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# Quantitative Comparisons of Gamma-Aminobutyric Acid Neurons and Receptors in the Visual Cortex of Normal and Dark-Reared Cats

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ABSTRACT

Physiological studies suggest that the function of the visual cortical gamma-aminobutyric acid (GABA) system is abnormal in cats reared in total darkness. The present study asked whether visual input is necessary for the normal postnatal anatomical development of the GABA system by comparing GABA neurons and receptors in the visual cortex of normal and dark-reared cats. Immunohistochemical techniques (anti-GABA) were used to localize GABA neurons. In both rearing conditions, GABA neurons were stained rather uniformly in all cortical layers. Counts of GABA cells indicated a marked increase in density in dark-reared compared to normal cats. Counts of total cellular density in cresyl-stained sections, however, indicated a comparable increase in dark-reared cats. When corrected for total cellular density, there were no differences between dark-reared and normal cats in the density of GABA cells per layer, or the relative proportion of GABA cells across cortical layers.

In vitro receptor binding of $^3$H-muscimol was used to compare $GABA_A$ receptors in the two rearing conditions. When corrected for total cellular density, saturation kinetics indicated no difference in the total number or affinity of receptors. Similarly, autoradiographic histology indicated no difference in the laminar distribution of receptors across cortical layers between dark-reared and normal cats.

These results indicate that the postnatal development of GABA neurons and receptors occurs normally in the absence of visual input.

**Key words: immunohistochemistry, receptor binding, visual development, visual pathways, visual deprivation**

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the visual cortex. Studies employing microiontophoresis of the GABA antagonist bicuculline have revealed several functions of this inhibition in normal and abnormal visual cortical physiology. In normal adult cats, GABA plays a role in determining orientation and direction tuning (Sillito et al., '75, '77, '79) and ocular dominance (Sillito et al., '80). In cats reared with abnormal monocular experience, GABA plays a role in the resultant abnormalities in visual cortical ocular dominance (Burchfiel and Duffy, '81; Sillito et al., '81; Mower et al., '84).

Studies of cats reared in total darkness provide a means of assessing whether visual input is necessary for normal development of the GABA system. Several aspects of visual cortical physiology in dark-reared cats suggest abnormal function of the GABA system. Visual cortical neurons in dark-reared cats lack the orientation and direction tuning of age-matched normal cats, and in many ways their physiology is more similar to that of newborn cats (Buisseret and Imbert, '76; Mower et al., '81; Mower and Christen, '85). When exposed to the visual environment, however, visual cortical neurons of dark-reared cats recover normal tuning properties (Cynader and Mitchell, '80; Mower et al., '81). Additionally, visual cortex of dark-reared cats shows susceptibility to abnormal monocular experience at much

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older ages than normal cats (Cynader and Mitchell, '80; Mower et al., '81). This capacity for delayed development and plasticity in dark-reared cats suggests that the GABA system is altered or fails to mature in the absence of visual input. Recently, direct microiontophoretic evidence has also suggested abnormal function of the GABA system in dark-reared cats (Tsumoto and Freeman, '87).

The present study asked whether visual input is necessary for the normal anatomical development of the visual cortical GABA system. In normal development, both GABA neurons and receptors show alterations during early postnatal life (Kalil et al., '85; Shaw et al., '85). We used immunohistochemical and in vitro receptor binding techniques to compare quantitatively GABA neurons and receptors in visual cortex of normal and dark-reared cats.

## MATERIALS AND METHODS

### Rearing conditions

Ten cats (five normal, five dark-reared [DR]) were used for quantitative comparisons in this study: six (three normal, three DR) for immunohistochemistry, four (two normal, two DR) for receptor binding. Normal cats were reared in a 12-hour light/dark cycle; DR cats were reared in total darkness from birth. These cats were reared in parallel and were 4–5 months of age at the time of sacrifice. Tissue from several other normal and DR animals was used to optimize conditions for each part of the study (immunohistochemistry, receptor binding) and for qualitative comparisons. Additionally, tissue from a newborn cat (3 days postnatal) was used for immunohistochemistry.

For quantitative analyses of both immunohistochemistry and receptor binding, matched comparisons were done. On a given day, one normal and one DR cat were perfused with the same solutions. Visual cortex from each hemisphere was blocked and all subsequent treatments were done together on tissue from one normal and one DR hemisphere.

### Immunohistochemistry

***Tissue preparation.*** Animals were perfused intracardially under deep barbiturate anesthesia (Nembutal, 50 mg/kg i.v.) with warm (38°C) phosphate-buffered saline (PBS: 0.9% NaCl in 0.1 M phosphate buffer, pH 7.4) followed by 3.8% paraformaldehyde and 0.2% glutaraldehyde in cold (4°C) phosphate buffer (PB: 0.1 M, pH 7.4). The brains were immersed in 30% sucrose in PB until they sank. They were then sectioned coronally on a freezing microtome, collected in PB, and stored at 4°C until processed. For each section processed for immunohistochemistry, an adjacent section was stained with cresyl violet. Properly fixed and stored, tissue could be kept for at least 2 months without degradation of immunolabeling.

***Tissue processing.*** To demonstrate GABA-immunoreactive cells, free-floating sections were incubated in anti-GABA antiserum (ImmunoNuclear, Inc., Stillwater, Minnesota) and processed by the avidin-biotin method of immunoperoxidase staining (Vectastain ABC kit, Vector Laboratories, Burlingame, California; R. Mize, personal communication; Sternberger, '79). In preliminary experiments, we manipulated various incubation parameters (primary antibody concentration, incubation times, blocking serum concentration, etc.) to optimize staining. Our final protocol was as follows:

1. PBS ..... 30 minutes
2. 1% sodium borohydride in PBS ..... 30 minutes
3. Rinse: PBS (7 changes) ..... 45 minutes
4. 10% normal goat serum (NGS) in PBS ..... 20 minutes
5. Primary antibody (anti-GABA 1/5,000 in PBS with 1% NGS and 0.1% Triton X-100) ..... 22 hours
6. Rinse: 5% NGS in PBS (5 changes) ..... 45 minutes
7. Biotinylated antibody and 1% NGS in PBS 30 minutes
8. Rinse: 5% NGS in PBS (5 changes) ..... 45 minutes
9. ABC reagent in PBS ..... 1 hour
10. Rinse: PBS (5 changes) ..... 45 minutes
11. DAB reaction (0.05% diaminobenzidine–4 HCl with 0.01% $H_2O_2$ in PBS) ..... 4–5 minutes
12. Rinse: PBS (4 changes) ..... 10 minutes

The sections were then mounted onto subbed slides, air dried, dehydrated through alcohols and xylene and coverslipped.

Two control procedures were used. In one, sections were incubated as described above, except that the antibody dilutant with no primary antibody was used in step 5. In all cases, this eliminated all specific staining and nearly eliminated background staining. This procedure is a control for the secondary antibody and the DAB reaction. The second control was to incubate sections as described above in primary antibody that had been preabsorbed with several concentrations of free GABA or free bovine serum albumin (the primary antibody was generated against a GABA–bovine serum albumin conjugate). With free GABA at $10^{-4}$ M, staining was markedly reduced; and at $10^{-2}$ M, it was eliminated almost completely. Bovine serum albumin at concentrations as high as 10% did not affect immunostaining. These results lend confidence to the specificity of the antibody for GABA. Additionally, the manufacturer provides data on the absence of cross-reactivity of the antibody with numerous neurotransmitters. Nevertheless, the possibility of antibody cross-reactivity cannot be excluded; therefore, the "GABA-like" immunoreactivity reported here will be referred to as "GABA" with this reservation in mind.

***Cellular density analysis.*** Counts of GABA-positive cells and total cells, using neuronal nuclei as test objects, were used quantitatively to compare normal and DR cats. For a particular hemisphere, these measures were made on an immunostained section and an adjacent cresyl-stained section. For counts of GABA cells, the section was projected at a magnification of ×254 onto a graph paper sheet (1 in = 100 μm). On each section, four randomly spaced 100-μm, wide strips, oriented vertically from surface to white matter in the medial bank of the lateral gyrus, were sampled. Every GABA-positive cell within these strips was marked. Landmarks of the section (surface, white matter, blood vessels) were also drawn on the sheet. The adjacent cresyl-stained section was then projected at the same magnification and laminar boundaries were drawn from the same region of the cortex. These raw data provided the number of GABA cells per 100-μm strip from surface to white matter and the number of GABA cells within the sampled area of each cortical layer. These numbers were converted to GABA cell density (cells/100 × 100 μm) for each cortical layer by dividing the number of GABA cells in a particular layer by the area of that layer that was sampled. This statistic aided comparison of GABA cell density with total cell density, as determined in the adjacent cresyl-stained sections (see below). Finally, the weighted means of these laminar densities (by the relative area of each layer) were used to estimate the average density of GABA cells through the total cortical thickness. Each of these measures was generated for each sampled strip and the mean was used as

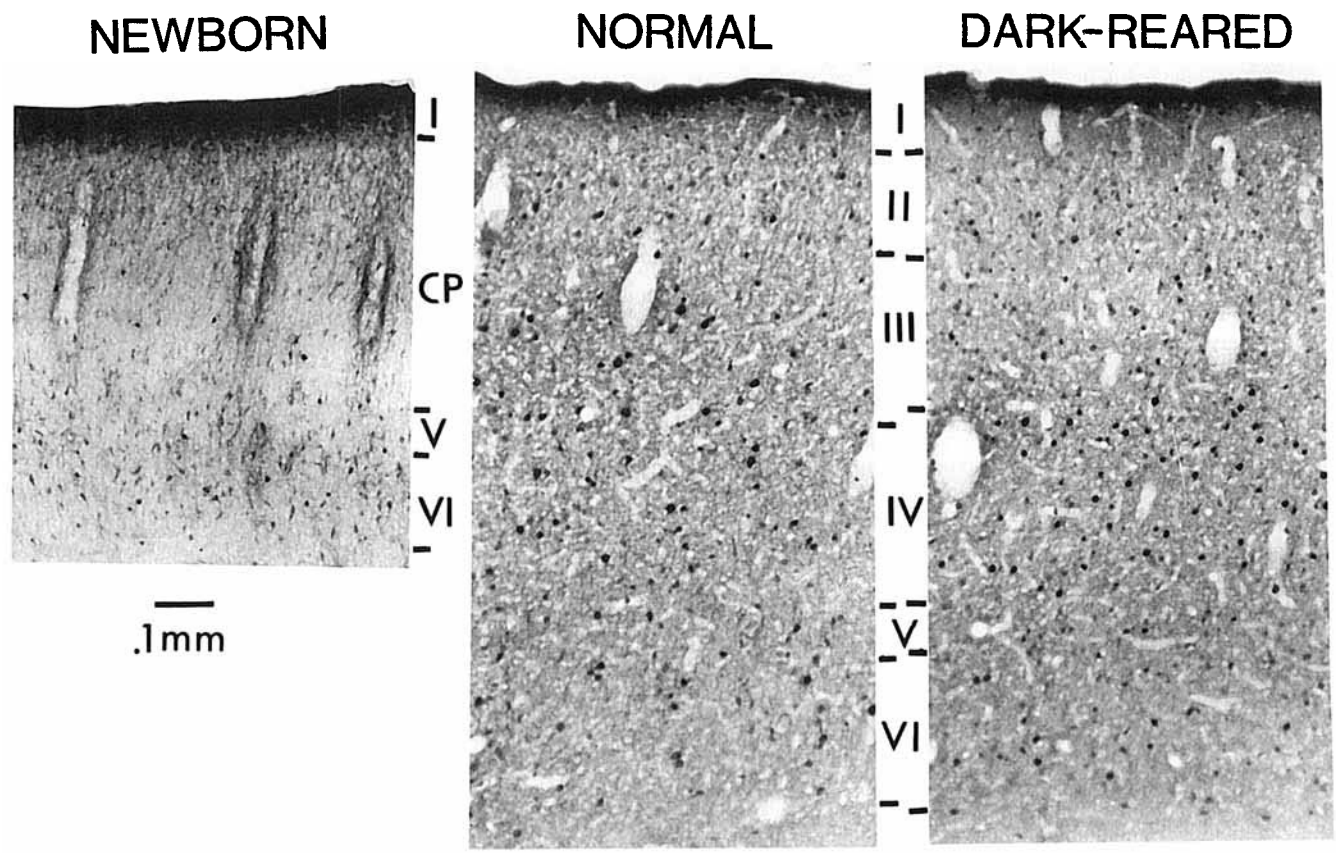


Fig. 1. The distribution of GABA-positive neurons in normal, dark-reared, and newborn visual cortex. Laminar boundaries (I–VI), as determined from adjacent cresyl-stained sections, are indicated. In the newborn cat, the laminar designations of Shatz and Luskin ('86) were used. CP indicates the cortical plate (histologically undefined layers of superficial visual cortex).

the value for that hemisphere. Subsequently, each hemisphere was treated as a single datum point.

The cresyl-stained section was also used to determine total cellular density. Because of the higher cellular density, these measures were made at a higher magnification (×400). Within each cortical layer, the cellular number in ten randomly spaced 100 × 100-μm areas was determined. The average of these counts was used as a measure of cellular density within each layer (cells/100 × 100 μm). Average cellular density across the full cortical thickness was determined from the weighted mean of these data by the relative width of each layer in that section. These numbers were used to correct each normal-DR comparison for cell density within each layer and to estimate the relative proportion of GABA cells within each layer. Each of these measures was made in four matched hemisphere comparisons from three normal and three DR cats.

In order to correct for split cell error in these cell counts, we used a method suggested by Abercrombie ('46). Sections were cut at 15 μm and 30 μm, and counts were made in each cortical layer at each tissue thickness. The difference between these counts was taken as the true number of nuclei in a 15-μm section. A correction factor (true counts/raw counts) was then calculated and applied to the cell counts. In the cresyl-stained tissue, the correction factor was consistent between layers and averaged .82 in normal cats and .86 DR cats. In the immunohistochemical tissue, very small differences in counts were found between 15- and 30-μm sections. This result suggests that the antibody did not penetrate through the sections and stained only cells close to the cut surfaces. This nature of the immunostaining had two effects. One was that it precluded determining the absolute numbers of GABA cells, since the incomplete antibody penetration necessarily leads to an underestimation of cell number. A second was that it necessitated a different approach to account for split cell error in the GABA cell counts. To do this, we measured the diameter (long axis) of 100 GABA-positive nuclei, distributed across all cortical layers, in both normal and DR tissue (at a magnification of ×650). The mean nuclear diameter of GABA cells in normal cats was 7.6±.2, in DR cats 6.6±.2. We used these measures to estimate the relative error in our GABA cell counts, based on the equations of Abercrombie ('46). If we assume the antibody penetrates 1 μm, this difference would lead to an overestimation in DR cats of 12%; if the antibody penetrates 15 μm, the overestimation would be 4%. We did not apply these correction factors to the presented data, since we could not determine the extent of antibody penetration. However, we assumed that any increase in GABA cell density of DR cats within the range of 4–12% was not significant. We also limited our analysis to relative comparisons (both between preparations and between laminae within preparations) since the absolute numbers are necessarily an underestimation.

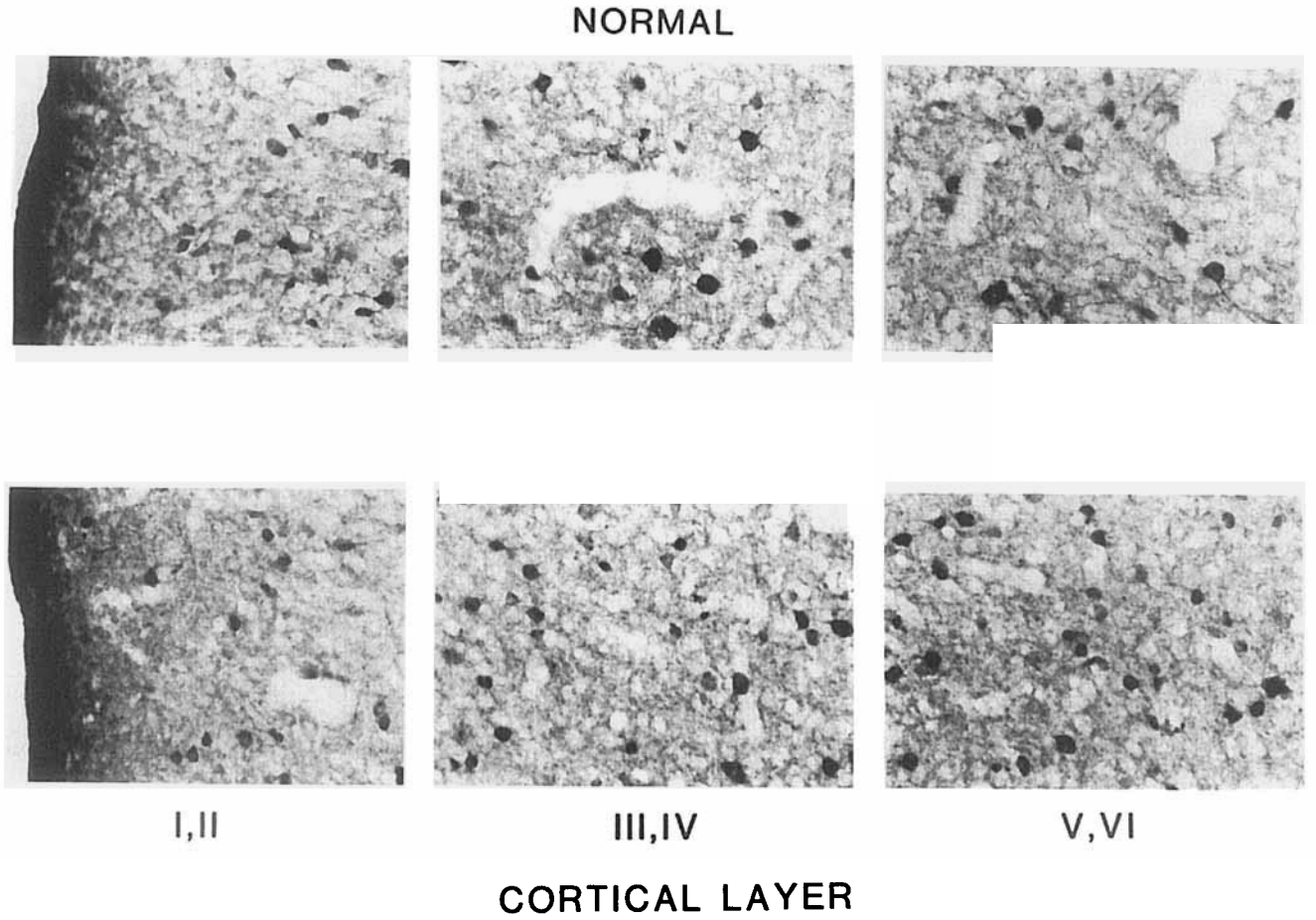


Fig. 2. Higher-power photomicrographs of GABA cells in different cortical layers of normal and dark-reared cats. Note generally smaller soma diameters and higher packing density of GABA cells in dark-reared tissue.

## Receptor binding

***Tissue preparation.*** The cats were anesthetized as above and perfused with 0.1% formaldehyde in PBS for a 5-minute period. The brain was quickly removed, and the visual cortex was blocked bilaterally and frozen onto microtome chucks, which were stored at −70°C. Tissue was cut coronally on a cryostat (14 μm), thaw-mounted onto subbed slides, air dried, and then returned to the freezer. Every section was saved over a distance of approximately 2.0 mm in each hemisphere. The anterior-posterior position of these sections was closely matched among brains to ensure comparable sampling regions and tissue areas.

***Tissue processing.*** In vitro receptor binding techniques (Palacios et al., '80, '81) were used to compare the number, affinity, and laminar distribution of GABA receptors as revealed by $^{3}$H-muscimol binding. In a previous study (Mower et al., '86) we had applied these techniques to cat visual cortex and made detailed analyses of association/disassociation rates, saturation kinetics, and pharmacological specificity. As the present study used essentially identical techniques, they will be described only briefly here.

The slides were brought to room temperature and preincubated in 0.31 M Tris citrate buffer, pH 7.1, at 4°C in an ice bath for 20 minutes. They were then transferred to an incubation solution of the same buffer containing various concentrations (1, 2, 4, 8, 16, 32, 64, 96, 144, 216 nM) of $^{3}$H-muscimol (8 Ci/mmol, Amersham, Arlington Heights, Illinois) for 40 minutes. A matched set of slides was incubated in an identical solution containing 1 mM unlabeled GABA to obtain values for nonspecific binding. After incubation, the slides were rinsed in the same buffer for 30 seconds. Tissue used for kinetic analyses of receptor binding was wiped off the slide onto small filter paper circles and assayed in a scintillation counter for radioactivity. Tissue used for autoradiographic histology was left on the slide and rapidly dried under a stream of nitrogen gas. The labeled receptors were histologically localized by apposition to LKB Ultrofilm $^{3}$H in x-ray cassettes stored at 22°C. Tritium standards (Amersham) were mounted onto selected slides and exposed with the tissue sections. Test slides of both tissue and standards were developed at various times to determine an exposure whereby all concentrations were resolvable and within the usable range of the film, as determined from the tritium standards (approximately 10 weeks). The film was developed in Kodak D-19 for 5 minutes at 20°C, rinsed in distilled water for 10 seconds, fixed in Kodak fixer for 5 minutes, and rinsed in distilled water for 20 minutes.

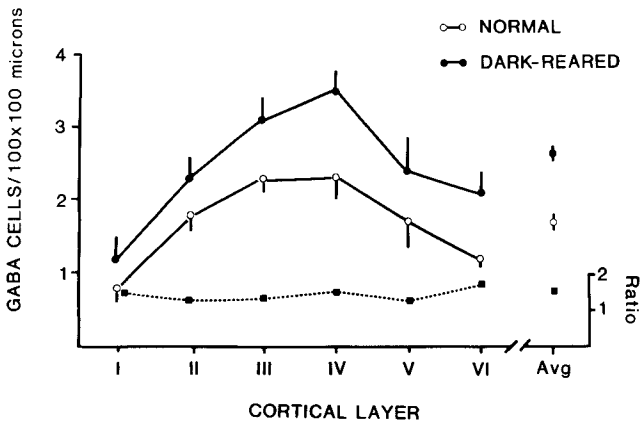


Fig. 3. Density of GABA cells (cells/100 × 100 μm) in different cortical layers of dark-reared and normal cats as determined by cell counts (described in Materials and Methods). The average GABA cell density across all layers (Avg) is the weighted mean of the individual laminar densities by the width of each layer. Each point is the mean ± standard error of four determinations. Also shown is the ratio (DR/normal) of GABA cell densities of the two preparations in each layer, indicating rather uniform elevations in dark-reared cats.

## RESULTS

### Immunohistochemistry

Figure 1 shows typical immunostaining results from normal, DR, and newborn cats. In the newborn cat, GABA cells were concentrated in the deep cortical layers and were rare above layer V. In the normal 4-month-old cat, GABA cells were found in all layers. Thus, there were marked postnatal changes in the distribution of GABA neurons. Dark-rearing did not markedly alter this developmental process: the staining pattern across cortical layers in DR cats was similar to that in normal cats.

Figure 2 shows higher power photomicrographs of GABA cells in different cortical layers of normal and DR cats. GABA cells in each preparation were nonpyramidal in morphology and were typically circular with radiating processes. Glial cells were not stained. In DR cats, GABA cells appeared to be smaller and more densely packed than in normal cats. This qualitative difference was confirmed by GABA cell counts in matched comparisons of four hemisphere pairs from three normal and three DR cats. Because of the nature of the immunostaining (discussed in Materials and Methods), these counts provided relative but not absolute measures of the numbers of GABA cells. Figure 3

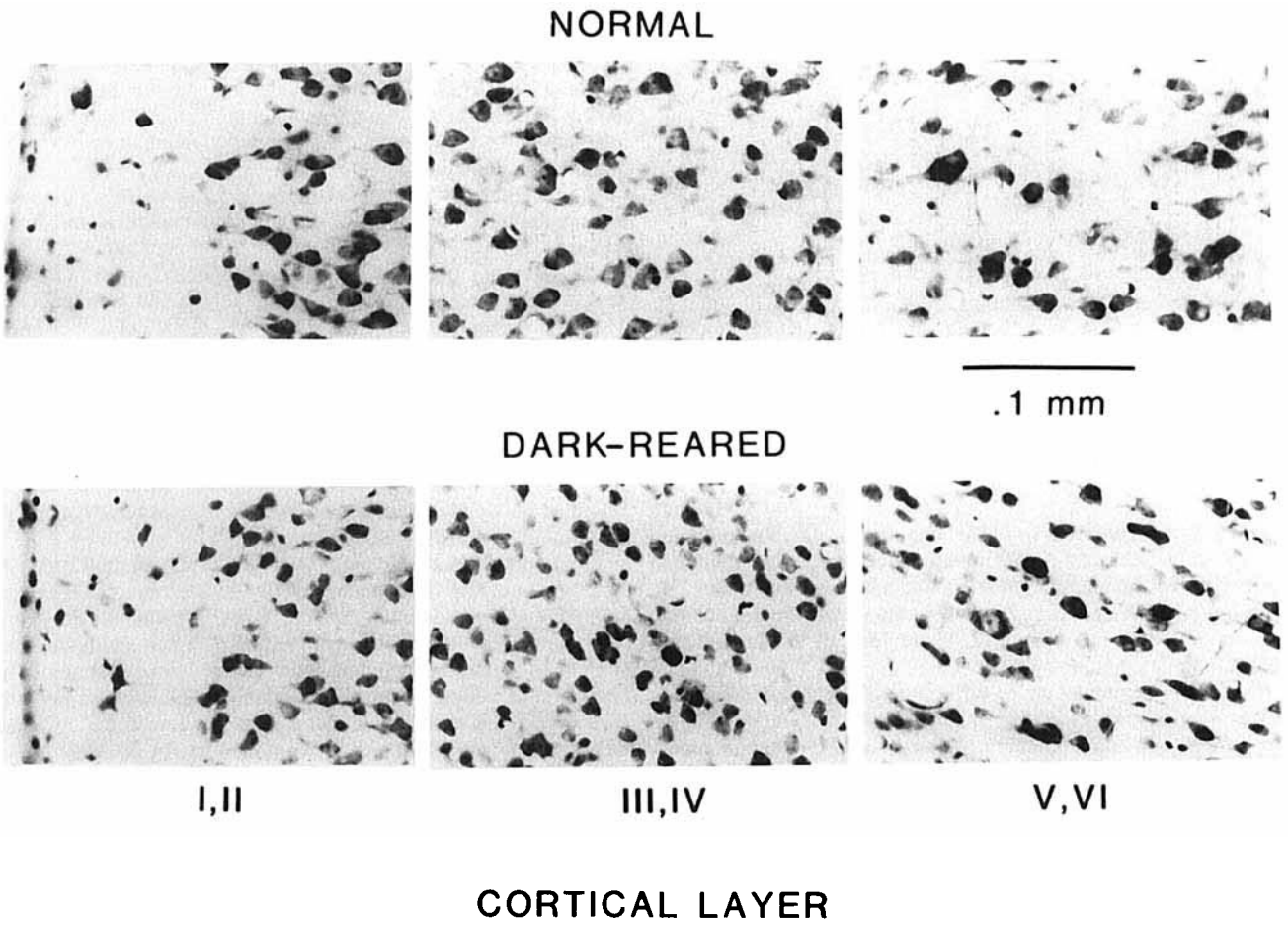


Fig. 4. Photomicrographs of total cellular density in different visual cortical layers of normal and dark-reared cats. Sections stained with cresyl violet. Note higher packing density in dark-reared cats.

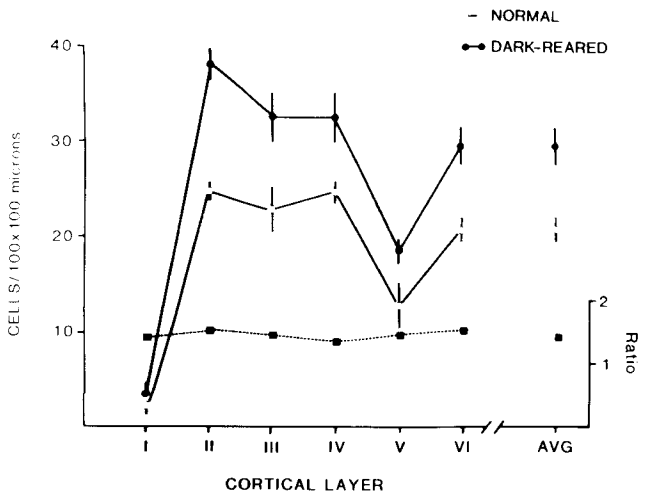


Fig. 5. Total cellular density (cells/100 × 100 $\mu$m) in different cortical layers of normal and dark-reared cats as determined by cell counts (described in Materials and Methods). Each point is the mean ± standard error of four determinations. Also shown is the ratio (DR/normal) of total cell densitites of the two preparations in each layer.

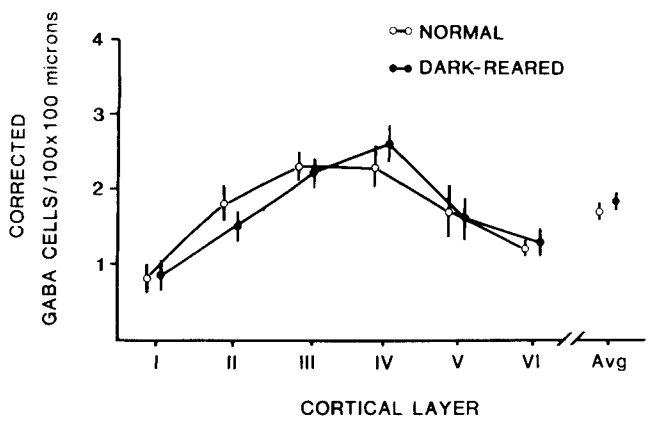


Fig. 6. Density of GABA-positive cells in dark-reared cats when corrected for differences in total cellular density. Data for normal cats replotted from Figure 3.

presents the relative laminar density of GABA neurons (cells/100 × 100 $\mu$m) in both preparations. GABA cell density was consistently higher in all layers in DR cats. The average GABA cell density across the total cortical thickness was 2.65±.11 in DR cats and 1.76±.13 in normal cats (Mann-Whitney U, $P$=.014).

The question arises as to whether the higher GABA cell counts in DR cats reflect a true increase in GABA cells or an increase in total cellular packing density. Inspection of cresyl-stained tissue suggested that cellular packing density was higher in DR than normal cats (Fig. 4). To quantitatively answer this question, we determined the total cellular density from the cresyl-stained sections, as described in Materials and Methods. Figure 5 shows the results. In DR and normal cats, the profile of total cellular density across cortical laminae was similar, but the values were higher in DR cats in all layers. In DR cats, the average cellular density across all cortical layers (cells/100 × 100 $\mu$m) was 29.5±2.1, in normals 20.7±1.4 (Mann-Whitney U, $P$=.014). This difference must be taken into account to compare the results of the above-described GABA cell counts in normal and DR tissue. To do this, the density of GABA cells in each layer of DR cats was corrected by the ratio of the total cellular density in a given layer of DR tissue to that in the matched normal tissue. These results are shown in Figure 6 with the results from normal cats of Figure 3 replotted.

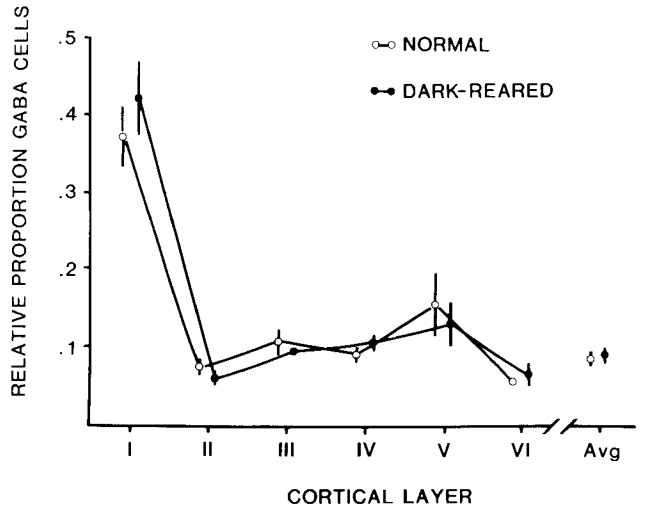


Fig. 7. Percentage of GABA neurons (GABA cell density per layer/total cell density per layer) in different layers of visual cortex in normal and dark-reared cats. Each point is the mean ± standard error of four determinations.

When corrected for differences in total cellular density, the relative densities of GABA cells between normal and DR cats are very similar and there are no significant differences between preparations in any cortical layer. The corrected average GABA cell density across the total cortical thickness of DR cats was 1.85±.08 cells/100 × 100 $\mu$m (compared to 1.76±.13 in normals). Thus, the increased GABA cell density in DR cats can be almost entirely accounted for by the increase in total cellular density in this preparation. This result indicates that there is no difference between normal and DR cats in the number of GABA neurons. The small residual difference (5%) can be accounted for by the smaller mean diameter of GABA cells in DR tissue (see Materials and Methods).

The above counts were also used to estimate the relative proportion of the total number of cells that are GABAergic in each cortical layer (GABA cell density/total cell density). These results are shown in Figure 7. This analysis also indicates no difference between normal and DR cats. In both preparations, layers I and V show the highest proportion of GABA cells, a reflection of the low total cellular density in these layers. The weighted mean of these laminar values (by the laminar thickness of that section) provides a summary statistic for comparing the proportion of GABA neurons in normal (8.9%±.8%) and DR (9.1%±.8%) cats. Because of the nature of the immunostaining in the present study, these values do not represent the absolute proportion of GABA cells. They do, however, indicate that there is no significant difference in the relative proportion of GABA cells between normal and DR cats (Mann-Whitney U, $P$=.44).

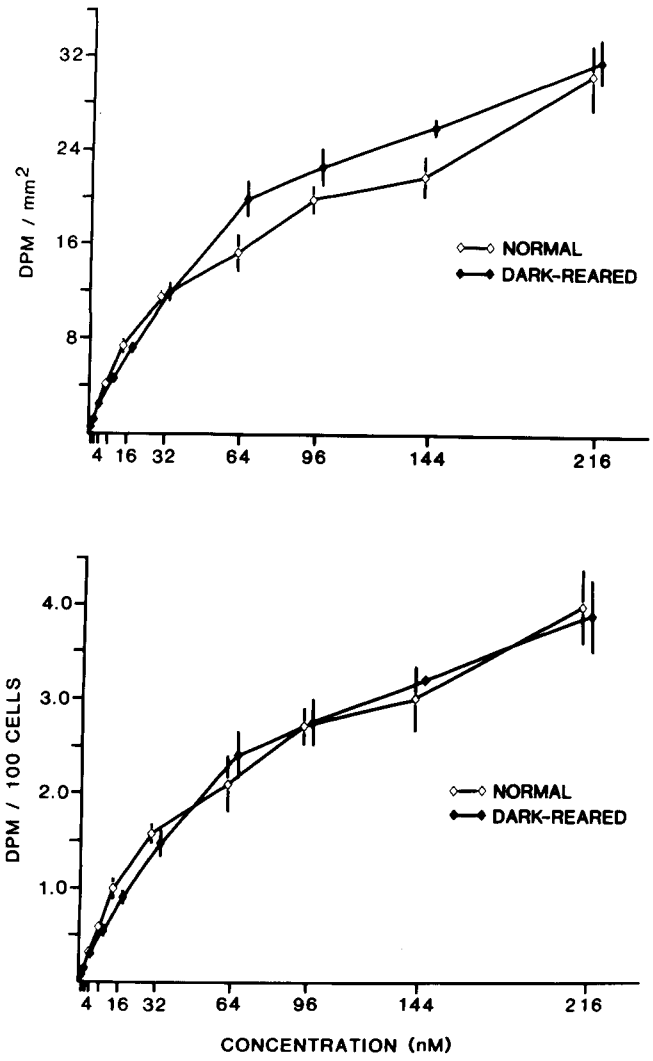


Fig. 8. Saturation kinetics of $^3$H-muscimol binding in normal and dark-reared cats. **Top:** Binding per mm$^2$ cellular area in the tissue sections. **Bottom:** Binding corrected for differences in total cellular density. See text for details. Each point is the mean ± standard error of four determinations.

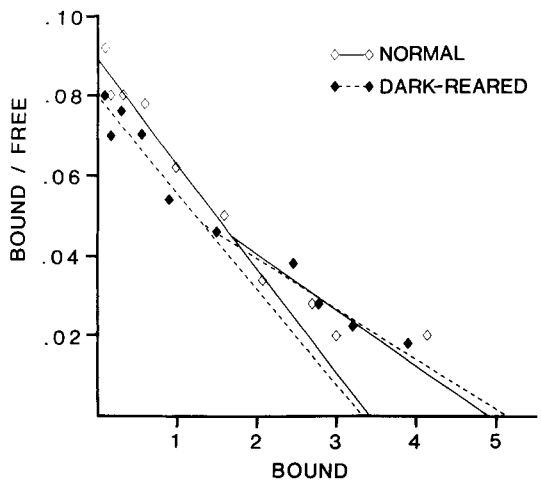


Fig. 9. Scatchard analysis of binding data (DPM/100 cells) in normal and dark-reared cats. Each point is the mean of four determinations at each concentration. Data fit assuming two binding sites.

## $GABA_A$ receptor binding

The goal of these studies was to determine whether there are differences between normal and DR cats in the number ($B_{max}$), affinity ($K_d$), or laminar distribution of GABA receptors as revealed by $^3$H-muscimol binding. Four paired comparisons were done (each hemisphere of two normal and two DR cats).

For comparison of ($B_{max}$) and $K_d$, closely matched sections from each preparation were concurrently incubated in varying concentrations of ligand. To provide directly comparable measures among cases, binding was expressed in terms of the cellular area of the tissue sections. Within each series of sections used for kinetic analysis, sections from the beginning, middle and end were stained with cresyl violet, their cellular region carefully traced, and the mean area calculated. Binding was expressed as DPM/mm$^2$ cellular area. Figure 8 (top) shows the results from the four paired comparisons which suggest a slight elevation of binding in DR cats (Mann-Whitney U, $P$ = .029). These results, however, do not account for increased cellular density in DR cats, as found above in tissue processed for immunohistochemistry. Cell counts were done on the cresyl-stained sections, as already described, to determine average cellular density across all cortical layers (cells/100 × 100 $\mu$m). In the relatively unfixed tissue used for receptor binding, there was a slight increase in cellular density in DR cats (13%±7%) but much less than that found in the fixed tissue used for immunohistochemistry (47%±8%). To account for the difference in packing density, binding was expressed as DPM/100 cells in each case ([DPM/10$^6$ $\mu$m$^2$]/[cells/10$^4$ $\mu$m$^2$] = DPM/100 cells). These results are shown in Figure 8 (bottom). When corrected for differences in packing density, $^3$H-muscimol binding in normal and DR cats is nearly identical. Scatchard analysis of these binding data also indicated no difference between normal and DR cats, as shown in Figure 9. These data were analyzed under a single site and a two site model (Table 1) and neither indicated a difference between preparations in $B_{max}$ or $K_d$.

Histological localization of binding sites also revealed no marked differences between normal and DR tissue at any concentration. Figure 10 shows representative autoradiograms at a concentration near saturation (144 nM) and the relevant portion of an autoradiographic standard. In both preparations, label appeared in all cortical layers and was nearly absent in the white matter. There was no evidence for a lateral or columnar organization of receptors. However, there were laminar differences in grain densities: labeling was highest in layers II and III, lowest in layer V, and intermediate in layers I, IV, VI. It should be noted, however, that label was far above background in all layers and the differences between layers were not extreme. This pattern was consistent at all ligand concentrations.

## DISCUSSION

The major conclusion of the present study is that with the methods employed, no differences in visual cortical GABA neurons or GABA receptors were found between normal and DR cats. When differences between normal and DR cats in total cellular packing density were accounted, neither GABA immunohistochemistry nor $^3$H-muscimol binding to $GABA_A$ receptors indicated differences between the two rearing conditions. During the early postnatal period, there are progressive changes in the laminar distribution of GABA-positive neurons (Kalil et al., '85; present results) and in the number and affinity of $GABA_A$ receptors (Shaw et al., '85). The present results indicate that these develop-

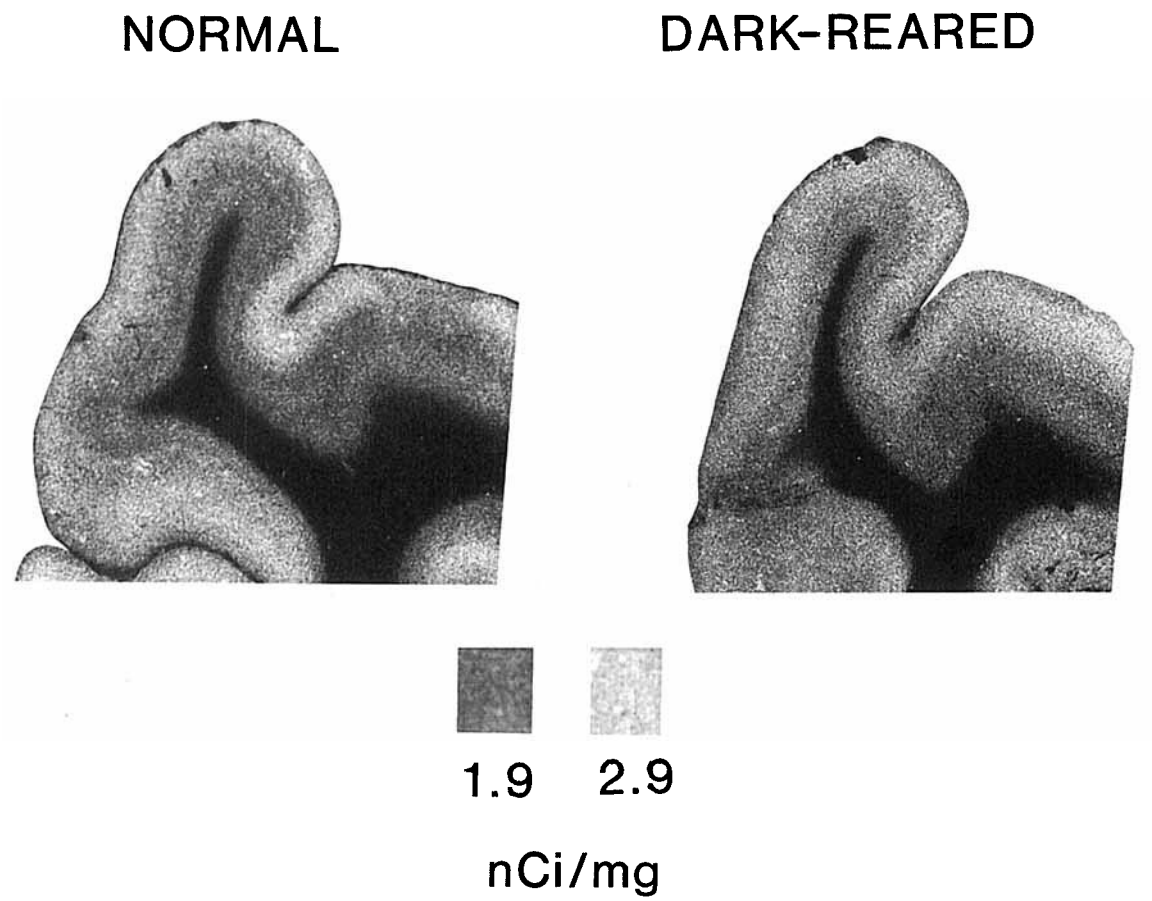


Fig. 10. Prints of Ultrofilm results showing histological localization of GABA receptors in normal and dark-reared cats. At the bottom is shown a portion of a calibrated standard which covers the range of the observed binding.

TABLE 1. Results of Scatchard Analysis of Binding Data (DPM/100 Cells) in Normal and Dark-Reared Cats (Each Value Is the Mean ± Standard Error of Four Determinations)[1]

| | | | Two-site model | | | |
|---|---|---|---|---|---|---|
| | Single-site model | | High affinity | | Low affinity | |
| Preparation | $B_{max}$ | $K_d$ | $B_{max}$ | $K_d$ | $B_{max}$ | $K_d$ |
| Normal (N = 4) | 4.6 ± .32 | 59.8 ± 6.3 | 3.5 ± .38 | 43.1 ± 7.6 | 5.4 ± .56 | 89.2 ± 8.8 |
| Dark reared (N = 4) | 4.8 ± .26 | 64.2 ± 4.9 | 3.8 ± .82 | 47.7 ± 10.3 | 5.2 ± .28 | 82.4 ± 7.4 |

[1]Paired t tests, $P > .1$ in all normal/dark-reared comparisons.

mental changes progress to their normal end point in the absence of visual input. This result does not preclude that there are differences in the temporal pattern of GABA system development; however, the endpoint appears the same with or without visual input.

Our results also demonstrated a marked increase in packing density of visual cortical neurons in DR cats. We do not believe that the increased cellular density in DR cats represents an increase in absolute cell number. Rather, the effect is probably due to decreased thickness of the cortical mantle in DR cats. In our sample, the average cortical thickness was 14.5±.6 mm in normal cats and 12.4±.6 mm in DR cats. This difference is sufficient to account for most of the observed change in cellular density between the two rearing conditions. Several factors could contribute to reduced cortical thickness in DR cats. O'Kusky ('85) reported effects similar to ours in cats placed in the dark after 70 days of normal vision, and he also found that mean cell diameter was reduced in these animals. Recently, Daw ('86) reported decreased myelin staining in DR compared to normal cats and this effect was specific to visual cortex. Thus, the increased packing density seems to result from a combination of smaller cell bodies and decreased myelin. We also found that the cellular density differences between normal and DR cats were less marked in the relatively unfixed tissue used for receptor binding. This result could

indicate a higher water content in visual cortex of DR cats. Whatever the cause of increased cellular packing density, the present results underscore the importance of considering this difference in comparisons of normal and DR tissue.

The present study adds to the literature describing GABA neurons in cat visual cortex (Fitzpatrick et al., '83; Bear et al., '85; Gabbot and Somogyi, '86). Qualitatively, our results are in good agreement with those of previous reports: GABA neurons appear in all cortical layers without any marked laminar distribution, they are nonpyramidal in morphology, and glial cells do not appear to be GABA immunoreactive. The present results also provide quantitative estimates of the distribution of visual cortex GABA neurons. We emphasize, in this regard, that our methods in thick sections (30 $\mu$m) provided only relative measures across layers and not absolute GABA cell densities. Recently, Gabbott and Somogyi ('86) made a quantitative description of cat visual cortex GABA neurons in semithin (.5 $\mu$m) sections, and found that 20% of visual cortex neurons are GABAergic. In terms of absolute numbers, our results (9%) are necessarily an underestimation owing to incomplete antibody penetration in our sections. The relative distribution of GABA neurons across cortical layers, however, is in good agreement between their study and ours. GABA cells are in highest proportion in layer I and uniformly lower in proportion in the other layers. Gabbott and Somogyi also reported the percentage of total GABA cells within each layer of cortex (number of GABA cells per layer/total number GABA cells). When analyzed in this way, our results (I, $5\pm.8$; II, $16\pm1.6$; III, $20\pm1.7$; IV, $35\pm2.4$; V, $7\pm.7$; VI, $16\pm2.6$) are also in good agreement with those of Gabbott and Somogyi.

$^3$H-muscimol binding revealed no differences between normal and DR animals in the number, affinity, or laminar distribution of $GABA_A$ receptors in visual cortex. In both preparations, receptors were most dense in layers II and III, least dense in layer V, and intermediate in layers I, IV, VI (see also Mower et al., '86). It is noteworthy, however, that the pattern of label can change with incubation parameters. Specifically, short rinse times (15 seconds) result in an elevation of label in layer IV (Needler et al., '84; Mower et al., '86), possibly owing to a lower affinity muscimol binding site. Saturation kinetics in the present study provided some support for this possibility by suggesting two muscimol binding sites. These results were the same in both preparations, however, again indicating no difference between normal and DR visual cortex.

Overall, the present results indicate that the maturation of the GABA system, at the light microscopic level, is normal in animals reared with total visual deprivation. Physiological studies, on the other hand, suggest an alteration in GABA function in DR cats, as described in the introduction. Additionally, a recent series of microiontophoretic studies analyzed the role of GABA in visual cortex of DR cats. Visual cortical cells were found to be equally sensitive to GABA in DR and normal cats (Tanaka et al., '87), a result consistent with the normal number, affinity, and distribution of GABA receptors reported here. However, microiontophoresis of the GABA antagonist bicuculline had abnormal effects in DR cats. Although it revealed responsiveness in many otherwise unmappable neurons, bicuculline disinhibition did not alter orientation or direction tuning (Tsumoto and Freeman, '87). Rather, its effects were similar to application of an excitatory amino acid (Ramoa et al., '87). These results suggest abnormal function of the GABA system in DR cats, because, unlike the case in normal cats, the effects of disinhibition are the same as those of increased excitability.

How can these physiological results be reconciled with the apparently normal anatomy of the GABA system? A similar inconsistency has been found in studies of monocularly deprived cats, where physiologically a GABA enhancement appears to play a role in the abnormal ocular dominance (Duffy et al., '76; Burchfiel and Duffy, '81) but anatomical studies reveal no abnormalities in GABA neurons or receptors (Bear et al., '85; Mower et al., '86). There are several possibilities. One is alterations that would not be detected with the methods we used. For example, in the immunohistochemistry, we made no quantitative analysis of labeled puncta, which were resolvable in our tissue at high magnification. Qualitative examination indicated that puncta were present in all layers, and there was no obvious difference between normal and DR cats. Quantitative analysis of synaptic densities, however, could reveal differences between normal and DR cats. In the receptor binding, we analyzed only the number, affinity, and laminar distribution of $GABA_A$ receptors. There could be differences between preparations in the distribution of these receptors across the cell membrane (e.g., clustering under GABA synapses) which would not be detected by our methods. Also, other subtypes of GABA receptors exist, including bicuculline-insensitive $GABA_B$ receptors (Bowery et al., '80) and GABA autoreceptors (Brennan and Cantrill, '79), and these could be altered by dark-rearing. Other possible effects in DR cats include functional abnormalities at the GABA synapse or in the physiological response properties or GABAergic neurons, either of which would produce abnormalities in the function of the GABA system. Perhaps the GABA system develops correct wiring in the absence of visual input, but requires visual activation, and the consequent development of mature response properties in GABAergic cells, before it can exert its normal physiological effect.

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