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# Rearing With Monocular Lid Suture Induces Abnormal NADPH-Diaphorase Staining in the Lateral Geniculate Nucleus of Cats

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ABSTRACT

We investigated the changes in NADPH-diaphorase staining that occur in the lateral geniculate nucleus of cats following rearing with monocular lid suture. This staining allows visualization of the synthesizing enzyme of nitric oxide, a neuromodulator associated with plasticity. In the lateral geniculate nucleus of normally reared cats, NADPH-diaphorase exclusively labels the axons and terminals of an input from the parabrachial region of the brainstem; no geniculate cells in the A-laminae are labeled. Early monocular lid suture has no obvious effect on the NADPH-diaphorase staining of parabrachial axons. However, this lid suture results in the abnormal appearance of NADPH-diaphorase staining for geniculate somata. These cells are located primarily in the nondeprived laminae. Double-labeling experiments indicate that these cells with abnormal NADPH-diaphorase reactivity are Y relay cells: NADPH-diaphorase staining is found in cells retrogradely labeled from visual cortex; it is found in cells labeled with a monoclonal antibody for CAT-301, which selectively targets Y cells; it is not found in cells labeled with an anti-GABA antibody, which targets interneurons. Also, NADPH-diaphorase labeled cells are among the largest cells in the nondeprived laminae, again suggesting that they are Y relay cells. We cannot suggest a specific mechanism for this induction of NADPH-diaphorase labeling, but it does not seem to be due to abnormal binocular competition induced by the monocular lid suture. 

**Key words: thalamus, Y cells, CAT-301, nitric oxide, visual deprivation**

Early monocular lid suture affects the development of the lateral geniculate nucleus and visual cortex (for reviews, see Movshon and Van Sluyters, 1981; Sherman and Spear, 1982). Most of these studies have been carried out with the cat, and they have concentrated on deprivation effects at the geniculate level that occur in the deprived laminae. Relay cells innervated from the deprived eye are smaller than normal (Wiesel and Hubel, 1963; Guillery and Stelzner, 1970; Guillery, 1972; Hickey et al., 1977); of the two main classes of relay cell, X and Y, deprived Y cells seem particularly sensitive to morphological, physiological, and metabolic effects of deprivation (Sherman et al., 1972, 1975; LeVay and Ferster, 1977; Wong-Riley, 1979; Lehmkuhle et al., 1980; Friedlander et al., 1982; Friedlander and Stanford, 1984; Kageyama and Wong-Riley, 1986; Sur et al., 1988; Guimarães et al., 1990); and axonal arbors of many deprived geniculate cells are abnormally small (Friedlander et al., 1991). It is also interesting to note that effects of monocular lid suture have also been described for cells in nondeprived geniculate laminae. Cell somata there and their axonal arbors in cortex grow to an abnormally large size (Guillery, 1973; Hickey et al., 1977; Friedlander et al., 1991). These effects in the lateral geniculate nucleus seem limited to relay cells, because interneurons appear unaffected by the lid suture (Robson and Martin-Elkins, 1985).

Although effects of lid suture are clearly seen on relay cells, it is not so clear generally how afferents to the lateral geniculate nucleus are affected. There is evidence that retinogeniculate axons from the deprived eye develop abnormal arbors (Sur et al., 1982; Garraghty et al., 1986), but most inputs to relay cells emanate from nonretinal sources, and virtually nothing is known about effects of lid suture on these nonretinal afferents. We have recently published evidence from normal cats that one important such input from the parabrachial region of the brainstem can be

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unequivocally identified. These axons stain positively for NADPH-diaphorase. We initially sought to determine if monocular lid suture leads to macroscopic changes in NADPH-diaphorase labeling between deprived and nondeprived laminae, which would suggest an effect of such visual deprivation on parabrachial input to the lateral geniculate nucleus. One reason to focus on this input has to do with evidence that NADPH-diaphorase labeling is associated with the presence of nitric oxide synthase, suggesting, in turn, that these parabrachial axons may release nitric oxide. In other neural systems, nitric oxide has been associated with a variety of neuroplastic phenomena, including developmental plasticity (Pow, 1992; Regidor et al., 1993; Wetts and Vaughn, 1993; Gonzáles-Hernández et al., 1993; Zhang et al., 1993). Thus it seemed plausible that an input using nitric oxide might play a role in the effects of monocular lid suture seen among relay cells, and, if so, this input itself may exhibit abnormal staining patterns.

However, although our initial rationale was to use NADPH-diaphorase staining to search for changes in parabrachial innervation of the lateral geniculate nucleus in monocularly lid-sutured cats, NADPH-diaphorase among parabrachial axons seemed quite normal. Instead, we obtained a string of completely unexpected results. We found that monocular lid suture caused NADPH-diaphorase labeling to be found within geniculate relay cells, mostly of Y cells, and such cellular labeling was not seen in normally reared cats. Furthermore, these abnormally labeled Y cells were found mostly in nondeprived laminae.

## MATERIALS AND METHODS

### Subjects

Our observations are based on a total of 13 adult cats. For all procedures, the NIH guidelines for the use of animals in research were assiduously followed. Six were normally reared controls used also for a previous study of parabrachial innervation of the lateral geniculate nucleus (Bickford et al., 1993). The remaining seven were reared with monocular lid suture from the first postnatal week (the time of normal eye opening) for varying periods from 8 months to 10 years. We induced monocular lid suture via suture of the lids of one eye in kittens that were anesthetized with halothane. At daily intervals, the animals were inspected for possible infection of the sutured lids. Such infections were rare and, when noticed, were effectively treated with ophthalmic antibiotics. The eyelids remained closed until sacrifice. In all of the normal and the monocularly lid-sutured cats, we performed histochemistry to reveal NADPH-diaphorase reactivity in the lateral geniculate nucleus. In five of the monocularly lid-sutured cats, we also applied immunocytochemical techniques to reveal cellular labeling for CAT-301 (which labels relay Y cells; see Hockfield et al., 1983; Hendry et al., 1984; Sur et al., 1988; Guimarães et al., 1990; Hockfield and Sur, 1990) or GABA (which labels interneurons; see Fitzpatrick et al., 1984; Montero and Zempel, 1985; Montero, 1986). Finally, for two of the above mentioned five monocularly lid-sutured cats, we also used retrograde transport of biocytin injected into visual cortex bilaterally to label relay cells.

### Retrograde tracer injections

We deeply anesthetized each of two monocularly lid-sutured cats with an intravenous injection of 15 mg/kg of sodium pentobarbital. The state of anesthesia was maintained with 5–10 mg supplements of the barbiturate every 2 hours throughout the entire 24-hour period needed for intracranial injection and transport of tracer. We placed each cat in a stereotaxic apparatus and prepared it for aseptic surgery. Its heart rate was monitored, and its body temperature was maintained at 37°C with a feedback-controlled electric blanket. We infused all wounds and pressure points with lidocaine. A craniotomy was performed bilaterally over the visual cortex and the dura reflected on both sides. We then made several insertions of a Hamilton syringe into the white matter below the visual cortex with the intent of severing axons there to permit retrograde transport of the biocytin. We have found (unpublished observations; see Izzo, 1991) that transport of biocytin is essentially only orthograde unless axons of passage are severed, in which case, excellent retrograde labeling is achieved. We intended to sever corticogeniculate axons from at least areas 17, 18, and 19. During the last penetration of the syringe, we injected 0.5 μl of a 5.0% solution of biocytin in saline into the white matter. After 24 hours, we perfused the animals as described below.

### Histochemistry and immunohistochemistry

All animals were deeply anesthetized with an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline followed by a fixative solution of 4.0% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate buffer (PB). After the perfusions, we removed the brains and placed them in the same fixatives for 6 hours. We then immersed the brains in a 30% sucrose solution in 0.1 M PB at 4°C. After sinking in this solution, the brains were cut on a freezing microtome into 70-μm-thick sections.

***NADPH-diaphorase.*** We rinsed the sections in 0.1 M Tris buffer for 30 minutes. We then incubated them for 15 minutes to 2 hours at 37°C with 0.1% NADPH, 0.025% nitro blue tetrazolium, and 0.2% Triton-X in 0.1 M Tris buffer at pH 7.1. The sections were subsequently mounted, air dried, and briefly dehydrated. Some of these sections treated for NADPH-diaphorase labeling were also processed for labeling of biocytin, GABA, or CAT-301 (see below). For experiments in which only NADPH-diaphorase labeling was investigated, we coverslipped the sections using DPX (Aldrich) as the mounting medium. For experiments in which NADPH-diaphorase labeling was combined with fluorescent labeling (see below), we coverslipped the sections using Vectashield (Vector Laboratories, Burlingame, CA) as the mounting medium.

***Biocytin.*** In the two experiments designed to label relay cells retrogradely, we incubated sections through the lateral geniculate nucleus in solutions to permit visualization of the biocytin. For sections in which only biocytin was to be revealed, they were incubated overnight at 4°C in a solution containing avidin and biotinylated horseradish peroxidase (HRP; Vector) in a 1:100 dilution, 1% normal rabbit serum in phosphate-buffered saline (PBS; 0.01 M PB and 1.8% NaCl), and 0.3% Triton-X. The next day, we rinsed the sections in PBS at room temperature and reacted them with a nickel-intensified, diaminobenzidine (DAB) solution (0.2% beta D-glucose, 0.04% ammonium chloride, 0.002% glucose oxidase, 1.5% nickel ammonium sulfate, and 0.05% DAB in 0.05 M sodium acetate, pH 6.0).

We also looked for cells that contained NADPH-diaphorase and were also retrogradely labeled with biocytin. For such double labeling of single cells, the sections containing biocytin were incubated with 1% avidin-D-fluorescein (Vec-

tor) in PBS overnight at 4°C. The next day, the sections were rinsed and processed for NADPH-diaphorase as described above.

*GABA.* We incubated sections through the lateral geniculate nucleus in which only GABA was to be revealed in 10% normal goat serum in PBS for 30 minutes and then placed overnight at 4°C into a solution containing 1% normal goat serum, 0.3% Triton-X, and a 1:1,000 dilution of a polyclonal anti-GABA antibody (Sigma, St. Louis, MO; made in rabbit). We then rinsed the sections in PBS and placed them for 1 hour at room temperature in a 1:100 dilution of biotinylated goat anti-rabbit antibody (Vector) in PBS containing 1% normal goat serum. We finally rinsed the sections in PBS and incubated them for 1 hour at room temperature with a 1:200 solution of avidin and biotinylated HRP in PBS followed by visualization of the anti-GABA antibody with DAB.

In some experiments, we searched for cells labeled both for GABA and NADPH-diaphorase. For such double labeling, we followed the steps described in the preceding paragraph up to the point of exposing the sections to the biotinylated goat anti-rabbit serum. We then rinsed the sections in PBS and transferred them into a 1:100 dilution of avidin-D-fluorescein in PBS for 1 hour. The sections were rinsed and reacted for NADPH-diaphorase as described above.

*CAT-301.* We rinsed the geniculate sections in PBS, incubated them in 10% normal mouse serum for 30 minutes, and then transferred them for overnight incubation at 4°C into an undiluted tissue culture medium containing CAT-301 (provided by Dr. Susan Hockfield). We then rinsed the sections in PBS and placed them for 1 hour at room temperature in a 1:50 solution of biotinylated rabbit anti-mouse antibody (Vector) in PBS with 1% normal mouse serum. After rinsing in PBS, we transferred the sections into a 1:100 dilution of avidin-D-fluorescein in PBS for 1 hour. The sections were rinsed and reacted for NADPH-diaphorase as described above.

# RESULTS

## Lateral geniculate nucleus of normal cats

We have previously published a detailed study of the pattern of NADPH-diaphorase staining in normally reared cats (Bickford et al., 1993) and shall briefly summarize the findings to serve as a framework for this staining in monocularly lid-sutured cats. We found dense labeling of axons and terminals throughout the lateral geniculate nucleus. We were able to show that this labeling represents cholinergic axons and terminals from the parabrachial region of the brainstem (Bickford et al., 1993). Figure 1a shows an example of this labeling. The labeled axons were of fine caliber with many en passant swellings or terminals (Fig. 1c). Labeled axons and terminals were most dense in the A-laminae and less dense in the C-laminae, in the interlaminar zones, and in the medial interlaminar nucleus. This difference of labeling density provided us with a convenient means to identify the main regions of the lateral geniculate nucleus, including the borders of the A-laminae.

In each of the six normal cats, virtually all of the lateral geniculate nucleus, including the A-laminae and medial interlaminar nucleus, were completely devoid of cells labeled for NADPH-diaphorase. However, we noted a region in the ventral C-laminae near the border with the optic tract in which we found a small number of NADPH-diaphorase positive cells with small somata. Figure 1d shows an example of such cells. We excluded these NADPH-diaphorase-positive cells from further analysis, because, in our comparison with monocularly lid-sutured cats, we concentrated on the A-laminae, where NADPH-diaphorase-positive cells were never found. Nevertheless, during the NADPH-diaphorase reaction, these cells, along with the labeled axons and terminals of the A-laminae, served as a control for the thoroughness and completion of tissue processing for both normal and monocularly lid-sutured cats.

## Lateral geniculate nucleus of monocularly lid-sutured cats

***Axon and terminal labeling.*** As can be seen in Figure 2a, NADPH-diaphorase staining of the geniculate neuropil in the monocularly lid-sutured cat showed no evidence of abnormality. For instance, the density of axon and terminal staining was not obviously different between the deprived and nondeprived A-laminae. Thus we conclude that early monocular lid suture does not qualitatively affect parabrachial innervation of the lateral geniculate nucleus.

***Cell labeling.*** Although the answer to our initial question regarding parabrachial inputs seems clear, we encountered a wholly unanticipated finding. We encountered many cells stained positively for NADPH-diaphorase in the lateral geniculate nucleus following early monocular lid suture. Figure 2b,c shows examples of this labeling. As is documented more thoroughly below, these labeled cells occur more commonly in nondeprived laminae in all regions of the nucleus. Here, they have large somata, display moderate staining in the cytoplasm, and have unstained nuclei (see Fig. 2c). Because there were no cells stained positively for NADPH-diaphorase in the A-laminae or medial interlaminar nucleus of the normal lateral geniculate nucleus, we considered the appearance of these NADPH-diaphorase-positive geniculate cells in the monocularly lid-sutured cat to be an abnormality induced by this visual deprivation.

Figure 3 illustrates the distribution of NADPH-diaphorase-positive cells in the lateral geniculate nucleus. Figure 3A serves as a reminder that few if any such geniculate cells are found in normal cats despite their common occurrence in monocularly lid-sutured cats (Fig. 3B). In the monocularly lid-sutured cats, cells positive for NADPH-diaphorase were found throughout the rostrocaudal and mediolateral extents of the lateral geniculate nucleus.

As noted above, most of the NADPH-diaphorase-labeled cells were in geniculate laminae innervated from the normal eye (i.e., nondeprived laminae), even though some were clearly found in deprived laminae. We counted cells stained for NADPH-diaphorase in matched regions of the binocular segment of laminae A and A1 in both deprived and nondeprived laminae. We measured four of these regions in each of three cats, and each region was 0.4 $mm^2$ in area. For nondeprived laminae, lamina A had 20 labeled cells per $mm^2$, and lamina A1 had 27 cells per $mm^2$; for deprived laminae, lamina A had 2.1 labeled cells per $mm^2$, and lamina A1 had 1.7 cells per $mm^2$. The differences in density of labeled cells seen between laminae A and A1 when both are nondeprived or deprived are not statistically significant ($P > 0.1$ on a Mann-Whitney U test). However, in every case, nondeprived lamina A or A1 had more NADPH-diaphorase-labeled cells than did its deprived counterpart ($P < 0.05$ on a Mann-Whitney U test). We emphasize the

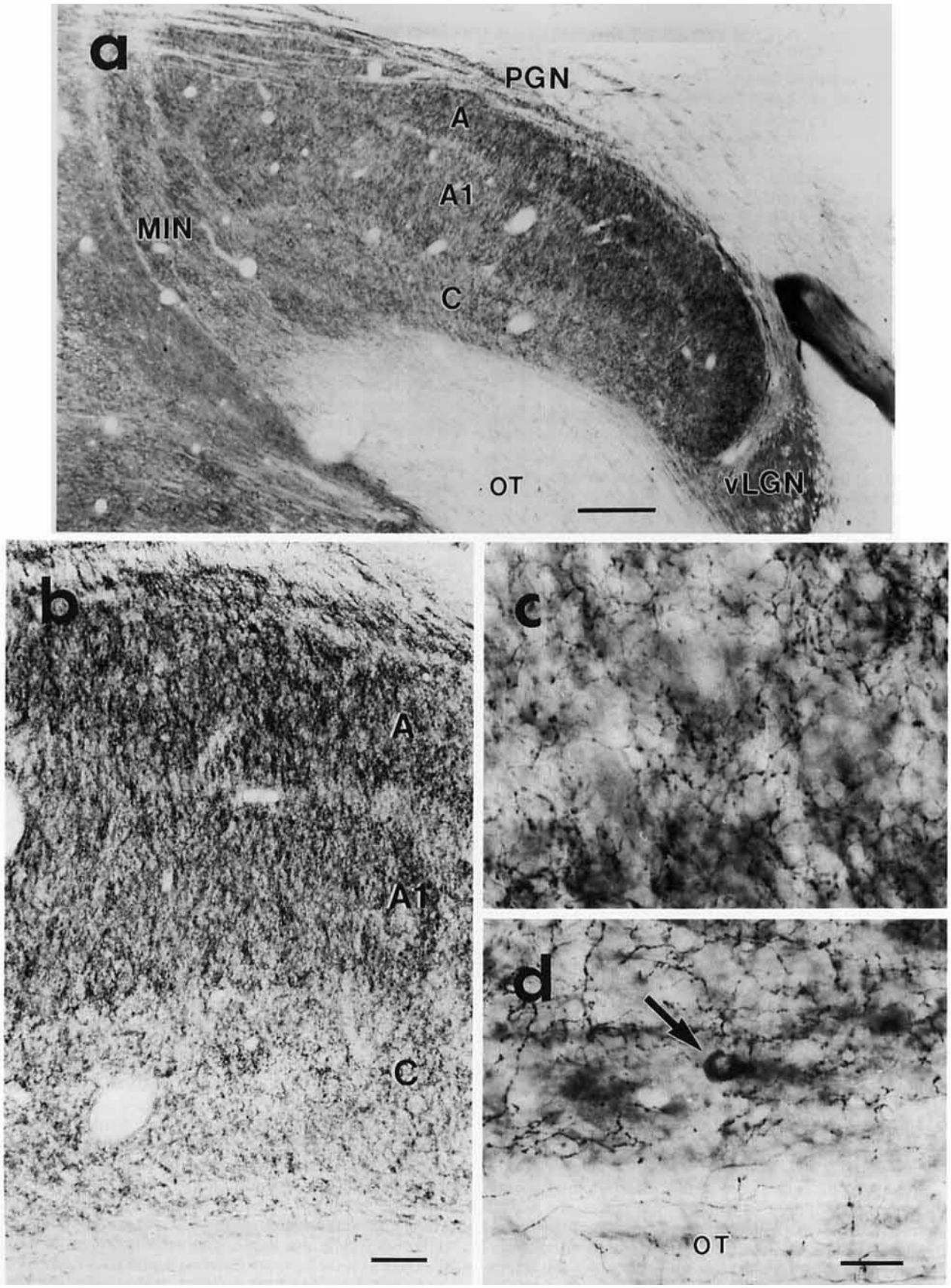


Fig. 1. NADPH-diaphorase staining in a coronal section through the right lateral geniculate and perigeniculate nuclei of a normally reared cat. **a:** Low-power view. **b:** Medium-power view of the A- and C-laminae. Note that the axons and terminals labeled with NADPH-diaphorase are denser in the A-laminae than in either the C-laminae or the interlaminar regions. **c,d:** High-power view of lamina A (c) and lamina C (d) showing NADPH-diaphorase positive axons and terminal swellings. The arrow in d points to a NADPH-diaphorase-positive cell found adjacent to the optic tract. See text for details. A, lamina A; A1, lamina A1; C, C-laminae; MIN, medial interlaminar nucleus; PGN, perigeniculate nucleus; OT, optic tract; vLGN, ventral division of the lateral geniculate nucleus. Scale bars = 500 μm in a, 125 μm in b, 25 μm in c,d.

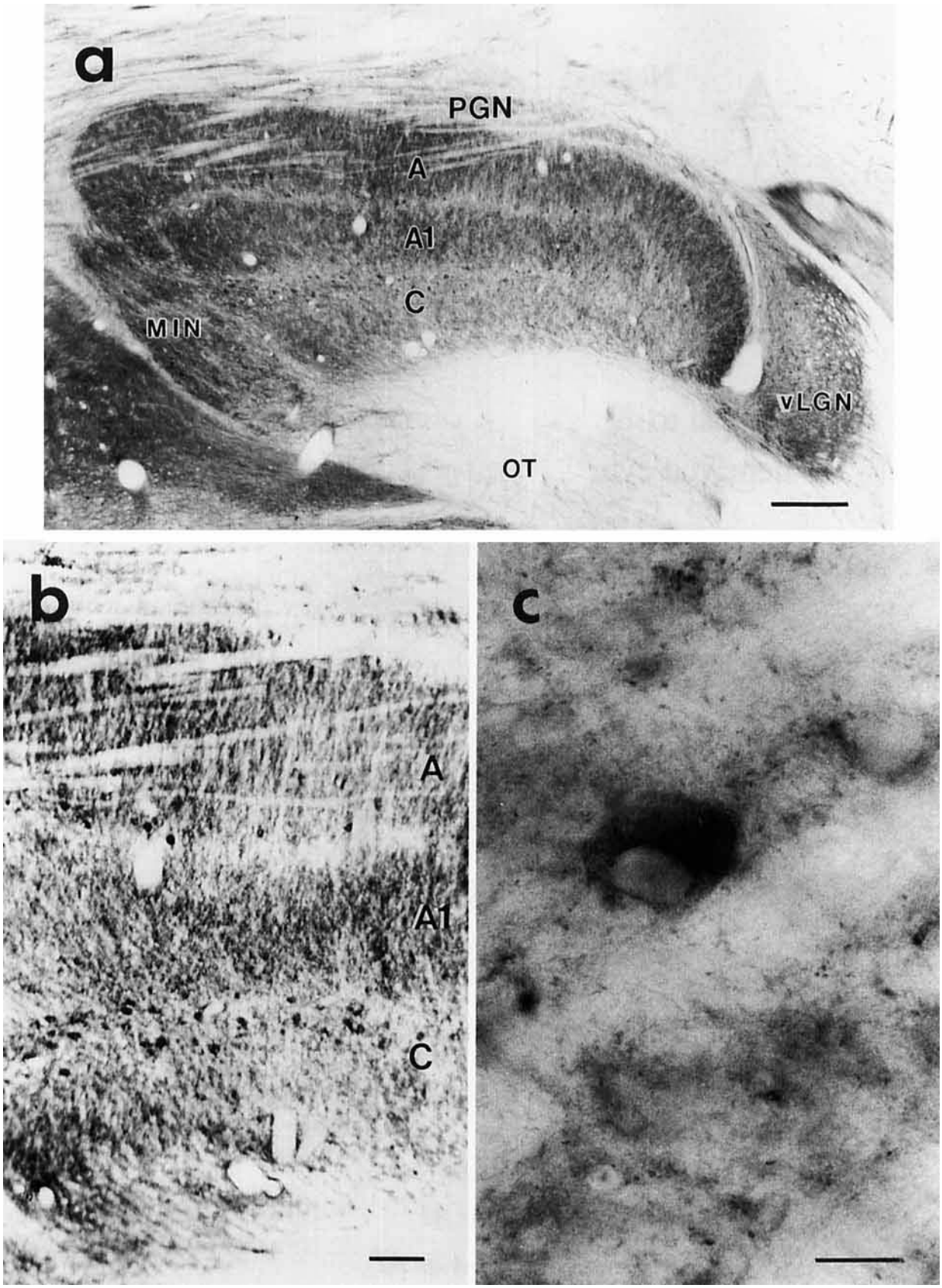


Fig. 2. NADPH-diaphorase staining in a coronal section through the right lateral geniculate and perigeniculate nuclei of a cat reared with monocular lid suture of the right eye (compare with Fig. 1). **a:** Low-power view. Note the shrunken size of the deprived lamina A1. **b:** Medium-power view showing somata stained positively for NADPH-diaphorase. **c:** High-power view of a cell in nondeprived lamina A stained positively for NADPH-diaphorase. Scale bars = 500 μm in a, 125 μm in b, 25 μm in c.

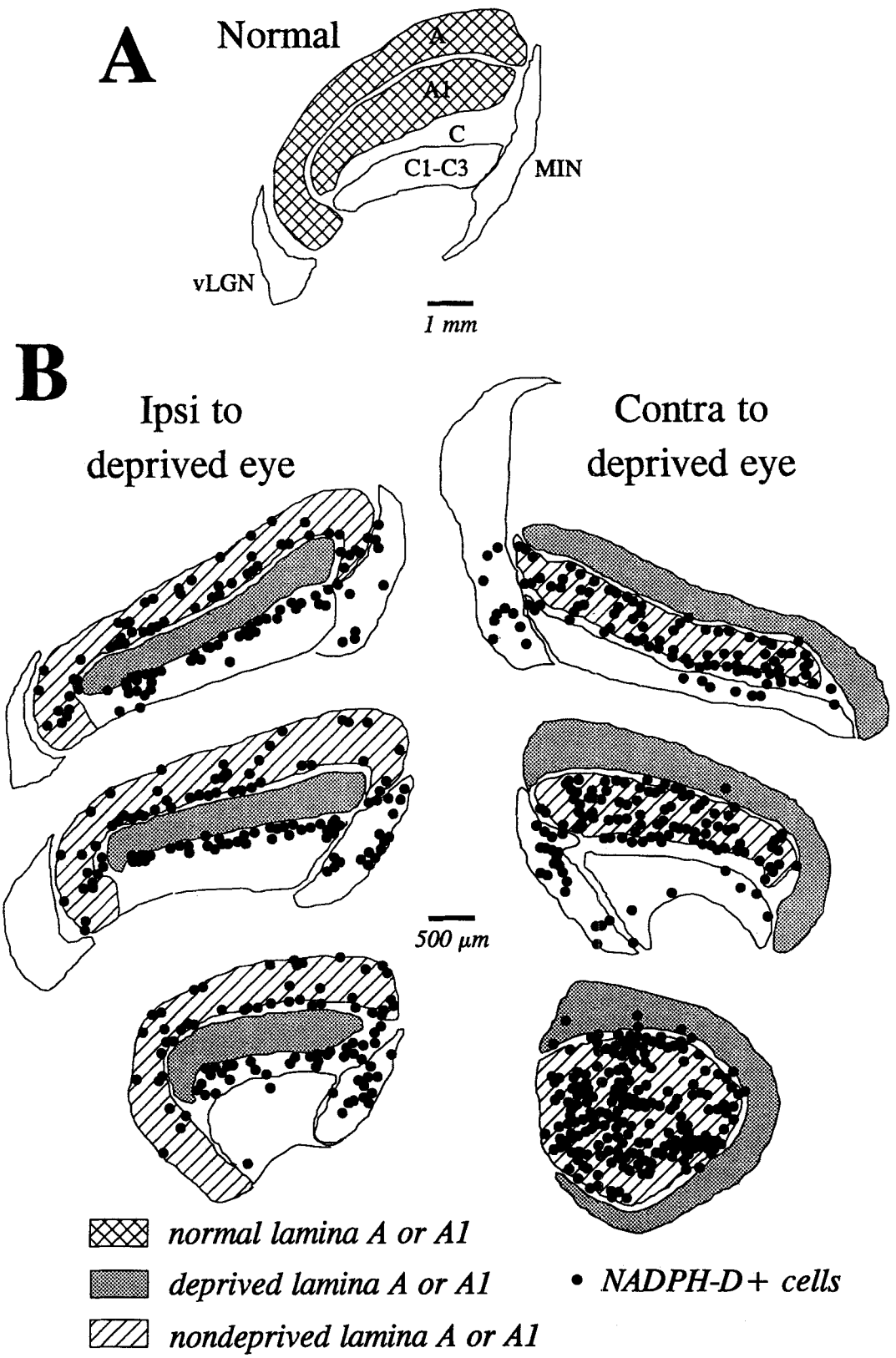


Fig. 3. The distribution of NADPH-positive cells in the lateral geniculate nucleus of normal and monocularly lid-sutured cats. Each filled dot represents a labeled cell. **A:** NADPH-diaphorase labeling in a normal lateral geniculate nucleus. No labeled cells were found in the A-laminae. **B:** Rostrocaudal series from a monocularly lid-sutured cat through the lateral geniculate nucleus ipsilateral (**left**) and contralateral (**right**) to the lid-sutured eye. Many cells positive for NADPH-diaphorase are found, especially in the nondeprived laminae. A, lamina A; A1, lamina A1; C and C1-C3, C-laminae; MIN, medial interlaminar nucleus; vLGN, ventral division of the lateral geniculate nucleus.

relative novelty of this observation (see also Discussion); heretofore, most effects of visual deprivation, especially those related to enzymatic or metabolic activity, have emphasized the deprived laminae as the site of abnormalities (Wong-Riley, 1979; Kageyama and Wong-Riley, 1986; Sur et al., 1988). Because we did not use special techniques to label ocular zones (i.e., zones innervated by one or the other retina) in the parvocellular C-laminae and medial interlaminar nucleus, this pattern is most obvious in our data for the A-laminae and magnocellular C-laminae (see Fig. 3B). However, by comparison with published studies of ocular zones in the parvocellular C-laminae and medial interlaminar nucleus (Hickey and Guillery, 1974; Guillery et al., 1980; Lee et al., 1984), the pattern of labeling in these regions is consistent with most NADPH-diaphorase-labeled cells being in nondeprived zones, although we cannot be certain from our material.

***Sizes of NADPH-diaphorase-labeled and other somata***

*NADPH-diaphorase-labeled cells.* Figure 4A,B shows the soma size distributions for geniculate cells labeled in the A-laminae with NADPH-diaphorase. Although it was simple to obtain a large sample of these cells for measurement from nondeprived laminae, we could not do so for deprived laminae, so fewer deprived cells were measured. Two points can be gleaned from Figure 4. First, in nondeprived laminae, the NADPH-diaphorase-labeled cells are generally quite large, averaging 508 ± 135 $\mu m^2$. Second, cells labeled in deprived laminae are smaller, averaging 302 ± 107 $\mu m^2$, and this is significantly smaller than the size distribution for the nondeprived cells ($P < 0.001$ on a Mann-Whitney U test).

*NADPH-diaphorase-labeled cells vs. those of identified relay cells.* To identify relay cells in the A-laminae of monocularly lid-sutured cats, we injected biocytin into cortical areas 17 and 18 to label these cells retrogradely. Figure 5 shows the resultant labeling, and it is clear that relay cells in nondeprived laminae are larger than are their deprived counterparts, confirming earlier observations (Wiesel and Hubel, 1963; Guillery and Stelzner, 1970; Guillery, 1972). This is due in roughly equal parts to abnormally small cells in deprived laminae and to hypertrophy of nondeprived cells (Guillery, 1973; Hickey et al., 1977).

Figure 6 displays soma sizes of relay cells and compares them with NADPH-diaphorase-labeled cells in the nondeprived and deprived A-laminae. In this analysis, the comparison is made between sections treated only for retrograde labeling and adjacent sections treated only for NADPH-diaphorase. Retrogradely labeled relay cells in nondeprived laminae are significantly larger than are those in deprived laminae (301 $\mu m^2$ ± 105 $\mu m^2$ for nondeprived vs. 189 $\mu m^2$ ± 50 $\mu m^2$ for deprived; $P < 0.001$ on a Mann-Whitney U test). Note also that, in both nondeprived and deprived laminae, soma sizes of NADPH-diaphorase-labeled cells mostly overlap the upper end of those of the largest relay cells. In both nondeprived and deprived laminae, NADPH-diaphorase-labeled cells are larger on average than are retrogradely labeled cells ($P < 0.001$ on a Mann-Whitney U test for each comparison). In other sections treated for both retrograde labeling and NADPH-diaphorase, many of the cells positive for NADPH-diaphorase also were retrogradely labeled with biocytin (see below). This suggests not only that these NADPH-diaphorase labeled cells are relay cells but also that most or all are relay Y cells, because the relay X cells are smaller (LeVay and Ferster, 1977; Friedlander et al., 1981; Stanford et al., 1983).

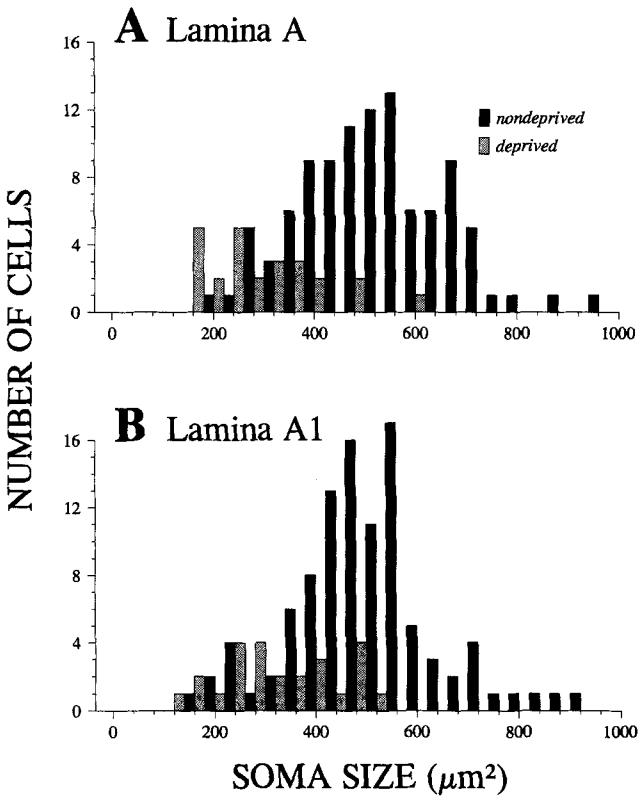


Fig. 4. Soma size frequency histograms of cells positive for NADPH-diaphorase. The measurements were made from one monocularly lid-sutured cat, using three sections in both hemispheres. **A:** Nondeprived and deprived lamina A. **B:** Nondeprived and deprived lamina A1.

*NADPH-diaphorase-labeled somata vs. those of CAT-301-positive cells and interneurons.* In a further attempt to identify the nature of the NADPH-diaphorase labeling, we used two other markers of geniculate neurons. The first marker is an antibody directed against GABA that selectively labels interneurons (Fitzpatrick et al., 1984; Montero and Zempel, 1985; Montero, 1986). Figure 7 shows the pattern of geniculate interneurons labeled in a monocularly deprived cat. As has been previously reported (Robson and Martin-Elkins, 1985), we found no evidence of effects of the visual deprivation on either the numbers or sizes of interneurons. The second marker is CAT-301, which is a monoclonal antibody directed against a surface antigen found on relay Y cells (Hockfield et al., 1983; Hendry et al., 1984; Sur et al., 1988; Guimarães et al., 1990; Hockfield and Sur, 1990). Figure 8 shows the CAT-301 staining pattern of geniculate cells in a monocularly lid-sutured cat. This staining does outline the reactive cells as expected for an antibody directed against a surface antigen. In agreement with previous findings (Sur et al., 1988; Guimarães et al., 1990), the number of cells stained for CAT-301 in the deprived laminae was significantly reduced in number and size when compared with those in the nondeprived laminae, and this is thought to reflect the relatively selective effect of early monocular lid suture on geniculate Y cells (Sherman et al., 1972, 1975; Sherman and Spear, 1982; Friedlander et al., 1982; Friedlander and Stanford, 1984).

Figure 9 quantitatively compares the soma size distributions of cells labeled for CAT-301, NADPH-diaphorase, and

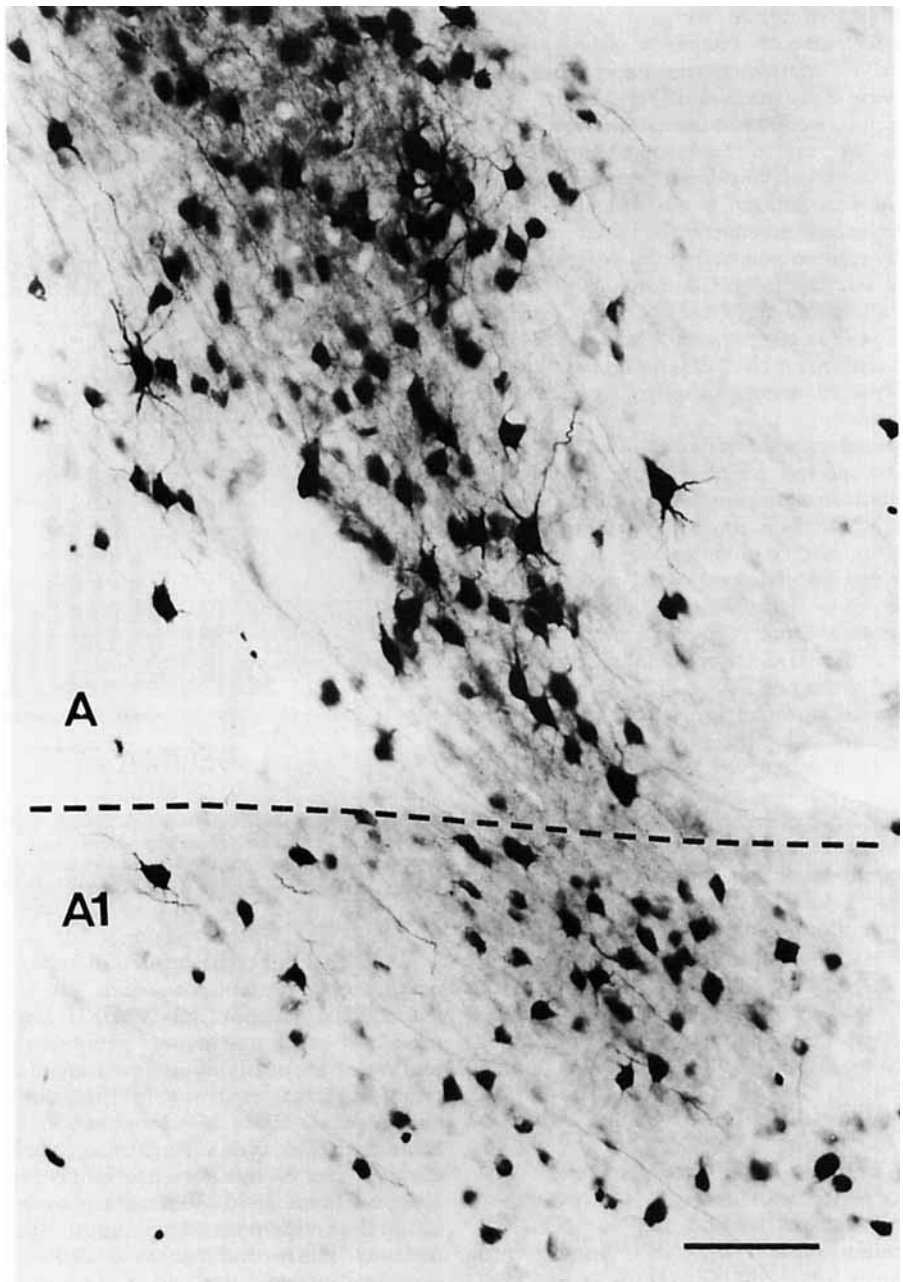


Fig. 5. Coronal section through the lateral geniculate nucleus of a monocularly lid-sutured cat showing relay cells labeled retrogradely from an injection of biocytin into the visual cortex. Note the larger size of labeled cells in nondeprived lamina A compared to those in deprived lamina A1. Scale bar = 100 μm.

GABA. Because there were very few cells labeled for either CAT-301 or NADPH-diaphorase in the deprived A-laminae, we limited our analysis to the nondeprived A-laminae. We used the CAT-301 staining as the marker for the nondeprived Y cells in the lateral geniculate nucleus of monocularly lid-sutured cats. The similar size distributions for the CAT-301 and NADPH-diaphorase labeling (Fig. 9A,B) is consistent with the possibility that these two cell populations largely overlap. Also, because there is very little overlap in size distributions between the NADPH-diaphorase-labeled cells and those labeled by the anti-GABA antibody (Fig. 9B,C), we conclude that few if any of the NADPH-diaphorase-labeled cells are interneurons.

***Double labeling with NADPH-diaphorase and CAT-301.*** Although the above data indirectly support the conclusion that the NADPH-diaphorase-labeled cells in the nondeprived A-laminae are relay Y cells, we sought more direct evidence for this by double labeling these nondeprived cells with both NADPH-diaphorase and CAT-301. This double labeling was relatively easy to recognize, because the fluorescent CAT-301 label, which labeled the cell surface, brightly outlined the cell, whereas NADPH-diaphorase labeled the

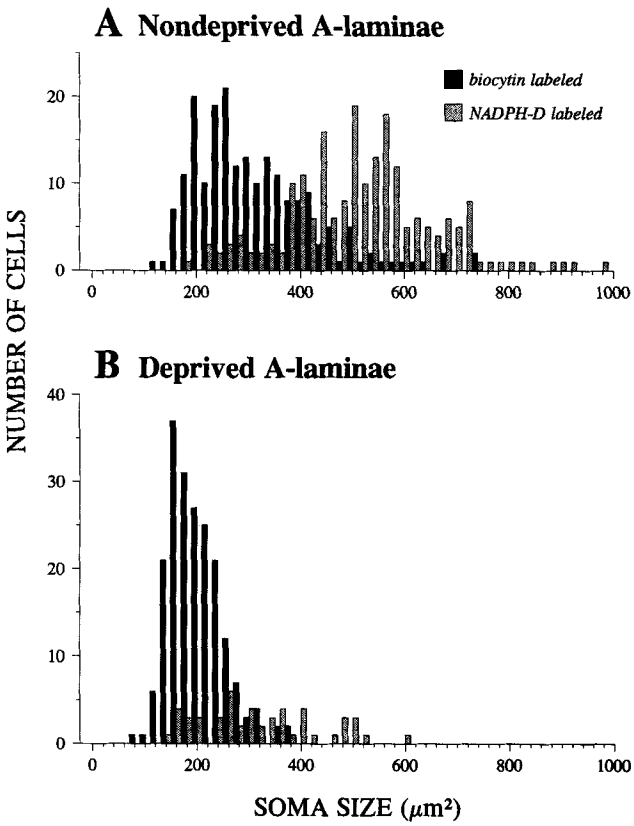


Fig. 6. Histograms showing soma size distributions of cells labeled retrogradely with biocytin and cells stained for NADPH-diaphorase. The measurements were made from four sections though each hemisphere, two each for biocytin labeling and NADPH-diaphorase labeling. **A:** Cells in the nondeprived A laminae. **B:** Cells in the deprived A laminae.

cytoplasm a dark blue. Figure 10 demonstrates an example of these double-labeled cells.

Quantitative analysis of this material revealed that 82% of the NADPH-diaphorase-labeled cells in the nondeprived A-laminae also stained for CAT-301. Because the CAT-301 antibody has a restricted penetration into brain sections, this percentage is probably an underestimate. Therefore, most, if not all, of these NADPH-diaphorase-labeled cells also stain for CAT-301, marking them as relay Y cells. However, an analysis of nondeprived cells stained for CAT-301 revealed that only 55% also labeled for NADPH-diaphorase. This suggests that only some of the Y cells in the nondeprived A-laminae exhibit NADPH-diaphorase labeling as a result of early monocular lid suture.

Although this is not illustrated here, we found that geniculate cells double labeled for NADPH-diaphorase and biocytin retrogradely transported from cortex were common. Also, we found no cell that was double labeled for NADPH-diaphorase and GABA. These observations further support the relay cell identity of the NADPH-diaphorase labeled cells. Finally, we found cells double labeled for NADPH-diaphorase and CAT-301 in the deprived A-laminae, but these were too few and scattered to permit quantitative evaluation.

***Role of binocular competition in NADPH-diaphorase labeling.*** We have shown that early monocular lid suture causes abnormal NADPH-diaphorase labeling of cells in the lateral geniculate nucleus, mostly in nondeprived laminae. In order to investigate the possible mechanisms for this feature, we tested the hypothesis that this abnormality results from unbalanced binocular competition (Guillery and Stelzner, 1970; Guillery, 1972; Sherman et al., 1972, 1975; Sherman and Spear, 1982). The rationale for this is as follows, and it is illustrated schematically in Figure 11. During early development, relay cells appear to compete with one another for synaptic contacts in cortex, and monocular lid suture upsets the competitive balance by conferring an advantage to neurons associated with the nondeprived eye. As a result, geniculate relay cells in monocularly lid-sutured cats hypertrophy in nondeprived laminae, and in deprived laminae they fail to grow to normal size; these effects seem most pronounced for Y cells (Sherman et al., 1972, 1975; Guillery, 1973, Hickey et al., 1977; Lehmkuhle et al., 1980; Friedlander et al., 1982; Friedlander and Stanford, 1984; Sur et al., 1988; Guimarães et al., 1990). The best evidence that these phenomena result from unbalanced binocular competition derive from comparisons of the binocular and monocular segments of lamina A. The binocular segment maps the more central, binocularly viewed region of visual field and thus receives inputs from both eyes: Only here can binocular interactions occur. The monocular segment maps the more peripheral, monocularly viewed sector of visual field, and only receives innervation from the contralateral eye: by definition, deprived cells here cannot suffer from a binocularly competitive disadvantage (see Fig. 11). Many authors have reported that developmental anomalies seen in monocularly deprived cats, including geniculate Y cell and soma size abnormalities, are limited to the deprived binocular segment (Guillery and Stelzner, 1970; Guillery, 1972; Sherman et al., 1972, 1975; Sur et al., 1988).

We looked for the potential role of unbalanced binocular competition in inducing NADPH-diaphorase labeling of nondeprived geniculate cells by estimating the relative numbers of these labeled cells in each of four separate areas: binocular and monocular segments of nondeprived and deprived lamina A. To minimize sampling biases, we chose sections from the anteroposterior middle of the lateral geniculate nucleus and selected areas in matched regions of lamina A. We counted every labeled cell in a 0.4 $mm^2$ area centered on each region. From these counts, we computed four different ratios of the numbers of NADPH-diaphorase-labeled cells; monocular to binocular segment in both deprived and nondeprived lamina A and deprived to nondeprived lamina A for both the binocular and monocular segment.

Figure 12 summarizes this analysis, and its main features can be predicted from Figure 3. As shown, we found no difference in the extent of NADPH-diaphorase labeling between the monocular and binocular segments, and this applies to both deprived and nondeprived lamina A ($P > 0.1$ on a two-way analysis of variance for each comparison). As noted above, if this labeling were due to abnormal binocular competition, significantly more labeled cells would be expected in the binocular segment of nondeprived lamina A. Furthermore, the nondeprived monocular segment contains many more NADPH-diaphorase-labeled cells than does its deprived counterpart ($P < 0.001$ on a two-way analysis), and, again, the hypothesis that binocular compe-

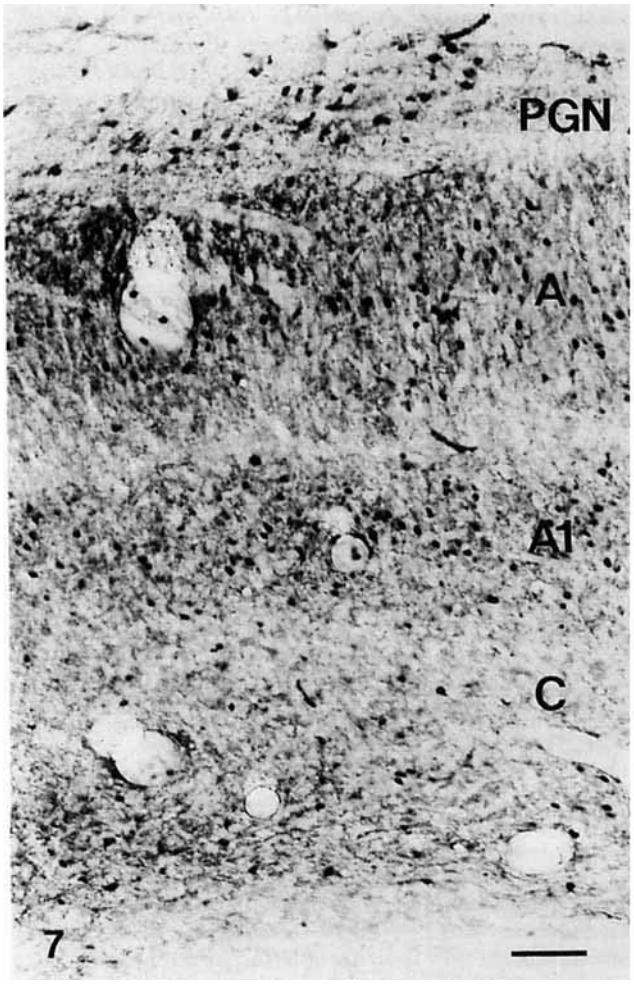


Fig. 7. Coronal section through the lateral geniculate nucleus of a monocularly lid-sutured cat showing interneurons reacted for GABA. The interneurons are distributed evenly throughout nondeprived lamina A, deprived lamina A1, and nondeprived lamina C. Perigeniculate cells stained positively for GABA are also visible dorsal to lamina A. A, lamina A; A1, lamina A1; C, lamina C; PGN, perigeniculate nucleus. Scale bar = 125 μm.

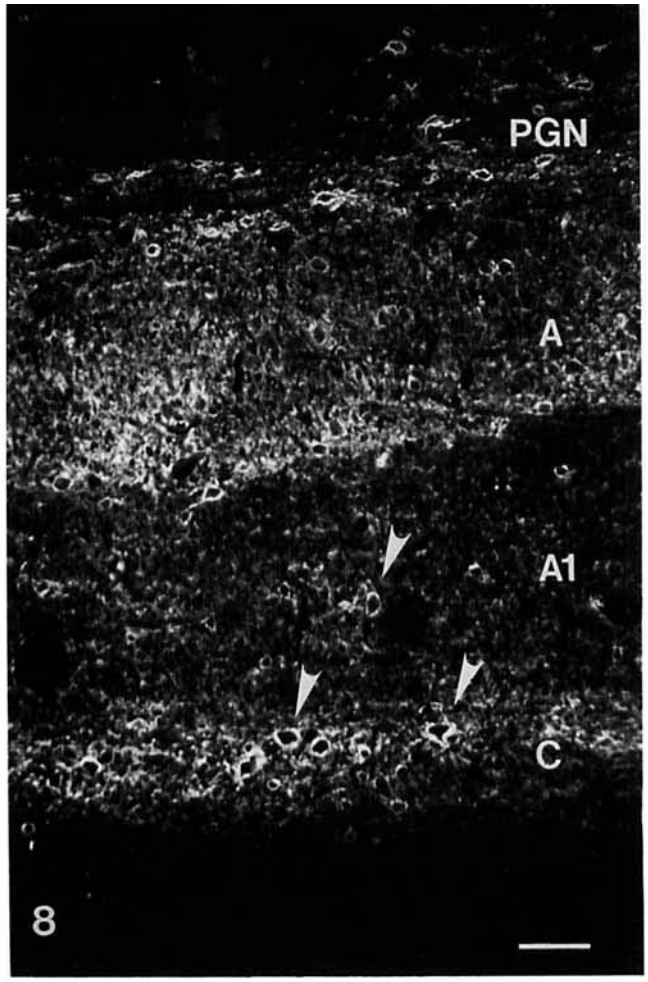


Fig. 8. Coronal section through the lateral geniculate nucleus of a monocularly lid-sutured cat showing cells labeled for CAT-301 tagged with fluorescein. Arrowheads indicate labeled cells. Lamina A1 is deprived; laminae A and C are nondeprived. A, lamina A; A1, lamina A1; C, lamina C; PGN, perigeniculate nucleus. Scale bar = 125 μm.

tition played a role in this would require that the nondeprived and deprived monocular segments were more similar.

## DISCUSSION

The initial goal of our experiments was to investigate the changes in the brainstem input from the parabrachial region to the lateral geniculate nucleus after monocular lid suture. In normal cats, this input stains positively for NADPH-diaphorase, and it is the only source of this staining in the lateral geniculate nucleus (Bickford et al., 1993). NADPH-diaphorase staining is highly correlated to nitric oxide synthase activity (Bredt et al., 1991; Dawson, et al., 1991; Hope et al., 1991), and its presence suggests that nitric oxide is available as a neurotransmitter or neuromodulator (Bredt et al., 1990). Qualitative evaluation of NADPH-diaphorase staining in geniculate laminae after monocular lid suture did not reveal any changes in the parabrachial input. However, we encountered a surprising phenomenon: Monocular lid suture led to the appearance of NADPH-diaphorase-positive cells in geniculate laminae, and such cellular staining was not seen in normally reared controls. We thus considered the appearance of this staining as an abnormality induced by monocular lid suture.

We determined that these cells in monocularly lid-sutured cats that stained positively for NADPH-diaphorase were located almost exclusively in the nondeprived laminae. We also obtained evidence that this abnormal staining is selective for geniculate cell type. First, we found only relay cells to be affected, because the interneurons did not stain for NADPH-diaphorase. Second, although two classes of relay cell exist in the A-laminae, known as X and Y cells, our data suggest that the effects are largely limited to the Y cells.

### Effect of monocular lid suture on nondeprived cells

***Effects in nondeprived vs. deprived laminae.*** It is well established that monocular lid suture leads to abnormali-

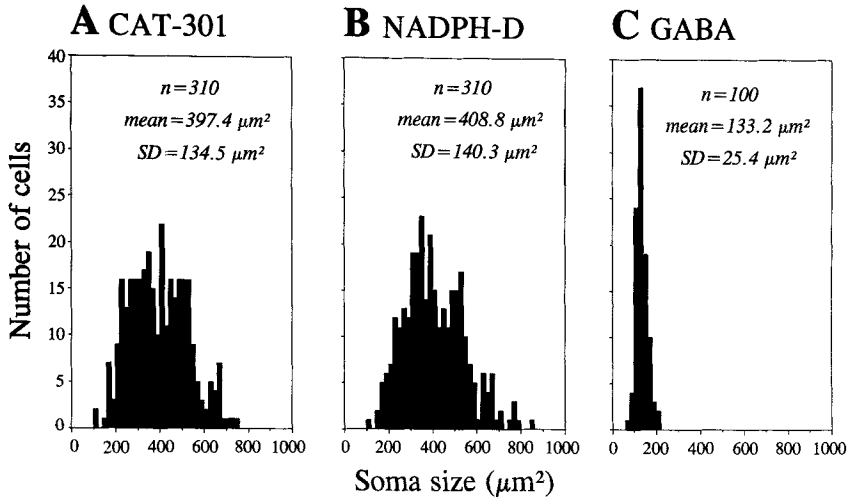


Fig. 9. Soma size frequency histograms of various geniculate cell populations in the nondeprived A-laminae of a monocularly lid-sutured cat. **A:** Cells labeled for CAT-301. **B:** Cells labeled for NADPH-diaphorase. **C:** Cells labeled for GABA.

ties not only of cells in deprived laminae of the lateral geniculate nucleus but also in nondeprived laminae. Effects on deprived cells include abnormally small somata (Wiesel and Hubel, 1963; Guillery and Stelzner, 1970; Guillery, 1972; Hickey et al., 1977), reduction of cytochrome oxidase and CAT-301 reactivity (Wong-Riley, 1979; Kageyama and Wong-Riley, 1986; Sur et al., 1988; Guimarães et al., 1990), reduction in the size of their axonal arbors in cortex (Friedlander et al., 1991), reduction in the number of functional Y cells (Sherman et al., 1972, 1975; LeVay and Ferster, 1977; Friedlander and Stanford, 1984), and abnormal somadendritic morphology (Friedlander et al., 1982). In contrast to the abundant evidence for deprived cell abnormalities, those for nondeprived cells are sparse. There are two previous lines of anatomical evidence describing the effect of monocular lid suture on nondeprived geniculate cells. First, Nissl staining displays a moderate hypertrophy of these cells (Guillery and Stelzner, 1970; Guillery, 1972; Hickey et al., 1977). Second, the cortical axon arbors of nondeprived Y cell axons are expanded, have larger boutons, and make ectopic synapses (Tieman, 1984; Friedlander et al., 1991). The current study reveals a third effect on the nondeprived cells: an abnormal presence of cellular NADPH-diaphorase reactivity.

With the exception of Nissl staining, previous histochemical studies of monocularly deprived cats focused on abnormalities only in deprived geniculate laminae. For instance, both cytochrome oxidase and CAT-301 labeling are reported to be reduced among deprived geniculate cells (Wong-Riley, 1979; Kageyama and Wong-Riley, 1986; Sur et al., 1988; Guimarães et al., 1990). However, in these studies, the control for staining in deprived laminae was staining in nondeprived laminae. In theory at least, this experimental design cannot distinguish among abnormally low reactivity for deprived cells, abnormally high reactivity for nondeprived cells, or both. Because both cytochrome oxidase and CAT-301 staining are found in normal geniculate cells, it is necessary to compare staining in monocularly lid-sutured cats with that in normal cats to distinguish unambiguously among these alternatives. Such a distinction was more readily made with respect to NADPH-diaphorase cellular staining, because comparable staining is not seen in normal cats.

It should be noted that we failed to find NADPH-diaphorase staining in cells of the normal lateral geniculate nucleus, but we cannot conclude from this that NADPH-diaphorase enzyme activity is normally completely absent in those cells. That is, it is possible that low levels of NADPH-diaphorase activity may exist, but our staining protocol is insufficiently sensitive to detect it. Thus we cannot unambiguously distinguish in these experiments between a de novo appearance of NADPH-diaphorase activity among nondeprived geniculate cells and a large increase in such activity from normally low but undetectable levels.

***Effects on nondeprived Y cells.*** Our results also establish that this abnormal NADPH-diaphorase staining in nondeprived laminae is largely limited to Y cells, although it appears that only a subset of these Y cells are affected. This is interesting in the context of many previous studies that suggest most effects in deprived laminae are also limited to Y cells (Sherman et al., 1972, 1975; LeVay and Ferster, 1977; Friedlander et al., 1982; Sherman and Spear, 1982; Friedlander and Stanford, 1984; Kageyama and Wong-Riley, 1986; Sur et al., 1988; Guimarães et al., 1990). This suggests that Y cells, both deprived and nondeprived, are especially sensitive to the effects of visual deprivation.

***Critical period of effect.*** Our results show that monocular lid suture causes abnormally high levels of NADPH-diaphorase activity in cells of nondeprived geniculate laminae, but we have not performed controls to determine whether or not this effect has a critical period associated with it. That is, these effects may be related to other effects of early visual deprivation (for reviews, see Movshon and Van Sluyters, 1981; Sherman and Spear, 1982), or they might result from any extensive period of monocular lid suture, even lid suture initiated after the end of the critical period. In our experiments, we started the lid suture at the time of normal eye opening. It will be important to determine if equal periods of lid suture during adulthood have the same effect on NADPH-diaphorase staining in the lateral geniculate nucleus. One recent study in monkeys reports that monocular enucleation in adults does not lead

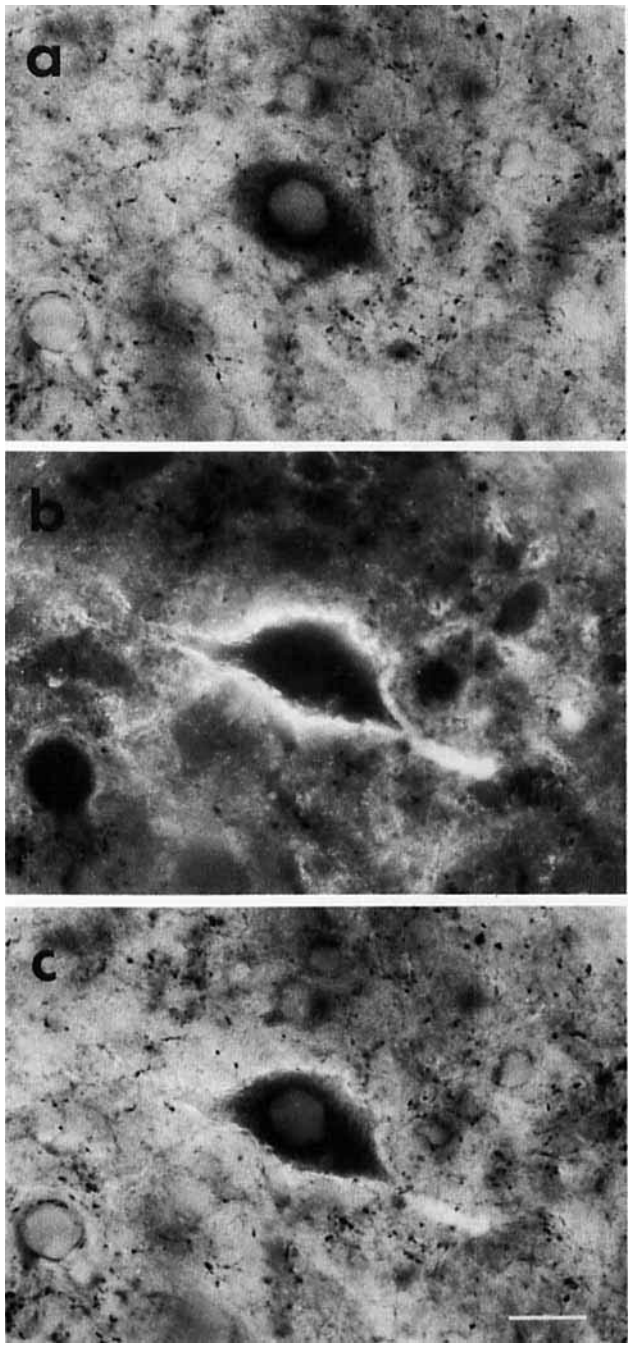


Fig. 10. Cell in nondeprived lamina A double labeled with NADPH-diaphorase and CAT-301. The CAT-301 antibody is tagged with a fluorescent label. **a:** Brightfield view. The NADPH-diaphorase label is clearly visible in the cytoplasm. **b:** Fluorescent view. The CAT-301 label outlines the same cell. **c:** Combined brightfield and fluorescent illumination. Scale bar = 125 μm.

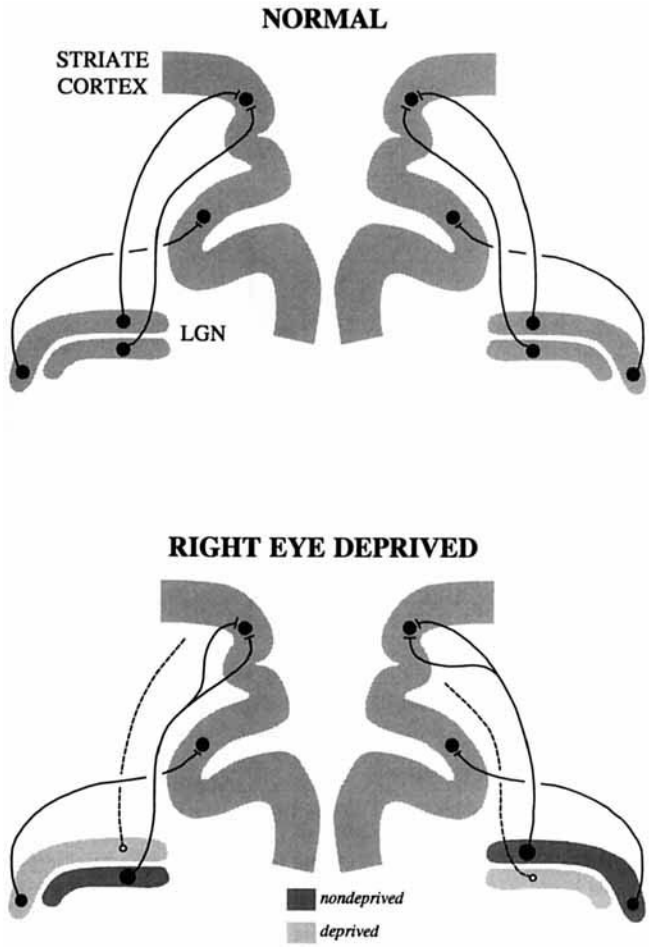


Fig. 11. Schematic depiction of binocular competition during development. Axons from retinotopically matched regions in the binocularly represented region of laminae A and A1 converge to innervate a single cell in striate cortex. In this schema, considerable growth occurs in the number and strength of geniculocortical synapses. During normal development (**top**), a competitive battle ensues for synaptic control of the cortical cell, but, because neither axon has a competitive advantage, a balance is struck, and cortical cells develop with binocular receptive fields. Monocular lid suture (**bottom**), by some as yet unexplained action, places the axons from the deprived lamina at a disadvantage. This results in a complete takeover of the cortical cell by the nondeprived axon. However, by definition, a deprived axon from the monocularly represented region of lamina A cannot be placed at such a competitive disadvantage, because there are no axons representing the nondeprived eye in this region. Thus deprived axons from the monocular segment develop relatively normal connections in cortex. See text for further details.

to NADPH-diaphorase labeling in cells of the lateral geniculate nucleus (Aoki et al., 1993), and this suggests that the effects we observed do have a critical period. However, this must be explicitly tested.

## Increases in NADPH-diaphorase activity

Our results indicate that NADPH-diaphorase, and thus nitric oxide synthase, can be abnormally induced by visual deprivation. Similarly, other studies have found that NADPH-diaphorase and/or nitric oxide synthase activity can be increased or induced de novo by changes to the central nervous system. Several different types of change can effectively elevate NADPH-diaphorase activity. Unilateral injury of the cerebral cortex and hippocampus in rats leads to the de novo appearance of NADPH-diaphorase reactivity in those same structures bilaterally (Regidor et al., 1993). Dehydration causes a large increase in the NADPH-diaphorase reactivity of supraoptic nucleus neurons in rats (Pow, 1992). Transient NADPH-diaphorase staining during development has been reported for geniculate neurons in ferrets (Cramer et al., 1993), dorsal root ganglia neurons (Wetts and Vaughn; 1993), and superior

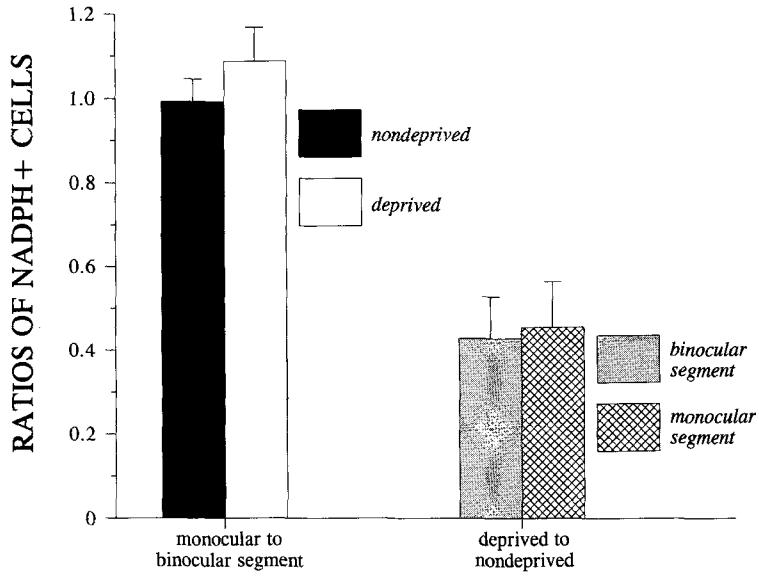


Fig. 12. Comparisons of the numbers of cells that stain positively for NADPH-diaphorase. The analysis was based on three sections from each hemisphere in each of four cats. The ordinate shows the ratios of labeled cells from matched, paired areas as indicated on the abscissa. From left to right, these ratios are: monocular to binocular segment of lamina A for the deprived lamina and the nondeprived lamina and deprived to nondeprived lamina A for its binocular segment and monocular segment.

colliculus cells in rats (Gonzales-Hernández et al., 1993). Finally, an induction of such activity after axotomy has been reported for rat motoneurons, but only during development (Clowry, 1993), indicating a combination of trauma and development in the control of NADPH-diaphorase activity. Thus there seems to be a wide variety of induced changes that can increase the neuronal level of NADPH-diaphorase activity, and the results reported here for monocular lid suture may be viewed in this larger context.

## Possible mechanisms for abnormal NADPH-diaphorase activity after monocular lid suture

Abnormalities found in deprived laminae after monocular lid suture are usually explained by one of two mechanisms (Sherman and Spear, 1982). One is competitive interactions, which include the phenomena related to binocular competition (see Results and Fig. 11). The other is visual deprivation per se, which causes abnormalities independent of any competitive imbalance that may ensue. These general mechanisms may be distinguished from one another on the basis of the distribution of the abnormalities present after monocular lid suture. For instance, abnormalities concentrated in the deprived binocular segment and extending less into the monocular segment suggest binocular competition (see Fig. 11); abnormalities found in both deprived segments suggest deprivation per se. Of these two general mechanisms, binocular competition seems a more likely candidate to affect nondeprived laminae, because, as cells in these laminae win the competition for development of properties such as synaptic contacts in cortex, they might very well hypertrophy and exhibit increased metabolic activity for various substances, including NADPH-diaphorase.

Thus we explicitly tested for the possibility that the increased NADPH-diaphorase labeling seen in nondeprived laminae was due to abnormal binocular competition. Our expectation was that this abnormal increase of NADPH-diaphorase activity would be limited to the binocular segment and not extend into the monocular segment. However, we found this not to be the case (see Fig. 12) and thus rule out binocular competition as a plausible mechanism for these effects. The other general mechanism, deprivation per se, also seems unlikely, because there is no obvious means by which this mechanism should affect cells in nondeprived laminae, which receive normal input from the open eye.

In fact, the distribution of cells labeled for NADPH-diaphorase suggests a third and more global process that is responsible for this effect of monocular lid suture. There seems to be a mechanism that upregulates NADPH-diaphorase fairly extensively for geniculate Y cells innervated by the nondeprived eye. We cannot yet be more specific about this presumptive mechanism, but it seems as if deprivation of one eye causes widespread effects in geniculate relay Y cells innervated by the other eye that cannot be explained by simple competitive mechanisms.

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