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Effects of Unilateral Visual Deprivation on the Developing Avian Brain

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The effect of varying states of visual deprivation on the development of the optic lobes and cerebral hemispheres has been studied in the chick where the visual pathways are totally crossed over. Unilateral eye extirpation of the new hatched chick resulted in arrested development of the contralateral but not ipsilateral lobe, as measured by weight, protein content and acetylcholinesterase activity. Similar effects but of smaller magnitude were observed in the cerebral hemispheres. Histologic and enzymic evidence revealed the absence of significant degeneration in the optic lobe contralateral to an eye removed 17 days previously. These results were observed in the optic lobes of operated animals maintained either in light or in darkness between 3 and 17 days after hatch. However, in the impaired cerebral hemispheres, differences could only be detected in birds kept in the light. The effects of unilateral eyelid suturing on the development of chick brain regions were also examined. In this group, all asymmetrical differences observed within paired brain regions were totally light dependent and confined to the cerebral hemispheres. The hemisphere contralateral to the sutured eye weighed less and had less acetylcholinesterase activity than its paired hemisphere. The cerebral hemispheres of monocularly treated birds manifested effects of similar magnitude whether the treatment was enucleation or suturing. This suggests that the complete development of the associative centers in avian cerebral hemispheres is dependent on both intact innervation and on the information content of the visual input.

Introduction

The determination of biochemical changes in the central nervous system, which are correlated with differential sensory input, is of primary interest in the study of the molecular basis of cerebral function. Experiments de-

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1 We thank Dr. P. Cancilla for very generous help in the preparation and interpretation of the histological material. We also thank Dr. S. Roberts (grant from the United Cerebral Palsy Research and Educational Foundation) and Dr. S. Zamenhof (NIH Grants HD01909 and NB-08723-01 and American Cancer Society Grant P-503A) for kind and generous support during the course of this study and Mrs. B. Morelos for expert technical assistance.
signed to investigate these changes are complicated by the difficulty of choosing appropriate controls, so that only one parameter is varied. However carefully conditions are selected, experimental and control animals remain different individuals subject to unintended variations which could overshadow subtle but significant differences in chemical composition. It would thus be desirable to be able to use each animal as its own simultaneous control. An experimental system which seems to meet this criterion is the avian visual system.

The complete decussation of the optic tract in many birds (10) suggests that unilateral manipulation of visual input could be expected to result in differential effects in the two optic lobes of the avian brain. Since one optic lobe can be compared to its contralateral partner, each bird can serve as its own simultaneous internal control. The two optic lobes of the chick are anatomically distinct and constitute a considerable fraction of the total weight of the brain. Any differences detected between the two lobes would presumably be the result of experimental manipulation of sensory input and not due to any systemic or humoral variations which would affect both lobes equally. Long-term unilateral histological changes occurring in the adult avian visual system after removal of one eye in the young bird were described many years ago (30). Autoradiographic studies of the long-term effects of this operation on the optic lobes of adult birds have also been described (1). However, little is known of the early, quantitative biochemical changes in various brain regions after unilateral eye removal in newly hatched birds (26).

We have examined the differential effects of unilateral visual deprivation for comparatively short times on the paired optic lobes and cerebral hemispheres of newly hatched chicks. We have investigated two types of monocular visual deprivation resulting from enucleation or eyelid suture over an intact eye. The role of incident light was also tested. A portion of this work has been published as a preliminary report (28).

Methods

Fertile chicken eggs from white leghorn strain K137 were obtained from Kimbler Farms (Pomona, Calif.) and incubated at 37.5°C in a forced-draft incubator until hatched. The effect of unilateral visual deprivation was studied in chicks anesthetized by an intramuscular injection of 1 mg Nembutal (Abbott) on the day after hatching. The eyelids of either the right or left eye were sutured with nylon monofilament and then painted with a collodion film to insure the eyelids remained sealed. Left or right enucleation was performed as described by Menaker (29). Mortality was less than 5%. Animals recovered from anesthesia and surgery in a warm chamber. They were then maintained in brooder cabinets with free access to food and wa-
ter. Two days after operation one brooder cabinet was transferred to a completely dark room.

After an additional 2 weeks the chicks were weighed and killed by decapitation. The brains were dissected into individual optic lobes and cerebral hemispheres as previously described (27), weighed, and frozen. The effects of light and dark before hatching and in the period immediately after hatch were investigated as follows. Eggs which had been incubated for 16 days were placed in an incubator in total darkness or subjected to continuous illumination from a 25-watt bulb at a distance of less than 30 cm. The embryos were not otherwise disturbed. One day before or 4 days after hatch the chicks were decapitated and brains dissected as described above. Butyrylcholinesterase activity at a substrate concentration of $4.4 \times 10^{-3}$ M and acetylcholinesterase activity at a substrate concentration of $4.7 \times 10^{-4}$ M were determined on homogenates of the frozen tissues (13). Enzyme activity is defined as $\mu$moles substrate hydrolyzed/minute/brain region. Fractions of the homogenates were taken for estimation of protein (24) with bovine serum albumin as a standard. The DNA was measured by a modification of the diphenylamine procedure (7, 27); RNA, by hydrolysis in 0.3 N KOH for 2 hours at 37°C followed by neutralization with HClO$_4$ (34) and precipitation of KClO$_4$ in the cold. The supernatant fluid was assayed for RNA by the orcinol method of Ceriotti (8) using cerebral ribosomal RNA (5) as a standard.

Brains were removed from decapitated birds and fixed in 20 vol of phosphate-buffered formalin for histological studies. They were transferred to fresh formalin after 24 hours and several days later were dehydrated in an ascending alcohol series (50-95%) terminating in dioxane. The tissues were then embedded in low-melting paraffin (52°C) and were sectioned at 8 $\mu$m through a plane which included both optic lobes and cerebral hemispheres. Sections were stained with Weil's method or hematoxylin and eosin.

Statistical evaluations were performed using the Wilcoxon signed rank test for paired replicates (37). The ratios reported in the tables represent the average of the individual ratios calculated for each bird.

**Results**

*Effects of Unilateral Enucleation on Weights of Optic Lobes and Cerebral Hemispheres of Chick Brain.* Chicks were enucleated 1 day after hatch and were then maintained under normal conditions of illumination (12 hours light, 12 hours dark) for a further 17 days. The optic lobes and cerebral hemispheres contralateral to the excised eye were referred to as the "blind" regions while those areas contralateral to the unoperated eye were referred to as "visual" regions.
Table 1A shows a considerable difference in the weights of blind and visual optic lobes from unilaterally enucleated birds exposed to normal illumination. A large difference was also found in birds that were maintained in total darkness from the third to the seventeenth day after surgery. Thus, removal of an eye with concomitant severance of the optic nerve rather than the presence or absence of light was the major factor determining lobe weight. The optic lobes innervated by the severed optic nerve gained no additional weight after enucleation (compare with Table 1C), while the lobes innervated by an intact optic nerve increased in weight to the same extent as optic lobes from control unoperated birds (Table 1B).

Unoperated birds were maintained in total darkness from the third to the seventeenth day after hatch. The optic lobes from these birds did not differ in weight from those of birds maintained under normal conditions of illumination, although the body weights of these two groups of chicks did differ greatly (Table 1B). This finding again suggested that major effects on the weight of the optic lobe did not occur merely in the absence of light but required severance of afferent innervation.
The paired weights of cerebral hemispheres from the enucleated birds were also compared internally with one another (Table 1A). A small but significant weight difference was found between the blind and visual hemispheres in the enucleated animals maintained under normal illumination. However, no difference was found between the hemispheres of the dark-maintained enucleated birds. This observation suggested that the difference observed in the light-maintained chicks was not directly related to removal of the eye but was secondarily derived from the differential activity of the two optic lobes. After unilateral visual stimulation in very young birds, potentials were observed in both the optic lobe and cerebral hemisphere on the contralateral side only (9). The absence of major functional interhemispheric connections in the immature avian brain, coupled with the absence of a corpus callosum, presumably reduces the degree of transfer of neural information between hemispheres (23). These phenomena may be the basis for the differential development of the two cerebral hemispheres in unilaterally enucleated birds. This concept of a qualitatively different effect of enucleation on cerebral hemispheres compared to that produced in the optic lobes is strengthened by the data on unilateral eyelid suturing (see following sections).

Unoperated control animals never manifested statistically significant differences in weight between regions dissected from the left and right sides of the brain.

Chemical and Enzymic Studies on Optic Lobes from Unilaterally Enucleated Chicks. The levels of protein, RNA, and cholinesterase were determined in the blind and visual optic lobes of chicks enucleated and maintained under conditions of normal illumination as described in the previous section.

The RNA and protein levels in the visual lobes were the same as those of 17-day-old unoperated controls. The blind lobes showed no accretion of total RNA or protein over the levels observed in 1-day controls corresponding to the age at which the eye was extirpated (Table 2). However, no difference in the DNA content of the visual and blind lobes was observed. Since the DNA content of the normal chick optic lobe increases only very slightly in the first 2 weeks after hatch (27), one would expect any interlobe difference in operated animals to be minor. The implication of this finding is that enucleation arrested cell growth in the optic lobe rather than altered total cell number. Maturation of the normal optic lobe between 1 and 17 days after hatch is largely characterized by increased cell volume and only a slight cellular proliferation.

Maturation has also been reported to result in an increase in the specific activity of certain enzymes related to nervous activity, such as specific acetylcholinesterase (36). The amount of this enzyme in the optic lobes of unoperated control birds rose between 1 and 17 days after hatch (Table 2).
In the visual lobes of enucleated birds, a normal rise was noted. However, in the blind lobes, enucleation (which totally suppressed the developmental rise in cell volume, protein and RNA in the blind lobe) also prevented the normal increase in the activity of acetylcholinesterase. The acetylcholinesterase content of specific cerebral regions may be related to the amount of acetylcholine in these regions (6). Since acetylcholine is thought to be a major neurotransmitter (12), this finding suggests that the number of synapses per unit area may increase during maturation of the optic lobe and that this increase is incomplete in the blind optic lobe.

The level of nonspecific cholinesterase was also examined in the optic lobes of operated animals (Table 2). This enzyme is thought to be confined to neuroglia cells in brain (15). Its activity was similar in both visual and blind lobes suggesting that enucleation did not result in significant gliosis in the blind lobe. The activity of butyrylcholinesterase was very low relative to acetylcholinesterase. To determine whether these activities were due to two different enzymes in chicken brain, we examined the effect of 284 C51 (Burroughs Wellcome), a specific acetylcholinesterase inhibitor (2). At $6.9 \times 10^{-5}$ M acetylcholinesterase was inhibited 71% and butyrylcholinesterase 20%, while at $6.2 \times 10^{-6}$ M acetylcholinesterase was inhibited 95% and butyrylcholinesterase 28%. This indicated that two different enzymes were being assayed and that acetylcholinesterase was not interfering with the cholinesterase assay by hydrolysis of butyryl choline.

**Histological Studies.** Histological examination of the optic lobes contralateral to the enucleated eye, showed the absence of normal myelination at the surface of the optic tectum. The superficial layers of the tectum were somewhat thinner than those of the partner lobe which appeared to
be indistinguishable in histological appearance from the optic lobes of control birds of the same age.

The blind optic lobes had less superficial myelin and slightly thinner marginal layers than the optic lobes of one day old chicks. This finding suggested that, in addition to a failure of normal development, the blind lobes might have undergone some transneuronal degeneration. No microscopic evidence for glial infiltration was obtained.

Studies of the Effect of Visual Deprivation by Procedures Not Involving Enucleation. Control animals maintained in total darkness from the third to the seventeenth day after hatch had brain weights and acetylcholinesterase levels identical to those in light-maintained chicks (Table 2). Since the increase in acetylcholinesterase levels of chick brain optic lobes is most rapid around hatch (36), a series of experiments on unoperated animals maintained in light or darkness at this period were performed one day before hatch, the acetylcholinesterase level of the optic lobes of chicks maintained in darkness continually from Day 16 of incubation was identical to the level of acetylcholinesterase from similar eggs maintained under continuous illumination for the same period (unpublished results, Bondy and Margolis). However, when chicks were maintained either in total darkness or in constant illumination from 16 days of incubation until Day 4 after hatch, the total activity of acetylcholinesterase in brain regions of the dark-maintained chicks was significantly lower than that in light-maintained chicks (Table 3). In contrast to the results of enucleation experiments, this difference was of similar magnitude in both cerebral hemispheres and optic lobes. Therefore, the level of acetylcholinesterase in the chick brain was in part determined by the state of illumination of the chick over this period. The absence of a specific anatomical locus for this effect suggested that it had a different basis from the differential effects of unilateral enucleation on optic lobe and cerebral hemisphere.

**TABLE 3**

<table>
<thead>
<tr>
<th>Protein/region (mg)</th>
<th>Light-maintained</th>
<th>Dark-maintained</th>
<th>Light/dark</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optic lobes</strong></td>
<td>5.69</td>
<td>5.47</td>
<td>1.04</td>
<td>N.S.*</td>
</tr>
<tr>
<td><strong>Cerebral hemispheres</strong></td>
<td>20.7</td>
<td>21.1</td>
<td>0.98</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Acetylcholinesterase activity/region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Optic lobes</strong></td>
<td>2.55</td>
<td>2.28</td>
<td>1.12</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td><strong>Cerebral hemispheres</strong></td>
<td>4.13</td>
<td>3.66</td>
<td>1.13</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

*N.S. = p > 0.05.

*Micromoles substrate hydrolyzed/region/min.
TABLE 4
EFFECT OF UNILATERAL EYE SUTURE AT 1 DAY ON WEIGHTS OF REGIONS OF 17-DAY-OLD CHICKS

<table>
<thead>
<tr>
<th>Region weight (mg)</th>
<th>Mean body weight (g)</th>
<th>Contralateral to nonsutured eye</th>
<th>Contralateral to sutured eye</th>
<th>Non-sutured sutured</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light-maintained</td>
<td>Optic lobes</td>
<td>88.3</td>
<td>85.2</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerebral hemispheres</td>
<td>353</td>
<td>340</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>Dark-maintained</td>
<td>Optic lobes</td>
<td>85.6</td>
<td>85.4</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerebral hemispheres</td>
<td>314</td>
<td>316</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*N.S. = p > 0.05.

A further series of experiments was carried out to determine whether the specific informational content of the visual input affected cerebral acetylcholinesterase levels. The eyelids of 1-day-old chicks from normally maintained eggs were unilaterally sutured. The birds were then kept under normal illumination until they were 3 days old, when half of them were transferred to total darkness. Seventeen days after hatch, the brains were assayed for protein and acetylcholinesterase. The cerebral hemispheres contralateral to the sutured eyes of the light-maintained chicks were significantly smaller than the hemispheres contralateral to the nonsutured eyes (Table 4). Surprisingly, no significant differences could be found in the corresponding pairs of optic lobes. Furthermore, this effect on cerebral hemispheres was totally light-dependent, since it was absent in the dark-maintained set of birds. Acetylcholinesterase values were always in proportion to lobe weights and significant differences were found only in the amount of this enzyme in the cerebral hemispheres from the light-maintained birds. These findings suggested that the major locus of the action of light on the brains of sutured birds was different from that in enucleated chicks.

Discussion

The anatomical arrangement of the avian brain permits the exploration of asymmetrical changes after unilateral modification of visual input. Marked metabolic differences between two symmetrically equivalent regions within a single animal may result, in the absence of procedures which involve intracranial surgery. No direct projections from the retina to the cerebral hemispheres exist. The principal site of termination of retinal fibers is in the op-
tic tectum (10). These characteristics, coupled with the complete decussation of the optic tract at the chiasma (10), resulted in the localization of major histological changes after enucleation within a single optic lobe. Furthermore, since a structure corresponding to the mammalian corpus callosum is absent, there is consequently a low degree of interhemispheric communication, in young birds especially (9). It would appear that effects of unilateral visual deprivation on the cerebral hemispheres may be predominantly confined to a single hemisphere. Moreover, in the present studies the paired cerebral hemispheres and optic lobes were bilaterally identical in control birds with regard to several chemical parameters. Levine (23) found that neither avian eye is dominant in learning various tasks.

The chicks used for these studies were at an age when the brains were maturing rapidly. At this period, the young brain is actively forming new synapses and is considerably more responsive to modification than the mature brain (19). However, cellular proliferation of the optic lobes and cerebral hemispheres (27) are largely completed at this time. This is also true for myelination (16, 35). The profound differences between the two optic lobes of a unilaterally enucleated bird appeared to be the result of at least two processes. The major mechanism was probably the localized failure of lobe development due to the elimination of retinotectal connections. Another mechanism was transneuronal degeneration. Pronounced differences between paired optic lobes occur as early as 7 days after enucleation (28) without histological or enzymatic evidence of gliosis. The present results appear to be primarily attributable to arrested development.

The major differences in the paired optic lobes after unilateral eye extirpation may have resulted from cessation of afferent nervous impulses and, thus, of electric activity of a large fraction of the optic lobe (31). Another causative factor may have been lack of substances normally secreted at the synaptic junctions by axons constituting the optic nerve. Axoplasmic flow in this nerve has been shown to have a fast-moving component (17, 20). Thus the absence of an essential neurohormone at the synapse could rapidly occur after nerve section. Development of the optic lobe after severance of the optic tract resembles the retarded maturation of the brain in the absence of thyroid hormone. Thyroid deficiency in the immature rat results in arrested cerebral development, which involves reduced cell size (3) and diminished dendritic growth (22). Similarly, our data on the enucleated chick demonstrate failure of normal attainment of cell size and acetycholinesterase content. Long-term effects of unilateral eye removal on the development of the optic lobes in the frog were similar to our findings in the chick but were also accompanied by hypoplasia and degeneration (4).

Unlike enucleation, maintenance of birds in total darkness at various stages of development had no effect on weight or protein content of cerebral
hemispheres or optic lobes. However, in chicks maintained in darkness from 16 days of incubation to 4 days after hatch, acetylcholinesterase levels rose significantly less than in light-maintained birds. In contrast to the region-specific effects of eye removal, this effect was equally marked in the hemispheres and lobes.

Several laboratories have reported differences in acetylcholinesterase levels in dark- and light-maintained animals (18, 21, 25). Some of these long-term effects have been found to be anatomically widespread (21) and to vary with the state of maturity of the animal (25). However, these studies, as well as those reported here comparing unoperated light- and dark-maintained animals, were not internally paired. Therefore, it may be that the effects of varying illumination were mediated by systemic hormones.

The reduction in activity of acetylcholinesterase implies reduction in the level of acetylcholine (6) and thus a reduction in the number of synaptic junctions in dark-maintained animals. Histological evidence suggests that the number of synapses in some areas of such animals may be reduced (11). The absence of any effect of rearing unoperated birds in the dark for 2 weeks (3–17 days after hatch) may be ascribed to the development of spontaneous retinal discharges in the chick at around 3 days after hatch, which were sufficient to maintain the optic lobe in a normal state. It is at this time that the electroencephalogram becomes identical to that of an adult bird (9).

It has been reported that when adult birds were blindfolded less dramatic changes occurred than those after enucleation (1). However, long-term experiments involving eye suture in the rat have been found to have an effect histologically identical to that resulting from enucleation (14). In the present studies, unilateral eye suture resulted in effects which differed from those of enucleation. After 17 days of the former treatment, differences in weight and acetylcholinesterase content between the internally-paired cerebral hemispheres were identical to those resulting from enucleation for the same period. This unilateral effect was never seen in dark-maintained animals. Astonishingly, no significant differences in weight, protein, or acetylcholinesterase activities could be found between the paired optic lobes. Thus, the major result was in the cerebral hemispheres, a region with little or no direct innervation from the optic tract (10, 33). Unilateral eyelid suture has advantages over enucleation in that it is reversible and offers greater versatility in studies of recovery after visual deprivation.

Visual stimuli received by the two optic lobes of monocularly sutured chicks differ not only in light intensity but also in the information content of impulses reaching each lobe. Visual correlation and control of activity can be accomplished only with the unsutured eye, almost exclusively via the contralateral optic lobe and cerebral hemisphere. It may be that monocular
suturaing still allows the contralateral optic lobe to receive sufficient stimulation albeit unpatterned to develop normally. However, the cerebral hemisphere, which contains the major associative area for visual information of the avian brain (32) and receives considerable innervation from the ipsilateral optic lobe (9), may be more dependent on the information content of afferent input.

Our data suggest the existence of several parameters which determine the final size and composition attained by a brain region. Some of these factors are endogenous to the developing chick and cannot be manipulated by varying sensory input to the brain. Thus, the development of adult acetylcholinesterase levels may be in large part independent of afferent nervous stimulation. Other factors may be indirectly dependent on sensory input and mediated by systemic hormones. The effects observed in dark-main­tained unoperated chicks shortly after hatch may be of this nature. Other factors which influence the development of a brain region may be more precisely localized and more directly related to the innervation of a specific brain region. The normal development of the optic lobe is dependent on the integrity of the optic nerve. If this nerve is sectioned, normal growth cannot take place under any circumstance. Thus, a systemic hormone cannot compensate for this effect. Finally, the complete development of certain brain regions may not only require nervous input, but this input may have to be encoded in a meaningful pattern. The complete development of the cerebral hemisphere of the chick may require that afferent stimulation is information-rich. The necessity for complex sensory input to permit full cerebral maturation has been suggested previously (21). The system described in the present investigations may permit the mechanisms whereby this is achieved to be separated and their individual contributions determined.

References


