Effects of the Duration of Early Strabismus on the Binocular Responses of Neurons in the Monkey Visual Cortex (V1)

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PURPOSE. To determine how the duration of early strabismus influences the severity of loss of disparity sensitivity in V1 neurons and the effects of extensive poststrabismus visual experience on the maintenance of functional binocular connections.

METHODS. Concomitant strabismus was optically simulated in 10 rhesus monkeys using a prism-rearing procedure. The onset of strabismus was kept constant at 4 weeks of age and the duration was maintained for 2, 4, or 8 weeks. In one group of monkeys (infants), the neurophysiological experiments were conducted immediately after the period of rearing with prisms. In another group (adults), after the termination of the prism-rearing regimen at either 8 or 12 weeks of age, the monkeys were kept in a normal housing environment until maturity and behavioral testing was conducted before the recording experiments to determine the animal’s monocular and binocular visual capacities. To assess the effects of the period of early strabismus on binocular interactions in V1, extracellular single-unit recording methods were used in anesthetized and paralyzed monkeys, and dichoptic sine-wave gratings were used as stimuli.

RESULTS. In all strabismic monkeys, the sensitivity of V1 units to interocular spatial phase disparity (disparity sensitivity) was significantly reduced, and the prevalence of binocular suppression was higher than that found in age-matched control animals. Although 8 weeks of strabismus resulted in a slightly larger loss of disparity sensitivity, the overall effects of the duration of strabismus were surprisingly small in infant strabismic monkeys. After poststrabismus visual experience, a small but significant, higher degree of disparity sensitivity was noted in V1 if prism-rearing was terminated after 4 weeks of strabismus (i.e., at 8 weeks of age), but not after 8 weeks of strabismus (i.e., at 12 weeks of age).

CONCLUSIONS. A brief period (2 weeks) of misalignment after the emergence of stereopsis is sufficient to drastically reduce the functional binocular connections in V1, and longer periods of strabismus result in little additional loss in disparity sensitivity. Clinically, these results suggest that taking corrective measures for infantile esotropes before the known onset age for stereopsis may be important for maintaining better binocular sensory function and better interocular alignment at later stages of development. (Invest Ophthalmol Vis Sci. 2002;43:1262-1269)

In normal infant monkeys, both the binocular and monocular response properties of neurons in the primary visual cortex (V1) are immature near birth, but rapidly improve during the first four postnatal weeks.1,2 These neurons exhibit near adult-like properties by 4 weeks of age (equivalent to 4 months of age in humans) and this rapid cortical maturation just precedes the age at which stereopsis emerges.3 However, the normal development of binocular functions can be readily disrupted by early abnormal visual experience (e.g., strabismus) due to a relatively high level of plasticity in the visual brain.4–6 The onset age and the duration of early strabismus are known to affect the severity of binocular response anomalies in the primary visual cortex.6–8 We have reported that a brief period (2 weeks) of optical strabismus causes more severe disruptions in V1 development if it occurs after rather than before, the known onset age (approximately 4 weeks) of stereopsis in monkeys.6 In the current study, we asked whether the duration of optical strabismus influences the development of the binocular response properties of V1 neurons when the onset age is kept constant at an age that corresponds to the normal emergence of stereopsis and whether visual experience after the termination of prism-rearing procedures has any effect on the maintenance of disparity sensitivity in monkey V1.

METHODS

All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee of the University of Houston.

Subjects

Concomitant strabismus was optically simulated in 10 rhesus monkeys (Macaca mulatta) using a prism-rearing procedure. Specifically, infant monkeys were fit with a lightweight helmet that held 15 and 12 dioptr (D) prisms oriented base-in in front of the right and left eyes, respectively. The total prismatic deviation exceeded the fusional vergence ranges of normal monkeys. To ensure the absence of fusion during prism-rearing, the prism for the right eye was rotated base-down by 15°. The optical-rearing procedure began at 4 weeks of age, and the duration of prism-rearing was maintained for 2, 4, or 8 weeks (i.e., the age at prism removal was 6, 8, or 12 weeks of age, respectively; Fig. 1). In one group of monkeys (infants), the recording experiments were conducted immediately at the end of rearing period, and in another

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To determine the strength and the nature of binocular interactions, responses were collected for dichoptic sine-wave gratings of the optimal spatial frequency and orientation as a function of the relative interocular spatial phase disparity of the grating pair (Fig. 2A). In addition, monocular stimuli for each eye and one zero-contrast control were included in each stimulus parameter file. For descriptive and analytical purposes, a single cycle of a sine wave was fit to each neuron’s phase-tuning function. The amplitude of the fitted sine wave was used to calculate the degree of binocular interaction (binocular interaction index [BII] = amplitude of the fitted sine wave/average response amplitude). Operationally, a unit was considered disparity sensitive if its BII was 0.3 or more.

To determine whether binocular signal interactions were excitatory or inhibitory in nature, the mean binocular response amplitude—dominant monocular response amplitude ratios (B/M) were calculated for each unit. If the ratio of the binocular response amplitude over the cell’s dominant monocular response amplitude was less than 0.8, the cell was operationally defined as exhibiting binocular suppression.

RESULTS

We quantitatively analyzed the binocular responses of V1 units of strabismic (n = 525) and normal monkeys (n = 496). In each monkey, the electrode traversed all neuronal layers of the oculomotor portion of V1 at similar angles to the cortical sur-

FIGURE 1. The timing of the prism-rearing regimen. (V), Ages at which the recording experiments were conducted. In three groups of infant monkeys, the recording experiments were conducted immediately after the prism-rearing (at 6, 8, and 12 weeks of age). In two other groups, prisms were removed at either 8 or 12 weeks of age, and daily behavioral testing was performed between 2 and 4 years of age. After completion of the psychophysical experiments, the neurophysiological experiments were conducted to determine the effects of poststrabismus visual experience.

group (adults), the monkeys experienced optical strabismus for a duration of either 4 or 8 weeks, and then the recording experiments were conducted after the completion of behavioral testing at approximately 4 years of age. The results from the experimental animals were compared with those from age-matched normal control animals.

Preparation

The surgical preparation and the recording and stimulation methods are described in detail elsewhere. Briefly, monkeys were anesthetized initially with an intramuscular injection of ketamine hydrochloride (15–20 mg/kg) and acepromazine maleate (0.15–0.2 mg/kg), and a superficial vein was cannulated. All subsequent surgical procedures were performed under thiopental sodium anesthesia. The animals were paralyzed by an intravenous infusion of pancuronium bromide (a loading dose of 0.1–0.2 mg/kg followed by a continuous infusion of 0.1–0.2 mg/kg · hr) and artificially respired with a mixture of 59% N2O, 39% O2, and 2% CO2. Anesthesia was maintained by the continuous infusion of pentobarbital sodium (2–4 mg/kg · hr). The core body temperature was kept at 37.6°C. Cycloplegia was produced by 1% atropine sulfate, and the animals’ corneas were protected with rigid, gas-permeable, extended-wear contact lenses. Retinoscopy was used to determine the contact lens parameters required to focus the eyes on the stimulus screens.

Recording and Response Analysis Procedures

Tungsten-in-glass microelectrodes were used to isolate the activity from individual cortical neurons. Action potentials were extracellularly recorded and amplified using conventional technology. For each isolated neuron, the receptive fields for both eyes were mapped using handheld stimuli. For the quantitative analysis of monocular tuning and binocular signal interactions, the receptive fields were projected onto the centers of two matched cathode ray tube (CRT) screens (P-3 phosphors). The CRTs had a space average luminance of 56 candelas (cd)/m2. The visual stimuli were drifting sine-wave gratings. The neuron’s responses were sampled at a rate of 100 Hz (10 msec bin widths) by a laboratory computer and compiled into peristimulus time histograms that were equal in duration to, and synchronized with, the temporal cycle of the sine-wave grating. The amplitudes and phases of the temporal response components in the peristimulus time histograms were determined by Fourier analysis. Responses to drifting sinusoidal gratings (TF, 3.1 Hz, contrast, 35%–45%) were measured to determine the orientation tuning, spatial frequency tuning, and direction selectivity of individual units. Cells were classified as simple or complex on the basis of the temporal characteristics of their responses to a drifting sine-wave grating of the optimal spatial frequency and orientation.

Binocular Response Properties

To determine whether binocular signal interactions were excitatory or inhibitory in nature, the mean binocular response amplitude—dominant monocular response amplitude ratios (B/M) were calculated for each unit. If the ratio of the binocular response amplitude over the cell’s dominant monocular response amplitude was less than 0.8, the cell was operationally defined as exhibiting binocular suppression.
face. We attempted to study every isolated unit in each penetration. The receptive fields of all neurons were located between 1.0° and 4.0° from the center of the fovea.

Infant Monkeys without Poststrabismus Visual Experience

The sensitivity of individual units to interocular spatial phase disparity (referred to as disparity sensitivity) was dramatically reduced and the prevalence of interocular suppression was higher in all strabismic monkeys compared with normal control monkeys. Figure 2 shows the binocular responses for representative units from a normal and a strabismic monkey. Compared with the unit from the normal 8-week-old monkey (Figs. 2A), the unit from the monkey that experienced 4 weeks of strabismus beginning at 4 weeks of age (Fig. 2B) showed low disparity sensitivity (BII = 0.19) and a relatively high degree of interocular suppression (i.e., mean B/M = 0.55).

Figure 3 compares the degree of disparity sensitivity loss for V1 units in normal monkeys and infant monkeys reared with three different durations of optical strabismus. For our popu-
lation of V1 units, both the proportion of disparity-sensitive units (BII ≥ 0.3) and the mean BIIs were clearly reduced in all strabismic infants compared with normal control monkeys (χ² test, P < 0.01; one-way ANOVA, P < 0.01, respectively). We were surprised that there were no significant differences in the proportion of disparity-sensitive units (Fig. 3A) or in the mean BIIs (Fig. 3B) between any of the three groups of prism-reared monkeys (χ² test, P > 0.1; one-way ANOVA, P > 0.05). Thus, if the onset of strabismus was at 4 weeks of age (i.e., the known onset age of stereopsis in monkeys), only 14 days of ocular misalignment was sufficient to cause a major deficit in the disparity sensitivity of V1 neurons.

Figure 4 plots the BIIs of individual units as a function of their ocular dominance. The ocular dominance of a given unit (ocular dominance index; ODI) was quantitatively determined by comparing the peak response amplitudes for the right and left eyes. In normal monkeys, the ocularly balanced units tended to show higher BIIs, which was most apparent in normal adults. The proportion of monocularly dominated units (ODI = 0–0.2 or 0.8–1.0) was higher in all strabismic infant monkeys, and the proportion of ocularly balanced units was lower. As expected, units having low BIIs (e.g., < 0.3) tended to be strongly dominated by one eye. Compared with the plots for the age-matched normal control animals, the units exhibiting extreme monocular dominance and very low BIIs were more likely to be found in monkeys that experienced the longest duration of strabismus (4-12 weeks).

In all strabismic groups, a higher proportion of units showed interocular suppression compared with age-matched normal control monkeys (χ² test, P < 0.01; Fig. 5). We operationally defined binocularly suppressive units as those having B/M ratios of 0.8 or less (a conservative criterion that is approximately 2 SEs below the mean B/M ratio of 1.0 in normal monkeys). In addition to the higher proportion of suppressive units, neurons having strong binocular facilitation (e.g., B/M ≥ 2.0) were very rare in strabismic monkeys (2%–4% in strabismic infant monkeys versus 16% in normal 8 week-old monkeys). Consequently, early strabismus also decreased the mean B/M ratios in all treated groups (one-way ANOVA, P < 0.01). However, there was no significant effect of the duration of strabismus on the prevalence or the magnitude of binocular suppression (one-way ANOVA, P > 0.5).

Effects of Poststrabismus Visual Experience

Because the sensitive period for binocular vision development in monkeys extends for more than 2 years after birth, we next...
asked whether poststrabismus visual experience promotes any recovery of disparity sensitivity in V1 units and, if it does, whether the duration of misalignment influences this recovery. In four additional monkeys, the prism-rearing was terminated at either 8 (n = 2) or 12 weeks of age (n = 2). The monkeys were reared under normal lighting conditions until behavioral testing was initiated at 2 years of age. Extensive monocular and binocular psychophysical tests took place over the next 2 years. The behavioral experiments involved measurements of spatial contrast sensitivity for each eye, binocular contrast summation, stereoacuity, and disparity vergence functions. After completion of the behavioral testing, we conducted the neurophysiological experiments.

Figure 6A shows the average BII for each monkey. If optical strabismus was continued for only 4 weeks between 4 and 8 weeks of age, we found small but significantly higher average BIIs in both adult strabismic monkeys with poststrabismus visual experience than in infants without recovery (one-way ANOVA, \( P < 0.01 \)). In contrast, if the prisms were removed at 12 weeks of age (i.e., 8 weeks of optical strabismus), subsequent visual experience had no effect on the mean BIIs (one-way ANOVA, \( P > 0.1 \)). Thus, the average BIIs for adult mon-
keys that experienced 4 weeks of strabismus were almost twice as high as those for adult monkeys that experienced 8 weeks of misalignment.

All the strabismic monkeys that were given poststrabismus visual experience exhibited a higher prevalence of binocularly suppressive units than did normal adults ($\chi^2$ test, $P < 0.001$) and their mean B/M ratios were lower than those of normal adult monkeys (one-way ANOVA, $P < 0.01$; Fig. 6B). The adult monkeys that experienced 4 weeks of strabismus showed a greater proportion of units exhibiting high B/M ratios (e.g., $>2.0$) than did strabismic infants (Fig. 6B, middle column). In addition, the mean B/M ratio for the adults that experienced 4 weeks of strabismus was higher than that for the infants that were treated for 4 weeks ($t$-test, $P < 0.05$). No comparable effect of poststrabismus visual experience was found if the prism-rearing continued until 12 weeks of age (Fig. 6B, right column).

**DISCUSSION**

The main finding of this study was that the duration of strabismus had a relatively small impact on the severity of binocular response deficits in V1 if its onset was at 4 weeks of age. Only 14 days of misalignment were sufficient to cause a great majority of the observed binocular anomalies in V1. In a related matter, we also found that poststrabismus visual experience had a small but positive impact on the maintenance of functional binocular connections in V1 only if the prism-rearing was terminated after 4 weeks of strabismus (i.e., at 8 weeks of age), but not after 8 weeks of misalignment (i.e., at 12 weeks of age). Both of these results highlight the importance of eliminating an interocular misalignment before 8 weeks of age (roughly equivalent to 8 months of age in humans) and preferably before 4 weeks of age (described later).
Comparisons to Previous Findings

In a previous study, we reported that 2 weeks of optical strabismus causes more severe disruptions in the binocular responses of V1 neurons if the misalignment is present after rather than before the onset of stereopsis in monkeys (~4 weeks of age). Figure 7 combines some of these previous results with some of the present findings from the infant monkeys that experienced optical strabismus between 4 and 6 weeks of age. In this comparison, the duration of strabismus is constant but the onset age (and thus, the age of prism removal) is different for each group. Regardless of whether we analyzed the proportion of disparity-sensitive units or the mean BIIs, the binocular response deficits were smallest in infant monkeys that experienced misalignment before 4 weeks of age ($\chi^2$ test and one-way ANOVA, $P < 0.01$). However, there was no significant difference between the two late-onset groups, both of which had optical strabismus after 4 weeks of age.

The data from these two studies suggest that the most sensitive period during which interocularly discordant signals affect the disparity sensitivity of V1 neurons is not immediately after birth but soon after the known onset age of stereopsis. By this time of postnatal development, V1 neurons have attained near adult-like tuning properties and responsiveness. Consequently, small imbalances in the signals between the two eyes are more readily detected by binocular neurons and as a result, may be more effective in disrupting the normal development of binocular response properties of V1 neurons. It is remarkable that during this critical time of development, only 14 days of strabismus is sufficient to cause major damage to the binocular signal processing mechanisms in primate V1.

Comparisons to Human Data

The onset age, the alignment age, and/or the duration of ocular misalignment impact the eventual sensory status of individuals who experience strabismus early in life. With respect to the management of infantile strabismus, it has been a matter of debate as to which of these timing factors has the greatest impact on a patient’s eventual binocular sensory states. Birch et al. and Fawcett et al. investigated the effects of onset age, the age of alignment, and the duration of strabismus on the prevalence of stereopsis and stereoacuity in human infants. These investigators found that the age of strabismus onset (which varied between 2 and 6 weeks of age in monkeys) had no significant effects. However, both the age of alignment and the duration of misalignment affected the prevalence of stereopsis and stereoacuity. Specifically, the shortest duration (less than 2 months) and the earliest alignment ages (between 3 and 5 months of age) were more likely to be associated with the preservation of stereopsis and the achievement of better stereoacuity. However, these investigators isolated duration as the more critical factor. Consequently, they concluded that the primary reason for better stereoacuity outcome in patients who had early surgical alignment is not because the alignment is achieved during the earliest period of visual maturation, but because the duration of misalignment is shorter in those patients.

Although the time course of the breakdown of stereoscopic vision in human infants may be somewhat different from that for disparity detectors in monkey V1, our present findings are generally consistent with the findings of Birch et al. and Fawcett et al. In our experiments, it was not possible to separate the duration of misalignment from the age of prism removal (alignment age in humans). However, the data in Figure 7 suggest that the onset age and/or the alignment age strongly influence the outcome if the duration is kept constant and relatively short. Again, we cannot separate the effects of onset age from alignment age in Figure 7, but the results of this study and those of our previous studies unequivocally support the importance of alignment before the known onset age of stereopsis. Thus, it is reasonable to conclude that in cases of infantile esotropia, alignment before 4 months of age (roughly 4 weeks in monkeys) would be most effective in increasing the odds of preserving disparity sensitivity and later alignment.

References


