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DIFFERENTIATION OF NEWLY BORN NEURONS AND GLIA IN THE DENTATE GYRUS OF THE ADULT RAT

H. A. CAMERON,* C. S. WOOLLEY, B. S. MCEWEN and E. GOULD

Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, U.S.A.

Abstract—In order to determine whether newly born cells in the dentate gyrus of the adult rat express the neuronal marker, neuron-specific enolase, or the glial marker, glial fibrillary acidic protein, we performed combined immunohistochemistry and autoradiography on brains from adult rats perfused at various times ranging from 1 h to four weeks following [^{3}H]thymidine administration. Light-microscopic examination revealed a negligible number of [^{3}H]thymidine-labeled cells showing neuron-specific enolase immunoreactivity during mitosis. However, by two weeks after [^{3}H]thymidine administration, a significant increase in the density of [^{3}H]thymidine-labeled neuron-specific enolase-immunoreactive cells was detected. Three weeks following [^{3}H]thymidine injection the majority of [^{3}H]thymidine-labeled cells (> 70%) were immunoreactive for the neuronal marker. At the four-week time-point, [^{3}H]thymidine-labeled neuron-specific enolase-immunoreactive cells were indistinguishable from neighboring granule cells. In contrast, glial fibrillary acidic protein immunoreactivity was observed in a small but significant number of [^{3}H]thymidine cells at the 1-h time-point and the proportion of labeled cells that were immunoreactive for this cell marker did not increase with time. [^{3}H]Thymidine-labeled cells that were immunoreactive for glial fibrillary acidic protein typically showed morphologic characteristics of radial glia at all time-points. At the 1-h time-point, the majority of [^{3}H]thymidine-labeled cells were observed in the hilus (> 60%) with the remainder being located in the granule cell layer. However, with a four-week survival-time most [^{3}H]thymidine-labeled cells (> 85%) were located in the granule cell layer.

The majority of newly born cells in the adult dentate gyrus differentiate into neurons. Some of these cells are born locally in the granule cell layer while others arise from the hilus and migrate to the granule cell layer. The presence of [^{3}H]thymidine-labeled presumptive radial glia observed in the adult dentate gyrus supports the contention that many of the newly born neurons migrate. These results set the stage for future studies aimed at determining the factors that regulate these processes.

In most regions of the mammalian brain, cell birth and migration occur only during a discrete embryonic period. The rat dentate gyrus is unusual in that the majority of its neurons (at least 85%) are born postnatally.[5,23] During the first two postnatal weeks, dentate gyrus granule cells arise from the hilus and migrate, presumably along radial glial fibers, to reside in the granule cell layer.[22,23] Following this period of maximal cell birth, this process continues at a much lower but significant level until at least 11 months postnatally.[12] Although it is clear that some newly born cells in the adult rat dentate gyrus are glia,[11–13] the following evidence supports the contention that a substantial proportion of these cells are neurons: (i) light-microscopic analysis of Nissl-stained brain sections has shown that the number of granule neurons increases in adulthood[7] (but see Ref. 8); (ii) light-microscopic analysis of [^{3}H]thymidine-labeled dentate gyrus cells has identified these cells as granule neurons on the basis of morphological characteristics and their location in the granule cell layer;[3,6] and (iii) electron-microscopic studies have identified [^{3}H]thymidine-labeled cells as neurons on the basis of their ultrastructural characteristics, including the presence of synapses on their cell bodies and dendrites.[12,13]

Although the occurrence of neurogenesis in the adult dentate gyrus has been demonstrated by these studies, several gaps in our knowledge of this phenomenon have impeded progress toward understanding its regulation (see Ref. 11). First, it is not known what proportion of newly born cells in the adult dentate gyrus become neurons. Second, detailed information regarding the birthplace, migration and timing of differentiation of newly born cells in the adult dentate gyrus is not available. In order to positively identify newly born cells in the adult dentate gyrus and to determine whether these cells migrate and survive, we performed a survival time-course of [^{3}H]thymidine autoradiography combined with immunohistochemistry for the neuronal marker neuron-specific enolase (NSE) and the glial marker glial fibrillary acidic protein (GFAP) in brains of adult rats.

*To whom correspondence should be addressed.

Abbreviations: GCL, granule cell layer; GFAP, glial fibrillary acidic protein; NSE, neuron-specific enolase; PBS, phosphate-buffered saline.

EXPERIMENTAL PROCEDURES

Animal treatments and histology

Eighteen adult (four month) male Sprague–Dawley rats (Charles River) were injected intraperitoneally with

5.0 μCi/g body weight [^{3}H]methyl thymidine in water; three at each of six time-points (1, 24 h, one, two, three or four weeks) prior to perfusion. The 1-h time-point was selected to determine the location of the progenitor population; 1 h is sufficient time for uptake of [^{3}H]thymidine by dividing cells but not for the completion of mitosis[15,20] or presumably migration. During development, the cell cycle of dentate gyrus granule cells is thought to be approximately 16 h.[19] Since cell cycles typically lengthen with age,[4,25] the 24-h time-point probably represents a time when [^{3}H]thymidine-labeled cells have divided once.

After the appropriate survival time, the rats were deeply anesthetized and transcardially perfused with 120 ml 4.0% paraformaldehyde in 0.1 M phosphate buffer with 1.5% (v/v) picric acid. The brains were dissected from the skulls and processed for combined immunohistochemistry, autoradiography and Nissl staining as described previously.[11] Briefly, following an overnight postfixation in the perfusate, the brains were cut in the coronal plane on a Vibratome (40 μm) into a bath of phosphate-buffered saline (PBS). The sections were rinsed in PBS and incubated for 24 h in a solution containing either polyclonal antisera to NSE (Polysciences, diluted 1:2000 in PBS) or monoclonal antibodies to GFAP (Boehringer Mannheim, diluted 1:50 in PBS). Previous studies have shown that in the brain, NSE is expressed exclusively by neurons,[17,24] while GFAP is expressed exclusively by glia.[9] The sections were rinsed in PBS and incubated for 2 h in a solution of biotinylated secondary antibodies in PBS directed against the appropriate species (anti-rabbit for NSE, anti-mouse for GFAP). Following this, the sections were rinsed again and incubated for 2 h in a solution of avidin–biotin–horseradish peroxidase in PBS. The sections were rinsed again and reacted with diaminobenzidine and hydrogen peroxide in PBS for 15 min. The sections were rinsed again, mounted on to gelatinized slides, dried, rinsed in distilled water, dried, dipped in photographic emulsion (NTB-2, Kodak) and stored in the dark at 4°C for four weeks. The slides were then developed in Dektol (Kodak), rinsed in distilled water, fixed in Ektaflo (Kodak), rinsed again, stained for Nissl using Cresyl Violet and coverslipped under Permount. Control sections were processed as described above with omission of the primary antisera and revealed no nonspecific staining of secondary antibodies.

Data analysis

The slides were coded prior to analysis and the code was not broken until the analysis was complete. Sections through the middle of the dentate gyrus, where the suprapyramidal and infrapyramidal blades are joined at the crest and the hippocampus is oriented horizontally beneath the corpus callosum, were selected for analysis. At least six sections were analysed per brain. For each side of each selected section, the numbers of [^{3}H]thymidine-labeled NSE-immunoreactive and non-NSE-immunoreactive or GFAP-immunoreactive and non-GFAP-immunoreactive cells were counted in the granule cell layer (GCL) and hilus. A cell was considered labeled if it had five or more silver grains over its nucleus (this value is $>20\times$ the background level). The cross-sectional areas of the GCL and the hilus were determined using a camera lucida drawing tube and a Zeiss Interactive Digitizing Analysis System. These data were expressed as densities (number of cells per 10^6 μm^2) as well as percentages of [^{3}H]thymidine-labeled cells that were NSE-immunoreactive and non-NSE-immunoreactive or GFAP-immunoreactive and non-GFAP-immunoreactive. In addition, densities and percentages were calculated for [^{3}H]thymidine-labeled cells categorized according to location in the hilus and GCL. The number of silver grains/cell was also determined for both the hilus and GCL of each brain. Means of these variables were obtained for each brain and these data were subjected to one-way ANOVA with Tukey HSD *post hoc* comparisons.

RESULTS

Location and number of [^{3}H]thymidine-labeled cells

Following a single dose of [^{3}H]thymidine, labeled cells were seen in the dentate gyrus of every brain examined. Quantitative analysis revealed significant differences in the overall density of [^{3}H]thymidine-labeled cells with time following injection ($P < 0.01$; Fig. 1). The density of [^{3}H]thymidine-labeled cells significantly increased from 1 h to one week following injection ($P < 0.01$). However, between one and two weeks following [^{3}H]thymidine injection, a significant decrease in the density of these cells occurred (Fig. 1).

At all time-points examined, [^{3}H]thymidine-labeled cells were observed both in the hilus and the GCL. [^{3}H]Thymidine-labeled cells located in the hilus were distributed throughout this region with some residing near the polymorph layer and others near the GCL. [^{3}H]Thymidine-labeled cells located in the GCL were also distributed throughout this region with no obvious preferential locations in suprapyramidal/infrapyramidal or medial/lateral planes. [^{3}H]Thymidine-labeled cells in the GCL, however, were preferentially located in the deep aspect of the layer although some of these cells were located superficially at all time points.

Quantitative analysis of the percentages of the total number of [^{3}H]thymidine-labeled cells that were located in each of these two regions showed significant changes with time ($P < 0.01$; Fig. 2); the percentage of [^{3}H]thymidine-labeled cells in the hilus decreased whereas the percentage of [^{3}H]thymidine-labeled cells in the GCL increased between 1 h and one week following injection. From one to four weeks, the relationship between the density of [^{3}H]thymidine-labeled cells in the hilus and the density of [^{3}H]thymidine-labeled cells in the GCL did not change; i.e. more labeled cells were always located in

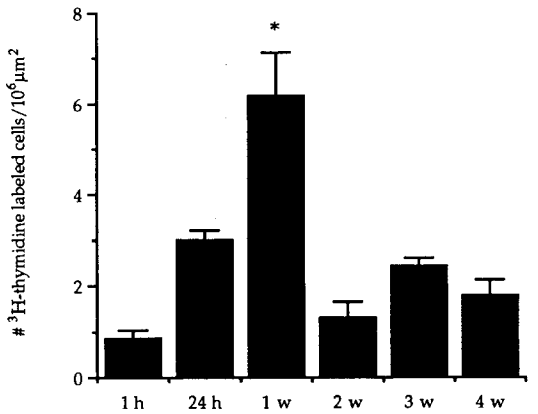

Fig. 1. Density of [^{3}H]thymidine-labeled cells at different survival intervals following [^{3}H]thymidine injection. Bars represent mean + S.E.M. The one-week (w) group was significantly different from the 1-h, two-, three- and four-week groups; asterisk indicates significant difference, $P < 0.05$.

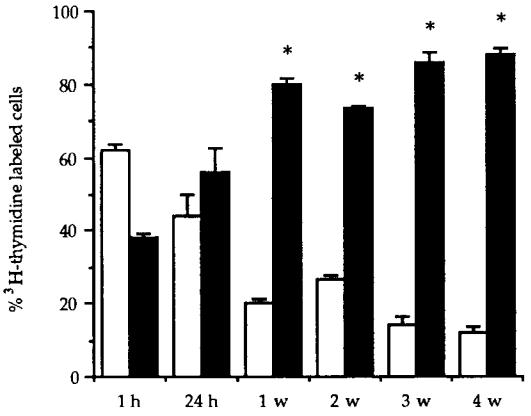

Fig. 2. Percentage of all [^{3}H]thymidine-labeled cells which are located in the hilus (open bar) and GCL (solid bar) at different survival intervals following [^{3}H]thymidine administration. Bars represent mean + S.E.M. Asterisks indicate significant difference from 1 h; $P < 0.01$. w, week.

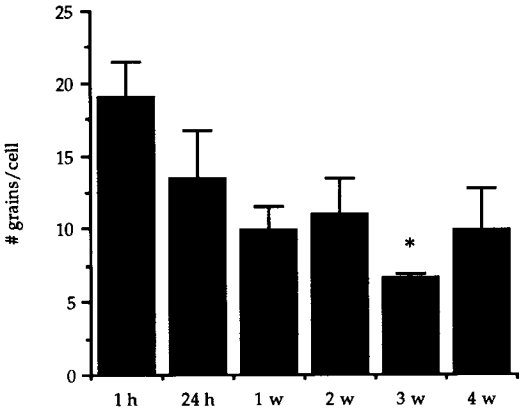

Fig. 4. Number of silver grains/[^{3}H]thymidine-labeled cell in the GCL at different survival times following [^{3}H]thymidine injection. Bars represent mean + S.E.M. Asterisk represents significant difference from 1 h; $P < 0.05$. w, week.

the GCL than in the hilus at these later time-points (Figs 2, 3).

The number of silver grains per [^{3}H]thymidine-labeled cell showed a significant decrease between 1 h and three weeks in the dentate gyrus ($P < 0.05$). Thereafter, no significant changes in the number of silver grains were observed. This difference occurred in the GCL; the number of silver grains/cell dropped significantly in this region ($P < 0.05$) (Fig. 4) and not in the hilus ($P > 0.1$).

Expression of neuron-specific enolase in [^{3}H]thymidine-labeled cells

Light-microscopic examination of sections processed for NSE-immunohistochemistry revealed moderate to intense staining of neurons in the GCL of the dentate gyrus. Although nearly all cells in the GCL expressed NSE, there were some cells, both with and without granule cell morphology, that were not immunoreactive for this neuronal marker. In general, [^{3}H]thymidine-labeled NSE-immunoreactive cells showed granule cell morphologies, i.e. medium-sized round or oval cell bodies (Fig. 5). The [^{3}H]thymidine-labeled non-immunoreactive cells showed varying morphologies; some cells were small with irregular shapes and others showed characteristic granule cell morphology (Fig. 5).

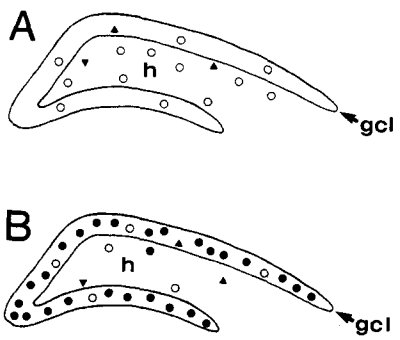

Fig. 3. Templates mapping the distribution of [^{3}H]thymidine-labeled cells in the hilus and GCL 1 h (A) and four weeks (B) following [^{3}H]thymidine administration. Values represent mean number of cells per brain (seven sections). Open circles represent non-immunoreactive [^{3}H]thymidine-labeled cells; solid circles represent NSE-immunoreactive [^{3}H]thymidine-labeled cells; solid triangles represent GFAP-immunoreactive [^{3}H]thymidine cells. gcl, granule cell layer; h, hilus.

Both the density and percentage of [^{3}H]thymidine-labeled cells that were NSE-immunoreactive increased with time ($P < 0.01$). At 1 h following [^{3}H]thymidine injection, a single [^{3}H]thymidine-labeled cell with weak NSE immunoreactivity was observed in the GCL. At later time-points following [^{3}H]thymidine injection the percentage of [^{3}H]thymidine-labeled cells that were NSE-immunoreactive increased ($P < 0.01$). Whereas at 24 h following injection fewer than 10% of the [^{3}H]thymidine-labeled cells were NSE-immunoreactive, at the three-week time-point, the majority of [^{3}H]thymidine-labeled cells (70%) were NSE-immunoreactive (Fig. 6). At four weeks following [^{3}H]thymidine injection, the majority of [^{3}H]thymidine-labeled NSE-immunoreactive cells were virtually indistinguishable from neighboring granule neurons. The vast majority of [^{3}H]thymidine-labeled NSE-immunoreactive cells (92%) were located in the GCL (Fig. 3). No significant change was observed in the ratio of NSE-immunoreactive [^{3}H]thymidine-labeled cells located in the hilus vs the GCL with time following injection. Very few heavily labeled NSE-immunoreactive cells were observed; NSE-immunoreactive cells typically showed fewer than 15 silver grains.

Expression of glial fibrillary acidic protein in [^{3}H]thymidine-labeled cells

In contrast to the staining pattern observed for NSE immunohistochemistry, GFAP immunostaining in the dentate gyrus was observed primarily outside the GCL. GFAP-immunoreactive cells in the dentate

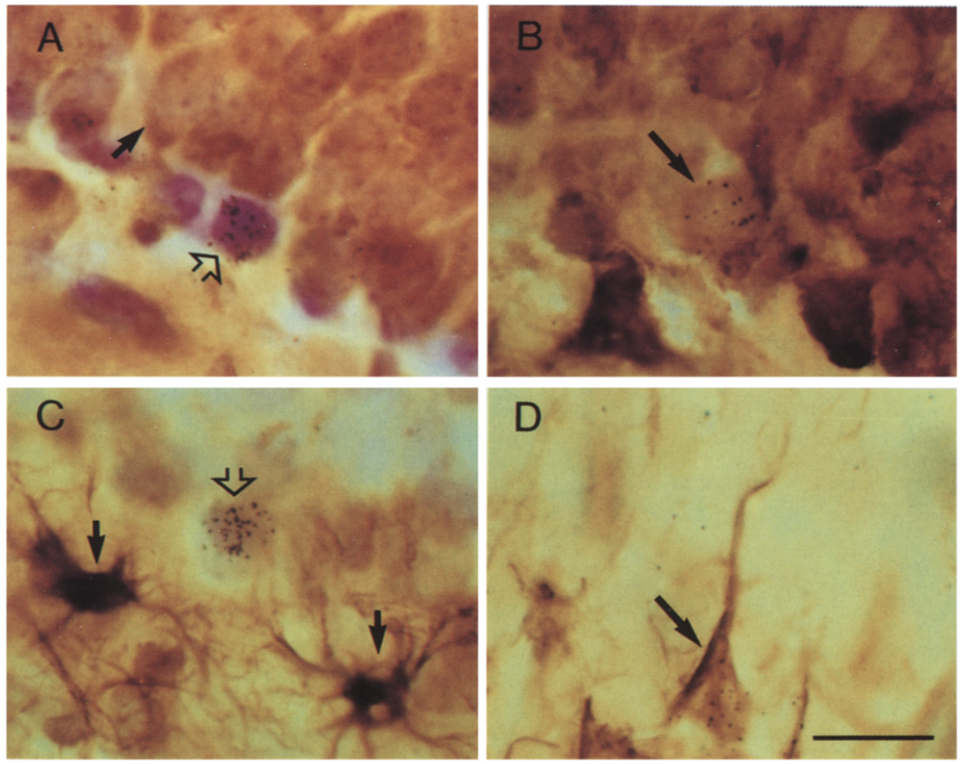

Fig. 5. Photomicrographs of [^{3}H]thymidine-labeled cells that are non-NSE-immunoreactive (A, open arrow), NSE-immunoreactive (B, long arrow), non-GFAP-immunoreactive (C, open arrow) and GFAP-immunoreactive (D, long arrow) in the dentate gyrus. Note the granule neuron morphology of [^{3}H]thymidine-labeled cells in A–C and the radial glial morphology of the [^{3}H]thymidine-labeled cell in D. NSE-immunoreactive non-[^{3}H]thymidine-labeled neuron in A and GFAP-immunoreactive non-[^{3}H]thymidine-labeled astrocytes in C are indicated by short arrows. Scale bar in D = 40 μm and applies to all frames.

gyrus were located mainly in the hilus and the molecular layer; the majority of these cells showed the morphology of mature astrocytes, i.e. small cell bodies with stellate processes. Most of GFAP-immunoreactive cells of the GCL and subgranular zone (the region between the GCL and hilus) showed the characteristic appearance of radial glia, i.e. triangular cell bodies with thin radial processes. The vast majority of [^{3}H]thymidine-labeled GFAP-immunoreactive cells showed the morphology of radial glial cells (Fig. 5). The [^{3}H]thymidine-labeled non-immunoreactive cells observed in GFAP-immunostained sections showed varying morphologies; some cells were small with irregular shapes whereas others had medium-sized round or oval somata (Fig. 5).

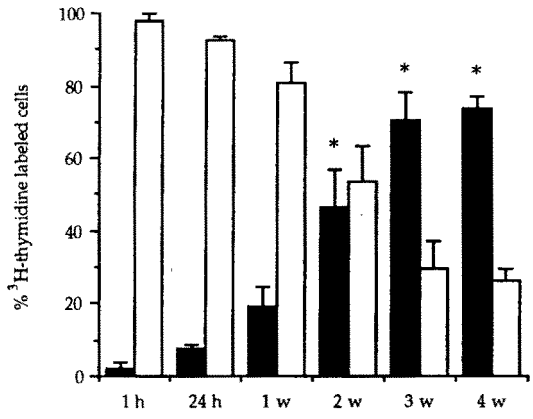

Fig. 6. Percentage of [^{3}H]thymidine-labeled cells in the dentate gyrus which were NSE-immunoreactive (solid bar) and non-NSE-immunoreactive (open bar) at different times following [^{3}H]thymidine administration. Bars represent mean + S.E.M. Asterisks indicate significant difference from 1 and 24 h; $P < 0.01$. w, week.

At 1 h following [^{3}H]thymidine injection, a few [^{3}H]thymidine-labeled cells were GFAP-immunoreactive. These cells were located in both the hilus and the GCL. Quantitative analysis of the density of [^{3}H]thymidine-labeled GFAP-immunoreactive cells in the dentate gyrus revealed significant changes with time ($P < 0.01$). A significant increase in the number of these cells was observed by one week and thereafter a significant decrease was noted ($P < 0.05$). However, due to increases in the numbers of other [^{3}H]thymidine-labeled cell types, i.e. NSE-immunoreactive and non-immunoreactive cells, the percentage of [^{3}H]thymidine-labeled cells that were GFAP-immunoreactive did not increase with time (Fig. 7).

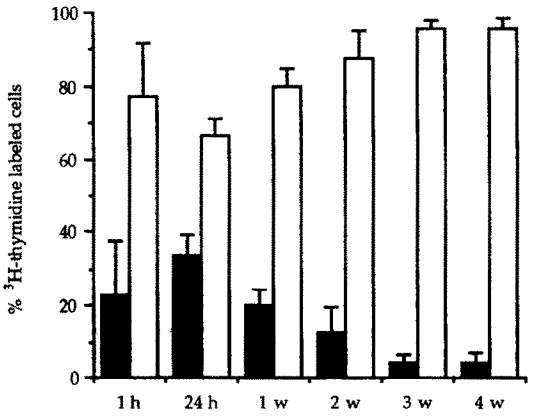

Fig. 7. Percentage of [³H]thymidine-labeled cells in the dentate gyrus which were GFAP-immunoreactive (solid bar) and non-GFAP-immunoreactive (open bar) at different times following [³H]thymidine administration. Bars represent mean + S.E.M. No significant differences were observed. w, week.

DISCUSSION

Fate of newly born neurons in the adult rat dentate gyrus

The results of this study demonstrate that the adult rat dentate gyrus produces a substantial number of new cells, the majority of which become neurons. Three weeks following [³H]thymidine injection the majority of labeled cells are immunoreactive for NSE; this marker has been shown to reliably and consistently stain only neurons in brain sections.[17,24] The constant number of these cells between three and four weeks following injection indicates that these cells do not undergo rapid degeneration. Rather, these cells appear to differentiate into functional granule cells: they express a neuronal marker shown by mature granule neurons, are morphologically identical to unlabeled granule cells and have previously been shown to possess postsynaptic densities.[12,13]

In all brains examined here, [³H]thymidine-labeled cells were observed; the mean number of [³H]thymidine-labeled cells observed was approximately seven per section, or 50 in each brain. It is likely that this value grossly underestimates the actual number of new cells being produced in this region for the following reasons: (i) since a single [³H]thymidine injection is available for uptake up to 2 h following injection,[20] only cells that are synthesizing DNA during this discrete time-period will become labeled; (ii) the emulsion can only detect [³H]thymidine in the top 3 μm of each 40-μm section;[10] and (iii) the dentate gyrus spans many 40-μm sections. Given these methodological considerations, the actual number of granule cells produced in adulthood must be large, and they probably comprise a significant proportion of the total granule cell population.

The observation that the density of [³H]thymidine-labeled cells increases seven-fold between 1 h and one week suggests that dentate gyrus cells are dividing multiple times. Between one and two weeks, however, a significant decrease in the density of [³H]thymidine-labeled cells occurred in the GCL. The most likely explanation for this observation is that [³H]thymidine-labeled cells are dividing multiple times such that the number of silver grains is diluted beyond detectable limits. The evidence that the number of silver grains per [³H]thymidine-labeled cell decreased significantly supports this hypothesis. Although it is also possible that the decrease in [³H]thymidine-labeled cell density results from cell death or migration outside of the dentate gyrus, this is unlikely due to the absence of degenerating or migrating [³H]thymidine-labeled cells in or around the dentate gyrus (Cameron, unpublished observations).

Although it is not possible to determine whether a specific subset of these cells undergoes multiple cell divisions, two lines of evidence suggest that it is primarily the cells with neuronal destiny that divide multiple times in a four-week period. First, the tremendous increase in the number of [³H]thymidine-labeled cells and the decrease in silver grains per cell occurs primarily in the GCL, a region that contains few glial cells.[14] Second, NSE-immunoreactive [³H]thymidine-labeled cells were rarely heavily labeled with silver grains. Since the number of silver grains would be reduced with each cell division, it is likely that lightly labeled cells, i.e. those immunoreactive for NSE, have divided multiple times.

Fate of newly born glia in the adult rat dentate gyrus

The results of this study indicate that glial cells are also produced in the dentate gyrus of the adult rat. We found a low number of GFAP-immunoreactive [³H]thymidine-labeled cells at every time-point examined indicating that glia born in the adult survive for at least four weeks following mitosis. Unlike the pattern observed for NSE-immunoreactive [³H]thymidine-labeled cells, the percentage of GFAP-immunoreactive [³H]thymidine-labeled cells does not increase with time. It is unlikely then that a population of non-GFAP-immunoreactive cells are born in the adult and differentiate into GFAP-expressing glia within four weeks. In general, immature glial cells have been shown to express vimentin and not GFAP.[21] Vimentin is typically expressed by radial glia, which are thought to differentiate into mature GFAP-expressing astrocytes after their function as migratory substrates has been served.[21] In contrast, radial glia in the adult rat dentate gyrus show both vimentin and GFAP immunoreactivity[11] (cf. present report). Vimentin-immunoreactive radial glial have also been shown to incorporate [³H]thymidine in the adult dentate gyrus.[11] Since the number of labeled cells and their morphological characteristics are virtually identical, it is likely that the population of GFAP-immunoreactive [³H]thymidine-labeled cells observed in the present study represents the same group of cells which are immunoreactive for vimentin (cf. Ref. 11). Collectively, this evidence suggests that

a population of GFAP- and vimentin-immunoreactive radial glia divide at a slow but steady rate in the adult dentate gyrus.

Migration of newly born cells in the adult rat dentate gyrus

The observation that the densities and percentages of [^{3}H]thymidine-labeled cells located in the hilus and the GCL change with time indicates that these cells are migrating. The majority of mitotic cells ([^{3}H]thymidine-labeled cells observed following 1 h survival) are located in the hilus; by one week post-mitosis the majority of [^{3}H]thymidine-labeled cells reside in the GCL. This pattern of migration is similar to that observed during postnatal development in this brain region. During the first two postnatal weeks of life in the rat, granule cell precursors arise from the secondary proliferative zone in the hilus and migrate into the GCL as quickly as one day following mitosis.[23] In the adult, proportionately more cells are born locally in the GCL than observed developmentally (cf. present results with Ref. 23). Moreover, a longer lag-time exists between [^{3}H]thymidine incorporation and migration to the GCL in the adult compared to the developing dentate gyrus. Migration from the hilus to the GCL in the adult appears to occur some time between 24 h and one week following [^{3}H]thymidine incorporation. If granule cells migrate only after completion of mitosis, this age-difference may reflect a longer cell cycle of older progenitor cells. Alternatively, cells born in the adult may migrate at a slower rate than those born during development.

These results indicate that, unlike other brain regions, the dentate gyrus of the adult rat maintains an environment conducive to neuronal migration. In most mammalian systems radial glia, which provide a substrate along which newly born cells migrate, are present only during a discrete developmental period, after which they differentiate into mature astrocytes.[21] However, the rat dentate gyrus maintains a significant population of these cells throughout adulthood.[11] Since adult-generated neurons appear to migrate from the hilus to the GCL through the subgranular zone, a region rich in radial glia, it is likely that these glial cells serve the same function in adulthood as during development. During development, a new population of radial glia appears in the dentate gyrus prior to each phase of neurogenesis and migration.[22] Despite the persistence of older radial glia in close proximity to proliferating cells, it appears that newly born granule neurons preferentially follow the migratory pattern provided by younger radial glia.[22] On the basis of this developmental pattern, it has been suggested that only recently formed radial glia provide the appropriate substrate for neuronal migration.[22] It is possible that radial glia participating in the migration of neurons born in adulthood are those born in adulthood as well. Our previous observation of [^{3}H]thymidine-labeled vimentin-immunoreactive cells and our observation reported here of [^{3}H]thymidine-labeled GFAP-immunoreactive cells with radial glial morphology indicate that the adult dentate gyrus does provide a population of newly born radial glia upon which neurons could migrate from the hilus into the GCL.

Location and characteristics of the granule cell precursors

These data indicate that fully differentiated granule neurons do not divide. We observed only a single [^{3}H]thymidine-labeled NSE-immunoreactive cell 1 h following [^{3}H]thymidine administration and it was not until two weeks later that a significant increase was observed in the number of [^{3}H]thymidine-labeled cells that were NSE-immunoreactive. Although it is possible that differentiated cells transiently suppress NSE expression during mitosis, this is unlikely as developmental studies have shown that neurons express this protein only after migration and synapse formation are complete, after their final cell division.[24] Thus, using the expression of NSE as a marker for differentiation, the lag-time between [^{3}H]thymidine incorporation and NSE expression in our study suggests that the population from which new granule neurons are derived consists of undifferentiated precursor cells.

The observation that cells are born in both the hilus and GCL in adulthood indicates that the distribution of the granule neuron progenitor cell population changes with developmental age. During the embryonic period, granule cells arise from the subependymal layer of the lateral ventricle and migrate across the hippocampal rudiment.[1,3,22,23] Postnatally, granule neurons arise from stem cells located predominantly in the hilus.[1,2,23] In adulthood, granule neurons arise from many precursor cells located in the GCL, as well as in the hilus, suggesting that during development some granule cell progenitors move toward the final destination of the granule neurons. If this hypothesis is correct, then the dentate gyrus of older adults may show dividing cells predominantly in the GCL itself. Kaplan and Bell[12] have shown that cell birth continues in the rat dentate gyrus up until at least 11 months of age. However, since a 20-day survival period following [^{3}H]thymidine injection was used in their study, it is not possible to determine the location of the progenitor population in these animals. Future studies in our laboratory will be aimed at locating and characterizing granule cell precursors in rats throughout their lifespan.

Implications and future directions

In general, neurons in the mammalian brain are unable to divide once development is complete. Consequently, the adult brain can not replace neurons lost as a result of injury, pathology, or aging. The key to inducing neurogenesis in the adult mammalian brain may be found by studying systems in which this

process continues throughout adulthood. Progress toward this end has been impeded by the lack of a mammalian system in which adult neurogenesis has been well established. The majority of studies on adult neurogenesis have focused on non-mammalian models, such as avian[18] and reptile brains.[16,26] The results of this report positively identify newly born cells in the adult rat dentate gyrus as neurons and show that many of these cells migrate to the GCL and have the morphologic characteristics of granule neurons. Moreover, the number of these cells generated in the adult dentate gyrus is surprisingly high. If the number of cells observed in this study continues to be produced every few hours throughout adulthood, it is likely that the functional consequences of such changes are large.

The dentate gyrus of the adult rat offers the opportunity to study the characteristics of cell birth and migration in a mammalian system in the absence of similar phenomena occurring simultaneously in related neuronal regions. Moreover, identification of the factors that control these processes is of great interest in an attempt to better understand the cues that normally terminate neurogenesis and migration in other adult mammalian systems. The results of the present report set the stage for future studies aimed toward these goals.

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