Histological studies of the visual system in monkeys with experimental amblyopia*

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Amblyopia can be produced in rhesus monkeys by suturing the lids of one eye (visual deprivation amblyopia) or by producing artificial esotropia (strabismic amblyopia) during visual immaturity. Sections from retinas, lateral geniculate nuclei (LGN), and areas 17 and 18 of the visual cortex from monkeys with behaviorally proved amblyopia and cortical neurophysiologic changes were examined histologically and compared with normal tissue. Other than significant reduction in cell section areas in all layers of the LGN that received input from the deprived or esotropic eye, we were unable to demonstrate anomalies elsewhere in the visual system. In spite of the difference in pathogenesis of visual deprivation and strabismic amblyopia, the similarity of findings in the LGN of monkeys with both types of amblyopia suggests a common mechanism. Since dissimilarity of visual input from the two eyes caused by unilateral lid suture has been shown to affect geniculate cell size in visually immature kittens, we surmise that binocular competition at the geniculate or cortical level is operative in both unilateral lid closure and strabismus and causes similar changes in the primate visual system.

Key words: amblyopia, visual deprivation, strabismus, lateral geniculate nucleus, monkeys, primates, esotropia.

In view of the severe behavioral and cortical neurophysiologic anomalies demonstrated in earlier studies on monkeys with amblyopia induced by unilateral lid closure or artificially produced strabismus, one may expect to find equivalent structural anomalies in the visual system. Indeed, such anomalies have been described in the retina or optic nerve, in the lateral geniculate nuclei (LGN), and in the visual cortex of lower animals in which various forms of visual deprivation have been produced. The methodology and selection of experimental species used by different investigators to cause visual deprivation have differed widely. It is not surprising, therefore, that a bulk of often contradictory information on the effect of visual deprivation on the morphology of the visual system has been accumulated.

Results of studies on the morphology of...
the visual system in primates with stimulus deprivation or strabismic amblyopia are not available in the literature. In this paper we will report on the histologic examination of retinas, the LGN, and parts of the visual cortex of rhesus monkeys (Macaca mulatta) with behaviorally proved amblyopia following unilateral lid suture or artificially produced esotropia early in life.

Material and methods

Surgical techniques for lid closure and production of strabismus in infant monkeys and procedures for testing visual acuity in monkeys were described in earlier studies. 1-2 The behavioral and experimental data regarding the animals used in this study are summarized in Table I. At the time animal B 35A was killed, our equipment limited visual acuity testing to a level of only 20/140. After the apparatus was modified, in all other animals a visual acuity of 20/39 was established in the sound eye before the previously sutured or deviated eye was tested. Our previous studies had established that amblyopia occurs in all animals a marked decrease in the number of cortical units that responded to binocular stimulation, an absence of cortical cells that could be driven from the deprived eye (VDM 3), and a preponderance of cells that responded only to stimulation of the nonamblyopic eye (VDM 4 and 6). 4 All animals were examined with an ophthalmoscope and the fundi were found to be normal. The retinas of the normal and amblyopic eyes from animals VDM 3 and 6 were examined light microscopically. The inner plexiform layer of the retina of the sound eye and the amblyopic eye and sections from areas 17 and 18 of the striate cortex from animal B 35A were examined with the electron microscope and compared with corresponding tissue from a normal monkey. The histologic techniques are summarized briefly.

Retina. The enucleated eyes were placed in formaldehyde and then sectioned horizontally. The central portion containing the pupil, the optic nerve, and the macula was dehydrated using alcohol and chloroform and then embedded in paraffin. The entire macula was sectioned serially to a thickness of 8 μ and the sections were stained with hematoxylin and eosin. Comparable sections of optic nerve were stained for axon cylinders (Bodian's stain) and myelin. Sections from the fovea, nasal and temporal parafoveal and peripheral retina as well as through the optic nerve of the amblyopic eye were compared with corresponding tissue from the normal eye of the same animal. Using a stage micrometer, the thickness of the various retinal layers was determined under the microscope in 10 consecutive sections from the following areas: 1 and 2 mm. temporally and nasally from the foveal center, respectively, and 6 mm. posteriorly from the nasal ora serrata.

Sectioned through the fovea, nasal and temporal parafoveal and peripheral retina as well as through the optic nerve of the amblyopic eye were compared with corresponding tissue from the normal eye of the same animal. Using a stage micrometer, the thickness of the various retinal layers was determined under the microscope in 10 consecutive sections from the following areas: 1 and 2 mm. temporally and nasally from the foveal center, respectively, and 6 mm. posteriorly from the nasal ora serrata. In addition, the relative density of retinal ganglion cells was determined in 10 subsequent sections 1 mm. nasally and temporally from the foveal center using a net micrometer.

The eyes of monkey B 35A were enucleated at the time of perfusion and prepared for electron microscopy using a method similar to that employed by Dubin. 24 Sections were obtained from the foveal and parafoveal regions of the sound and amblyopic eyes. Photomontages of the entire thickness of the inner plexiform layer were prepared, giving a final magnification between x17,000 and x21,000. The area of the inner plexiform layer surveyed extended from the proximal edge of the nuclear layer to the distal edge of the ganglion cell layer. A total area of 10,820 μ² was surveyed in the right eye and 11,000 μ² in the left eye, and the relative density of bipolar and amacrine synapses was determined. The synapses were identified according to the criteria employed by Dubin 24 and Dowling 25 (Fig. 1).

Lateral geniculate nuclei. LGN's were removed after perfusion of the animals with 10 per

| Table I. Behavioral characteristics of monkeys used for histologic study |
|------------------|------------------|------------------|------------------|
| Animal          | Procedure        | Age at surgery (days) | Visual acuity |
| B 35A           | Lid closure R.E. | 12                | Light perception 20/140 |
| VDM 3           | Lid closure R.E. | 7                 | Light perception 20/39 |
| (E 2911)        | Surgical esotropia of 24° R.E. | 7 | Light perception 20/39 |
| VDM 4           | Surgical esotropia of 32° L.E. | 1 | 20/360 | 20/39 |

*Highest level of tested visual acuity.*
cent formalin. The specimens were embedded in celloidin, cut coronally, and stained with thionine. The primate LGN contains six well-differentiated laminae numbered in a ventrodorsal direction from 1 to 6. The cells in laminae 3 to 6 are considerably smaller than those in layers 1 and 2. Laminae 2, 3, and 5 receive input via the uncrossed optic fibers from the homolateral eye, and laminae 1, 4, and 6 via the crossed fibers from the contralateral eye. The LGN is a uniquely suitable structure for the study of the effects of unilateral visual deprivation or strabismus since affected and unaffected layers are available for comparison in the same specimen. The retinal projection on the sections of LGN which were studied includes an area extending from the fovea approximately 6 to 12° into the nasal and temporal periphery and 10° into the superior and inferior periphery. Since foveal fibers project only on the posterior portion of the LGN, foveal cells were not included in these sections.

Using the following technique, the cell section area was measured in all layers that receive their input from the amblyopic eye and the normal eye. One hundred cells from the central portion of each lamina were selected for measurement (Fig. 2) and photographed. The selection was made on the basis of a sharply outlined nucleus and lack of overlay by other cells. The negatives were projected on white paper with a photographic enlarger, and each cell and its nucleus were traced with a sharp pencil. The section area of 100 cells from each layer was determined with a planimeter and recorded in \( \mu^2 \). The \( \chi^2 \) test was employed to establish whether statistically significant differences existed in the frequency of occurrence between the cell section areas in corresponding layers of the right and left LGN.

**Visual cortex.** At the age of five years, animal B 35A was anesthetized and perfused through the left carotid artery with glutaraldehyde and paraformaldehyde as fixatives. The brain was removed and thin sections were taken from corresponding aspects of areas 17 and 18 on the right and left sides. For purposes of comparison, corresponding specimens were obtained from the left visual cortex of a 7-year-old normal monkey perfused in a similar manner.

To study the morphology of synapses, photomicrographs were obtained with a total magnification of \( \times 41,650 \). The area to be photographed was selected on the basis of the quality of tissue definition, and not more than four photographs were taken from each grid in order to survey as large an area as possible and to avoid duplication. Fifty synapses were selected for analysis from different levels of areas 17 and 18 from the right and left cortex of the experimental animal and the left cortex of the normal animal. The appropriate areas were identified using the lunate sulcus as a landmark. Selection of synapses to be studied was made on the basis of good tissue definition; synapses with curved or broken membranes were not included.

In the analysis of each synapse, the following
Fig. 3. Asymmetric synapse in area 18 of the visual cortex of normal monkey. Typical synaptic elements consist of presynaptic and postsynaptic processes and membranes, vesicles in the presynaptic process, and mitochondria.

Factors were considered: (1) length of thickened apposition between presynaptic and postsynaptic membranes; (2) presence or absence of mitochondria in presynaptic and postsynaptic processes; (3) presence or absence of spine apparatus; (4) number of vesicles in the presynaptic processes; (5) classification into symmetrical and asymmetrical types; (6) distance between the presynaptic and postsynaptic membranes. Fig. 3 shows a typical asymmetrical synapse with round vesicles and mitochondria in the presynaptic and postsynaptic processes.

Various sections from the right and left areas 17 and 18 of deprived cortex and from the left side of the normal cortex were studied in order to obtain an estimate of relative synaptic density throughout the thickness of the visual cortex. Using a magnification of x20,800, photomicrographs of adjacent tissues from four different areas of each sample were obtained and a photomontage was prepared. The total tissue area thus surveyed from each sample was 16,500 μm². Only those synapses with clearly identifiable vesicles in the presynaptic process and the characteristic membrane thickening of the synaptic region were included in the count. Fields containing large cell bodies, blood vessels, or bundles of myelinated fibers were avoided.

Results

Retina. The results of measurements of the thickness of various retinal layers and of the relative density of ganglion cells were influenced by uncontrollable artifacts. Varying degrees of obliquity of the sections and tissue distortion precluded a meaningful analysis of the data. No conclusions regarding subtle variation in thickness of the layers and density of ganglion cells could be drawn one way or the other, even though the sections from normal and amblyopic eyes appeared grossly comparable in all respects. Special stains of the optic nerve showed no evidence of optic atrophy. The total number of synapses and the ratio between the ribbon and conventional synapses in the inner plexiform layer of the normal and amblyopic eyes are summarized in Table II. Amacrine synapses were found abundantly in the retinas of both normal and amblyopic eyes. The ratio between bipolar and amacrine synapses showed no difference between the two specimens and was similar to that reported by Dubin in the monkey retina. The total number of all synapses counted in both specimens was highest in the normal eye; however, this apparent increase can be attributed to the slightly larger area surveyed in the normal eye and, thus, is not significant.

Lateral geniculate body. Examination of the LGN of VDM 3 and 4 at low mag-
Fig. 4. Right (A) and left (B) LGN of monkey VDM 3 (lid closure). Note poor staining characteristics in all layers receiving input from the deprived eye (d) compared with those receiving input from the normal eye (n). (×17½.)

Table II. Inner plexiform layer synapses in unilaterally deprived monkey (B 35A)

<table>
<thead>
<tr>
<th>Total area surveyed (in microns)</th>
<th>Normal eye</th>
<th>Deprived eye</th>
<th>Bipolar</th>
<th>Amacrine</th>
<th>Rational (bipolar : amacrine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11,100</td>
<td>10,820</td>
<td>197</td>
<td>771</td>
<td>1:3.7</td>
</tr>
</tbody>
</table>

nification revealed poor staining characteristics in all deprived layers (Figs. 4 and 5). At higher magnification the difference in the size of cell sections becomes more obvious (Fig. 6). A comparison of 100 cell-section areas between normal and affected layers in the animal with unilateral lid closure (VDM 3) and the one with strabismic amblyopia (VDM 4) are shown in Figs. 7A and 7B. Statistical analysis of the data from both specimens reveals a highly significant shift toward areas with smaller cell sections in all layers containing the terminals from the amblyopic eye. This difference appeared to be more marked in VDM 3, as can be seen in Fig. 8, in which we have compared the difference in median cell-section area between the normal and affected layers of both animals.

Visual cortex. The comparison between data obtained from the right and left sides of the visual cortex from the amblyopic animal, as may be expected, yielded identical results and will not be considered further in this report. We shall discuss only the results of comparison between the left sides of the visual cortex from the amblyopic and normal monkeys.

A comparison between the length of synaptic apposition in areas 17 and 18 of the normal and deprived cortex is shown in Table III. No significant difference exists between these groups. The distribution of the data collected for different parameters of synaptic characteristics is also strikingly uniform (Table IV). Asymmetrical synapses were predominant in all specimens. The distribution of mitochondria in presynaptic and postsynaptic processes was similar in deprived and nondeprived visual cortex. This was true also for the mean number of vesicles identified in the presynaptic processes, even though the large variation in the number of vesicles in individual synapses (5 to 50) did not permit a meaningful statistical analysis of these data. The distance between presynaptic and postsynaptic membranes (not included in Table IV) was constant in all samples and ranged with great uniformity be-
Fig. 5. Right (A) and left (B) LGN of monkey VDM 4 (esotropia). Staining characteristics are poor in all layers receiving input from the esotropic eye (e) compared with those receiving input from the normal eye (n). (×17½.)

Table III. Length of synaptic apposition in areas 17 and 18 of normal and deprived cortex

<table>
<thead>
<tr>
<th>Area 17</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Deprived</td>
<td>0.352±0.0642</td>
<td>p = 0.05</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.352±0.0714</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Area 18</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Deprived</td>
<td>0.306±0.0678</td>
<td>p = 0.05</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.303±0.0615</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

between 260 and 280 Å. There was no significant difference between the relative density of synapses in areas 17 and 18 of deprived and normal cortex (Table V).

Discussion

The influence of experimentally restricted visual experience early in life on the development of visual function of the adult organism has been the subject of numerous investigations. Experimental conditions of inducing visual deprivation have ranged from raising newborn or visually immature animals in complete darkness, to suturing one or both eyelids at various periods during their life, and structural alterations have been reported in the retina, the LGN, and the visual cortex. Furthermore, with the exception of Chow, Riesen, and Newell, who reared chimpanzees in the complete dark, all these studies were performed in subprimate species, including mice, rats, cats, and dogs. No previous investigation has dealt with experimentally produced strabismic amblyopia, nor have quantitative behavioral data in terms of visual acuity been correlated with neuroanatomic findings. Therefore, a meaningful comparison of our data with those found in the literature is not possible.

From previous investigations, the retina seems to be least affected by visual deprivation, although atrophy of ganglion cells and optic nerve fibers and...
Fig. 6. Cell sections in layer six of the right (A) and left (B) LGN of VDM 3. Note difference in section area between nondeprived (A) and deprived (B) layers. (x2,100.)

thinning of the inner plexiform layer reportedly have been caused in dark-reared cats and monkeys. Other workers have described a significant increase of amacrine synapses in the inner plexiform layer of unilaterally visually deprived rats. On gross inspection of our specimens, the thickness of the inner plexiform layer was comparable in normal and amblyopic eyes, and atrophy of ganglion cells or the optic nerve fibers was absent. No difference existed in the distribution of bipolar and amacrine synapses in the inner plexiform layer of normal and amblyopic eyes.

In view of the severe cortical neurophysiologic and behavioral anomalies observed in cats and monkeys with experimental amblyopia, it is reasonable to expect analogous morphologic alterations in the visual cortex. Several reports indicate that such changes may exist in dark-reared mice, rats, and rabbits but the results are contradictory. Even though the tissue samples which we studied were small, they should have been sufficient to demonstrate gross changes such as synaptic deformities, decreased synaptic density, or increase in length of synaptic apposition which other investigators have reported to exist in deprived visual cortex. Again, that our data do not confirm these findings could be due to the difference in experimental design and the selection of species. On the other hand, the absence of abnormalities of the cortical tissues examined does not necessarily mean that the visual cortex was morphologically normal since existing anomalies may have been subtle or located in areas other than those examined.

The finding of atrophy of ganglion cells in the LGN of the animal with unilateral lid suture during visual immaturity is in accordance with numerous studies on lower animals. This is of interest since atrophic changes in the LGN of primates and humans have previously been related to complete peripheral deafferentation following enucleation of one eye or to longstanding blindness with optic atrophy.
Fig. 7. Frequency distribution of 100 cell-section areas measured in six layers of the right and left LGN. Histograms of cells connected with the normal and amblyopic eyes are superimposed for better comparison. (A) VDM 3 [lid closure], and (B) VDM 4 [esotropia].
There is no precedent for demonstration of morphologic changes in strabismic amblyopia in the visual system of any species including the human. Our findings are especially noteworthy inasmuch as a fundamental difference exists between the pathogenesis of amblyopia caused by visual deprivation and that of strabismus. The first type can be related to form-vision deprivation during visual immaturity. Conversely, with strabismus the deviated eye at no time is deprived of normal light or form-vision stimulation. Images are formed on the fovea and the retinal periphery, and the retina participates in visual activity to the same degree as the normal eye. However, due to the deviation, the visual message imprinted on the retina of the deviated eye is different from that received by the fixating eye. In view of the etiologic difference between both forms of amblyopia, the similarity of the structural changes which we observed in the LGN is remarkable. At the present stage of our knowledge, any explanation for this finding must be considered hypothetical.

Since only diffuse light can enter the eye after lid closure, the only common factor shared by deprivation and strabismus appears to be the dissimilarity of input received by the two eyes. In the case of visual deprivation this may lead to competition, and perhaps interaction on a geniculate or cortical level, between the input received by the occluded eye and the nonoccluded eye. With strabismus, the visual message received by the deviated eye perhaps competes in a similar manner with that of the fixating eye. This phenomenon is known to occur in man in the form of retinal rivalry. Certain evidence exists from experiments in cats that binocular competition may indeed affect geniculate cell size, and possibly the changes in the LGN in our monkeys will be explained in a similar manner as additional information becomes available.

Whether binocular interaction occurs at a geniculate or cortical level is not clear at this time since both possibilities exist. There is ample evidence, at least in cats, that interlaminar geniculate interaction is a factor in the processing of visual information. On the other hand, the geniculate changes may well be secondary to inhibition at a cortical level since cortico-geniculate pathways exist and geniculate cells atrophy after experimental lesions in the visual cortex. 

There is no direct evidence that the amblyopia is caused by cell shrinkage in the LGN since these morphologic changes may merely reflect the effect of abnormal binocular interaction during visual immaturity and the seat of amblyopia could be elsewhere in the visual system. Studies of the LGN of monkeys with alternating exotropia and without amblyopia are currently under way to clarify this important point.

The decrease of cell area sections in the affected layers of the LGN could be caused by arrest of normal cell growth or by shrinkage of formerly larger cells. Hubel and Wiesel, who studied receptive fields in very young and visually inexperienced kittens, reported highly differentiated visual functions in these animals and favor the theory that after deprivation disruption of already well-developed visual connec-
Table IV. Synaptic characteristics in areas 17 and 18 of normal and deprived visual cortex

<table>
<thead>
<tr>
<th>Area 17:</th>
<th>Deprived</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
<td>Asymmetrical</td>
<td>Symmetrical</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Area 18:</th>
<th>Deprived</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
<td>Asymmetrical</td>
<td>Symmetrical</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>—</td>
</tr>
</tbody>
</table>

Table V. Relative density of synapses in areas 17 and 18 of normal and deprived cortex

<table>
<thead>
<tr>
<th>Area 17</th>
<th>Total number</th>
<th>Mean</th>
<th>S.D.</th>
<th>Significance (x²-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deprived</td>
<td>471</td>
<td>117.75</td>
<td>± 13.3</td>
<td>p = 0.4</td>
</tr>
<tr>
<td>Normal</td>
<td>440</td>
<td>110.0</td>
<td>± 10.5</td>
<td></td>
</tr>
<tr>
<td>Area 18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deprived</td>
<td>514</td>
<td>128.5</td>
<td>± 16.4</td>
<td>p = 0.2</td>
</tr>
<tr>
<td>Normal</td>
<td>516</td>
<td>129.0</td>
<td>± 7.35</td>
<td></td>
</tr>
</tbody>
</table>

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our understanding and clinical management of these disorders in humans.

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