Limits of Neurogenesis in Primates

Abstract. *Systematic analysis of autoradiograms prepared from postpubertal rhesus monkeys given single and multiple injections of tritium-labeled thymidine and killed 3 days to 6 years later displayed a slow turnover of glial cells but failed to reveal any radiolabeled neurons. Therefore, unlike neurons of some nonprimate species, all neurons of the rhesus monkey brain are generated during prenatal and early postnatal life. A stable population of neurons in primates, including humans, may be important for the continuity of learning and memory over a lifetime.*

The frequently stated assumption that the adult human brain lacks the capacity to generate new neurons has never been tested in the adult of any primate species (*1*). Repeated reports of replacement and addition of neurons in the central nervous system of mature fish, amphibians, birds, and rodents (*2*) has led to the supposition that neurogenesis may occur in the human adult (*3*). The functional implications of even a minute turnover or accretion of neurons in the brains of normal and brain-injured human juveniles or adults is enormous and justifies a search for newly formed cells with the [^{3}H]thymidine autoradiographic technique.

Twelve rhesus monkeys ranging in age from 6 months to 11 years were injected, from one to seven times, with [^{3}H]thymidine (10 mCi per kilogram of body weight) and killed after intervals ranging from 3 days to more than 6 years (Table 1). The longer intervals between injections and death allow dividing cells to differentiate into specific classes, and the shorter intervals eliminate the possibility of overlooking neurons that may have divided several times after injection. Nine animals were pregnant at the time of injection, and the presence of heavily labeled neurons in the brains of their offspring served as a test of the technique and of the amount of radioactivity (which indicates DNA replication). In the remaining animals, control tissues were taken from the skin, cornea, intestine, liver, spleen, or other renewing epithelia.

All major structures and subdivisions of the brain including the visual, motor, and association neocortex, hippocampus, olfactory bulb, basal ganglia, thalamus, retina, cerebellum, brain stem, and spinal cord were analyzed. Between 500 and 1000 sections from each animal were first scanned at low power in dark-field illumination, and cells suspected of being radioactively labeled were examined in detail in bright field or with Nomarski differential interference contrast illumination with a ×63 oil immersion objective. This procedure permitted a survey of an estimated 10^8 cells in each specimen. The DNA labeling indicative of cell division (usually more than 15 to 30 silver grains per nucleus) was determined for each case separately by counts of grains present over postmitotic glial and endothelial cells in the brain, or over dividing cells in renewable nonbrain tissues of the same specimens (Fig. 1). The same criterion is commonly used in autoradiographic analyses of developing mammalian brain (*4*, *5*).

Not a single heavily labeled cell with the morphological characteristics of a neuron was observed in the brain of any adult animal. In contrast, one could invariably observe a number of heavily labeled parenchymal cells in the other organs (Fig. 1, A to C). Apparently, [^{3}H]thymidine passed easily through the blood-brain barrier as it was incorporated into the nuclear DNA of dividing fibrillary, protoplasmic, and satellite astrocytes within the central nervous system (Fig. 1, D and E, and Fig. 2). Oligodendrocytes were labeled mostly in the 6- and 18-month-old specimens and were found predominantly in the white matter of the forebrain and within the pyramidal tract (Fig. 1E). Only occasionally were labeled oligodendrocytes observed in postpubertal animals.

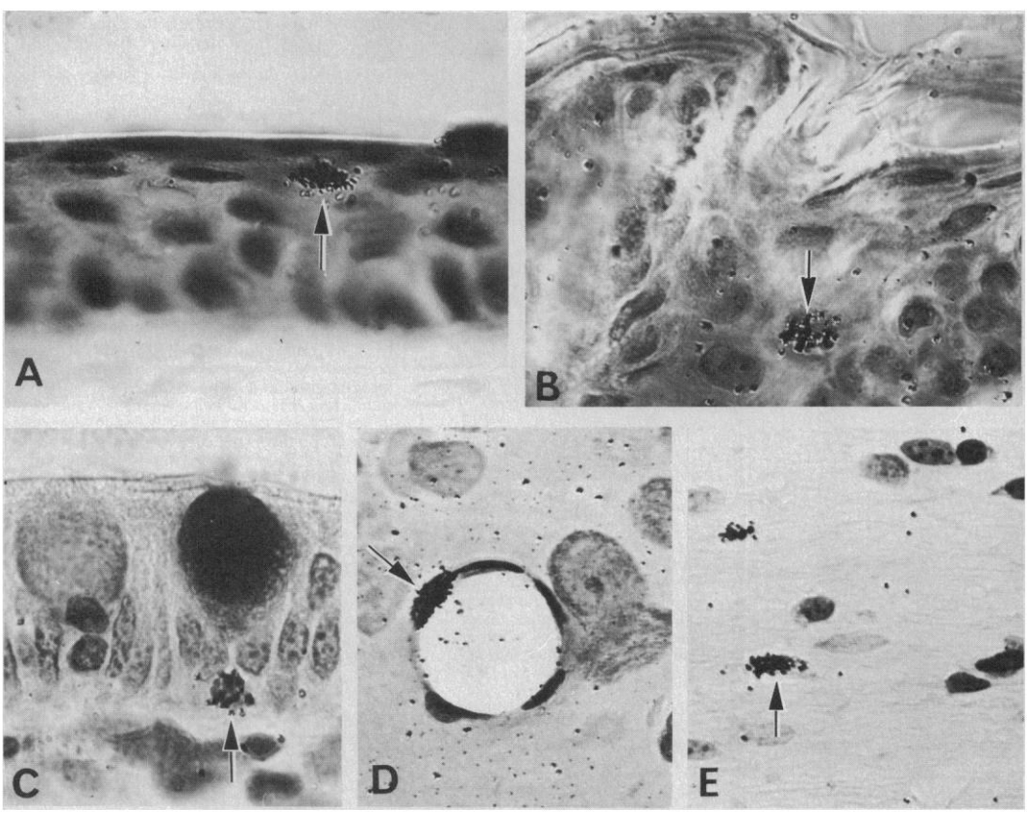

Fig. 1. Interference (Nomarski) contrast photomicrograph of the autoradiograms prepared from various renewing cell populations in mature monkeys exposed to [^{3}H]thymidine before death. (A) Heavily labeled corneal endothelial cell (arrow) in a 5-year-old monkey exposed to [^{3}H]thymidine 3 months before death. (B) Facial epidermis with heavily labeled basal cell in a 1½-year-old animal. (C) The lining of the small intestine in the 1½-year-old monkey showing radiolabeled epithelial cell (arrow) in the villi. (D) Heavily labeled vascular endothelial cell in the brain of the 7-month-old monkey injected with [^{3}H]thymidine 1 month earlier. (E) Radioactively labeled oligodendrocyte in the corpus callosum of the same specimen.

Table 1. Experimental schedule. The sets of six numerals indicate day, month, and year of [^{3}H]thymidine injections and death. The tritium's half-life of 12 to 14 years was adequate even for the longest survival intervals. After being fixed, brains were blocked, embedded in paraffin, and cut serially at 8 μm; every second to tenth section (depending on size of the region) was processed for autoradiography (*4*). Vibratome sections of the hippocampal region from selected monkeys were treated with antibodies against glial fibrillary acidic protein and processed for immunocytochemistry at light and electron microscopic levels to determine the glial or neuronal nature of labeled cells (*6*). The tissue and control samples from other organs were processed for light microscopic autoradiography. Exposure of sections ranged from 12 to 18 weeks for light microscopy and more than 24 weeks for electron microscopic autoradiography. Abbreviations: y, years; m, months; d, days.

Monkey	Date of death	Age at death	Date of [^{3}H]thymidine injection							Interval between injection and death
			1	2	3	4	5	6	7	
1	030783	7m	013183							35d
2	031683	19m	020983							35d
3	022576	6y	051674							1.8y
4	090978	6y	101475							2.9y
5	100779	6y	111273							5.8y
6	031280	8y	110573							6.3y
7	050269	4y	043069							3d
8	021583	17y	101176							6y
9	100777	6y	031673	062374						3.2y
10	012980	10y	062775	030676	040877					2.8y
11	012376	10y	010573	102373	072174	052774	011976			14d
12	112883	5y	082683	082783	082883	082983	083083	083183	090183	3m

Some small cells labeled in the caudate nucleus and hippocampal dentate gyrus of the 6-month-old monkeys were difficult to classify, and the use of routine autoradiograms counterstained with the cresyl violet method could not exclude the possibility that some of the radiolabeled cells were granule neurons. However, fewer of these cells were present in the dentate gyrus and none in the caudate of the adult monkeys, even after multiple injections of [^{3}H]thymidine. Furthermore, immunocytochemistry at the light and electron microscopic levels with antibody to glial fibrillary acidic protein showed that none of the radiolabeled cells in the mature dentate gyrus had neuronal properties (*6*).

Minimal radioactivity (fewer than five or six grains) was occasionally observed over a neuron. This is not likely to be the result of dilution of radioactivity by multiple divisions of initially heavily labeled neurons since monkeys killed shortly after [^{3}H]thymidine injections did not contain any heavily labeled neurons. It is more reasonable to assume that the presence, observed in an occasional cell, of slightly more grains than the background number may reflect a technical artifact or DNA repair, particularly in the cases subjected to multiple injections of the isotope.

Thus, unlike nonprimate species that may display variable degrees of postdevelopmental neurogenesis, the full complement of neurons in the primate central nervous system seems to be attained during a restricted developmental period ending shortly after birth. Previous [^{3}H]thymidine analyses of more than 25 neuronal classes carried out exclusively on embryonic and early postnatal monkeys showed that, except for some granule cells of the cerebellum and hippocampus that continue their genesis for several months after birth, the majority of neurons examined in the rhesus monkey are produced within the first half of gestation (*4*). Experiments conducted by the method of supravital DNA synthesis on fetal tissue as well as comparison of various cytological and biochemical criteria of maturation indicate that human neurogenesis probably has similar sequences and time limitations (*7*).

What is the biological significance of the early genesis of neurons and strict limits of neurogenesis in adult primates? Most neuronal precursors enter the irreversible G_1 phase of their division cycle in the proliferative centers before migrating (*4*); the large, synaptically interconnected adult brain may not normally permit redistribution of new neurons and subsequent growth of axons to distant destinations. Indeed, postdevelopmental neurogenesis is most prominent in species having a considerable capacity for axon regeneration, such as fish and amphibians, or in the avian brain with particular seasonal changes in brain structures related to song production (*2*). However, the brain of primates as well as some other species may be uniquely specialized in lacking the capacity for neuronal production once it reaches the adult stage. One can speculate that a prolonged period of interaction with the environment, as pronounced as it is in all primates, especially humans, requires a stable set of neurons to retain acquired experiences in the pattern of their synaptic connectivity.

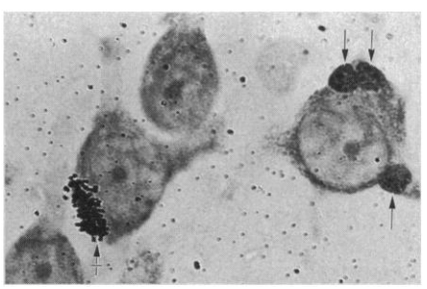

Fig. 2. Interference (Nomarski) contrast microphotograph of a heavily labeled glial cell in the neocortex of the frontal lobe in the 7-month-old monkey that was exposed to [^{3}H]thymidine at 6 months of age. The radioactively labeled cell (crossed arrow) is classified easily as glial in cresyl violet counterstained autoradiograms on the basis of its size, shape, staining properties, and satellite position to the neuron. Three unlabeled satellite glial cells (simple arrows) associated with the other neuron are a useful control.

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References and Notes

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Ecological Character Displacement in Darwin's Finches

Abstract. *Character displacement resulting from interspecific competition has been extremely difficult to demonstrate. The problem was addressed with a study of Darwin's ground finches (*Geospiza*). Beak sizes of populations of* G. fortis *and* G. fuliginosa *in sympatry and allopatry were compared by a procedure that controls for any possible effects on morphology of variation among locations in food supply. The results provide strong evidence for character displacement. Measurement of natural selection in a population of* G. fortis *on an island (Daphne) lacking a resident population of* G. fuliginosa *shows how exploitation of* G. fuliginosa *foods affects the differential survival of* G. fortis *phenotypes.*

Ecological character displacement occurs when morphological differences between coexisting species are enhanced as a result of competitive interactions between them (*1*). Despite the wide range of conditions under which character displacement is predicted by coevolutionary models (*2, 3*) there are few good examples of its occurrence in nature (*4, 5*). Most evidence suggesting ecological displacement derives from a comparison of differences between species in locations of sympatry and allopatry. The main assumption used in inferring character displacement from such comparisons is that there are no other factors influencing morphological differences between locations—for example, differences in food supply. This assumption may often be false.

Darwin's finches provide one of the most familiar examples of apparent character displacement (*6*) (Fig. 1). *Geospiza fortis* and *G. fuliginosa* are sympatric on most Galápagos islands (for example, Santa Cruz), where they are very different in beak and body size. Their beak sizes are intermediate where they occur alone, on the islands of Daphne Major (*G. fortis*) and Los Hermanos (*G. fuliginosa*).

We reexamine this example of apparent character displacement by two approaches: (i) we compare the morphology of the species in sympatry and allopatry, controlling for any differences that might arise because of variation in food supply, and (ii) we use observations of selection pressures on one species in allopatry to show how the differential survival of phenotypes is affected by the use of foods exploited elsewhere by the second species. The results support the original interpretation of character displacement (*6*).

Detailed studies on the Galápagos show that seed supply strongly determines the distribution and survival of finch phenotypes (*7–11*). We can investigate the possibility that morphological differences in allopatry and sympatry result simply from variation in food supply (*12*) by quantitatively describing food characteristics of individual islands in terms relevant to the differential survival of finch phenotypes (*13*). The resulting "adaptive landscapes" (*14*) permit predictions of mean beak sizes for *G. fuliginosa* and *G. fortis* on different islands on the assumption that morphology is determined by food supply alone (*15*). Greater morphological differences in sympatry than expected from food supply would constitute evidence for character displacement.

The procedure for quantifying an island's food supply as an adaptive landscape (*13*) involves the estimation of expected density of a solitary finch population on an island as a function of mean beak depth. First, we identify those seeds that would be included in the diet of a finch population with a given mean beak depth by noting all edible seeds on a given island with size greater than a specific lower bound and with hardness softer than a specific upper bound. The size-hardness limits, determined from empirical data on 21 *Geospiza* populations, are both increasing functions of mean beak depth (*13*). Second, we compute the summed density of seeds on the island that fall between these two limits (*13*). Third, we estimate the number of individual finches which may be supported by that density of seeds, using an empirical relation between the density of finches in individual populations on islands and the available standing crop of the seeds they eat (*13*). These three steps are repeated for 0.05-mm increments of mean beak depth between 5.5 and 15 mm. The resulting curves describe expected population density on each island over the range of beak sizes spanned by actual populations of *G. fuliginosa* and *G. fortis* (*16*).

This procedure was used to calculate expected finch density as a function of beak size on eight Galápagos islands where both *G. fortis* and *G. fuliginosa* are present (Pinta, Marchena, San Salvador, Fernandina, Rábida, Plazas, Santa Fe, and Santa Cruz) as well as the single-species islands of Daphne and Los Hermanos. Almost all seed supply data were collected during visits in the dry season (June to January) when food supply limits finch density (*8, 9*). The exception is Plazas, where seed supply data are available for only the wet season (March). The essential features of expected density curves are not sensitive to seasonal or annual variation in food supply, as shown by analysis where repeated measurements of seed data are available (*17*).

Expected population density curves for Santa Cruz, Daphne, and Los Hermanos (Fig. 1) show two important features: first, there are distinct local maxima (peaks) in expected density and, second, a given island often has more than one maximum—that is, the distributions are polymodal.

Polymodality results from wide gaps in the frequency distribution of seed size and hardness classes on an island. A finch population with mean beak size directly under a given maximum has a higher expected density than a slightly smaller species because it is able to crack and consume one or more seed types that are too hard for a smaller finch to deal with. A finch population with a mean beak size to the right of this peak has lower expected density than a population at the peak because it includes no additional hard seeds in its diet, and it may include fewer smaller seeds. A population of larger finches has a lower expected density than a population of smaller finches when supported by the same biomass of seeds (*13*).

Expected density curves are important