

DEVELOPMENT OF VISUAL ACUITY IN EXPERIMENTALLY STRABISMIC MONKEYS

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Summary—1. The purpose of this study was to document the time course for the development of strabismic amblyopia in infant monkeys, and to evaluate the factors involved in the production of amblyopia. Twenty-one monkeys were raised with experimentally produced strabismus; 14 had esotropia surgically induced and 7 had esotropia induced by injection of *Botulinum A* neurotoxin into the lateral rectus muscle. The ages of induction of esotropia ranged from 1 to 15 weeks.

2. Amblyopia, defined as a difference in grating acuity between the eyes of greater than one octave (factor of two), developed in 67% of these monkeys.

3. The time course of amblyopia development was different on average for the two groups of esotropes. In the surgical group, the most common time course for amblyopia development showed a period of continued normal, parallel acuity development in both eyes for a period of weeks following surgery before amblyopia began to appear in the deviated eye. In the neurotoxin group, amblyopia generally appeared soon after the induction of esotropia.

4. The development of amblyopia was most clearly associated with the pattern of fixation. Animals that adopted a unilateral fixation pattern were more likely to develop amblyopia. A multiple regression analysis revealed that age of esotropia onset, refractive error, and alternation percentage together accounted for 39% of the variance in the extent of the acuity difference between the eyes for the entire group of monkeys.

5. A separate multiple regression analysis was performed to analyze the variance in the extent of amblyopia for the group of 14 amblyopic monkeys. The analysis revealed that the size of the esotropic deviation and the refractive error of the deviated eye accounted for a significant proportion of the variance.

Key words—Visual development; amblyopia; strabismus; acuity; macaque monkey.

INTRODUCTION

Strabismus is a misalignment of the visual axes, which may be constant or intermittent and may occur in conjunction with a variety of other visual disorders. The condition occurs with a frequency of 2–5% in human infants and children (Fledelius, 1976; Simons and Reinecke, 1978); it occurs naturally in monkeys with a frequency of about 4% (Kiorpes *et al.*, 1985a). Strabismus that has an onset during infancy and early childhood is often associated with amblyopia, a deficit in visual acuity in the deviating eye which is not due to any obvious organic cause. Adults who develop strabismus do not develop amblyopia. There is therefore a *sensitive*

period for this deficit. There is no very clear definition of this sensitive period in humans, although it is thought to extend up to about the age of 8 years (von Noorden, 1980).

The factors involved in the production of amblyopia are not well understood. Strabismic amblyopia is reported to occur in 35–50% of children with strabismus; however, the incidence of amblyopia varies with the type of strabismus (Costenbader *et al.*, 1948; Costenbader, 1961; see also von Noorden, 1980). Constant monocular strabismus is most likely to lead to the development of amblyopia. The additional presence of high refractive errors or a difference in refractive error between the eyes increases the likelihood of amblyopia. The age of onset of strabismus is also an important factor, with amblyopia being more likely to develop if the strabismus occurs early in the sensitive period. Historically, it has been difficult to determine the relative importance of these various factors to the development of

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strabismic amblyopia because the published data are primarily retrospective analyses of case histories. In many such cases, important factors such as the actual age of onset are unknown.

It is important both for clinicians, attempting to prevent and treat strabismic amblyopia, and basic scientists, attempting to study the neural basis of amblyopia, to understand the factors involved in its production. We have conducted a study of amblyopia development in an animal model in order to evaluate the relative importance of various factors such as age of onset, refractive error, and size of the strabismic deviation. We chose to use the macaque monkey as our animal model because of the demonstrated similarity between visual system structure and function of macaque monkeys and man (see Boothe, 1981). In particular, infant macaque monkeys show a progression of visual acuity development that is similar to that of human infants, but is approximately four times faster than acuity development in human infants (Teller and Boothe, 1979). In addition, infant monkeys develop amblyopia under conditions that are similar to those that produce amblyopia in human infants (e.g. von Noorden and Dowling, 1970; Kiorpes and Boothe, 1980; Harwerth *et al.*, 1983).

We have studied the time course for the development of monocular grating acuity in infant monkeys who had strabismus experimentally induced at ages ranging from 1 to 15 weeks. Two methods for induction of esotropic strabismus were used: surgical alteration of the horizontal rectus muscles, and injection of *Botulinum A* neurotoxin into the lateral rectus muscle. These two methods were used in an attempt to model both nonparalytic and paralytic types of esotropia. As in the human population, not all of our monkeys developed amblyopia. We examined refractive error, size of deviation, age of induction, and pattern of fixation as possible predictors of amblyopia development. The development of amblyopia was most clearly associated with the pattern of fixation. The primary factors associated with the degree of amblyopia produced were the size of the esotropic deviation and the refractive error of the deviated eye. This paper presents in detail the acuity development data from these monkeys, and an analysis of the factors commonly associated with amblyopia. Some of these data have been presented briefly elsewhere (Kiorpes *et al.*, 1984; Boothe *et al.*, 1985; Kiorpes *et al.*, 1985b).

METHODS

Subjects

Subjects in this experiment were 21 *Macaca nemestrina* monkeys from the Washington Regional Primate Center. The infants were separated from their mothers within a few days after birth and were housed in the nursery facilities of the Infant Primate Research Laboratory. All care of the infants was conducted in accordance with the protocols of the Regional Primate Center and conformed to the NIH guidelines for research animal welfare.

Clinical methods

For each monkey, we evaluated refractive error, angle of deviation, and fixation pattern. Refractive error was measured by cycloplegic retinoscopy; cycloplegia was induced with a combination of 1% cyclopentolate and 10% phenylephrine. All except the youngest animals were lightly sedated with ketamine hydrochloride for the period of ophthalmic examination. The angle of deviation and fixation pattern were evaluated from photographs of corneal light reflexes. Photographs were taken at 2–4 week intervals between the age of esotropia induction and 20–40 weeks. The Hirschberg method was used to estimate the angle of deviation from a series of photographs taken at each age; the accuracy of the method was about 5 prism diopters (Δ). In addition, a fixation preference percentage was calculated for each eye from all photographs of each animal. Specifically, we calculated the percentage of the total number of frames taken in which each animal was fixating with the deviated (left) eye. 5 to 10 frames were analysed for each animal at each age.

Esotropia was induced by one of two methods: surgically or by *Botulinum A* neurotoxin injection. The surgical group consisted of monkeys whose esotropia was induced at ages ranging from 1–15 weeks; the neurotoxin group consisted of seven monkeys whose esotropia was induced at ages ranging from 1–4 weeks. The surgical procedure involved transection of the lateral rectus muscle and resection of the medial rectus muscle of the left eye. The medial rectus was, in addition, advanced to the limbus. The neurotoxin procedure involved injection of *Botulinum A* neurotoxin (Oculinum) into the lateral rectus muscle of the left eye (Scott *et al.*, 1973). The lateral rectus was exposed by dissection of the conjunctiva, and the neurotoxin

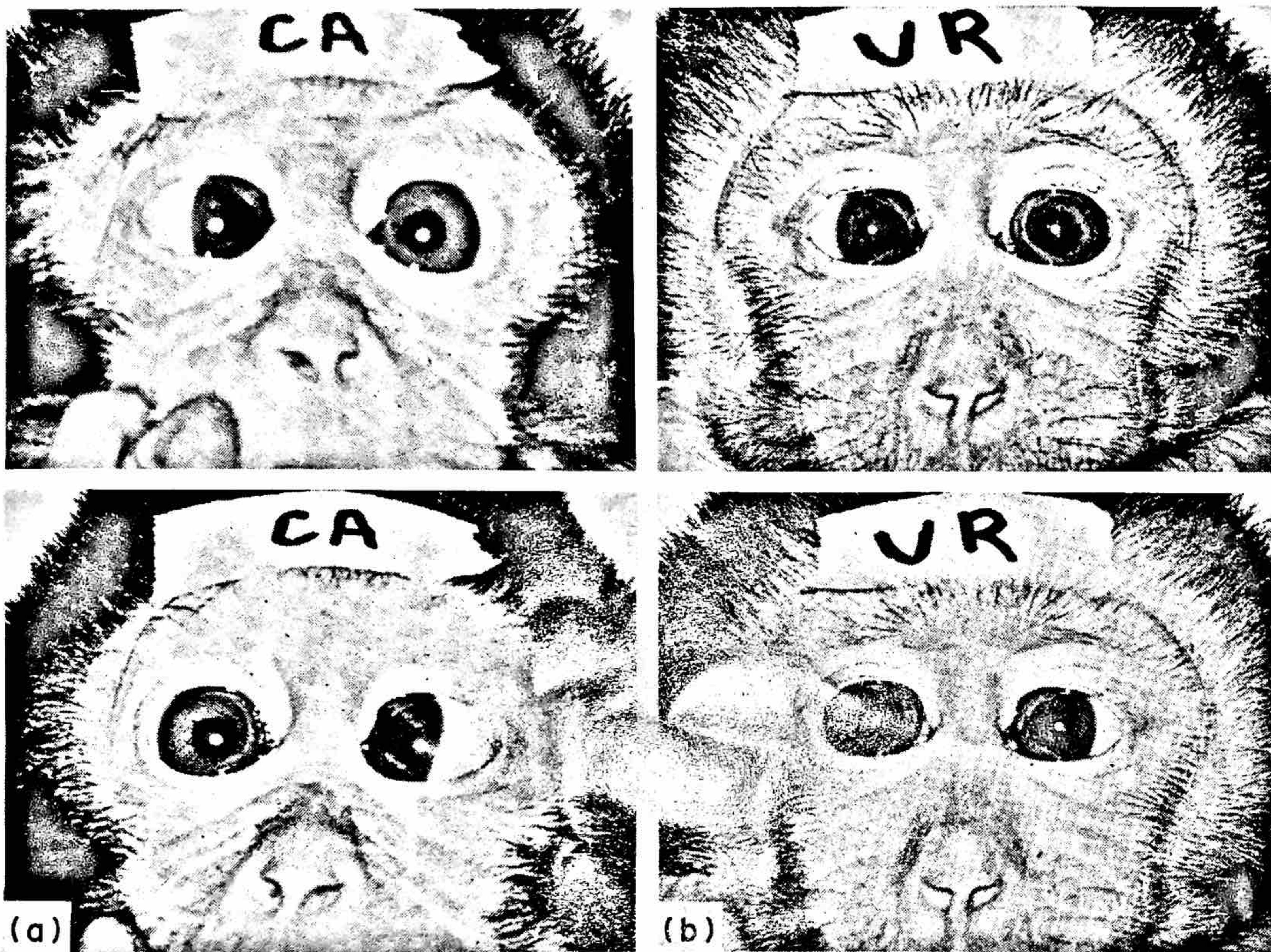


Fig. 1. Photographs of two experimentally esotropic monkeys, one from the surgical group (a) and one from the neurotoxin group (b). The top photographs show the monkeys holding fixation with the operated eye; the bottom photographs show fixation with the unoperated eye.

was injected under visual control. Dosages of *Botulinum A* ranged from 3.75 to 10 units per injection. Both methods for creation of esotropia were conducted under ketamine hydrochloride anesthesia, using sterile surgical techniques. The resulting esotropia in both cases was generally of moderate extent, ranging from 10 to 55 prism diopters. In two animals, the neurotoxin injection produced smaller deviations, 5–10^Δ. Resulting ocular motility was reasonably good; all animals could hold fixation, in adduction, with their operated eye (always left). Figure 1 shows photographs of one monkey from each group (a = surgical; b = neurotoxin), fixating with the operated (top) and the unoperated (bottom) eye. The neurotoxin group recovered grossly normal motility within 2 weeks of injection. Two animals from the neurotoxin group, IM and CT, developed a ptosis (drooping of the eyelid) that at least partially obscured the pupil for the duration of the period of paralysis (13 and 10 days, respectively).

Visual acuity testing

Monocular grating acuity was assessed using a combination of the forced-choice preferential looking procedure described by Teller (1979) and operant techniques. From near birth to about 15 weeks, we used the preferential looking procedure, which is described briefly as follows. A human observer holds the monkey in front of a grey screen which contains two circular apertures. On each trial, one aperture contains a high contrast square wave grating and the other contains an homogeneous field which is matched in space-average luminance to the grating and the screen. The holder observed the animal's face via a video camera and monitor. On the basis of the animal's looking behavior, the observer makes a forced-choice judgement as to whether the grating stimulus appears on the right or left side of the display on each trial. The observer is blind as to the position and identity of the grating stimulus, both of which were randomized from trial to trial. Feedback is provided as to whether each judgement is correct or wrong.

The preferential looking apparatus used for this study has been described previously (Kiorpes and Boothe, 1980; Gunderson and Sackett, 1984). The grey screen was viewed by the monkey from a distance of 36 cm. Each aperture subtended 14 deg of visual angle, with a separation of 58 deg. The grating stimuli were

photographically produced, with a contrast of 85%. The luminance of the display was 150 cd/m²; testing was conducted in an otherwise darkened room. During testing, the monkey subject wore an adhesive eye patch over one eye and was wrapped snugly in a cloth diaper.

Estimates of acuity were obtained following method of constant stimuli. For each estimate, we presented in a randomized order four grating sizes (spatial frequencies), separated by 0.5 or 1.0 octave steps, chosen to span the observer's performance range from chance to near 100% correct. Thirty trials were collected at each grating frequency. Threshold (acuity) was taken to be the spatial frequency at which the observer's performance was 75% correct. The threshold values and standard errors of estimate were obtained by probit analysis (Finney, 1971). Estimates of acuity for each eye at each age were obtained in counterbalanced order over periods of not more than 7 days. Interocular differences in acuity of greater than one octave (factor of two) were considered to be indicative of amblyopia.

The development of acuity was assessed by preferential looking at intervals of approximately 2 weeks, continuing up to 12–15 weeks of age. Most animals were later trained to perform an operant two-alternative forced-choice discrimination task so that we could evaluate long term deficits in acuity (ages ranged from 15 to 66 weeks). The animals were trained to discriminate a square-wave grating stimulus of 50% contrast from an homogeneous field of equal space-average luminance, for an apple juice reward. The training and testing procedures have been described in detail previously (Williams *et al.*, 1981). For operant testing, the stimuli were generated on CRT display monitors (Tektonix 602 with P31 phosphor); the luminance of the displays was 27 cd/m². All aspects of stimulus generation and data collection were under computer control (PDP11/10). The methods for threshold estimation were the same as for preferential looking except that the estimates were based on 5 spatial frequencies and at least 50 trials per frequency. Probit analysis was once again used to estimate the 75% correct performance level and standard errors of estimate.

RESULTS

The clinical data for each animal are presented in Table 1, along with the age of

Table 1. Summary data for (A) Surgical (B) Oculinum groups. For each subject, the age of esotropia induction (onset age), the extent of the interocular acuity difference at last test (IAD), refractive error (diopters), the extent of the esotropic deviation (prism diopters), and the percent of fixation with the left eye (percent alternation) are listed. The refraction and deviation measures are those taken nearest the age of onset. Animals are ranked in order of increasing acuity deficit

Subject	Onset age (days)	IAD (octaves)	Refractive error		Deviation angle (Δ)	% Alt
			OD	OS		
(A)						
HB-84046	107	0.25	+6.25	+6.00	29	0
GH-84022	45	0.44	+2.00	+1.75	19	19
EX-83430	57	0.60	+4.00 + 2.00 \times 20	+3.50 + 1.75 \times 160	38	26
LS-84192	26	0.84	+2.00	+2.00	17	50
CA-83330	29	0.92	+3.00 + 0.25 \times 180	+3.00 + 0.25 \times 180	21	36
NN-84250	20	1.12	+0.25	+0.25	26	20
DP-83381	23	1.12	+2.50	+2.25 + 0.25 \times 90	23	39
FS-84005	36	1.68	+4.00	+3.50	19	46
HC-84047	86	2.19	+2.00	+2.25 + 0.50 \times 180	11	39
FT-84006	22	2.24	+2.50	+2.25	25	6
HT-84067	73	2.28	+3.50 + 0.50 \times 180	+3.25 + 0.50 \times 180	19	0
UY-83105	13	2.77	+1.25 + 0.50 \times 75	+5.50	35	0
VN-83126	20	2.98	+2.00 + 0.25 \times 180	+2.50 + 0.25 \times 180	42	0
VC-83111	12	4.44	+3.50	+3.50	55	0
(B)						
VR-83134	13	0.03	+2.50	+2.50	28	44
IH-84082	27	0.30	+1.75 + 0.50 \times 180	+1.25 + 1.00 \times 180	20	50
NI-84244	32	1.19	+1.00 + 0.25 \times 165	+1.00 + 0.50 \times 10	6	11
FV-84008	18	1.28	+3.50	+3.50	9	40
LU-84195	24	1.68	-0.25 + 0.50 \times 160	0.00 + 0.75 \times 20	6	25
IM-84086	19	1.94	+3.50 + 0.50 \times 170	+3.50 + 0.50 \times 10	26	0
CT-83353	12	3.24	+4.50	+4.50	23	28

esotropia induction (onset age). Anisometropia greater than 1.0 diopter (D) (spherical equivalent refractive error) was present in only one of the subjects, UY. Thus, for the other animals, anisometric amblyopia can be ruled out as a confounding factor in amblyopia development. The refractive errors of these monkeys were unremarkable relative to normally raised infant monkeys. Follow-up assessment of refractive errors was made for some of the subjects, only three of which showed a change of greater than 1.0 D. There was no evidence to suggest a consistent change in refractive error as a result of the intervention.

The initial angle of deviation, evaluated within 1–4 weeks of esotropia induction, ranged from 11–55 $^{\Delta}$ for the surgical group and 6–28 $^{\Delta}$ for the neurotoxin group. The two groups on average showed different directions of change in the angle of deviation over time. In the surgical group, the deviations tended to get larger over time, while those in the neurotoxin group tended to get smaller. These trends are shown in Table 2, where the initial and final (evaluated between 20 and 40 postnatal weeks) deviations are listed for each monkey for which angle of deviation changed more than 5 $^{\Delta}$ during the period of study. In the surgical group, 6/9 showed an increased angle of deviation, whereas

3/4 in the neurotoxin group showed a decreased angle.

The pattern of monocular grating acuity development is shown in Fig. 2 for several representative monkeys. The functions in Fig. 2a, b and c demonstrate the range of developmental patterns shown by the surgical group; Fig. 2d

Table 2. Initial and final angles of deviation for those monkeys who showed a change greater than 5 diopters over the course of the study. Initial deviation angle was evaluated within 1–4 weeks of esotropia induction; final deviation angle was evaluated between 20 and 40 weeks post-natal, depending upon the age of esotropia induction. Direction of change is indicated in the last column. Data for the surgical and neurotoxin groups are shown in A and B, respectively

Subject	Initial angle (Δ)	Final angle (Δ)	Direction
(A)			
HB	29	40	+
GH	19	11	-
EX	38	50	+
CA	21	51	+
HT	19	39	+
FS	19	25	+
UY	35	47	+
VN	42	34	-
VC	55	32	-
(B)			
IH	20	11	-
NI	6	0	-
IM	26	5	-
CT	23	32	+

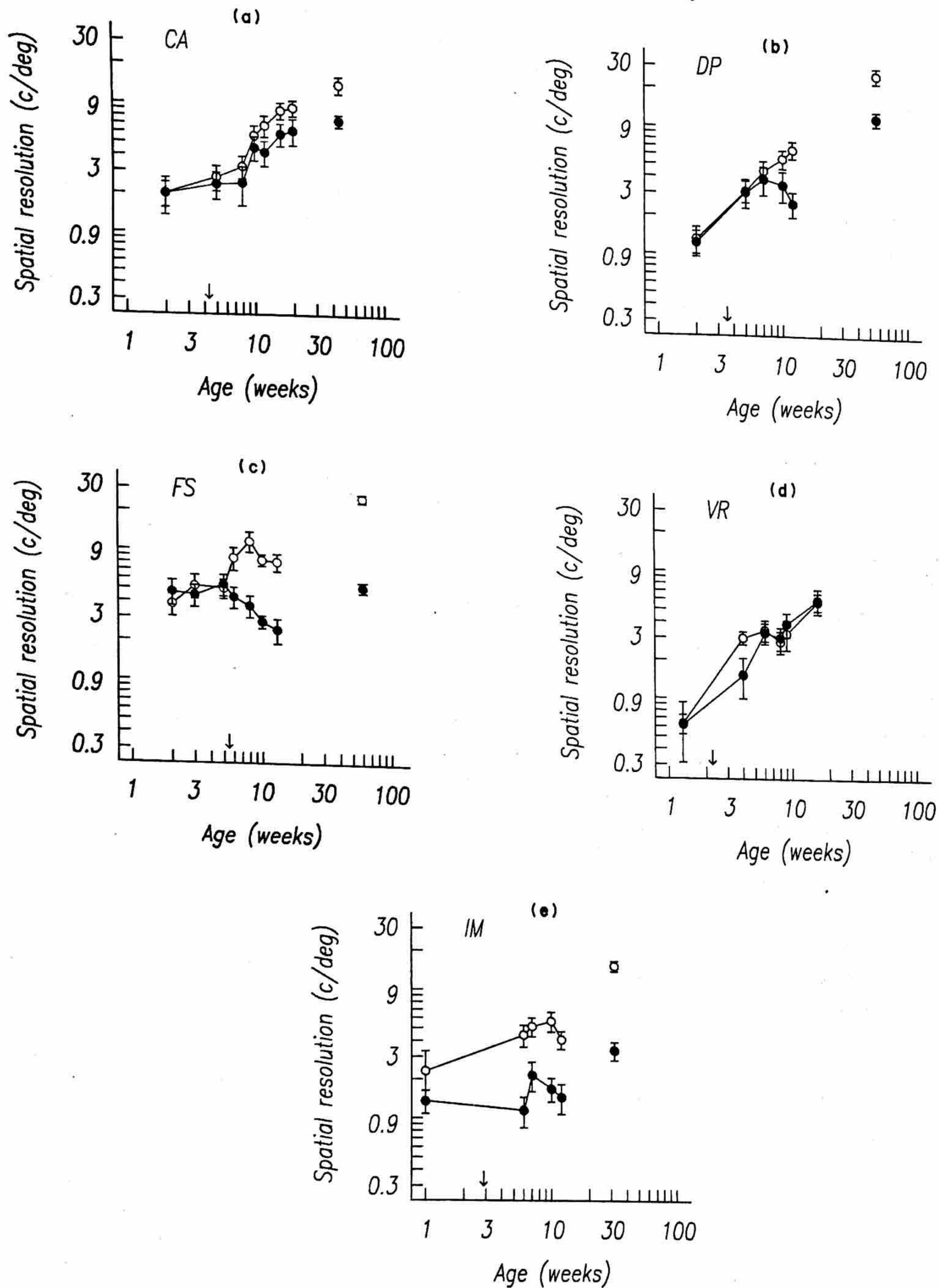


Fig. 2. The pattern of monocular grating acuity development for five experimentally esotropic monkeys: (a) surgical monkey CA whose final interocular acuity difference (IAD) was less than one octave; (b) surgical monkey DP whose final IAD was greater than one octave; (c) surgical monkey FS whose final IAD was greater than one octave; (d) neurotoxin monkey VR whose final IAD was less than one octave; (e) neurotoxin monkey IM whose final IAD was greater than one octave. Open and filled symbols represent data from the nondeviated and deviated eyes, respectively; the arrow on the abscissa points to the age of esotropia induction. The breaks in the curves separate data collected with preferential looking from data collected with operant methods. The data from monkeys DP (b) and IM (e) demonstrate the typical patterns of acuity development for amblyopic monkeys from their respective groups.

and e show representative developmental patterns from the neurotoxin group. In all figures, the open and closed circles represent data from the nondeviated and the deviated eye, respectively;

the arrow on the abscissa points to the age of esotropia induction. The first data set (Fig. 2a) shows a developmental pattern from one surgically esotropic monkey that did not

develop amblyopia; her interocular acuity difference (IAD) was less than one octave (factor of two) at all test ages. This was the typical pattern for the nonamblyopic monkeys, although one monkey, GH, exhibited an IAD greater than one octave at one test age (not shown). The most common pattern of acuity development for the surgically esotropic monkeys that developed amblyopia is represented in Fig. 2b. The important feature of this data set is the continued parallel, uninterrupted acuity development of the two eyes for several weeks *after* the induction of esotropia. Thus, the development of a deficit in acuity was not necessarily coincident with the onset of the esotropia. Amblyopia in these cases developed between 3 and 12 weeks after surgery. There were three monkeys in the surgical group that demonstrated an IAD of greater than one octave at the first post-operative test (VN, FS, HT). Representative data from one of these monkeys, FS, is shown in Fig. 2c.

The data presented in Fig. 2d and e are representative of the acuity development patterns demonstrated by the neurotoxin group. Figure 2d shows data from one of the neurotoxin monkeys that did not develop amblyopia. This monkey never demonstrated a difference in acuity between the eyes beyond the first post-injection test. The temporary decline in acuity for the deviated eye at the first post-injection test is probably a result of the period of paralysis (see below). Figure 2e illustrates the most common pattern of acuity development for the monkeys in the neurotoxin group that developed amblyopia. Most of these monkeys showed a large IAD at the first post-injection test, which was maintained to a greater or lesser degree through the final evaluation. For two of these monkeys, IM (Fig. 2e) and CT, the esotropia was accompanied by a large ptosis, which at least partially obscured the pupil for 10–13 days. These two monkeys showed the largest deficits in acuity in the neurotoxin group; however, the pattern of acuity development shown in Fig. 2e was not a direct function of the ptosis. The same pattern of acuity development was shown by monkeys that had no accompanying ptosis.

The difference between the patterns of acuity development for the surgical and neurotoxin groups is probably due to the initial period of paralysis for the neurotoxin monkeys. Even VR (Fig. 2d), who did not develop amblyopia, showed an acuity deficit at the first post-

injection test. The initial period of paralysis seriously restricted the ability of the animal to use the deviated eye for the duration of the paralysis, although in many cases the monkeys used a head-turn in order to view with the deviated eye. However, these initial deficits are probably not an artifact due to the preferential looking method used for testing. We were careful not to collect data during the period of paralysis, when the monkeys would have had difficulty viewing the grating patterns with the deviated eye. Also, the paralysis would have made it very difficult for the observer to judge the position of the grating stimulus.

Adopting the standard criterion of a one octave interocular difference in acuity for the presence of amblyopia, 67% (14) of the 21 monkeys developed amblyopia. The majority of monkeys with the earliest ages of intervention developed amblyopia: 12/16 with onset between 1 and 5 weeks (7/9 surgical, 5/7 neurotoxin). Less than half of those that were older than 5 weeks when esotropia was induced developed amblyopia (2/5 surgical). In order to determine what factors discriminated between those monkeys that developed amblyopia and those that did not, we analyzed four factors for their potential contribution to the development of amblyopia using a multiple regression/correlation analysis (Cohen and Cohen, 1975). The factors examined were intervention age, alternation percentage, refractive error of the deviated eye, and initial size of the deviation; the analysis was done in stages. In the first stage of the analysis we included the first three of the factors listed above in the regression equation; they were included first on the basis of clinical data linking them to amblyopia development. In the second stage of the analysis we added the initial size of the deviation to the equation; the size of the deviation is generally accepted to be a poor predictor of amblyopia development. A hierarchical strategy was then used to determine the proportion of the variance associated with each factor in the equation. In a hierarchical analysis the factors are added sequentially, in a prescribed order, and the increase in R^2 is calculated following each step. The increase in R^2 at each step is the contribution to the total explained variance accounted for by the particular factor added on that step. The order of inclusion of the variables was (1) intervention age, (2) refractive error, (3) percent alternation, (4) initial deviation.

The results of the analysis revealed that age

of intervention, refractive error and alternation percentage together explain 39% of the variance in IAD ($F = 3.66$; d.f. = 3,17; $P < 0.05$). Adding the size of the deviation, in the second stage of the analysis, increased the explained variance by less than 1%, which is a nonsignificant increase. A significant proportion of the explained variance, 22%, was accounted for by alternation percentage ($F = 6.04$; d.f. = 1,17; $P < 0.05$), suggesting that fixation pattern was a primary factor in the development of amblyopia. The remainder of the explained variance was attributed to intervention age and refractive error, although the unique variances associated with these factors were not themselves statistically significant. The variance associated with the size of the deviation was also not significant; moreover, the addition of the deviation factor to the regression equation did not reduce the significance of the proportion of variance explained by percent alternation.

Scatterplots of the variation in interocular acuity difference as a function of each of the four factors examined are presented in Fig. 3(a-d); variation as a function of the final angle of deviation is presented in Fig. 3e.

In each panel of the figure, data from the surgical and neurotoxin groups are represented by the filled circles and filled triangles, respectively; the dashed line through each graph is drawn at the level of one octave acuity difference between the eyes. The trend toward smaller interocular acuity differences with greater alternation is apparent from inspection of Fig. 3a.

Further inspection of Fig. 3 reveals another interesting feature of the data: among the animals that developed amblyopia, the extent of amblyopia varies over a 4 octave range. It is of interest, then, to evaluate what factors contribute to the variation in the extent of amblyopia. A second multiple regression/correlation analysis was done with the data from the amblyopic monkeys alone (all points above the dashed lines in Fig. 3; $n = 14$); this analysis was also done in stages. In the first stage, a simultaneous regression was done with the same four factors that were examined for the previous analysis. In the second stage, the final size of the deviation was added to the equation. A hierarchical strategy was then used to determine the proportion of the explained variance

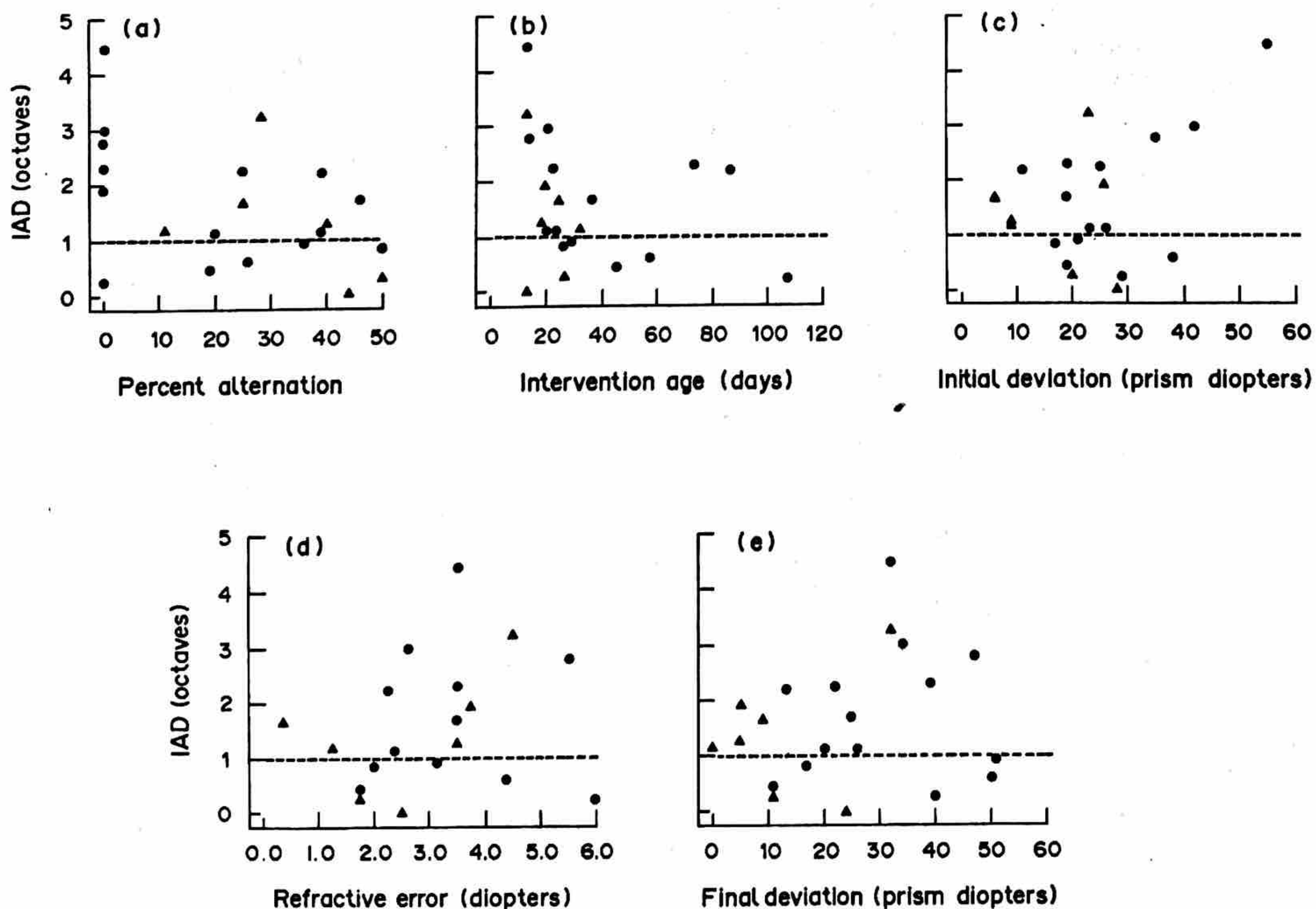


Fig. 3. Scatter plots of the distribution of IADs for all monkeys in the surgical and neurotoxin groups combined as a function of (a) percent alternation, (b) intervention age, (c) initial extent of the esotropic deviation, (d) spherical equivalent refractive error of the operated eye, and (e) final extent of the deviation. The dashed line in each panel delimits amblyopes and nonamblyopes on the basis of an IAD greater than or less than one octave. Data from monkeys in the surgical group are represented by filled circles and those from monkeys in the neurotoxin group are represented by filled triangles.

associated with each factor. The order of inclusion of the variables was (1) intervention age, (2) refractive error, (3) percent alternation, (4) initial deviation, (5) final deviation.

The results of the regression analysis for the amblyopic monkeys revealed that the first equation, including initial size of the deviation, refractive error of the deviated eye, age of intervention and percent alternation, explained 67% of the variance in IAD ($F = 4.50$; d.f. = 4,9; $P = 0.03$). Addition of the final size of the deviation, in the second stage, increased the explained variance by less than 1%, which is a nonsignificant increase. The initial size of the deviation and the refractive error of the deviated eye each accounted for significant proportions of the variance in the extent of amblyopia, 23% ($F = 6.14$; d.f. = 1,9; $P < 0.05$) and 25% ($F = 7.41$; d.f. = 1,10; $P < 0.05$), respectively. These trends can be seen in the associated scatterplots (Fig. 3c and d). The remainder of the explained variance was attributed to percent alternation and intervention age, although the unique variances associated with these factors were not themselves statistically significant.

To summarize, the statistical analyses revealed several points of interest. First, there was no single factor that alone determined whether or not amblyopia developed. Similarly, there was no single factor that regulated the depth of amblyopia. In both cases, there was a constellation of inter-related factors that contributed to the development of amblyopia and the extent of amblyopia. Among the factors we examined, the development of amblyopia was most closely associated with percent of alternation, although age of intervention and refractive error of the deviated eye also contributed to the process. The extent of amblyopia was closely associated with both initial size of the deviation and the refractive error of the deviated eye; age of intervention and percent alternation also contributed to the extent of amblyopia.

Our criterion for the presence of amblyopia was based on the difference in grating acuity between the nondeviated and deviated eye. There is an inherent assumption that the nondeviated eye is "normal". However, there have been a number of recent reports that the preferred eyes of strabismic amblyopes do not have normal spatial vision (e.g. Levi and Klein, 1985, humans; Holopigian and Blake, 1983, cats; Chino *et al.*, 1983, cats). Therefore, we compared the spatial resolution of the nondeviated eyes of all of the esotropic monkeys to resolu-

tion data from normal monkeys. This comparison is shown in Fig. 4 where the triangles represent data from neurotoxin group monkeys and the circles represent data from surgical group monkeys. The dashed lines in Fig. 4 delimit the range of spatial resolution data obtained from normal monkeys tested monocularly over the same age ranges using the same techniques. During the early postnatal weeks, the data from the strabismic monkeys nearly completely overlap the data from the normal monkeys. However, beyond about 10–15 weeks of age the normal monkeys' acuities are in general superior to the nondeviated eyes of the strabismic monkeys.

DISCUSSION

The results of this study show that a majority of animals with experimental esotropia developed amblyopia, although the predominant patterns of amblyopia development were different depending on the method used to create the esotropia. For the monkeys that failed to develop amblyopia, there was a tendency for the nonoperated eye to have slightly higher acuity than the deviated eye; however, the difference between the eyes was less than one octave. An evaluation of a number of factors that potentially contributed to the development of amblyopia suggested that the fixation pattern adopted primarily discriminated those animals that developed amblyopia from those that did not. There was a greater tendency toward alternation of fixation among the animals that did

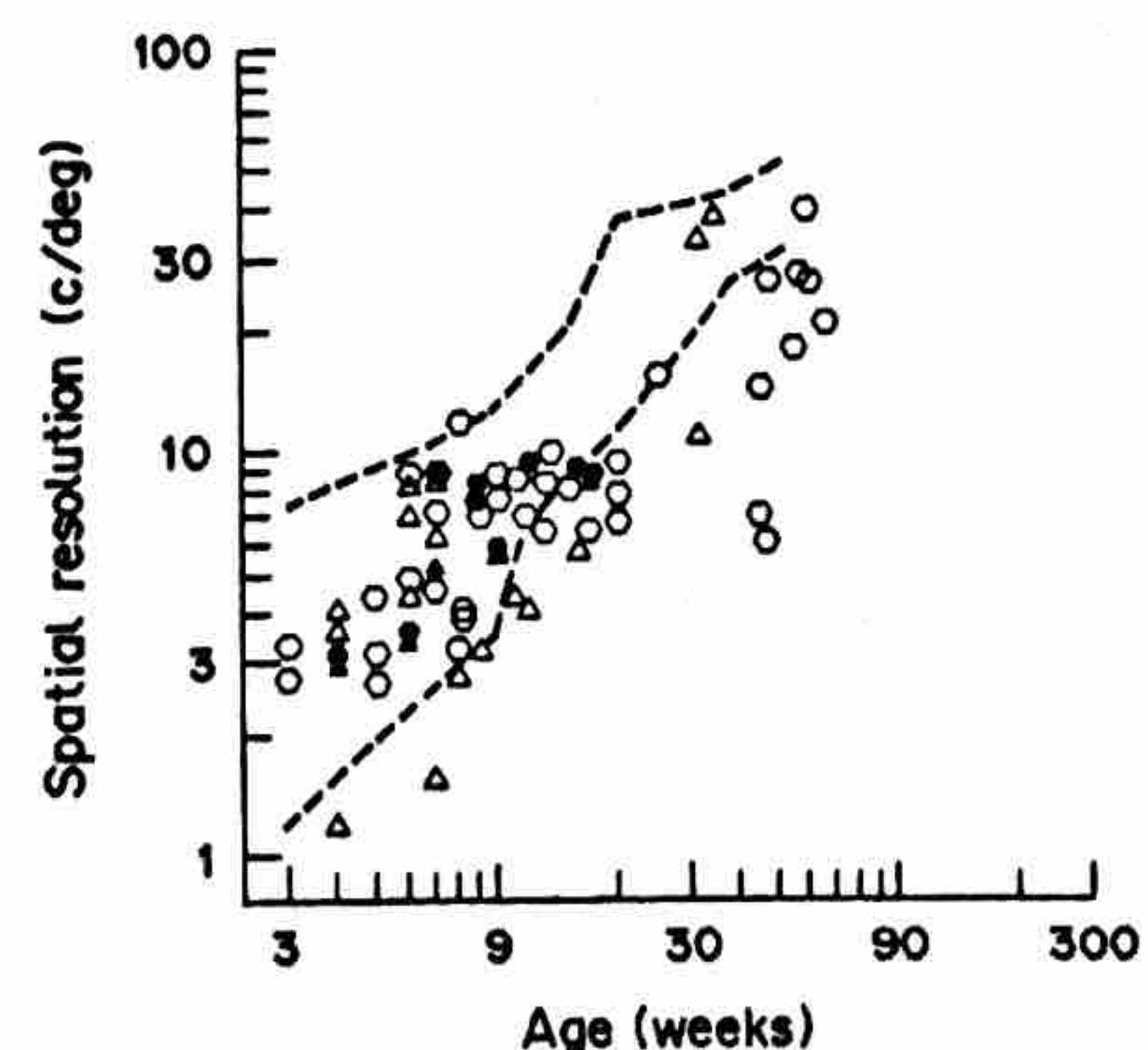


Fig. 4. The development of acuity for the nondeviated eyes of the experimentally strabismic monkeys as compared with that of normal monkeys. Circles represent data from surgical group and triangles represent data from the neurotoxin group. The dashed lines delimit the range of data from 38 normal monkeys tested cross-sectionally using the same techniques over a similar period of time. The data from the esotropic monkeys fall outside the range of the normal monkeys beyond 10–15 weeks postnatal.

not develop amblyopia. An additional analysis of factors related to the extent of amblyopia suggested that larger deviations and refractive errors primarily contributed to a greater depth of amblyopia.

The difference between the patterns of amblyopia development for the two experimental groups is interesting in view of the tendency for fixation pattern to discriminate the eventual development of amblyopia. The neurotoxin injections were followed by a period of oculomotor paralysis that lasted up to 2 weeks. Most of the animals in this group showed a deficit in acuity for the deviated eye at the first post-injection test (which was conducted after the paralysis subsided); in most cases the deficit was maintained thereafter. The animals in the surgical group recovered motility within 1 or 2 days of the surgery, although the range of motility was restricted as a result of the surgery. The majority of animals in this group showed a delay of 3–12 weeks between the induction of esotropia and the appearance of amblyopia. It seems reasonable to suppose, then, that the limitation of ocular motility of the deviated eye may be an important determinant of fixation pattern, which in turn may contribute to the eventual development of amblyopia. Possibly those monkeys in the surgical group that showed a deficit in acuity at the first post-surgical test had more restricted motility than the other animals in the group. Since we had no objective or quantitative test of ocular motility this remains speculative. It is interesting to note, though, that in cases where the surgery to create experimental strabismus produced extremely limited ocular rotation (e.g. von Noorden and Dowling, 1970; Crawford and von Noorden, 1979; Harwerth *et al.*, 1983) the resulting visual deficits tended to be of greater extent than those found in most of our animals. In addition, animals studied physiologically, within a few days of strabismus surgery, show large, virtually immediate shifts in the distribution of ocular dominance in striate cortex (Crawford and von Noorden, 1979).

One of the purposes of this study was to document the sensitive period for the development of strabismic amblyopia in monkeys. We would actually need to study more monkeys with older intervention ages in order to be able to quantify the time course for the decline of the sensitive period. However, there are still some general statements that can be made. First, it is clear that the majority of animals with early intervention developed amblyopia. Given the

criterion of a one octave IAD for amblyopia, 12/16 animals that had esotropia created before the age of 5 weeks developed amblyopia. Thereafter, only two monkeys developed amblyopia. Although only 2/5 monkeys who had esotropia created after 5 weeks developed amblyopia, it would be unreasonable to conclude that the sensitive period is over by 15 weeks. Given the variability among the older monkeys, and the demonstration that spatial vision is susceptible to degradation by other forms of deprivation beyond 15 weeks (Harwerth *et al.*, 1986), it is likely that the development of strabismic amblyopia can occur beyond 15 weeks as well.

Our finding that the nondeviated eyes do not develop normally in the experimentally strabismic monkeys confirms previous similar findings in humans and cats (Levi and Klein, 1985; Holopigian and Blake, 1983). The basis for the deficit in the nondeviated eyes is not clear; however, our data show that the effect is not an immediate consequence of the esotropia. In most cases the resolution of the nondeviated eye was within the normal range until 10–15 weeks; thereafter the poorer performance became obvious. Similarly, Kiorpes (1989) reported that the development of spatial vision was slower overall in naturally strabismic monkeys than in normal monkeys, although in the early post-natal period their spatial resolution was similar to normal monkeys. It is possible that the presence of strabismus, natural or experimentally produced, slows the rate of visual development. If so, this effect could account for the fact that, in humans, strabismic amblyopia can be successfully treated beyond the age when spatial vision in normal children reaches adult levels, which is about five years of age (e.g. Mayer and Dobson, 1982; Bradley and Freeman, 1982).

Our analysis of factors such as refractive error, angle of deviation and age of onset did not show any single factor that determined whether or not amblyopia developed. However, the analysis revealed a constellation of factors that are associated with the development of amblyopia and we can comment on the relative contributions of these factors. Clearly, an alternating pattern of fixation is less likely to lead to amblyopia than a unilateral pattern of fixation. Similarly, an earlier onset of strabismus is more likely to result in amblyopia than later onset. Given an early age of onset and a unilateral fixation pattern, a large refractive error is likely to contribute to the development of amblyopia. The association of these particular factors with

the development of amblyopia in monkeys is especially interesting in that they are consistent with trends found in the human population on the basis of clinical data (see Introduction). Our analysis of the factors associated with the depth of amblyopia also showed a constellation of factors that are important for determination of the extent of amblyopia. Clearly, large deviations and large refractive errors are likely to contribute to the development of large interocular acuity differences. However, age of onset and fixation pattern also contribute to the extent of amblyopia. Since the pattern of fixation is a major factor in discriminating amblyopes from nonamblyopes, and also contributes to the depth of amblyopia, a quantitative analysis of oculomotor behavior in the early period following the onset of esotropia could provide important information about this developmental process.

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