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**ORIGINAL ARTICLE**

# Early Versus Delayed Correction of Infantile Strabismus in Macaque Monkeys: Effects on Horizontal Binocular Connections in the Striate Cortex

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**ABSTRACT** *Objective:* To determine how the duration of infantile strabismus influences the development of horizontal connections in V1. *Methods:* Six infant macaques were fitted with prisms, which were removed after 3 weeks (wks.), 3 months (mos.), or 6 mos. Two control monkeys wore plano lenses. The number of horizontal connections was determined using neuroanatomic techniques. *Results:* The 3-wks animal had equal number of monocular (51%) and binocular (49%) connections. In the 6-mos. animal, however, monocular connections (76%) were three times more abundant than binocular (24%) connections ($p<0.001$). *Conclusions:* Longer duration of infantile strabismus causes greater deficit of binocular connections. Early correction of infantile strabismus is supported.

**KEYWORDS** Binocular connections; infantile strabismus; ocular dominance columns; V1; primary visual cortex

## INTRODUCTION

The striate cortex (V1) is arranged into a series of ocular dominance columns, with one column representing the right eye, and an adjacent column representing the left eye. Neurons within one column connect, via axonal projections, to columns belonging to the same eye (i.e., monocular connections), and also to columns belonging to the opposite eye (i.e., binocular connections). These binocular connections allow sharing of information between the two eyes, and form the basis for binocular vision.

In normal primates, monocular and binocular connections in area V1 are equally prevalent; however, in primates with naturally occurring infantile esotropia, binocular connections are sparse.[1] These deficits are most prominent in layers 2, 3, and 4b of V1 known to be important for fine stereopsis and motion processing,[2–5] providing the neuroanatomical basis for the clinical findings seen in infantile esotropia.[6,7] Our laboratory

previously reported that early binocular image decorrelation in primates causes abnormal stereopsis and ocular motor deficits.[8,9] As a neuroanatomic correlate to these behavioral findings, the present study aimed to determine how the duration of early-onset binocular image decorrelation influences the development of horizontal binocular connections in area V1.

## MATERIALS AND METHODS

### Animal Rearing

All of the animal care and experimental procedures were performed in compliance with the Association for Research in Vision and Ophthalmology resolution on the use of animals in research and were approved by the Washington University Animal Care and Use Committee. Experiments were performed on 8 normal infant macaque monkeys (*Macaca mulatta*) born at the Yerkes Regional Primate Research Center in Atlanta, Georgia. Optical strabismus was induced on the first day of life by fitting each monkey with prism goggles. The goggles were removed after 3 wks. (i.e., early correction), or after 3 or 6 mos. (i.e., delayed correction), emulating surgical repair of strabismus in humans at 3 mos., 6 mos. and 24 mos. of age, respectively. Detailed descriptions of animal rearing methods are available in reports previously published by our laboratory.[9] Herein, we report preliminary data from an early (3 wks.) and delayed (6 mos.) correction monkey.

### Overview of Anatomic Methods

At age 2–3 years, monocular and binocular connections in V1 were examined using a double-labeling technique. A transneuronal tracer (wheat germ agglutinin-horseradish peroxidase complex (WGA-HRP) or [$^3$H] proline) was injected into one eye to label ocular dominance columns (ODCs) using a protocol adapted from Horton et al.[10] Biotinylated-dextran-amine (BDA) was then injected directly into V1 cortex to label horizontal connections between ODCs. Following euthanasia, the V1 cortex was excised, flattened, and sectioned on a freezing microtome to a thickness of 40 $\mu$m on a plane tangent to the pial surface. Alternate brain slices were prepared for visualization of ODCs (i.e., processed for WGA-HRP or [$^3$H] proline) or horizontal connections (i.e., processed for BDA).

### Tissue Processing

To reveal ODCs labeled with [$^3$H] proline, sections were mounted on gelatinized slides, dried and dipped into NTB-2 emulsion (Eastman Kodak, Rochester, NY). The emulsion was exposed for 10 weeks and developed using D19 developer (Eastman Kodak, Rochester, NY). To reveal ODCs labeled with WGA-HRP, floating sections were processed for tetramethylbenzidine (TMB) histochemistry using the protocol by Mesulam [11]as modified by Gibson et al.[12] To reveal transported BDA, sections were incubated with avidin-biotin-HRP complexes and reacted with 0.05% diaminobenzidine (DAB) and 0.05% hydrogen peroxide according to the protocol by Jiang et al.[13] Stained tissue was mounted on gelatin-coated slides, dehydrated in ethanol, cleared in xylene, and coverslipped with DPX.

### Data Analysis

Stained sections were photographed using a microscope-mounted monochrome CCD camera for computer imaging using Photoshop CS (Adobe Systems, San Jose, CA). Digital images of BDA- and WGA-HRP- or [$^3$H]-proline-stained sections of layer 4b in area V1 were superimposed and aligned, using blood vessels as anatomic landmarks. ODC boundaries apparent on WGA-HRP- or [$^3$H] proline-stained sections were traced and overlaid on corresponding images of BDA-stained sections.

BDA injection sites chosen for analysis were contained within a single ODC. BDA uptake centers were 0.2–0.3 mm in diameter and thus smaller than the average width of ODCs (0.5 mm).[14] For each BDA injection site, the number of horizontal connections per unit area (i.e., BDA-labeled boutons and cell bodies) that fall within a same eye's (i.e., monocular connections) or a fellow eye's (i.e., binocular connections) ODC was counted. To minimize the impact of over-staining near the injection center or overlap with neighboring injection sites, areas that lay closer than 1mm or further than 2 mm from the BDA injection center were not analyzed. The percentage of monocular and binocular connections was compared between the early and delayed correction monkeys using the chi-square test, with significance set at $p < 0.05$.

## RESULTS

All normal control and 3-wks. (i.e., early correction) monkeys had normal eye alignment, while the 3-mos.

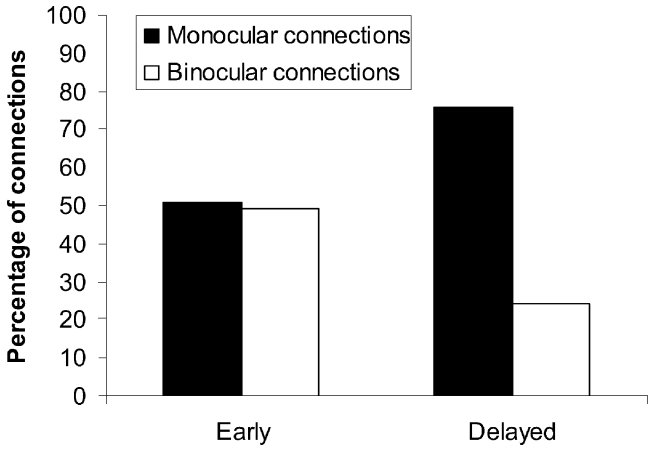


**FIGURE** **Percentage of monocular and binocular horizontal connections in the striate cortex of early (3 wks.) and delayed (6 mos.) correction monkeys.**

and 6-mos. (i.e., delayed) correction monkeys displayed horizontal and vertical strabismus. Spatial sweep VEPs indicated the absence of amblyopia in all animals. Cycloplegic refractions documented a refractive error of less than +3.00 spherical equivalent in each monkey.

The Figure shows the percentage of monocular and binocular horizontal connections in the striate cortex of an early (3 wks.) and a delayed (6 mos.) correction monkey. In the 3-wks. correction animal, BDA-labeled axonal projections terminated approximately equally in same-eye (51%) and opposite-eye (49%) ODCs. In the 6-mos. correction animal, however, horizontal connections terminated over 3 times more frequently in same-eye (76%) as opposed to opposite-eye (24%) ODCs ($p < 0.001$).

## DISCUSSION

The major finding presented in this report is that longer periods of binocular image decorrelation during the critical period of visual development produce greater deficits in horizontal binocular connections in area V1. Optical strabismus of 6 months duration led to a significant reduction in binocular connections in V1, consistent with the degree of deficit observed in monkeys with unrepaired, naturally occurring infantile strabismus.[1] In contrast, early correction of the optical strabismus at age 3 weeks resulted in equal numbers of monocular and binocular connections.

Non-correspondence of retinal images induced by prism rearing causes desynchronized activity in right and left eye ODCs in area V1. This desynchronized activity may lead to excessive pruning of binocular connections in V1 during the critical period of development.[15–17] Because the absolute number of horizontal connections could not be determined using the current technique, the relative difference in binocular versus monocular connections observed in our monkeys may be due to a decrease in connections to opposite-eye ODCs, or an increase in connections to same-eye ODCs, or a combination of both. Nevertheless, these preliminary results suggest that early correction of human infantile esotropia is critical for the normal development of binocular connections in the primary visual cortex. Our results further reinforce the importance of restoring normal eye alignment in infancy within a short period of time.

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