first detected in 45-day-old animals, and by 60 days of age the regenerating nerves had well-established mechanosensory fields. In these animals, however, the medial DCN-T13 nerve fields were now no different from those of normal control animals of the same age, and the number of touch domes now supplied by the medial DCN-T13 was normal (Table 1). In 60-day-old animals in which regeneration of the cut nerves was deliberately prevented by ligation of their central stumps, the isolated fields and their population of touch domes remained enlarged (Fig. 2 and Table 1). The regenerating nerves appeared therefore to have replaced the extra population of nerve endings sprouted by intact nerves. In keeping with this result, in the 60-day animals with regeneration of adjacent nerves, the mechanical stimulation of identified domes that had been reinnervated during the critical period by sprouts from intact axons now evoked impulses only in regenerated ones. The recapture of skin and its touch domes by the regenerated nerves occurred sometime between 40 and 60 days of age, commencing about 35 days after the nerves were cut. Interestingly, "foreign" regenerating axons, for example of DCN-T12 or L1, seemed as competent as those of the original nerve (the lateral branch of DCN-T13) in displacing sprouted endings. Regenerating high-threshold nerves have also been shown to recapture skin from sprouted nerves in the rat (*8*).

These results are in marked contrast to findings in the salamander, in which the Merkel cells occur singly, scattered throughout the epidermis (*10*); after their denervation they seem to become permanently captured by the first axon to reach them, whether this is the original axon or a foreign one, and the endings are not thereafter displaced (*11*). In the rat, the target character of the Merkel cell seems to be not totally suppressed upon innervation by sprouted nerves since the cells are apparently still recognized by regenerating axons. Furthermore, only the synapses established by sprouted endings on the Merkel cells in touch domes outside the normal territory of the parent nerve are vulnerable to competition from regenerating nerves; the synapses within the normal field are not. The sizes and maturity of the individual terminal fields of axons, their usage, and the distance between arriving nerve endings, have all been hypothesized to affect the stability of synapses, either during development (*12*), or during the functional replacement by regenerating nerves of other regenerated nerves or of sprouted ones (*8, 13*). In the light of the spatial constraints on collateral sprouting, it is interesting that the seemingly vulnerable endings of low-threshold mechanosensory nerves are those located in the former field of a neighboring nerve, even one within the parent dermatome, suggesting the additional possibility that territorial preference too may be of importance in determining the relative stability of synaptic connections.

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6. We have evidence similar to that described here indicating that in the adult rabbit, too, low-threshold mechanosensory nerves do not sprout into adjacent denervated skin [P. C. Jackson, thesis, McMaster University (1980)]. In earlier studies on nerve sprouting in salamander skin (*1*), we showed that colchicine blockade of axoplasmic transport resembled nerve section in evoking the sprouting of neighboring, untreated, nerves (the colchicine-treated ones did not sprout). We have also compared colchicine blockade to nerve section in adult rabbits [P. C. Jackson and J. Diamond, *Brain Res.* **130**, 579 (1977)], reporting only, however, on the physiological and morphological characteristics of the treated nerves, and not on the possibility that neighboring nerves might have sprouted [compare with M. C. Brown, R. L. Holland, W. G. Hopkins, *Annu. Rev. Neurosci.* **4**, 17 (1981)]. We now know that such sprouting would not have occurred in these adult rabbits even if the colchicine-treated nerves had died.
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Development of Visual Centers in the Primate Brain Depends on Binocular Competition Before Birth

Abstract. *Removal of one eye before birth permanently changes the cellular organization and synaptic connectivity of visual centers in the primate brain. The most notable alterations are (i) the lateral geniculate nucleus develops only two cellular layers and one interlaminar fiber band instead of the normal six layers and five bands, (ii) aberrant synaptic connections are formed between the intact eye and the geniculate neurons that have lost their normal input, and (iii) ocular dominance columns fail to develop in the visual cortex.*

It has been known for some time that monocular enucleation or sensory deprivation in the neonatal period causes functional and structural alterations in the visual system of mammals (*1*) including primates (*2, 3*). The effect is particularly prominent in the dorsal lateral geniculate nucleus (LGd), which in most Old World primates as well as in humans consists of six horseshoe-shaped cellular layers separated by five fiber-rich interlaminar bands (Fig. 1A). Three of the layers (1, 4, and 6) receive input from the contralateral eye (Fig. 1B) and the remaining three (2, 3, and 5) from the ipsilateral eye (*4*). The LGd neurons subserving each eye project to layer IV of the visual cortex (Fig. 2D) in the form of separate and alternating ocular dominance columns (*5*). The two ventralmost layers of the LGd contain large cells and are termed "magnocellular," while the upper four layers have smaller cells and are called "parvocellular" (Fig. 1A).

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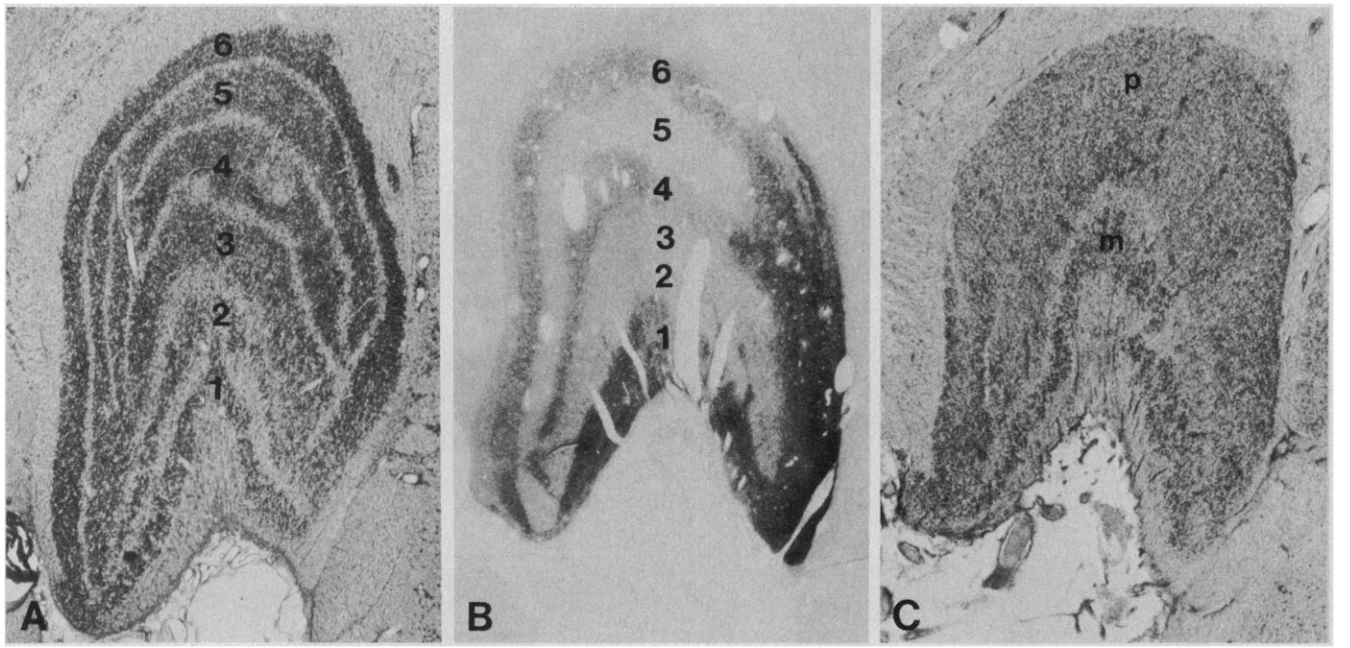

Fig. 1. (A) Nissl-stained coronal section of the lateral geniculate nucleus (LGd) in a normal adult monkey, showing six cellular layers (1 to 6) and five interlaminar bands. (B) Autoradiograph of the LGd of a normal adult monkey showing labeling of layers 1, 4, and 6 after injection of the contralateral eye with radioactive tracer. (C) Nissl-stained LGd in a monkey of the same age from which one eye was removed at the second fetal month showing the presence of the magnocellular (*m*) and parvocellular (*p*) moiety and the absence of the normal six-layered pattern.

The parvocellular and magnocellular moieties of the monkey LGd project to different sublayers of the cortex (*6*) and belong respectively to the X- and Y-like systems, which among their other functional properties are presumably related to color and noncolor vision (*7*). Although these features of LGd organization in primates are not fully understood, the developmental mechanisms and the biological significance of cell segregation into separate layers and functional subsystems have been of considerable practical and theoretical interest (*3, 4, 8*).

This study was initiated after pilot experiments in the monkey indicated that enucleation of one eye before birth had even more profound effects on the structure of the LGd than when performed postnatally (*9*). This can be expected, since in primates all LGd neurons are generated (*10*) and their basic connections established before birth (*11*). It should be emphasized, however, that retinogeniculate projections from the two eyes overlap before sorting out into three alternating layers in the LGd during the second half of gestation (*11*). Furthermore, the geniculocortical terminals are also initially intermixed before becoming segregated into ocular dominance columns (*3, 11*), and the projections from magno- and parvocellular layers of the LGd are unseparated before becoming distributed into appropriate sublayers of layer IV (*11*). Thus, enucleation of one eye before birth can reveal the extent to which competition between axons originating from two eyes may influence the development of binocular or X- and Y-like neuronal systems (or both) in the absence of any visual experience.

Twelve monkeys (*Macaca mulatta*) were studied. Two animals in the second month and two in the third month of pregnancy were subjected to hysterotomy; the fetuses were temporarily removed from the uterus and, after eye enucleation, replaced in the uterus (*11*). Pregnancies were carried to full term (5½ months), when each fetus was delivered by cesarean section and allowed to develop to the ages of 2 months to 1 year. In two of these animals, a mixture of [^{3}H]proline and [^{3}H]fucose (total of 1.0 to 1.5 mCi) was injected into the vitreous body of the intact eye 14 days before they were killed. As a control, four normal fetuses corresponding in age to that at the time of eye enucleation and four postnatal monkeys corresponding in age to that at death were processed in a similar way. In each brain, a 0.5- to 1.0-mm thick coronal slice was dissected from the middle level of the LGd contralateral to the eye injection and processed for electron microscopic analysis. The remainder of each brain was processed for autoradiography (*11*).

In the two monkeys in which one eye was enucleated during the second fetal month, the LGd was located in its usual position and was normal in size and shape (Fig. 1C). Although the cell packing density may have been slightly altered, the number of neurons was not significantly diminished. The most dramatic finding was the absence of six cell layers and all but one of the interlaminar bands in the LGd (Fig. 1C). The single remaining interlaminar band was situated between the magno- and parvocellular moieties and presumably corresponds to the space between layers 2 and 3 that normally receives input from the same eye. In the two monkeys in which one eye was enucleated at the end of the third fetal month, the LGd also attained a normal position and external shape, but the dimensions of the nucleus were smaller than in the animals operated on 1 month earlier (*12*). In addition, these specimens contained some indication of layers comparable to the stage of lamination that existed at the fetal age when the surgery was performed. Thus, the ingrowth of projections from both eyes during the first half of gestation is essential for both the initiation and completion of cellular lamination in the monkey LGd. However, the segregation into magno- and parvocellular moieties of the nucleus proceeds normally in the absence of one eye.

When radioactive tracer was injected in the remaining eye of the adult monkeys that had been enucleated at fetal month 2, anterogradely transported label was distributed over the entire LGd (Fig. 2A). Thus, the neurons in the positions normally occupied by layers 1, 4, and 6, which should receive projections from the removed, contralateral eye, received

ipsilateral input equivalent to that received by neurons situated in positions corresponding to the appropriate layers 2, 3, and 5. Furthermore, electron microscopic examination revealed that typical retinal synapses were present at positions of all presumed layers (Fig. 2, B and C). Therefore, the axons that originated in the remaining eye probably formed synapses with all LGd neurons. The functional significance of this abnormal synaptic arrangement is unknown, but the changes seem to be long-lasting since they were observed in a 1-year-old monkey lacking one eye from the early fetal age.

When one eye was removed at fetal month 3 and the remaining eye injected with radioactive tracers after birth, label was also distributed rather diffusely over the LGd. In this case, however, small, elongated, less densely labeled territories were visible within the posterior pole of the nucleus in a pattern similar to that described in normal 3-month-old fetuses (*11*). Therefore, even after the process of separation of the terminals originating from two eyes has begun, it is arrested if one eye is removed during the formative period.

In the visual cortex, transneuronally transported radioactive label injected into the eye of mature monkeys that were monocularly enucleated at prenatal periods formed continuous horizontal sheets localized mainly within layer IV without any indication of the alternating ocular dominance columns (Fig. 2E) that are characteristic of the normal visual cortex in this species (Fig. 2D) (*3*). The vertical segregation of the parvocellular and magnocellular moieties of the LGd input into sublayers IVA, IVCα, and IVCβ was still achieved, however (Fig. 2E).

These results indicate that the integrity of projections from the two eyes during prenatal development is necessary for the establishment of normal cell distribution and synaptic organization in the primate visual system. More specifically, competition between the two eyes is essential since neurons and their interconnections formed abnormally even

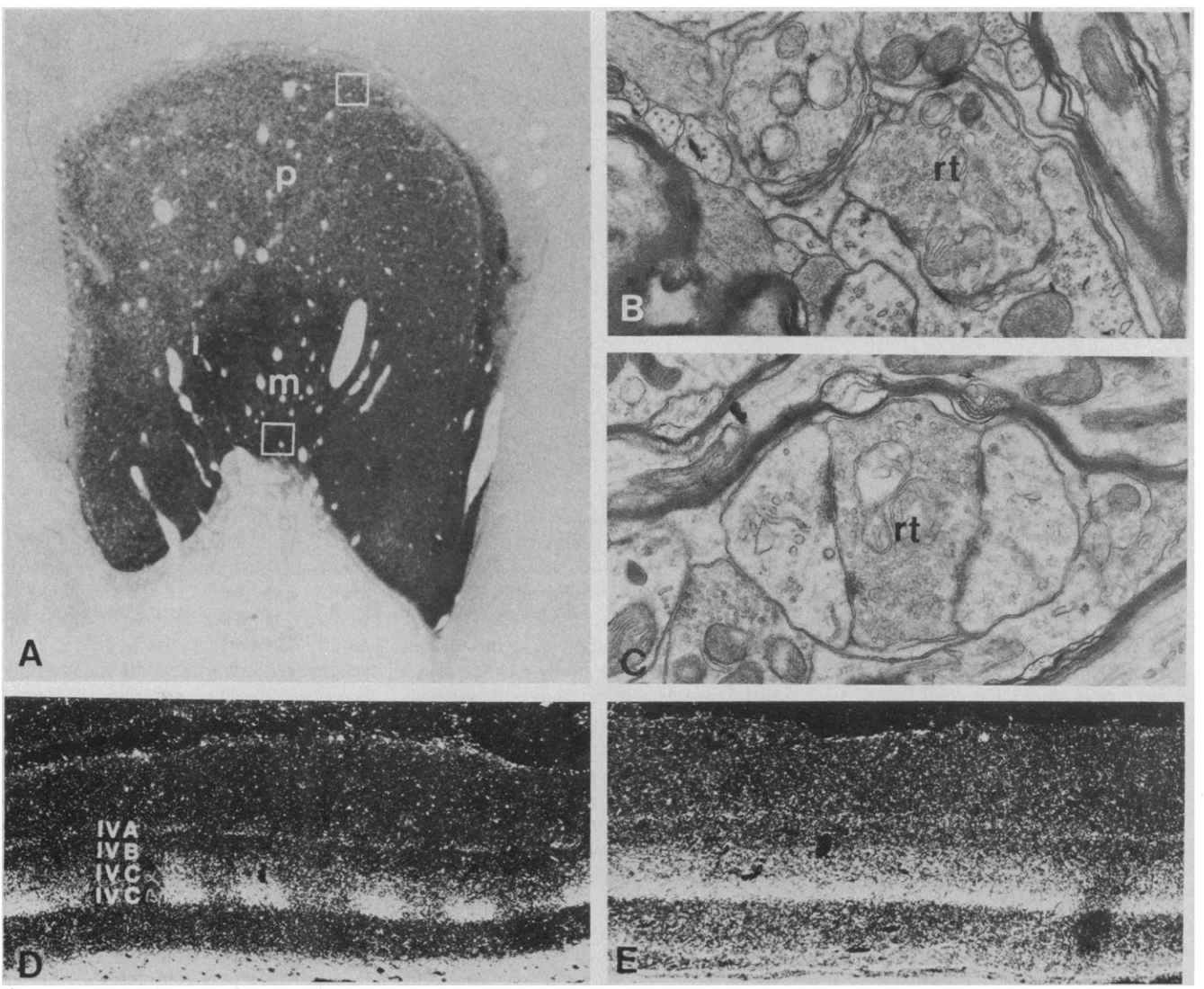

Fig. 2. (A) Autoradiograph of the LGd in a 2-month-old monkey from which one eye was removed at the second fetal month, showing the spread of radioactive retinal input over the entire nucleus. Although the magnocellular moiety (*m*) receives more dense input than the parvocellular (*p*), just as in the control monkeys (Fig. 1B), the layering pattern is not discernible. (B and C) Electron micrographs of retinal axon terminals (*rt*) in the left LGd in a monkey whose right eye was enucleated in the second fetal month. Such terminals are uniformly distributed and found even in the territories of presumptive layers 1 and 6 (indicated by rectangles in A), which normally receive input from the contralateral (removed) eye. (D) Dark-field autoradiograph of the primary visual cortex in a normal adult monkey. Transneuronally transported label is distributed in the form of alternating ocular dominance columns and over sublayers IVA, IVCα and IVCβ. (E) Autoradiogram of the primary visual cortex of a 2-month-old monkey from which one eye was removed at the second fetal month and the remaining eye injected with tracer 2 weeks before death. The label forms horizontal, uniform sheets without indication of ocular dominance columns, but segregated input into sublayers IVA, IVCα, and IVCβ is established.

though LGd neurons received morphologically normal synaptic input from the single remaining eye. This study further emphasizes that the binocular competition critical for the initial formation of the normal visual system does not require visual experience since it exercises its influence before birth. Finally, the dependence of normal development on prenatal binocular competition is selective; segregation of the LGd into magno- and parvocellular moieties and the laminar distribution of their terminals in the cerebral cortex developed normally in all experimental animals. This example of how an error in development of a single structure can alter distant but related structures in the complex primate brain may offer insight into various abnormalities of lamination or connections that occur in congenital malformations in humans.

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12. The smaller size of the LGd in the monkeys enucleated at later gestational ages may be due to neuronal atrophy, loss of neurons, or both. This effect may be related to the higher dependence of already committed, more mature LGd neurons on the proper retinal input.
13. Supported by PHS grant EY 02593.

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Axonal Elongation into Peripheral Nervous System "Bridges" After Central Nervous System Injury in Adult Rats

Abstract. *The origin, termination, and length of axonal growth after focal central nervous system injury was examined in adult rats by means of a new experimental model. When peripheral nerve segments were used as "bridges" between the medulla and spinal cord, axons from neurons at both these levels grew approximately 30 millimeters. The regenerative potential of these central neurons seems to be expressed when the central nervous system glial environment is changed to that of the peripheral nervous system.*

The inability of axons to elongate for more than a few millimeters within the damaged central nervous system (CNS) is believed to be a fundamental feature of the failure of regeneration in the brain and spinal cord of adult mammals. On the other hand, axons from transected peripheral nerves successfully regrow over long distances when they become associated with Schwann cells in the distal nerve stump or in a nerve graft. Although many mechanisms are probably involved (*1*), there is increasing evidence that differences in the capacity of certain damaged axons to elongate in the CNS and peripheral nervous system (PNS) are more dependent on the environment in which these axons are located than upon intrinsic properties of neurons. This hypothesis, proposed by Cajal (*2*), has received additional support from recent studies. (i) Experiments in adult mammals have demonstrated that, although PNS axons, with a known capacity to regenerate, fail to lengthen in a milieu of CNS glia (*3*), the axons from intrinsic CNS neurons grow into peripheral nerve segments transplanted into the transected spinal cord (*4*). (ii) Tissue culture investigations show that Schwann cells, other nonneuronal cells, and factors in the culture media exert various trophic influences on neurons (*5*). Using a new experimental model, we provide evidence that axons from nerve cells in the injured spinal cord and brainstem can elongate for unprecedented distances when the CNS glial environment is replaced by that of peripheral nerves.

In adult Sprague-Dawley rats weighing between 250 and 350 g, segments of autologous sciatic nerve 35 mm long were used as "bridges" between the medulla oblongata and the lower cervical or upper thoracic spinal cord (Fig. 1A). These "bridges" were placed extraspinally in the subcutaneous tissues along the back of the animal. One end of the graft was inserted through a laminectomy into the dorsolateral spinal cord, and the other end was introduced into the lower medulla through a small opening made across the craniocervical junction. We ensured the penetration of the graft endings into the CNS by using a glass rod with a 150-μm tip. Retaining 10-0 sutures were placed at both ends of the graft. The grafting procedure resulted in local damage at the site of insertion of the nerve into the dorsal brainstem and spinal cord, but the rest of the neuraxis was left intact. Because axons from spi-

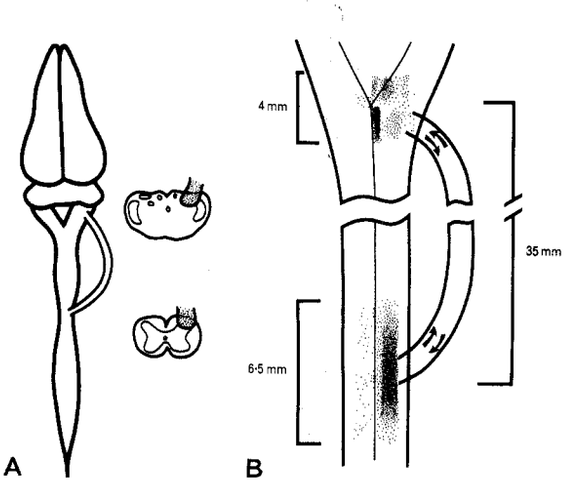

Fig. 1. (A) Diagram of the dorsal surface of the rat CNS, showing a peripheral nerve "bridge" linking the medulla and the thoracic spinal cord. Cross sections depict the region where the ends of the nerve graft were inserted. The origin of axons innervating the graft was determined by retrograde labeling with HRP. Axonal elongation was measured between the site of HRP application and that of the labeled cells in the CNS. For this purpose, when neurons were sought in the medulla, the tracer was applied after sectioning the nerve at a level situated approximately 30 mm from the brainstem and 5 mm from its caudal insertion into the cord (group A, Table 1). Conversely, when the growth of axons from the spinal neurons was assessed, the graft was cut close to the brainstem (group B, Table 1). The short stumps of these nerve grafts were also used for anterograde labeling. (B) Approximate rostrocaudal position of 1472 labeled CNS neurons (dots) demonstrated in seven grafted rats. In the brainstem the territory occupied by 450 of these cells extended along 4 mm, whereas 1022 labeled neurons were scattered along a 6.5-mm segment of the spinal cord.

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