Functional Plasticity in the Immature Striate Cortex of the Monkey Shown by the [^{14}C]Deoxyglucose Method

Abstract. *Autoradiographic representation of the local rates of cerebral glucose utilization and local cerebral functional activity by means of the [^{14}C]deoxyglucose technique reveals the existence of the ocular dominance columns in the striate cortex of the monkey in the first day of life. In contrast to the stability of these columns in more mature brain, monocular deprivation for 3 months from the first day of life results in their complete disappearance and a reversion of the autoradiographic pattern to that seen in animals with normal binocular vision. These results are consistent with a reorganization of the representation of the visual fields of the two eyes in the striate cortex and provide additional evidence of the plasticity of the striate cortex of the monkey in early life.*

By the end of gestation the striate cortex of the monkey has attained much of the complex structural and functional organization that characterizes that of the mature animal. Multiplication of neuronal cells has long since been completed, and their migration has resulted in a distribution of the cells in the six cytoarchitectural layers characteristic of this region of the cortex (*1*). Even in the absence of visual experience, cohorts of cells grouped perpendicularly to the surface have developed the capacity to respond selectively to linear visual stimuli of a given orientation (*2*). Also the anatomic substrate for binocular vision is advanced. Geniculocortical axon terminals carrying input from the two retinae are segregated into alternate zones in layer IV, marking the location of the ocular dominance columns (*3, 4*). Although the central visual pathways thus appear to have attained considerable maturity in the absence of visual stimulation, the deprivation of such stimulation in the immediate postnatal period has consequences to both function and subsequent structural development. Monocular deprivation in particular may have profound effects. Single-cell recordings have indicated that the ocular dominance columns develop asymmetrically if one eye is occluded in the second or third week of life (*3*). In such animals the width of the column having input from the intact eye enlarges at the expense of the adjacent column innervated by the deprived eye. These alterations in column width inferred from neurophysiologic studies have their structural counterparts in both histologic and autoradiographic studies (*3, 4*).

We have recently described a technique for the simultaneous measurement of the rates of glucose utilization in most of the macroscopic functional and structural components of the brain (*5–8*). In this method [^{14}C]deoxyglucose is used as a tracer for glucose consumption along with an autoradiographic procedure that provides a pictorial representation of the local rates of glucose utilization throughout the brain. Because local functional activity and energy metabolism appear to be closely linked in brain, the method has proved useful for mapping localized regions of altered cerebral functional activity in response to experimentally induced changes in physiological state (*6–8*). The method has been used to study the binocular visual system of the mature monkey and has provided direct visualization of the nature, dimensions, and distribution of the ocular dominance columns (*8*). We now report the application of the method to monkeys in early prenatal life and examine the effects of short-term and long-term monocular deprivation on the distribution of functional activities in the striate cortex. Our results provide autoradiographic confirmation of the existence and functional activity of the ocular dominance columns at birth and demonstrate that, in contrast to the effects in the mature brain, prolonged monocular deprivation results in a functional reorganization of the striate cortex in which the ocular dominance columns essentially disappear and the regions normally representing the occluded eye are taken over by and respond to the input of the functional eye.

Newborn and preadolescent rhesus monkeys of both sexes were studied. The newborn animals were delivered to the laboratory 6 to 24 hours after birth. The preadolescent animals, whose exact ages were unknown, weighed between 2.5 and 3.5 kg and were considered mature with respect to visual function. All animals were studied in the conscious state by means of the [^{14}C]deoxyglucose technique either within a few hours of their arrival in the laboratory or 3 months later. The procedures used for the application of the [^{14}C]deoxyglucose technique to monkeys, the sectioning of the brains, and the preparation of the autoradiographs were identical to those previously described (*8*).

Both the newborn and preadolescent monkeys were studied under three conditions of visual function: (i) both eyes open (four preadolescent, four newborn); (ii) one eye occluded for 3 hours before and during the experimental procedure (three preadolescent, two newborn); and (iii) one eye occluded continuously for the previous 3 months and during the experimental procedure (one preadolescent, one newborn). Long-term visual occlusion was accomplished by suturing of the lids of one eye together. For the short-term occlusions an opaque plastic disk was inserted under the lids before they were sutured. All of the animals were seated before an illuminated screen with a black and white geometric pattern on it which rotated around their heads during the experimental period. This stimulus induced continuous opticokinetic nystagmus in the mature animals, but in the newborn animals the nystagmus was only intermittent.

The autoradiographs obtained with the [^{14}C]deoxyglucose technique provide a pictorial representation of the relative rates of glucose utilization in the various structural components of the brain; the greater the optical density, the higher the rate of glucose utilization (*5, 6*). Because of the close relation between local energy metabolism and functional activity in the brain (*6*), the technique can be used to map the regions of the brain with altered functional activity in response to experimentally induced alterations in local functional activity (*6–8*). In our studies the autoradiographs clearly reflect the differences in the patterns of functional activity in the striate cortex under the various conditions of visual stimulation.

As was reported (*8*), glucose utilization in the preadolescent animal with normal binocular vision is not uniform but is distributed in a laminar fashion throughout the striate cortex (Fig. 1A). A centrally placed band of high density parallel to the cortical surface corresponds to layer IV, and deep to this band are a light band and then a moderately dark band corresponding to layers V and VI, respectively (*8*). Just superficial to the central dark band representing layer IV is an additional very narrow dark band (not readily seen in the reproduction) which probably corresponds to layer IVa. Still more superficial is a broad band of intermediate density traversed by layers I, II, and III; this band cannot be separately distinguished. In the autoradiograph of a comparable coronal section through area 17 of a newborn monkey with normal binocular vision a similar demarcation of the separate laminae is seen (Fig. 1D). Except for a narrower

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dark band corresponding to layer IV, the pattern in the newborn animal is similar to that of the more mature animal.

In the animals with monocular deprivation for only 3 hours, the laminar pattern of variable density described above is altered. Alternating stripes of high and low density, each measuring 0.3 to 0.4 mm in width, are seen perpendicular to the cortical surface in the autoradiographs from the preadolescent animals (Fig. 1B). The dimensions and arrangement of these transverse stripes conform to the descriptions of the ocular dominance columns (*9, 10*). In the newborn animal with short-term monocular deprivation, the pattern is similar (Fig. 1E) except that the interruptions in the dark band corresponding to layer IV are not as sharply defined as in the mature animal with monocular occlusion. Indeed, the separations between the dark and light columns in layer IV are incomplete in many regions where there are only narrowed segments. Thus, there is a pattern of beading rather than of equidistant separations. In the zone corresponding to layers I, II, and III, the light stripes, corresponding to the columns of the occluded eye, are wider than those marking the columns of the intact eye, the reverse of the pattern seen in layer IV.

The autoradiographs of coronal sections through area 17 of a preadolescent monkey which had had one eye occluded for 3 months prior to the experimental procedure (Fig. 1C) are qualitatively similar to those of the preadolescent animals with monocular occlusion for only 3 hours. Curiously, layer IV is represented in two distinct dark layers which border a central lighter layer. The stripes marking the ocular dominance columns are clearly visible in all layers, as is seen in the autoradiographs from the animal with acute monocular deprivation. In contrast to the relatively small effects in the more mature animals, monocular visual deprivation for 3 months in the immediate postnatal period alters the pattern in the striate cortex. In the animal studied under these conditions layer IV reappears as a dark solid homogeneous layer with neither segmentation nor interruption (Fig. 1F). The layers both deep and superficial to layer IV are relatively free of any regularly spaced stripes although there are some faint irregularly spaced markings in the superficial layers that may represent slight persistence of the columns. In general, however, the pattern is almost identical to that of a newborn animal with intact binocular vision, the major difference being a greater width of the band marking layer IV, which may reflect normal development in the first 3 months after birth.

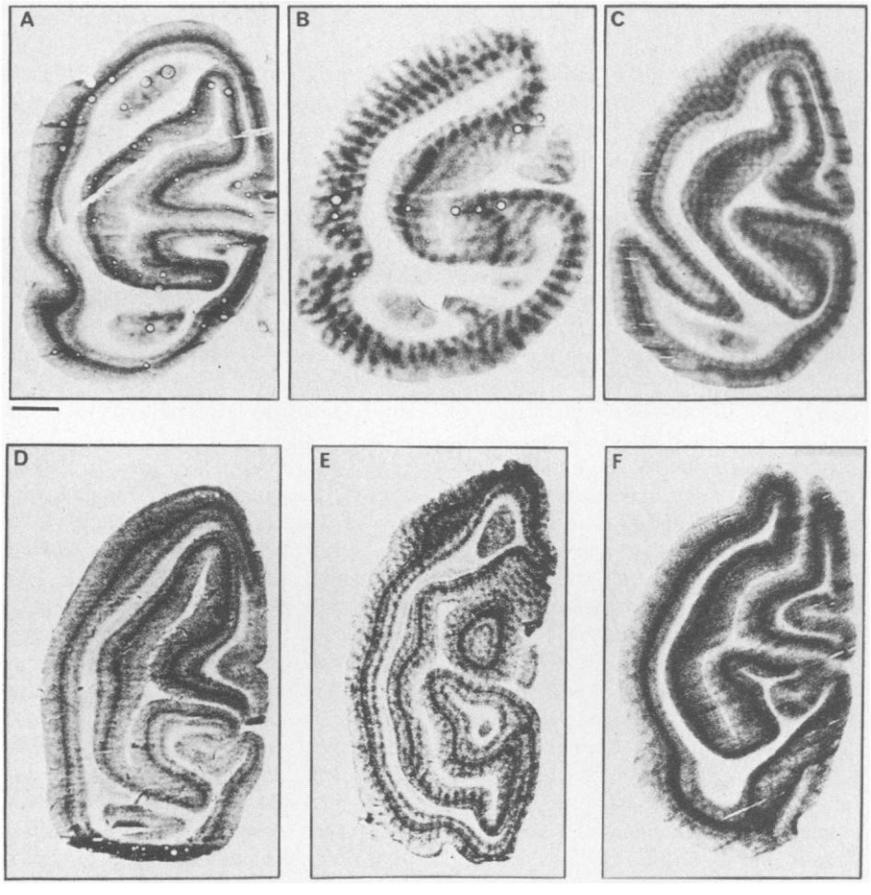

Fig. 1. Autoradiographs of coronal sections of monkey striate cortex obtained with the [^{14}C]deoxyglucose technique. Panels A, B, and C are from preadolescent animals: (A) both eyes intact; (B) monocular occlusion of 3 hours duration; (C) monocular occlusion of 3 months duration. Panels D, E, and F are from neonates: (D) studied on the first day of life with intact binocular vision; (E) studied on the first day of life 3 hours after occluding one eye; and (F) studied at 3 months with monocular occlusion having been initiated on the first day of life. Artifacts of tissue processing are; (i) small bubbles in (A) and (B); (ii) gross cracks in the tissue due to freezing; (iii) linear folds and gaps parallel to the plane of sectioning seen variably in all sections. These can be distinguished from true columns, which tend to be perpendicular to the cortical surface. Scale bar is equal to 3 mm.

Our studies provide additional evidence of the relative maturity and also of the functional plasticity of the striate cortex of the newborn monkey. The autoradiographic pattern produced by the [^{14}C]deoxyglucose technique in the striate cortex of the newborn monkey with normal binocular vision is similar to that found in the mature animal and indicates varying rates of local glucose utilization in the various cortical layers. Our studies also confirm anatomic and physiologic observations that the ocular dominance columns are, indeed, functionally active at birth (*3, 4*) and, therefore, support the inference of others (*3, 4*) that they are not dependent on visual input for their development. Although the present limits of resolution of the [^{14}C]deoxyglucose method do not allow exact comparison of the dimensions of the ocular dominance columns in the newborn and more mature animals, they do appear to be slightly smaller and less sharply defined in the newborn. Anatomic studies have shown that during fetal life the nerve terminals of the geniculocalcarine pathway from the monocular laminae of the lateral geniculate body are distributed in layer IV in an overlapping manner (*4*). As the fetus matures, there appears to be a shrinkage or retraction of the contribution from each eye with consequent reduction in overlapping that is incomplete even as gestation approaches termination (*4*). Such overlapping could explain the less distinct separation of the ocular dominance columns that we observe in the autoradiographs from the newborn animals as compared with those from the more mature animals.

The complete disappearance of the ocular dominance columns after monocular deprivation for the first 3 months of postnatal life is not entirely surprising. Hubel, Wiesel, and LeVay (*3*) have studied long-term monocular occlusion begun at 2 weeks and at 3 weeks of age. In both instances it was observed that, while the combined width of adjacent columns remained the same, there was an enlargement of the width of the column representing the intact eye and a corresponding reduction in the width of the column of the deprived eye. A greater discrepancy in column widths was found when the monocular deprivation was initiated at 2 weeks rather than at 3 weeks of age, and it was suggested that the difference in severity of the effects of monocular deprivation was related more to the age at which the monocular deprivation was initiated rather than to the total period of deprivation. The newborn animals that we studied were monocularly deprived on the first day of life. The complete disappearance of the ocular dominance columns and the functional preemption of the territory normally occupied by the column representing the occluded eye by that of the intact eye may reflect this earlier deprivation.

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References and Notes

1. P. Rakic, *Science* **183**, 425 (1974).
2. T. N. Wiesel and D. H. Hubel, *J. Comp. Neurol.* **158**, 307 (1974).
3. D. H. Hubel, T. N. Wiesel, S. LeVay, *Cold Spring Harb. Symp. Quant. Biol.* **40**, 581 (1976).
4. P. Rakic, *Nature (London)* **261**, 467 (1976).
5. L. Sokoloff, M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, M. Shinohara, *J. Neurochem.* **28**, 897 (1977).
6. L. Sokoloff, *ibid.* **29**, 13 (1977).
7. C. Kennedy, M. H. Des Rosiers, J. W. Jehle, M. Reivich, F. Sharpe, L. Sokoloff, *Science* **187**, 850 (1975).
8. C. Kennedy, M. H. Des Rosiers, O. Sakurada, M. Shinohara, M. Reivich, J. W. Jehle, L. Sokoloff, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4230 (1976).
9. D. H. Hubel and T. N. Wiesel, *J. Comp. Neurol.* **146**, 421 (1972).
10. S. LeVay, D. H. Hubel, T. N. Wiesel, *ibid.* **159**, 559 (1975).
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Brain Noradrenergic Systems as a Prerequisite for Developing Tolerance to Barbiturates

Abstract. *Mice treated with 6-hydroxydopamine before they were chronically fed phenobarbital did not develop functional barbiturate tolerance, measured by duration of the loss of righting reflex and hypothermia. Injection of 6-hydroxydopamine caused significant depletion of brain norepinephrine, while brain dopamine levels were not significantly depleted. Intact brain noradrenergic systems seem to be necessary for developing tolerance to the hypnotic and hypothermic effects of the barbiturates.*

Continual ingestion of sedative hypnotics such as barbiturates or ethanol results in the development of central nervous system (CNS) tolerance to and physical dependence on these drugs (*1*). The neuronal systems that participate in developing this tolerance and dependence have not been defined. We now report that intact brain noradrenergic systems may be necessary to develop tolerance to the hypnotic and hypothermic effects of barbiturates. Male mice of the C57Bl/6 strain (22 to 24 g) were injected intraventricularly with 10 μl of a solution (*2*) containing 50 μg of 6-hydroxydopamine (6-OHDA, free base), or 10 μl of an artificial cerebrospinal fluid (CSF) (vehicle). Seven days after injection, the mice were divided into four groups. Group 1, the vehicle/control group, consisted of mice pretreated with artificial CSF, housed individually, and offered an unlimited diet of ground Purina mouse chow and water. Group 2, the 6-OHDA/control group, consisted of mice pretreated with 6-OHDA, and housed and fed as group 1. Group 3, the vehicle/barbiturate group, consisted of mice pretreated with artificial CSF, housed individually, and offered a diet of ground Purina mouse chow containing phenobarbital (free acid). The drug was present in the diet at a concentration of 3.5 g per kilogram of food for the first 3 days of continuous feeding, and at 4 g per kilogram of food for the next 3 days. Group 4, the 6-OHDA/barbiturate group, consisted of animals first treated with 6-OHDA and then fed the same barbiturate-containing diet as group 3. After 6 days, all mice were fed the control diet (withdrawal).

Table 1. Effect of pentylenetetrazol injection in barbiturate-withdrawn and control animals.

Group	No convulsions	Convulsions
Vehicle/control	11*	1
6-OHDA/control	10	2
Vehicle/barbiturate	1	6
6-OHDA/barbiturate	1	9

*Number of animals exhibiting particular symptoms. Animals having no symptoms or only tremors were placed in the no convulsions category, while the convulsions category included animals showing clonic and/or tonic convulsions, or dying after convulsions. Comparisons of vehicle/control and 6-OHDA/control, $P = .39$ [Fisher exact probability test (*9*)]; vehicle/barbiturate and 6-OHDA/barbiturate, $P = .51$; vehicle/control and vehicle/barbiturate, $P = .002$; 6-OHDA/control and 6-OHDA/barbiturate, $P = .001$; vehicle/barbiturate and 6-OHDA/barbiturate, $P = .51$.

Blood levels of phenobarbital were determined daily while the mice were consuming the phenobarbital-containing diet and every 4 hours after withdrawal began (*3*). Mice were also weighed each morning, and assessed for intoxication by monitoring locomotor behavior and coordination (*4*). Phenobarbital was removed from the diet on the morning of the seventh experimental day (*5*). The mice were kept at 22° ± 1°C and observed at 4-hour intervals for overt signs of withdrawal hyperexcitability; rectal temperature was monitored during each observation period (*4*). After 24 hours of withdrawal, several animals (Table 1) from each group were injected with pentylenetetrazol (50 mg per kilogram of body weight, intraperitoneally), and their behavior (*6*) was continuously monitored for the next 30 minutes. Animals that had been injected with pentylenetetrazol were not used in any subsequent studies.

Some of the remaining mice were injected intraventricularly (*7*) with 200 μg of sodium phenobarbital 44 hours after withdrawal, and the duration of loss of righting reflex (sleep time) and barbiturate-induced hypothermia were monitored (*8*) (Table 2). Upon regaining the righting reflex, these mice were immediately decapitated, and their brains were removed and analyzed for phenobarbital (*3*). Other animals were injected intraperitoneally with sodium barbital (300 mg/kg), and the duration of the loss of righting reflex was monitored. Brain levels of norepinephrine (NE), dopamine (DA), and serotonin in mice of all four experimental groups were determined 44 hours after withdrawal (*8*, *9*).

Mice in the vehicle/barbiturate and 6-OHDA/barbiturate groups had similar

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