
Anisometropic amblyopia in *Macaca nemestrina* monkeys produced by atropinization of one eye during development

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Two macaque monkeys were reared with daily administration of the cycloplegic drug, atropine, to one eye until 8 months after birth. Behavioral testing of contrast sensitivity functions in the atropinized eyes during the rearing period demonstrated that this rearing procedure caused the treated eyes to be chronically defocused. Measurement at the age of 6 or 8 months of contrast sensitivity while optically correcting the defocus demonstrated that an amblyopia had developed in the treated eyes. Furthermore, the relative magnitude of the amblyopia across spatial frequencies was similar to the relative magnitude of the defocus. Subsequent testing at 1 year of age demonstrated that the deficits persisted for several months after termination of the defocus rearing. This rearing procedure appears to provide a reasonable primate model with which to study anisometropic amblyopia in monkeys. (INVEST OPHTHALMOL VIS SCI 22:228-233, 1982.)

Key words: amblyopia, atropinization, animal model, defocus, contrast sensitivity function, acuity, development

Numerous studies have demonstrated that infant humans and monkeys have poorer spatial resolution than adults, as reflected in measurements of acuity and contrast sensitivity.¹⁻³ The developmental processes by which vision reaches adult levels have important clinical and theoretical implications. Ab-

normal development can lead to a permanent impairment in spatial resolution, i.e., an amblyopia.³

Important factors that influence this development are the spatial properties of the visual stimuli to which the developing organism is exposed. For example, lid suture deprives the eye of contrast at all spatial frequencies. Closure of an infant monkey's eyelid during development results in poor acuity in that eye later in life.⁴

Optical defocus, on the other hand, produces a differential loss of contrast across spatial frequencies. Optical defocus has been produced in developing animals by surgical removal of the lens from an infant monkey eye,⁵ by use of an external defocussing lens in kittens,⁶ and by daily administration of a cycloplegic drug to kittens.⁷ Physiologic recordings from the dorsal lateral geniculate nuclei and the visual cortices of these animals

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indicate that defocus during rearing affects both ocular dominance and spatial tuning of single neurons. Thus defocus rearing may provide a method for producing anisometric amblyopia. However, behavioral results have not been reported to date that demonstrate that the animals raised with defocus are in fact amblyopic.

In this article we present behavioral measures of contrast sensitivity in monkeys reared with daily administration of the cycloplegic drug, atropine, to one eye. Our purposes in doing this experiment were (1) to determine whether defocus during early rearing does in fact lead to an amblyopia and, if it does, (2) to examine the relative magnitude of the contrast sensitivity loss across spatial frequencies. For example, since the optical defocus will produce more contrast attenuation at high spatial frequencies than at low, it might be expected that contrast sensitivity loss would also be more severe at high spatial frequencies.

Methods

Subjects. Two infant pigtail monkeys (*Macaca nemestrina*) were separated from their mothers and reared in the nursery facilities of the Infant Primate Laboratory at the University of Washington. Beginning at 10 days of age, one drop of 1% atropine sulfate was given to one eye (referred to in this paper as the "treated" eye) twice a day. Drops were administered to the right eye of monkey LD and to the left eye of monkey TC. This atropinization was continued until approximately 8 months of age.

Behavioral testing. Methods for generating and calibrating the visual stimuli as well as behavioral methods for training and testing the monkeys are described in detail elsewhere.⁸ Briefly, the animals were trained on an operant visual discrimination task while in a specially designed face-mask cage. Shutters in front of each eye hole in the face mask allowed either eye to be tested separately. Each animal was tested on a spatial two-alternative forced-choice discrimination task. On each trial the monkey had to discriminate a sinusoidally modulated grating from a homogeneous field of equal average luminance. The method of constant stimuli was used to present four or five contrast levels at each of a number of spatial frequencies.

Data analysis. Methods used are described in

more detail elsewhere.⁸ First, probit analysis was used to estimate contrast threshold (and standard errors of this estimate) at each spatial frequency. Then the results were summarized in the form of contrast sensitivity functions (CSFs) in which contrast sensitivity ($1/\text{contrast at threshold}$) is plotted as a function of spatial frequency (see Figs. 1 and 2). The smooth curves in these figures are best-fitting double-exponential functions.

Assessment of refractive error. We typically find that cycloplegic infant monkeys have an apparent hyperopia. (This finding may be caused by the small eye artifact.⁹) The magnitude of the hyperopia decreases over the first several postnatal months. This uncertainty about refractive state makes it impossible to specify exactly the amount of defocus produced by the cycloplegia. Nevertheless, we refracted our animals periodically to provide an estimate of defocus.

Monkey LD was refracted with retinoscopy at 2 weeks of age (OD = +5.25 +1.25 × 80°; OS = +5.50 +1.50 × 70°) and at 6 months of age (OD = +0.50; OS = +0.50 +1.00 × 90°). Monkey TC was refracted only once with retinoscopy, at 9 months of age (OD = +2.50 +1.25 × 180°; OS = +4.00 +0.50 × 90°).

Refractive state was also assessed behaviorally by measurement of contrast sensitivity to high-frequency gratings viewed through a series of spherical lenses. The lenses resulting in optimum performance for monkey LD when tested at a viewing distance of 120 cm were: OD = +1.00 D; OS = +1.50 D. These values are within one diopter of the predicted spherical equivalent values at this viewing distance based on the retinoscopy results at the same age. The lens values for monkey TC, when refracted behaviorally under the same conditions at 7½ months, were: OD = +1.25 D; OS = +2.00 D. These values are two to three diopters less than would have been predicted on the basis of the retinoscopy conducted 6 weeks later at 9 months of age. The reasons for this discrepancy are unknown but may be related to the small eye artifact⁹ or to a large depth of focus.¹⁰

All of the estimates of refractive error, retinoscopic and behavioral, indicate that both eyes of both monkeys were hyperopic to various degrees throughout the rearing period. This hyperopia should not have had any defocussing effect on the normal eye, since it could potentially overcome the hyperopia through accommodation. The treated eye, on the other hand, was constantly defocused for targets at all viewing distances, with the greatest blur occurring for close objects.

Pupil size. Pupil sizes of the treated eyes during

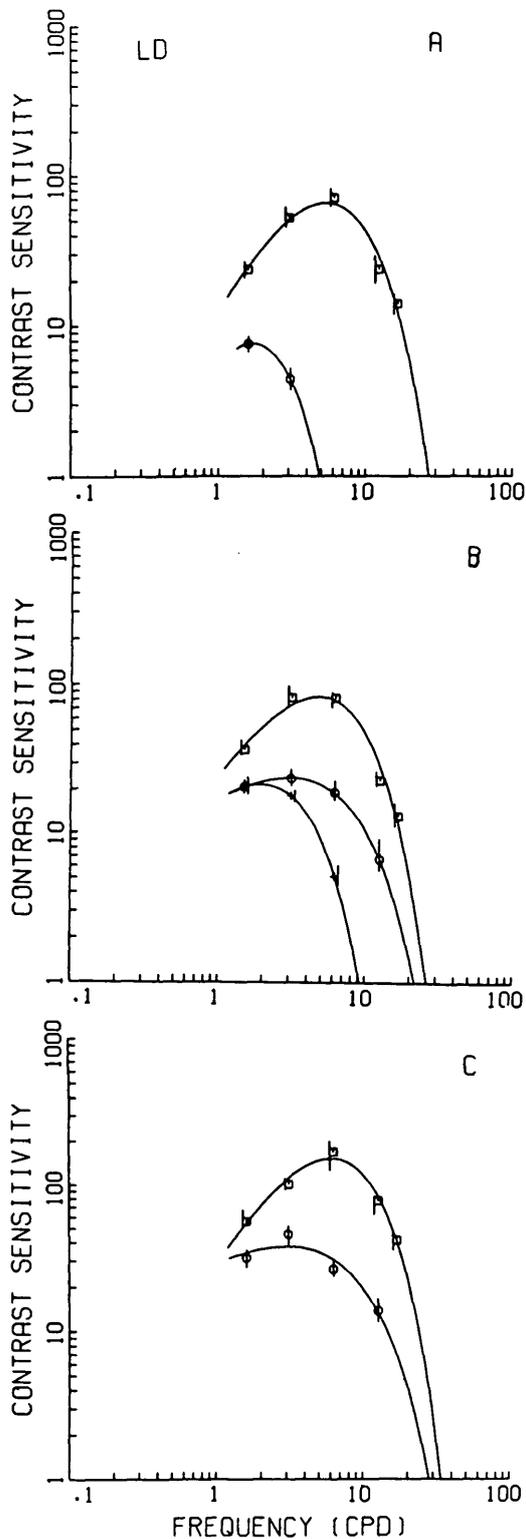


Fig. 1. For legend see right column.

rearing and of both treated and normal eyes during cycloplegic behavioral testing were approximately 10 mm. Pupil sizes of the normal eyes when tested under natural viewing conditions were approximately 6 mm.

Results

CSFs obtained from monkeys LD and TC are shown in Figs. 1 and 2. Figs. 1, *a*, and 2, *a*, show results obtained during the rearing period at four and five months of age respectively. The CSFs from the normal eye, tested under natural viewing conditions, showed peak contrast sensitivities near 6 cy/deg and extrapolated high-frequency cutoffs near 25 cy/deg. These results are consistent with binocular results obtained from normally reared monkeys in this age range.^{3, 11}

The results from the treated eyes, on the other hand, showed much poorer contrast sensitivity and demonstrate that the treated eyes could not see these grating targets clearly during the rearing period. The treated eyes showed an overall decrease in absolute sensitivity to contrast, as evidenced by downward shifts of the CSFs. They also showed

Fig. 1. Contrast sensitivity functions obtained from the treated and normal eyes of monkey LD at various ages. The data points show the monkey's sensitivity to contrast of sinusoidally modulated grating stimuli measured at a number of spatial frequencies. Error bars indicate ± 1 S.E. of the sensitivity measures. **A**, Results obtained during the atropine rearing period at 4 months of age with no optical correction. *Squares*, Results obtained from the normal eye under natural viewing conditions; *circles*, results obtained from the treated eye tested while under cycloplegia. **B**, Results obtained during the rearing period at 6 months of age. *Squares*, Results from the normal eye; *circles*, results from the treated eye. Both were tested under cycloplegia as the monkey viewed the gratings through an optical correcting lens. *Cross symbols*, Performance of the treated eye tested under cycloplegia and with no optical correction. **C**, Results obtained after the end of the rearing period at 1 year of age. Both eyes were tested under natural viewing conditions. *Squares*, Normal eye; *circles*, the previously treated eye.

lateral shifts of the CSFs toward lower spatial frequencies. CSF peaks shifted down to less than 2 cy/deg for monkey LD and down to about 3 cy/deg for monkey TC. High-frequency cutoffs, which are analogous to measurements of acuity, shifted down to about 5 cy/deg for monkey LD and about 10 cy/deg for monkey TC.

We repeated these measurements on monkey LD's treated eye at 6 months of age. The results are shown in Fig. 1, *b*. Comparison of the results from the treated eye at the two ages reveals that there had been some improvement with age, but contrast sensitivity remained much worse than that in the normal eye. The CSF peak was still less than 3 cy/deg and the high-frequency cutoff was less than 10 cy/deg.

It is impossible to tell from these data whether the monkeys had developed an amblyopia at these early ages or whether the poor results from the treated eyes are simply a reflection of the amount of optical degradation and defocus produced by the large pupils and relaxed accommodation. To answer this question we tested while optically correcting the defocus. These results are shown in Figs. 1, *b*, and 2, *b*. The two eyes of both monkeys were tested while under cycloplegia but with correcting spherical lenses that brought the grating target into optimum focus. Clearly, performance of the treated eyes remains poorer than that of the normal eyes even when the defocus is corrected optically.

Fig. 2. Contrast sensitivity functions obtained from the treated and normal eyes of monkey TC. Symbols refer to the same conditions as in Fig. 1. **A**, Results obtained during the rearing period at 5 months of age with no optical correction. The treated eye was under cycloplegia. The normal eye was tested under natural viewing conditions. **B**, Results obtained near the end of the rearing period at 8 months of age. Both eyes were tested under cycloplegia as the monkey viewed the gratings through an optical correcting lens. **C**, Results obtained after the end of the rearing condition at 1 year of age. Both eyes were tested under natural viewing conditions.

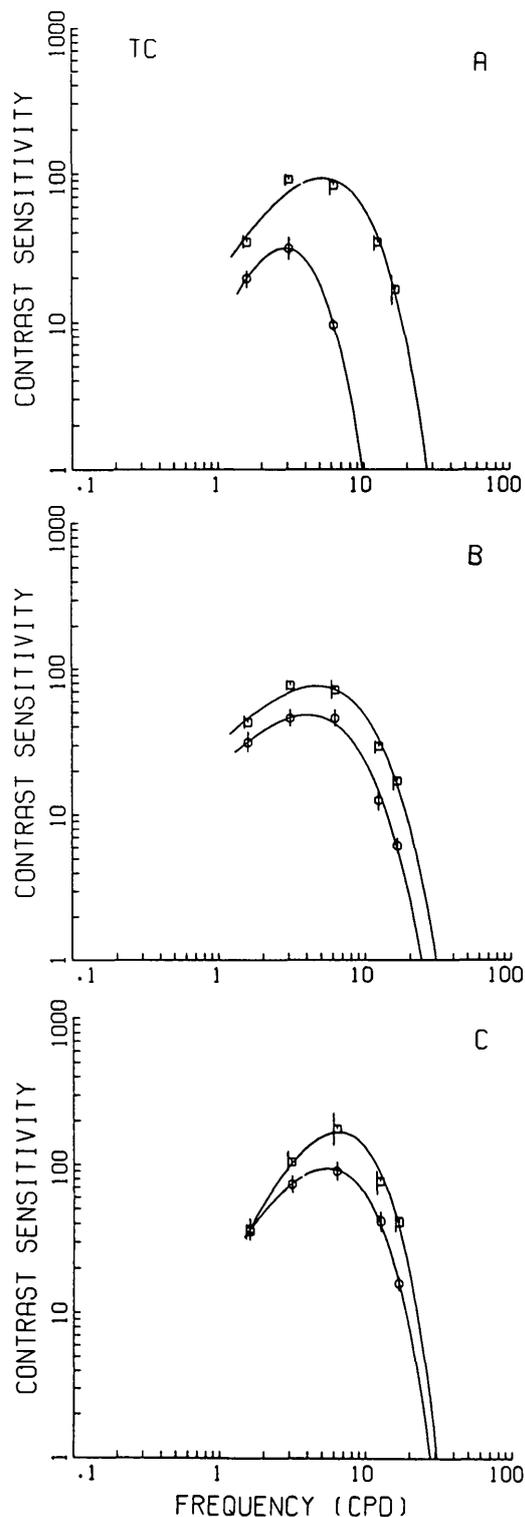


Fig. 2. For legend see left column.

The data for monkey LD (Fig. 1, *b*) were obtained at 6 months of age. Results from the normal eye were very similar to those obtained under natural viewing conditions, but the treated eye, under these conditions of optimum focus, still showed substantially reduced sensitivity, which was apparent at all spatial frequencies tested.

Similar results obtained from monkey TC under corrected viewing conditions at 8 months of age are shown in Fig. 2, *b*. Contrast sensitivity was substantially poorer at all spatial frequencies tested in the treated eye. This same experiment was replicated on monkey TC viewing through 5.5 mm artificial pupils so that comparisons could be made with conditions more similar to natural viewing through normal-sized pupils. The results obtained showed a difference between the two eyes of the same magnitude as that shown in Fig. 2, *b* (results not shown).

The atropine drops were discontinued at about 8 months of age. At 1 year of age we retested both monkeys to see if a long-term sensitivity loss had been produced. Prior to this time pupil size had returned to normal in the treated eye and, since accommodation returns much faster than pupil size after atropinization, both animals should have been capable of accommodating to the display with both eyes. Results are shown in Figs. 1, *c*, and 2, *c*. It is apparent from these data that the decreased contrast sensitivity produced by the rearing condition is stable for at least 4 months after termination of the atropine treatment. Long-term follow up will be required to determine whether these effects are permanent.

Both treated eyes showed a long-term loss in contrast sensitivity at all spatial frequencies above 1.5 cy/deg. The magnitude of the amblyopia was larger at mid and high spatial frequencies than at low. The magnitude of the sensitivity loss was greater for monkey LD than TC, a finding that is consistent with Figs. 1, *a*, and 2, *a*, which show a larger amount of functional defocus during the rearing period for monkey LD than for TC.

Discussion

This study demonstrates that monkeys raised with chronic defocus in one eye develop an amblyopia. The amblyopia mimics the rearing condition in that the mid and high spatial frequencies, which were most affected by defocus, show the greatest loss of contrast sensitivity. It was also found that the animal with the most functional defocus during the rearing period developed the largest amblyopia.

The magnitude of the effects produced in these animals appear to be stable and long lasting. However, we cannot be sure that the long-term effects we measured are entirely caused by amblyopia. The possibility exists that the chronic atropinization produced an impaired accommodative capacity as well as an amblyopia.

This atropine rearing paradigm with monkeys provides a reasonable method for producing anisometric amblyopia in an animal model. Daily administration of the atropine is relatively easy, it is noninvasive, and its defocusing effects can be reversed optically for either short or long periods. The pigtail monkey is a particularly good model to use for making comparisons with human development, since the time courses for acuity development in the two species can be essentially superimposed by charting age in weeks for the monkeys and in months for the humans.^{2, 3}

Because the present experiments involved defocus of only one eye, the resulting amblyopia may be caused by binocular interactions between the two eyes at a more central site. However, physiologic experiments in the kitten dorsal lateral geniculate nucleus have shown changes in spatial tuning properties of single cells after monocular or binocular atropinization.⁷ In the monkey it has also been shown that at least some kinds of defocus can produce changes in contrast sensitivity that are independent of binocular competition. For example, meridional amblyopia can be produced in monkeys through binocular cylindrical defocus.¹²

Both cylindrical defocus^{12, 13} and the spher-

ical defocus of the present experiments produce greater losses in contrast sensitivity at mid to high spatial frequencies than at low. This may be related to the fact that both kinds of defocus produce the most contrast attenuation at high spatial frequencies.¹⁴ Alternatively, it may be that deprivation of normal spatial input during development always results in relatively more sensitivity loss at high frequencies than at low. Measurements of contrast sensitivity loss at low or mid frequencies have not been reported for lid-sutured monkeys in which contrast attenuation is approximately constant across spatial frequencies or for monkeys reared under conditions producing selective loss of only low or mid spatial frequencies. Such experiments should be able to further determine the extent to which amblyopia is specific for the spatial frequencies at which the eye is deprived.

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