tetanus was applied. Therefore, it is unlikely that the effects of the initial stimulation protocol on subsequent LTP induction were due to "saturation" of LTP processes (*8*). Indeed, the difference between control and test pathways was significant regardless of whether LTP was expressed as a percent of the baseline response immediately before the strong tetanus (Fig. 1B) or as a percent of the baseline response before the weak tetani (that is, the total potentiation during the entire experiment) (Fig. 1C). In addition, the inhibition of LTP induction was not permanent because a strong tetanus that was incapable of generating LTP when applied 10 min after the repetitive weak tetani induced LTP when given 50 to 80 min later (Fig. 1D) (six of eight experiments; mean potentiation = 34.5 ± 5%, n = 8).

To test whether activation of NMDA receptors is required for the subsequent inhibition of LTP induction, we applied the NMDA receptor antagonist D-2-amino-5-phosphonovalerate (D-APV) (25 to 50 μM) during the inactivation protocol of the previous experiment. Blocking NMDA receptors prevented the inhibition of LTP by prior repetitive synaptic activity (11 of 11 experiments) (Fig. 2A) in that no differences were observed between the two pathways in either the magnitude or the time course of the potentiation elicited by a strong tetanus (Fig. 2, B and C). We also applied weak tetani at different times to both test and control pathways, each in the presence or absence of D-APV. Again, the presence of D-APV (25 μM) during the weak tetani prevented the subsequent inhibition of LTP induction whereas the same weak tetani applied to the other pathway after washout of D-APV inhibited LTP induction (three of three experiments).

To further test whether prior activation of NMDA receptors was sufficient to inhibit LTP induction, we ionophoretically applied NMDA directly at one recording site while monitoring a control EPSP at another site in the same slice (*9*). Thus, the LTP-inducing tetanus was applied to both pathways at the same time with the same stimulating electrode. Like a weak tetanus, NMDA ionophoresis evoked STP (*5, 10*) and when applied repetitively at 4- to 10-min intervals caused a transient inhibition of LTP induction at nearby synapses (Fig. 3, A and B) (9 of 13 experiments). As in the experiments with repetitive weak tetani, the total potentiation at the control recording site was

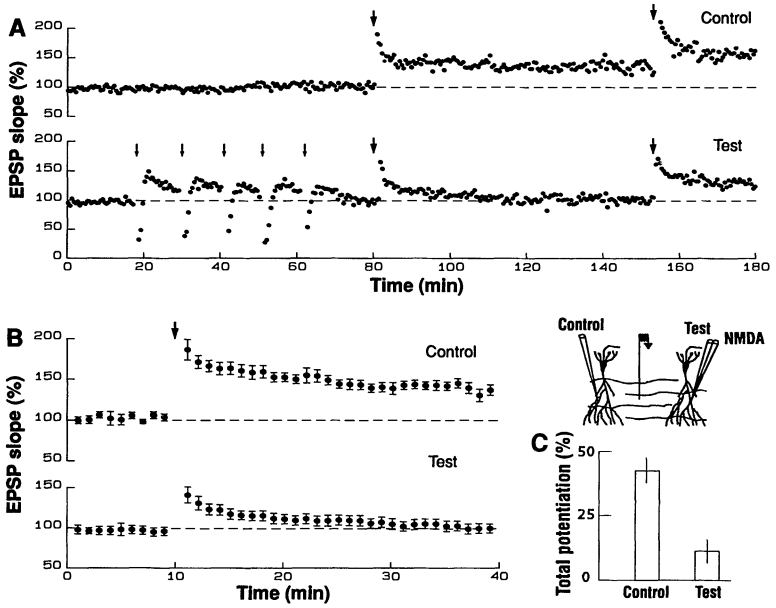

Fig. 3. Inhibition of LTP induction by prior ionophoretic application of NMDA. (**A**) Plot of a typical experiment in which NMDA was repetitively applied at the test recording site (small arrows; 150 nA, 10 s) before the LTP-inducing stimulus (large arrows; 100 Hz, 1 s). The tetanus was given a second time, 80 min after the first tetanus, and induced LTP in the test pathway. (Inset) The stimulating and recording arrangement. (**B** and **C**) Summary showing that prior NMDA ionophoresis inhibited LTP induction (B) and that the total potentiation (C) was significantly greater in the control pathway.

significantly greater than that where NMDA was applied (Fig. 3C) (control, 42 ± 5%; test, 10 ± 4%; $P < 0.01$; $n = 13$).

Our results suggest that repetitive activation of NMDA receptors, sufficient to induce STP but not LTP, can lead to a transient inhibition of LTP induction at the activated synapses. To explore this phenomenon without changing stimulation parameters or applying NMDA directly, we investigated LTP induction at different synapses on a single cell using intracellular and whole-cell patch-clamp recording (*11*). To elicit STP in the test input but not the control input, we paired synaptic stimulation of the test input with intracellular depolarizing current pulses given at a frequency that generally elicits STP but not LTP (*6, 12*). Following this input-specific pairing procedure, we attempted to induce LTP at the same time in both pathways using the identical LTP induction protocol (*12*). Consistent with previous results, the test input exhibited less potentiation than the control input (Fig. 4, A and B) ($n = 13$) and the total potentiation in the control pathway (Fig. 4C) (104 ± 13%) was significantly larger than that in the test pathway (62 ± 11%) ($P < 0.05$, $n = 25$) (*13*).

Still, LTP was not completely inhibited in the test pathway (Fig. 4), perhaps because a strong induction protocol was used when recording from single cells (*12*). Consistent with this proposal, we found that the inhibition of LTP induced by repetitive weak tetani (Fig. 1) could be overcome if the LTP-inducing tetanus was given repetitively (two to three times) with increased stimulation strength (four of seven experiments). These results suggest that the consequences of prior NMDA receptor activation may not be an inhibition of LTP per se but rather an increase in the threshold amount of synaptic activation required to elicit LTP.

Our results demonstrate that physiological stimuli can inhibit subsequent LTP induction. They suggest that each synapse is transiently influenced by prior NMDA receptor activation that raises the threshold for the induction of LTP. Previous results may be considered consistent with this hypothesis (*14*). The mechanism responsible for this inhibition of LTP induction is not known but could operate by changing NMDA receptor properties or the buffering of intracellular Ca^{2+}, or could involve any of the biochemical processes implicated in LTP. This mechanism may be particularly important in preventing positive feedback and "runaway" potentiation that might be induced by the additional depolarization provided by transiently potentiated EPSPs during STP.

Although we have experimental support for the activity-dependent adjustment of the threshold of synaptic modification that is incorporated into some neural network models, the effects we have observed appear to be synapse-specific rather than generalized to all inputs onto the postsynaptic neuron as predicted by theory (*3*). Thus, the threshold for LTP induction at active inputs may be higher than the LTP threshold for quiescent inputs synapsing on the same cell. An analogous synapse-specific phenomenon occurs at mixed synapses between eighth nerve fibers and the goldfish Mauthner cell, although in this system both the threshold and direction of long-lasting synaptic changes are influenced by initial synaptic efficacy (*15*).

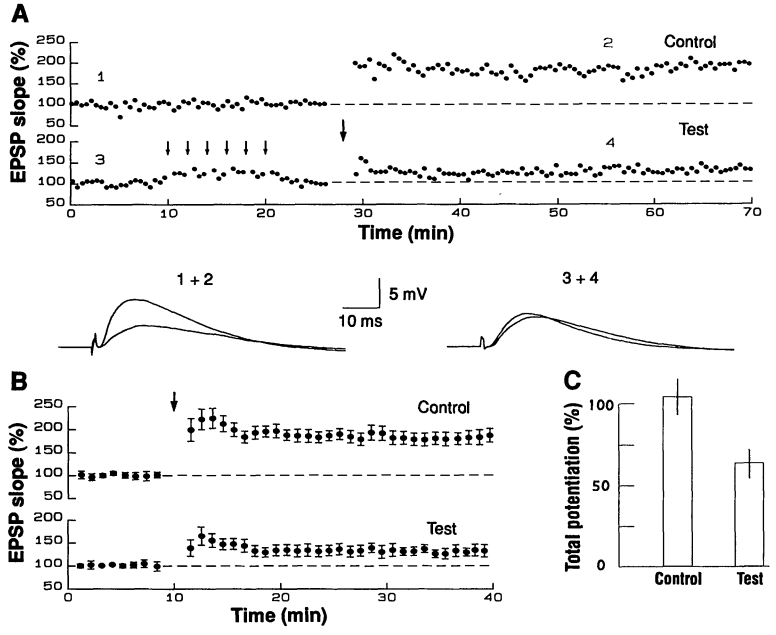

Fig. 4. Inhibition of LTP induction in a single cell by pairing depolarization with synaptic stimulation prior to LTP induction. (**A**) Plot of a typical experiment in which depolarizing current pulses were paired with afferent stimulation (small arrows) in the test pathway prior to LTP induction (large arrow) (*12*). Sample EPSPs (average of four successive sweeps) below the graph were taken at the times indicated by the numbers above the graphs. (**B** and **C**) Summary of the single-cell experiments.

In the hippocampus, specific patterns of synaptic activity can either facilitate (*16*) or, as shown here, inhibit LTP induction. Further clarifying the rules that govern the generation of LTP will be necessary for a comprehensive understanding of the role of LTP in nervous system function and in addition should provide important information for biologically based neural network models that incorporate Hebbian synaptic modifications.

REFERENCES AND NOTES

1. D. O. Hebb, *The Organization of Behavior* (Wiley, New York, 1949); T. H. Brown, E. W. Kairiss, C. L. Keenan, *Annu. Rev. Neurosci.* **13**, 475 (1990).
2. M. A. Gluck and D. E. Rumelhart, Eds., *Neuroscience and Connectionist Theory* (Erlbaum, Hillsdale, NJ, 1990).
3. E. L. Bienenstock, L. N Cooper, P. W. Munro, *J. Neurosci.* **2**, 32 (1982); M. F. Bear, L. N Cooper, F. F. Ebner, *Science* **237**, 42 (1987).
4. Standard procedures were used to prepare rat hippocampal slices [R. A. Nicoll and B. E. Alger, *J. Neurosci. Methods* **4**, 153 (1981); R. C. Malenka, J. A. Kauer, R. S. Zucker, R. A. Nicoll, *Science* **242**, 81 (1988)], which we transferred to a recording chamber and submerged beneath a continuously superfusing solution saturated with 95% O_2 and 5% CO_2. The composition of this solution was 119 mM NaCl, 4 mM KCl, 4 mM $CaCl_2$, 4 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 11 mM glucose, and 0.1 mM picrotoxin. The connection between CA3 and CA1 was severed to prevent propagation of burst discharges. Drugs were obtained from Sigma (picrotoxin, NMDA) and Cambridge Research Biochemicals (D-APV). Recording techniques were the same as those described previously (*6*). Stimulating electrodes were placed on opposite sides of the recording electrode (Fig. 1C, inset) and alternately stimulated at 0.2 Hz. The site receiving the test stimulation protocol was moved randomly (closer to either CA3 or the subiculum). We adjusted the stimulus intensity so that the EPSP slope was approximately the same in both inputs and this was not changed when tetani were applied. The independence of the two populations of afferents was tested as described (*6*), and data were collected and analyzed on-line (*6*). We constructed averaged summary graphs by (i) normalizing each experiment according to the average value of all points on the 10-min baseline before LTP induction, (ii) aligning the points with respect to the time of LTP induction, and (iii) dividing each experiment into 60-s bins and averaging these across experiments. For all graphs, the first one to three points after the LTP-inducing tetanus (during posttetanic potentiation) are not shown because these values were routinely well above the majority of values plotted. We calculated total potentiation for each experiment by averaging all EPSP slope values 25 to 30 min after LTP induction and comparing this value with the average of all EPSP slope values during the initial 10 min of the baseline. A two-tailed *t* test was used to test for significant differences between groups.
5. R. C. Malenka, *Neuron* **6**, 53 (1991).
6. A. Colino, Y.-Y. Huang, R. C. Malenka, *J. Neurosci.* **12**, 180 (1992).
7. W. C. Abraham, B. Gustafsson, H. Wigstrom, *Neurosci. Lett.* **70**, 217 (1986).
8. T. V. P. Bliss and T. Lomo, *J. Physiol. (London)* **232**, 331 (1973); B. L. McNaughton, R. M. Douglas, G. V. Goddard, *Brain Res.* **157**, 277 (1978).
9. NMDA (100 mM, pH 8) was applied (100 to 400 nA, 5 to 20 s) from a double-barreled electrode to ensure that the recorded field EPSPs originated within the synaptic region receiving NMDA (*10*).
10. J. A. Kauer, R. C. Malenka, R. A. Nicoll, *Nature* **334**, 250 (1988).
11. Standard intracellular or whole-cell patch-clamp recording techniques were used (*5, 6*), and EPSPs were recorded under current clamp with an Axoclamp 2A (Axon Instruments). Cells were held at −75 to −90 mV throughout the course of the experiment. We observed no significant differences between cells recorded with either technique, so we have combined results from all cells.
12. To activate the NMDA receptor during depolarization in the test but not control input, we gave a 400- to 800-ms depolarizing current step (0.4 to 1.0 nA) 5 to 10 ms before stimulation of the test input. Before LTP induction, this was repeated either (i) once every 90 s, six to ten times or (ii) three to six times at 0.1 Hz, which was then repeated four to five times at 3- to 7-min intervals. We induced LTP by depolarizing the cell (from −20 to 0 mV) with continuous current injection and applying 20 to 30 stimuli to both pathways at 0.25 Hz. A strong induction protocol was used to ensure that the occurrence of LTP in either pathway could be measured above the baseline, which is inherently noisier when recording single-cell EPSPs rather than field EPSPs.
13. The initial pairing protocol sometimes caused increases in both the test and control EPSPs that lasted for over 10 min. Therefore, in Fig. 4B, cells (n = 13) were included only if the net change in the test pathway EPSP following the inactivation protocol was not significantly different (<10%) from any net change in the control pathway. This may have resulted in the selection of cells in which the probability of generating LTP was intrinsically less in the test input than the control input, independent of any experimental manipulation. Therefore, for Fig. 4C, we included all cells (n = 25), even those in which the initial pairing protocol caused a stable increase in the test EPSP. If the differences in Fig. 4B were due to the selection process, then the total potentiation in the two inputs (Fig. 4C) would not be significantly different.
14. E. J. Coan, A. J. Irving, G. L. Collingridge, *Neurosci. Lett.* **105**, 205 (1989); S. Fujii, K. Saito, H. Miyakawa, K. Ito, H. Kato, *Brain Res.* **555**, 112 (1991).
15. X.-D. Yang and D. S. Faber, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4299 (1991).
16. J. Larson and G. Lynch, *Science* **232**, 985 (1986); J. Winson and D. Dahl, *ibid.* **234**, 985 (1986).

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Chondroitin Sulfate as a Regulator of Neuronal Patterning in the Retina

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Highly sulfated proteoglycans are correlated with axon boundaries in the developing central nervous system which suggests that these molecules affect neural pattern formation. In the developing mammalian retina, gradual regression of chondroitin sulfate may help control the onset of ganglion cell differentiation and initial direction of their axons. Changes induced by the removal of chondroitin sulfate from intact retinas in culture confirm the function of chondroitin sulfate in retinal histogenesis.

DURING THE EARLY STAGES OF VERtebrate retinal histogenesis, undifferentiated neuroepithelial cells in the eye undergo a change in cytodifferentiation predominantly toward a committed ganglion cell fate (*1, 2*). It is believed that retinal ganglion cells cease dividing when their somata are located at the ventricular surface, that those located near the optic fissure achieve maturity first, and that differentiation proceeds in a center-to-periphery sequence (*2*). At some time near the last cell division, ganglion cells project axons directly toward the optic fissure (*3, 4*). Although the relationship between morphogenesis of the optic fissure and egress of axons from the eye is known (*5, 6*), the mechanisms that control retinal ganglion cell differentiation and direct the growth of axons back toward the fissure are unknown.

Molecules that potentially promote axon growth in the retina are not distributed in a way that could impart precise directional information. Thus, molecules that are repulsive to axon growth may be instrumental in neural patterning (*7*). Structurally diverse proteoglycans are abundant in the developing central nervous system (*8–10*) and are found in the embryonic retina (*11, 12*). When sulfated proteoglycans are enriched relative to growth-promoting molecules in the same territory, a boundary is formed that inhibits advancing growth cones (*13, 14*). We have localized a chondroitin sulfate–containing proteoglycan within the embryonic rat retinal extracellular spaces that may help determine aspects of ganglion cell differentiation such as the polarity of retinal ganglion cell bodies and the initial direction of their axons.

In the rat, the retina is devoid of ganglion cells until day 12.5 of embryonic development (E12.5). At this time, the first retinal ganglion cells with axons appear just dorsal to the optic fissure. Thereafter, axons emerge from ganglion cell bodies located progressively more peripherally. This process continues until E16.5 when the vitreal (inner, facing the lens) surface of the retina is completely populated with fasciculated axons. On tissue sec-

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