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## The time course for the development of strabismic amblyopia in infant monkeys (*Macaca nemestrina*). LYNNE KIORPES AND RONALD G. BOOTHE.

The time course for the development of acuity was followed in two experimentally esotropic infant monkeys (Macaca nemestrina). Surgical esotropia was produced at 6 days postnatal. Acuity values for both eyes of both infant monkeys were found to be normal through 4 weeks postoperative. After this period of delay, amblyopia began to emerge. The deviated eyes showed poorer acuity than the nondeviated eyes at every subsequent age tested. These data support a developmental hypothesis for the emergence of strabismic amblyopia.

Previous behavioral research on strabismic amblyopia in animals has utilized an experimental paradigm which involves producing a strabismus at an early age, then testing for amblyopia a year or more later. Such studies have demonstrated that amblyopia can be induced by surgically creating esotropia in young kittens<sup>1</sup> or monkeys<sup>2</sup> during the first 3 months after birth. However an esotropia produced at older ages does not lead to amblyopia. It is also known that acuity is developing fairly rapidly during the time period in which both cats<sup>3</sup> and monkeys<sup>4</sup> are maximally susceptible to the deleterious effects of strabismus.

The fact that acuity is in an immature state during the time period when experimental esotropia leads to amblyopia suggests two types of hypotheses about the subsequent development of amblyopia. The first, which we will call a developmental hypothesis, is that the presence of esotropia disrupts the normal development of acuity. An example of a developmental disruption would be a simple arrest of acuity development at the onset of esotropia, as reported by Jacobson and Ikeda.<sup>1</sup> However, clinical data suggest that in many cases amblyopia resulting from early esotropia is more than an arrest of development.<sup>5</sup> A more complicated disruption of development would be necessary to explain the outcome of these cases. There are any number of more complicated disruptions of development which could lead to reduced acuity levels in the adult.

The second hypothesis, which we will call an adult-deterioration hypothesis, is that acuity develops normally, approaching adult levels, but then deteriorates some time later. For example, amblyopia could result from some kind of suppression which is not manifest until adult acuity levels would normally be reached, even though the esotropia had been present throughout.

In order to distinguish between these two hypotheses, it is necessary to look at the time course for the development of amblyopia following the onset of esotropia. In the present experiment we have followed the development of acuity for each eye of two monkeys made esotropic 1 week after birth. Our results are consistent with a developmental hypothesis but reflect a disruption of acuity development that is more complicated than a simple arrest. Some of these data have been reported previously.<sup>6</sup>

*Methods.* Seven infant monkeys (*Macaca ne-mestrina*) were used in this study. All were reared according to normal protocol for this laboratory.

Esotropia was produced surgically in the right eye of two infant monkeys on the sixth postnatal day. Refraction under cycloplegia (1% Kupfers solution) prior to surgery revealed no anisometropia in either monkey. The surgical method was similar to that reported by von Noorden and Dowling.<sup>2</sup> Briefly, the medial rectus was not resected but

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Fig. 1. Development of acuity for experimentally esotropic monkey TS. Upper panel, Resolution threshold (acuity)  $\pm 1$  S.E. as a function of age.  $\uparrow$ , Age at surgery. The connected points were obtained by the FPL method, and the single point by operant. Operant data could not be obtained for the deviated eye. Lower panel, IAD as a function of age.  $\blacktriangle$ , Normal control monkeys;  $\blacklozenge$ , monkey, TS; range found for normal monkeys is between the dotted lines. One octave is equal to one division of the ordinate in the upper panel.

advanced to the limbus, and the lateral rectus was excised. The extent of the deviation was assessed by a corneal reflex method (Hirschberg test)<sup>5</sup> regularly throughout the course of the experiment. Examination of the photographs revealed that fixation was always with the unoperated eye (nondeviated) whereas the operated eye (deviated) always deviated nasally. The deviation was consistently found to be 13° for monkey TS and 25° for monkey IO.

Five infant monkeys with normal eye movements (by inspection) served as controls. Three were chosen at random from the nursery population, and two were assigned to other studies of visual development. One of the control animals was tested twice, at 5 and 9 weeks. The others were tested only once. Four of the five were shown to have visual acuity that was within normal limits for infant pigtail macaques.<sup>4</sup> The fifth animal, tested at 7 weeks, showed acuity values that were about 1 octave poorer than normal.

During the first 6 months after birth, visual acuity was assessed by means of the forced-choice preferential looking method (FPL), which has been described in detail elsewhere.<sup>4, 7</sup> Briefly, an observer holds the monkey in front of a stimulus field which contains a high-contrast square-wave grating on either the left or right side. The observer, who is blind as to the position of the stimulus and the stripe width on every trial, views the monkey's face through a periscope. On the basis of the monkey's orienting behavior and eye movements, the observer makes a judgment as to the stimulus position, left or right.

In the present experiments, the observer was required to maintain a criterion performance of at



Fig. 2. Development of acuity for experimentally esotropic monkey IO. Upper panel, Resolution threshold (acuity)  $\pm 1$  S.E. as a function of age. Symbols are the same as in Fig. 1. The connected points were obtained by the FPL method, and the single points by operant. Lower panel, IAD as a function of age. Symbols are the same as in Fig. 1.

least 80% correct on the largest stripe width for any single session. Data from sessions in which this criterion was not met were discarded. Since the observer was blind as to which trials were control trials, the criterion made it impossible for the observer to bias the results on the basis of expectations of differences between the two eyes of an animal.

Acuity estimates were based on 20 to 40 trials at each of four stripe widths near resolution threshold. The stripe widths were chosen so that performance on the narrowest was expected to be near chance and performance on the widest was expected to be near 100%. Probit analysis<sup>8</sup> was applied to these data to obtain estimates of acuity (here defined as the stripe width which would result in 75% correct performance) and standard errors of this estimate.

An operant method of visual acuity assessment<sup>9</sup> was used to test the monkeys beyond 6 months of age. The monkey, who is in a face-mask cage,

looks out through the eyehole(s) at two cathode ray tube (CRT) screens. The monkey is trained to pull either a left or right grab bar corresponding to the position of a computer-generated grating pattern presented on the left or right screen. The other screen is blank but of space-average luminance equal to that of the pattern. Correct responses are rewarded with apple juice; wrong responses result in a time out.

Monkey IO was tested with square-wave gratings, and his estimate of acuity was obtained with the same methods as for the FPL data. Monkey TS was tested more extensively with sine-wave gratings at a number of spatial frequencies in order to measure the entire contrast sensitivity function. The contrast sensitivity function was extrapolated to its high-frequency cutoff in order to obtain the estimate of acuity presented in this paper. We find good agreement between the two methods for estimating acuity in our laboratory. Results at lower spatial frequencies will be presented elsewhere. Acuity estimates by both methods, FPL and operant, were obtained from each eye tested separately in counterbalanced order. The data for each pair of acuity estimates were collected within a 5 to 7 day period.

**Results and discussion.** The time course for the development of acuity in the two eyes of esotropic monkeys TS and IO are shown in Figs. 1 and 2, respectively. The patterns of acuity development for the two animals were qualitatively similar.

The time courses for acuity development of the nondeviated eyes were found to be similar to each other and also similar to that for normal binocular acuity development.<sup>4</sup> Improvement in acuity was about 0.25 octave/week during the first 8 weeks, followed by a more gradual improvement at later ages. (A 1 octave improvement would be equivalent to a halving of the minutes of arc resolution level, e.g., improvement from 16 to 8 min arc).

For at least the first 4 weeks after the surgery, the acuity values for the deviated eyes were similar to those for the nondeviated eyes. After 5 weeks of age, the developmental time courses for the two eyes of both monkeys became different. For every pair of acuity values obtained thereafter for both monkeys, the deviated eyes showed poorer acuity than the nondeviated eyes. TS's deviated eye showed some improvement in acuity after 13 weeks of age, whereas IO's did not. FPL data for IO's deviated eve after 8 weeks were not available because the monkey would not consistently perform above 80% on any stimulus with that eve. However, the acuity value obtained by operant methods at 35 weeks was similar to that obtained by FPL at 8 weeks.

The interocular acuity differences (IADs) found at each test age for the two esotropic monkeys are shown in the bottom portions of Figs. 1 and 2, respectively, together with the IADs from the control monkeys. The IAD for each control monkey is plotted at the age when tested. Through 5 weeks of age, the IAD values for monkeys TS and IO were within the normal range but thereafter fell outside the normal range in the predicted direction.

Similar patterns of acuity development have recently been reported by von Grunau<sup>10</sup> for esotropic kittens. He found a period of continued normal development, of 2 to 3 weeks' duration, after the onset of esotropia before acuity differences between the two eyes became apparent.

Monkeys TS and IO both showed a delay of at least 4 weeks between the production of esotropia and the emergence of amblyopia. The present data do not reveal whether the delay is fixed, i.e., always 4 to 7 weeks, or whether there is a particular age when amblyopia emerges. The onset of esotropia for both monkeys in the present study was 6 days postnatal. It is possible that amblyopia would emerge at about 6 weeks postnatal regardless of when, prior to that age, the esotropia was produced. Additional data are necessary to sort out this causal question.

The relation of these behavioral data to physiological results is interesting. Crawford and von Noorden<sup>11</sup> reported that within 1 or 2 weeks after the production of an experimental esotropia in visually immature rhesus monkeys there was already a shift in cortical ocular dominance away from the deviated eye. The fact that we found at least 4 weeks' delay before amblyopia emerged suggests that the physiological changes in ocular dominance precede the emergence of behavioral amblyopia. A pattern such as this would be consistent with the clinical theory of suppression amblyopia, which suggests that amblyopia is a carryover of suppression from binocular into monocular vision.<sup>5</sup> The cortical effects on ocular dominance could result from suppression during binocular vision, and the delay for behavioral amblyopia could represent the time necessary for the carrvover into monocular vision. Whatever the case, more concurrent physiological and behavioral investigations need to be done to fully understand the development of amblyopia.

In summary, the development of acuity proceeded differently in the deviated and nondeviated eyes of our experimentally esotropic infant monkeys. For each of these monkeys, both eyes showed normal acuity development for at least 4 weeks after the onset of the esotropia. Then there was a disruption of the normal pattern, after which the acuity development of the deviated eyes followed different time courses. These data support a developmental hypothesis for the emergence of amblyopia: esotropia leads to a disruption of the normal course of development of acuity in the deviated eye. However, our results indicate a more complicated disruption than a simple arrest of acuity development.

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