# Adult-generated hippocampal and neocortical neurons in macaques have a transient existence

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**Previously we reported that new neurons are added to the hippocampus and neocortex of adult macaque monkeys. Here we compare the production and survival of adult-generated neurons and glia in the dentate gyrus, prefrontal cortex, and inferior temporal cortex. Twelve adult macaques were injected with the thymidine analogue BrdUrd, and the phenotypes of labeled cells were examined after 2 h, 24 h, 2 wk, 5 wk, 9 wk, and 12 wk by using the following immunocytochemical markers: for immature and mature neurons, class III β-tubulin (TuJ1); for mature neurons, neuronal nuclei; for astrocytes, glial fibrillary acidic protein; and for oligodendrocytes, 2′,3′-cyclic nucleotide 3′ phosphodiesterase. We found that the dentate gyrus had many more BrdUrd-labeled cells than either neocortical area. Furthermore, a greater percentage of BrdUrd-labeled cells expressed a neuronal marker in the dentate gyrus than in either neocortical area. The number of new cells in all three areas declined by 9 wk after BrdUrd labeling, suggesting that some of the new cells have a transient existence. BrdUrd-labeled cells also were found in the subventricular zone and in the white matter between the lateral ventricle and neocortex; some of the latter cells were double-labeled for BrdUrd and TuJ1. Adult neocortical neurogenesis is not restricted to primates. Five adult rats were injected with BrdUrd, and after a 3-wk survival time, there were cells double-labeled for BrdUrd and either TuJ1 or neuronal nuclei in the anterior neocortex as well as the dentate gyrus.**

In many species of vertebrates, new granule neurons are added to the dentate gyrus of the hippocampal formation in adulthood (1–4). Beginning with the work of Altman (5, 6), this phenomenon of adult neurogenesis in the hippocampus has been studied in most detail in rodents where the new cells have been shown to receive synaptic input (7), express several neuronal markers (8–13), and extend axons into the CA3 region, a normal target area of granule cells (14–16). Most of these cells are generated in the hilus and subgranular zone of the dentate gyrus and then migrate the short distance to the granule cell layer (8). Recent results indicate that ≈9,000 new cells are produced each day in the dentate gyrus of the adult rat, most of which develop neuronal characteristics (17). Many of these new cells appear to die within several weeks of their birth (8, 18, 19). Adult-generated neurons may play a role in learning and memory (18, 20–24).

New cells that express neuronal markers also have been demonstrated in the dentate gyrus of adult primates including marmosets (25), macaques (26, 27), and humans (28) but have not been studied in any detail in these species.

A few early studies suggested that new neurons were added to the cerebral neocortex in adult mammals. Altman (5, 29, 30) made this claim for both rats and cats on the basis of labeling with [$^3$H]thymidine and light microscopy. Combining thymidine autoradiography and electron microscopy, Kaplan (31) reported a small number of new cells with the ultrastructural characteristics of neurons added to the neocortex of adult rats.

Recently, we found new cells with neuronal characteristics in the neocortex of intact adult macaque monkeys (32, 33). Using the thymidine analogue BrdUrd, a marker of DNA synthesis, to label proliferating cells and their progeny (17) and cell-type specific markers to determine the phenotypes of the new cells, we found that neurons were added to prefrontal cortex, posterior parietal cortex, and inferior temporal cortex. Some of these cells extended axons locally.

The present study compares the production, differentiation, and survival of adult-generated neurons and glia in three forebrain structures of the macaque monkey, namely, the dentate gyrus, prefrontal cortex, and inferior temporal cortex. The animals were injected with BrdUrd and then perfused from 2 h to 12 wk later. We assessed the phenotypes of the new cells with the following immunohistochemical markers: for immature and mature neurons, class III β-tubulin (TuJ1) (34); for mature neurons neuronal nuclei (NeuN) (35); for astrocytes, glial fibrillary acidic protein (GFAP) (36); and for oligodendrocytes, 2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNP) (37). We found that the number of new cells in all three areas had declined by 9 wk after their generation, suggesting that some have a transient existence, as appears to be the case for rodents (8, 18, 19). In addition to these results from macaques, we provide evidence, using similar techniques, for neocortical neurogenesis in adult rodents.

## Materials and Methods

**Monkeys.** Twelve sexually mature male *Macaca fasicularis* were used. They were obtained from Primate Products (Redwood City, CA) or Sierra Biomedical Products (Sparks, NV) and had been purpose-bred in Indonesia. They weighed between 2.9 and 6.2 kg (mean 3.9 kg) and, according to the birth dates provided by the suppliers, they were between 5.0 and 5.7 years old (mean 5.5) at the time of BrdUrd injection. They had never been used for any type of experiment or surgery. After arrival they were housed individually. They were exposed to a conventional daily schedule of "enrichment" such as food-foraging toys and mirrors that they could manipulate.

After being anesthetized with a mixture of ketamine (10 mg/kg, i.m.) and acepromazine (0.1 mg/kg, i.m.) the animals were injected i.p. with 100 mg/kg BrdUrd. Two animals were perfused under deep nembutal anesthesia (60 mg/kg) with 4.0% paraformaldehyde in 0.1 M phosphate buffer after each of the following post-BrdUrd injection intervals: 2 h, 24 h, 2 wk, 5 wk, 9 wk, and 12 wk. The 2-h survival time was chosen because it is sufficient time for uptake of BrdUrd in S phase but not for mitosis or migration to occur (17). The 24-h survival time was chosen because it was probably sufficient time for the labeled cells to undergo a single mitotic division (17). The 2-wk group

Abbreviations: TuJ1, class III β-tubulin; NeuN, neuronal nuclei; GFAP, glial fibrillary acidic protein; CNP, 2′,3′-cyclic nucleotide 3′ phosphodiesterase; svz, subventricular zone.

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**Table 1. Number of BrdUrd-labeled cells**

| Animal no. | Postinjection survival time | Dentate gyrus | | Prefrontal | | Temporal | |
|---|---|---|---|---|---|---|---|
| | | per mm$^3$ | % cells $\times 10^{-3}$ | per mm$^3$ | % cells $\times 10^{-3}$ | per mm$^3$ | % cells $\times 10^{-3}$ |
| 43 | 2 hr | 82.9 | 8.5 | 2.9 | 0.9 | 8.1 | 1.9 |
| 50 | 2 hr | 51.9 | 5.3 | 1.3 | 0.4 | 4.7 | 1.1 |
| 46 | 24 hr | 184.5 | 18.9 | 7.4 | 2.3 | 7.0 | 1.7 |
| 47 | 24 hr | 174.5 | 17.9 | 3.9 | 1.2 | 15.0 | 3.6 |
| 35 | 2 wk | 254.8 | 26.2 | 11.4 | 3.6 | 28.5 | 6.9 |
| 40 | 2 wk | 285.7 | 29.3 | 13.9 | 4.4 | 16.0 | 3.9 |
| 34 | 5 wk | 419.2 | 43.0 | 4.4 | 1.4 | 5.7 | 1.4 |
| 41 | 5 wk | 293.8 | 30.2 | 3.0 | 0.9 | 6.9 | 1.7 |
| 38 | 9 wk | 134.5 | 13.8 | 0.7 | 0.2 | 6.3 | 1.5 |
| 39 | 9 wk | 160.4 | 16.3 | 2.5 | 0.8 | 7.6 | 1.8 |
| 44 | 12 wk | 149.4 | 15.3 | 4.7 | 1.5 | 5.8 | 1.4 |
| 48 | 12 wk | 84.2 | 8.6 | 6.5 | 2.1 | 7.5 | 1.8 |

was to replicate our previous results under more standard conditions (32). The longer intervals were chosen to determine the longevity of the various adult-generated cell types.

**Rats.** Five adult male Sprague–Dawley rats were injected with BrdUrd (200 mg/kg). After 3 wk, the rats were deeply anesthetized with Nembutal and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer.

**Histological Procedures.** For monkey brains, 40-$\mu$m sections through the frontal lobe and the temporal lobe (containing the dentate gyrus) were cut on an oscillating tissue slicer. To obtain stereological estimates of the total numbers of BrdUrd-labeled cells (38), tissue was processed immunohistochemically for BrdUrd alone with peroxidase methods. The tissue was heated in 0.1 M citric acid and incubated in 2 M HCl and then overnight in primary mouse anti-BrdUrd (1:250) and 0.5% Tween-20 (1:20) and then for 1 h in biotinylated anti-mouse antibody (1:200) plus normal horse serum (1:100). The sections were then incubated in avidin-biotin-horseradish peroxidase and then in diaminobenzidine. After a final PBS rinse they were mounted onto slides, counter-stained with cresyl violet, and cover-slipped under permount.

For immunofluorescence, the tissue was pretreated for BrdUrd staining followed by incubation in primary rat anti-BrdUrd (1:250) and 0.5% Tween-20 (1:200 and one of the following antisera: NeuN, TuJ1, GFAP, and CNP). Next, it was incubated in biotinylated anti-rat (1:250) and then incubated

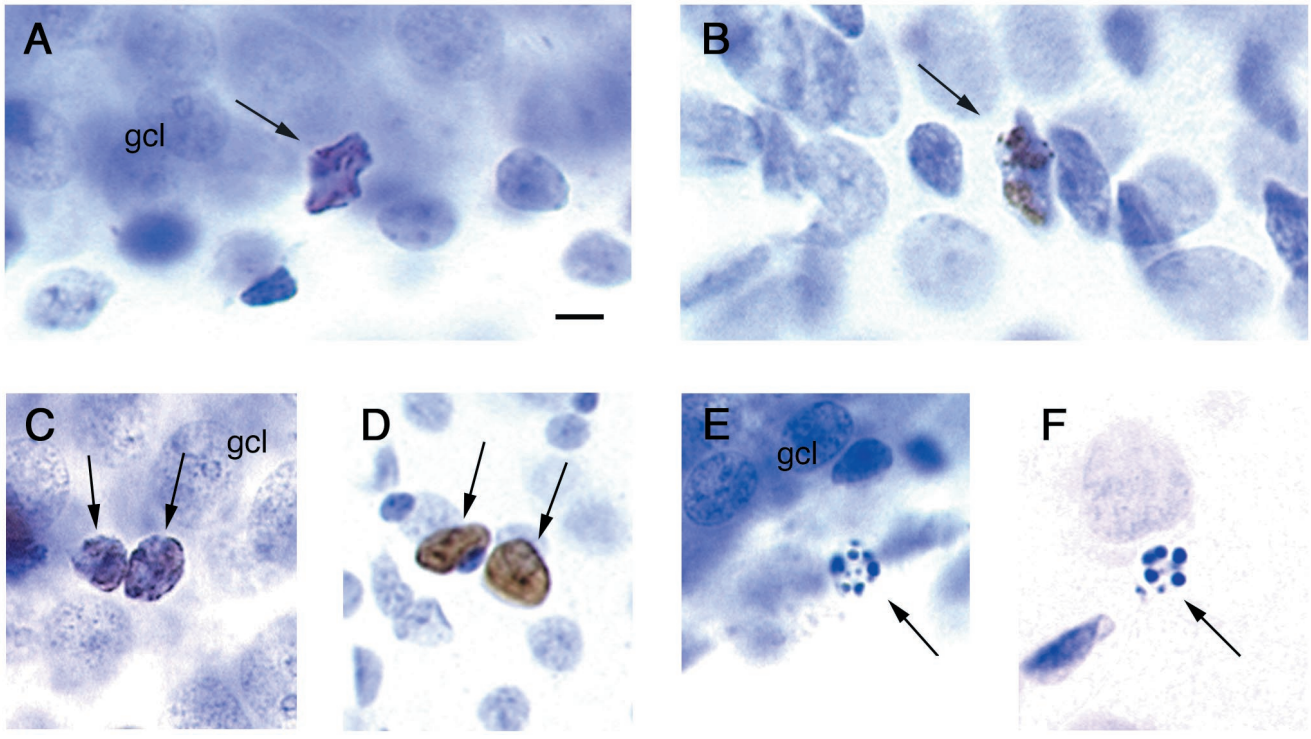


**Fig. 1.** Evidence for new cells and dying cells in adult macaque brains. Light photomicrographs of cresyl violet-stained tissue. (*A* and *B*) BrdUrd-labeled mitotic figures (arrows) in the (*A*) dentate gyrus and (*B*) svz 24 h after BrdUrd injection. Cells appear to be in anaphase. (*C* and *D*) Closely adjacent BrdUrd-labeled cells with the nuclear morphology of neurons (arrows) in (*C*) dentate gyrus and (*D*) prefrontal cortex 2 wk after BrdUrd injection. (*E* and *F*) Pyknotic cells (arrows) in (*E*) dentate gyrus and (*F*) prefrontal cortex. gcl, granule cell layer. Note that the new cells in the dentate gyrus and prefrontal cortex are similar to each other as are the dying cells in the two areas. (Scale bar = 10 $\mu$m.)

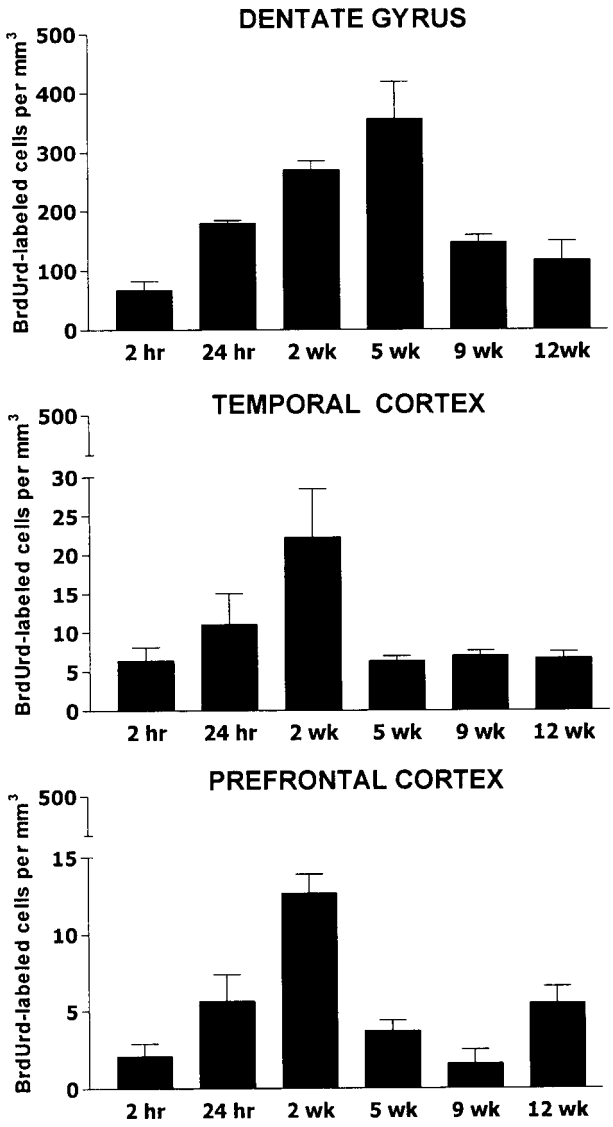


**Fig. 2.** Density of BrdUrd-labeled cells (number of BrdUrd-labeled cells per mm$^3$) in the dentate gyrus (*Top*), inferior temporal cortex (*Middle*), and prefrontal cortex (*Bottom*) at different survival times after a single injection of BrdUrd. There were two adult monkeys at each time point.

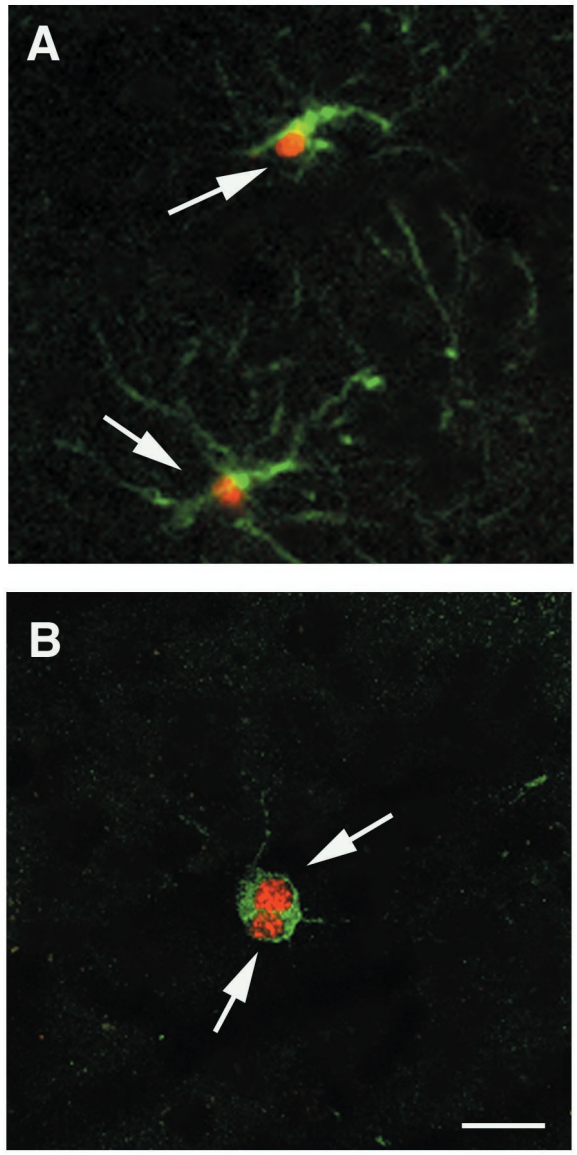


**Fig. 3.** Confocal laser scanning microscope images of glia in prefrontal cortex of a macaque 2 wk after BrdUrd injection. (*A*) Two astrocytes (arrows) double-labeled with BrdUrd (red nuclear stain) and GFAP (green cytoplasmic stain). (*B*) Two adjacent oligodendrocytes double-labeled with BrdUrd (red) and CNP (green cytoplasmic stain). (Scale bar = 5 $\mu$m.)

with streptavidin Alexa 568 (1:1,000) and either goat anti-mouse Alexa 488 (1:250) for NeuN, TuJ1, or CNP or donkey anti-mouse Alexa 488 (1:250) for GFAP.

The rat brains were cut in 40-$\mu$m sections on an oscillating tissue slicer, and the tissue was processed for BrdUrd and NeuN or BrdUrd and TuJ1 with immunofluorescent methods as described above.

**Data Analysis.** A modified version of the stereological optical dissector method (38) with STEREOINVESTIGATOR (MicroBrightField, Colchester, VT) was used on the peroxidase-stained monkey tissue. For every 20th section through the dentate gyrus, the principal sulcus (in prefrontal cortex) and the occipito-temporal sulcus (in inferior temporal cortex), the number of BrdUrd-labeled cells was determined with a Olympus BX-60 light microscope ($\times$1,000). The total volume of the dentate gyrus, both banks of the principal sulcus, and both banks of the occipito-temporal sulcus were estimated with the STEREOINVESTIGATOR program, and the data were expressed as the number of BrdUrd-labeled cells/mm$^3$. Estimates of the total number of cresyl violet-stained cells in each area were obtained by using the optical fractionator method. Immunofluorescent tissue was viewed with a Olympus BX-60 fluorescence microscope, and the percentage of a sample of 15–25 BrdUrd-labeled cells that were positive for each of the cell-type specific markers was determined. A confocal scanning microscope (Zeiss 510 LSM) was used to verify double-labeling with UV, HeNe, and argon lasers. Z-sectioning was carried out at 0.5- to 1.0-$\mu$m intervals, and cells were rotated in orthogonal planes to verify double-labeling.

Rat tissue was analyzed with a confocal microscope for the presence of cells double-labeled with BrdUrd and NeuN or BrdUrd and TuJ1. Labeled cells were rotated in orthogonal planes and z-sectioned at 0.5- to 1-$\mu$m intervals to verify double-labeling. Quantitative analysis was not carried out on this tissue.

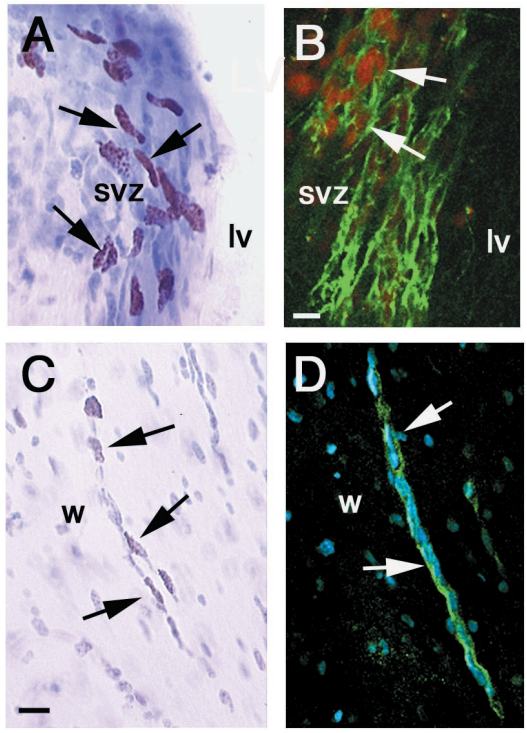


**Fig. 4.** Immature cells that appear to be migrating in the svz and white matter between the lateral ventricle and prefrontal cortex of macaques. (*A*) Light photomicrograph of BrdUrd-labeled cells (arrows) in the svz 2 h post-BrdUrd injection. lv, lateral ventricle. (*B*) Confocal image of cells (arrows) double-labeled with BrdUrd (red) and TuJ1 (green cytoplasmic stain) in the svz 2 h post-BrdUrd injection. (*C*) BrdUrd-labeled cells (arrow) that appear to be migrating in the white matter (w) 2 wk after BrdUrd injection. (*D*) Confocal image of a stream of TuJ1 (green)-positive cells (arrows) that appear to be migrating in the white matter of a prefrontal section. The blue nuclear stain is the DNA dye Hoechst 44323. (Scale bars = 5 $\mu$m.)

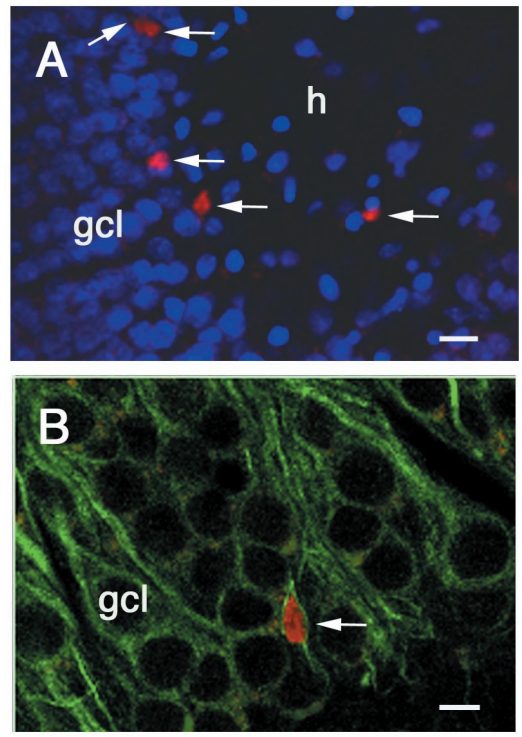


**Fig. 5.** Confocal images of new neurons in dentate gyrus of adult macaques 5 wk after BrdUrd injection. (*A*) Granule cells (arrows) labeled with BrdUrd (red). Other granule cells are labeled with the DNA dye Hoechst 44323 (blue). (*B*) Granule cell double-labeled with BrdUrd (red) and TuJ1 (green). [Scale bars = 20 $\mu$m (*A*) and 5 $\mu$m (*B*).]

## Results

**Number and Location of BrdUrd-Labeled Cells.** After a single dose of BrdUrd, labeled cells were seen in the dentate gyrus, prefrontal cortex, and inferior temporal cortex of all of the brains at each postinjection survival time (Table 1, Fig. 1). Other brain areas were not systematically examined in this study. The density of labeled cells varied with the brain site and the postinjection interval (Fig. 2; two factor ANOVA with repeated measures on one factor, df = 2, $F = 200$, $P < 0.0001$ for site; df = 5, $F = 12.6$, $P < 0.004$ for interval). The dentate gyrus had a density of labeled cells ≈20 times that of the temporal cortex, which had almost twice the density of the prefrontal cortex, (matched *t* test, $P < 0.0001$, $P < 0.006$, respectively). A similar statistical picture emerged when the ratio of the density of BrdUrd-labeled cells to the density of total cells was used to compare the three brain areas at each of the postinjection intervals (Table 1).

For all three sites, the density of labeled cells increased from 2 h to 2 wk postinjection and then fell after 5 wk for the dentate gyrus and after 2 wk for temporal and frontal cortex (Fig. 2). The density fell to an apparently asymptotic level by 9 wk postinjection for the dentate gyrus and 5 wk for the other areas. In all three brain regions, mitotic figures and pyknotic cells, some of which were BrdUrd-labeled, were observed (Fig. 1). After the longer postinjection intervals, in all three areas there were many pairs of closely adjacent BrdUrd-labeled cells that had the morphological characteristics of either neurons (Fig. 1 *C* and *D*) or glia (Fig. 3).

BrdUrd-labeled cells also were observed in the subventricular zone (svz) in the frontal and temporal sections at the 2-h, 24-h, and 2-wk time points but were rare in this location at later time points (Figs. 1 and 4). In the dentate gyrus, at the 2-h time point, the majority of the BrdUrd-labeled cells were in the hilus or subgranular zone rather than the granule cell layer. Many of them were located in clusters at this time point. The number of labeled cells in the hilus rose in the first 24 h after BrdUrd injection, presumably as the result of mitosis of the originally labeled population; it then decreased by 2 wk, whereas the number of labeled cells in the granule cell layer increased (Fig. 5).

**Expression of Neuron-Specific Markers.** At 24 h after the BrdUrd injection, new cells expressing TuJ1, a marker for both immature and mature neurons, were found in the svz and in the white matter of frontal and temporal sections (Fig. 4) where they were sometimes arrayed along blood vessels. In addition, at this postinjection time, in each animal in each of the three brain regions a small number of cells were found colabeled for BrdUrd and TuJ1 (Table 2). This proportion became larger with increasing time after BrdUrd injection (Fig. 5).

In all three areas, the proportion of new cells that were double-labeled for BrdUrd and TuJ1 increased from 24 h to 2 wk after the BrdUrd injection and remained about the same through the 9-wk postinjection. The proportion of BrdUrd and TuJ1 double-labeled cells was similar in the three areas at the postinjection intervals examined (Table 2).

At both 2 wk and 9 wk postinjection, approximately half of the BrdUrd-labeled cells sampled in the dentate gyrus expressed NeuN, a marker for mature neurons. In the neocortical areas at both postinjection times the proportion was less, approximately one-quarter (Table 2). Colabeling with BrdUrd and NeuN was verified by rotating cells in orthogonal planes (Fig. 6) and by

**Table 2. Percent cells colabeled with BrdUrd and cell-specific marker**

| Animal no. | Postinjection survival time | Dentate gyrus | | | | Prefrontal | | | | Temporal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NeuN | TuJ1 | CNP | GFAP | NeuN | TuJ1 | CNP | GFAP | NeuN | TuJ1 | CNP | GFAP |
| | | Percentage colabeled with BrdUrd (sample $n = 25$) | | | | | | | | | | | |
| 35 | 2 wk | 40 | 32 | 33* | 8 | 15 | 38 | 16 | 24 | 16 | 40 | 33* | 0 |
| 40 | 2 wk | 64 | 26* | 20 | 4 | 20 | 32 | 24 | 24 | 20 | 28 | 4 | 4 |
| 34 | 5 wk | — | 50 | — | — | — | — | — | — | — | — | — | — |
| 41 | 5 wk | — | 48 | — | — | — | — | — | — | — | — | — | — |
| 38 | 9 wk | 52 | 68 | 12 | 4 | 12 | 15 | 27 | 8 | 40 | 20 | 24 | 0 |
| 39 | 9 wk | 84 | 36 | 12 | 16 | 36 | 28 | 12 | 12 | 28 | 44 | 16 | 0 |

*Based on a sample $14 < n < 25$.

z-sectioning at 0.5- to 1-$\mu$m intervals (Figs. 6 and 7). Rarely, a BrdUrd cell was observed in such close proximity to the nucleus of a NeuN-positive cell that the two cells might have been confused for a single double-labeled cell if the pair had not been examined throughout their extents (Fig. 7).

**Expression of Glial Markers.** At 2- and 5-wk postinjection times some of the new cells expressed GFAP, a marker for astrocytes (Fig. 3*A*), or CNP, a marker for oligodendrocytes (Fig. 3*B*). The percentages of BrdUrd-labeled cells that expressed either of these markers were similar across the three brain regions at both these postinjection times (Table 2).

**Neurogenesis in the Rat.** We found cells colabeled with BrdUrd and NeuN (Fig. 8) or BrdUrd and TuJ1 in the anterior neocortex as well as in the dentate gyrus of the rat. Although quantitative analyses were not carried out on this tissue, it was clear that many fewer new cells with neuronal staining characteristics were located in the neocortex than in the dentate gyrus.

## Discussion

The present results confirm our previous reports of neurogenesis in the dentate gyrus, prefrontal cortex, and inferior temporal cortex of adult macaques (26, 32). However, in the previous studies the animals were heterogeneous in age, sex, species,

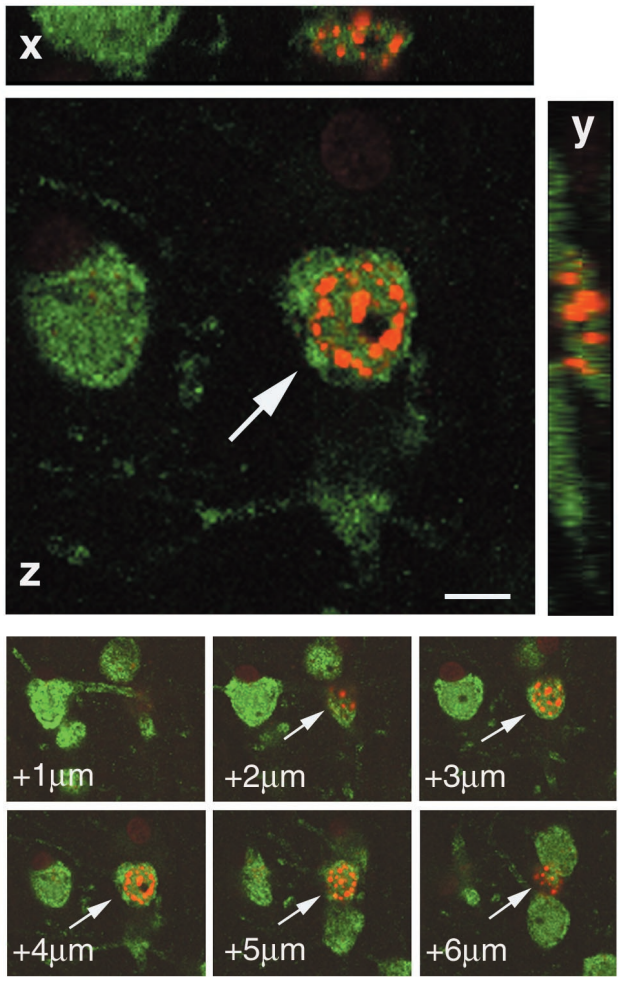


**Fig. 6.** Confocal image of a cell in prefrontal cortex of a macaque double-labeled with NeuN (green nuclear and cytoplasmic stain) and BrdUrd (red) rotated in orthogonal planes (*x*,*y*,*z*) and z-sectioned to verify double-labeling throughout its extent. Note the double-labeled cell begins at +2 $\mu$m. The monkey was perfused 2 wk after the BrdUrd injection. (Scale bar = 5 $\mu$m.)

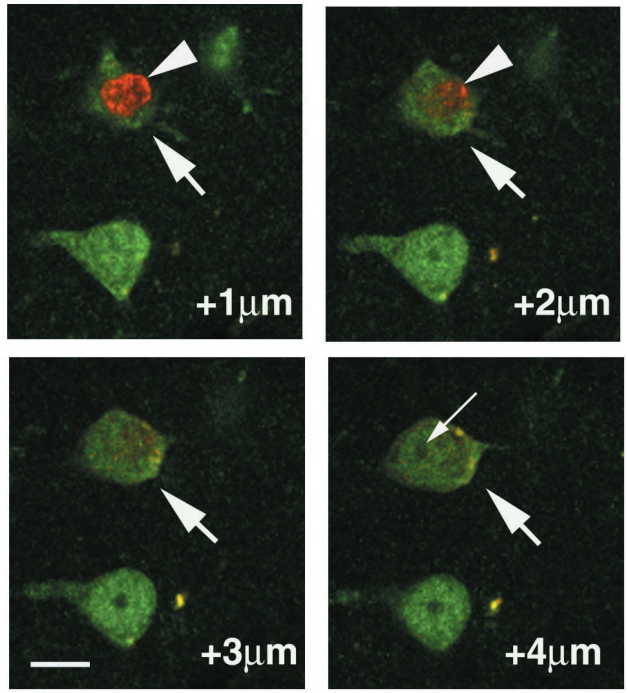


**Fig. 7.** Confocal z-sections through two closely adjacent cells in prefrontal cortex of a macaque 2 wk after BrdUrd injection. The neuron (arrow) is labeled with NeuN (green), and the satellite cell (arrowhead) is labeled with BrdUrd (red). If only the +1-$\mu$m and +2-$\mu$m sections were examined they might have been incorrectly interpreted as containing a cell double-labeled with NeuN and BrdUrd. The nucleolus (thin arrow) in a section that does not have BrdUrd labeling indicates that these are two separate cells. (Scale bar = 10 $\mu$m.)

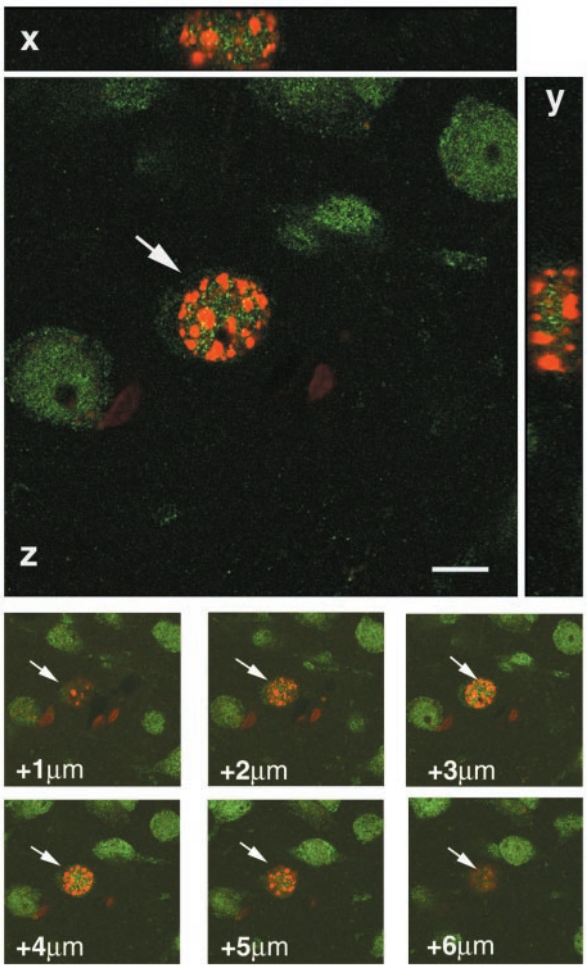


**Fig. 8.** Confocal image of a cell in anterior cortex of an adult rat double-labeled with NeuN (green) and BrdUrd (red) rotated in orthogonal planes and z-sectioned to verify double-labeling throughout its extent. The rat was perfused 3 wk after the BrdUrd injection. (Scale bar = 5 $\mu$m.)

experience, and the number of BrdUrd injections they received, making differences among them difficult to interpret.

**Evidence for Adult-Generated Cells.** The finding of BrdUrd-labeled cells in the dentate gyrus and regions of the neocortex indicated the presence of adult-generated cells in these structures. It is likely that the cells labeled with BrdUrd did so in preparation for division rather than while undergoing DNA repair for two reasons. First, the number of BrdUrd-labeled cells in the dentate gyrus and neocortex increased between 2 h and 24 h and then further by 2 wk. Second, BrdUrd-labeled cells did not show the characteristics of mature neurons until 2 wk or more after the BrdUrd injection. These data imply expansion of the initially labeled population by division and subsequent differentiation into neurons.

Probably because of the known deleterious effects of BrdUrd on embryonic and neonatal rats, most investigators have been conservative in the doses they used in studying neurogenesis in the dentate gyrus of adult animals, the modal dose being 50 mg/kg (e.g., refs. 19, 27, and 39–45). Recently, Cameron and McKay (17) systematically studied the effect of higher doses in adult rats. They found that doses up to 600 mg/kg did not have adverse effects on the animals or their labeled cells. Furthermore, the number of new cells labeled in the dentate gyrus increased markedly between doses of 50 mg/kg and 300 mg/kg. At the latter dose they estimated that 9,000 new cells were added to the dentate gyrus every day, most of which became neurons. Although little is known about the similarity of rats and macaques in the clearance of BrdUrd and the ability of BrdUrd to cross the blood-brain barrier, these results raise the possibility that our count of new adult-generated cells in the macaque dentate gyrus and neocortex may be an underestimate.

**Evidence for the Phenotype of Adult-Generated Cells.** As in our previous studies of neurogenesis in the adult macaque (26, 32) we found new cells in all three areas that expressed the marker for astroglia, GFAP. In addition, there were new cells in each region that expressed the marker for oligodendrocytes, CNP. Some cells expressed neither of these glia markers nor the two markers specific for neurons, NeuN and TuJ1. This residual population could have been progenitor cells, neurons, or glia that did not stain for these markers or undifferentiated cells that eventually would become neurons or glia. At all postinjection times examined, a larger proportion of the sample of new cells expressed a marker for neurons than for glia.

In our previous study of neurogenesis in the adult macaque hippocampus (26), we proposed that many of the adult-generated cells labeled with BrdUrd were neurons because (*i*) they had the light microscopic characteristic of neurons, (*ii*) they expressed a marker for mature neurons [NeuN, NSE (neuron-specific enolase) or the calcium-binding protein calbindin] or the marker for immature neurons TOAD-64 (turned-on-after-division 64-kDa protein) now known as TUC-4, and (*iii*) they did not express GFAP, a marker for astroglia.

Similarly, in our previous study of neocortical neurogenesis in the adult macaque (32), we argued that many of the adult-generated neocortical cells labeled with BrdUrd were neurons because (*i*) they had the light microscope characteristic of neurons, (*ii*) they expressed a marker for mature neurons [NeuN, NSE, or MAP-2 (microtubule-associated protein-2)] or the marker for immature neurons now known as TUC-4, (*iii*) they could be filled with the retrograde tracers Fast Blue and Fluoro-Emerald injected into local targets, and (*iv*) they did not express GFAP, a marker for astroglia.

In the present study we added two lines of evidence that some of the adult-generated cells in the dentate gyrus and neocortex were neurons. First, they expressed the marker for immature and mature neurons, TuJ1. Second, they did not express the marker for oligodendrocytes, CNP.

**Site of Origin of Adult-Generated Cells.** In the dentate gyrus, the distribution of BrdUrd-labeled cells at different time points after injection strongly suggests that the new cells originate from the hilus or subgranular zone. These new cells appear to arise from progenitor cells located in these regions and move the short distance to the granule cell layer where they differentiate into neurons. Although little is known of the progenitor population in the dentate gyrus, it may be significant that cells with radial glial morphology have been observed in this region. These cells, which stain for vimentin or GFAP, have irregularly shaped cell bodies in the subgranular zone and processes that extend through the granule cell layer (46, 47). Because radial glial cells recently have been implicated as cortical progenitor cells in the ventricular zone during gestation (48), these cells may serve as precursors to immature neurons in the adult dentate gyrus as well.

The site of origin of adult-generated neocortical cells is less clear. One possibility is the svz, the source of new neurons that travel through the rostral migratory stream to the olfactory bulb in the monkey (26, 49, 50) as well as rat (51–53). We previously reported observing adult-generated cells in the svz and white matter of frontal sections. Some of these cells were fusiform in shape, and those that were TUC-4-positive had leading and

trailing processes characteristic of migrating cells (54). We raised the possibility that these may have been immature neurons generated in the svz and migrating through the white matter to the cerebral cortex. In the present study we obtained additional evidence for this possibility: some of the BrdUrd-labeled cells in the white matter between the lateral ventricle and cortex expressed the marker for immature and mature neurons, TuJ1. Others expressed the marker for oligodendrocytes, CNP.

In the three areas examined we also found examples of pairs of BrdUrd-labeled cells that had the characteristics of either neurons or glia. This observation raises the possibility that cells may arise from division in neocortex itself as well as migration from the svz. This hypothesis is supported by the increase in BrdUrd-labeled cells between 2 and 24 h postinjection, too short a period for migration of the new cells from the svz to cortex. Furthermore, in all three brain areas, we observed a few BrdUrd-labeled cells that expressed TuJ1 in the 24-h postinjection group. This observation indicates that new neurons as well as glia may have been generated locally as well as in the svz.

**Longevity of Adult-Generated Cells.** There was a decline in the number of BrdUrd-labeled cells between 5 and 9 wk in the dentate gyrus and between 2 and 9 wk in the neocortical areas. This change may reflect either death of the new cells or dilution of the BrdUrd label as a result of continuing proliferation of the labeled cells. It is likely that at least some of the decrease in the number of new cells reflects cell death because pyknotic cells, both BrdUrd-labeled and not, were observed in all three regions (Fig. 1 *E* and *F*). Furthermore, fewer BrdUrd-labeled cells that expressed neuronal markers were found with increasing postinjection survival times. Because cells that express a mature neuronal marker are unlikely to continue dividing, label dilution is not a likely explanation for their decrease. There was no further decline between 9 and 12 wk. One interpretation of this pattern is that by 9 wk many of the new cells have died and a fixed but smaller percentage of them continue to survive. The long-term survival of at least some adult-generated neurons is suggested by the findings of Eriksson *et al.* (28) of cells in the human hippocampus double-labeled with BrdUrd and a neuronal marker many months after BrdUrd injection.

**Are Primates Unique?** Adult neurogenesis in the hippocampus appears to be qualitatively similar in the rat and macaque. In both cases new cells arise in the hilus and subgranular zone and migrate to the granule cell layer. It is difficult to make a quantitative comparison between the species, however. Not only are the doses and number of injections often different, but, more important, nothing is known of the relative bioavailability of BrdUrd or its ability to cross the blood-brain barrier and be available for uptake by dividing cells in the two species.

As mentioned in the Introduction, there have been early reports of adult-generated neurons in the neocortex of rats that used thymidine labeling (5, 29–31). Using higher than usual doses of BrdUrd (i.e., 200 mg/kg) we found a few cells in the anterior neocortex of adult rats double-labeled with BrdUrd and NeuN or BrdUrd and TuJ1, thereby confirming these results. As in the macaque monkeys there were many fewer new cells with neuronal characteristics in the neocortex than in the dentate gyrus.

**Environmental Effects on Adult-Generated Cells.** In rodents and birds experience in an "enriched environment" (i.e., relatively less deprived than the usual laboratory housing) enhances neurogenesis and the survival of adult-generated hippocampal neurons (21, 39, 40, 55). This could be caused by a number of factors such as the increased intensity and variety of visual, auditory, tactile, social, and sexual stimulation, increased locomotor activity, and increased opportunities for learning. Furthermore, specific experience on hippocampal-dependent learning tasks increases the number of adult-generated hippocampal neurons (18, 56, 57). These effects of experience might be true of macaques as well. All of the monkeys in the present study were individually housed in stainless steel cages. Although they were exposed to an enrichment program, their environments remained exceedingly deprived relative to a free-ranging life. Perhaps this contributed to the short longevity of a majority of the adult-generated cells. If so, animals of the same species, age, and sex living in a more natural environment would be expected to have a higher proportion of long-surviving adult-generated cells.

**Functions of Adult-Generated Neurons.** As Altman (58) and Nottebohm (20, 21) proposed some time ago and we have discussed in detail elsewhere (22, 23), postnatally generated neurons may play a role in learning and memory. Several lines of evidence support this view. First, new neurons are added to structures important for learning and memory such as the dentate gyrus, the higher vocal center in birds, and primate association neocortex (1, 20, 32). Second, several conditions that decrease dentate neurogenesis impair hippocampal-dependent learning such as stress and increased level of glucocortocoids (22, 59). Third, several conditions that enhance dentate neurogenesis also enhance hippocampal-dependent learning such as increased estrogen level and increased environmental complexity (22). Fourth, specific learning experience on hippocampal-dependent tasks increases the number of new hippocampal cells (18, 56, 57). Fifth, reduction of neurogenesis in the rodent dentate gyrus with a toxin for proliferating cells (methylazoxymethanol acetate, MAM) has a specific deleterious effect on hippocampal-dependent learning (24).

Adult-generated neurons may posses some properties similar to those found in embryonic and early postnatal neurons that could make them particularly well suited to play a role in learning and memory (22, 23). For example, during development new neurons extend axons even during migration (60). Some evidence suggests that this is the case for adult-generated neurons as well (16). Developing hippocampal granule cells show long-term potentiation of greater duration than mature granule cells (61). Furthermore, granule cells that are thought to have been generated in adulthood have a lower threshold for long-term potentiation induction and produce greater short-term facilitation (62, 63). Finally, as Nottebohm (20, 21) and we (23) have proposed, it is the transient nature of most adult-generated neurons that may make them appropriate to play a role in some of the processes that underlie the formation of memories.

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