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10. Cells were grown in medium consisting of 15 percent (by volume) fetal calf serum (Irvine Scientific) and 85 percent Ham's F-10 (Gibco) without antibiotics. The cultures were maintained in a humidified atmosphere of 5 percent CO_2 at 37°C. The B104 cells were first trypsinized [0.1 percent trypsin in Hanks balanced salt solution (Gibco) for 10 minutes at 37°C and plated onto various Falcon tissue culture plastic plates at a concentration of 1 to 2×10^5 cells per square centimeter of surface area, where they were allowed to grow to confluency. Four days prior to the start of coculture, C6 cells were similarly trypsinized and plated at a low cell density. On each of the following 3 days, fresh medium supplemented with 2 μCi of [^{3}H]methyl thymidine (New England Nuclear, 60.83 Ci/mmole) per 10 milliters was added to the growing cells. On day 4 ($t = 0$) these labeled C6 cells were again trypsinized, mixed with unlabeled trypsinized C6 cells in a ratio of about 1:5 (labeled: unlabeled), and plated at a concentration of 5 to 6×10^5 cells per square centimeter (equal to one-fourth of their normal confluent density). From this point on, medium was changed each day to avoid differences in nutrient levels and *p*H between the C6-only, B104-only, and C6/B104 cocultures.
11. Cells were fixed in 4 percent glutaraldehyde-phosphate buffered (*p*H 7.2, 0.01*M*) saline solution overnight at 4°C. After they were extensively washed in water and dried, the plates were processed for "high speed scintillation autoradiography" [B. G. M. Durie and S. E. Salmon, *Science* **190**, 1093 (1975)], Spectrafluor (Amersham/Searle) being used as the scintillator.
12. The DNA was assayed by the method of J. M. Kissane and E. Robins [*J. Biol. Chem.* **233**, 184 (1958)] as modified by F. Setaro and C. G. D. Morley [*Anal. Biochem.* **71**, 313 (1976)] with calf thymus DNA (Sigma) used as a standard.
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15. The method of M. Lucas and J. Bockaert [*Mol. Pharmacol.* **13**, 314 (1977)] was modified as described below. Cells, grown on Falcon 3024 plates (75 cm^2), were washed three times with 0.9 percent (weight to volume) NaCl at room temperature. The cell surface was then scraped with a rubber policeman into a total volume of 7.5 ml of 25 m*M* tris buffer (*p*H 8.0) with 5 m*M* EDTA (0°C) and sonicated on ice with an EM/C Sonicator at power 6 for 1 minute. To remove nuclei and whole cells, the suspension was centrifuged at 1000*g* for 10 minutes at 0°C and the pellet discarded. The supernatant was then spun at 105,000*g* for 1 hour and the pellet resuspended in 500 μl of 100 m*M* tris buffer (*p*H 8.0) with 1 m*M* EDTA and 5 m*M* $MgSO_4$. Into each assay tube, 20 μl of this suspension was added to 80 μl, such that the final binding mixture consisted of 100 m*M* tris-HCl (*p*H 8.0), 1 m*M* EDTA, 5m*M* $MgSo_4$, 1 m*M* cyclic AMP, 0.2 m*M* adenosine triphosphate, 0.2 mg of creatine phosphokinase (Sigma, type I) per milliliter, 20 m*M* phosphocreatinine, and various concentrations of [^{3}H]DHA (New England Nuclear, 48.6 Ci/mmole). After 10 minutes of incubation at 30°C, the binding mixture was passed through two GF/C filters (Whatman), the upper filter was dried at 50°C for 1 hour, and then the ^{3}H bound to this filter was counted in a mixture of 8.0 ml of 0.5 percent (weight to volume) 2,5-diphenyloxazole (PPO, Sigma), 10 percent (by volume) Triton X-100, 20 percent (by volume) Triton X-114 in toluene. For assessment of nonspecific binding, 10 μM *l*-alprenolol (Sigma) was included in the binding mixture. Typically, nonspecific binding comprised about 10 percent of the total counts bound.
16. Although the numbers of β receptors per C6 cell reported here are several times the number published by other groups *15*), several pieces of evidence confirm that these specific [^{3}H]DHA binding sites are indeed β-receptors: (i) the apparent dissociation constant [calculated as in (*17*)] for *l*-propranolol (Ayerst Labs) is $2.3 \times 10^{-8} M$, while that of *d*-propranolol (Ayerst Labs) is $27.1 \times 10^{-8} M$—suggesting stercospecificity of binding, and (ii) the specific binding of [^{3}H]DHA is linear with respect to the amount of protein added. Possible reasons for the discrepancy in receptor number include differences in the particular subclone of C6 used, the source of serum, or various modifications used in the preparation of membrane fractions.
17. The K_d values for [^{3}H]DHA were calculated directly from Scatchard plots of [^{3}H]DHA binding data. The K_d values for isoproterenol were calculated by first determining the concentration of isoproterenol required to inhibit 50 percent of specific [^{3}H]DHA binding to membranes. This concentration multiplied by the K_d of [^{3}H]DHA and divided by the concentration of [^{3}H]DHA used (20 n*M*) in the competition study, yields the K_d for isoproterenol.
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Neuronal Plasticity in Primate Telencephalon: Anomalous Projections Induced by Prenatal Removal of Frontal Cortex

Abstract. *When the dorsolateral prefrontal cortex in one hemisphere of a rhesus monkey is resected 6 weeks before birth and the fetus survives to postnatal ages, neurons of the corresponding cortex in the intact hemisphere issue a greatly expanded projection to the contralateral caudate nucleus in addition to a normal projection to the ipsilateral caudate. The enhancement of the crossed prefronto-caudate pathway after prenatal neurosurgery provides direct evidence for lesion-induced neuronal rearrangement in the primate telencephalon.*

Rearrangement of synaptic connections is potentially the most important biological mechanism underlying recovery of function after brain injury. Most of the evidence for such rearrangement in mammals has been obtained from studies of focal ablation of sensory, motor, and limbic structures in developing (*1*) and mature (*2*) rodents or carnivores. It is not known, however, whether and to what degree such neuronal plasticity can occur in the primate brain at maturity or at any stage of development. Primates, including humans, exhibit remarkable sparing of behavioral function after circumscribed brain injuries, particularly those occurring early in life (*3*, *4*). Knowledge of the capacity for axonal redistribution in the primate order is essential for understanding the mechanisms of both reversible and permanent consequences of brain damage. I now report that neocortical neurons in a nonhuman primate can alter their locus of termination in response to a focal brain lesion.

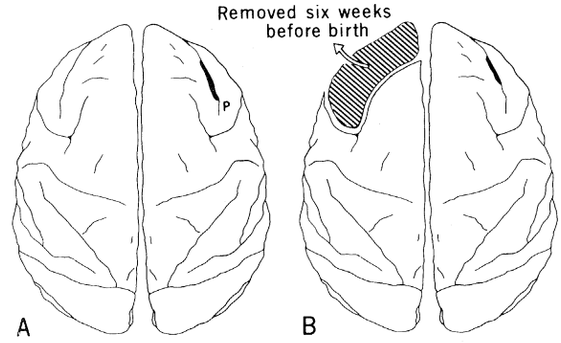

Fig. 1. Injections sites in (A) a control monkey and (B) the monkey whose prospective dorsolateral cortex in the left hemisphere was resected before birth and whose right hemisphere was injected after birth with [^{3}H]proline and [^{3}H]leucine. Injection sites were reconstructed from serial sections through the labeled area. They were defined by extremely dense labeling of the middle portion of the dorsal rim and about half the height of the dorsal bank of the principal sulcus (*P*) in the experimental animal and in both controls. The comparability of the cortical territory labeled in all cases was further corroborated by the similar distribution of labeled cortico-thalamic fibers in the appropriate sector (parvocellular division) of the dorsomedial nucleus which reciprocates projections to the principal sulcus (not shown) as well as by correspondence in the topographic location of ipsilateral cortico-caudate connections shown in Fig. 2. Although the injection site of the control animal was somewhat larger than that of the experimental animal, the autoradiograms (Fig. 2) did not display evidence of anomalous crossed projections.

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Unilateral resection of the frontal lobe in the region of the prospective dorsolateral prefrontal cortex was performed on a fetal rhesus monkey 6 weeks before birth on the 119th embryonic day (E119). The fetus was exposed after laparotomy and hysterotomy (*3*). Cortical tissue in the anterior bank of the primordial arcuate sulcus and in both banks of the developing principal sulcus as well as all cortex lying on the dorsal convexity superior to the principal sulcus was ablated. After the resection was completed, the fetus was returned to the uterus and subsequently delivered around term, which on the average occurs at E165. On the fifth postnatal day, a single injection of a mixture of [^{3}H]proline and [^{3}H]leucine (35 μCi/μl) was placed in the middle of the dorsal rim of the principal sulcus in the hemisphere opposite to that resected prenatally. One week later the monkey was killed and its brain was embedded in paraffin, sectioned at 10 μm, and processed for autoradiographic analysis of axonally transported radioactive label. In two additional monkeys that had not undergone prior surgery, similar quantities of tritiated amino acids were injected into the same region of the cortex at 5 days; the monkeys were killed 1 week later (Fig. 1). Autoradiograms from these animals provided data on normal prefrontal connections in neonatal monkeys.

After radioactive tracers were injected into the dorsal bank of the principal sulcus in the control neonatal monkeys, labeled axons were revealed in the white matter under the injection site and in the ipsilateral caudate nucleus, entering via the subcallosal fasciculus and internal capsule. A high concentration of label is present in the anterodorsal quadrant of the head of the caudate nucleus in the ipsilateral hemisphere. In agreement with previous studies (*5, 6*), the cortico-striatal terminals form multiple clusters or patches, some of which encapsulate core label-free areas. Another class of axons from the prefrontal cortex cross to the opposite hemisphere via the corpus callosum and terminate in columnar bands throughout the homotopic and, to a lesser extent, heterotopic cortical areas (*7*). Transported label was not at first detected in the contralateral caudate nucleus, even in autoradiograms exposed as long as 4 months. After extended examination and quantification by grain counts, however, it was possible to discern small territories in the contralateral nucleus which contained grain densities that could be distinguished from the background. The label is so faint, however, that it cannot be readily seen in low-power photomicrographs (Fig. 2A). A crossed projection from the prefrontal cortex to the contralateral caudate nucleus has not been described in previous studies of frontal lobe connections (*5, 8*).

In the monkey whose dorsolateral prefrontal cortex was resected in the prenatal period, the projection to the ipsilateral caudate nucleus in the neonatal period is similar in topography and configuration to that observed in normal animals at the same age or older (Fig. 2B). However, the projection to the contralateral caudate nucleus stands in marked contrast to that found in normal animals. In the animal operated on prenatally, dense concentrations of label can be traced in consecutive serial sections thoughout the head of the nucleus, which extends for more than 10 mm in the anteroposterior plane. The anomalous contralateral pathway is present in that portion of the caudate that normally receives ipsilateral projections from the cortical area that was resected. Thus, the abnormal input from the contralateral cortex seems to have a predilection for the territories deafferented by the prenatal resection. Moreover, the crossed fibers are distributed in the form of multiple aggregates, smaller in size and number and lacking distinct label-free cores, but clearly reminiscent of the patchlike pattern found in the ipsilateral caudate nucleus of the experimental and normal monkeys (Fig. 2B.).

The anomalous crossed prefrontocaudate pathway represents a considerable alteration in the connectivity of the primate brain. Several mechanisms could account for such reorganization.

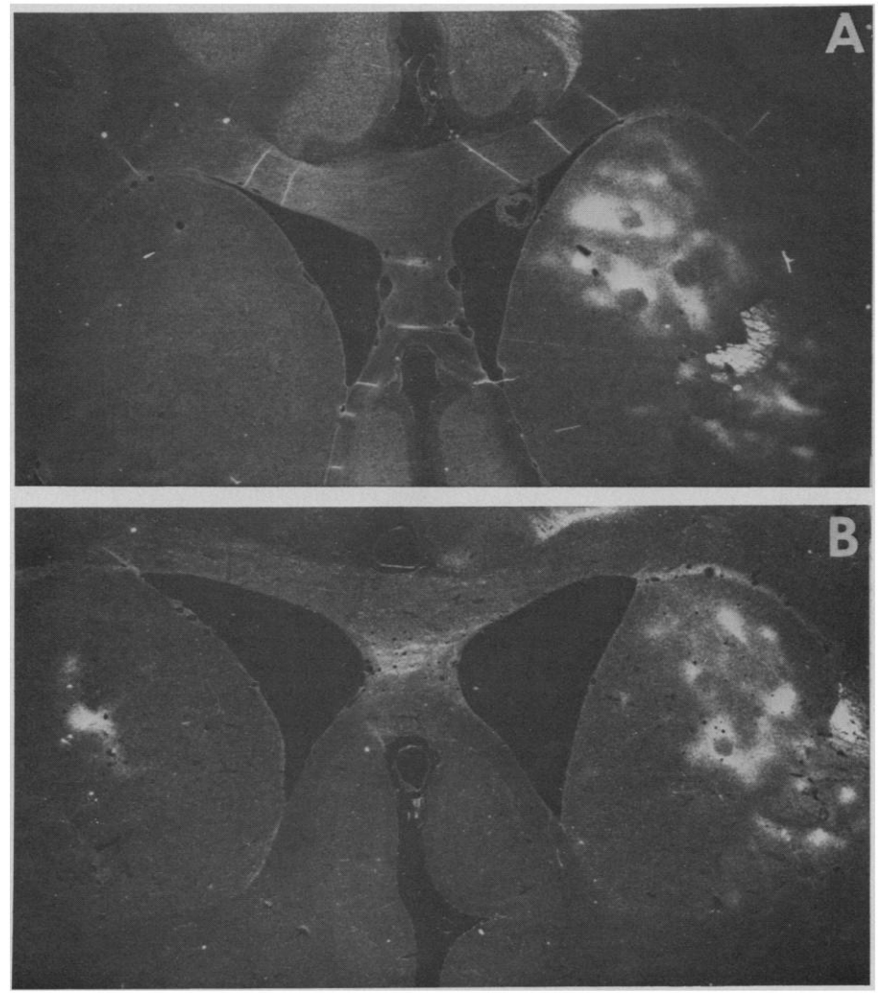

Fig. 2. Photographs of the head of the caudate nuclei in left and right hemispheres under dark-field illumination. (A) A normal monkey. The autoradiogram, exposed 16 weeks, shows an intricate and dense pattern of labeling in the right (ipsilateral) caudate nucleus; the cingulum bundle, internal capsule, and putamen are also densely labeled. However, label in the contralateral nucleus is too faint to be resolved in the photograph. (B) The monkey whose prospective dorsolateral prefrontal cortex in the left hemisphere was resected before birth and whose prefrontal cortex in the right hemisphere was injected with tritiated amino acids. The autoradiogram was exposed 13 weeks. Note both the intricate pattern of grains in the right (ipsilateral) caudate nucleus and also a distinct projection to the left (contralateral) caudate.

The most obvious possibility is that the anomalous axons belong to the class of efferent neurons that normally issue a minor projection to the contralateral caudate nucleus. As proposed for other systems (*1, 2*) these cortico-striatal neurons may expand their terminal fields to occupy vacated synaptic space on the caudate neurons that were deprived of their normal input from the ipsilateral cortex. Another possibility is that the anomalous axons belong to callosal neurons, which, in the absence of their homotopic target cells, are attracted to and invade the caudate nucleus subjacent to the lesion to join with the normally meager complement of crossed cortico-caudate fibers. Still another possible explanation for the anomalous projection is that cortico-caudate projections may be bilateral at embryonic stages and become primarily ipsilateral by selective elimination of a large proportion of crossed projections during development. If so, many contralateral fibers may fail to retract in the absence of competition from the resected ipsilateral cortico-striatal system. Such a mechanism has been hypothesized for the unequal widths of ocular dominance stripes in the visual cortex of monkeys subjected to eye enucleation or monocular deprivation during critical stages of development (*9*). Obviously, overlapping of the prefronto-caudate pathway across the midline would involve a more drastic reorganization during development than that which occurs between adjacent cortical areas within a single hemisphere in the visual system.

The rearrangement of connections in the monkey is comparable to that described in nonprimate mammals (*1, 2*). It is probably important that this high degree of plasticity can be obtained in a primate when a lesion is made before birth. Major developmental events such as neuron genesis, cell migration, and elaboration of basic connections, which in rodents continue to a considerable extent after birth, occur largely prenatally in the monkey (*10*). Neuronal rewiring has been described in mature animals of several nonprimate species (*2*); definition of age limits for the neuroplastic phenomenon reported here requires further investigation.

The dramatic structural rearrangement is relevant to the evidence for sparing or recovery of function after brain damage incurred during early stages of development, particularly for lesions sustained unilaterally. The finding in a primate that neurons from an intact neocortex of one hemisphere can expand their terminal distribution to structures of a damaged hemisphere may provide a long awaited clue to a possible neural mechanism for sparing of associative and linguistic competence after early unilateral brain damage or hemispherectomy in humans.

PATRICIA S. GOLDMAN

Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, Maryland 20014

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Caste in a Primitive Ant: Absence of Age Polyethism in *Amblyopone*

Abstract. *Polyethism, the divison of labor among members of a colony, is based on worker age and size in ants. In the ponerine species* Amblyopone pallipes *worker behavior is independent of age, therefore temporal castes, or groups of age-related task specialists, do not exist. This primitive caste system, previously unknown in ants, appears to be correlated with the peculiar characteristics of the life history and ecology of* Amblyopone.

Caste structures are nearly universal in the social insects. Although the most fundamental difference is between the reproductive queen and the sterile worker caste, within the latter category morphological and temporal subgroups often coexist and provide a finely tuned division of labor within a colony. These two common phenomena, physical polymorphism and age polyethism, are well documented in ants (*1*). In age polyethism, workers change roles in a predictable fashion, and the sequence of worker behavior shifts progressively from nursing to foraging with increasing age. Although this pattern of age-dependent behavior is consistent among the higher subfamilies of ants, age castes in primitive species have remained uninvestigated (*2*). I report here on the apparent lack of temporal division of labor in the ponerine ant *Amblyopone pallipes*, which appears to have the most primitive caste system yet documented in ants.

The genus *Amblyopone* contains the most diverse and widely distributed array of species in the ponerine tribe Amblyoponini (*3*). This assemblage, together with the genera *Myrmecia* and *Nothomyrmecia* contains, on behavioral and morphological grounds, the most primitive ants and represents the closest living approximation to ancestral forms. *Amblyopone pallipes* ranges throughout the northeastern United States and Canada, and populations are dense and local in distribution (*3*). The population structure is unicolonial; colony subunits typically consist of one or more queens and 9 to 16 workers (*4*). The behavior of *Amblyopone* contrasts sharply with the scavenging and homoptera tending habits of most ant species. These ants are exclusively hypogaeic and carnivorous, their predaceous habits being restricted to live linear arthropods with soft cuticles, such as centipedes and beetle larvae, which solitary huntresses paralyze by stinging and then drag to the nest. Both adults and larvae feed directly on the prey; regurgitation is totally absent. The life-cycle data of 28 colonies show that a single brood per year is reared to maturity, from which both sexuals and workers eclose in early August. Callow workers escape from their cocoons unassisted and behave precociously from the time of their eclosion.

The behavior of callows is not directed solely toward queen attendance or egg and larval grooming, as might be expected from comparative studies of other ants, and ethogram studies reveal a distinct, novel pattern for workers of this age. Callows differ from older workers in pigmentation only in a lighter coloration

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