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14. A 7.5-kb Eco RV fragment of the *Hoxb-1* gene from cosmid 8D (*25*) was inserted into the pKS+ vector. Constructs 1, 2, and 12 to 20 contain the *lacZ* gene in frame in the first exon. In cn 12 to 20, a 2.0-kb r3/r5 enhancer (*11*) from *Hoxb-2* was inserted at the 3′ end. Constructs 13 to 15 were digested with Apa I, Sac I, and Spe I at their 5′ ends, respectively. In cns 16, 17, 19, and 20, fragments were inserted into the Spe I site of cn 15. Construct 18 contains point mutations in the RARE (*19*). Constructs 10, 11, 19, and 20 have fragments of chick *Hoxb-1* inserted into the Spe I site of cn 15. Constructs 3 (sense), 7, 10, and 11 contain regulatory regions in the promoterless vector pZA (*26*). For cns 3 (antisense), 4 to 6, 8, and 9, fragments were inserted into the Spe I site of pGZ40, which uses the human β-globin promoter (*26*). In cn 17, a 750-bp fragment from the mouse anti-Müllerian hormone gene was inserted in cn 15.
15. Animal experiments were done according to procedures regulated under the project licenses issued by the United Kingdom Home Office and by the Genetic Manipulation Advisory Group. Generation and analysis of transgenic mice from F_1 hybrids (CBA × C57BL/6) were as described (*8*, *16*, *27*, *28*). The anaesthetic in embryo transfer was Avertin at a dose of 0.015 to 0.017 ml of a 2.5% solution per gram of body weight, injected intraperitoneally. RA was administered to pregnant females (7.5 dpc) by gavage application of 200 μl of sesame seed oil containing all *trans*-RA for a final dose of 20 mg per kilogram of body weight, as described (*8*).
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18. The EMSAs were done with purified human RARα and RARγ and with human RXRα, as described (*29*).
19. The point mutations in the RARE were generated by inverse polymerase chain reaction (PCR). Oligos used were: 5′-ACCCTTACCTGCCTGGACTTGCCCTAGCTC and 5′-ACCGGGAGGGATCTCCGTAAACAGTGGC. The PCR product was purified and self-ligated and mutations were confirmed by complete sequencing. The fragment containing the point mutations replaced the Apa I–Sac I fragment of cn 13.
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30. We thank P. Chambon and H. Gronemeyer for the RXR and RAR proteins, D. Guerrier for spacer DNA, Z. Webster and L. Jones for animal husbandry, W. Hatton for histology, and members of the lab for helpful discussions and comments. H.M. and parts of this work were supported by a Human Frontiers in Science Program collaborative grant. M.S. was supported by postdoctoral fellowships from the Swiss National Foundation and the European Molecular Biology Organization (EMBO), and H.P. was supported by an EMBO fellowship.

25 April 1994; accepted 3 August 1994

Control of Thalamocortical Afferent Rearrangement by Postsynaptic Activity in Developing Visual Cortex

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The formation of specific connections in the developing central nervous system is thought to result from mechanisms that increase the strengths of synapses at which pre- and postsynaptic activity are correlated and decrease it otherwise. In the visual cortex, initially widespread inputs normally sort out into eye-specific patches during early life. If only one eye can see during this period, its patches are much larger than normal, and patches from the occluded eye become much smaller. Anatomical experiments here show that closed-eye inputs expand within a region of cortex that is silenced, establishing that inhibition of common target cells gives less active inputs a competitive advantage.

Ocular dominance columns (alternate patches of visual cortex that receive input from the two eyes) form in normal development by a selective loss of the initially widespread and overlapping branches of the geniculocortical afferents and the selective growth of new arbor in territory dominated by one eye or the other (*1*). During this period of segregation, cortical neurons and their input afferents show dramatic plasticity in response to changes in the visual environment (*2*). Closing the lid of one eye during this period in early postnatal life causes most visual cortical neurons to lose response to the deprived eye and respond instead exclusively to the nondeprived eye (*3*). Such monocular deprivation also causes an anatomical expansion of the cortical territory into which geniculocortical afferents carrying information from the nondeprived eye terminate, as well as a complementary shrinkage of territory serving the deprived eye (*4*).

Experiments in which neural activity in visual cortex was suppressed by the sodium channel blocker tetrodotoxin demonstrated that ocular dominance plasticity requires neural activity in the cortex (*5*). Furthermore, microelectrode experiments indicated that the suppression of postsynaptic visual cortical activity by cortical infusion with the γ-aminobutyric acid type A ($GABA_A$)–receptor agonist muscimol during the period of monocular deprivation shifted ocular dominance toward the deprived eye (*6*). This result suggested that postsynaptic activity in the cortex plays a crucial role in ocular dominance plasticity, but it was not clear whether it revealed mechanisms that normally operate during development or was, instead, a pharmacological curiosity. Because the formation of cortical columns in normal development ultimately involves anatomical plasticity, the present study aimed to determine whether the pharmacological control of postsynaptic activity also controlled the anatomical rearrangements of presynaptic geniculocortical afferents. For this purpose, we labeled geniculocortical afferent termination in regions of layer IV of the visual cortex in which postsynaptic activity was inhibited during the period of monocular deprivation, and we compared the patterns of labeling with those in the control cortex.

Postsynaptic activity in one hemisphere of the primary visual cortex of 4-week-old kittens was inhibited by infusing the cortex with muscimol solution. Four weeks is midway through the process of ocular dominance column segregation. Two days after starting the infusion, kittens were deprived of vision in the contralateral eye by eyelid suture. Geniculocortical afferent termination in the visual cortex was labeled by transneuronal transport of [^{3}H]proline, which was injected into one eye (*7*). In normal older animals, the patches of cortex labeled transneuronally were almost precisely complementary, which allows one to infer the distribution of the unlabeled eye's inputs in experimental material (Fig. 1). After 2 or 4 weeks of muscimol infusion, the region inactivated by muscimol was delineated physiologically by mapping the activity of cortical cells with microelectrodes (Fig. 2A). Drug infusion was then stopped, and the ocular dominance of cortical cells was determined after the effects of muscimol had subsided (*8*). Thereafter, the ani-

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mal was perfused and the tissue was processed to show ocular dominance columns by conventional autoradiography (9, *10*).

After 4 weeks of monocular deprivation during which the spike activity of cortical cells was suppressed by muscimol, the ocular dominance of visual cortical cells had shifted in favor of the closed eye within the inactivated area (Fig. 2B). Injection of [^{3}H]proline revealed clear transneuronal labeling in layer IV of the primary visual cortex in both muscimol-treated and control hemispheres (Fig. 3A). However, the pattern of labeling was quite different between these two hemispheres. In the control hemisphere, as reported (*4*), the labeling was nearly continuous except for narrow gaps, which reflected the expansion of the cortical territory within which the geniculocortical afferents serving the open eye terminate. In contrast, labeling in the muscimol-treated hemisphere showed clear periodic fluctuations of density. This result indicates that the expansion of the afferent termination of the open eye was prevented in the muscimol-treated cortex.

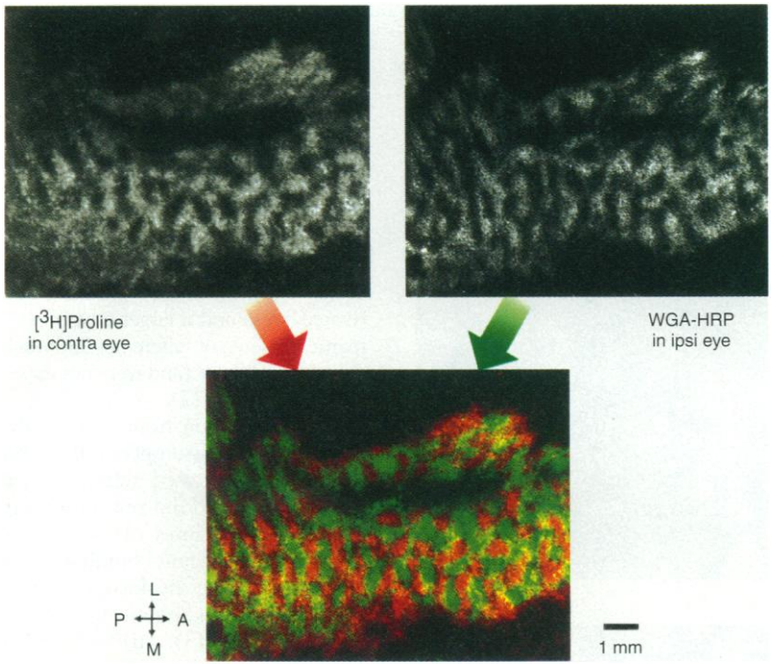

Fig. 1. Ocular dominance columns in normal visual cortex. Different transneuronal tracers, [^{3}H]proline and wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), were injected into the two eyes of a 7-week-old kitten. Alternate sections of flattened cortex were processed for conventional autoradiography and the 3,3',5,5'-tetramethylbenzidine reaction to visualize the geniculocortical afferent termination serving the two eyes. A pair of neighboring sections showing contralateral (contra) eye columns ([^{3}H]proline, above left, red) and ipsilateral (ipsi) eye columns (WGA-HRP, above right, green) are superimposed below to show their complementary pattern. L, lateral; A, anterior; M, medial; P, posterior.

To reveal the columnar organization of afferent termination over a larger area, we flattened the occipital cortex region from five animals. Two flattened cortices of a kitten in which [^{3}H]proline was injected into the open eye (*11*) is shown in Fig. 3B. In a manner similar to that in Fig. 3A, the labeling was almost confluent over most of the visual cortex in the control hemisphere, indicating the expansion of cortical territory for the open eye. In contrast, in the region around the cannula in the muscimol-treated hemisphere, the labeled area formed small discrete patches separated by unlabeled gaps that were larger in size, whereas in the anterior and posterior regions of the cortex far from the cannula, the labeled patches were much thicker than the unlabeled gaps and were often confluent. Two flattened cortices from a kitten in a complementary experiment in which [^{3}H]proline was injected into the closed eye are shown in Fig. 3C. In the control hemisphere, labeling formed small discrete patches as reported (*4*), which demonstrates that the cortical territory for the closed eye had been reduced. However, in the muscimol-treated cortex, the labeled patches were more prominent than the unlabeled gaps in the region around the cannula, revealing that the cortical territory for the closed eye had expanded. This expansion of the closed eye territory in the muscimol-treated cortex was found only in the region around the cannula. Far from the cannula (for example, the posterior part of the cortex in Fig. 3C, left), in the region in which the activity of cells had not been inhibited by muscimol, the labeling resembled that in the control hemisphere.

Although physically flattening the cortex reveals the overall pattern of labeling, flattening is always accompanied by a risk of distortion. To exclude this potential artifact, we explored the labeling pattern in sagittal sections of the visual cortex in three animals. Photomontages showing the medial bank of the muscimol-treated and the control hemispheres of two kittens in which [^{3}H]proline was injected into the open or the closed eye are shown in Fig. 4. The patterns of labeling were similar to those observed in the flattened cortex.

Measurements of the fraction of cortical territory occupied by afferents from the labeled eyes confirmed the visual impressions

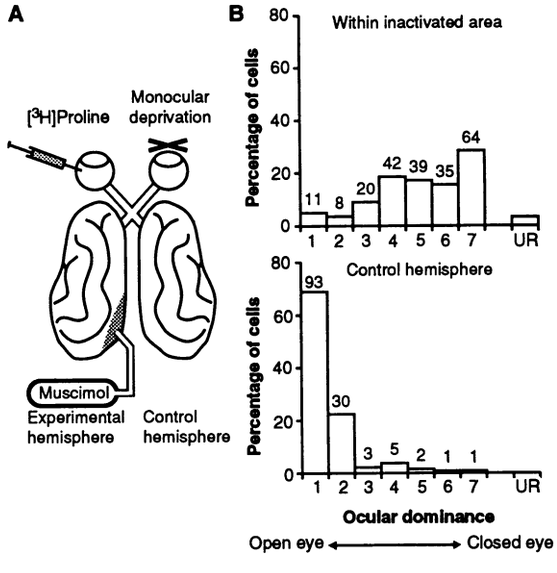

Fig. 2. Ocular dominance shift toward the closed eye in the cortex after long-term treatment with muscimol. (**A**) Schematic illustrating experimental procedures. (**B**) Ocular dominance histograms compiled from single-unit responses recorded in area 17. Results are plotted as if the eyelid suture was always ipsilateral to the hemisphere in which the recording was made. That is, ocular dominance of 1 indicates a cell driven exclusively by the open eye, and ocular dominance of 7 indicates a cell driven exclusively by the closed eye. Cells in category UR showed no visual responses. The upper histogram was compiled from responses recorded (total n = 226; UR, n = 7) within the muscimol-inactivated area of four kittens in which the visual cortex had been infused with muscimol solution for 4 weeks. The lower histogram was compiled from responses (total n = 135; UR, n = 0) in the control hemisphere of the same four kittens.

described above. In two muscimol-treated hemispheres in which the closed (contralateral) eye was injected, the area occupied by labeled afferents was 81 and 82% near the cannula, but 33 and 36% far from the cannula. Both of these are out of the range of similar measurements, 57 and 50%, in two hemispheres from normal kittens in which the contralateral eye was labeled. In five muscimol-treated hemispheres in which the open (ipsilateral) eye was labeled, the median area occupied was 43% near the cannula and 80% far from the cannula, whereas in four hemispheres from normal kittens, a median of 46% ipsilateral label was measured. During the period of afferent segregation, the cortical territory covered by each eye's afferents shrinks (*1*, *4*). Open-eye afferent territory in the area near the cannula also shrank, but not significantly more than normal. Closed-eye afferent territory near the cannula had clearly expanded and resembled open-eye afferent territory in the area far from the cannula.

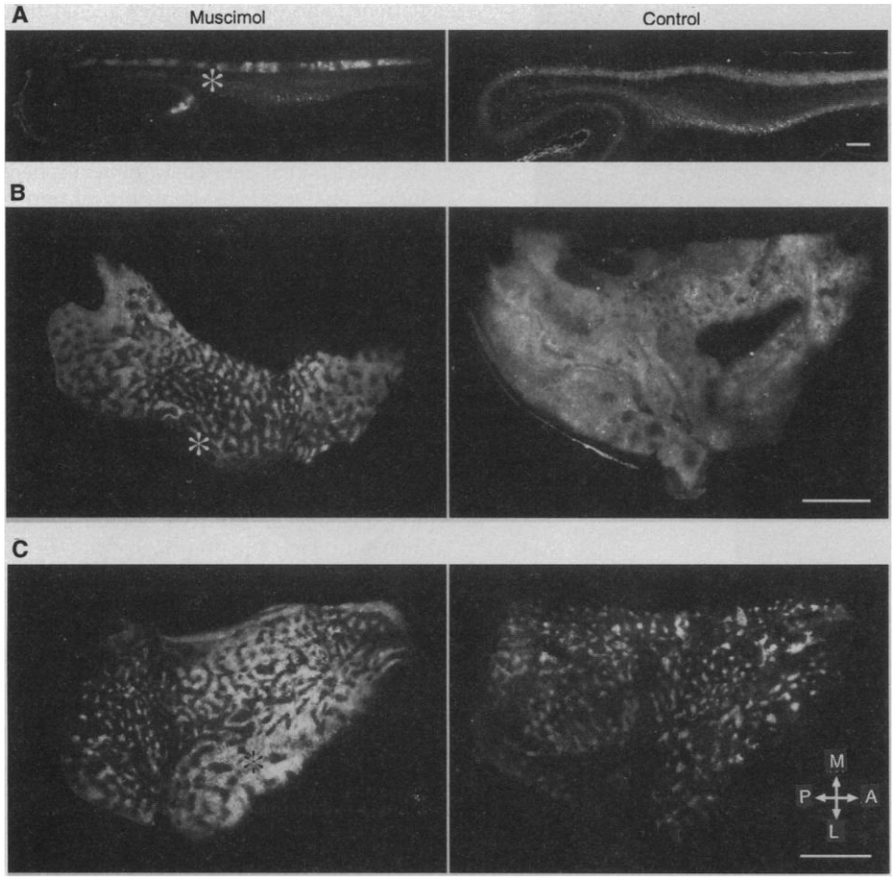

Fig. 3. Geniculocortical afferent termination in the muscimol-treated cortex (left) and in the control cortex (right). (**A**) Examples of horizontal sections obtained from a kitten in which the open eye was labeled with [^{3}H]proline. The bright area reveals the autoradiographic label. Scale bar, 1 mm. (**B** and **C**) Photomontages of labeling in layer IV made from sections of flattened cortex of two kittens in which the open eye (B) or closed eye (C) was labeled. Scale bar, 5 mm. In the left panels of (A) to (C), the * indicates the position of the cannula that infused the muscimol solution. In (B), the large black area without labeling in the control hemisphere is a region that was not flattened successfully.

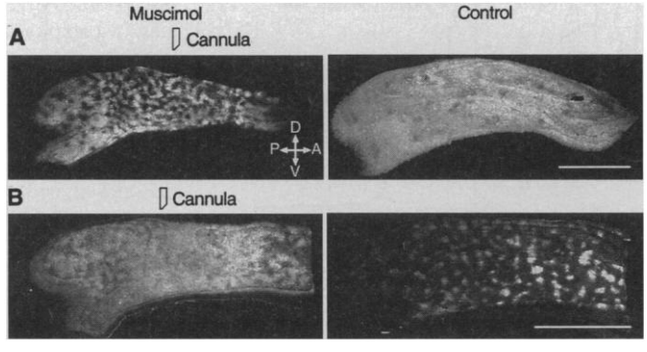

Fig. 4. Surface view of geniculocortical afferent termination in the medial bank. Photomontages of labeling in layer IV were made from sagittal sections obtained from kittens in which the open eye (**A**) or closed eye (**B**) was labeled. Scale bar, 5 mm. D, dorsal; A, anterior; V, ventral; P, posterior.

The observation that the labeling showed a different pattern on the cortex depending on the distance from the cannula infusing muscimol strongly suggests that the rearrangement of geniculocortical afferents was modified in response to the effective concentration of muscimol. If so, one expects a monotonic relation between the size of the inactivated area determined by mapping and the size of the cortical area where the rearrangement of afferents was reversed. In three animals, the distances from the cannula of the borders of physiologically inactivated areas and the areas showing reverse anatomical plasticity were (i) 5 mm physiology and 2 mm anatomy, (ii) 7 mm and 5 mm, and (iii) 10 mm and 6 mm. When a larger cortical region had been inactivated, we found a larger area of the reverse rearrangement of afferents, although these two values did not (and were not expected to) match precisely (*12*).

Our conclusion from these findings depends on the assumption that muscimol selectively suppressed activity of the postsynaptic cells and did not have presynaptic effects. Several lines of evidence support this idea: (i) Muscimol binding studies demonstrate binding in kitten visual cortex with a single binding affinity and $GABA_A$ pharmacology (*13*). (ii) Whereas GABA binds both pre- and postsynaptically, the presynaptic binding site is reported to be a $GABA_B$ receptor (*14*). Finally, if muscimol has an effect on the viability of presynaptic fibers or on neurotransmitter release, such an effect should (iii) cause a degradation of presynaptic afferents for both eyes or (iv) disrupt all plasticity, as was observed in the tetrodotoxin-treated cortex in which both the pre- and postsynaptic elements were inactivated (*5*). The present finding that afferents in muscimol-treated cortex showed plasticity (albeit in the opposite direction from normal) argues strongly against the last two possibilities.

Identical patterns of input activity caused opposite plasticity depending on whether the response of the postsynaptic cortical cells was inhibited. Thus, postsynaptic activity plays a crucial role in the anatomical plasticity of afferent inputs to the developing visual cortex. Although it is not obvious what parameter of the postsynaptic activity is critical for geniculocortical afferent rearrangement, it is clear that spike activity in postsynaptic cells was absent and therefore not necessary for this type of plasticity, which suggests that membrane potential or conductance or both are important in the determination of the direction of plasticity.

Postsynaptic activity was recently shown

to be important in the developmental plasticity of thalamocortical afferents in the mammalian somatosensory system (*15*). In the rat S1 cortex, the excitatory neurotransmitter antagonist D-2-amino-5-phosphonovaleric acid (APV) blocked (suppressed) the rearrangement in the somatotopic patterning of thalamocortical afferents induced by the destruction of a row of vibrissae follicles, but reverse plasticity was not observed. Reverse physiological plasticity in kitten visual cortex was observed only with the strongest $GABA_A$ agonists (*16*, *17*).

Previous physiological studies (6) did not answer whether the ocular dominance shift toward the closed eye in the muscimol-treated cortex had been caused by the strengthening of inputs to cortical cells from the closed eye or by the weakening of inputs from the open eye. The present findings suggest that both these changes take place in the anatomy, although they do not provide information about events at the synaptic level that might precede the changes in afferent arborizations and determine the synaptic strength. Control of at least four types of anatomical plasticity is now evident: When the postsynaptic cells can respond, there is the well-known (i) increase in the territory covered by arbors of more active afferents with (ii) a complementary decrease of the less active afferents. When postsynaptic responses are prevented, (iii) the territory of less active afferents increases with (iv) a less than complementary decrease in that of the more active afferents. These findings suggest a push-pull mechanism of synaptic plasticity—that without postsynaptic activity, less presynaptic activity gives an input the advantage, whereas with a strong postsynaptic response, the contrary is true.

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7. All surgical procedures were performed with the animals under an anesthetic of N_2O:O_2 (2:1) and halothane (1.5 to 2.5%). A cannula (33-gauge stainless steel needle) connected to an osmotic minipump (Alzet 2002 or 2ML4, Alza) was implanted into one hemisphere of the primary visual cortex of 11 4-week-old kittens (P27-35), and muscimol solution was infused continuously (10 mM in saline, 2.5 μl/hour for 4 weeks or 20 to 30 mM, 0.5 μl/hour for 2 weeks) until the terminal experiment described in (*8*). For anatomical demonstration of the geniculocortical afferent termination, we injected [^{3}H]proline (1.7 to 2.1 mCi in 20 μl of saline) into one eye 6 to 14 days before the experiment.
8. Animals were anesthetized with N_2O:O_2 (2:1) and Nembutal (2 to 4 mg per kilogram of body weight per hour) during recording experiments. Spike activities of cortical cells were recorded extracellularly with a tungsten microelectrode at various distances anterior to the cannula that was infusing muscimol solution. When no spike activity except for injury discharges was recorded while the electrode was advanced to the depth of 2000 μm at least, we considered the site inactivated by muscimol. The inactivated area extended between 5 and >10 mm anterior to the cannula. After mapping the inactivated area, muscimol infusion was stopped. After a recovery period of 16 to 19 hours, we recorded spike activity and vigorous visual responses in the area of the cortex that had previously been inactivated.
9. Animals were perfused transcardially with saline and then with 4% paraformaldehyde or 2% glutaraldehyde in 0.1 M phosphate buffer. A block of the brain containing the lateral geniculate nucleus was cut (thickness, 50 μm) on the vibratome in the frontal plane. Blocks containing visual cortex were cut (30 μm) on the frozen microtome in the horizontal or sagittal plane. In five animals, the caudal part of the cortex, which includes the primary visual cortex, was unfolded and flattened between two glass slides [J. Olavarria and R. C. Van Sluyters, *J. Neurosci. Methods* **15**, 191 (1985)]. The flattened cortex was then cut tangentially (40 μm) on the frozen microtome.
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11. Photomontages of labeling in layer IV were made from several sections in which the intensity of labeling was not necessarily uniform, as is evident in Fig. 3A. Because it was necessary to adjust contrasts of labeled ocular dominance patches in different regions to match one another to make the photomontages, one cannot infer the absolute intensity of labeling from photomontages presented in this report. The montages do represent the areas of ocular dominance patches accurately.
12. The size of the inactivated area of cortex was evaluated in animals placed under general anesthesia, a treatment that enhances GABA-mediated inhibition. The inactivated area was probably smaller in alert animals. In the other eight animals, we could not compare these two measures, either because the inactivated area was too large to be located within the part of area 17 accessible to our microelectrode penetrations or because it was difficult to measure the distance on the cortex after flattening the cortex.
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16. Other less potent $GABA_A$ agonists, 3-aminopropane-sulfonic acid and isoguvacine, prevented ocular dominance plasticity but did not cause reverse plasticity [R. Feldman, thesis, University of California, San Francisco (1993)].
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18. We thank S. Harris for her help during the experiments. This work was supported by NIH grant EY02874 to M.P.S. and by Human Frontiers Science Program long-term fellowship to Y.H.

20 April 1994; accepted 22 July 1994