Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3950–3953, June 1981
Neurobiology

Changes in protein synthesis underlying functional plasticity in immature monkey visual system

(autoradiography/central nervous system plasticity/lateral geniculate nucleus/monocular deprivation)

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Contributed by Louis Sokoloff, March 10, 1981

ABSTRACT **Local rates of cerebral protein synthesis were determined in newborn rhesus monkeys subjected to either acute or chronic monocular visual deprivation. Chronic monocular deprivation resulted in decreased rates of protein synthesis in the laminae of the lateral geniculate nuclei innervated by the deprived eye whereas rates of protein synthesis were normal in geniculate laminae innervated by the functioning eye. Acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the geniculate laminae.**

In animals with binocular vision the projections from the retinae of the two eyes are partly crossed at the optic chiasma in such a manner that the retinal outputs representing the right and left visual fields of both eyes are collected and distributed more or less exclusively to the left and right geniculate bodies, respectively. However, the information from the two eyes remains segregated, and the optic tracts terminate in six well-defined cellular laminae in each of the lateral geniculate bodies: laminae 1, 4, and 6 receive input from the contralateral eye, and laminae 2, 3, and 5 are served by the ipsilateral eye. The segregation of the information from the two eyes and for both visual fields is retained in the organization of the pathways from the lateral geniculate bodies to the striate cortex.

Each geniculate body projects to the ipsilateral striate cortex consigning the information for each visual field to the contralateral visual cortex. The cell bodies in the laminae of each geniculate body project to the ipsilateral striate cortex in an organized pattern in which the information for a given point in the visual field converges to two adjacent visual cortical columns, one pair of columns for each point in the visual field and one column for each of the two eyes. These so-called ocular dominance columns are most prominent and discrete in layer 4 of the striate cortex but are perpendicularly arranged and traverse all the other cytoarchitectural layers (1). In the young adult rhesus monkey they are approximately 400 μm in width (1).

The organization of the binocular visual system is largely although not completely developed in the monkey at birth (2). The ocular dominance columns are present, but they are less discretely defined, and there is some overlapping of representation of the two eyes in adjacent columns (2, 3). The system also exhibits a considerable degree of plasticity. If, in a young monkey, binocular visual balance is chronically impaired—for example, by unilateral enucleation, insertion of an opaque lens, or lid suture—then the ocular dominance columns representing the functioning eye extend their boundaries and broaden at the expense of the adjacent columns representing the deprived eye; eventually, most of the striate cortex may be incorporated into a monocular visual system that serves only the undeprived eye (3, 4). The organization of the geniculate body, however, remains normal (2).

The reorganization of the visual system that follows chronic unilateral visual deprivation in the immature brain clearly reflects the overgrowth of axonal terminals from the geniculo-cortical pathways to the ocular dominance columns of the functional eye into the adjacent columns with the formation of synapses on cellular elements normally reserved for the deprived eye. The nature of the processes involved is unclear. There may be an accelerated growth of nerve terminals or sprouting from the functional columns into the adjacent nonfunctional columns, a reduced growth and consequent retraction of the terminals in the nonfunctional pathway and columns, or a combination of both. Axonal sprouting is dependent on continued protein synthesis, a process that is confined to the cell bodies of origin of the axons. The perikarya of the axonal terminals of interest in the striate cortex are situated in well-defined laminae segregated for the right and left eyes in the lateral geniculate bodies.

In the present studies we used a newly developed autoradiographic method for measuring local rates of protein synthesis in the nervous system (5) to examine the changes that occur in the newborn monkey during reorganization of the visual system induced by chronic unilateral visual deprivation. The results demonstrate that chronic visual deprivation results in reduced rates of protein synthesis in the geniculate cell bodies of the deprived pathway and suggest that the loss of the ocular dominance columns for the deprived eye is the result of inadequate growth or maintenance of axonal terminals with consequent default of synaptic connections to the normally maintained and sprouting axonal terminals of the functional pathway.

METHODS

Animals. Six full-term newborn rhesus (*Macaca mulatta*) monkeys were taken from their mothers on the day of delivery and reared in an animal nursery. Two of the monkeys were used on their second day of life for measurement of local cerebral protein synthesis; one, with normal intact bilateral vision, served as a control, and the other was subjected to unilateral lid suture approximately 3 hr before the experimental procedure.

The four other monkeys continued to be reared in the nursery until 25 days of age when they were used for measurement of local rates of cerebral protein synthesis. One was left with intact bilateral vision, one was subjected to unilateral lid suture approximately 3 hr before the period of measurement; the remaining two had been subjected to unilateral lid suture, one on the left side and the other on the right, on their second day of life.

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Measurement of Local Cerebral Protein Synthesis. Local rates of protein synthesis were determined by a recently reported autoradiographic technique (5). Like the autoradiographic deoxyglucose technique for the measurement of local cerebral glucose utilization (6), this method is based on a kinetic model of the exchange of the labeled substrate between plasma and tissue, its metabolism in the tissue, and its flux through the metabolic pathway of interest. In this case the labeled substrate was the essential amino acid [1-^{14}C]leucine. Leucine was selected because, other than incorporation into protein, it has a single simple pathway of metabolic degradation in which it is rapidly decarboxylated. The use of [*carboxyl*-^{14}C]leucine ensures that the label is lost from any metabolically degraded [^{14}C]leucine. Any ^{14}C remaining in the tissue exists then in only two chemical forms, free [^{14}C]leucine and the product of interest, [^{14}C]leucine incorporated into protein. This feature greatly simplifies the analysis of the model and the determination of [^{14}C]leucine incorporated into the protein in the tissues by quantitative autoradiography.

The kinetic model has been mathematically analyzed to derive an operational equation that defines the local rate of leucine incorporation into protein in terms of measurable variables and determinable rate constants. The variables to be measured in each experiment are the final local tissue ^{14}C concentrations at the end of the experimental period and the time courses of the [^{14}C]leucine and endogenous leucine concentrations in the arterial plasma from the time of administration of the [^{14}C]leucine to the end of the experimental period. The rate constants are determined in a separate group of animals, but their influence on the final result is minimized if the [^{14}C]leucine is administered as a pulse at zero time and the experimental interval is extended long enough for the plasma and tissue free [^{14}C]leucine to be cleared to negligible concentrations. An additional advantage of the use of a pulse followed by an extended experimental period is that the autoradiographs then reflect almost exclusively the labeled protein product and therefore become pictorial maps of the relative rates of protein synthesis in the various structures of the brain.

The procedure to measure local cerebral protein synthesis was as follows. Polyethylene catheters were inserted into one femoral artery and vein (animal under light halothane anesthesia). In the experiments with acute unilateral visual occlusion, the eyelids were sutured at the same time. The animals were then allowed at least 3 hr to recover from the effects of anesthesia. The experimental period was initiated by the administration of an intravenous pulse of [1-^{14}C]leucine (59 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels; Amersham); the dose, 100 μCi/kg of body weight, was contained in 0.5–1.0 ml of physiological saline. Timed arterial blood samples were then drawn for determination of the time courses of the [^{14}C]leucine and leucine concentrations in the plasma. The blood samples were immediately centrifuged to remove the erythrocytes and the plasma was stored on ice and assayed later. At 60 min after the pulse, the animal was killed by an intravenous dose of thiopental followed by a saturated solution of KCl in sufficient amount to stop the heart. The brain was removed as rapidly as possible and frozen in isopentane (−50°C). The frozen brain was cut in the Horsley–Clarke plane into 20-μm sections in a cryostat maintained at −22°C. The frozen sections were dried on a hot plate at 60°C and then autoradiographed exactly as described for the [^{14}C]deoxyglucose technique (6).

Local tissue ^{14}C concentrations were determined by quantitative densitometry as described (6). The arterial plasma samples were deproteinized with 5% sulfosalicylic acid, and [^{14}C]leucine and leucine concentrations were assayed by liquid scintillation counting calibrated with internal standards and by quantitative amino acid analysis (Beckman amino acid analyzer, model 121 MB, Beckman Instruments, Fullerton, CA), respectively.

RESULTS

In order to facilitate interpretation of the autoradiographs, a cresyl violet-stained histological section of the lateral geniculate nucleus is presented (Fig. 1) in which the laminae of the geniculate body are clearly visualized and numbered. Laminae 1, 4, and 6 are known to be the sites of termination of the optic tract fibers from the contralateral eye; laminae 2, 3, and 5 are innervated by the ipsilateral eye (2). These laminae are clearly represented in the autoradiographs of the lateral geniculate bodies from a normal 25-day-old monkey with intact binocular vision (Fig. 2*A*). The autoradiographs represent the relative rates of protein synthesis in the various structures visualized; the darker the region, the greater the rate of protein synthesis. It is apparent that the rates of protein synthesis in all the laminae of the lateral geniculate bodies are more or less the same and are higher than those in any other structures represented in the autoradiographs; they are, in fact, among the highest in the brain at this age.

Acute visual occlusion had no differential effects on the rates of protein synthesis in the laminae of the lateral geniculate bodies; the autoradiograph from the animal studied 3 hr after unilateral lid suture (Fig. 2*B*) was indistinguishable from that of the animal with intact bilateral vision (Fig. 2*A*). These results obtained in 25-day-old monkeys were not noticeably different from those obtained in the monkeys studied at 2 days of age (autoradiographs not shown).

Chronic unilateral visual deprivation, however, resulted in marked changes in the appearance of the autoradiographs of the lateral geniculate nuclei. The laminae innervated by the occluded eye (1, 4, and 6, contralaterally; 2, 3, and 5, ipsilaterally) exhibited lower optical density compared to the laminae served by the still-functioning eye, and the autoradiographic patterns of the laminae were inverse rather than bilaterally symmetrical (Fig. 2 *C* and *D*). Quantification of the rates of protein synthesis indicated that the rates were entirely normal in the laminae innervated by the intact eye but were decreased by about 20–25% in the deprived laminae. Examination of autoradiographs of sections through other parts of the visual system of monocularly

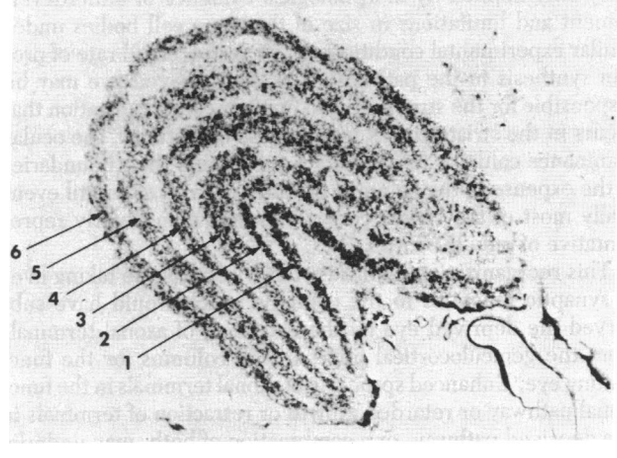

FIG. 1. Cresyl violet-stained coronal section at the level of the lateral geniculates of a young adult monkey with intact binocular vision. Crossed fibers of the optic tract terminate in laminae 1, 4, and 6; uncrossed fibers terminate in laminae 2, 3, and 5. (×12.)

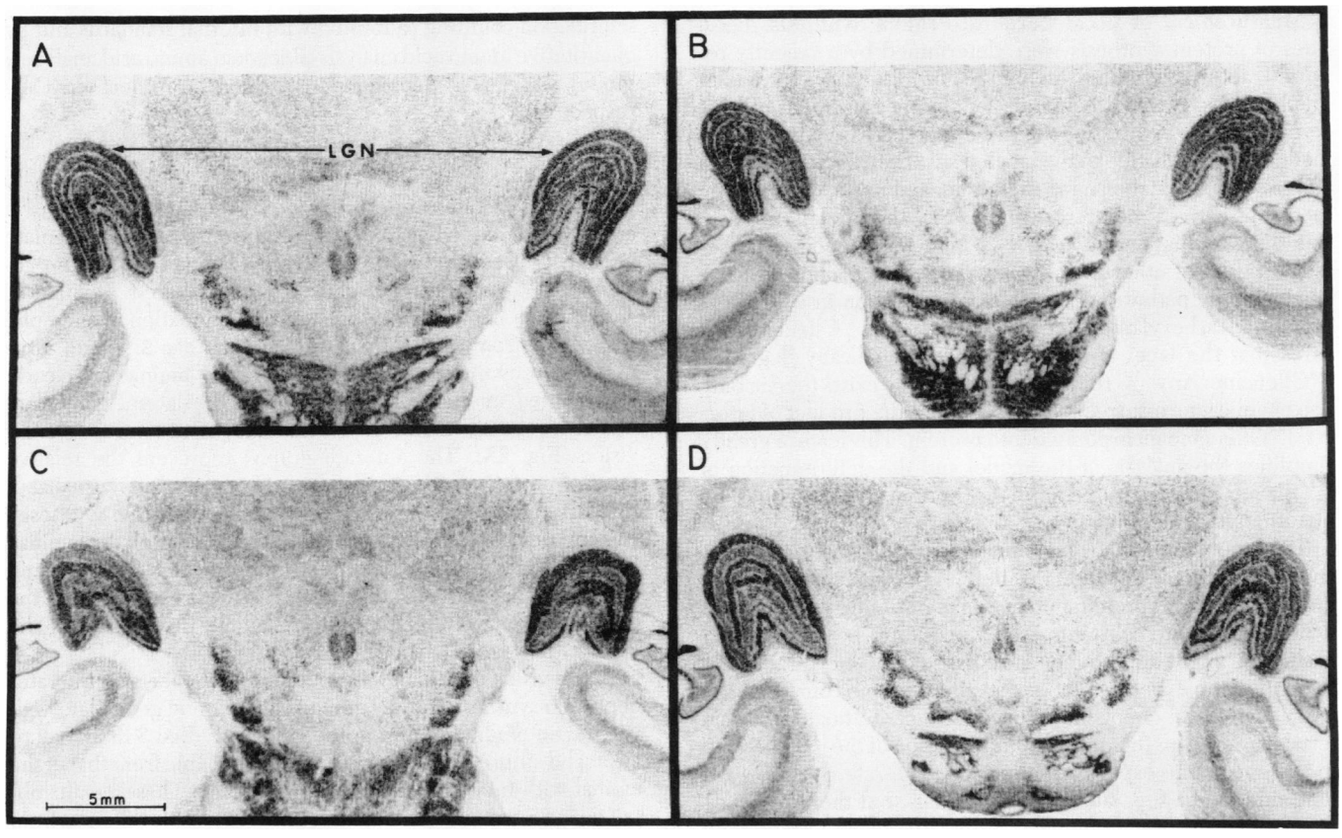

FIG. 2. Autoradiographs of coronal sections of monkey lateral geniculate nuclei (LGN) obtained with the [^{14}C]leucine method. The left side of the brain is on the left side of the autoradiographs. (*A*) Twenty-five-day-old monkey with intact binocular vision. (*B*) Twenty-five-day-old monkey with acute occlusion of the left eye. (*C*) Twenty-five-day-old monkey with chronic occlusion of right eye initiated on second day of life. (*D*) Twenty-five-day-old monkey with chronic occlusion of left eye initiated on second day of life.

deprived animals, except for the retina and optic nerves which were not examined, provided no convincing evidence of a change in pattern from that of the normal 25-day-old monkey.

DISCUSSION

The results of the present study demonstrate that chronic monocular deprivation in the newborn monkey results in reduced rates of protein synthesis in the cell bodies of the lateral geniculate nuclei to which the optic tract fibers from the deprived eye project. These results, therefore, confirm what was previously only implied by morphological evidence of underdevelopment and limitations in size of the same cell bodies under similar experimental conditions (2, 7). A decreased rate of protein synthesis in the pathway from the deprived eye may be responsible for the structural and functional reorganization that occurs in the striate cortex under these conditions. The ocular dominance columns for the intact eye extend their boundaries at the expense of the columns for the deprived eye until eventually most of the striate cortex becomes monocularly representative of only the intact eye.

This reorganization almost certainly reflects the taking over of synaptic junctions in the columns which would have subserved the deprived eye by the extension of axonal terminals from the geniculocortical fibers in the columns for the functioning eye. Enhanced sprouting of axonal terminals in the functional pathway or retarded growth or retraction of terminals in the deprived pathway, or a combination of both, may underlie this reorganization. The growth of axonal terminals is dependent on the continued synthesis of protein in the perikaryon which is transported axonally to the terminals. The cell bodies for the terminals in the striate cortex reside in discrete laminae in the lateral geniculate bodies segregated for the two eyes. The present findings of normal rates of protein synthesis in the lateral geniculate laminae for the functional eye and decreased protein synthesis in the geniculate cell bodies of the deprived pathway provide evidence that inadequate growth and maintenance of axonal terminals in the geniculocortical projections of the deprived pathway are responsible for the functional reorganization of the striate cortex in the newborn monkey. It is possible that a different mechanism may underlie the plasticity observed by LeVay *et al.* (7) in more mature monkeys.

The findings in the present study are also in accord with the hypothesis of Hubel *et al.* (2) who proposed that column asymmetry arising from chronic monocular deprivation is the result of the lesser ability of the growing neuropil of the deprived column to maintain its territory in the face of competition from terminals arising from the functional column. The idea of such competition in visual deprivation was originally suggested to account for the finding that binocular deprivation produced defects in cortical cell responses that were milder than predicted by monocular deprivation (8). In addition, the monocular segment of the striate cortex that represents only the contralateral temporal crescents of the visual fields retains normal function on the side contralateral to the closed eye (2). Thus, simple disuse can be ruled out as the explanation for the unresponsiveness of cells subserving a deprived eye. Further evidence for such competition is the observation that, once column asymmetry has been established by chronic monocular deprivation, normal columnar organization cannot be reestablished by restoration of vision in the previously deprived eye unless the previously functional eye is subsequently deprived (7). The present results suggest that depressed protein synthesis in the

deprived pathway may underlie the loss of competitive efficacy.

In contrast to the effects of chronic monocular deprivation, acute unilateral visual occlusion had no specific differential effects on the rates of protein synthesis in the laminae of the lateral geniculate bodies. A lack of effect on protein synthesis in the lateral geniculate bodies has also been observed in the rat approximately 3 hr after unilateral enucleation (unpublished data). It appears then that, unlike glucose utilization which is closely coupled to functional activity in the nervous system (9), the rate of protein synthesis does not appear to reflect the level of functional activity. Protein synthesis is apparently altered only indirectly and after a relatively long delay during functional deprivation.

We thank Jane Jehle for technical assistance, J. D. Brown for preparing the photographs, and Ruth Bower for preparing the typescript.

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