Bilateral Form Deprivation in Monkeys
Electrophysiologic and Anatomic Consequences

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The response characteristics of neurons in the striate cortex are described for rhesus monkeys that underwent bilateral form deprivation by surgical closure of the eyelids starting within the first month of life and lasting for 2, 6, 7, 13, or 16 weeks. The monkeys had been tested for visual deficits resulting from these experimental deprivations. Single-unit recordings from the striate cortices of these animals showed a single significant abnormality; the absence of excitatory binocular input. Whereas, 76% of the neurons in the foveal striate cortex of the normal animals were binocular, fewer than 20% of the neurons in the experimental monkeys were binocular. However, each eye was well represented by monocular cells. As demonstrated in oblique microelectrode penetrations, the cortical eye-dominance zones for each eye appeared to be of equal width with sharp transitions at the monocular boundaries. The sizes of the cells of the lateral geniculate nuclei were smaller (~15%) than those in controls. Binocular form deprivation early in life has its most obvious effect on the physiology and function of cortical binocular neurons and secondarily on the size of neurons of the lateral geniculate nucleus. Invest Ophthalmol Vis Sci 32:2328–2336, 1991

The behavioral, electrophysiologic, and anatomic consequences of incongruous visual input on the immature visual system of experimental animals were the subject of numerous studies in recent years.1–38 Conditions known to produce amblyopia in humans were simulated by raising monkeys with unilateral lid suture,†7,11–14,18,20,31–33,35,39–49 surgically or prismatically induced strabismus,22,49 or anisometropia.55–58 Two amblyopiogenic factors were identified: abnormal binocular interactions as a result of unequal visual inputs and deprivation of form vision.33 The contribution of each of these factors to clinically different types of amblyopia was recognized, and the period of susceptibility, during which the normal development of various visual functions can be altered by abnormal visual stimulation, was defined in experimental animals and humans.13,15,17,25,39–65 However, little is known about the effects of bilaterally and equally decreased visual input on the immature afferent visual pathways of primates. Such information from experimental animals would be clinically interesting in connection with the optimal management of bilateral congenital cataracts, surgical aphakia, or isohypermetropic refractive errors.

We report the electrophysiologic and anatomic consequences of bilateral form deprivation by surgical lid closure in visually immature Macaca mulatta. Extensive behavioral testing of these animals66 showed that none of the bilaterally form-deprived monkeys had binocular vision, using measurements of binocular summation or stereodetection, even if the animal had normal monocular visual functions. Moreover, all monkeys reared with bilateral form deprivation for 7 weeks or longer had reduced spatial contrast sensitivity for both eyes.

Materials and Methods

Subjects

Data were gathered from 14 rhesus monkeys, M. mulatta (five bilaterally deprived, three control animals for electrophysiology, and six control animals for the morphology of the lateral geniculate nucleus). All animals were obtained, cared for, and used according to all applicable regulations and the ARVO Resolution on the Use of Animals in Research. The lids of both eyes of monkeys to be deprived of form vision were fused surgically when they were about 1 month of age (±3 days) and then parted after various periods of deprivation (2, 6, 7, 13, or 16 weeks). The behavioral responses of these monkeys during deprivation, the events preceding behavioral testing, and
the behavioral results are described in detail in the companion paper.66

**Electrophysiologic Procedures**

At the end of the extensive behavioral testing period of about 2.5 years, the monkeys were prepared for electrophysiologic recordings from single cells of the striate cortex, using standard techniques. The monkeys initially were anesthetized with ketamine hydrochloride (15 mg/kg), the scalp was encircled by injections of lidocaine, an incision made along the midline, the bone exposed over the striate cortex, and a hole 8 mm in diameter was trephined over the foveal representation. The dura mater was removed carefully, and the hole was filled with warm bone wax. A head holder was attached to the skull at the anterior midline position, and a tracheostomy was done. The monkey was paralyzed by an infusion of pancuronium bromide 0.4 mg/kg/hr, and anesthesia was maintained throughout by an infusion of thiopental 8 mg/kg/hr. Respiration was maintained at about 20 strokes/min to keep the expired CO₂ between 4.5–5.0% with adjustments of the stroke volume of the respirator. The monkey was kept at a normal core temperature of 38°C as it faced a tangent rear-projection screen positioned 1 m in front of the eyes. The monkey’s refractive errors were determined by retinoscopy and corrected for the viewing distance with contact lenses. The foveal representations on the screen for each eye were identified using a reversible ophthalmoscope, which projected a line of sight from the fovea through the center of the pupil to the screen.

As the tungsten electrode was advanced by a Burroughs microdrive (Burleigh Instruments, NY) along an oblique penetration path, variably sized slits of light from a hand-held projector were used to stimulate and map the receptive fields of isolated single neurons in the striate cortex. In some instances, square-wave luminance gratings of 80% contrast were projected through a dove prism and swept to and fro by a galvanometer-driven mirror. All judgments for orientation specificity and degree of eye dominance were made during the recording session by at least two observers who inspected the neuronal response on oscilloscopes and listened to the amplified response on a speaker. The criterion for preferred stimulus orientation was that orientation of a slit of light, dark bar, or grating that produced the highest response rate of the neuron being tested. Orientation tuning was defined as the range of stimulus orientations that produced any response above background discharge of the neuron. Eye dominance of each cell was assigned according to the seven-category classification scale of Wiesel and Hubel,36 where cell categories 1 and 7 are strictly monocular cells driven by the ipsilateral or the contralateral eye, respectively. The number of binocular cells of categories 2–6 were summed to yield the percentages of binocular cells for the overall neuronal sample. Neuronal responses were sampled about every 50 μm along the electrode path; the angle and direction were selected to intercept as many eye-dominance columns as possible. All recordings were through striate cortex representing the central 1–2° of the visual field. Data are presented for the five bilaterally deprived monkeys and for three control monkeys.

**Histologic Procedures**

At the end of the extensive behavioral testing period (24–36 hr), the monkeys were anesthetized deeply with pentobarbital 50 mg/kg and perfused with 1–2 l of a mixture of 2% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer. The lateral geniculate nuclei (LGN) were blocked and embedded in nitrocellulose, sectioned at 30 μm, stained by thionin, and individual cell bodies projected through a Zeiss microscope tracing tube (Carl Zeiss, Thorawood, NY) and drawn at 1800X magnification. Using a small computer and a digital tablet, the average sizes and standard deviations for cell samples (n = 50 cells each) from each of the LGN layers were calculated. Data are presented here for the five bilaterally lid-sutured monkeys and compared with data from six normal monkeys.

**Results**

The most dramatic finding in binocularly visually deprived monkeys was the loss of excitatory binocular neurons from the foveal striate cortex (Fig. 1A). In the sample of 319 cortical cells recorded from the striate cortices of three control monkeys, 76% had excitatory input from both eyes. All experimental animals showed reductions of excitatory binocular inputs. Examination of the 517 neurons recorded from the five experimental animals showed that the relative proportions of binocular to monocular cells in striate cortex was reversed from those of normal animals; 82% of the sample was monocular, with only 18% of the cells retaining functional excitatory binocular connections.

This loss of excitatory binocular cells is shown in the average eye-dominance response histogram for cortical cells (Fig. 1A). There was a dramatic reduction of the number of binocular cells in the middle of the distribution where many balanced binocular cells normally are found. Moreover, the absence of binocularly balanced neurons was consistent for all of the experimental monkeys, regardless of the duration of the deprivation. This is shown clearly in the individual eye-dominance histograms in Figure 1B where the
two eyes are well represented in the striate cortex by monocular cells.

One of the experimental monkeys (BD-2) had significant amblyopia in the left eye, and it was interesting to see if there was a correlated reduction in the number of neurons in the cortex serving that eye. Of a sample of 127 units recorded from the striate cortex of BD-2, most (83%) were monocular, and only slightly fewer cells were dominated by the amblyopic (42%) eye than the nonamblyopic eye. The inset in Figure 1B shows the eye-dominance histogram for BD-2 compared with the average histogram of the experimental monkeys. Both eyes of this monkey were well represented, and the individual eye-dominance profile was not different from the other experimental monkeys. Therefore, although bilateral form deprivation greatly reduced the number of excitatory binocular neurons, we did not find the large shift in eye dominance away from the amblyopic eye and toward the better eye commonly found with monocular deprivation and unilateral strabismus.

For each of the experimental animals, the monocular eye-dominance domains appeared to be of approximately equal width, as judged by the oblique electrode excursions through the striate cortex. Moreover, the transition between tangential monocular domains of one eye to the monocular domain of the other eye occurred within a very short distance (usually within 50 μm), attesting to a loss of binocular neurons that normally have their highest concentrations directly above and below the transition zones of monocular inputs in layer 4C. It was as though the exclusively monocular eye-dominance boundaries had been extended outside layer 4C for the full thickness of cortex. In 53 electrode transitions from one monocular eye-dominance domain to the other, the average tran-

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**Fig. 1.** The average eye-dominance response profiles of striated cortical neurons recorded from normal and binocularly deprived monkeys (top). Although the distribution for the control subjects shows a fortuitously larger sample of cells dominated by the ipsilateral eye, it is the significantly high percentage of binocular neurons (76%) that is compared to the experimental group which had only 18% binocular cells. The individual eye-dominance profiles for the five experimental monkeys are shown in bottom figure. The figure inset in the bottom figure shows that the response profile of monkey BD-2 does not differ in eye-dominance compared to the other experimental monkeys.
position distance was about 90 μm (standard deviation, 60). In 18 (33%), those transitions occurred within 50 μm, or from one electrode sampling position to the next.

The stimulus orientation preferences for neurons of the striate cortex did not differ from normal in any obvious way. As the electrode progressed along an oblique penetration path, the best orientation of a stimulus slit of light, dark bar, or grating shifted systematically, as has been described for normal monkeys.\textsuperscript{69-71} Figure 2A displays the individual orientation preferences for the 517 neurons recorded from the five experimental monkeys. A wide variety of orientation preferences were found.

The precision of the orientation tuning was determined for the sample of cortical neurons and is shown in Figure 2B for both the monocular and the small sample of binocular neurons. It was generally the case that the full range of orientation tuning was seen in the experimental animals, with only a slightly higher percentage of the sample having broad tuning functions. Our conclusion was that bilateral form deprivation has minimal permanent effect on the precision of orientation tuning in striate cortex.

Finally, when the average size of the principal relay neurons of the LGN were measured and compared with those in normal monkeys, they were found to be consistently smaller. Figure 3 shows that the average size of the cells (n = 50) of each of the 12 magno- and parvocellular layers of the LGN were significantly smaller than normal by 15% (paired one-tailed student t-test; t = −7.5; df = 11; \( P < 0.001 \)). Therefore, bilateral form deprivation, which reduced the number and function of cortical binocular neurons, also resulted in smaller cells in the LGN.

Discussion

The absence of binocular form stimulation early in life leaves the primate visual cortex with very few neurons excited by binocular stimulation, sufficient reason to account for the absence of binocular summa-
Binocular Deprivation Group

Individual Orientation Analysis (n=517)

Percent of Cells [%]

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Fig. 2. The percentage of cortical neurons having different optimal stimulus orientations for each of the five experimental monkeys (A). Five best orientation classes are indicated along the bottom (with reference to the face of a clock) along with the relative percentages of neurons from each animal which showed no stimulus orientation bias (*). The average orientation tuning classification for 517 binocular and monocular cells recorded from the striate cortex of the binocularly deprived animals (B). VF = very fine tuning, ±5 deg.; F = fine tuning, ±10 deg.; M = moderate tuning, ±20 deg.; B = broad tuning, any response bias; and * = no measurable bias in tuning.

The dramatic reduction of excitatory binocular input to striate neurons after bilateral form deprivation is sufficient to explain the absence of binocular summation and failure of stereopsis in these animals. However, because we did not test for them, we do not know whether inhibitory binocular interactions also may play a role.

We did not find a significant association between the psychophysical results on contrast sensitivity and amblyopia and the numbers or precision of monocular neurons in the striate cortex (BD-2). That is, there was only a minor shift in cortical eye dominance away from an eye with substantial amblyopia in favor of the better eye. Moreover, there was no systematic effect of the duration of the binocular form deprivation on the numbers of either binocular neurons or neurons controlled by each eye. For example, animal BD-2 had amblyopia in one eye, but that eye was well represented in the cortical neuronal sample. This is unlike the condition of monocular deprivation where only 2 weeks of eyelid closure produced a clear shift in eye orientation and for the stereoblindness described for these monkeys. These results are consistent with earlier reports where bilateral form deprivation by lid fusion was used on monkeys and kittens. The effects in kittens appear to be less severe and transient with some recovery of binocular cell connections after a period of binocular visual experience. This is evidently not the case with binocularly deprived primates, who have persistent behavioral, physiologic, and anatomic defects after bilateral deprivation despite subsequent exposure to normal binocular conditions for as long as several years. These defects were retained during binocular viewing and in the absence of any observable strabismus or oculomotor abnormalities. Therefore, in primates, the effects of bilateral form vision deprivation must be considered permanent.
dominance in favor of the nondeprived eye. However, in this case where 2 weeks of bilateral form deprivation preceded the conditions inducing the amblyopia, both eyes were likely to be weakened in their absolute control over cortical neurons, allowing substantial amblyopia to develop in one eye without a concurrent gain in dominance over cortical cells by the opposite weakened viewing eye. It may be that the depth of amblyopia is determined as much by the robustness of the normal eye during development as by the disadvantage of the affected eye. Finally, we did not measure the spatial resolution of these monocular cortical neurons, as was done in other studies where they were shown to be less acute than normal. A blunted resolution of cortical neurons, presumed to have occurred here, would be sufficient to explain the amblyopia found in the behavioral experiments.

Earlier studies commented on the increased numbers of cortical neurons unresponsive to visual stimulation after bilateral closure. However, our results do not reliably show this to be the case. We found most neurons to be normally responsive to visual stimulation, but most were controlled by only one eye. Therefore, it would appear that the experience of binocular form deprivation is inhospitable to the nurture and development of binocular cells, failing to support the congenital binocular connections, and leaving the striate cortex populated by a high proportion of functionally monocular neurons.

The finding that the cells of the LGN were smaller than normal illustrates the effect of binocular sensory disuse, rather than changes produced by binocular competition, which is usually of greater magnitude. Here the average reduction in cell size was about 15%, relatively small compared with the 30–40% shrinkage reported after monocular deprivation. The argument that LGN cells shrink after some binocular competition was supported indirectly by the smaller effect seen here with binocular form deprivation. It seems that the effects of both sensory disuse and binocular competition function in monocular deprivation; binocular deprivation produces a somewhat smaller shrinkage of cell size, presumably through sensory disuse alone. Others made similar
Fig. 3. The average cell sizes of principal cells of the magno- (M) and the parvo-cellular (P) divisions of the lateral geniculate nucleus of normal (± 1SD shown in hatched area) compared to the binocularly deprived monkeys. The cells from the binocular deprived monkeys were significantly smaller than normal by an average 15% for the 12 LGN layers (one tailed t-test for paired samples, df = 11, t = 7.5, P < 0.001).

measurements in two monkeys bilaterally form deprived from birth for 65 or 376 days and killed immediately thereafter. Compared with those of normal animals, these authors found robust effects in magnocellular layers (~30%) but only slight effects in parvocellular layers after 65 days. After 376 days, deprivation effects as large as that reported for monocular deprivation (~30%) were reported for all LGN layers. These mixed results are difficult to compare with our study because we initiated the bilateral form deprivation when the monkeys were 30 days of age, and there was an intervening 2-year period of bilateral visual experience before termination of the experiment. Any recovery of function during this time may have ameliorated the initial deprivation effects on LGN cell size.

Another earlier study concluded that bilateral form deprivation at birth arrested the growth and development of LGN cells, although the data from two monkeys presented in that study did not support such a claim. As monkey LGN cells reach full size well before 30 days of age, the smaller cells found in our study must represent a shrinkage, rather than a failure of normal cell growth. Because cortical binocular neurons are lost consequent to either monocular or bilateral form deprivation, the role of binocular cortical feedback to the LGN deserves further study.

From clinical experience, it is well known that there are several conditions occurring during visual immaturity that may interfere with the development of normal stereopsis and lead to the debilitating condition of amblyopia. In addition to those that create a competitive stimulus situation and thus disrupt congruous binocular visual input, such as in strabismus, symmetric visual deprivations from bilateral congenital cataracts or after bilateral occlusion also are known to impair stereopsis. Even milder forms of bilateral visual deprivation, such as those that occur in patients with uncorrected high hypermetropia without strabismus, have a devastating effect on stereopsis.

What are some of the clinical implications of these physiologic and anatomic results? The most obvious comparison of these animal results is with the visual capacity of humans after early treatment for infantile cataracts. Several studies measured visual acuity, contrast sensitivity, and stereopsis in children who had therapy for bilateral cataracts during infancy. The physiologic results from monkeys in our study can account only for the absence of stereopsis and binocular summation after bilateral form deprivation, whether by bilateral cataracts in children, or bilateral lid suture in monkeys. The latter destroys the excitatory binocular input to striate cortex, and it is likely that there are similar defects in the cortical visual system of children with similar form deprivation from cataracts.

Relative to the devastating effects of monocular form deprivation where the normal eye dominates the vast majority of cortical cells, bilateral form deprivation leaves large numbers of striate cortex neurons responsive to stimulation of each eye. Moreover, for comparable durations of form deprivation, the resultant amblyopia after bilateral deprivation is relatively mild. Therefore, under conditions in infancy where form deprivation is unavoidable, it would be better that the deprivation be binocular rather than monocular in the interest of preventing amblyopia. Stereopsis may be impaired or destroyed in any event because cortical binocular connections seem to be the most fragile of neonatal neural connections.

Key words: amblyopia, monkeys, binocular neurons, visual cortex, visual disuse, binocular competition, visual deprivation

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