Studies of Strabismus and Amblyopia in Infant Monkeys

Ronald G. Boothe, Ph.D.
Atlanta, Georgia
Lynne Kiorpes, Ph.D.
New York, New York
Melvin R. Carlson, M.D.
Seattle, Washington

ABSTRACT

Longitudinal studies of grating acuity have been conducted with strabismic infant monkeys. Representative data from several different monkey models of strabismus are presented. Acuity in the fixating eyes of these monkeys developed to adult levels similarly to acuity development in normal infant monkeys. Acuity in the non-fixating eyes often lagged behind, and in some cases never reached normal levels, resulting in a permanent amblyopia.

Introduction

The pioneering behavioral work on monkey models of strabismic amblyopia was conducted by von Noorden and co-workers.[1,2] These studies demonstrated that an esotropia produced surgically during the first three months after birth can lead to a permanent amblyopia in the deviated eye later in life.

The experimental protocol used by von Noorden was to produce the strabismus early in life, and then test behaviorally a year or more later to determine whether an amblyopia had developed. The time course over which the amblyopia developed was not determined in these studies. The amblyopia might have resulted from an arrest of normal development, deterioration after having previously reached some normal level, or some more complicated abnormal time course of acuity development.

In recent years our laboratory has been conducting longitudinal studies of acuity development in normal and in strabismic monkeys. Our preliminary results from infant monkeys made surgically esotropic at six days of age demonstrated that amblyopia results from an abnormal development of acuity in the deviated eye.[3] We have extended these observations to additional strabismic monkeys and have investigated a variety of models of strabismus. The present report provides an overview of these studies and summarizes our data collected to date.

Methods

Infant macaque (*Macaca nemestrina*) monkeys were used as subjects in all of these experiments. We have been studying three separate kinds of monkey models of strabismus. We refer to these models according to the way the strabismus originated: 1) Surgically, 2) by Neurotoxin injection, and 3) Naturally.

We use unilateral transection of the lateral rectus and resection of the medial rectus to produce the surgical model. The Neurotoxin model is produced by injecting the lateral rectus muscle with botulinum neurotoxin Type A.[4] In both cases these procedures result in moderate angles of esotropia (typically 30 to 60 prism Diopters). All animals have maintained the ability to abduct the operated or injected eye to at least the primary position. The Natural model consists of infants screened from our breeding colony that have a naturally occurring strabismus.[5,6]

From the Yerkes Primate Center, Emory University, Atlanta, Georgia (Dr. Boothe); the Department of Psychology, New York University, New York, New York (Dr. Kiorpes); and the Department of Ophthalmology, University of Washington, Seattle (Dr. Carlson).

This report is based on a presentation by Drs. Kiorpes, Carlson and Boothe at the Annual Meeting of the Association for Pediatric Ophthalmology and Strabismus, Vail, Colorado, February 1984. Research projects described in this report are supported by EY03956.

Manuscript received March 16, 1984. Accepted for publication October 1, 1984.

Requests for reprints should be addressed to Ronald G. Boothe, Ph.D., Yerkes Primate Center, Emory University, Atlanta, Georgia 30322.

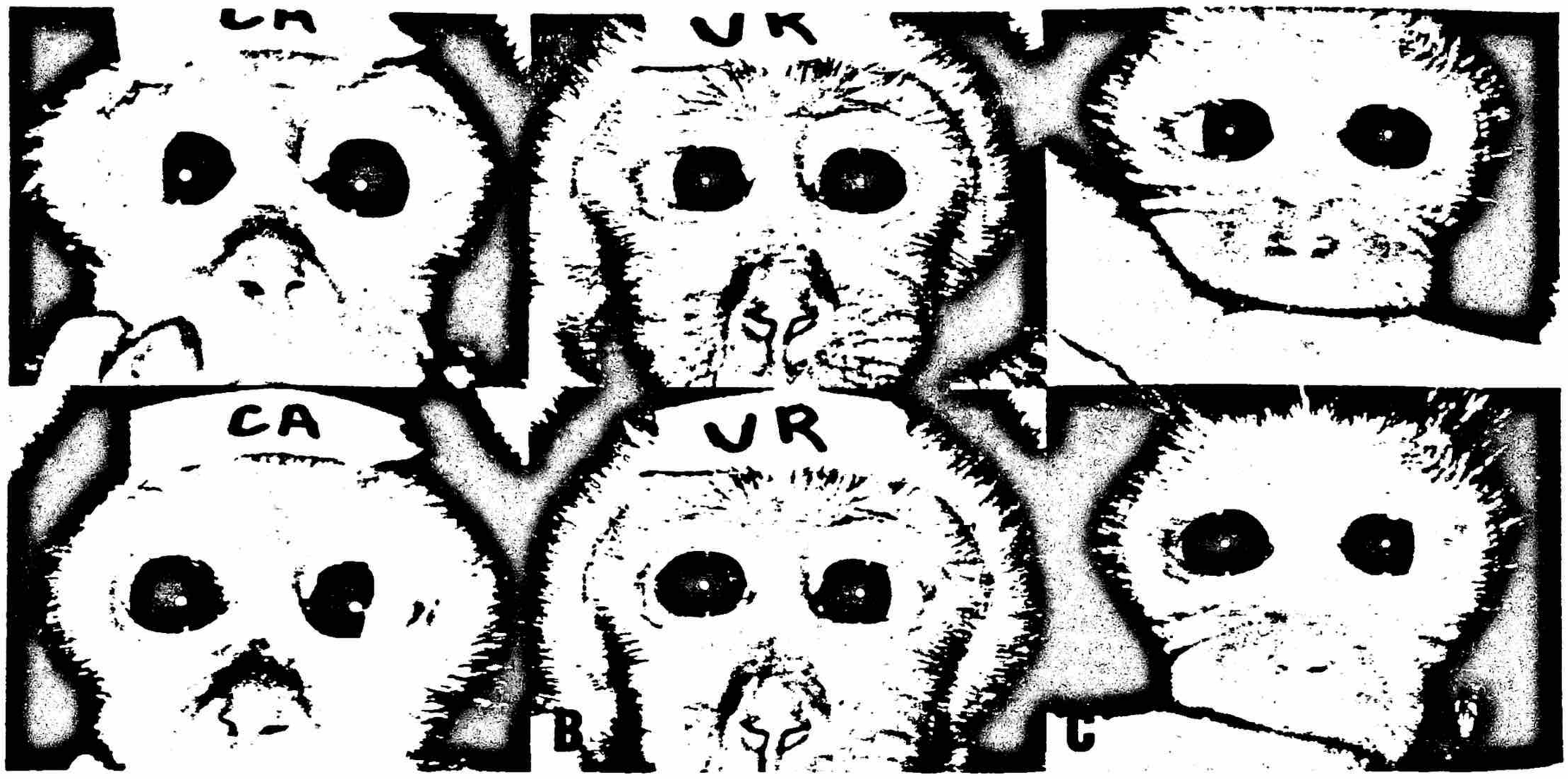

FIGURE 1: Photographs of strabismic monkeys. Figure 1A shows surgically strabismic monkey CA who had the left eye operated on at 29 days of age. Figure 1B shows neurotoxin strabismic VR whose left eye was injected at 13 days of age. Figure 1C shows naturally strabismic monkey VP. These photographs illustrate that all of our models of strabismus can abduct either eye to at least the midline, and can hold fixation with either eye.

The photographs in Figure 1 illustrate the nature and magnitudes of the squints present in our animals. Figure 1A shows surgically strabismic monkey CA, Figure 1B is neurotoxin monkey VR, and Figure 1C is naturally strabismic VP.

Two methods have been used to measure the time course of grating acuity development in these monkeys. At the youngest ages, acuity estimates were obtained with Forced-Choice Preferential-Looking.[7] At older ages the monkeys were trained and then tested with operant methods. Detailed descriptions of our methods for stimulus generation, behavioral testing, and data analysis have been presented previously.[3,8,9]

Results

The data in Figure 2 summarize the time courses of acuity development in normal monkey infants and in normal human infants. This figure summarizes results from a number of studies conducted in our laboratory and in the laboratories of Dr. D. Teller and Dr. V. Dobson.[8,10,11]

Several important points about normal acuity development can be noted from Figure 2. Acuity levels are similar in neonatal monkeys and humans. Acuity levels are also similar in adult monkeys and humans. The time courses of acuity development in humans and monkeys can be made to look reasonably similar by plotting age in terms of weeks

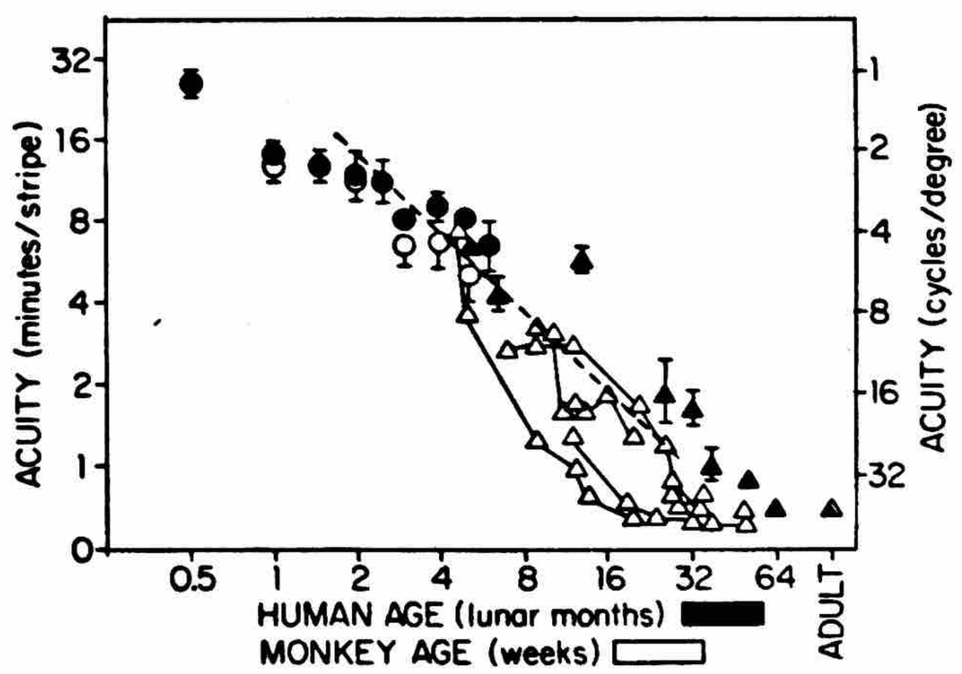

FIGURE 2: Acuity is plotted as a function of age for both normal humans and normal monkeys. Age is specified in months for humans and in weeks for monkeys. The left ordinate specifies grating acuity in terms of minutes/stripe where a 1-minute stripe corresponds to 20/20 Snellen acuity. The right ordinate specifies acuity in terms of cycles/degree.

for monkeys and in terms of months for humans. It is worth noting that when acuity development is specified in terms of cycles/degree (see right side legend in Figure 2), then acuity values between 2 and 30 cycles/degree are approximately equal to age in weeks for monkeys. This rule of thumb, indicated by the dashed line in Figure 2, is not

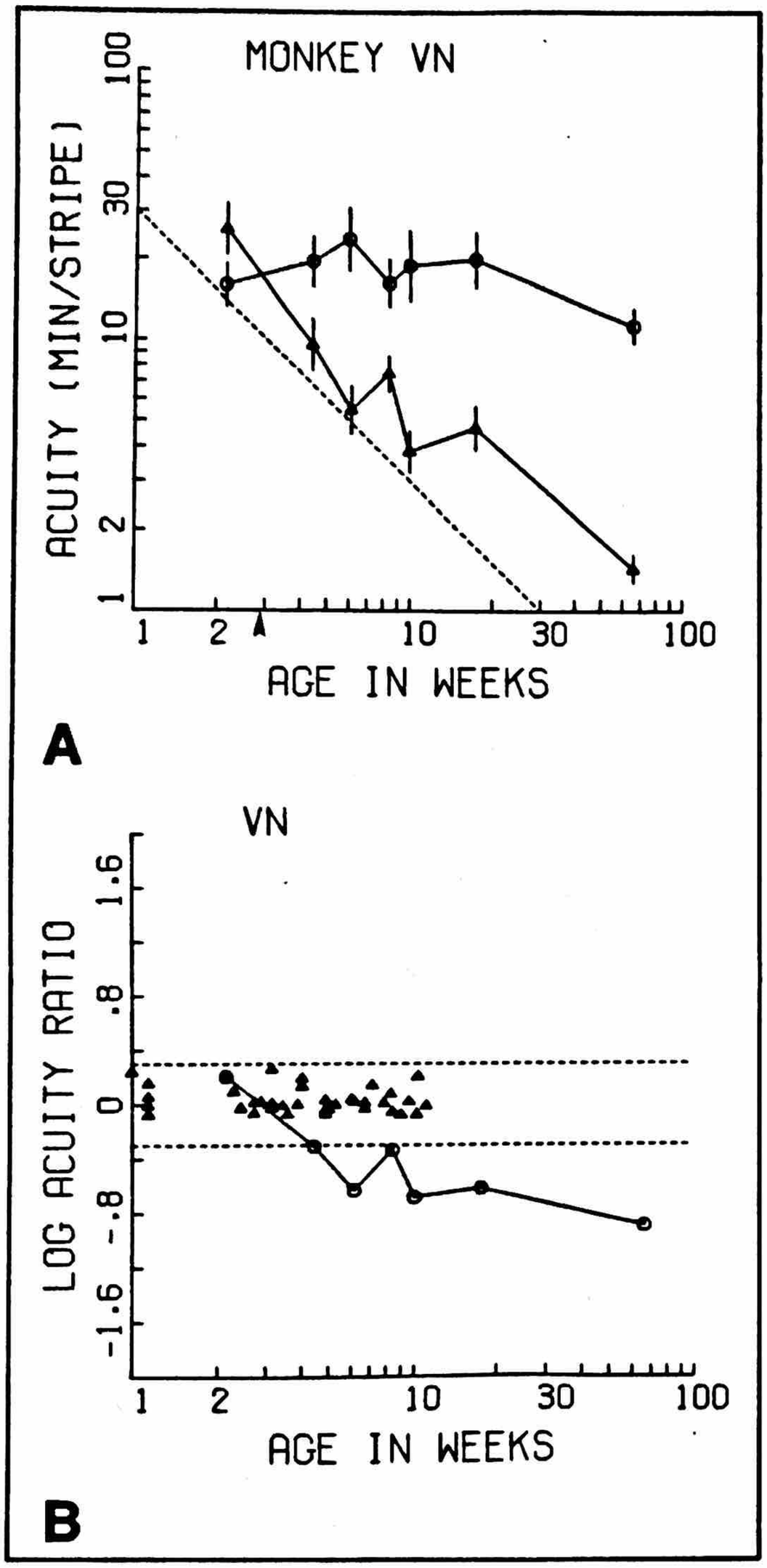

FIGURE 3: Acuity development in monkey VN. In Figure 3A, acuity is plotted for each eye as a function of age. An acuity value of 1 minute corresponds to 20/20 Snellen. Circles are for the left eye; Triangles are for the right eye. The dashed line demonstrates the rule of thumb of expected binocular acuity development in normal monkeys. The arrow on the abscissa indicates the age that esotropia was induced. In Figure 3B, the log of the ratio between left and right eye acuities are shown. If the two eyes had identical acuities then the log of the ratio would be zero. The triangles in this figure are control data obtained from normal monkeys. The circles show results from monkey VN. Positive difference values indicate that acuity is better for the left eye; negative values indicate that the right eye is better.

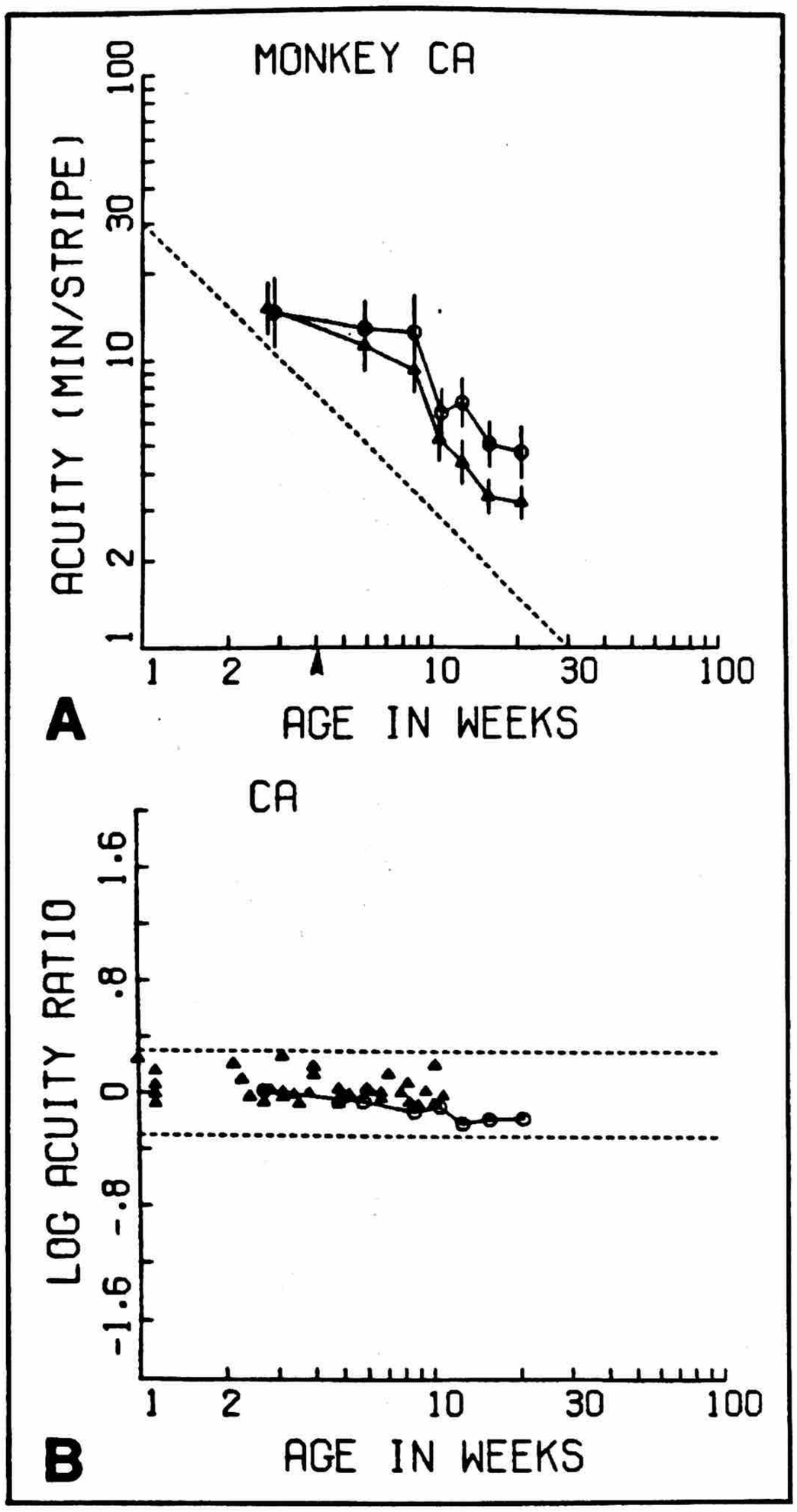

FIGURE 4: Acuity development in surgically strabismic monkey CA. Legends and symbols are the same as in Figure 3.

exact, but provides a useful mnemonic for remembering approximate acuity values during the mid-range of normal development. This rule of thumb is repeated by the dashed lines in Figures 3-7 in order to facilitate comparisons between acuity development in normal and strabismic monkeys.

A final observation to be made from Figure 2 is that both age and acuity are plotted on log axes. Westheimer[12] has discussed the reasons that a log axis is the most appropriate for specifying acuity. By similar reasoning, it might be argued that a log axis is also the most appropriate for age.

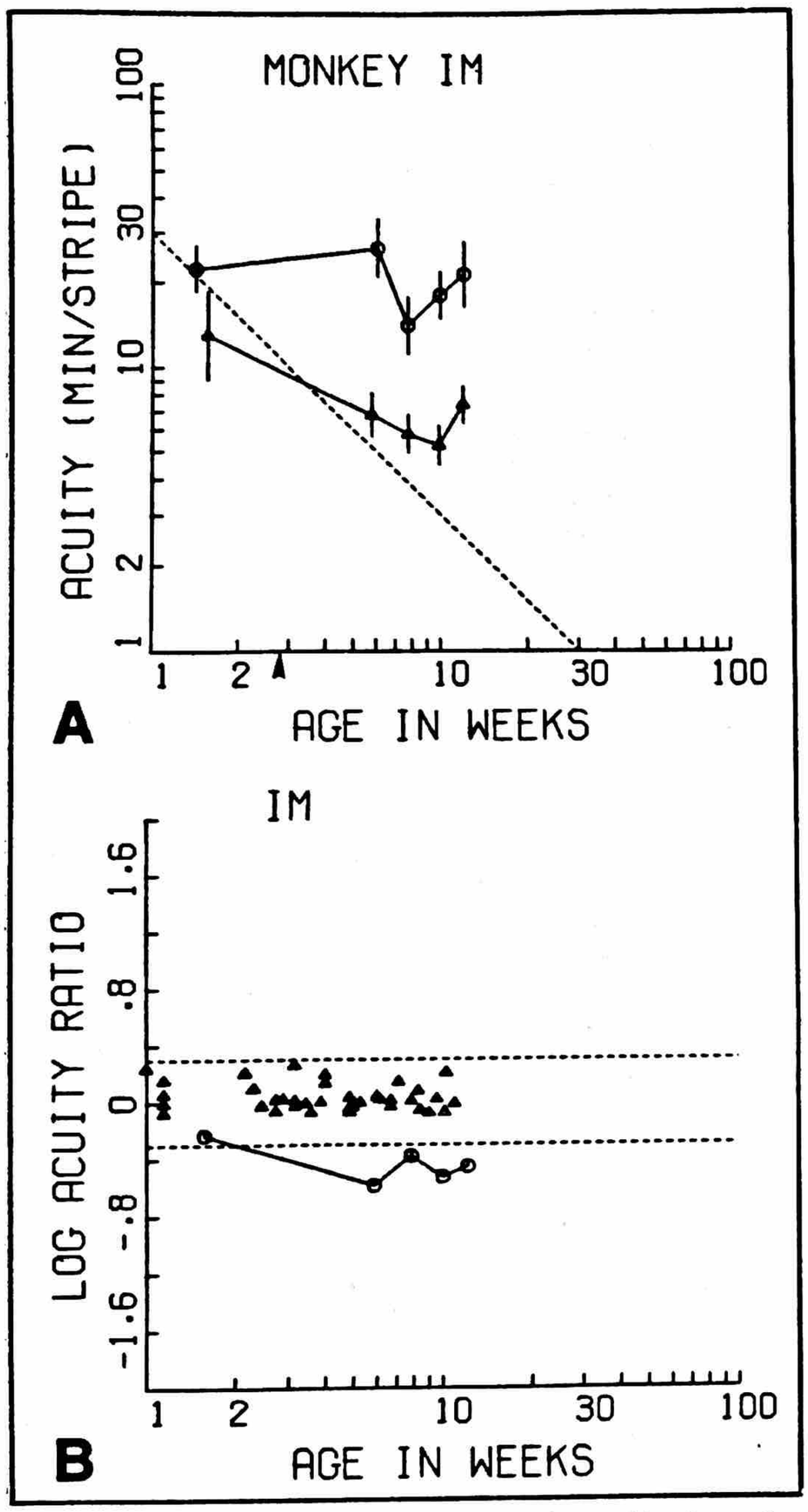

FIGURE 5: Acuity development in neurotoxin strabismic monkey IM. Legends and symbols are the same as in Figure 3.

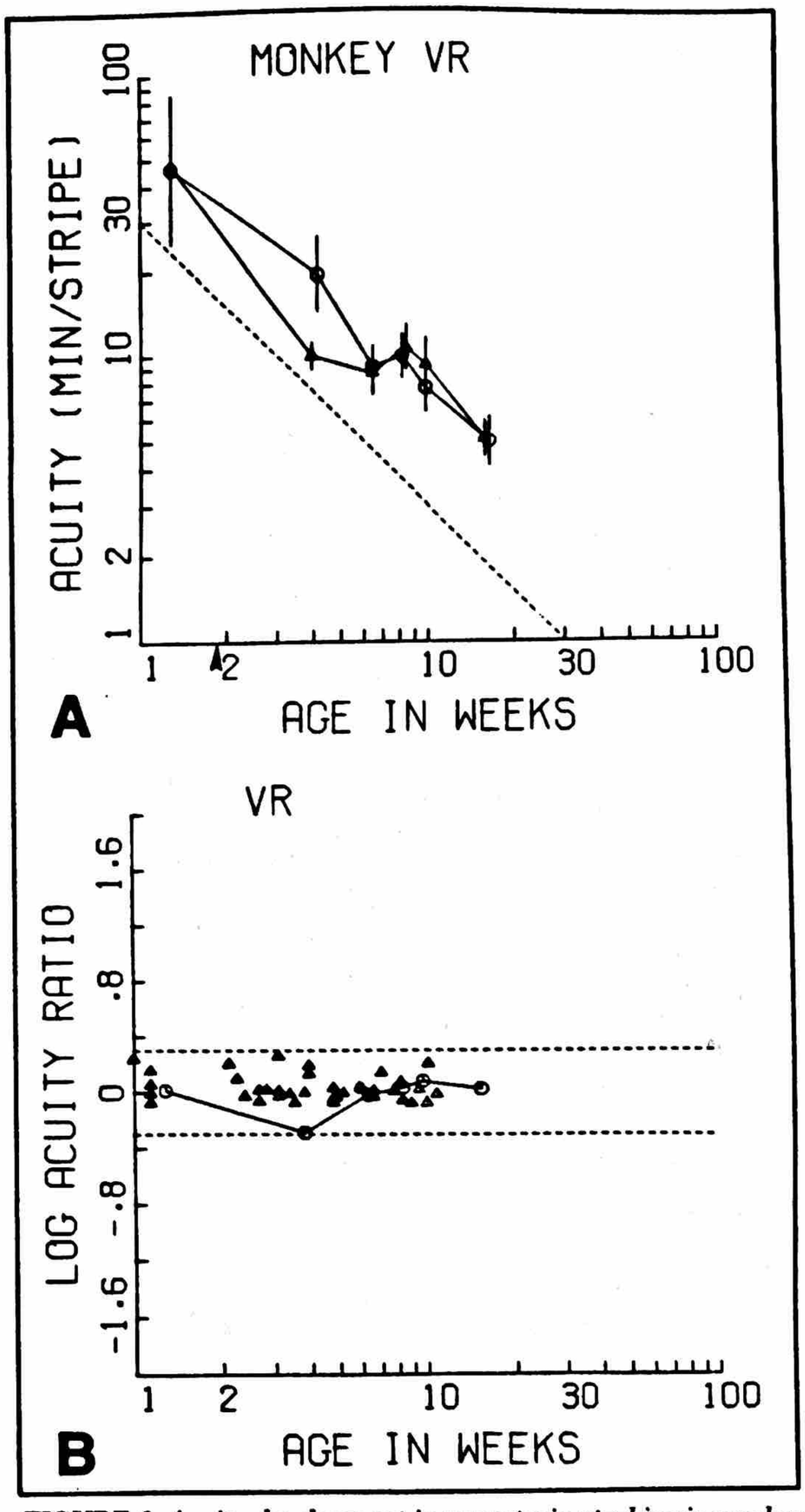

FIGURE 6: Acuity development in neurotoxin strabismic monkey VR. Legends and symbols are as in Figure 3.

On a linear age scale, constant amounts of improvement in acuity will be associated with different amounts of time at different ages. Note however that in Figure 2, constant amounts of acuity improvement between 2 and 30 cycles/degree are associated with nearly constant increments in age. The sensitive periods associated with abnormal acuity development are probably also best described in terms of log age.

The range of results we have obtained from our surgically strabismic monkeys are illustrated by the results from monkeys VN and CA shown in Figures 3 and 4. Surgery was conducted on VN's left eye at 20 days of age. This monkey can hold fixation with either eye, but usually fixates with her right eye. Figure 3A shows estimates of acuity for each eye of monkey VN, along with standard errors of these estimates, as a function of age. These results can be compared to binocular acuity development in normal monkeys by reference to the dashed line that shows our rule of thumb discussed above. Prior to surgery, VN's left eye was actually slightly better than her right eye. This small difference is probably not significant, but it does demonstrate that there was no predisposition for the right eye to

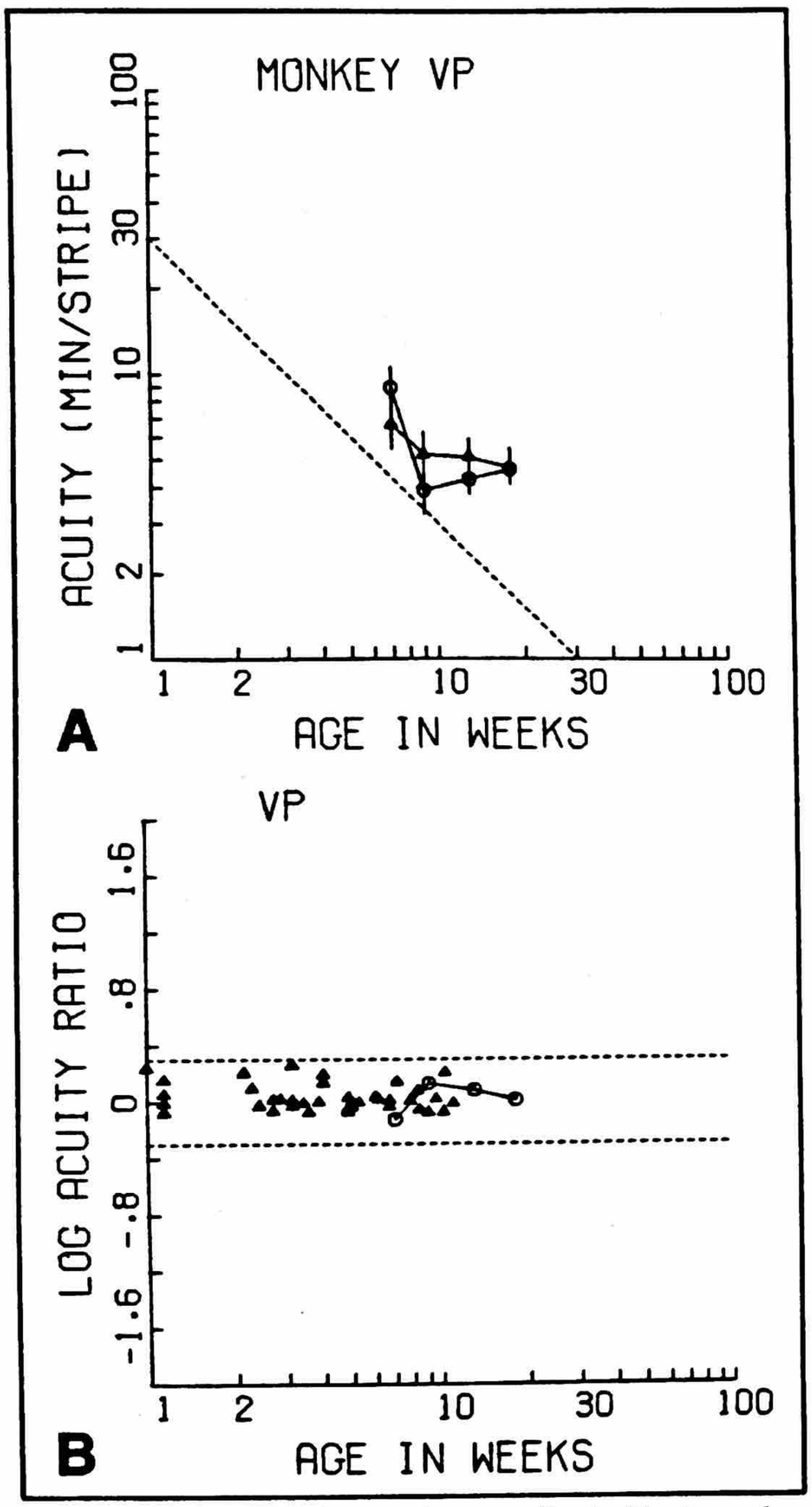

FIGURE 7: Acuity development in naturally strabismic monkey VP. Legends and symbols are as in Figure 3.

have better acuity. Following the surgery, acuity in the untreated (right) eye continued to improve, but there appears to have been an arrest of acuity development in the deviated (left) eye.

A comparison of the relative acuity differences between the two eyes of monkey VN as a function of age are shown in Figure 3B. Control data (triangle symbols) are shown as a standard of reference in this figure. These control data are from normal animals tested in our laboratory and demonstrate that the range of acuity differences between the eyes of normal monkeys fall within plus or minus one octave (.3 log units). We therefore define amblyopia as an intraocular acuity difference of at least one octave. The normal range of intraocular acuity differences is demarcated by the dashed horizontal lines in Figure 3B. Strabismic monkey VN is within the normal range prior to surgery, falls near the edge of the normal range at first test after surgery, and thereafter consistently exhibits an intraocular difference that falls outside of the range for normal monkeys.

Results obtained from a second surgically strabismic monkey, CA, are shown in Figure 4. A left esotropia was produced at 29 days of age. This monkey prefers to use the right eye for fixation, but can hold fixation with either eye. Figure 4A shows estimates of acuity development for each eye, along with standard errors of these estimates, as a function of age. Acuity levels were similar in the two eyes prior to surgery. The development of acuity progressed slowly in both eyes for about four weeks following the surgery. After eight weeks of age, both eyes progressed at more normal rate toward normal acuity levels. However, the development of acuity in the treated (left) eye lagged slightly behind development in the untreated (right) eye. Figure 4B demonstrates that the intraocular differences found for monkey CA do not fall outside the range found in normal animals. Experiments with monkey CA are continuing to determine whether an amblyopia will eventually develop.

A similar range of results have been obtained from our monkeys with neurotoxin induced strabismus and we show representative examples from monkeys IM and VR in Figures 5 and 6. Neurotoxin was injected into the lateral rectus of the left eye of monkey IM at 19 days of age. He initially preferred the right eye, but at nine weeks of age showed a pattern of alternating fixation. Grating acuity development for each eye of IM is shown in Figure 5A. The right eye acuity was slightly better than the left eye prior to surgery, but Figure 5B demonstrates that this difference is not outside the normal range. Acuity development for the left eye has consistently lagged behind that for the right eye. Figure 5B demonstrates that all of the post-injection intraocular acuity differences are outside of the normal range.

Results obtained from another neurotoxin monkey, VR, are shown in Figure 6. Neurotoxin was injected into the lateral rectus of the left eye of monkey VR at 13 days of age. This monkey alternated fixation, and showed no eye preference. Behavioral testing has demonstrated that acuity developed roughly in parallel in the two eyes of this monkey, as can be seen in Figure 6A. Figure 6B demonstrates that none of the intraocular acuity differences for this monkey are outside of the normal range.

The individual data presented above are representative of the ranges of results we have seen for our monkeys with experimentally induced strabismus. All of the monkeys studied fell between these extremes. Of the nine monkeys with strabismus surgically induced between two and seven postnatal weeks, seven developed amblyopia (log acuity ratios of −0.3 to −0.6) three or more weeks post-surgery. Two showed small intraocular acuity differences (log acuity

ratios of −0.18 and −0.25), but the differences were within the normal range, e.g., CA. Of five monkeys with strabismus induced by injection of neurotoxin between one and four postnatal weeks, two developed amblyopia (log acuity ratios of −0.4 to −1.0) within a few weeks of the injection. Two showed small intraocular acuity differences (log acuity ratios of −0.17 to −0.24) that were within the normal range, and one, VR, showed no difference.

An example of data from a naturally strabismic monkey, VP, appears in Figure 7. Additional information about this monkey and other naturally strabismic monkeys is presented elsewhere.[6] We detected VP's strabismus at four weeks of age during routine screening of our breeding colony. The strabismus may have been congenital. VP shows a constant esotropia of about 45 prism Diopters. During the first four months after birth she alternated fixation with no apparent preference. At older ages we noted an increased preference for fixating with the left eye.

Developmental acuity measurements obtained during the first four months demonstrated that acuity developed roughly in parallel in the two eyes (Figure 7A). Grating acuity differences between the two eyes were all within the normal range during this period (Figure 7B).

However, when VP was tested more extensively on a contrast sensitivity task, it was determined that a small but significant intraocular difference had developed in this monkey's right eye. The contrast sensitivity results are shown in Figure 8. In normal monkeys the error bars for data obtained from the two eyes overlap at high frequencies.[13] Note that for VP the contrast sensitivity for the left eye is consistently better than that for the right eye at all spatial frequencies higher than the peak (3 cy/deg).

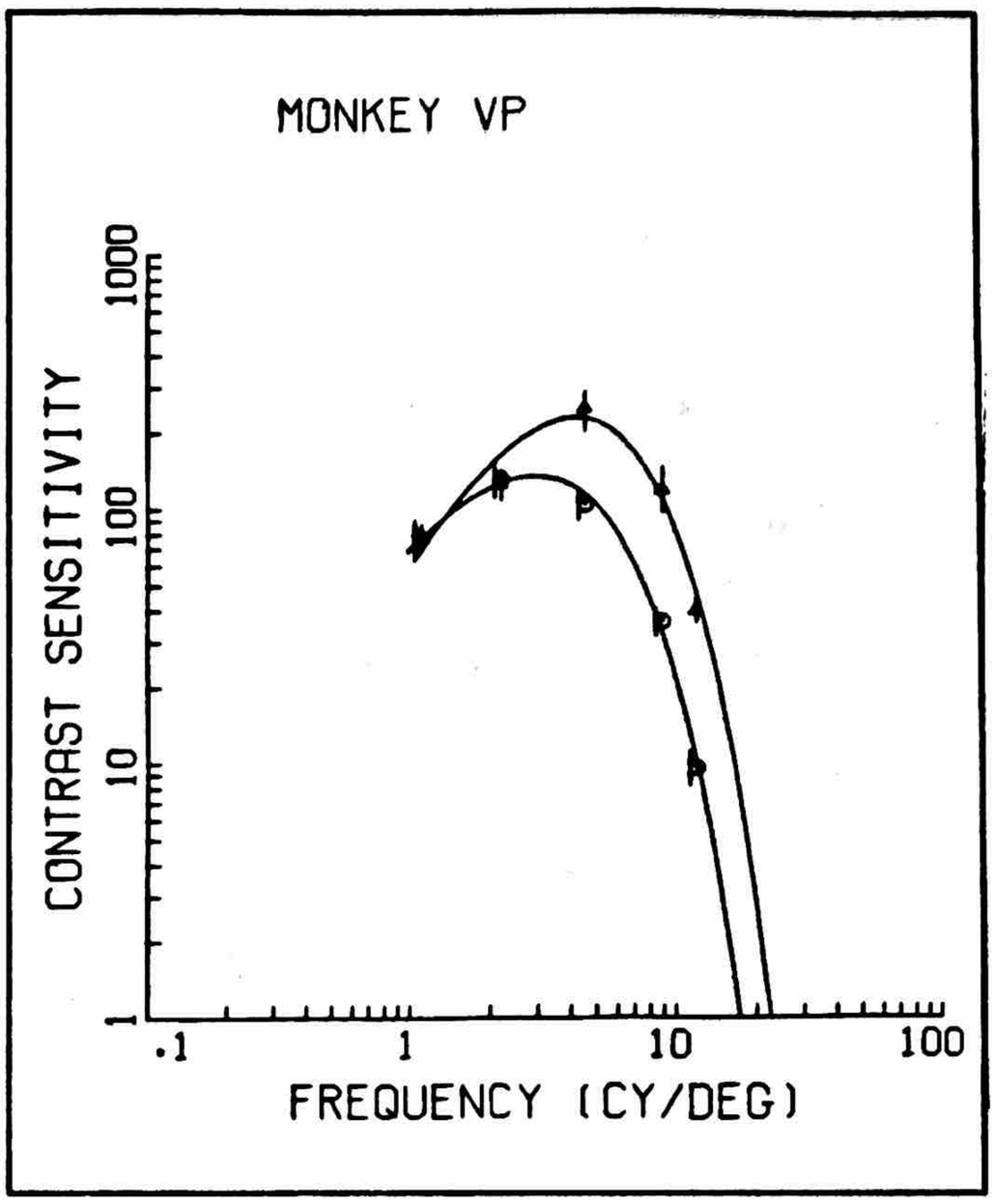

FIGURE 8: Contrast Sensitivity results for naturally strabismic monkey VP. The abscissa indicates the frequency of a spatial sinusoidally modulated grating. The ordinate indicates the sensitivity (1/contrast at threshold) of the monkey to the grating target. These results were obtained from monkey VP at 1.5 years of age.

Discussion

In this paper we have presented representative acuity development data from several monkey models of strabismus. We have found that in most cases intraocular differences in acuity are present at various times during development. However, these developmental differences do not always result in permanent intraocular differences large enough to be considered amblyopia. We are conducting experiments such as these on a number of strabismic monkeys, in whom the strabismus originated at different ages. This is an ongoing project and we do not have the answers yet, but our long-term goal is to try to determine the relative time courses over which amblyopia develops during the sensitive period in these animals, and also to try to determine why it is that some strabismic monkeys never develop an amblyopia. Reference to data such as shown in our Figure 2 should allow results about the time course of amblyopia development in monkeys to be related to human infants.

Grating acuity is not necessarily the most sensitive measurement for picking up small intraocular differences, and it is possible that other measurements such as optotype acuity or contrast sensitivity might reveal an abnormality in some of our animals where the intraocular grating acuity differences were not outside of the normal range. This might be the case, for example, with the results we presented for naturally strabismic monkey VP. It will be necessary to use multiple measures on individual animals to try to sort out this question.

Another issue that warrants discussion is the magnitude of the individual differences from animal to animal in susceptibility to deprivation. A comparison of our Figures 3 with 4 and 5 with 6 illustrates the range of results that we have obtained with our two experimental models of strabismus. There are a large number of factors that could be contributing to these differences (e.g., age at which the strabismus is induced, magnitude of the deviation, refractive errors), and we will need to collect data on additional subjects before we can draw a definitive conclusion about this question. In this regard, monkeys are probably a good model for the human clinical cases where patients with similar kinds of strabismus can differ widely in the amount of amblyopia present.

We can summarize our primary conclusions to date as follows: Strabismic monkeys that develop an alternating fixation pattern do not develop an amblyopia. Their eyes develop acuity roughly in parallel. On the other hand, in

monkeys that preferentially fixate with one eye, the development of acuity does not always follow the same time course in both eyes. Acuity development in the non-fixating eye lags behind. In some monkeys an intraocular acuity difference becomes apparent almost immediately after the strabismus is induced. In other monkeys, there is a delay of from several weeks to several months following onset of the strabismus before an intraocular acuity difference or amblyopia develops.

References

1. Von Noorden GK: Experimental amblyopia in monkeys. Further behavioral observations and clinical correlations. *Invest Ophthalmol Vis Sci* 1973; 12:721-726.
2. Von Noorden GK, Dowling JE: Experimental amblyopia in monkeys. II. Behavioral studies of strabismic amblyopia. *Arch Ophthalmol* 1970; 84:215-220.
3. Kiorpes L, Boothe RG: The time course for the development of strabismic amblyopia in infant monkeys (Macaca nemestrina). *Invest Ophthalmol Vis Sci* 1980; 19:841-845.
4. Scott AB, Rosenbaum A, Collins CC: Pharmacologic weakening of extraocular muscles. *Invest Ophthalmol Vis Sci* 1973; 12:924-927.
5. Kiorpes L, Boothe RG: Naturally occurring strabismus in monkeys. *Invest Ophthalmol Vis Sci* 1981; 20:257-263.
6. Kiorpes L, Boothe RG, Carlson MR, Alfi D: Frequency of naturally occurring strabismus in monkeys. *J Pediatr Ophthalmol Strabismus*, to be published.
7. Teller DY: The forced-choice preferential looking procedure: A psychophysical technique for use with human infants. *Infant Behavior and Development* 1979; 2:135-153.
8. Boothe RG: Development of spatial vision in infant macaque monkeys under conditions of normal and abnormal visual experience. In: Aslin R, Alberts J, Petersen M (eds), *The Development of Perception: Psychobiological Perspectives* 1981; 2:217-241.
9. Williams R, Boothe R, Kiorpes L, Teller D: Oblique effects in normally reared monkeys (Macaca nemestrina) meridional variations in contrast sensitivity measured with operant techniques. *Vision Res* 1981; 21:1253-1266.
10. Teller DY, Boothe RG: The development of vision in infant primates. *Trans Ophthalmol Soc UK* 1980; 99:333-337.
11. Mayer DL, Dobson V: Visual acuity development in infants and young children, as assessed by operant preferential looking. *Vision Res* 1982; 22:1141-1152.
12. Westheimer G: Scaling of visual acuity measurements. *Arch Ophthalmol* 1979; 97:327-330.
13. Kiorpes L: The development of spatial vision in naturally strabismic monkeys. Doctoral Dissertation, University of Washington.

Announcements & News

10th Annual Pediatric Ophthalmology Day. Sainte-Justine Hospital, Montreal. October 25, 1985. Sponsored by the Department of Ophthalmology. A full day of lectures and presentations of cases on pediatric ophthalmology and strabismus. Guest speakers: Arthur Jampolsky, MD, Director, Smith-Kettlewell Institute of Visual Sciences, San Francisco, California, and Robert Ellsworth, MD, The New York Hospital-Cornell Medical Center, New York. Neither registration nor tuition are required. Further information: Jean Milot, MD, Department of Ophthalmology, Hôpital Sainte-Justine, 3175 Côte Ste-Catherine, Montreal, Quebec H3T 1C5, Canada. (514) 731-4931.

Seventh Symposium. International Society on Metabolic Eye Disease. Parma, Italy. May 11-14, 1986. Theme: Neonatal Aspects. Session I. Retinal Vascular Disease; Session II, Metabolic Cataract; Session III, Metabolic Corneal Dystrophies and Congenital Glaucoma; Session IV, Metabolic and Genetic Disorders. For free communications send a topic and a 500-word abstract, no later than November 1, 1985, to Heskel M. Haddad, MD, Program Chairman, 1125 Park Avenue, New York, New York 10128. (212) 427-1246.

Perspectives in Ophthalmology. The Cleveland Clinic. December 6 & 7, 1985. Information: Center for CME, The Cleveland Clinic Educational Foundation, 9500 Euclid Avenue, Room TT3-101, Cleveland, Ohio 44106. (800) 762-8173. In Ohio (800) 762-8172.

Computers in the Practice of Ophthalmology. Duke University Eye Center. September 6 & 7, 1985. Information: Ms. Carol Vilas, Program Coordinator, Duke Eye Center, Durham, North Carolina 27710. (919) 684-6743.