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N-Methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map

(retinocollicular projection/visual system development/axon elimination/retinal ganglion cells/topographic maps)

DAVID K. SIMON*, GLEN T. PRUSKY†, DENNIS D. M. O'LEARY*‡, AND MARTHA CONSTANTINE-PATON†

*Molecular Neurobiology Laboratory, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037; and †Department of Biology, Yale University, Kline Biology Tower, P.O. Box 6666, New Haven, CT 06511

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ABSTRACT **The topographic ordering of retinal connections in the rat superior colliculus emerges during early postnatal life from an initially diffuse projection. Disruption of *N*-methyl-D-aspartate (NMDA) receptor activity in the superior colliculus during this period interferes with map remodeling. In rats chronically treated with NMDA receptor antagonists during the first two postnatal weeks, aberrant axons remain and arborize at topographically incorrect sites. These results indicate that, at a stage preceding visually evoked activity, normal NMDA receptor function is important for the development of an ordered neural map in the mammalian brain.**

The generation of meaningful behavioral responses to visual stimuli requires the appropriate topographic ordering of synaptic connections in the central nervous system (1). Topographic maps are a fundamental organizational feature throughout the visual pathway and in many other brain regions. It has been postulated that an activity-dependent component of visual map development exists across vertebrates (2–5). The mechanism is believed to operate even before the retina is fully differentiated, using only correlations in spontaneous activity among neighboring retinal ganglion cells (6–8). In the central nervous system, activation of *N*-methyl-D-aspartate (NMDA) receptors is hypothesized to be a mechanism to detect temporal correlations among afferents and to initiate maintenance of simultaneously active synapses (5, 9). This hypothesis predicts that interfering with activation of NMDA receptors, even prior to sensory stimulation, should influence the organization of neural maps in the brain.

The NMDA receptor hypothesis is supported by experimental evidence from cold-blooded vertebrates that axonal rearrangements, such as the eye-specific terminal segregation in "dually innervated" tecta (10), the refinement of topography after optic nerve regeneration (11), the maintenance of existing topography (12), and the alignment of ipsilateral and contralateral visual maps (13), are mediated by NMDA receptors. However, map development in these vertebrates is quite different from that in mammals. In cold-blooded vertebrates, retinal axons grow directly to topographically appropriate regions of the tectum, where they subsequently arborize (14–18). This phase of map development is independent of activity (19–22). All subsequent synaptic remodeling, including the refinement and maintenance of topography, occurs when the retina is fully functional and generates mature visual responses (23).

In rats, by contrast, developing retinal axons commonly form branches or arbors in topographically incorrect positions in the superior colliculus (SC), resulting in an initially disordered retinocollicular projection. The establishment of topographic order requires a major remodeling that involves not only local axon rearrangements but also the large-scale elimination of these aberrantly positioned branches and arbors (24, 25). This remodeling stage takes place while the retina is still differentiating and incapable of vision.§ Nevertheless, blockade of ganglion cell action potentials during this stage interferes with the remodeling process, indicating that, as in the chick (29, 30), map remodeling is at least partially dependent on retinal activity (31). Demonstrations of NMDA receptor involvement in mammalian visual development have been obtained in studies of the laminar segregation of retinogeniculate axon arbors according to similarities among ganglion cells (32) or in studies of later developmental interactions in visual cortex, when pattern vision clearly plays a role (33–36). It is not known whether the NMDA receptor is critical during development for the topographic ordering of retinal connections in their central targets, which occurs prior to eye opening and can rely only upon position-dependent correlations in the spontaneous activity of developing ganglion cells.

Here, we demonstrate a developmental role for NMDA receptors in the initial establishment of topographic order in visual connections in the mammalian brain, using the retinocollicular projection as a model system.¶ We show that blockade of NMDA receptor function interferes with the elimination of aberrantly positioned retinal ganglion cell arbors in the SC and thus disrupts the normal establishment of a topographic map.

METHODS

Animals. Sprague–Dawley rats ranging in age from P0 to P19, obtained from timed pregnant females (Harlan–Sprague–Dawley or Sasco, Omaha, NB), were used for these experiments. The first 24 hr after birth is designated P0.

Elvax Preparation. Elvax was prepared as described by Silberstein and Daniel (38). Briefly, Elvax-40W beads (DuPont) were dissolved in 10 times their volume of methylene chloride (Sigma). A volume of 10 μl of the appropriate drug, at a concentration of 10 mM for MK-801, L-2-amino-5-phosphonovalerate (L-AP5; the inactive stereoisomer of AP5), or dihydro-β-erythroidine; either 10 mM or 100 mM for DL-AP5 (a mixture of active and inactive stereoisomers of

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Abbreviations: AP5, 2-amino-5-phosphonovalerate; DiI, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; NMDA, *N*-methyl-D-aspartate; SC, superior colliculus.

‡To whom reprint requests should be addressed.

§Rat retinal axons first reach the SC on embryonic day 16 (5–6 days before birth) (26) and establish a topographically ordered map prior to postnatal day 12 (P12) (24, 25). The outer segments of photoreceptors develop beginning on P5. Synapses are not found in the inner plexiform layer until P12. P12 is the earliest age that a retinal response can be recorded on an electroretinogram (27) and the earliest time that light-evoked responses can be recorded in the SC (28). Rats do not open their eyes until after P12.

¶In rats, the SC is the primary target of retinal axons. In mature rats, virtually all retinal ganglion cells project to the SC (37).

AP5); and 1.0 M for D-AP5 (the active stereoisomer of AP5), was added to this methylene chloride/Elvax solution. In a separate preparation, 10 μl of water (the carrier for the drugs) was added to the methylene chloride/Elvax solution. The methylene chloride was then allowed to slowly evaporate as the Elvax polymerized, resulting in a final drug concentration in the Elvax of 0.1 mM (for MK-801, L-AP5, the lower concentration of DL-AP5, or dihydro-β-erythroidine), 1.0 mM (for the higher concentration of DL-AP5), or 10 mM (for D-AP5). Sections of Elvax (90 μm) were cut on a cryostat at −25°C. Drug concentrations in this paper refer to these final drug concentrations in the Elvax.

Elvax Implantation. Surgeries to implant the Elvax were performed on P0. The rat pups were anesthetized with hypothermia and the skin and skull overlying the SC were retracted. The exposed dura was incised and a circular piece of Elvax (4–5 mm in diameter; 90 μm thick) was placed on the pia covering the colliculus and tucked under the posterior cortex. The bone flap was replaced and the skin was sutured and sealed with Vetbond (3M Corp., St. Paul) or cyanoacrylate ester (Locktite, Cleveland). The animals recovered on a warm heating pad before being returned to the mother.

In some cases the SC was slightly deformed in the Elvax-implanted rats. The deformity consisted of a shallow depression across the SC, or a widening of the depression along the midline separating the left and right SC. However, such deformities showed no correlation with the presence of mispositioned arbors at P12 or P19 in MK-801- or DL-AP5-treated rats, and a normal-appearing topographic map developed in control rats with similar deformities. Thus, although the implantation of Elvax over the SC may cause slight abnormalities in the gross structure of the SC, this procedure alone does not alter the normal development of a topographically ordered retinocollicular projection.

Axon Labeling. The axon labeling methods have been described in detail (24, 25). Briefly, a 5–10% solution of the fluorescent axon tracer (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) (Molecular Probes) in dimethylformamide (Sigma) was pressure injected into the retina of anesthetized rats [open metofane (Pitman–Moore, Washington Crossing, NJ) or intraperitoneal injection of 3.5% chloral hydrate (Fisher), 10 mg/kg of body weight, in 0.9% saline] on P9 or P16 through a glass micropipette with a Picospritzer II (General Valve, Fairfield, NJ). Approximately 0.01 μl of DiI solution was injected. After a survival period of 3 days (on P12 or P19), the rats were deeply anesthetized and perfused transcardially with buffered 4% paraformaldehyde.

Analysis. After perfusion with fixative, the injected retina and the dorsal midbrain, including the SC contralateral to the injected eye, were whole-mounted and examined with rhodamine optics on a fluorescence microscope. The positioning of the Elvax was determined during dissection to verify that it covered the SC contralateral to the injected eye. The SC was embedded in 3.5% agar/8% sucrose and sectioned sagittally at 200 μm on a Vibratome. Every section was mounted serially on glass slides and examined under fluorescence. The retina was carefully examined with a fluorescent microscope using rhodamine optics to verify that all labeled axons that entered the optic disc arose from the injection site in temporal retina. Since the site of origin in the retina of the labeled axons was known, this method allowed us to accurately predict their topographically appropriate termination site in the SC. Therefore, this labeling method, in conjunction with Elvax implants, allowed us to observe changes in the topographic accuracy of the positioning of axons and arbors in the SC following treatment with NMDA receptor antagonists. For this study, we define an "arbor" as a focus of branching from a single labeled axon including at least three secondary or tertiary branches (at least one of which *must* be a tertiary branch) within 150 μm of each other.

RESULTS

Sheets of Elvax, a slow-release polymer, containing one of two NMDA receptor antagonists, AP5 or MK-801, were placed over the SC of rats within the first 24 hr after birth, an age when the retinocollicular map is topographically disordered. Later, a small number of retinal axons were labeled with focal injections of DiI in temporal retina and viewed in the contralateral SC on P12, by which age a topographically precise projection is normally established (24, 25), or on P19.

To verify that the normal development of topographic order was not altered by the surgical placement of Elvax, control rats received Elvax containing L-AP5 (the inactive stereoisomer of AP5) or water alone. A mature topographic ordering of retinal axons comparable to that seen by P12 in the SC of normal rats was observed in the SC of P12 or P19 control rats implanted with Elvax containing L-AP5 or water on P0 (Fig. 1*A*). As in normal animals, aberrantly positioned axons, branches, and arbors were rare; in 14 control-Elvax cases examined at P12 or P19, we observed no aberrantly positioned arbors. Thus, implanting of Elvax over the SC did not interfere with the remodeling of the initially diffuse retinocollicular projection.

The NMDA receptor antagonist MK-801 can also block nicotinic receptors (39). Therefore, in an additional set of control experiments, we examined the effect of dihydro-β-erythroidine, a nicotinic receptor antagonist, on map remodeling, using the same procedures as with the NMDA receptor antagonists. We found that this treatment had no effect on the development of a topographically ordered retinocollicular projection; no aberrant arbors were found in the SC in four cases examined at P12 or P19. Thus, interfering with nicotinic receptor function does not alter the establishment of a topographically ordered map.

Observations made on P12 or P19 rats implanted with Elvax containing 0.1 mM MK-801 (n = 7 rats), a noncompetitive antagonist of NMDA receptors, or 0.1 mM AP5 (n = 9 rats), a competitive antagonist to NMDA receptors, showed that these NMDA receptor blockers interfered with map development (Fig. 1 *B* and *C*). An increased number of topographically mistargeted axons persisted and some extended numerous side branches, as many as 10 along a 500-μm segment of primary axon, a feature not observed in normal (24, 25) or control-Elvax-treated rats at P12. Most striking was an abnormal maintenance of well-developed arbors at topographically inappropriate positions in the SC, when the map normally would have achieved its mature topographic order (Fig. 1*D*). The topographically aberrant arbors could persist at any location in the SC, including its caudal edge. The laminar position of the aberrant arbors also varied; many were positioned in the superficial gray region, where retinal axons normally arborize, but others were positioned deeper, in the stratum opticum. The finding of aberrantly positioned arbors at P12 following disruption of NMDA receptor activation shows that normal NMDA receptor activation is important for the elimination of aberrantly positioned arbors. Further, the similarities in the labeling patterns at P12 and P19 suggest that disruption of normal NMDA receptor function prevents rather than slows the elimination of aberrantly positioned branches and arbors.

In the same P12 or P19 rats treated with AP5 or MK-801, a dense zone of arborizations developed at the topographically correct location near the rostral edge of the SC (Fig. 1 *B* and *C*). This observation suggests that some aspects of map formation may be independent of NMDA receptor-mediated mechanisms. Alternatively, the amount of antagonist in the tissue may have been insufficient to achieve a maximum

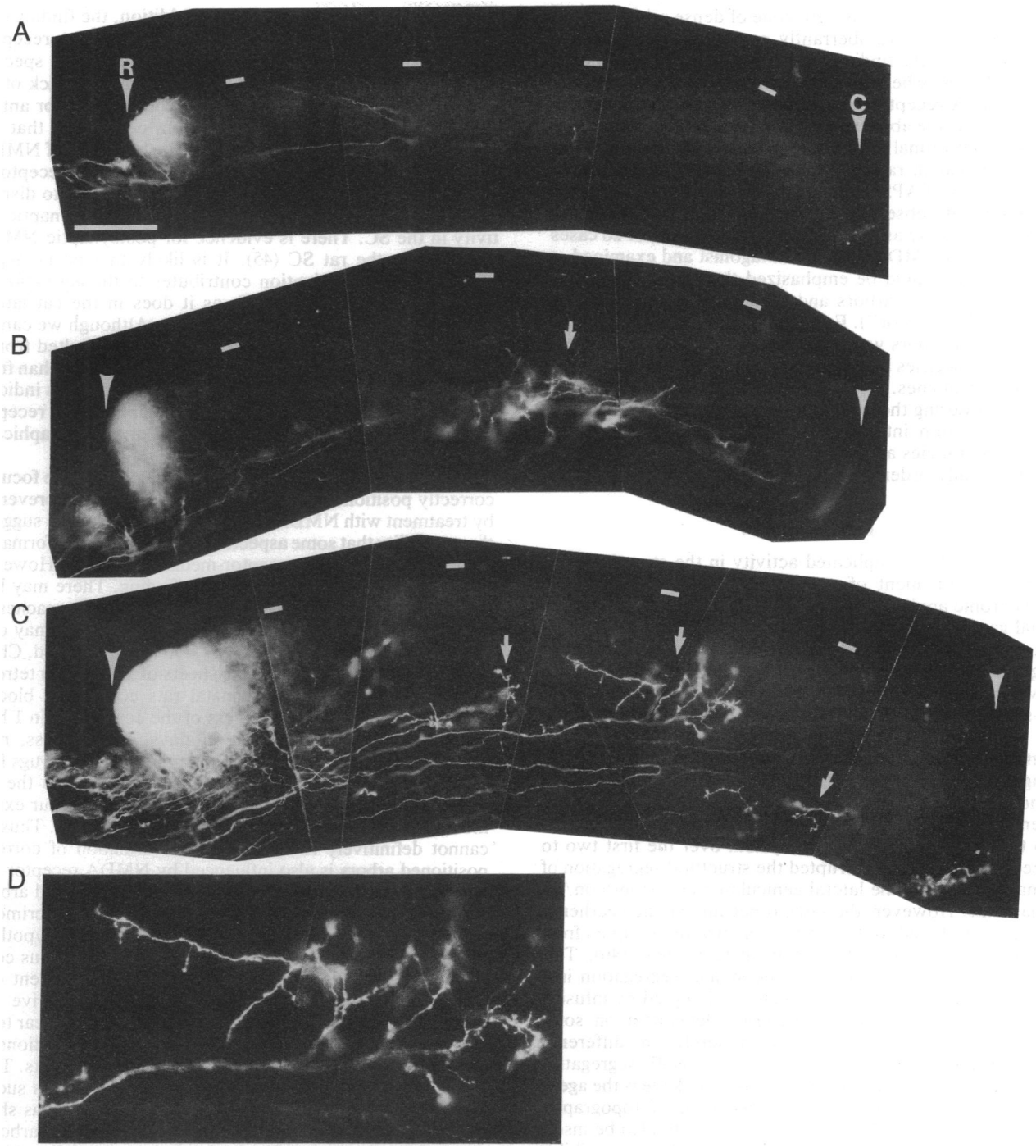

FIG. 1. NMDA receptor antagonists interfere with map development. Axons labeled by a focal injection of DiI in temporal retina are shown in parasagittal sections of the contralateral SC. (*A*) Control: parasagittal section of the SC from a P12 rat implanted with Elvax containing L-AP5, the inactive isomer of AP5, over the SC on P0. The normal development of a topographically ordered projection is unaltered by the surgical procedure. By P12, as in unoperated rats (12), a dense focus of arborizations of labeled axons is apparent at the topographically appropriate position near the rostral (R) border of the SC. Few axons extend and branch beyond this terminal zone. Arborizations in aberrant positions in the SC are very rare. (*B*) Effect of NMDA receptor blockade: SC of a P12 rat treated with MK-801 since birth. A dense focus of correctly positioned arbors is present at the rostral end of the SC, as in controls. But in addition, aberrantly positioned branches and arbors are present in caudal SC. (*C*) SC of a P19 rat treated with AP5 since birth, showing a focus of correctly positioned arbors at the rostral edge along with aberrantly positioned branches and arbors in other regions of the SC. The percentage of the SC occupied by the correctly positioned arbors varies from case to case with the size of the retinal injection site but is not observably different between experimental and control groups. The rostral–caudal length of the sections varies with the position of the section along the medial–lateral axis of the SC, as well as with the age of the animal. Arrows in *B* and *C* indicate aberrantly positioned branches and arbors. Arrowheads in *A–C* indicate the rostral (R) border to the left and the caudal (C) border to the right. Dashes indicate the dorsal surface of the SC. (*D*) Higher-power view of an aberrantly positioned arbor from the AP5-treated case illustrated in *C*. [Bar = 250 μm (*A–C*) or 100 μm (*D*).]

block of NMDA receptors. To address this issue, sheets of Elvax containing 1.0 mM AP5 (n = 5 rats) or 10 mM AP5 (n = 5 rats) were implanted over the SC of other newborn rats. Despite this 10-fold or 100-fold increase in dose, which presumably resulted in a greater concentration of antagonist in the SC, no differences were observed in the effect on map

development. At P19, a discrete zone of dense arborizations was apparent, as were aberrantly positioned branches and arborizations. These findings suggest that the lower concentration of AP5 may be sufficient to produce a saturating effect on the NMDA receptor-dependent effect.

In summary, the abnormal maintenance of topographically mispositioned retinal arbors and branches in superior colliculi was similar in rats treated with MK-801 or with three concentrations of AP5. When combining the data from these experiments, we observed at least one aberrantly positioned arbor, and as many as four aberrant arbors, in 18 of 26 cases treated with an NMDA receptor antagonist and examined at P12 or P19. It should be emphasized that we distinguished between elaborated arbors and branches (see *Methods* for definition of an "arbor"). Experimental animals not found to have aberrant arbors were nonetheless distinguishable from typical control cases by the presence of numerous aberrantly positioned branches. Thus, treatment with NMDA receptor antagonists during the period of remodeling of the retinocollicular projection interferes with the elimination of topographic aberrancies and therefore with the development of a topographically ordered map.

DISCUSSION

Many studies have implicated activity in the structural and functional refinement of vertebrate visual projections by using chronic application of tetrodotoxin to block activity in retinal ganglion cells (2–5). Studies in amphibians and goldfish, conducted when pattern vision was well established, suggested the NMDA receptor as the detector of the relevant activity patterns, since blockade of this receptor disrupted eye-specific segregation and the refinement of topography. The first studies to indicate a role for NMDA receptors in mammalian visual plasticity showed that NMDA receptor antagonists blocked the shift of ocular dominance induced by monocular deprivation of patterned vision (33–36). More recently, Hahm *et al.* (32) demonstrated that infusion of AP5 into the ferret central nervous system over the first two to three postnatal weeks disrupted the structural segregation of retinal afferents in the lateral geniculate nucleus into on/off sublaminae. However, the same procedure applied earlier in development failed to disrupt the segregation of inputs from the two eyes in the lateral geniculate nucleus (40). This finding was surprising because the similar segregation into eye-specific laminae in the cat can be disrupted by infusion of tetrodotoxin (41) and is therefore dependent on some aspect of activity. A possible explanation for the difference between sublaminar segregation and eye-specific segregation in their sensitivities to NMDA receptor blockade is the age of treatment. By analogy, the establishment of topographic maps in mammals, which occurs early, might also be insensitive to NMDA receptor blockade, despite the susceptibility of ocular dominance plasticity to this treatment (33–36). However, our findings show that the normal activation of postsynaptic NMDA receptors during the early postnatal period is indeed important for the development of a topographic map. Chronic treatment with NMDA receptor antagonists interferes with the preferential elimination of entire retinal axon arborizations positioned in topographically inappropriate regions of the SC.

Since the blockers used in these experiments, AP5 and MK-801, are potent and selective antagonists for NMDA receptors (42), it is unlikely that our results are due to a blockade of non-NMDA receptors. If 0.3% of the initial amount of the drug is released from the Elvax each day (see 12), and if this drug accumulates in the SC, then after 24 hours, the drug concentration in the SC would be in the low micromolar range, well below concentrations of AP5 shown to selectively block NMDA receptors in slices of rat visual cortex (43) or hippocampus (44). In addition, the finding of a similar effect with two different classes of NMDA receptor antagonists suggests that our results are due to the specific blockade of NMDA receptors. Furthermore, the lack of an effect of dihydro-β-erythroidine, a nicotinic receptor antagonist, on map remodeling supports our conclusion that the effects of AP5 or MK-801 are due to the blockade of NMDA receptors rather than from an effect on nicotinic receptors.

It seems likely that the effect of the blockers is to disrupt a component of NMDA receptor-mediated postsynaptic activity in the SC. There is evidence for postsynaptic NMDA receptors in the rat SC (45). It is likely that postsynaptic NMDA receptor activation contributes to the activation of the target neurons in the SC, as it does in the cat lateral geniculate nucleus (46) and cortex (47). Although we cannot yet rule out the possibility that our findings resulted from a disruption of postsynaptic activity in general rather than from a specific blockade of NMDA receptors, our results indicate that, in the developing rat SC, normal NMDA receptor function is important for the formation of a topographically ordered map.

We also find, though, that the formation of a dense focus of correctly positioned arborizations in the SC is not prevented by treatment with NMDA receptor antagonists. This suggests the possibility that some aspects of topographic map formation do not require NMDA receptor-mediated activity. However, other factors could account for this finding. There may be a delay after Elvax implantation before the drug reaches an effective concentration, or the drug concentration may drop below effective levels prior to P12. On the other hand, Chiaia *et al.* (48) found that implanting sheets of Elvax with tetrodotoxin over the cortex of neonatal rats completely blocked activity throughout the thickness of the cortex within 1 hr of the surgery, and for at least 11 days. Nonetheless, rapid clearance or barriers to diffusion may prevent the drugs from reaching their maximum levels of effectiveness in the SC. Therefore, even the highest dose of AP5 used in our experiments may not completely block NMDA receptors. Thus, we cannot definitively rule out that the formation of correctly positioned arbors is also influenced by NMDA receptor activation, as is the elimination of aberrantly positioned arbors.

Our results, together with those of earlier experiments, support the broad generalizations of the activity hypothesis for developmental synaptic remodeling. Spontaneous correlations in activity patterns of ganglion cells, present even before differentiation of the circuitry that will drive their activity in adulthood is functional (6–8, 27, 28), appear to use NMDA receptor activation to register these correlations and restructure their connections in central visual targets. These interactions produce not only local rearrangements such as the remodeling of individual arbors (32) but also, as shown here, the preferential elimination of entire retinal arborizations that are incorrectly positioned in the SC, which is required for establishing an accurate topographic map of visual space. A similar mechanism relying on "spontaneous" activity could be important for establishing topographic neural connections throughout the brain.

A role for NMDA receptors in mediating the activity-dependent patterning of synaptic connections is not limited to development. Temporal relationships in the activity of afferents are also important in models of associative learning (49). The induction of some forms of long-term potentiation, a prominent model for associative learning, is blocked by NMDA receptor antagonists (11, 50). Thus, NMDA receptors may subserve a general cellular mechanism for activity-dependent plasticity both during development and in adulthood.

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1. Northmore, D. P. M. (1984) *Exp. Neurol.* **84,** 109–125.
2. Fawcett, J. W. & O'Leary, D. D. M. (1985) *Trends Neurosci.* **8,** 201–206.
3. Udin, S. B. & Fawcett, J. W. (1988) *Annu. Rev. Neurosci.* **11,** 289–327.
4. Schmidt, J. T. & Tieman, S. B. (1989) *Comments Dev. Biol.* **1,** 11–28.
5. Constantine-Paton, M., Cline, H. T. & Debski, E. (1990) *Annu. Rev. Neurosci.* **13,** 129–154.
6. Maffei, L. & Galli-Resta, L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2861–2864.
7. Meister, M., Wong, R. O. L., Baylor, D. A. & Shatz, C. J. (1991) *Science* **252,** 939–943.
8. Shatz, C. J. (1990) *Neuron* **5,** 745–756.
9. Wigstrom, H. & Gustafsson, B. (1985) *Acta Physiol. Scand.* **123,** 519–522.
10. Cline, H. T., Debski, E. A. & Constantine-Paton, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4342–4345.
11. Schmidt, J. T. (1990) *J. Neurosci.* **10,** 233–246.
12. Cline, H. T. & Constantine-Paton, M. (1989) *Neuron* **3,** 413–426.
13. Scherer, W. & Udin, S. B. (1989) *J. Neurosci.* **9,** 3837–3843.
14. Holt, C. E. & Harris, W. A. (1983) *Nature (London)* **301,** 150–152.
15. Holt, C. E. (1984) *J. Neurosci.* **4,** 1130–1152.
16. Sakaguchi, D. S. & Murphy, R. K. (1985) *J. Neurosci.* **5,** 3228–3245.
17. Fujisawa, H. (1987) *J. Comp. Neurol.* **260,** 127–139.
18. Stuermer, C. A. O. (1988) *J. Neurosci.* **8,** 4513–4530.
19. O'Rourke, N. A. & Fraser, S. E. (1990) *Neuron* **5,** 159–171.
20. Harris, W. A. (1980) *J. Comp. Neurol.* **194,** 303–317.
21. Harris, W. A. (1984) *J. Neurosci.* **4,** 1153–1162.
22. Stuermer, C. A. O., Rohrer, B. & Munz, H. (1990) *J. Neurosci.* **10,** 3615–3626.
23. Reh, T. A. & Constantine-Paton, M. (1983) *J. Neurosci.* **4,** 442–457.
24. Simon, D. K. & O'Leary, D. D. M. (1990) *Dev. Biol.* **137,** 125–134.
25. Simon, D. K. & O'Leary, D. D. M. (1992) *J. Neurosci.* **12,** 1212–1232.
26. Bunt, S. M., Lund, R. D. & Land, P. W. (1983) *Dev. Brain Res.* **6,** 149–168.
27. Weidman, T. A. & Kuwabara, T. (1968) *Arch. Ophthal.* **79,** 470–484.
28. Itaya, S. K. & Molitchnikoff, S. (1990) *Neurosci. Res. Commun.* **7,** 75–82.
29. Nakamura, H. & O'Leary, D. D. M. (1989) *J. Neurosci.* **9,** 3776–3795.
30. Kobayashi, T., Nakamura, H. & Yasuda, M. (1990) *Dev. Brain Res.* **57,** 29–35.
31. Simon, D. K. & O'Leary, D. D. M. (1990) *Soc. Neurosci. Abstr.* **16,** 1287.
32. Hahm, J., Langdon, R. B. & Sur, M. (1991) *Nature (London)* **351,** 568–570.
33. Kleinschmidt, A., Bear, M. F. & Singer, W. (1987) *Science* **238,** 355–358.
34. Gu, Q., Bear, M. F. & Singer, W. (1989) *Dev. Brain Res.* **47,** 281–288.
35. Bear, M. F., Kleinschmidt, A., Gu, Q. & Singer, W. (1990) *J. Neurosci.* **10,** 909–925.
36. Rauschecker, J. P., Egert, U. & Kossel, A. (1990) *Int. J. Dev. Neurosci.* **8,** 425–432.
37. Linden, R. & Perry, V. H. (1983) *Brain Res.* **272,** 145–149.
38. Silberstein, G. B. & Daniel, C. W. (1982) *Dev. Biol.* **93,** 272–278.
39. Ramoa, A. S., Alkondon, M., Aracava, Y., Irons, J., Lunt, G. G., Deshpande, S. S., Wonnacott, S., Aronstam, R. S. & Albuqerque, E. X. (1990) *J. Pharmacol. Exp. Ther.* **254,** 71–82.
40. Smetters, D. K., Hahm, J. & Sur, M. (1991) *Soc. Neurosci. Abstr.* **17,** 1136.
41. Shatz, C. J. & Stryker, M. P. (1988) *Science* **242,** 87–89.
42. Monaghan, D. T., Bridges, R. J. & Cotman, C. W. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29,** 365–379.
43. Artola, A. & Singer, W. (1987) *Nature (London)* **330,** 649–652.
44. Hablitz, J. J. & Langmoen, I. A. (1986) *J. Neurosci.* **6,** 102–106.
45. Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1991) *Nature (London)* **354,** 31–37.
46. Funke, K., Eysel, U. T. & FitzGibbon, T. (1991) *Brain Res.* **547,** 229–238.
47. Fox, K., Daw, N., Sato, H. & Czepita, D. (1991) *Nature (London)* **350,** 342–344.
48. Chiaia, N. L., Fish, S. E., Bauer, W. R., Bennett-Clarke, C. A. & Rhoades, R. W. (1992) *Dev. Brain Res.* **66,** 244–250.
49. Hebb, D. O. (1949) *Organization of Behavior* (Wiley, New York).
50. Collinridge, G. L. & Singer, W. (1990) *Trends Pharmacol. Sci.* **11,** 290–296.