

# Effects of Early Unilateral Blur on the Macaque's Visual System. I. Behavioral Observations

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**We raised 8 macaque monkeys with chronic atropinization of one eye throughout the first 6–10 months after birth. This rearing procedure produces retinal image blur, with the most pronounced contrast attenuation occurring at high spatial frequencies. Measurements of contrast sensitivity were made using behavioral methods in 6 monkeys and evoked potential methods in 2 monkeys. The results showed that this rearing procedure produced long-term deficits in the contrast sensitivity and spatial resolution of the atropinized eye, which were not due to residual losses in accommodative capacity. There was considerable interanimal variation in the magnitude of the effects on visual performance. Similar losses in visual performance are seen in some forms of human amblyopia. Rearing monkeys with chronic instillation of atropine therefore provides a nonhuman primate model for studying the underlying neural mechanisms of anisometropic amblyopia.**

The development of visual function depends on visual experience. Neonatal human and nonhuman primates have relatively poor vision, which improves gradually over the first weeks or months of life to reach adult levels; this improvement is not limited by the quality of the visual optics and must therefore be due to maturation of the visual nervous system (see Dobson and Teller, 1978; Boothe, 1983; Boothe et al., 1985). Abnormal early visual experience impedes or arrests this process of development, leading to a functional visual deficit known as *amblyopia*, as well as a variety of physiological and morphological changes in the visual pathways (see Movshon and Van Sluyters, 1981; Sherman and Spear, 1982; Boothe et al., 1985).

In humans, amblyopia frequently occurs as a result of *anisometropia*, a condition in which the refractive power of the 2 eyes is unequal. This amblyopia presumably develops as a result of the persistently blurred image in one eye (Sen, 1980; Kivlin and Flynn, 1981). The principal effect of blur is to eliminate fine detail by attenuating high spatial frequency components in the retinal image; patterned visual input is provided by the low- and middle-frequency components of the image. In animal models, however, visual deprivation is usually achieved by suturing together the lids of one eye, which abolishes virtually all patterned stimulation of the retina and reduces retinal illumination by 1–4 log units (Crawford and Marc, 1976). Lid-suture leads to the development of a very profound amblyopia in the deprived eye, as well as a striking set of physiological and morphological changes in the central visual pathways (von Noorden et al., 1970; Hendrickson et al., 1977; Hubel et al., 1977; Blake-more et al., 1978; Harwerth et al., 1981, 1983).

Total form deprivation is rarely encountered clinically and is a rather extreme experimental model for the environmental disruption of visual development. We wished to study animals raised under conditions that more closely approximate those experienced by human anisometropes. Accordingly, we chose to degrade the vision of one eye by daily instillation of atropine (Crawford, 1978; Ikeda and Tremain, 1978; von Noorden, 1981). Atropine has 2 principal effects on the eye: it paralyzes accommodation and causes the pupil to dilate fully. The enlargement of the pupil causes a modest reduction in retinal image quality (see, for example, Campbell and Gubisch, 1966); however, the paralysis of accommodation has more severe consequences for the visual image. When combined with the hyperopia typical of young monkeys, paralysis of accommodation blurs the images of all objects viewed through the treated eye.

This paper reports the results of behavioral and electrophysiological measurements of visual performance in 8 monkeys whose vision in one eye was degraded with daily administration of atropine from birth through the age of at least 6 months. In preliminary experiments, we showed that chronic atropinization during the first 6–8 months after birth produces deficits in contrast sensitivity and acuity that are similar to those found in human amblyopes (Boothe et al., 1982); similar results obtained using optical methods to blur the image in one eye have been reported by Smith et al. (1985). In the present paper, we report the results of more extensive studies in which we sought to establish whether the deficits produced by chronic atropinization are long-lasting and the degree to which they vary from animal to animal. The behavioral results show that this treat-

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**Table 1. Summary data for atropine-reared monkeys**

Characteristic	Subject								
	LD	TC	DH	NW	GO	GZ	OH	OL	
Rearing history									
Eye treated	R	L	R	L	L	R	R	R	R
Atropine begun (d)	14	10	5	10	6	13	14	14	2
Atropine ended (mo.)	7	8	10	8	6	7	6	6	6
Sacrificed (mo.)	30	25	22	13	9	10	12	12	14
Initial refraction									
Right eye	+5.25	—	+5.00	+5.00	+5.00	+4.50	+3.00	+3.00	+3.00
Left eye	+5.50	—	+5.00	+4.50	+7.00	+4.50	+1.50	+1.50	+2.00
Experimental procedures									
Behavior	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
EP recording <sup>a</sup>	No	No	No	No	Yes	No	No	No	Yes
Physiology	VI	V1, V4	V1	V1	No	No	No	No	LGN
2dG stimulation <sup>b</sup>	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Retinal morphology	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
LGN cell size	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Cytochrome oxidase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

<sup>a</sup> Evoked potential (see Materials and Methods).

<sup>b</sup> 2-deoxyglucose autoradiography (see Hendrickson et al., 1987).

ment does indeed produce a permanent visual deficit and that the magnitude of the deficit varies from rather mild to severe, probably depending on the degree of blur experienced in early life. In order to understand the neural changes that underlie atropine-induced amblyopia, we conducted morphological and physiological studies of the visual pathways of these animals; the results of these studies are described in the 2 following papers (Hendrickson et al., 1987; Movshon et al., 1987). We have briefly presented some of these results elsewhere (Hendrickson et al., 1982).

## Materials and Methods

**Subjects.** In these experiments we used 8 pigtailed macaque monkeys (*Macaca nemestrina*). Each monkey was separated from its mother within a few days after birth and reared in the nursery facilities of the Infant Primate Laboratory at the University of Washington. Within the first 2 weeks after birth, the refractive state of the monkey's eyes was determined by retinoscopy, and atropine treatment of one eye (hereafter the "treated eye") was begun. Cycloplegia for retinoscopy was obtained by administration of 1% cyclopentolate (Cyclogyl; Alcon) to both eyes (3 drops to each eye, given at 5 min intervals). If a refractive difference between the eyes was found, the eye showing the greater hyperopic error became the treated eye. Atropine (1 drop of 1% atropine in sulfate sterile saline) was administered twice daily, at 8 a.m. and 6 p.m. The age at treatment onset ranged from 2–14 d; treatment was continued without interruption until at least 6 months of age. Details of the rearing history for each monkey appear in Table 1. All animals received normal visual stimulation in a colony room maintained on a 13 hr/11 hr light–dark cycle during and after the atropine treatment. At least 3 times each week the monkeys were given a "play period" in a large exercise room with other infant monkeys, as was routine for all infant monkeys in the lab.

**Visual stimulation during rearing.** Infant pigtailed monkeys are typically 3–5 diopters (D) hyperopic near the time of birth; this changes gradually towards emmetropia during the first postnatal months (F.A. Young, personal communication). Part of this apparent hyperopia may be due to errors that occur when performing retinoscopy on small eyes (Glickstein and Millodot, 1970), and it is therefore difficult to specify the precise refractive state during the period of atropinization. However, we used behavioral refractions as well as retinoscopy at various times during and after the rearing periods to obtain estimates of refractive error. Behavioral refractions were obtained by measuring contrast sensitivity for a single spatial frequency, chosen to be near the peak of the contrast sensitivity function, while the monkey viewed the sinusoidal

grating stimulus through a trial lens. We repeated this procedure with a series of lenses (in steps of 0.25–0.5 D) until we identified the optimal lens: the lens value which allowed the highest contrast sensitivity. In cases where behavioral refractions were obtained from the untreated eye, 1 drop of atropine was administered to that eye 30 min prior to each test session for the duration of the lens testing sequence.

All of the estimates of refractive error, both during and after the rearing period, indicated that the eyes of all but one monkey were hyperopic to various degrees throughout the duration of the experiment. The hyperopia that was present should not have had any defocusing effect on the untreated eyes, since these eyes could (and presumably did) overcome the hyperopia through accommodation. The treated eyes, on the other hand, were constantly defocused during the rearing period for targets at all viewing distances, with the greatest blur occurring for close objects.

The pupils of the monkeys' untreated eyes during behavioral testing under natural viewing conditions were approximately 6 mm in diameter. The pupils of the treated eyes during rearing and of both treated and untreated eyes during cycloplegic behavioral testing were approximately 10 mm in diameter. In 2 monkeys (LD and TC), we measured the axial length of the eyes before the recording experiments using B-scan ultrasonography; in 2 other animals (DH and NW), we measured the length of the eyes *post mortem* with vernier calipers.

**Behavioral testing methods.** The monkeys were trained and then tested on an operant visual discrimination task in a specially designed face-mask cage, using methods that are detailed elsewhere (Boothe, 1981; Williams et al., 1981). Briefly, on each trial the monkey was required to discriminate a CRT display containing a stationary vertical sinusoidal grating from an identical display containing a homogeneous field of equal mean luminance (26 cd/m<sup>2</sup>, P31 phosphor). The 2 displays were side by side, surrounded by a panel of similar color and luminance to the display. The display on which the grating appeared was randomly switched from trial to trial. Shutters placed in front of each eye hole in the face mask allowed either eye to be tested separately; the eyes were tested in counterbalanced order. Lenses and small artificial pupils could also be positioned in front of either eye hole in order to bring the retinal image into good focus during behavioral testing. Testing was generally conducted at a viewing distance of 1.2 m, where the CRT screens subtended 4°. However, shorter viewing distances were used as necessary for animals with deeper amblyopia.

The method of constant stimuli was used to measure contrast sensitivity at a number of spatial frequencies. We normally spaced the test spatial frequencies 1 octave apart, except at the highest spatial frequencies, where we used a half-octave spacing. Four or 5 contrast levels were presented for each spatial frequency tested, with a minimum of 40 trials at each stimulus value. Trials were presented in a pseudo-

**Table 2. Refraction and eye length data**

Subject	Eye	Initial refraction	Final refraction	Length (mm)
LD	UE	+5.50	+1.50	20.07
	TE	+5.25	+2.75	19.98
TC	UE	—	+3.25	19.37
	TE	—	+3.25	19.37
DH	UE	+5.00	0.00	20.30
	TE	+5.00	+6.00	19.30
NW	UE	+5.00	+2.00	19.12
	TE	+4.50	+5.00	18.80
GO	UE	+5.00	—	—
	TE	+7.00	—	—
GZ	UE	+4.50	+1.00	—
	TE	+4.50	+2.50	—
OH	UE	+1.50	—	—
	TE	+3.00	—	—
OL	UE	+2.00	+1.50	—
	TE	+3.00	+3.50	—

UE, untreated eye; TE, treated eye.

random sequence of intermixed spatial frequencies and contrasts. The resulting forced-choice data were used to prepare a psychometric function for each spatial frequency. These functions were subjected to probit analysis (Finney, 1971) to obtain an estimate of contrast threshold at each spatial frequency, which we take to be the lowest contrast supporting performance at the 0.75 level. These probit thresholds define *contrast sensitivity functions* in which contrast sensitivity (the inverse of contrast at threshold) is plotted as a function of spatial frequency.

*Visual evoked potential recording.* In 2 animals that were not tested behaviorally, we estimated overall spatial resolution and contrast sensitivity by measuring the cortical potentials evoked by sinusoidal grating targets. These measurements were made after the animals had been anesthetized and paralyzed and prepared for electrophysiological recording as described in the third of these papers (Movshon et al., 1987). We recorded signals differentially through stainless steel screws placed in the skull over the foveal representation of each striate cortex. The animal's foveas were reflected onto the centers of 2 identical CRTs using a haploscopic arrangement, and each eye was separately stimulated with vertical gratings produced on the CRTs by a PDP11 computer; the gratings varied in spatial frequency and contrast. The CRTs subtended 6° and had a mean luminance of 40 cd/m<sup>2</sup> (P31 phosphor). All the stimuli to both eyes were presented together in a pseudorandom sequence, and the phase at which the gratings appeared on the screen was randomly varied from trial to trial. The gratings' contrast was modulated in time with a 1 Hz square wave (that is, their phase reversed every 500 msec). Potentials were bandpass-filtered between 0.2 and 40 Hz, sampled at 167 Hz, and averaged by the PDP11. We used the analysis devised by Snyder and Shapley (1981), which estimates the evoked potential by comparing those response components occurring at even harmonics of the stimulus frequency with those occurring at odd harmonics. Response curves constructed from these estimates as a function of stimulus contrast were extrapolated to zero response to obtain the contrast sensitivity of the evoked potential, in the manner of Campbell and Maffei (1970).

*Analysis of contrast sensitivity functions.* To unify the treatment of contrast sensitivity functions, we numerically fit the data with a double-exponential function (Williams et al., 1981). We also experimented with fitting other functions, such as a difference of exponentials, but found the double exponential to provide a consistently superior fit. It must be understood that we attribute no particular significance to this choice of functional form, but simply use it as a convenient tool for data reduction. There are certain unusual circumstances under which the fitted functions may represent the data poorly, and we make occasional note of this below. When we wished to use estimates of visual acuity, we took the

spatial frequency at which the value of the fitted function fell to 1 as an acuity estimate.

## Results

The rearing histories, initial refractions, and experimental histories for each monkey in the study are presented in Table 1.

### *Physiological optics*

Refractive errors determined to be present at the beginning and end of the study appear in Table 2 along with the available eye length measurements. The initial refractions were obtained from all monkeys within the first 2 postnatal weeks, at which time these monkeys showed hyperopia typical of young monkeys. In all but 2 cases, GO and OH, the refractive errors for both eyes were similar, within 0.5 D. These data for monkey TC are unavailable. Six monkeys were also refracted at the termination of the experiment. In all but one case, the treated eye was more hyperopic than the untreated eye; the differences in refractive errors ranged from 1.25 D (LD) to 6.00 D (DH), with TC showing no difference. These final refractions suggest that atropine-rearing may have interfered with the normal process of emmetropization that occurs during postnatal development.

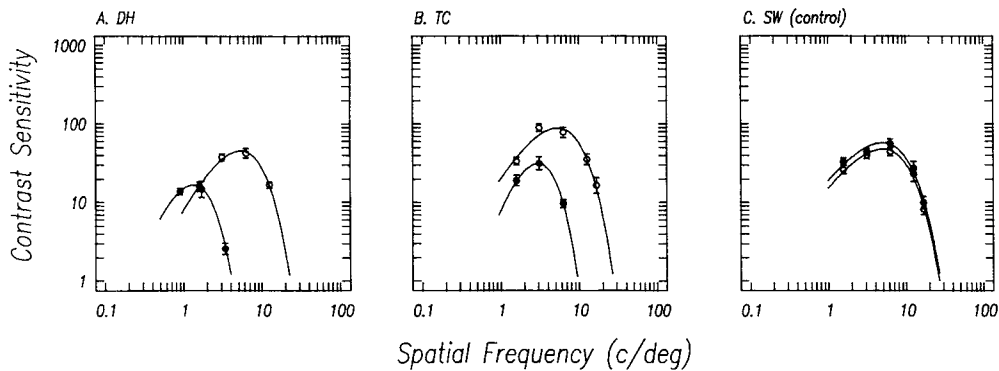
Axial length measurements were made on both eyes of 4 of the monkeys around the time of physiological recording and are included in Table 2. These measurements, made by ultrasound (LD and TC) or with calipers (DH and NW), reflect in basic degree the cycloplegic refraction. The more hyperopic eye in each case was found to be shorter to a greater or lesser degree in accordance with the measured refractive error. TC demonstrated no difference between his eyes on either measure. These data support the notion that the rearing procedure in some way attenuated the normal process of eye elongation for the deprived eyes.

Inspection of each monkey throughout the course of the experiment suggested that normal eye alignment was preserved. No obvious strabismus was seen in any of these monkeys as determined by observation during the daily administrations of atropine, corneal reflex photography (Hirschberg Test), or cover test. However, the possibility that microstrabismus existed in these animals remains open.

### *Behavioral measurements*

Training and testing for 3 (LD, TC, DH) of the 6 monkeys assessed using behavioral methods was begun during the rearing period in order to determine the extent of the deprivation. This initial testing was done without use of artificial pupils or optical correction. Thus, the visual targets were in good focus for viewing with the untreated eye, but the treated eye continued to be exposed only to blurred targets. Representative data obtained from 2 of our monkeys during the rearing period are shown in Figure 1, *A* and *B*. Data from a normally reared monkey are presented in Figure 1*C* for comparison.

Contrast sensitivity functions for both eyes of monkey DH obtained during rearing, at 17 weeks of age, are shown in Figure 1*A*. In all plots, open circle symbols represent results obtained from the untreated eye and filled circles represent results from the treated eye; error bars represent the SE of estimate. Peak sensitivity for the untreated eye occurred around 5 c/deg and spatial resolution for this eye was 23 c/deg. When viewing the target through the cycloplegic eye, DH showed extremely depressed contrast sensitivity. Peak sensitivity for this eye occurred near 1 c/deg, and acuity was below 5 c/deg.



**Figure 1.** Contrast sensitivity functions obtained during the rearing period. *Open circles* represent data for the untreated eyes; *closed circles*, the treated eyes. The untreated eyes were tested under natural viewing conditions; treated eyes were tested while cycloplegic. No optical corrections or artificial pupils were used for either eye; viewing distance was 1.2 m. *A*, Results obtained from monkey DH at 17 weeks of age; *B*, Results obtained from monkey TC at 24 weeks of age. *C*, Results obtained from a normally reared monkey tested at 1 year of age under natural viewing conditions. *Open* and *closed circles* in this case represent data from the right and left eyes, respectively.

Data for a second monkey, TC, obtained during the rearing period at 24 weeks of age, are shown in Figure 1*B*. Similar to monkey DH, the contrast sensitivity function from TC's untreated eye showed maximal sensitivity between 3 and 6 c/deg; spatial resolution was 28 c/deg. The contrast sensitivity function from TC's treated eye reflected a more moderate deficit in contrast sensitivity throughout the mid- to high-spatial frequency range, with the largest deficits occurring at high frequencies. For this eye, peak sensitivity was about 3 c/deg and spatial resolution was near 10 c/deg.

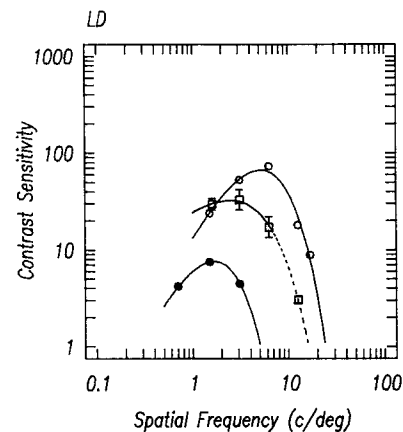
There was no evidence that the contrast sensitivity of the untreated eyes of any of our monkeys was subnormal. Contrast sensitivity functions for both eyes of a normally reared monkey are presented in Figure 1*C*. As is typical of normal monkeys in our testing paradigm, these functions show peak sensitivity occurring between 3 and 6 c/deg and extrapolated acuities near 30 c/deg. The level of maximal sensitivity is usually near 100.

The results shown in Figure 1 demonstrate the quality of vision experienced during the rearing period by the cycloplegic eye for objects at a distance of 1.2 m. It is important to note that the depressed contrast sensitivity for the treated eyes shown in Figure 1 was due to an uncertain combination of the degraded retinal image of the cycloplegic eye and any amblyopia that had developed by that age. We can, however, discern the amount of defocus produced by the atropine treatment and the relative extent of the contrast sensitivity deficit in the treated eye for one monkey, LD. The open and closed circles in Figure 2 represent data collected from the untreated (with natural viewing conditions) and treated (cycloplegic and uncorrected) eyes of LD, respectively, during the rearing period as in Figure 1, *A* and *B*. In addition, the open squares represent data collected with the *untreated eye* while cycloplegic and uncorrected. Comparison of the functions defined by the open circles (untreated eye with normal viewing) and the open squares (untreated eye while cycloplegic) reveals the degree of contrast sensitivity loss that is attributable directly to defocus. The additional loss of sensitivity shown by the treated eye (closed circles) is indicative of amblyopia. It should be noted that cycloplegic refraction obtained at this time showed the 2 eyes to have equal refractive error.

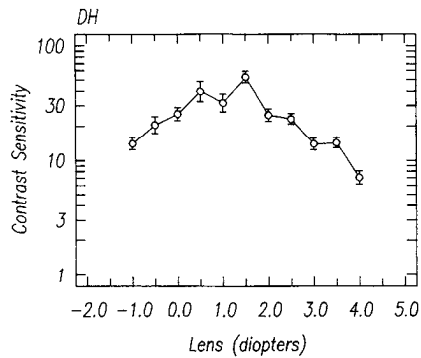
Near the end of the rearing period the 3 monkeys tested during rearing were behaviorally refracted (see Materials and Methods) so that the treated eye could be tested with optical correction. In this way, we could assess the degree of amblyopia that had developed by this stage in the experiment. The procedure for

determining the optimal correcting lens is graphically illustrated in Figure 3, where contrast sensitivity for a single sinusoidal grating is plotted as a function of lens value. These data, collected for DH's untreated eye using a grating of 3.4 c/deg and a 65 cm viewing distance, showed a reduction in contrast sensitivity with changes in lens value in both the positive and negative direction from +1.5 D. Thus, the optimal correcting lens for this eye, when viewing targets at the shortened viewing distance, was +1.5 D.

Representative contrast sensitivity data obtained from each of the 3 monkeys near the end of the rearing period, while they viewed the display through optimal correcting lenses, are shown in Figure 4. The data in Figure 4*A* were obtained from monkey DH, at 40 weeks of age, who showed the largest deficit of the 3 monkeys tested at this time. These data were collected at a shortened viewing distance (65 cm); both eyes were cycloplegic and no artificial pupils were used. The contrast sensitivity function from the untreated eye under these conditions showed a peak sensitivity near 3 c/deg and a spatial resolution of 18 c/deg. Despite the optical correction, the treated eye showed markedly depressed contrast sensitivity and spatial resolution was still about 5 c/deg. The function obtained from the untreated eye at this age reflects slightly inferior sensitivity relative to that ob-



**Figure 2.** Contrast sensitivity data from monkey LD collected around 20 weeks of age. *Open circles* represent data from the untreated eye under natural viewing conditions; *closed circles*, data from the treated eye while cycloplegic, with no optical correction or artificial pupils; *open squares* represent data from the untreated eye tested under the same conditions as those for the treated eye (cycloplegic and uncorrected).



**Figure 3.** Contrast sensitivity for a 3.4 c/deg grating as a function of lens value. Data were collected from monkey DH while viewing with his untreated eye through a series of correcting lenses. Viewing distance was 65 cm and a 6 mm pupil was used. Maximal sensitivity occurred with a 1.5 D lens. The eye was cycloplegic during testing.

tained earlier under natural viewing conditions (Fig. 1A). While we have no satisfactory explanation for this, it is possible that the large pupil could have caused the apparent depression in sensitivity; a similar depression was not apparent for the other 2 monkeys.

Data obtained from monkey LD at 26 weeks of age appear in Figure 4B. Again, both eyes were cycloplegic and artificial pupils were not used; viewing distance was the usual 1.2 m. The untreated eye's contrast sensitivity function showed normal peak sensitivity, near 5 c/deg, and a spatial resolution of 27 c/deg. The treated eye, on the other hand, had depressed contrast sensitivity levels across all spatial frequencies tested, with the largest deficits occurring at the higher frequencies. Peak sensitivity was near 2 c/deg and spatial resolution was 18 c/deg. It should be noted that the high-frequency portion of this function was inadequately constrained by the data and thus the extrapolated acuity value should be treated with caution.

Monkey TC (Fig. 4C) showed the smallest deficits during rearing both with and without optical correction (refer to Fig. 1B). The data shown in Figure 4C were collected under the same conditions as those for monkey LD (Fig. 4B), at 35 weeks of age. The contrast sensitivity functions for both eyes were similar in form, although that for the treated eye was depressed relative to the untreated eye in the high-frequency range. Peak sensitivity for both eyes occurred near 5 c/deg; the extrapolated acuities for the untreated and treated eyes were 31 and 24 c/deg, respectively.

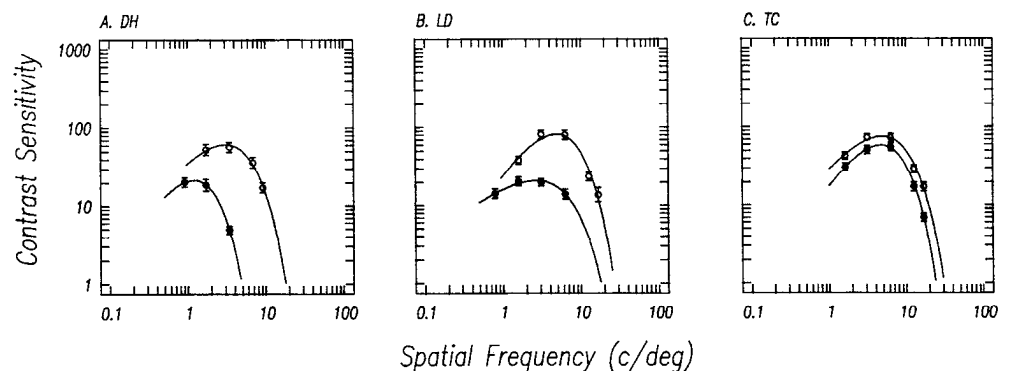
By the end of the rearing period, an amblyopia had developed in the treated eye of each of these monkeys, as demonstrated

by the results presented in Figure 4. Differences between the extrapolated acuity values for the eyes of normally reared monkeys tested by us and others (see Smith et al., 1985) are found to be 3 c/deg or less. The wide range of individual differences in the magnitude of amblyopia was readily apparent at the end of the rearing period, with DH showing a considerable loss of contrast sensitivity and spatial resolution and TC showing only a modest deficit in high-frequency sensitivity and spatial resolution. LD demonstrated an intermediate degree of sensitivity loss. The administration of atropine was discontinued at ages ranging from 6 to 11 months (see Table 1). Six monkeys were then tested, beginning several weeks to several months following the termination of the rearing period, in order to determine whether amblyopia would persist in the treated eyes. Representative contrast sensitivity data from each monkey are shown in Figure 5.

As in previous figures, data for the untreated eye are represented in Figure 5 by the open circles and for the treated eye by closed circles. All tested animals showed persistent deficits in contrast sensitivity and spatial resolution for the treated eye. There continued to be large individual differences in the magnitude of the deficits. Monkeys NW, GZ, and DH (Fig. 5, A–C) all showed large sensitivity deficits across the entire range of spatial frequencies tested. The spatial resolution values for their treated eyes fell between 3 and 6 c/deg. In these cases, the low-frequency portion of the function was lower than could be measured with our display. Monkeys LD, TC, and OH (Fig. 5, D–F) demonstrated more moderate deficits, with TC and OH showing the most modest reductions in spatial resolution and contrast sensitivity, respectively. Examination of the data for those monkeys tested at the end of the rearing period, as well as after recovery from atropine treatment, revealed an improvement in contrast sensitivity for both eyes of LD and TC during the posttreatment period. For DH, though, a further reduction of sensitivity occurred during the interim.

The data presented in Figure 5 also demonstrate that the magnitude of the sensitivity deficits for the treated eyes of these monkeys decreased at lower spatial frequencies. In fact, for TC, sensitivity was similar for his 2 eyes at the lowest frequency tested. It is possible that the other monkeys would also show no sensitivity difference between their eyes if tested at a low enough frequency, although this does not seem likely for the more severely affected animals. It is also worth noting that the contrast sensitivity function for the untreated eye of each of these monkeys was similar to that for normally reared monkeys (refer to Fig. 1C).

The final measured spatial resolution, spatial frequency at the



**Figure 4.** Contrast sensitivity results obtained near the end of the rearing period. Both eyes were tested while cycloplegic and viewing through correcting lenses; no artificial pupils were used. Open circles are untreated eye data; closed circles, treated eye data. A, Monkey DH at 40 weeks of age; viewing distance was in this case 65 cm. B, Monkey LD at 26 weeks of age; viewing distance was the usual 1.2 m. C, Monkey TC at 35 weeks of age; viewing distance, 1.2 m.

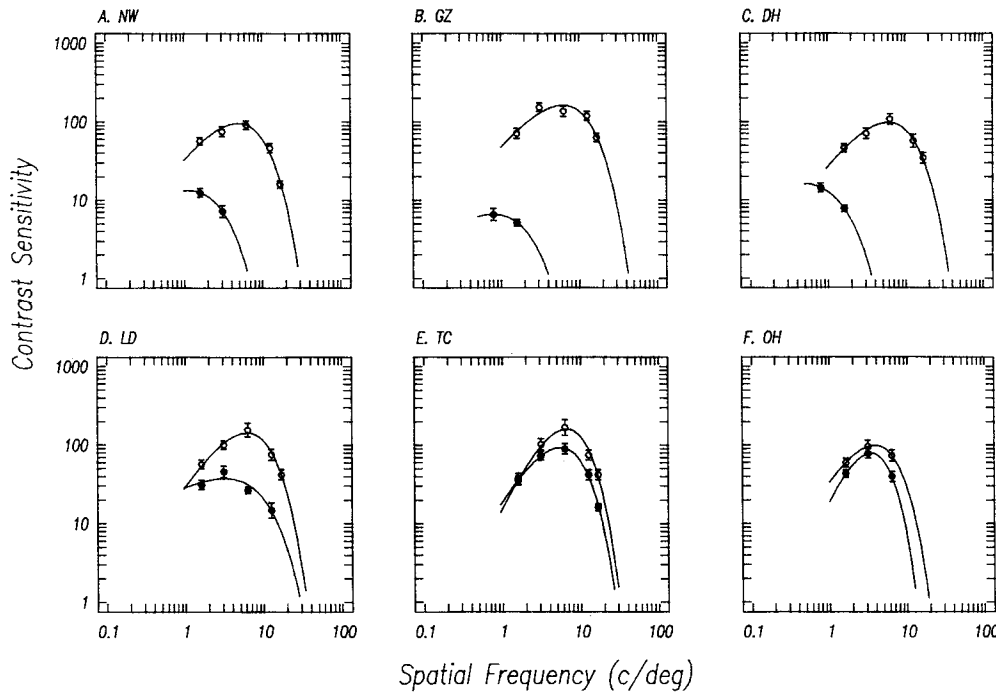


Figure 5. Long-term follow-up contrast sensitivity functions obtained from each of the experimental monkeys after the end of the atropine rearing period. Open circle symbols show results obtained under natural viewing conditions with the untreated eyes; closed circles show results obtained under natural viewing conditions for the treated eyes. Viewing distance was 120 cm except for the treated eyes of DH and GZ, which were tested at 60 cm. The monkeys were 36 weeks or older at the time of testing. Peak frequency, sensitivity at the peak, and spatial resolution values from this figure are listed in Table 3.

peak, and peak sensitivity values for each eye of each monkey tested are listed in Table 3. In addition, the extent of the differences between the eyes on each of these measures, expressed in log units, is included in the table (Log Difference). As already discussed, all 6 monkeys had higher extrapolated acuity for the untreated eye than the treated eye, although the differences were quite small for LD and TC. Examination of the other measures, contrast sensitivity at the peak and peak spatial frequency, showed the treated eye to be poorer than the untreated eye in each case, although the extent of the differences varied considerably across animals on these measures as well.

It is possible that the long-term deficits demonstrated by these monkeys were due to persisting accommodative defocus in the treated eyes, produced as a long-term side effect of the chronic atropinization. In order to explore this possibility, one monkey, DH, was retested at long-term follow-up with optimal correcting lenses in place. In this case, the treated eye viewed the display through a +4.75 D lens. The results are shown in Figure 6, where the data from Figure 5C are replotted along with data from the treated eye tested with optical correction (filled diamonds). The optimal correcting lens improved sensitivity somewhat, indicating that the chronic atropinization did lead to a slight deficit in accommodative capacity in the treated eye of this monkey. However, the improvement was much too small to eliminate the differences between the treated and untreated eyes, suggesting that the bulk of the difference was due to neural rather than optical factors. It may be argued that the importance of the accommodative deficit might have been relatively greater in animals (such as TC) that showed smaller deficits. However, testing with the eyes correctly refracted revealed no smaller a deficit than that seen under free viewing conditions (Fig. 4). It should be recalled that the animals with smaller deficits tended to have smaller hyperopic refractive errors than more severely affected animals; this would tend to reduce the visual effects of a slight accommodative insufficiency.

#### Evoked potential measurements

Two animals, GO and OL, were not tested behaviorally, but their contrast sensitivity was measured using evoked potential methods immediately prior to sacrifice. The results of these

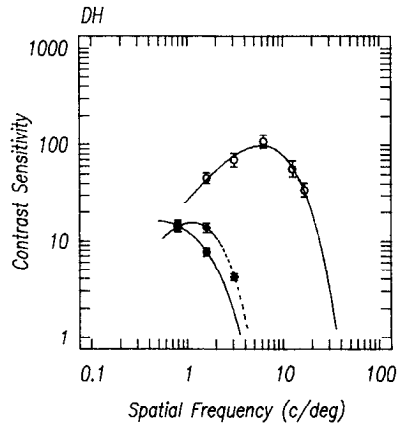
Table 3. Contrast sensitivity and resolution data

Subject	Eye	Peak CS	Log Diff	Peak SF	Log Diff	Cutoff SF	Log Diff
LD	UE	144	0.58	6.0	0.29	34.9	0.08
	TE	38		3.1		29.3	
TC	UE	159	0.23	6.5	0.08	31.5	0.05
	TE	94		5.4		28.1	
DH	UE	98	0.77	6.0	0.74	36.9	1.00
	TE	17		1.1 <sup>a</sup>		3.7	
NW	UE	95	0.85	4.8	0.64	29.6	0.65
	TE	13		1.1 <sup>a</sup>		6.7	
GO	UE	35	0.03	4.2	0.30	57.5 <sup>b</sup>	0.49
	TE	33		2.1		18.7	
GZ	UE	162	1.39	6.2	0.89	43.0	1.01
	TE	7		0.8 <sup>a</sup>		4.2	
OH	UE	99	0.10	3.7	0.04	19.3 <sup>b</sup>	0.16
	TE	79		3.4		13.3 <sup>b</sup>	
OL	UE	54	0.20	3.0	0.30	22.8	0.63
	TE	34		1.5		5.3	

Contrast sensitivity and spatial frequency at the peak, and extrapolated acuity values for both eyes of each monkey, determined from the functions in Figure 5. Differences between the values for the eyes of each monkey, expressed in log units, are also listed. UE, untreated eye; TE, treated eye. CS, contrast sensitivity; SF, spatial frequency.

<sup>a</sup> Peak poorly defined.

<sup>b</sup> High-frequency portion of function poorly defined.



**Figure 6.** Contrast sensitivity measurements for monkey DH replotted from Figure 5 along with data for the treated eye with optical correction. *Open* and *closed circles* are as for previous figures; *closed diamonds* represent DH's treated eye, under natural viewing conditions with the addition of a +4.75 D correcting lens. Viewing distance was 60 cm for both treated eye functions.

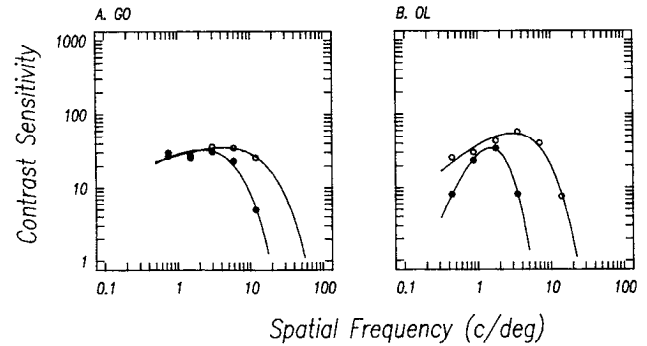
measurements are shown in Figure 7. Because these data were taken in anesthetized animals using temporally modulated gratings, they are not directly comparable to those obtained behaviorally. Nonetheless, the data show many of the same general features as those obtained behaviorally.

One animal, GO (Fig. 7A), showed a relatively modest difference in sensitivity between the eyes except at the highest spatial frequencies tested; in fact, for this animal, there was no detectable sensitivity difference for spatial frequencies below 3 c/deg. The contrast sensitivity functions for this animal are relatively flat, presumably because of the temporal modulation of the stimulus (Robson, 1966). The function for the untreated eye shows a broad optimum between 3 and 6 c/deg; the extrapolated resolution limit for this eye was 57 c/deg, but this value is poorly specified by the data set and should be treated cautiously. The treated eye showed no clear optimum in spatial frequency; its resolution limit was about 19 c/deg. The second animal, OL, showed a more marked effect. The untreated eye's sensitivity was best near 7 c/deg, and the resolution limit was 23 c/deg; the treated eye's peak sensitivity was near 2 c/deg, and its resolution limit was only 5.3 c/deg. Despite this loss in extrapolated acuity, at spatial frequencies between 1 and 2 c/deg this eye's sensitivity was only slightly worse than that for the untreated eye.

## Discussion

The results of this study demonstrate that chronic atropinization of one eye during early visual development can lead to a permanent and, in some cases, severe loss of contrast sensitivity and spatial resolution in that eye. The contrast sensitivity functions from the untreated eyes of all of our monkeys exhibited sensitivity and resolution levels similar to those observed in normal monkeys tested at these same ages (Fig. 1C; see also Williams et al., 1981; R. G. Boothe, L. Kiorpes, R. A. Williams, and D. Y. Teller, unpublished observations). The treated eyes showed deficits in contrast sensitivity that are similar in form and magnitude to those shown by human anisometric amblyopes (Levi and Harwerth, 1977; Hess, 1979; Bradley and Freeman, 1981).

We found substantial individual differences in the treated eye's performance among the experimental monkeys. The extent



**Figure 7.** Contrast sensitivity functions measured using the evoked potential method for monkeys GO and OL. *Open* and *closed circles* represent untreated and treated eye data, as for previous figures. Measurements were made at the time of physiological recording (see Materials and Methods for details).

of these differences is reassuring in that human amblyopes also vary widely in visual performance. The monkey therefore quite accurately models the human condition, and we naturally would like to know the source of the variation. It is reasonable to suspect that the more degraded the retinal image is in early life, the more profound will be the amblyopia that results.

While we cannot establish with precision the amount of defocus experienced by each animal during its rearing, we do have accurate data on the final refractive state of the eyes for 6 of the monkeys (see Table 2). The amount of defocus experienced is directly related to the hyperopic refractive error in the treated eye; thus, we might expect that those animals with the smallest refractive error in their treated eyes at the end of the experiment would also have the smallest contrast sensitivity deficits. This hypothesis has some support in that, of the 4 animals that showed the largest deficits, 3 had substantial refractive errors (at least +3.5 D) in their treated eyes. The others had smaller refractive errors for their treated eyes and/or no difference between the eyes.

Sen (1980) reported a significant correlation between the degree of anisometropia in humans and the depth of amblyopia. It is worth noting in this regard that all 4 monkeys with large sensitivity deficits showed at least 1.5 D refractive difference between their eyes at the end of the experiment, while those with smaller deficits had 0.75 D difference or less. In order to determine the actual relationship between the magnitude of amblyopia and the degree of defocus it would be necessary to have data on the time course of the change in refractive error during the rearing period. Comprehensive data of this sort were not obtained; however, the differences between behaviorally measured refractive errors for the eyes of each of the monkeys tested at the end of the atropine-rearing period are informative. TC and LD, the monkeys that showed moderate sensitivity deficits, had refractive differences less than 1.0 D. DH, on the other hand, showed considerable loss of contrast sensitivity and had a refractive difference of 3.0 D.

These rather large individual differences would seem to mandate that behavioral or electrophysiological measurements of visual performance be conducted on each animal in studies of this kind before embarking on anatomical or physiological experiments. Otherwise, it is impossible to evaluate whether the individual differences seen morphologically and physiologically are due to differences in the magnitude of the amblyopia produced or to other factors.

The form of the deficit in contrast sensitivity shown by our monkeys with moderate degrees of amblyopia (TC, OH, LD) was similar to that reported by Smith et al. (1985) for rhesus monkeys reared with optically induced anisometropia. They raised monkeys wearing helmets in which plano lenses were fitted in front of one eye and  $-10$  D lenses in front of the other. Their monkeys developed moderate deficits in contrast sensitivity when rearing periods extended from 30 d postnatally to either 90 or 120 d postnatally. Shorter periods of deprivation produced little or no behavioral deficit. Given the later onset and shorter duration of the deprivation used by Smith et al. (1985), it is not clear whether the more uniformly moderate deficits found in their study were due to the use of the optical model rather than chronic atropinization or the particular period of deprivation used.

Harwerth et al. (1983) raised 2 monkeys with chronic unilateral cycloplegia and found little or no effect of the treatment on contrast sensitivity. Although the period of treatment included the first 7 postnatal months, the data were similar to those reported by Smith et al. (1985) for the shortest deprivation group. The rearing procedure employed by Harwerth et al. (1983) was different from ours in that they administered only 1 drop of atropine per day and restricted the visual environment by draping the cages with white canvas. Either of these aspects of the rearing protocol could have reduced the severity of the deprivation.

Consideration must be given to the concern that the chronic atropinization of one eye may have caused organic damage to the optics of the eye or the accommodative control system in our monkeys. We found no evidence for damage to the eye itself or the optics, when inspected at the time of physiological recording. Differences in eye length were noted that correlated with refractive differences between the eyes of the monkeys examined. Atropine has previously been found to attenuate the process of eye elongation in some macaque species (see Raviola and Wiesel, 1985). The attenuation of the normal process of eye elongation in our monkeys is interesting in view of the finding that optical defocus during rearing in rhesus monkeys, produced with  $-10$  D lenses, resulted in excessive elongation of the treated eyes (Smith et al., 1985).

Smith et al. (1984) reported long-term effects on pupil size in kittens reared with chronic instillation of atropine. All kittens, whether treated monocularly or binocularly, showed permanent reductions in pupil size as a result of the chronic mydriasis. Some of our monkeys showed permanent changes in pupil size, but they were of the opposite form: The pupils were permanently enlarged (1–2 mm). However, the use of artificial pupils during testing failed to produce improvements in contrast sensitivity in these monkeys. Kiorpes and Boothe (1984) reported normal accommodative capacity in both eyes of a monkey reared with chronic bilateral atropinization but found accommodative deficits in monkeys who were amblyopic as a result of unilateral atropinization or unilateral esotropia. We observed a small residual loss of accommodative capacity in the treated eye of 1 monkey in the present study (Fig. 6), but the accommodative deficit was much too small to account for the deficit in contrast sensitivity. Since the observed changes in pupil size and accommodative capacity had a negligible effect on contrast sensitivity, we consider the demonstrated contrast sensitivity and resolution deficits to be due to neural factors.

Amblyopia that results from defocus in humans is produced by conditions in which one retinal image persistently contains

reduced contrast at high spatial frequencies but normal contrast at lower spatial frequencies. The blurred eye's image in an atropinized monkey also has this character, and the resulting selective deprivation of high spatial frequencies is reflected in the behavioral deficits. Like human anisometropic amblyopes (Levi and Harwerth, 1977; Hess, 1979; Bradley and Freeman, 1981), atropine-reared monkeys show behavioral contrast sensitivity deficits that are greatest at high spatial frequencies. This suggests that the amblyopia is primarily due to the abnormal development of visual mechanisms selectively sensitive to these frequencies. Interestingly, the neural effects of this rearing are largely confined to elements in the visual pathway that are responsible for the relay of signals concerning high spatial frequencies; the nature of these effects is the subject of the 2 following papers (Hendrickson et al., 1987; Movshon et al., 1987).

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