Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9246–9249, December 1990
Neurobiology

Binocular competition in the control of geniculate cell size depends upon visual cortical *N*-methyl-D-aspartate receptor activation

(synaptic plasticity/excitatory amino acid receptors/neural development)

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Communicated by Leon N Cooper, August 29, 1990 (received for review June 1, 1990)

ABSTRACT **The lateral geniculate nucleus relays visual information from the retina to cortex. One well-known anatomical consequence of monocular deprivation during early postnatal development is a shrinkage of neurons in the lamina of the lateral geniculate nucleus that receive input from the deprived eye. This is thought to reflect the competition of afferents subserving the two eyes, possibly at the level of the visual cortex. We find that blockade of *N*-methyl-D-aspartate receptors in kitten visual cortex disrupts this process of binocular competition. These data provide direct evidence that postsynaptic activation of cortical neurons is required for competitive changes in lateral geniculate cell size and suggest a role for *N*-methyl-D-aspartate receptors in anatomical as well as physiological plasticity in the mammalian visual system.**

Monocular deprivation (MD) of kittens during the second postnatal month leads to a striking change in the physiological organization of visual cortex such that few cortical neurons remain responsive to stimulation of the deprived eye (1, 2). One correlate of this change in cortical physiology is a shrinkage of the neurons in the lateral geniculate nucleus (LGN) that relay information to visual cortex from the deprived retina (3). Because the morphological changes in the LGN are most prominent in the binocular segment, it has been proposed that they reflect a process of binocular competition. The classic test of this hypothesis was performed by Guillery in 1972 (4). By destroying part of the central retina of the nondeprived eye he created a region of the LGN that was free of the effects of binocular competition. In this "critical segment" MD caused far less shrinkage of LGN neurons. Evidently, the activity in the open eye promotes the shrinkage of cells subserving the closed eye. Left unanswered by this and subsequent work, however, was the site and mechanism of the competition leading to LGN cell size changes.

Cortical responses to afferent LGN activity are mediated by excitatory amino acid receptors, of which *N*-methyl-D-aspartate (NMDA) receptors are a subclass. Recently, it has been shown that blockade of visual cortical NMDA receptors disrupts the ocular dominance modifications that normally occur after MD (5–7). Left unanswered by this work, however, was the question of whether anatomical as well as physiological plasticity is affected by cortical NMDA-receptor blockade.

In the present paper we address these important unanswered questions. We find that the local infusion of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5) into the striate cortex of monocularly deprived kittens creates a critical segment of LGN that is resistant to the effects of MD. Our results provide direct evidence that the shrinkage of LGN neurons after MD depends upon postsynaptic cortical activity and strengthen the hypothesis that the site of competition is the visual cortex. Further, this work provides additional support for the hypothesis that visual cortical NMDA-receptor activation plays a central role in the mechanisms, both anatomical and physiological, that subserve binocular competition.

MATERIALS AND METHODS

Kittens were reared normally until ≈5 weeks of age (Table 1) at which time they were fitted bilaterally with osmotic minipumps (Alzet 2001) that were attached via polyethylene tubing to 30-gauge needles implanted directly into the visual cortex. These minipumps deliver their contents at a rate of 1 μl per hr for up to 10 days. In the experimental group the pumps contained 50 mM D,L-AP5; in an age-matched control group the pumps contained vehicle solution only (sterile Ringer's solution). At the same time as the pump implant, the animals were monocularly deprived by lid suture.

The extracellular concentration of AP5 declines exponentially with increasing distance from the infusion cannula; at 6 mm the steady-state concentration is estimated to be ≈150 μM and is sufficient to block the ocular-dominance shift measured electrophysiologically (7). To identify the geniculate segment that projects to this region of striate cortex, 0.2–0.3 μl of a 20–30% horseradish peroxidase (HRP) solution was injected into the striate cortex 6 mm anterior to the infusion site on the eighth day of the infusion (Fig. 1*a*). After allowing 2 days for retrograde transport of the HRP, the animal was deeply anesthetized with sodium pentobarbital and perfused through the ascending aorta with saline followed by 1.25% glutaraldehyde/1% paraformaldehyde/0.1 M sodium phosphate buffer, pH 7.4. The brains were removed and cryoprotected for several days in cold 15% sucrose in phosphate buffer. The brains were then quick-frozen in −50°C 2-methylbutane and sectioned in the sagittal plane by using a cryostat; section thickness was 60 μm. The sections were treated for HRP in 0.05% 3,3′-diaminobenzidine (DAB)/0.01% hydrogen peroxide/0.1 M phosphate buffer, pH 7.4, for 30 min. The sections were then mounted onto microscope slides, stained for Nissl substance with cresyl violet, dehydrated in ethanol, cleared in xylene, and coverslipped. The DAB method of HRP histochemistry was chosen specifically because it is compatible with good quality cresyl violet staining.

In a 600-μm-wide column within the HRP-labeled LGN segment (Fig. 1*b*, shaded region marked a), each Nissl-stained neuron with a clearly recognizable nucleus and nucleolus was drawn at a final magnification of ×660 with a camera lucida. The drawings were digitized to yield cross-sectional areas. During the entire quantification process the

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Abbreviations: NMDA, *N*-methyl-D-aspartate; AP5, 2-amino-5-phosphonovaleric acid; LGN, lateral geniculate nucleus; MD, monocular deprivation; HRP, horseradish peroxidase.

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Table 1. Effect of 10-day MD on LGN ipsilateral to deprived eye

Case	Age, days	Anterior A1 cell size, μm^2	Anterior A cell size, μm^2	Anterior A1/A	Posterior A1 cell size, μm^2	Posterior A cell size, μm^2	Posterior A1/A
			Ringer-treated				
1	53	227.1 ± 10.7	274.0 ± 14.7	0.83	245.7 ± 19.6	281.9 ± 18.7	0.87
3	50	264.7 ± 14.6	331.6 ± 17.9	0.80	231.0 ± 19.0	358.6 ± 22.5	0.64
5	41	269.0 ± 20.9	325.0 ± 17.1	0.83	247.9 ± 17.3	300.0 ± 19.3	0.83
Mean ± SEM		253.4 ± 13.2	310.2 ± 18.2	0.82 ± 0.01	241.5 ± 5.3	313.5 ± 23.17	0.78 ± 0.07
			AP5-treated				
2	54	322.1 ± 19.7	273.0 ± 13.1	1.18	283.6 ± 24.2	285.5 ± 18.4	0.99
4	51	354.9 ± 16.3	325.3 ± 15.7	1.09	241.0 ± 15.7	284.6 ± 15.5	0.85
6	42	273.4 ± 10.6	240.0 ± 10.1	1.14	236.4 ± 13.6	300.4 ± 11.7	0.79
8	41	305.7 ± 16.0	236.9 ± 9.7	1.29	204.9 ± 10.7	273.2 ± 10.7	0.75
Mean ± SEM		314.0 ± 17.0	268.8 ± 20.5	1.17 ± 0.04	241.5 ± 16.2	285.9 ± 5.6	0.84 ± 0.05
			Normal (no MD)				
9L	45	305.3 ± 12.1	264.9 ± 9.7	1.15			
9R	45	349.6 ± 11.6	276.0 ± 11.7	1.27			
Mean ± SEM		327.4 ± 22.1	270.4 ± 5.5	1.21 ± 0.6			

L, left; R, right.

experimenter was "blind" to the nature of the cortical drug treatment. Cells were sampled in each lamina of each LGN until the standard error (SEM) was <10% of the mean (n per LGN ranged from 58 to 123). These means were then considered as independent observations in the statistical comparisons between experimental groups (Tables 1 and 2).

RESULTS

Neurons in lamina A of the LGN receive input from the contralateral retina; those in lamina A1 receive input from the ipsilateral retina. It has been noted previously that in normal animals, cells in lamina A1 are, on average, slightly larger than those in lamina A (10–12). Our measurements of cell size in the two geniculates of a normal kitten confirm this to be the case; the ratio of A1 to A cell size is ≈1.2 (Table 1). Lamina A and A1 cells also differ in their response to brief periods of MD. In the Ringer-treated animals, lamina A1 neurons ipsilateral to the deprived eye were clearly shrunken and less darkly stained after 10 days of MD (Figs. 2 and 3); the A1/A cell size ratio was only 0.82 ± 0.01 (Table 1), and the mean cross-sectional area of A1 neurons was significantly smaller than that of A cells ($P < 0.02$, paired t test). It is worth noting that A1 cells were significantly smaller than A cells in each case considered individually (case 1, $P < 0.02$; case 3, $P < 0.01$; case 5, $P < 0.05$; t test), confirming that our sample of cells was sufficient to detect changes across LGN lamina. On the other hand, after only 10 days of MD, shrinkage was far less obvious in lamina A contralateral to the deprived eye (Table 2); here the A1/A cell-size ratio (1.28 ± 0.06) was similar to that found in the normal geniculates. This finding was not completely unexpected because hemispheric differences in the effects of MD have been noted before (13, 14). For this reason, the detailed analysis of AP5 effects was restricted to the hemispheres ipsilateral to the deprived eye.†

In contrast to the cases in which cortex was infused with Ringer's solution, neurons in the HRP-labeled segment of deprived lamina A1 appeared unaffected by MD in cases where cortex was treated with AP5 (Figs. 2 and 3). The mean cross-sectional area of A1 neurons ipsilateral to the deprived eye was actually larger than that of lamina A cells ($P < 0.02$, paired t test) and was very similar to the value for A1 cells in the normal LGN (314 ± 17 μm^2 in AP5-treated kittens as compared with 327 ± 22 μm^2 in the normal animal; see Table 1). Accordingly, the A1/A cell size ratios are significantly different for the AP5- and Ringer's solution-treated groups (1.17 ± 0.04 vs. 0.82 ± 0.01, respectively; $P < 0.002$, unpaired t test). Thus, the data clearly indicate that intracortical infusion of AP5 prevents the shrinkage of LGN neurons that normally results from 10 days of MD.

Microperfusion of AP5 does not block the NMDA receptors in all regions of visual cortex; rather, the effect is confined to the vicinity of the infusion site. This provided us with a useful internal control because the posterior segments of the LGN in the same sections already quantified had projections to a region of striate cortex that was ≈15 mm from the infusion site (Fig. 1 a and b; region marked p). Subsequent analysis of cell size showed that, in the AP5 group, A1 neurons in the posterior LGN were significantly smaller than those in the anterior, HRP-labeled segment ($P < 0.05$, paired t test). This regional difference was also reflected by the A1/A cell size ratio (0.84 ± 0.05 in the posterior segment vs. 1.17 ± 0.04 in the anterior segment; $P < 0.005$, t test). These data are summarized in Fig. 1c and lend strong support to the interpretation that AP5 exerted its effects on cell size in the anterior LGN by blocking cortical NMDA receptors.

DISCUSSION

Important considerations for the interpretation of this study are that (i) clear morphological effects in the LGN require

Table 2. Effect of 10-day MD on LGN contralateral to deprived eye

Case	A cell size (deprived), μm^2	A1 cell size (nondeprived), μm^2	A/A1
	Ringer-treated		
1	233.6 ± 6.0	273.2 ± 8.1	0.85
3	305.9 ± 8.8	420.2 ± 12.4	0.73
5	214.2 ± 2.0	279.7 ± 13.1	0.77
	AP5-treated		
2	294.6 ± 7.8	330.8 ± 9.4	0.89
4	302.5 ± 11.1	412.3 ± 12.4	0.73
8	228.4 ± 6.2	288.1 ± 12.0	0.79
	Normal (no MD)		
9L	264.9 ± 9.7	305.3 ± 12.1	0.87
9R	276.0 ± 11.7	349.6 ± 11.6	0.79

L, left; R, right.

†Another way to assess shrinkage is to compare lamina A cell size contralateral to the deprived eye with that ipsilateral to the deprived eye, in the same animals (cf. ref. 12). This approach is useful when the brain is sectioned in the coronal plane, and, consequently, both hemispheres are processed for histology identically. However, in the present experiments the brains were sectioned in the sagittal plane, and each hemisphere was processed for histology independently, usually on different days. Differential shrinkage of the two hemispheres made it impractical to perform within-animal interhemispheric comparisons.

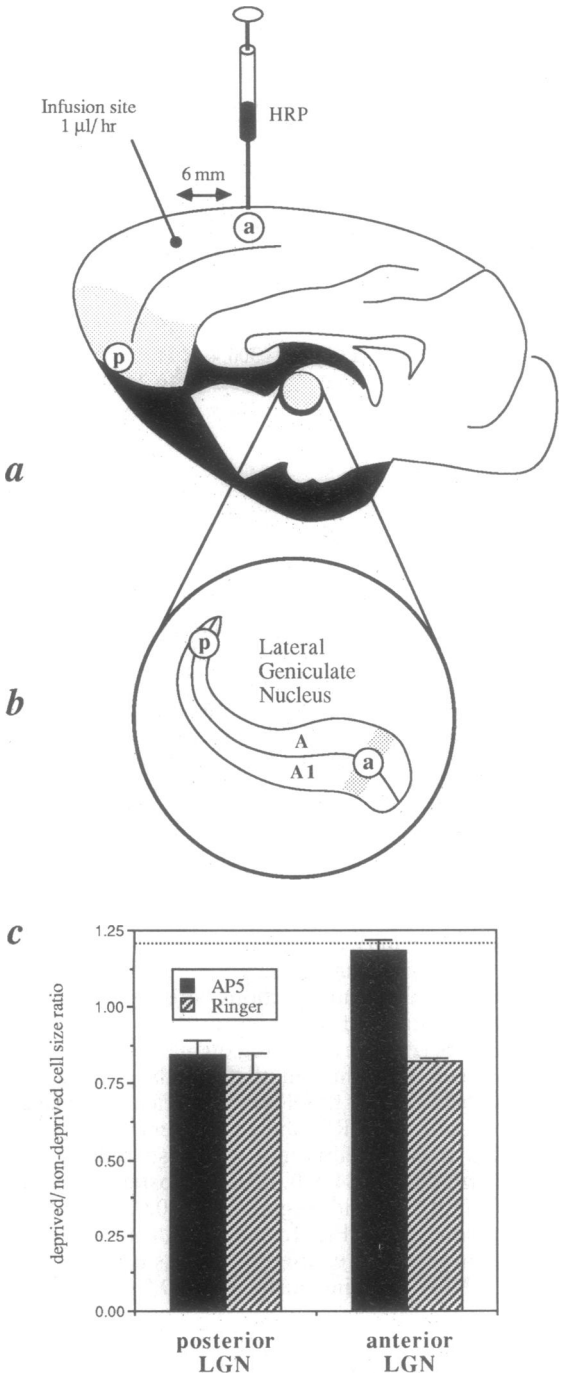

FIG. 1. Experimental design and results. (*a*) Schematic midsagittal view of a kitten brain to illustrate the site of infusion. Through needles chronically implanted at this location, either 50 mM AP5 or Ringer's solution was infused into the visual cortex at 1 μl per hr for 10 days. The ipsilateral eyelid was sutured closed for the duration of infusion. On the eighth day, an injection of HRP (0.2–0.3 μl of 20–30% solution) was made in visual cortex at the location labeled ⓐ, 6 mm anterior to the infusion needle. (*b*) Schematic parasagittal view of the LGN. Cells in lamina A and A1 receive synaptic input from the contralateral and ipsilateral (deprived) retinae, respectively. The lightly shaded region marked ⓐ indicates the approximate location of neurons retrogradely labeled after cortical HRP injection. The cross-sectional area of each Nissl-stained LGN neuron with a clearly recognizable nucleus and nucleolus was measured in a 600-μm-wide strip extending through regions of lamina A and A1 identified as HRP-positive. The experimenter was blind to the type of drug treatment during all phases of measurement. Cells were also measured in the region of the posterior LGN marked ⓟ. Neurons in this segment of the LGN project to a region of visual cortex (marked ⓟ in *a*)—that is ≈15 mm from the infusion site, as estimated by visual field maps of LGN and cortex (8, 9). (*c*) Plotted is the ratio of lamina A1 (deprived) to lamina A (nondeprived) cell size in the Ringer- and AP5-treated groups in both segments of the LGN that were quantified (ⓐ and ⓟ in *b* correspond to anterior and posterior LGN, respectively). The A1/A cell size ratio in the anterior LGN segment of the AP5 group differs significantly from both segments in the control group, as well as from the posterior segment in the AP5 group. Dashed line indicates the mean A1/A cell size ratio in the two LGN of a normal kitten (see Table 1).

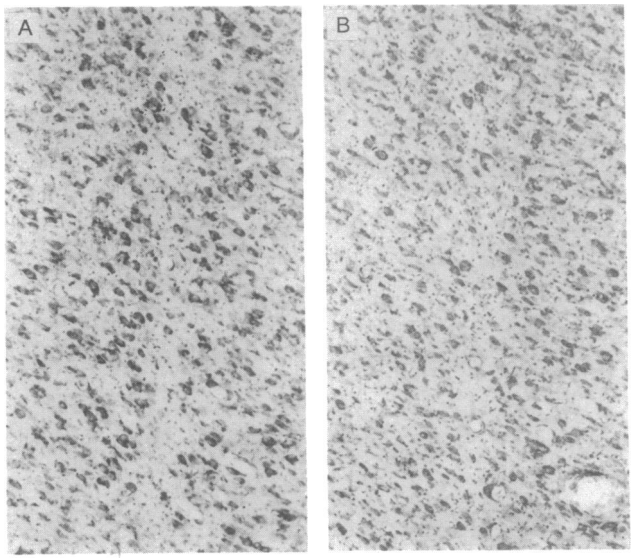

FIG. 2. Photomicrographs of the anterior LGN in age-matched, monocularly deprived kittens (cases 3 and 4, Table 1) treated with AP5 (*A*) or Ringer's solution (*B*) infusion into the visual cortex. In both *A* and *B* lamina A1 is at left and lamina A is at right. In *A*, cells in lamina A1 are as large and darkly stained as those in lamina A. In *B*, lamina A1 neurons are visibly shrunken and less darkly stained. (×60.)

many days of MD, (*ii*) morphological effects are more severe and occur more rapidly in the hemisphere ipsilateral to the deprived eye, and (*iii*) the duration of AP5 infusion is limited by the volume and flow rate of the osmotic minipumps. The pumps used in this study necessarily limited the duration of the MD to 10 days; quantification revealed that this brief deprivation produced unequivocal anatomical effects only in the LGN ipsilateral to the deprived eye. Therefore, this hemisphere was used to investigate differences between AP5-treated and control groups. This investigation revealed that blockade of cortical NMDA receptors completely prevented the shrinkage of LGN neurons that normally results from MD.

It has always been assumed that binocular competition in the control of LGN cell size occurs at a site where inputs from the two eyes converge onto a common target. The visual cortex was considered the probable location, although interlaminar interactions within the LGN could not be excluded (4, 15). The present results provide direct evidence that visual cortex is necessary for binocular competition and strengthen the hypothesis that changes in LGN cell size after MD reflect competition of geniculocortical afferents for synaptic space [probably in layers IV and VI (16)]. Further, because NMDA receptors are thought to be entirely postsynaptic in the visual cortex, these results support the idea that postsynaptic activation of cortical neurons plays a central role in the mechanisms of binocular competition (17, 18).

A debate continues to rage in the literature about whether the effects of AP5 on ocular dominance plasticity are due solely to the suppression of visually evoked postsynaptic activity in area 17 (5–7, 19, 20). Although the present results do not directly bear upon this question, two points should be

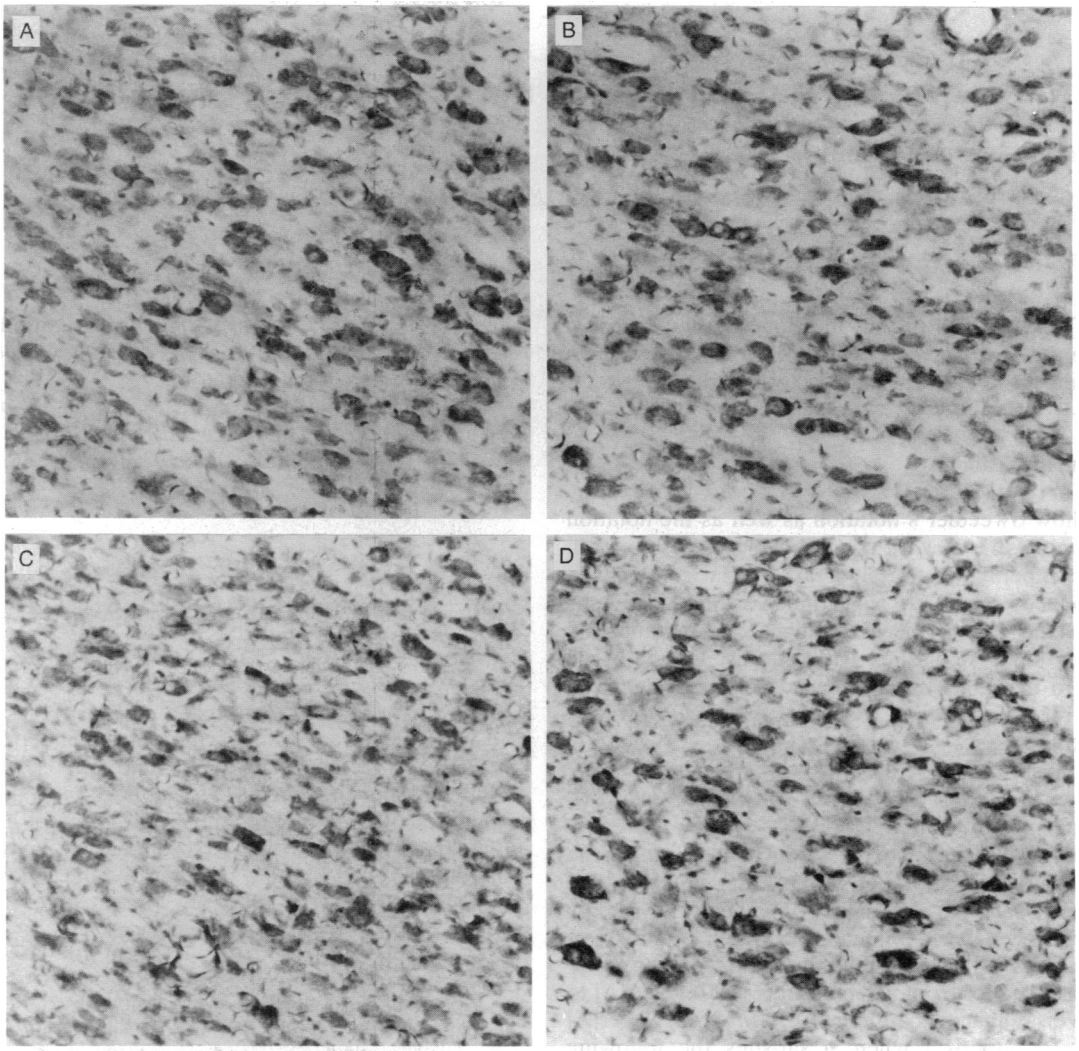

FIG. 3. Higher magnification photomicrographs of LGN neurons from the same cases as in Fig. 2. (*A* and *B*) Cells in lamina A1 and A, respectively, from the AP5-treated case. (*C* and *D*) Cells in lamina A1 and A, respectively, from the Ringer's solution-treated case. (×100.)

considered. (*i*) Our LGN cell-size measurements demonstrate that binocular competition is disrupted as far away as 6 mm from the site of AP5 infusion; here it appears that all agree that most cortical neurons are responsive to visual stimulation (5, 7, 20). (*ii*) Geniculocortical afferents are thought to compete in cortical layers IV and VI, and here visual responses appear relatively more resistant to NMDA receptor blockade than in other layers (19). In any case, the present results clearly support the view that cortical responses generated in the absence of adequate NMDA receptor activation are not sufficient to support binocular competition in the control of LGN cell size.

We thank Sarah Packman and Christine Lydon for technical assistance, Dr. N. Lange for consultations on the statistical analyses, and Dr. M. Paradiso for critically reading the manuscript. This work was supported by grants from the National Eye Institute and Office of Naval Research.

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