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"Limits of Neurogenesis in Primates"

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SCIENCE

Limits of Neurogenesis in Primates

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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.

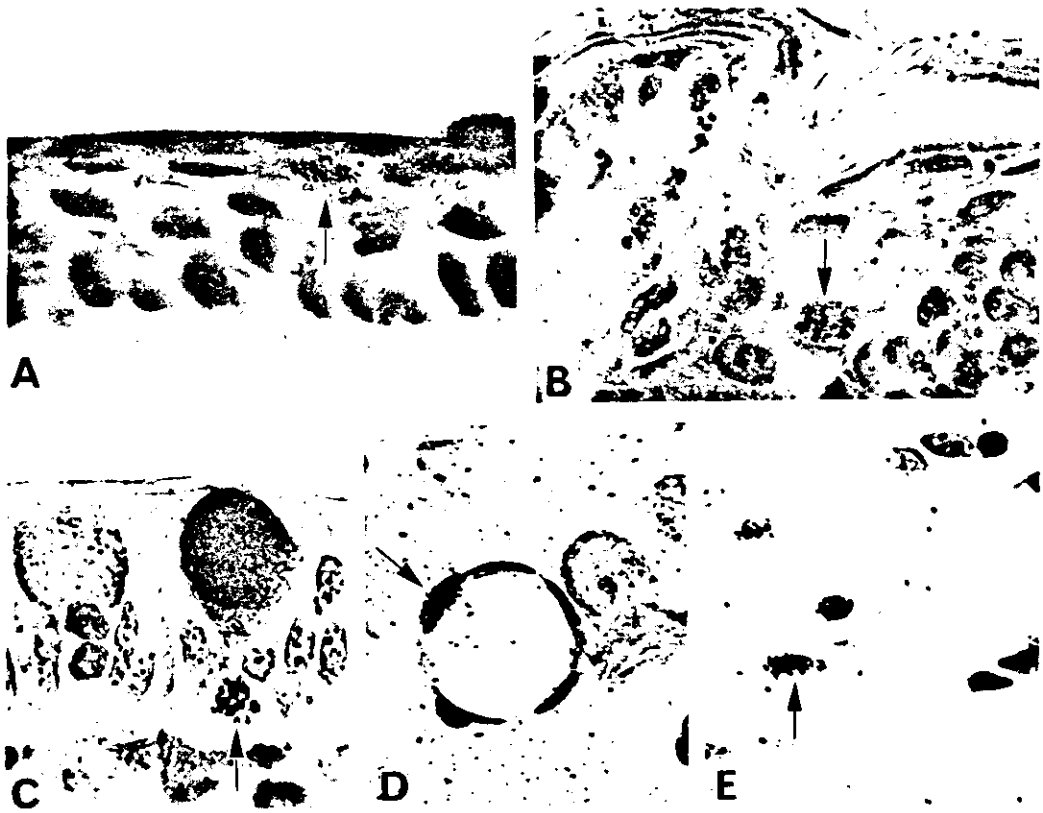

Fig. 1. Interference (Nomarski) contrast photomicrograph of the autoradiograms prepared from various renewing cell populations in mature monkeys exposed to [^{3}H]thymidine before death. (A) Heavily labeled corneal endothelial cell (arrow) in a 5-year-old monkey exposed to [^{3}H]thymidine 3 months before 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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