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Functional organization of the cat's visual cortex after prenatal interruption of binocular interactions

(visual development/monocular enucleation)

B. L. SHOOK*, L. MAFFEI†, AND L. M. CHALUPA*

*Department of Psychology, University of California, Davis, CA 95616; and †Istituto di Neurofisiologia del Consiglio Nazionale delle Ricerche, Via S. Zeno, 51, Pisa, Italy

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ABSTRACT **The functional consequences of interrupting *in utero* binocular interactions were studied by recording from single cells in area 17 of adult cats that had one eye removed at least 2 wk before birth. In these animals all cortical neurons could be driven by the remaining eye, and in tangential microelectrode penetrations, sequences of neurons containing a full 180-degree cycle of preferred orientations were encountered. Other response properties of cortical neurons in the prenatally enucleated animals were also normal with the notable exception that the dimensions of receptive fields were significantly smaller when compared with those of control animals. Our results indicate that orientation columns in the visual cortex can develop independently of ocular dominance columns, and they suggest that interruption of binocular interactions during prenatal development of the visual pathways may enhance the resolving power of the remaining eye.**

In adult mammals with highly developed binocular vision, inputs from each retina are segregated in the geniculostriate system. Retinal ganglion cell axons emanating from the right and left eyes terminate in alternate layers of the dorsal lateral geniculate body (LGd). In turn, the axons of LGd neurons terminate in spatially alternate clusters in areas 17 and 18 (1–3). Alternate clusters represent projections from the same eye, and this provides the anatomical substrate for ocular dominance columns (4).

During the early course of development, ocular segregation emerges only gradually from a diffuse projection pattern. Thus, axons representing the two eyes show an initial overlap of their terminal zones within the LGd, superior colliculus, pretectum, and visual cortex (5–9) but progressively sort out to form the distinctive patterns characteristic of the adult visual system. In cat and monkey, segregation of retinofugal projections occurs largely before birth (6–9), whereas this occurs postnatally in the geniculocortical pathway (10, 11).

The factors underlying the remarkable transformation of early visual connections are not well understood. However, it is known that binocular interactions play a critical role in this process. This was discovered by Wiesel and Hubel (12), who showed that monocular deprivation shortly after birth alters the size of cortical ocular dominance columns. It is now known that binocular interactions are also critical for the establishment of ocular domains during fetal development because removal of one eye before birth results in widespread projections from the remaining eye. Thus, ganglion cell axons from the remaining eye project to the entire ipsilateral and contralateral LGd (13, 14). In turn, principal cells of the LGd of prenatally enucleated cats and monkeys innervate layer IV of the visual cortex in a continuous rather than an alternating pattern (13, 15). In addition, prenatal unilateral enucleation attenuates naturally occurring ganglion cell and axon loss in the remaining retina and optic nerve and prevents the formation of distinct cell laminae in the LGd (13, 16–18).

In view of the substantial anatomical reorganization that occurs in the visual system after early eye removal, we sought to determine the functional consequences of interrupting *in utero* binocular interactions. Recordings were made from single neurons in the visual cortex of adult cats that had one eye enucleated at least 2 wk before birth, and the results were compared to those obtained from normal animals.

MATERIALS AND METHODS

Procedures used to time gestational age and the surgical methods used to enucleate one eye of fetal cats have been described in previous publications from this laboratory (14, 16, 18). In brief, anesthesia was induced in pregnant cats with 4% halothane vapor in oxygen. The animals were intubated, and anesthesia was maintained with halothane (1.0–1.5%) and a 70% nitrous oxide/30% oxygen mixture. Under aseptic conditions, a midline abdominal incision exposed the uterine horns, and a small opening made over the nonplacental portion of the uterus revealed the fetal head. The fetal palpebrae were separated, the extra-ocular muscles severed, and the entire globe was removed. The uterine and abdominal incisions were closed with absorbable suture and anesthesia was discontinued. This procedure was performed in one fetus in each of three litters of embryonic (E) days E40, E49, and E51. The litters were permitted to come to term normally.

Experiments were performed when the cats with prenatal monocular enucleation were at least age 1 yr. These animals, as well as three normal adult cats, were premedicated with atropine (0.08 mg) and anesthetized with ketamine hydrochloride (15 mg/kg of body weight). Following tracheotomy and cannulation of the saphenous vein, the animals were placed in a stereotaxic instrument, and a small opening was made in the skull over the visual cortex (area 17). Pupillary mydriasis was achieved by topical application of atropine sulfate, the nictitating membranes were retracted with phenylephrine hydrochloride, and clear plastic lenses were applied to protect the corneas. The animals were paralyzed with a mixture of gallamine triethiodide (10 mg/kg per hr) and tubocurarine chloride (0.5 mg/kg per hr) in a 5% dextrose solution and were ventilated artificially with 70% nitrous oxide/30% oxygen. Anesthesia was maintained by the addition of sodium pentabarbital (2 mg/kg per hr) to the infusion mixture. End-tidal CO_2 levels, cortical electroencephalogram, body temperature, and heart rate were monitored throughout each experiment. The eyes were focused with supplementary lenses on a tangent screen placed 57 cm in front of the animals. The optic disk was projected and plotted on the screen to check for residual eye movements and also to serve as a reference for the location of the area centralis

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Abbreviation: LGd: dorsal lateral geniculate body.

representation (19) to which the position of receptive fields were related.

Both tangential and vertical penetrations were made into the medial bank of the marginal gyrus with tungsten microelectrodes. Extracellular single-unit recordings were carried out in the conventional manner. The luminance of the tangent screen was 0.16 cd/m^2; the stimuli used, usually bars of light (1.5 degrees in width and between 5 and 40 degrees in length), were 1.9 logarithmic units above background. Cells were sampled at intervals between 50 and 100 μm. We first assessed the orientation selectivity of each cell by systematically varying the angle of the moving and flashing bars of light. The preferred orientation was that angle of the bar that yielded the greatest number of spike discharges as judged by listening to the audio monitor, although post-stimulus-time histograms were also available to verify the qualitative impressions. After the preferred orientation was determined, the borders of the receptive field were carefully delimited and plotted on a tangent screen. This was achieved by using luminous bars at the preferred orientation, which were flashed or moved from outside the receptive field while gradually approaching its center. The width dimension was obtained by marking the position of the long leading edge where a response was first detected. Similarly, the length dimension was determined by introducing the short edge of the stimulus into the receptive field orthogonal to the preferred orientation and marking the region that yielded an initial response.

In each penetration at least two small electrolytic lesions (0.5 μm·A for 1 sec) were made to facilitate reconstruction of electrode tracks. At the end of the recording session, animals were deeply anesthetized and perfused transcardially with phosphate-buffered saline followed by a paraformaldehyde/gluteraldehyde mixture and then a 10% sucrose buffer solution. The brains were blocked *in situ* and sectioned frozen at 50 μm. Every third section was stained with cresyl fast violet and every fourth with hematoxylin.

RESULTS

We made tangential microelectrode penetrations that were from 2.5 to 3.0 mm in length; therefore, the electrode transversed a region of cortex that in normal cats was usually occupied by a number of discrete ocular dominance columns. Twelve long penetrations were made in the medial bank of the marginal gyrus in three cats with one eye enucleated prenatally, and the responses of 287 cells were recorded. No differences among these three animals were noted in the results. Seven penetrations were made in a corresponding region of area 17 of normal animals and 88 cells were studied.

In the cats with prenatal unilateral enucleation of an eye, all of the neurons were driven by the remaining eye, and the activity did not show any signs of waxing and waning in

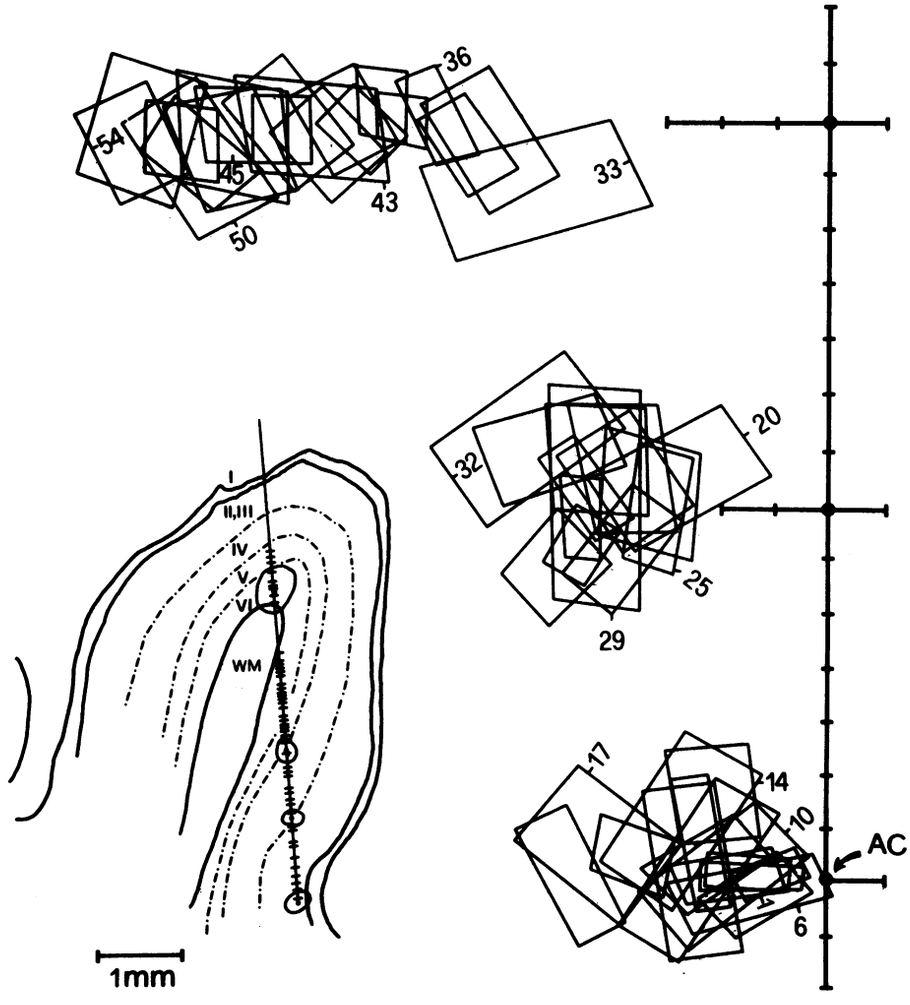

FIG. 1. Typical electrode penetration into area 17 of an adult cat that was monocularly enucleated on day E49, illustrating the orderly topography of receptive fields in this region of the marginal gyrus. The numbers associated with receptive fields indicate the sequence in which cells were isolated. Each division along the ordinate equals 1 degree and is referenced to area centralis (AC). (*Inset*) A reconstruction of the electrode track where each line represents one neuron. Circles along the track are electrolytic burns.

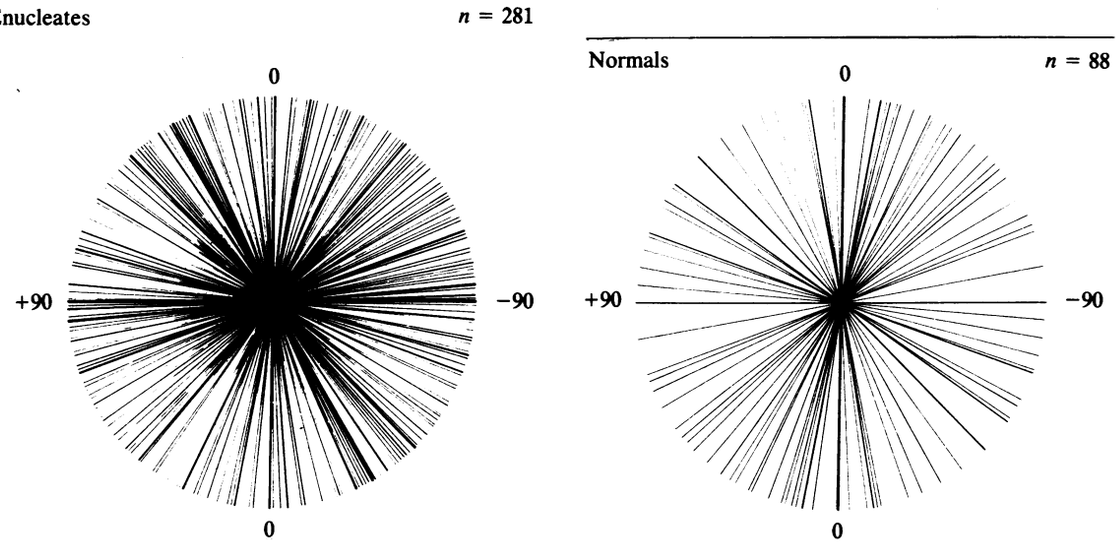

FIG. 2. Orientation selectivity of neurons in area 17 of normal and prenatally enucleated cats. Each line corresponds to the orientation preference of a single cell. Thick lines indicate that more than one cell preferred that particular orientation. Note that all stimulus orientations are clearly represented in both normal and prenatally enucleated cats.

responsiveness. In all penetrations an orderly shift of receptive field positions was observed as the electrode was advanced through the cortex (see Fig. 1). As in the case of normal animals, all but a few of the neurons were orientation selective (281 of 287), and a full complement of orientation preferences was encountered (Fig. 2). It is particularly noteworthy that cells exhibited an orderly progression in orientation selectivity in the prenatal enucleates. In several tangential penetrations, we isolated sequences of neurons containing a full 180-degree cycle of preferred orientations (Fig. 3). This finding is characteristic of hypercolumns in the visual cortex of normal cats (20), where a single hypercolumn contains cells representing both the right and left eyes. Therefore, our finding indicates that orientation columns can develop independently of ocular dominance columns.

Responses to properly oriented bars of light were as brisk in the prenatally enucleated cats as in normal animals; thus, we had no difficulty in plotting secure receptive field borders

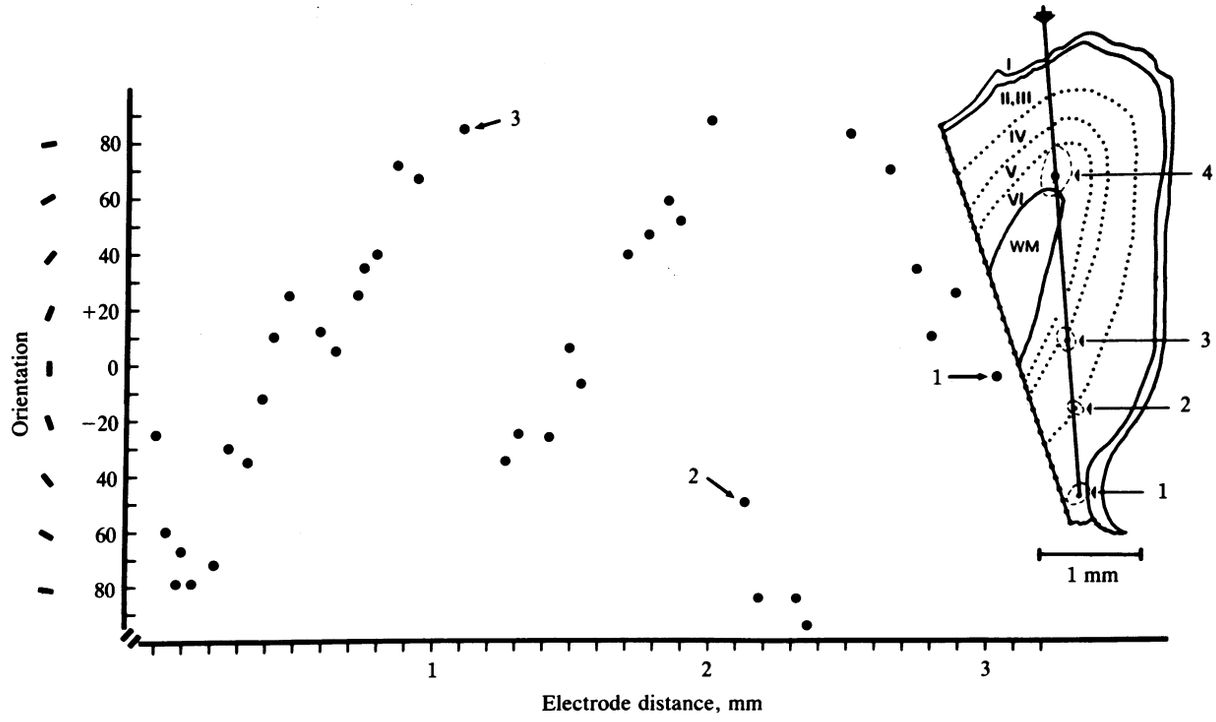

FIG. 3. An example of hypercolumns in area 17 of a prenatally enucleated cat. Stimulus orientation is indicated along the ordinate, while electrode distance is represented along the abscissa (each division equals 100 μm). (*Inset*) Reconstruction of the electrode track. Numbers along the *Inset* and in the figure refer to the electrolytic lesions made as the electrode was being withdrawn from the cortex. The first cell was recorded 823 μm below burn number 4. Note the orderly shifts in preferred orientations resulting in full 180-degree cycles as the electrode was advanced approximately 1 mm into the cortex. This is characteristic of hypercolumns in normal cats. Because of variation in the angle of the tangential penetrations, it was not possible to compare precisely the widths of the hypercolumns of the prenatally enucleated cats with those of normal animals.

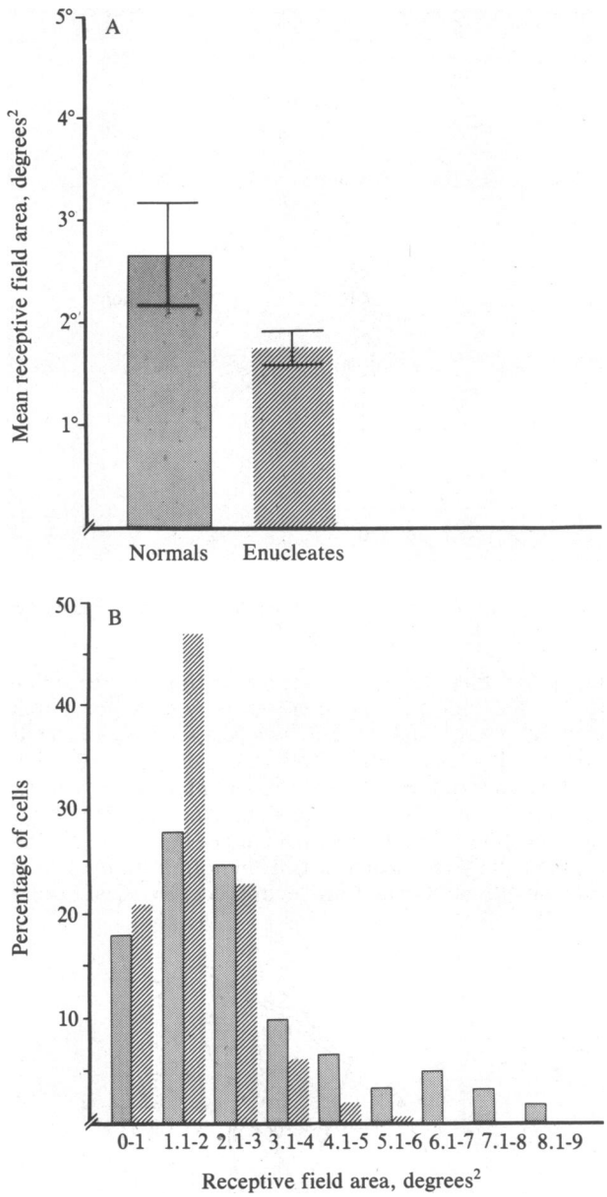

FIG. 4. (*A*) The mean receptive field area of neurons in area 17 of normal (□) and prenatally enucleated (□) cats. Only cells with receptive fields within 5 degrees of the area centralis representation were included in this analysis, which is based on a sample of 60 cells from normal cats and 145 neurons from animals that had an eye removed *in utero*. The error bar denotes the 95% confidence interval. (*B*) Breakdown of the data presented in *A* showing the percentage of cells with a given receptive field area. Note that the prenatal enucleates have a higher percentage of cells with receptive field areas between 1.1 and 2.0 degrees² with a concomitant decrease of cells with field areas greater than 4.1 degrees².

in both groups of animals. Although not obvious during the course of these experiments, a subsequent analysis of receptive field dimensions revealed an unexpected result. The average receptive field area (1.76 degrees²) was considerably smaller in the cats that had one eye removed *in utero* than in normal animals (2.67 degrees²) (Fig. 4*A*). This difference is statistically significant beyond the 0.001 level (t = 4.54; degrees of freedom = 203). (Statistically significant differences also were obtained between the two groups of animals when receptive field width and length were used as measures of receptive field size.) Most of our electrode penetrations sampled a region of area 17 around the area centralis representation, and all of the receptive fields included in the foregoing analysis were within 5 degrees of area centralis.

Fig. 4*B* shows the distribution of receptive fields as a function of field size. It may be seen that, in the prenatally enucleated animals, there was an increase in the proportion of cells with receptive fields between 1.1 and 2.0 degrees² and a concomitant decrease in neurons with fields larger than 4.0 degrees². This implies that termination of prenatal binocular interaction influences the distribution of selective classes of cortical cells, and further study will be necessary to clarify this point. In normal cats different layers of area 17 have unique functional cell types (21, 22). A reconstruction of our electrode penetrations ruled out the possibility that differential laminar sampling may have accounted for the result depicted in Fig. 4. In normal cats 31% of the neurons we studied were in layer IV, 23% in layer V, and 40% in layer VI. The comparable distribution in prenatally enucleated cats was 25% in layer IV, 28% in V, and 36% in VI.

DISCUSSION

The present study provides information about the functional consequences of terminating *in utero* binocular interactions in the visual cortex. All of the neurons we studied in the prenatally enucleated cats could be driven by the remaining eye. Furthermore, retinotopy and orientation columns appeared normally organized. However, within 5 degrees of the area centralis representation, receptive fields were significantly smaller in the animals that had an eye removed before birth than in normal cats.

Our finding that orientation columns can develop independently of ocular dominance columns is, to some extent, not surprising since Hubel and Wiesel (20) observed that, when a microelectrode crossed from a left-eye-dominated to a right-eye-dominated region of the normal cat's cortex, there was no noticeable disturbance in the sequence of orientation columns. However, other investigators have claimed that the two-columnar systems of the visual cortex, ocular dominance and orientation, are not completely independent (23–25). In particular, Fregnac *et al.* (23) reported that monocular enucleation of postnatal cats resulted in a selective preference for vertical and horizontal orientations. This was not found in the present study (see Fig. 2), and it is unclear whether this is due to differences in the ages of the animals at the time of enucleation or to other factors.

What could account for the smaller receptive fields in the visual cortex of the prenatally enucleated cats? One possible explanation is that the dendritic fields of retinal ganglion cells may be less extensive in these animals than in normals. This is suggested by a recent study from our laboratory (18), which showed that the remaining retina of the prenatally enucleated cat has about 30,000 more ganglion cells than normal, although retinal area is not expanded. The higher density of ganglion cells following early eye removal could result in smaller dendritic trees because of exacerbated dendrodendritic competition (26, 27). It should be relatively straightforward to test the validity of this hypothesis with anatomical and physiological methods. Other factors, such as increased density of geniculo-cortical terminations and altered intracortical connections also may contribute to this phenomenon.

Since receptive field size has been related inversely to measures of acuity (28), our results suggest that, in some respects, the prenatally enucleated cats may have supernormal vision. In this context, it is of interest to note that, in proposing the binocular competition hypothesis, Wiesel and Hubel (12) briefly considered the possibility that the eye put at a competitive advantage during development could show enhanced "effectiveness" because of its expanded control of cortical neurons. The prenatally enucleated cat provides a particularly appropriate opportunity to examine this intrigu-

ing concept. It may be possible to demonstrate enhanced performance in one area of the brain as a consequence of early damage to a related brain region.

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