Binocular Cross-Orientation Suppression in the Primary Visual Cortex (V1) of Infant Rhesus Monkeys

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PURPOSE. To better understand the course of cortical maturation during early development, the phenomenon of binocular cross-orientation suppression in neurons of the primary visual cortex (V1) in young infant monkeys was investigated.

METHODS. Extracellular single-unit recordings were made in anesthetized and paralyzed monkeys ranging in age between 6 days and 8 weeks. Orthogonally oriented, dichoptic sine-wave gratings were used as visual stimuli.

RESULTS. V1 neurons in young infant monkeys showed a higher prevalence and greater magnitude of binocular cross-orientation suppression than in adult monkeys. Binocular suppression decreased and reached an adult level between 4 and 8 weeks of age, the presumed onset-age for stereopsis in monkeys.

CONCLUSIONS. During the first 4 weeks of life, the functional connections that are necessary for initiating binocular cross-orientation suppression exist in the monkey primary visual cortex. This finding is consistent with the view that before the abrupt onset of stereopsis, human infants may detect the differences between interocularly iso-oriented gratings and orthogonal gratings. (Invest Ophthalmol Vis Sci. 2000;41:4022–4031)

The binocular visual capacities of primates are severely limited near birth.1–3 Stereopsis, a highly sensitive indicator of the functional status of binocular vision, is absent in subhuman primates at birth but suddenly emerges around 4 to 6 weeks of age.4 Similarly stereopsis emerges at about 4 to 6 months of age in humans.5,6 Many of the neural connections in V1 that are required for binocular functions in primates are present at birth7 and functionally emerge without extensive visual experience.8 However, before 8 weeks of age, the monocular receptive-field properties of V1 units are immature, and their overall responsiveness is lower than in adult monkeys.8,9 The immature monocular responses of V1 neurons are likely to limit the processing of disparity information and delay the onset of stereopsis.8 However, because the presence of disparity sensitive binocular units in the extrastriate areas may be necessary for stereopsis,10 the functional connections in the extrastriate visual areas may be poorly developed during the first several weeks of life, which may also constrain the emergence of stereoscopic vision.

A recent VEP study using dichoptic masking methods in human infants revealed another immaturity in how binocular signals are combined in the visual cortex.11 Specifically, these investigators found that although qualitatively adultlike, binocular VEP responses were present in infants ranging in age between 5 and 15 months (roughly equivalent to 5–15 weeks in monkeys), binocular cross-orientation suppression was weaker in these infants than in adults.11 To gain insight into the origins of this reduced interocular cross-orientation inhibition in the visual brain of infants, we determined whether individual V1 neurons in infant monkeys exhibit adultlike binocular inhibitory interactions in response to orthogonally oriented, dichoptic sine-wave gratings.

METHODS

All experimental procedures conformed to the National Institute of Health guidelines for use of animals in research and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Subjects and Surgical Preparation

The preparation and recording methods have been described in detail elsewhere.8,12 The ages of the infant monkeys (Macaca mulatta) at the time of recording experiments were 1 (n = 3), 2 (n = 2), 4 (n = 2), or 8 weeks (n = 2). The 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, including a tracheotomy and a small craniotomy and durotomy over the operculum of V1, were carried out under sodium thiopental anesthesia (2.5% solution). Each animal was given an initial injection of between 10 and 15 mg/kg. During the surgical procedures small amounts of the anesthetic were given every 5 to 10 minutes to maintain a deep level of anesthesia, that is, the corneal blink reflex and the withdrawal reflex produced by a paw-pad pinch.

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were completely suppressed. After all surgical procedures, the animals were paralyzed by an intravenous (i.v.) infusion of pancuronium bromide (a loading dose of 0.1–0.2 mg/kg, followed by a continuous infusion at the rate of 0.1 to 0.2 mg/kg/h). The animals were artificially respired with a mixture of 59% N₂O, 39% O₂, and 2% CO₂ to maintain an end-tidal CO₂ between 4.0% and 4.5%. The core body temperature of the monkeys was kept at 37.6°C. Throughout the recording session, the anesthesia was monitored and maintained by the continuous i.v. infusion of sodium pentobarbital (2–4 mg/kg/h). Cycloplegia was produced by 1% atropine sulfate, and the animal’s 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 activity from single cortical neurons. Action potentials were extracellularly recorded and amplified using conventional technology. The neuron’s responses, converted into standard pulses by a window discriminator, were sampled at a rate of 100 Hz (10-msec bin widths) by a laboratory computer and compiled into peristimulus time histograms (PSTHs) 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 PSTHs were determined by Fourier analysis. In all experiments, the stimuli were presented multiple times in a randomly ordered sequence.

For each isolated neuron, the receptive fields were mapped for both eyes and ocular dominance was determined using handheld stimuli. These procedures were followed by quantitative measurements using drifting sine-wave gratings (temporal frequency = 3.1 Hz; contrast = 0.5). Cells were classified as simple or complex on the basis of the temporal characteristics of their responses to a drifting sinusoidal grating of the optimal spatial frequency and orientation.

The optimal orientation and preferred direction of stimulus drift were determined from the unit’s orientation response function obtained with a near-optimal spatial frequency. At the optimal orientation and direction, a spatial frequency response function was measured to determine the cell’s optimal spatial frequency.

**Measurement of Binocular Suppression**

The details of the methods for determining the prevalence and the magnitude of binocular suppression are illustrated in Figure 1. Briefly, responses were collected for dichoptic sine-wave gratings of the optimal spatial frequency as a function of the relative interocular spatial phase of the gratings pairs. To create interocularly rivaling stimuli, the orientation of the gratings presented to the nondominant eye was rotated by 90°. 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.

To determine whether binocular signal interactions were suppressive in nature, we calculated the ratio of the mean binocular response amplitude/dominant monocular response amplitude (B/M). Because the great majority of V1 neurons in adult monkeys show B/M ratios near a value of 1.0 when interocularly orthogonal stimuli are presented, we operationally defined “suppressive” units as those having B/M values of 0.8 or less (an arbitrary, conservative criterion that was 2 SEs below the mean B/M value of 1.0 for adult monkeys). In addition, the amplitude of the fitted sine wave was used to calculate the degree of binocular interaction (BII), binocular interaction index = amplitude of the fitted sine wave/the average response amplitude.

**Histology**

To identify our recording sites, small electrolytic lesions were produced at several locations along the electrode track by passing current through the electrode (5 μA for <5 seconds, electrode tip negative). At the end of the recording experiments, an overdose of sodium pentobarbital (100 mg/kg) was...
administered intravenously to induce a deep level of anesthesia, and the animals were killed by a perfusion through the heart with an aldehyde fixative (2% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4). The brain was removed immediately and kept overnight in fixative with 20% sucrose. The tissue was cut in 40-μm sections on a freezing microtome in the tangential, frontal, or sagittal plane. The sections were used to identify recording sites for laminae analysis and to investigate the histochemical changes that occur during early development in V1. Cytochrome oxidase (CO) staining and Nissl staining techniques were used to reconstruct our electrode tracks and to estimate the location of individual cells with respect to the neuronal layers in V1.

RESULTS

High Prevalence of Binocular Cross-orientation Suppression in Infants

The majority of V1 neurons in young infant monkeys exhibited clear interocular cross-orientation suppression. For the representative cell from a 2-week-old infant monkey shown in Figure 2A, the binocular responses to an iso-oriented grating pair (left) varied systematically with the relative interocular spatial phase disparity as in mature monkeys, and the mean binocular response amplitude was greater than the dominant monocular response amplitude, showing unambiguous binocular cross-orientation suppression (B/M = 1.32).

Suppressive interactions were occasionally found even for iso-oriented gratings in 2- and 4-week-old infants. For example, the complex cell from a 2-week-old monkey in Figure 2B was not disparity sensitive, but the binocular response amplitudes for all interocular spatial phase disparities were clearly lower than the better monocular response amplitude (B/M = 0.53). The ocular dominance of this unit was 1.0 (apparently “monocular” and contralateral-dominant). However, dichoptic gratings initiated a very strong interocular suppression at all spatial phases. The data indicate that the influence of the left eye was subthreshold and inhibitory. Subthreshold binocular interactions, excitatory or inhibitory, are common in V1 units of cats and monkeys. Presenting an orthogonally oriented grating to the nondominant eye did not alter the cell’s disparity sensitivity and produced binocular cross-orientation suppression that was similar in magnitude (B/M = 0.58) to that found for iso-oriented stimuli.

A higher proportion of V1 units in young infant monkeys exhibited interocular cross-orientation suppression than in adults. The population data in Figure 3 show the distributions of units as a function of the B/M values for the interocularly iso-oriented (left) and orthogonal (right) conditions. The cell in the top panel (A) showed normal binocular interactions for iso-oriented stimuli, that is, clear disparity sensitivity (BII = 0.91) and excitatory interactions (B/M = 1.32). For the orthogonal condition, however, the B/M ratio was nearly half as large as the dominant monocular amplitude (B/M = 0.51). The cell in the lower panel (B) showed strong binocular suppression for both the iso-oriented and orthogonal conditions. The format and conventions are as in Figure 1B.
age, the prevalence of binocular cross-orientation suppression (40%) was just slightly higher than that in normal adults. Approximately 30% of the units in adults showed binocular cross-orientation suppression, which is similar to the proportion previously reported for adult cats. The differences between adult monkeys and 1-, 2-, and 4-week-old infants were significant (χ² test, *P* < 0.027 for 1 week, *P* < 0.0001 for 2 and 4 weeks). However, the distribution of units for 8-week-old infants was not significantly different from that for adults (χ² test, *P* > 0.2). The differences between the iso-oriented and orthogonal stimulus conditions within the same age were significant for 1-, 2-, and 4-week-old infants (χ² test, *P* < 0.188, *P* < 0.005, *P* < 0.001, respectively) but not for 8-week-old infants or adults (χ² test, *P* > 0.1 and *P* > 0.7, respectively). Interestingly, approximately 20% of the units in 1-week-old infants showed relatively strong excitatory binocular interactions (i.e., B/M > 1.2) for interocularly orthogonal stimuli. However, the overall proportions of binocularly suppressive versus facilitatory units in 1-week-old infants were not significantly different from those for 2-week-old monkeys (χ² test, *P* = 0.085).

To understand the nature of binocular cross-orientation suppression in V1 units of young infant monkeys, we manipulated the degree of interocular orientation differences by presenting pairs of gratings that differed in orientation by 45°...
between the two eyes in addition to pairs of orthogonally oriented gratings (Fig. 4). We found that the magnitude of binocular suppression was closely associated with a cell’s monocular orientation tuning and could be predicted by how well the binocular stimulus orientations matched the preferred orientation for each cell. The data for a complex cell from a 4-week-old monkey that are shown in Figure 4A demonstrate that a relatively small deviation from the optimal orientation for the dominant eye was always kept at the cell’s optimal orientation. (B) A scatter plot showing the relationship between orientation bandwidth and binocular interaction bandwidth for a population of cells from 2- and 4-week-old monkeys ($r = 0.72, P < 0.01$).

**Eye Dominance versus Binocular Suppression in Simple and Complex Cells**

There is considerable interest in the ocularity of cortical neurons that are suppressed by interocularly rivaling stimuli in adults. Figure 5 shows that for all ages, binocular cross-orientation suppression is strong regardless of an unit’s ocular dominance index (ODI), that is, whether a cell is strongly dominated by one eye (ODI = 0.0 or 1.0), binocularly balanced (ODI = 0.5), showing contralateral eye dominance (0.5 < ODI > 0.0) or ipsilateral eye dominance (1.0 < ODI > 0.5). However, before 8 weeks of age relatively higher proportion of complex cells were binocularly driven, and these cells generally exhibited a higher degree of interocular suppression than simple cells.

**Neuronal Layers and Binocular Suppression**

Figure 7 shows the average (±SE) binocular/monocular ratios of individual neurons in response to iso-oriented (open circles) and orthogonal (filled circles) gratings for different neuronal layers. The neuronal layers were estimated from the normalized depths of the recording sites and the Nissl- and CO-stained histologic sections of V1 (Fig. 8). The most striking finding was that in infant monkeys, cells in and around layer IVB consistently showed a large degree of binocular suppression for orthogonal stimuli.

**DISCUSSION**

The most important finding of this study was that binocular cross-orientation suppression is far more prevalent in monkey V1 before 8 weeks of age than in adulthood.

**Origins of Binocular Suppression in Infant V1**

In the adult cat visual cortex, interocular cross-orientation suppression was thought to originate in the LGN. The present results indicate that the observed interocular suppression in young infant monkeys originates within the striate cortex. Specifically, binocular suppression was more common and stronger in complex cells than in simple cells of 2- and 4-week-old infants (Figs. 5 and 6), and neurons in layer IVB exhibited the most consistent and largest interocular suppression to interocularly orthogonal stimuli although the same units showed typically high degrees of binocular facilitation for iso-oriented stimuli (Fig. 7). Moreover, the magnitude of the reduction in the monocular/binocular response ratio for a given unit was correlated with the unit’s orientation tuning (Fig. 4). All these findings are consistent with the hypothesis that the observed interocular suppression in monkeys originates at or after the binocular combination of signals in V1.

Why do V1 units of young infant monkeys show a higher prevalence of binocular cross-orientation suppression than those in adult monkeys? The major reason may be found in the relative immaturity of the excitatory binocular connections in

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**Figure 4.** (A) Orientation tuning (left) and binocular interaction (right) functions for a complex cell from a 4-week-old monkey. The tuning bandwidth was defined as the full width at one half of the peak value. The binocular interaction functions were obtained by plotting the ratios of the maximum (■) or mean (○) binocular response amplitudes over the dominant monocular amplitudes as a function of the interocular differences in stimulus orientation. The stimulus orientation for the dominant eye was always kept at the cell’s optimal orientation. (B) A scatter plot showing the relationship between orientation bandwidth and binocular interaction bandwidth for a population of cells from 2- and 4-week-old monkeys ($r = 0.72, P < 0.01$).
infant monkeys before 4 to 6 weeks of age. Specifically, the excitatory connections from the two eyes, whether they are the feed forward receptive-field generating projections or the intrinsic-local and long-range horizontal connections, are relatively immature compared with those in adults. However, the generally orientation “insensitive” inhibitory connections, particularly the intrinsic long-range horizontal connections, have been reported to function relatively more vigorously during early infancy. Consequently, changing stimulus orientation from a neuron’s preferred stimulus orientation may reduce the overall binocular excitatory drive without a concomitant reduction in binocular inhibitory signals. As a result, the binocular response rate would decrease as the stimulus orientation disparity between the two eyes increases (Fig. 4). A similar imbalance between binocular excitatory and inhibitory inputs was proposed to explain the high prevalence of interocular suppression in V1 neurons of strabismic cats and monkeys.

In a related matter, the distribution of B/M values under orthogonal conditions for 1-week-old monkeys appears to be different from those for other young infants, specifically, 20% of the units showed relatively strong excitatory binocular interactions (i.e., B/M > 1.2). This may be also due to immaturities in the receptive-field properties of many V1 units in 1-week-old monkeys. Specifically, the spatial tuning of V1 units is broader and their direction/orientation selectivity is exceptionally low in neonates compared with older infants and adults. The lower orientation/direction selectivity of V1 units may arise because of subnormal spatial summation of geniculate signals, which may be associated with the immature afferent LGN axon arbors in neonates and young infants. As a result, stronger excitatory signals from the two eyes may be more readily summated in those particular units, despite interocular differences in stimulus orientation. Our finding of reduced direction/orientation selectivity in V1 units is consistent with a report that neonates fail to discriminate the direction of moving stimuli and a recent finding by Birch et al. that the nasotemporal MVEP asymmetry, prevalent in young infants, is absent in neonates.

VEP Studies in Infants

The present findings substantially differ from the results of a recent VEP study that showed that human infants between 5 and 15 months of age (roughly equivalent to 5 and 15 weeks of age in monkeys) showed much weaker interocular suppression than adults. The source of this discrepancy between the two studies is not immediately clear. The most

FIGURE 5. Scatter plots showing the B/M values of individual units as a function of their ODI values (ocular dominance index) for each age group. ODI values of 0.0 represent monocular cells exclusively excited by the contralateral eye, whereas 1.0 signifies ipsilateral monocular dominance. The ODI values were calculated by comparing the peak monocular response amplitudes for right and the left eye stimulation during the measurement of the spatial frequency tuning functions. Open bars signify the average values for units within 0.2 intervals. Note that before 8 weeks of age complex cells (●) in infant monkeys exhibited more balanced ODI values and greater binocular suppression than simple cells (△).
obvious difference between the two studies is that their experiments were conducted in awake human infants, whereas our study was done in anesthetized and paralyzed monkeys. Although both studies used sine-wave gratings of relatively high contrast, the VEP study used contrast-reversal compared with the drifting gratings used in this study. However, it is somewhat difficult to conceive of how these technical differences may result in the differing results.

A more likely explanation may be found in the differences in the nature of signals that are recorded in the VEP versus single-unit studies. Previously, in the kitten visual cortex, monocular cross-orientation inhibition was shown to be present in almost all units as early as 4 weeks of age, whereas another study using VEP in cats could not reveal cross-orientation inhibition until 6 weeks of age. In addition to the fact that the VEP integrates signals from a large population of cells, VEP responses reflect changes in dendritic and somatic membrane potentials and thus may not necessarily correlate well with cross-orientation inhibition measured with action potentials from individual units. A similar explanation may apply here for the differences between the two studies with respect to the prevalence of binocular cross-orientation suppression in young infant primates.

Implications for Perceptual Binocular Rivalry in Infants

The inability to demonstrate binocular cross-orientation inhibition with the VEP has been taken as evidence that young infants do not experience perceptual binocular rivalry. This differs significantly from the claims that were made in earlier psychophysical studies. Our experiment was not designed to resolve the controversy regarding whether young infants perceive binocular rivalry. However, the results from this study may clarify some of the important issues associated with binocular rivalry in infants.

In adults, there is considerable disagreement among investigators concerning how perceptual rivalry is generated. Binocular rivalry has traditionally been considered to be an integral part of binocular visual functions because it was thought to reflect an interocular competition for perceptual eye dominance. According to this idea, spontaneous alterations in interocular suppression of neuronal activity at early stages of binocular processing (e.g., V1 and V2) are required for experiencing binocular rivalry. On the other hand, an increasing number of studies indicate that rivalry is a “multistable phenomenon” of perceptual dominance, not only between the two eyes but also within the same eye, and thus, it requires higher level “perceptual” processes beyond the binocular signal combination found at early cortical sites. There is a general agreement among investigators, however, that suppressive neuronal interactions between at least two groups of neurons at some stage of cortical processing, either between the two

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**FIGURE 6.** Scatter plots showing the B/M values of individual units as a function of the BII (binocular interaction index) values. △, simple cells; ○, complex cells. Data points that fall on the graph frame indicate values that exceeded the maxima for the x and y axes.
eyes or within the same eye, are required for perceptual rivalry. Our data indicate that neurons of V1 in young infant monkeys are capable of initiating interocular suppression whenever confronted with interocularly unmatched stimuli. Moreover, this interocular cross-orientation suppression in V1 is more prevalent and stronger in young infants than in adults. The bottom line is that before the emergence of stereopsis, infants may detect the differences between interocularly iso-oriented gratings and orthogonal gratings.

**FIGURE 7.** The average R/M values (±SE) as a function of laminar position for each age group. The relative recording depth was used to estimate a cell’s neuronal layer (Fig. 8). ○, the responses of units to interocularly iso-oriented stimuli; ●, the responses of the same units to interocularly orthogonal stimuli.
FIGURE 8. Changes in histochemical reactivity in V1 as a function of age. For each age group, Nissl (left) and cytochrome oxidase (right)-stained sagittal sections are illustrated. Neuronal layers are marked according to Hässler (left) and Brodmann (right). △, the location of layer IVcβ (4β) in 1-, 2-, and 4-week-old monkeys.
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