INTERNATIONAL ATOMIC ENERGY AGENCY
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
34100 TRIESTE (ITALY) - P.O.B. 586 - MIRAMARE - STRADA COSTIERA 11 - TELEPHONE: 2240-1
CABLE: CENTRATOM - TELEX 460392 - I

SMR/302-76

COLLEGE ON NEUROPHYSICS:
"DEVELOPMENT AND ORGANIZATION OF THE BRAIN"
7 November - 2 December 1988

"Prenatal Genesis of Connections Subserving Ocular Dominance in the Rhesus Monkey"

Pasko RAKIC
Yale University
School of Medicine
Section on Neuroanatomy
New Haven, CT
USA

Please note: These are preliminary notes intended for internal distribution only.

(Reprinted from Nature, Vol. 261, No. 5560, pp. 467–471, June 10, 1976)

Prenatal genesis of connections subserving ocular dominance in the rhesus monkey

Pasko Rakic

Department of Neuropathology, Harvard Medical School, and Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts 02115

In foetal monkey brain neuronal projections carrying input from the two eyes initially overlap; they segregate during the second half of gestation and become fully separated in subcortical visual centres and partially separated in the cortex three weeks before birth and thus before visual experience.

DEVELOPMENT of the binocular visual system has been a major topic of research since the demonstration more than a decade ago that neuronal function can be altered in kittens deprived of vision in one eye[1]. More recently, similar observations in the rhesus monkey[2] have underlined the relevance of this research to an understanding of mechanisms of amblyopia *ex* anopsia in children. In several respects, the development of the visual system is similar in man and monkey. For example, in both species all neurones that comprise the visual system are generated during the first two-thirds of gestation[3–5]. In both species, approximately one-half of the axons from the retinal ganglion cells of each eye are distributed at the optic chiasma to both the ipsilateral and the contralateral dorsal lateral geniculate bodies (LGd). In both man and monkey, the LGd has 6 laminae. Three laminae (1, 4 and 6) receive direct inputs from the contralateral eye and the remaining three (2, 3 and 5) from the ipsilateral eye[6].

The recently developed autoradiographic method of orthograde axoplasmic[7] and transneuronal transport[8,9] provide a simple and powerful method for exploring the structural development of the binocular visual system by tracing the distribution of radioactive label following injection of isotopes into one eye. Radioactivity transported to the appropriate laminae of the LGd and transneuronally to the primary visual cortex (area 17 of Brodmann) is distributed in the sublayers IVA and IVC over columns 350 μm wide that alternate with columns of the same width containing low grain counts[9]. The autoradiographic method thus confirms earlier more traditional anatomical and electrophysiological evidence for columnar organisation. It has also provided the first anatomical evidence for a segregated representation of each eye in the superior colliculus (SC), where fibres from the two eyes terminate in complementary, alternating territories 0.1–0.5 mm wide[10,11].

Autoradiography has also been used to determine the anatomical consequences of monocular deprivation at early postnatal ages. This method reveals a reciprocal expansion and contraction of the ocular dominance columns in layer IV of the visual cortex that are related to non-occluded and occluded eyes, respectively[12]. It has been suggested that the basic connections subserving binocular vision are determined innately, even though, as mentioned above, changes in the width of columns can be induced by an imbalance in visual experience during the first few postnatal months[12]. This interpretation, however, has not been fully accepted by some investigators who argue that visual experience rather than genetic factors may play a fundamental inductive role in establishing functional connections. (For a recent review on the subject, see ref. 13.) An analysis of the prenatal development of the circuitry involved in ocular dominance in the monkey may therefore help to resolve the issue.

Pregnant rhesus monkeys (*Maccaca mulatta*) were subjected to laparotomy and hysterotomy under halothane–oxygen anaesthesia at appropriate gestational intervals (Table 1). Equal amounts of ^{3}H-proline and ^{3}H-fucose (total 1.0–1.5 mCi) were injected into the vitreous body of one eye of each foetus. After injection, the foetuses were returned to the uterus, and the chorio-allantoic membrane, uterine and abdominal walls were sutured in layers. Fourteen days later, after an interval sufficient for concentration of tracer in LGd, SC and visual cortex by axonal and transneuronal transport, the living foetuses were removed from the uterus by a second caesarian section and perfused intracardially with a buffered paraformaldehyde–glutaraldehyde mixture. The brains were embedded in polyester wax, sectioned serially at 8 μm and processed by the standard autoradiographic method.

Retinogeniculate projections

The youngest foetus in this series was injected at E64 (embryonic day 64) by which time all LGd neurones have been generated[14]. This foetus was killed at E78, before the LGd becomes laminated[5]. Thus, this experiment was designed to determine whether retinal ganglion cell axons from each eye are segregated in the LGd before it develops laminae.

Radioactive tracer, transported in an orthograde direction from ganglion cells in the injected eye to the optic nerve, through the optic chiasma and the optic tracts, is concentrated in approximately equal amounts in both LGds. Evidently, equal numbers of crossed and uncrossed retinal fibres are already distributed to each LGd at this foetal age. Although there are areas with slightly higher or lower grain densities, segregation into separate layers is not discernible (Fig. 1*a*). Rather, grains are distributed more or

Table 1 Experimental protocol for three foetal monkeys injected at various times for autoradiography

Animal	Embryonic day (E) when injected	Embryonic day (E) when killed	Exposure time for LGd and SCsg (weeks)	Exposure time for cortex (weeks)
(1) 052775	E64	E78	2, 4	2, 4, 8, 16, 20, 24
(2) 021075	E110	E124	2, 4	2, 4, 8, 16, 20, 24
(3) 061275	E130	E144	2, 4	2, 4, 8, 16, 20, 24

Three foetal monkeys were injected once each at the specified embryonic (E) day into one eye with equal parts of ^{3}H-proline and ^{3}H-fucose After injection, each foetus was replaced in the uterus and killed 14 d later.

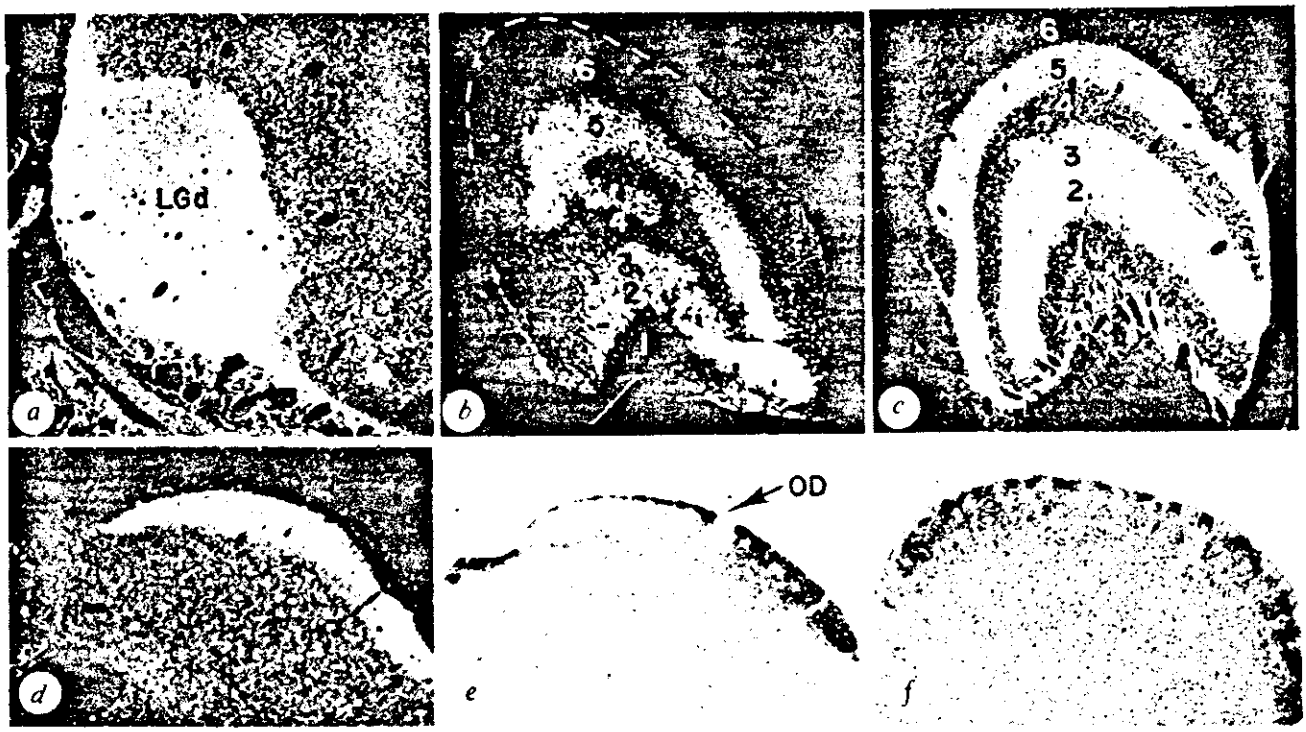

Fig. 1 Dark-field and bright-field illumination photographs of autoradiograms from foetuses exposed by hysterotomy and injected into the vitreous body of one eye with a mixture of ^{3}H-proline and ^{3}H-fucose at different embryonic (E) days. Foetuses were replaced in the uterus and killed 14 d later by a second caesarian section. All photographs are the same magnification (× 20). The terms "contralateral" and "ipsilateral" are used with respect to the injected eye. *a*, Contralateral LGd in the foetus injected at E64 and killed at E78. The radioactive label does not suggest a laminar pattern. *b*, Ipsilateral LGd in the foetus injected with comparable doses of the same isotopes at E110, killed at E124. Label is concentrated over the emerging laminae 2, 3 and 5. *d*, Dark-field photograph of a coronal section of the ipsilateral superior colliculus (SC) of the foetus injected at E64 and killed at E78. Label is uniformly distributed over the entire nucleus. *e*, Bright-field photograph of a sagittal section of the contralateral SC in the foetus injected at E110 and killed at E124. Retinotectal projections are clearly segregated into ocular dominance patches indicated by alternating areas of high and low grain counts. The large, empty zone corresponds to the optic disk (OD) representation. *f*, Bright-field photograph of a coronal section of the ipsilateral SC in the foetus injected and killed at slightly later gestational ages (E130–E144). Retinotectal projections are concentrated into discrete patches of dense labelling that correspond to areas devoid of grains in the contralateral SC. The optic disk representation is not present at this level.

less diffusely throughout the entire ipsilateral and contralateral LGd, indicating the possibility that at an early stage of development (around E64) projections from both eyes basically overlap in their distribution among neurones of the LGd with no evidence of the laminar segregation characteristically seen in the adult nucleus.

The second foetus in this series was injected in one eye with a mixture of ^{3}H-proline and ^{3}H-fucose at E110, that is, at an age when the characteristic laminar pattern of the LGd is beginning to emerge[5]. Fourteen days later, at E124, when this foetus was killed, the six-layered pattern of cells of the LGd, although still somewhat irregular, is already clearly developed. The laminae 2, 3 and 5 of the LGd are found to be intensely labelled ipsilateral to the injected eye (Fig. 1*b*) whereas laminae 1, 4 and 6 are labelled in the contralateral nucleus. The laminar segregation of ipsilateral and contralateral retinal inputs to LGd therefore occurs in the interval between E64 and E110 when laminar segregation of their target cells is also achieved. In both LGd, the more ventral laminae (one contralaterally and two ipsilaterally) are more intensely labelled than the more dorsal ones.

The distribution of radioactive label in a third foetus injected and killed at slightly later gestational ages (E130–E144) is essentially the same, except that the distribution of grains over the appropriate laminae resembles closely the adult patern (Fig. 1*c*); that is ,the laminar organisation of cells in the LGd is more regular and more sharply outlined than seen in younger foetuses.

Retinotectal projections

Substantial amounts of radioactivity were transported through the brachium of the superior colliculus into the tectum in all three foetuses. In the youngest foetus, which was injected at E64 and killed at E78, the tracer is concentrated uniformly over the superficial layer of both SC with no evidence of complementary segregation of projections from the ipsilateral and contralateral eyes (Fig. 1*d*). The amount of label is noticeably greater on the contralateral side, however. Fibres from the two eyes therefore initially overlap in the SC, as well as in the LGd.

By contrast, in the foetus injected at E110 and killed at E124, the radioactive label is distributed in a pattern of alternating dense and light concentrations (Fig. 1*e*). Although not as sharply defined, these correspond closely in number and pattern to the ocular dominance 'clumps' observed in the mature monkey[11]. The alternating pattern is most evident in the posterior region of the SC where the retinal axons penetrate more deeply into the superficial grey. The width of the 'clumps' in the foetal monkey, ranging from 0.07 to 0.3 mm, is substantially smaller than that of the adult monkey (0.1–0.5 mm)[11], presumably reflecting the smaller size of the SC in the foetus. As in the adult, the pattern of grain distribution on both sides fits like pieces in a puzzle: that is, territories of the contralateral SC which contain low concentrations correspond to territories of the ipsilateral SC which contain high concentrations. In addition, a sector which contains no label in the contralateral SC represents the position of the optic disk at this foetal age (OD in Fig. 1*e*).

The oldest foetus in this study, injected at E130 and killed at E144, shows essentially the same pattern of alternating grain distribution in the SC. The contrast between heavily and lightly labelled territories is more distinct and the boundaries of these territories are defined as sharply as in the adult monkey (Fig. 1*f*).

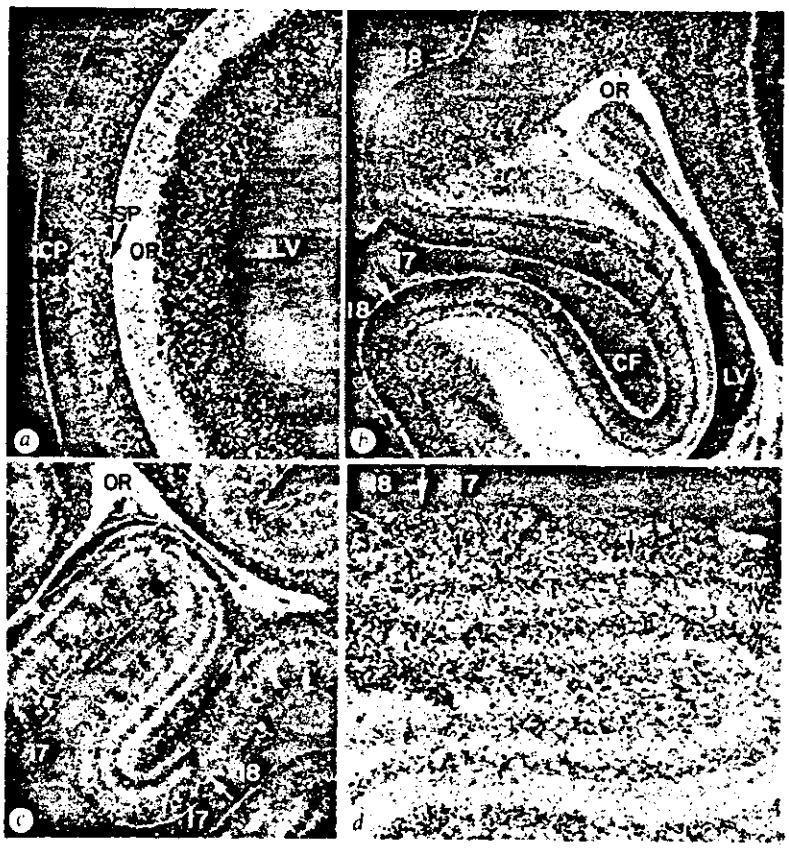

Fig. 2 Dark-field photographs of the cerebral wall and cortex at the level of the occipital lobe in the foetus injected with a mixture of ^{3}H-proline and ^{3}H-fucose at various foetal ages and killed 14 d later. The high grain background in these autoradiograms is due in part to the long exposure (Table 1) and partly to the high dose of ^{3}H-proline–^{3}H-fucose mixture that produces unspecific labelling similar to background levels found in autoradiograms of liver, kidney, spleen and other organs prepared from the same foetuses. Therefore, only a concentration of grains well above background in the autoradiograms of the cerebrum is considered to represent radioactivity in the axons of the visual pathways. *a*, A coronal cut across the cerebral wall in the foetus injected at E64 and killed at E78. The large lateral vesicle (LV) is on the right. The optic radiation (OR) is heavily labelled, but fibres stop at the subcortical zone (SP) and do not invade the developing cortical plate (CP). Approximately ×27. *b*, Occipital lobe of the foetus whose one eye was injected with the same mixture of labels at E110 and killed at E124. The optic radiation (OR) surrounds the lateral ventricle (LV) and emanates axons only to area 17 of the calcarine fissure (CF). Projections to the cortex stop sharply at the borderline between area 17 and area 18 (arrows). Some axons and/or terminals have entered the cortical plate but are distributed uniformly over layers IV and VI. Approximately ×8.7. *c*, Visual cortex of the foetus injected and killed at a slightly older age (E130–E144). More axons and/or terminals have invaded the cortex and are concentrated over layers IV and VI in area 17. ×12. *d*, Higher magnification photograph of the visual cortex demonstrates segregation of axons and/or terminals over sublayers IVA and IVC, as well as alternating regions of higher (black arrowheads) and lower grain counts. The limits of zones with high grain densities in both layers occur sharply at the borderline to area 18. ×30.

Geniculocortical projections

Radioactive label was transported transneuronally into neurones of the LGd and the geniculocortical fibres of the three foetuses as revealed by a high concentration of label of the optic radiations (OR in Fig. 2*a*). Because the amount of radioactivity transferred transneuronally is less than 3% of that transported through the primary pathway[4], autoradiographic exposures were lengthened appropriately for demonstration of geniculocortical projections (Table 1). As expected, this was done at the expense of relatively low background (Fig. 2).

The youngest foetus was injected at E64 and killed at E78, that is when only a fraction of neurones destined for layer IV of the visual cortex are generated[3], and many of these neurones which have already undergone their last cell division have not yet reached their final position and are still migrating towards the cortex through the fibre plexus of the intermediate zone[4]. The autoradiograms show that fibres from the optic radiation (OR in Fig. 2*a*) have reached the occipital lobe, but they have not entered the developing cortex in any substantial number, and most of the label is concentrated in the subcortical intermediate zone of the developing cerebral wall. There is an abrupt decline in grain density at the external border of this zone (SP in Fig. 2*a*) which is situated external to the optic radiation but internal to the developing cortex. The distribution of grains in the intermediate zone forms a continuous, uniform band without periodic variations. Thus, at this stage in the telencephalon, there is no evidence of segregation of fibres derived from the two eyes. This is not unexpected since, in the same foetus, axons of the retinal projections from the two eyes are not yet segregated in the LGd (see above).

The second foetus was injected at E110 and was killed at E124, that is, after all cortical neurones are generated and also after they have completed their migration to the cortex[4]. In this foetus, the location of LGd axons are clearly identified by a dense concentration of grains over the optic radiation (OR) in the occipital lobe (Fig. 2*b*). Numerous fibres pass from the optic radiation towards the cortex of area 17, but stop abruptly at the border of this field with area 18 (Fig. 2*b*). Although the great majority of these fibres is concentrated in the intermediate zone below the primary visual area, small numbers of them do enter the cortex, where they are uniformly distributed over layers IV and VI (Fig. 2*b*). There is, however, no horizontal bilaminar concentration of silver grains over sublayers IVA and IVC and no alternating radially aligned concentrations of grains that might indicate the incipient formation of ocular dominance columns.

In the third foetus that was injected and killed at a slightly later age (E130–E144) more grains are concentrated over layers IV and VI presumably because more geniculocortical fibres have invaded the cortex. In this specimen, a bilaminate pattern of grain concentration can be discerned over sublayers IVA and IVC in autoradiograms exposed 12 to 24 weeks (Fig. 2*c*). The boundaries between these two sublayers are not as sharp as in the adult, however. By and large, the grains over sublayers IVA and IVC at this foetal age are distributed as a continuous sheet, although there are sectors in which an alternating light and dense pattern of grain concentration seems to emerge (Fig. 2*c*). This pattern is of low contrast and, although difficult to see in photomicrographs, it is clearly present when a grain count is performed (Fig. 3). Sectors with higher counts in sublayers IVA are in register with those of IVC, and the same is true of sectors with lower counts (Figs 2*c* and 3). In all likelihood, these represent the emerging ocular dominance columns. Presumably, only the sectors, 100–150 μm wide, which contain the highest grain counts (underlined numerals in Fig. 3) and those with the lowest grain counts (numerals between outlines) correspond to territories occupied predominantly by terminals derived from the injected and uninjected eye, respectively. Therefore, the width of the sectors with high concentration of grains is not 50% of the double columns (Fig. 3) which indicates that there is

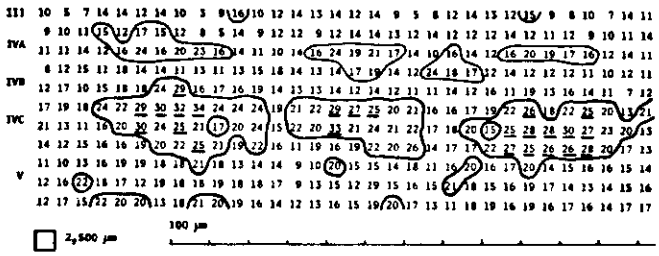

Fig. 3 Results of grain counts over a 0.8-mm² area of layers III, IVA, IVB, IVC and V of the primary visual cortex in monkey foetuses injected at E130 and killed at E144. The autoradiogram is photographed in dark-field illumination and its negative projected by a Prado (Leitz GMBH) apparatus on to grid paper in which each square corresponds to 2,500 μm² area of the cortex. Grains were counted in 352 unit areas along a 1.6-mm length of the calcarine cortex. Areas with 15 grains or more per 2,500 μm² in layer IVA are outlined by a thin line. Areas with 20 grains or more per 2,500 μm² in layer IVC are outlined by a thick line. Areas with 25 grains or more are underscored by a short, thick line. The distance between the two regions of highest grain counts that correspond to the pair of ocular dominance columns is approximately 500 μm. Note that the width of areas with higher grain counts is not half of the double column. Presumably, territories of terminals from the uninjected eye still considerably overlap the territories of injected eye and only a narrow, approximately 100–150-μm wide zone of highest and lowest grain counts may be considered to correspond predominantly to areas which receive projections derived predominantly from one eye.

still considerable overlap between the terminals derived from the two eyes. The combined width of an ipsilateral and contralateral ocular dominance column as determined by the distance between two peaks in grain concentrations is 500–600 μm. The comparable figure for the width of two columns in the mature monkey is 700–800 μm (ref. 9). The difference in width may be explained by a substantial increase in the cortical surface area between E144 and maturity.

Sequence of developmental events

The topographical interrelationship of the central connections in the adult visual system is the result of multiple, complex cellular interactions and events which occur during ontogeny. During the extended prenatal period of primates, young neurones change their position relative to one another; many of them migrate long distances to attain their final locations; and their dendritic and axonal processes differentiate and are deployed while the entire brain changes its external and internal form dramatically[4,5,14]. These cellular activities are coordinated in space and in time and may play a fundamental role in the formation of complex cytoarchitectonic patterns and point-to-point connectivity of widely separated visual centres.

These observations indicate that cellular events fundamental to the prenatal development of the visual system in the rhesus monkey may be separated into two broad phases: in the first phase, axons derived from each eye invade target structures, and their endings are distributed in an overlapping manner; in the second phase, the terminal axons derived from the two eyes become segregated from each other in their target structures. Although injections of isotopes at closer intervals and shorter survival times are needed to define the duration of each phase, the available evidence suggests that the first phase may be relatively short, even in the protracted development of the rhesus monkey, whereas the second phase may last for several weeks. Thus, in the foetus injected at E110 and killed at E124, afferents from the LGd are distributed in an overlapping manner over the entire IVth layer of the visual cortex. Even 6 weeks later, on the seventh postnatal day, the segregation into monocular columns is far from complete[12]. It is important to emphasise that although differentiation of pathways begins later in the cortex than in subcortical structures, the initial segregation of binocular connections in the cortex nevertheless begins well before birth—that is, before visual experience.

Thus the effect of monocular deprivation on the relative size of territories corresponding to each eye[17] may not necessarily involve the induced expansion of territories related to the functional eye. Rather, since domains of ocular representation overlap with each other during ontogeny (Fig. 3), it is possible that the territory of the functional eye remains arrested at a given developmental stage, while the territories of the deprived eye shrink. In this context, our developmental data support the hypothesis that alterations in the relative width of ocular dominance columns could be degradative rather than inductive.

Recently, autoradiographic studies have demonstrated that radial columnar organisation is not confined to the primary visual cortex but is a generalised characteristic of the mammalian neocortex[15,16]. For example, in the motor and prefrontal cortex of the neonatal monkey, callosal and cortico–cortical connections form well developed, sharply delineated columns whose development at this age is much more advanced than that of the ocular dominance columns of the visual cortex[16]. The precocious development of columns in the frontal lobe of the monkey adds further support to the notion that columnar organisation of the cortex may be largely specified innately. The significant alteration of monocular dominance columns by unbalanced visual stimulation during early stages of postnatal life in primates may occur because columns are not yet fully developed.

This work was supported by a grant from the US National Institutes of Health. I thank the New England Regional Primate Research Center, for making timed pregnancies and surgical facilities available. Caesarian sections by Dr P. K. Sehgal as well as expert advice for technical improvements in intrauterine surgery by Dr B. T. Jackson are gratefully acknowledged.

Received April 2; accepted April 14, 1976.

1 Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, **26**, 1004–1017 (1963).
2 Hubel, D. H., and Wiesel, T. N., *J. comp. Neurol.*, **158**, 307–318 (1974).
3 Rakic, P., *Science*, **183**, 425–427 (1974).
4 Rakic, P., in *Brain Mechanisms in Mental Retardation* (edit. by Buchwald, N. A., and Brazier, M.), 3–40 (Academic, New York, 1975).
5 Rakic, P., *Phil. Trans. Roy. Soc. Lond.*, B (in the press).
6 Polyak, S., *The Vertebrate Visual System* (University of Chicago Press, Chicago, 1957).
7 Cowan, W. M., Gottlieb, D. E., Hendrickson, A. E., Price, J. L., and Woolsey, T. A., *Brain Res.*, **37**, 21–51 (1972).
8 Grafstein, B., *Science*, **172**, 177–179 (1971).
9 Wiesel, T. N., Hubel, D. H., and Lam, D. M. K., *Brain Res.*, **79**, 273–279 (1974).
10 Graybiel, A. M., *Brain Res.*, **96**, 1–23 (1975).
11 Hubel, D. H., LeVay, S., and Wiesel, T. N., *Brain Res.*, **96**, 20–40 (1975).
12 Hubel, D. H., Wiesel, T. N., and LeVay, S., *Cold Spring Harb. Symp. quant. Biol.*, **40**, 581–589 (1976).
13 Barlow, H. B., *Nature*, **258**, 199–204 (1975).
14 Rakic, P., *Anat. Rec.*, **184**, 507–508 (1976).
15 Jones, E. G., Burton, H., and Porter, R., *Science*, **190**, 572–574 (1975).
16 Goldman, P. S., and Nauta, W. J. H., *Brain Res.* (in the press).

Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset