MEMOIR

THE DISCOVERY OF ADULT MAMMALIAN NEUROGENESIS

JOSEPH ALTMAN

I completed writing this manuscript in January, 2008 and submitted it then to Tatsunori Seki for inclusion in an edited book, entitled: *Neurogenesis in the Adult Brain I: Neurobiology*, 1 DOI 10.1007/978-4-431-53933-9_1,

ABSTRACT

Visualizing proliferating cells with ^{3}H-thymidine autoradiography, we discovered in the early 1960s that the microneurons (granule cells) of the hippocampal dentate gyrus and the olfactory bulb continue to be produced through adulthood. We later demonstrated that the precursors of hippocampal granule cells proliferate in the dentate subgranular zone, and that this secondary germinal matrix is far more prominent in a carnivore (young cats) than in rodents (rats and guinea pigs). By destroying these proliferating precursor cells with low-level X-rays, we prepared rats that lacked 85 percent of the normal complement of granule cells, the same proportion that we found to be generated postnatally with quantitative histology and autoradiography. Behavioral tests established that these "degranulated" rats displayed abnormalities comparable to those following extensive hippocampal lesions. We also showed that the granule cells of the olfactory bulb are generated in the persisting subependymal layer of the anterior forebrain and migrate to the olfactory bulb by way of a hitherto unidentified structure, the rostral migratory stream. We discuss why the neuroscience community may have refused to accept these multipronged demonstrations and our laboratory lost its public financing by the mid-1980s.

CONTENTS

1.1. SERENDIPITY

Although I decided to become (what is now called) a neuroscientist as a teenager in the early 1940s, and pursed that ambition through difficult times with a pretty clear idea what I hoped to accomplish, the problem of neurogenesis was not the subject I was planning to study. My original idea was to seek a better understanding of human behavior, observing it at its worse in the terrible times I lived, and thought that the best approach to that was to study experimentally the neural foundations of the psychological mechanisms that guide behavior. After engaging in an informal and formal "armchair" study of various topics in human and animal behavior, it was in the late 1950s that I succeeded in getting predoctoral training in neurophysiological techniques (working at Mount Sinai Hospital in New York City with the neurosurgeon, Leonard Malis) and postdoctoral training in neuroanatomy (working at the College of Physicians and Surgeons of Columbia University with Malcolm Carpenter). That work led to articles I published on the ascending and descending connections of the feline superior colliculus, and the physiological properties of single collicular neurons. I also published papers on certain facets of the visual behavior (locomotor activity, day-night activity cycle, light aversion) of rats with stereotaxic lesions of the superior colliculus.

While I received my neurophysiological and neuroanatomical training, I also began exploratory work on mapping regional differences in the protein metabolism of the brain following visual stimulation and induced motor activity. The first of these exploratory studies was carried out in collaboration with the neurochemist, Abel Lajtha, who at that time worked at the College of Physicians and Surgeons. We covered one eye of newly hatched chicks, and placed the animals overnight into a box lined with flashing Christmas tree bulbs. The next morning the chicks were injected with a ^{14}C-labeled amino acid and, after a short interval, their brains were removed and the rate of incorporation of the amino acid into a protein fraction was compared in the two dissected optic lobes (the avian homologue of the superior colliculus). The preliminary studies (which we did not think merited publication) suggested a higher rate of protein metabolism in the optic tectum contralateral to the stimulated eye. In the next study, which I carried out in a small laboratory I was establishing at the New York University Medical School, I turned to the use of the novel autoradiographic technique to determine if differences could be obtained in the optic pathways of adult pigeons whose one eye was either blindfolded or removed before the systemic administration of ^{3}H-glycine. While I could not detect laterality differences in the optic pathways in the unilaterally blindfolded pigeons, there were profound differences in the uptake of the radiochemical in the optic tract and the stratum opticum of the optic lobe of the enucleated pigeons contralateral to the removed eye (Altman and Altman 1962). I also observed that the elevated protein metabolism in the affected optic pathway was associated with an increase in the total number of glial cells.

After moving to MIT, I began the autoradiographic approach to the study of brain-behavior relationships by first mapping the regional pattern of CNS protein metabolism in rats injected intraperitoneally with ^{3}H-leucine (Altman 1963a). The quantitative microdensitometric results indicated bilaterally consistent differences in regional label density in various regions throughout the neuraxis. Using the same approach, I also observed elevated uptake of ^{3}H-leucine in motor neurons of the spinal cord in rats that were forced to run at a comfortable

speed in a motor-driven activity wheel in comparison with resting rats and rats that were injected with the radiochemical some time after they stopped running (Altman, 1963b). In another study with Gopal Das (who joined my laboratory as a graduate student), we observed increased uptake of ^{3}H-leucine in the degenerating optic tract of unilaterally enucleated adult rats in combination with a great increase in the concentration of glial cells (Altman and Das, 1964a). However, I was troubled by the inconsistent finding of increased protein metabolism with motor activation but not with visual stimulation, the relative non-specificity of amino acid uptake by single neurons in the exercised rats (such as increases in sensory structures), and by the great variability in individual animals. Accordingly, in an attempt to improve the autoradiographic technique, I also experimented with a more selective radioactive marker than amino acids, i.e.,. I used the latter to investigate the cellular dynamics of the lesion-induced glial proliferation that we observed histologically with labeled amino acids. Thymidine is the selective and metabolically stable precursor of DNA, and it is incorporated into the nucleus of a cell in large quantities only when the cell is undergoing division. (The early evidence was reviewed in Altman 1969b.) Using ^{3}H-thymidine, I expected to tag the newly forming reactive glial cells. In a pilot study, I made stereotaxic lesions in the lateral geniculate body of rats and concurrently injected a small dose of ^{3}H-thymidine into the lesion site (Altman 1962a). Pairs of animals were then killed 1 day, 1 week, 2 weeks, and 2 months after the operation. The observations revealed that the concentration of intensely labeled proliferating glial cells was highest in those animals that survived for 1 day after ^{3}H-thymidine administration but declined thereafter; however, the number of lightly-labeled cells increased up to 1 month after the injection. This suggested continued division of the already labeled glial cells as a facet of the degenerative and/or regenerative process. Labeled (newly-formed) astrocytes and microglia predominated around the lesion site whereas labeled oligodendrocytes were most numerous in the fiber tracts associated with the damaged lateral geniculate body.

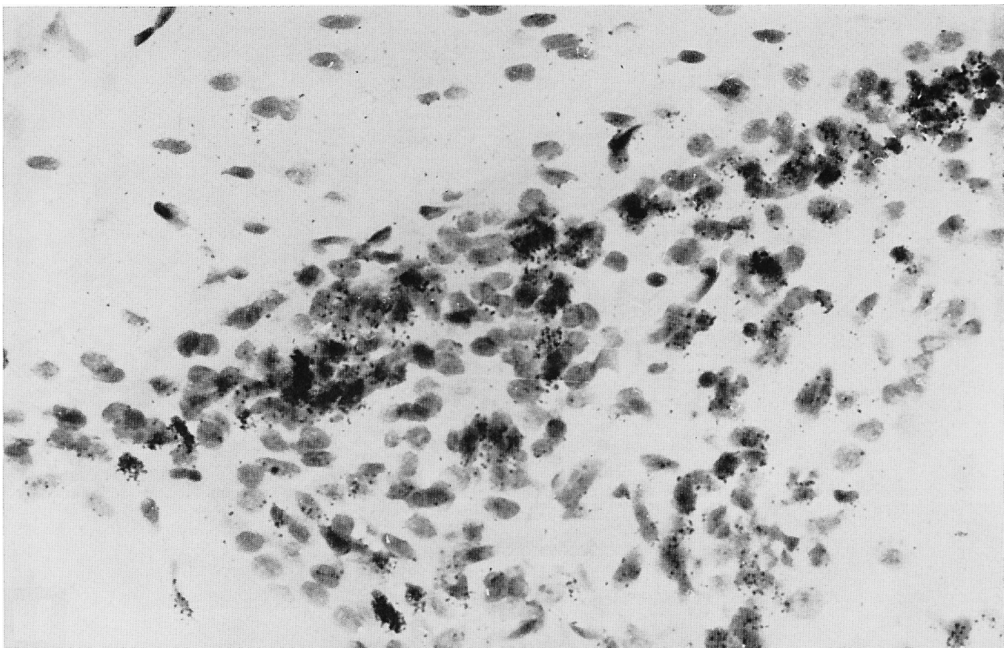


Figure 1 Autoradiogram showing labeled cells in the subependymal layer of the forebrain of an adult rat that was injected intraperitoneally with a single dose of ^{3}H-thymidine (flash labeling) two weeks before it was sacrificed. Reproduction of Fig 5 in Altman (1963c)

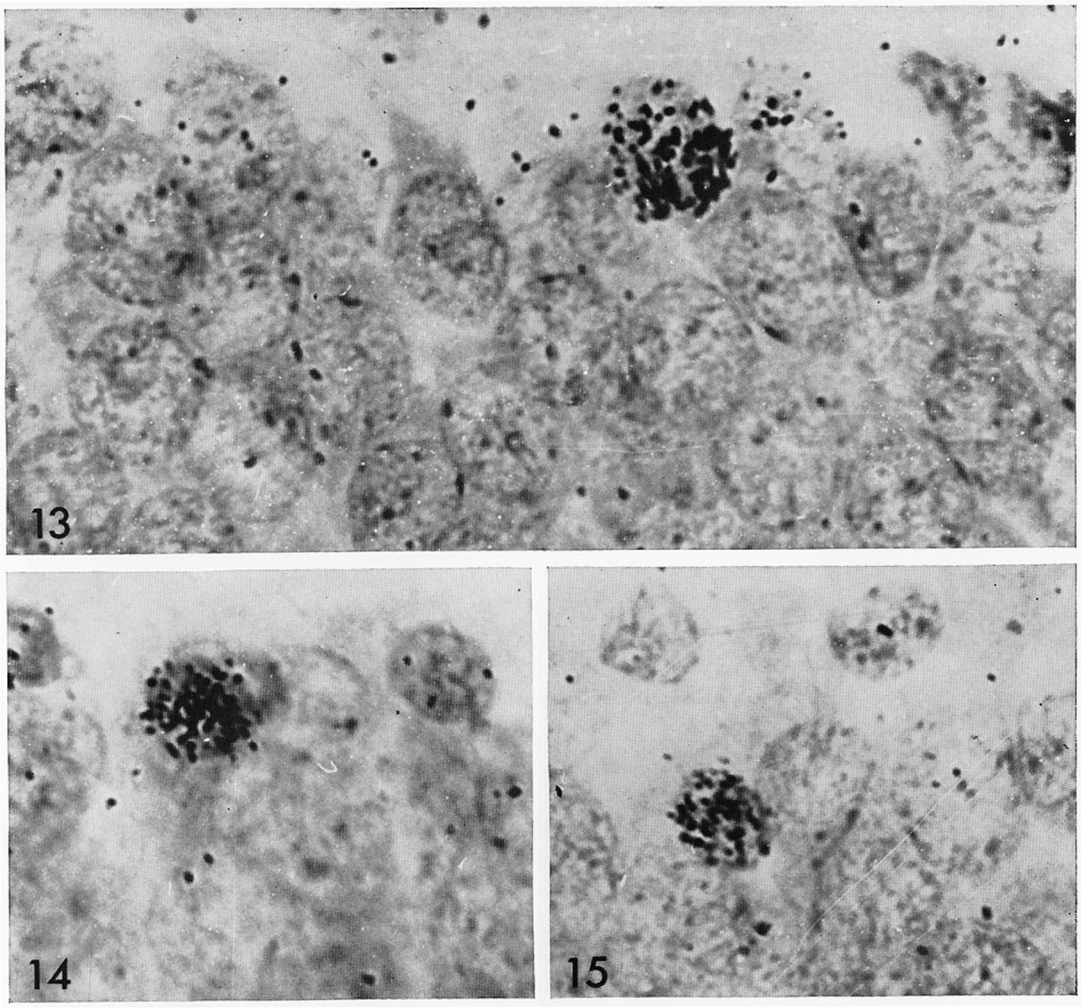


Figure 2. Autoradiograms of labeled granule cells near the hilus of the hippocampal dentate gyrus of an adult rat that was injected intraperitoneally with ^{3}H-thymidine and was killed two weeks afterwards. Reproduction of Figs 13-15 in Altman (1963c)

While these results were expected, I was surprised and confused to find a small number of neurons that appeared to be labeled at sites not connected with the lateral geniculate body (Altman 1962b). Is it possible that new neurons are formed in the adult mammalian brain? To clarify this I subsequently injected ^{3}H-thymidine intraperitoneally into normal adult rats and cats (Altman, 1963c). In the rats, the autoradiograms showed a variable number of labeled glia cells throughout the brain and spinal cord, and a high concentration of labeled cells in the subependymal layer in the roof of the anterior horn of the lateral ventricle (**Fig 1**). In addition, radioactive labeling was consistently present in all of the sections over a few granule cells in the dentate gyrus of the hippocampus (**Fig. 2**). In the cats, likewise, there were many labeled glial cells; but, in addition, the nuclei of some small cortical neurons also appeared to be labeled (**Fig. 3**). However, I argued that labeling in the latter (but not in the dentate granule cells) might have been due to the tagging of proliferating perineuronal glia in close proximity of neurons.

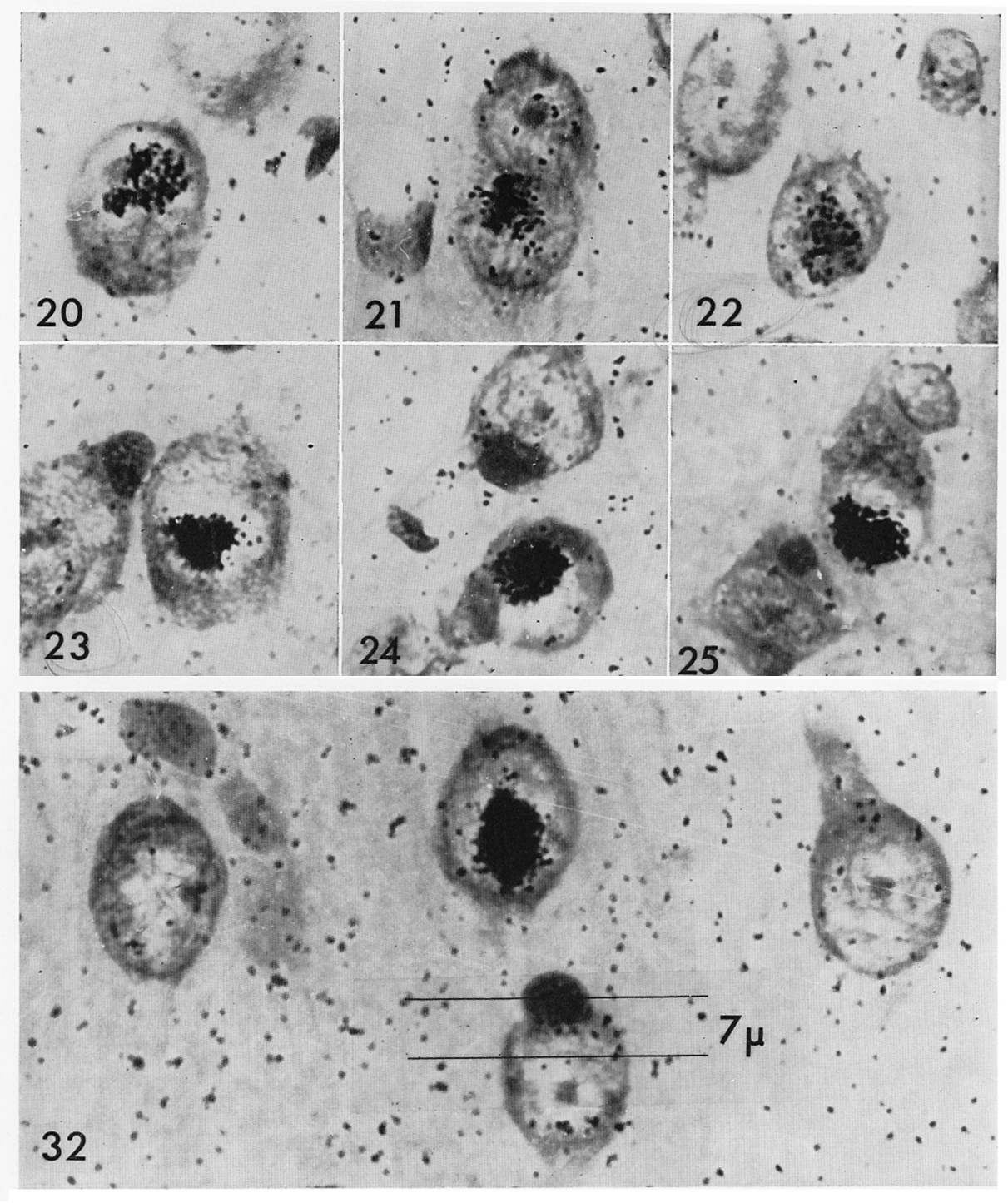


Figure 3. *Upper panel*. Autoradiograms of apparently labeled neocortical (lateral gyrus) neuronal nuclei in an adult cat that was injected intraventricularly with ^{3}H-thymidine. *Lower panel*. Autoradiogram to illustrate the possibility that in the 7μm-thick sections used, the labeling of some of the neurons may have come from closely apposed perineuronal glia. Reproduction of Figs 20-25 and 32 in Altman (1963c)

1.1.2 SYSTEMATIC INVESTIGATION OF POSTNATAL AND ADULT GLIOGENESIS AND NEUROGENESIS

1.2.1. FROM EXPLORATORY TO SYSTEMATIC INVESTIGATIONS OF POSTNATAL NEUROGENESIS.

The initial studies that I carried out at MIT were supported by grants to the Psychology Department (headed by Hans-Lukas Teuber). But in short order I was fortunate to be awarded several research grants to establish an independent laboratory. The first award I received was from the U.S. Atomic Energy Commission, as part of its program for the peaceful use of radioisotopes. Thereafter, the laboratory received generous research grants from the National Institute of Mental Health and the National Science Foundation. I was also fortunate in having from the outset the technical help of Elizabeth Altman and William J. Anderson, and in succession the cooperation of Gopal D. Das, several undergraduate and graduate students, and some postdoctoral fellows.

One of our first studies with ^{3}H-thymidine autoradiography dealt with the problem of adult gliogenesis. Since perineuronal glia are involved in the metabolism and functions of neurons, and perifascicular glia may play a role in the electrical conduction of nerve fibers, we posed the question: Might glial proliferation in the adult brain play some role in brain functions associated with behavioral activation and learning? To that effect we compared the rate of glial proliferation in the brains of adult rats that were raised in “impoverished” or “enriched” environments (Altman and Das 1964b). The impoverished rats were reared in isolation in a small cage with opaque walls and devoid of any manipulable objects. The enriched rats lived communally in a large multilevel enclosure where access to food, water, and other amenities was changed every second or third day. This required the animals to climb or jump from one floor to another, move through difficult passageways, run in an activity wheel, and engage in some other skilled activities. While the body weight of the exercising rats was consistently below the isolated rats, the brain weight of the enriched animals turned out to be higher when killed at 4.5 months of age. Moreover, there was a great increase in the number of ^{3}H-thymidine-labeled glia cells in all neocortical areas in the enriched rats when compared with the impoverished rats. This suggested higher rate of glial proliferation in the behaviorally more active animals. Another study dealt with possible differences in the proportion of newly formed dentate granule cells in the hippocampus between enriched and impoverished rats. However, due to serious ethical problems with the student involved in this study, we opted not to publish the results.

We followed these exploratory studies by a large-scale, normative histological and autoradiographic investigation of postnatal neurogenesis. In the first phase of this research (carried out for several years at MIT and continued from 1968 onward at Purdue University) we paid particular attention to postnatal neurogenesis in three brain regions with a cortical organization: the cerebellum, the hippocampus, and the cerebrum (Altman and Das 1965a, 1965b, 1966; Altman 1966, 1969). Large groups of rats ranging in age from neonates to adults were injected intraperitoneally with ^{3}H-thymidine and killed at intervals ranging from two hours (short-survival), several days (intermediate survival), up to eight months (long survival).

The analysis of short-survival autoradiograms showed that the proliferating cells of the external germinal layer of the cerebellum are consistently labeled with ^{3}H-thymidine in up to postnatal day 21 (when this layer disappears). Sequential- and long-survival autoradiograms showed that the labeled cells migrate into the granular and molecular layers where they differentiate as the microneurons of the cerebellar cortex: the granule, basket, and stellate cells.

In contrast to the limitation of postnatal cerebellar neurogenesis to the juvenile period, hippocampal neurogenesis was found to persist through adulthood (Altman and Das 1965). In short-survival autoradiograms, heavily labeled small and dark precursor cells were located at all ages (albeit in decreasing numbers in older animals) in the subgranular zone and hilus of the dentate gyrus (**Fig. 4,** top). After longer survival, the heavily and lightly labeled cells were identified as typical granule cells of the dentate gyrus (**Fig. 4,** bottom). Observations revealed that the differentiating granule cells settle in a regular order in the granular layer, with the early-differentiating (heavily labeled) neurons positioned outside near the alveus, and the later-generated neurons (lightly labeled due to the dilution of the radiochemical after repeated multiplication) inside near the hylus (**Fig 5**). Quantitative studies established that the number of undifferentiated precursor cells increased up to postnatal day 15, and then declined, whereas the number of differentiated granule cells began to increase by day 8 and remained very high as late 300 days (**Fig 6**). In adult rats injected with ^{3}H-thymidine the percentage of labeled precursor cells was highest 4 days after injection and declined by 2 weeks; in contrast, the percentage of labeled granule cells remained relatively constant up to 8 months after injection (**Fig 7**). With reference to the reduction in the addition of granule cells to the dentate gyrus as a function of age, a sharp decline was noted in the number of and percentage of labeled granule cells between days 10 and 30, and a steady decline between days 30 and 240 (**Fig 8**). Finally, in an attempt to determine whether postnatal hippocampal neurogenesis is something unique to altricial rats (rodents with a relatively short period of intrauterine development), we also studied postnatal neurogenesis in the precocial guinea pig, a rodent with a prolonged fetal life (Altman and Das, 1967), and in a carnivore, the cat. Importantly, we found that the subgranular zone containing the proliferating precursors of dentate granule cells is more prominent in the guinea pig than in the rat and, even more so, in the cat (**Figs 9, 10**). The postnatal production of hippocampal granule cells appeared to be a general phenomenon in mammals.

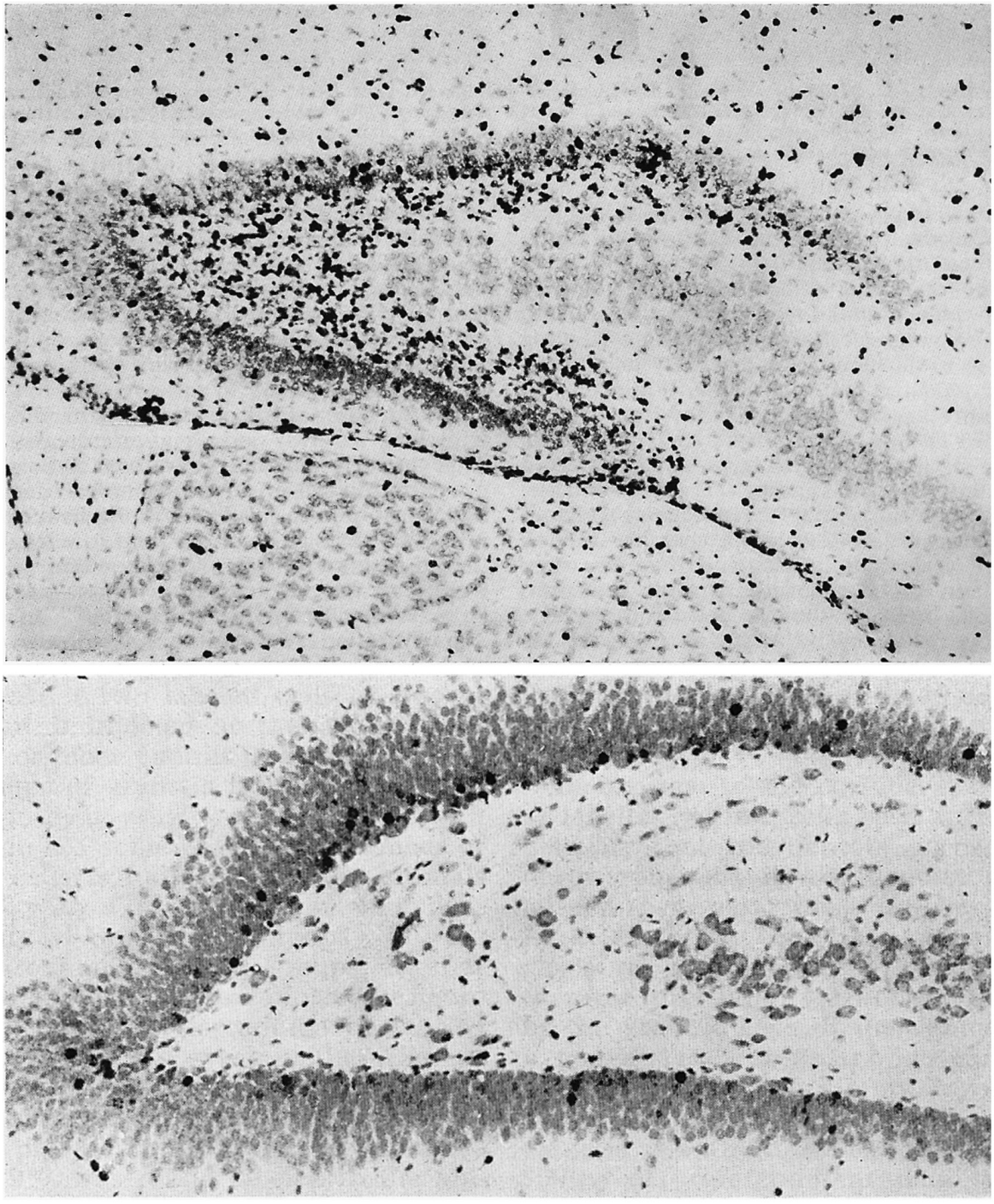


Figure 4. *Top*. Autoradiogram of labeled cells in the hippocampal subgranular zone and hilus of a rat pup repeatedly injected with ^{3}H-thymidine on the fifths, sixth, seventh and eights days (cumulative labeling), and killed on the ninth day. Note the relative thinness of the dentate gyrus at this age with many unlabeled precursor cells and a few labeled granule cells. *Bottom*. Autoradiogram of labeled "deep" granule cells (near the hilus) in a rat injected with ^{3}H-thymidine on postnatal day 10 (flash labeling) and killed two months later. Note the high proportion of unlabeled "superficial" granule cells (near the alveus). These are assumed to have been generated before postnatal day 10. Reproduction of Fig 21 in Altman (1996a), and Fig 1A in Altman and Das (1965)

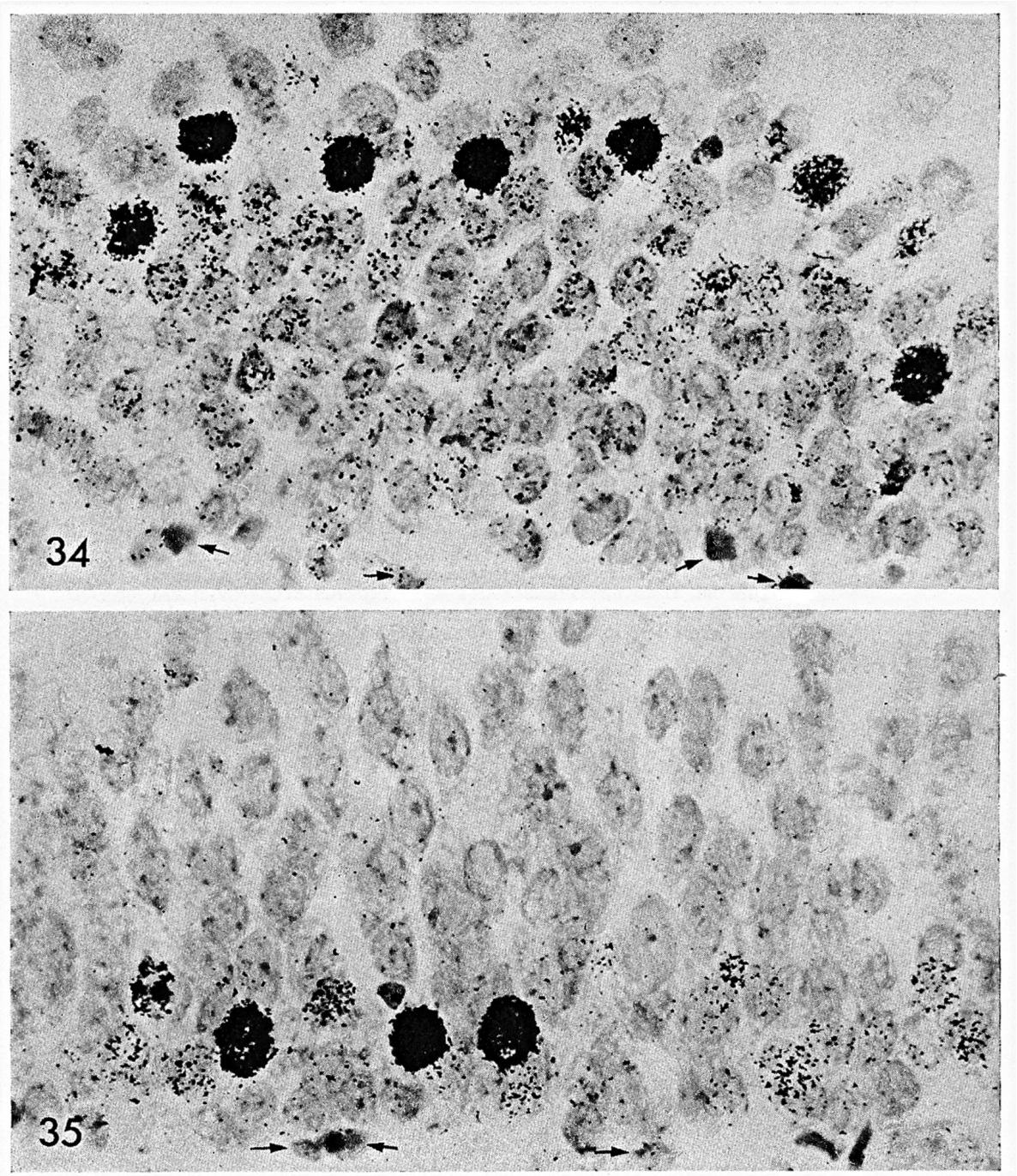


Figure 5. Autoradiograms showing the outside-in pattern of settling of the postnatally generated granule cells. *Top*. Labeling pattern of dentate granule cells in a young-adult rat that was injected with a single dose of ^{3}H-thymidine on postnatal day 2 and killed 2 months later. Note that the outermost granule cells are unlabeled. These are the granule cells that were generated before the injection. The next row of heavily labeled cells are the progeny of precursor cells that differentiated (became postmitotic) soon after the injection. The lightly labeled granule cells below them were generated later, as indicated by the dilution of the administered radioactive thymidine, as the daughter cells underwent further divisions before differentiation. *Bottom*. Pattern of granule cell labeling in a rat that was injected on postnatal day 13 and killed 2 months later. Note that the earlier generated outer granule cells are unlabeled, and heavily and lightly labeled granule cells are confined to the deep row close to the hilus. Arrows point the small, dark precursor cells of the subgranular zone. Reproduction of Figs 34-35 in Altman (1966a)

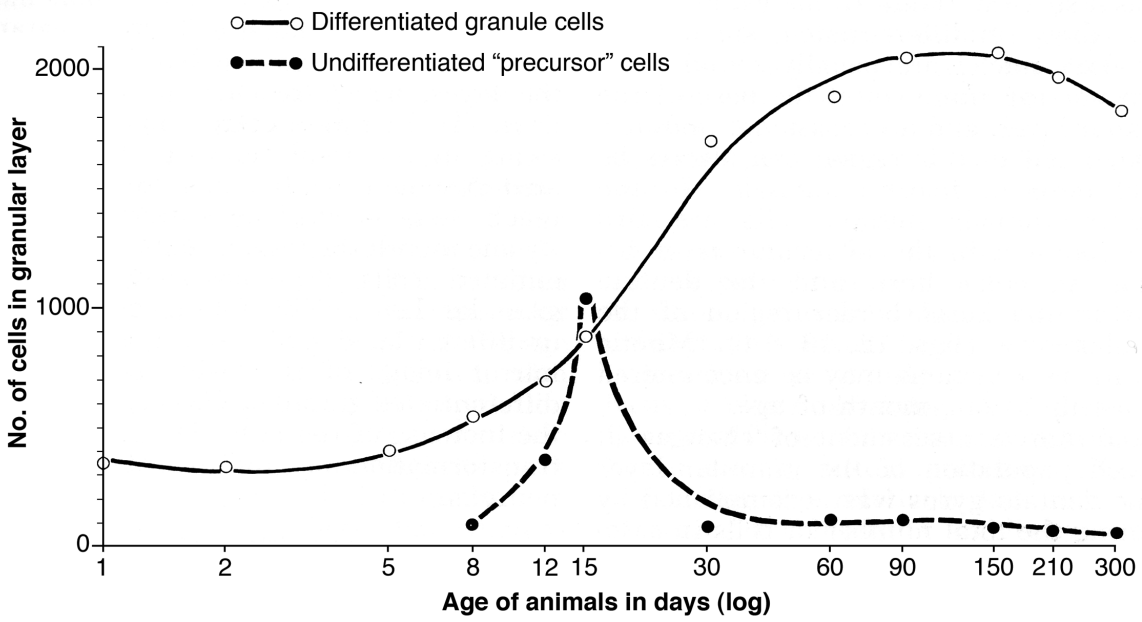


Figure 6. Number of undifferentiated precursor cells and of differentiated granule cells in 25 μm^2 sample areas in the hippocampal dentate gyrus, as a function of age. Note the great increase in the number of granule cells from the second week onward. Reproduction of Fig 7B in Altman and Das (1965a)

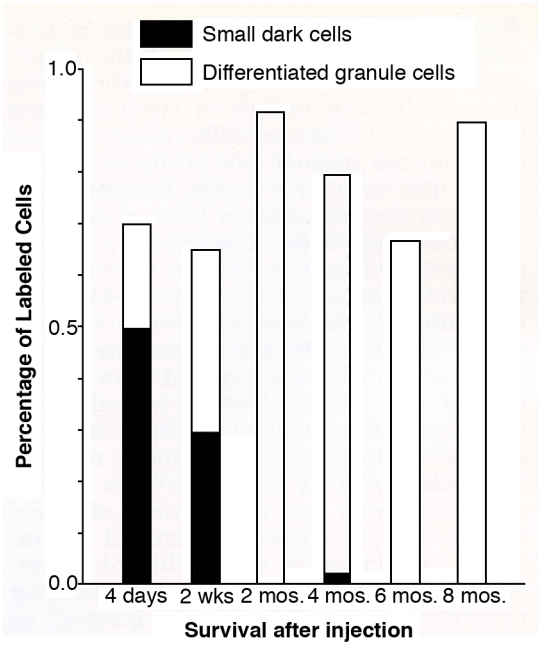


Figure 7. Percentage of labeled precursor cells and granule cells in the hippocampal dentate gyrus of rats injected with ^{3}H-thymidine as adults (4 months) and survived for periods ranging from 4 days to 8 months. Reproduction of Fig 5 in Altman and Das (1965a)

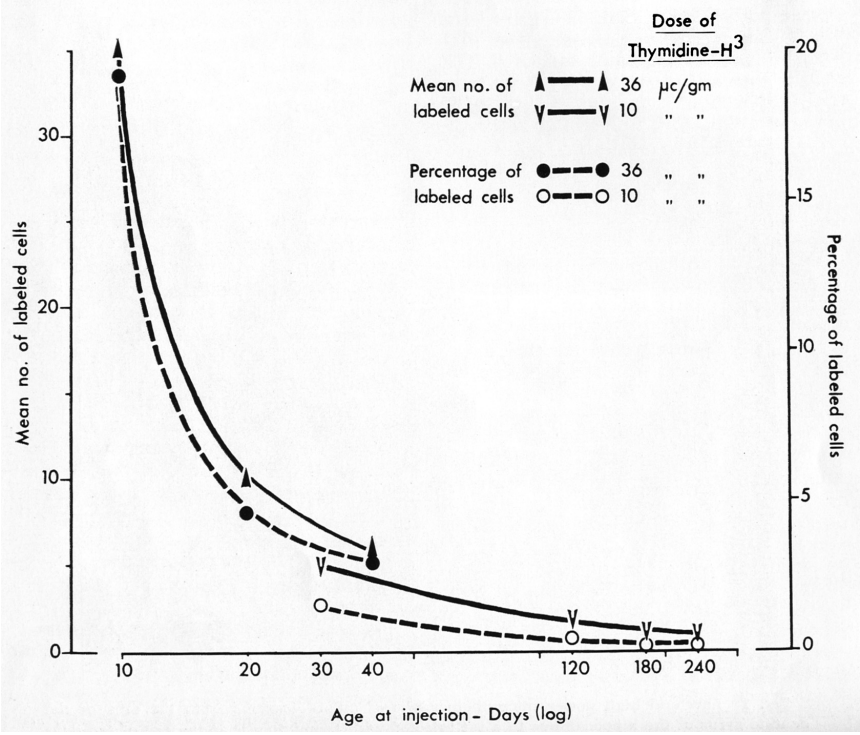


Figure 8.
Number and percentage of labeled granule cells in the hippocampal dentate gyrus as a function of postnatal age at the time of injection. Reproduction of Fig 2 in Altman and Das (1965a)

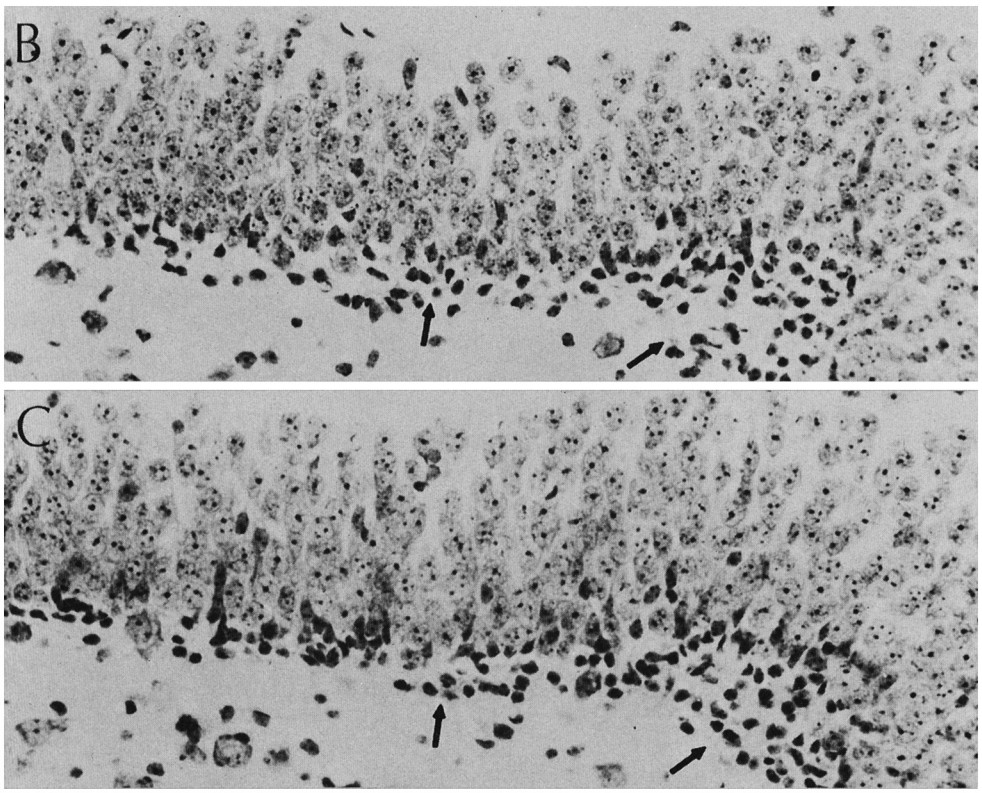


Figure 9. The prominent subgranular zone of the hippocampal dentate gyrus (arrows) in 6 day-old (*top*) and 18 day-old (*bottom*) guinea pigs. Reproduction of Figs 7B-C in Altman and Bayer (1975)

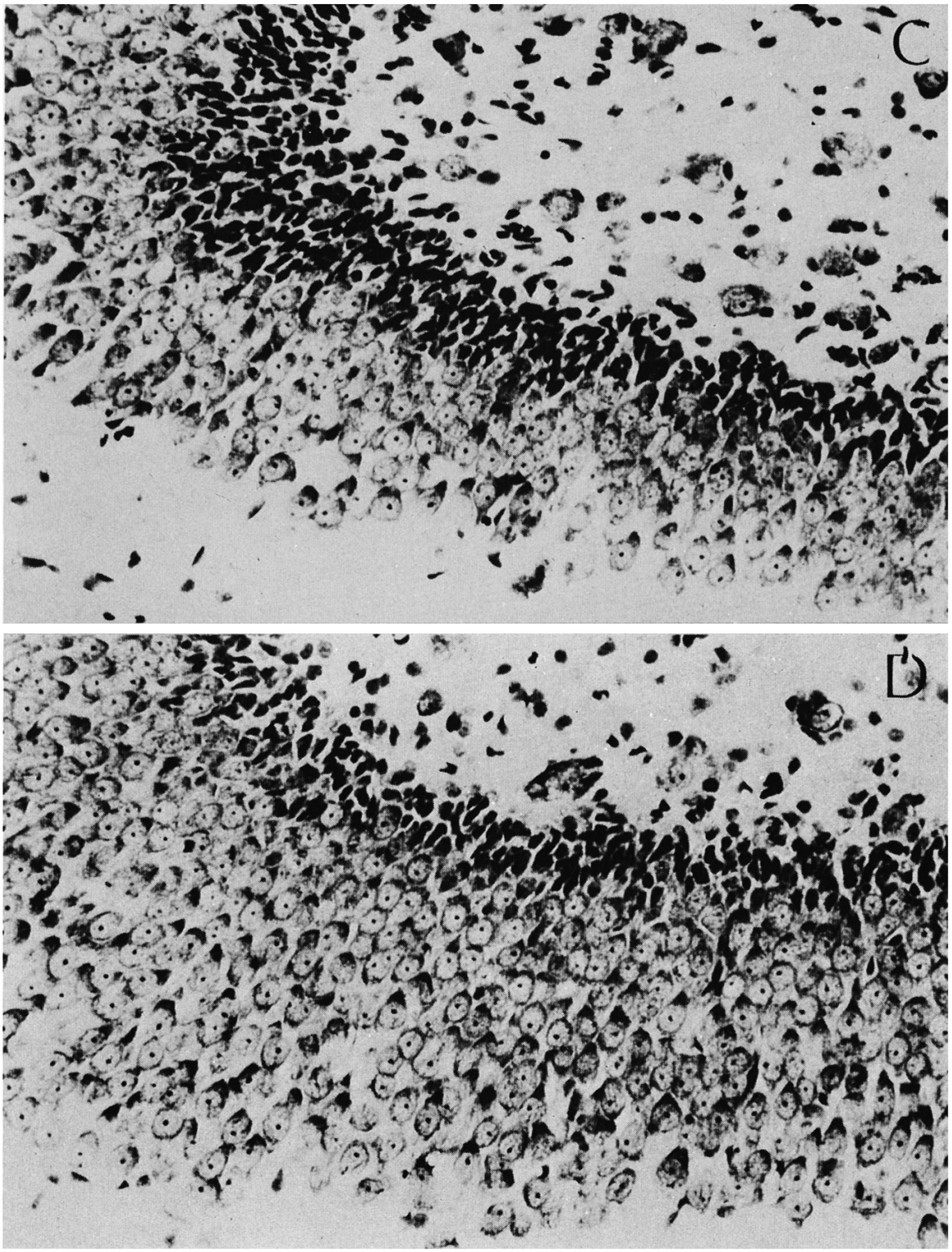
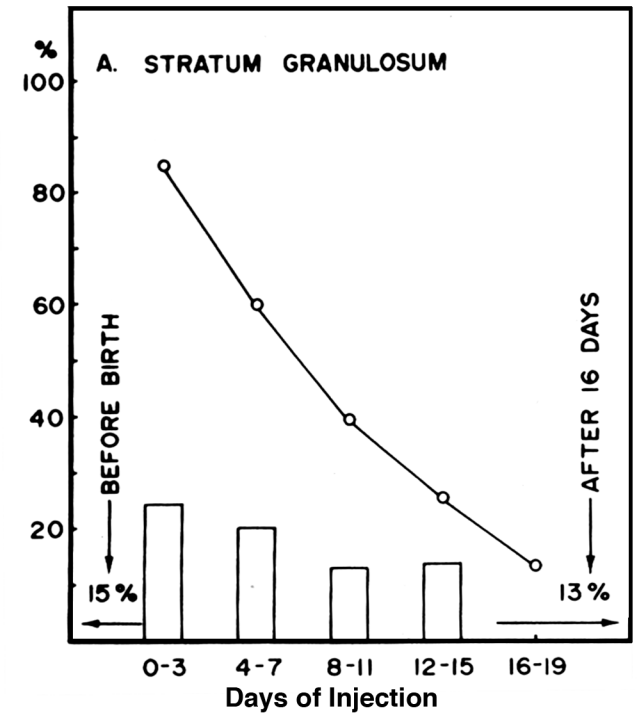


Figure 10. The prominent subgranular zone of the hippocampal dentate gyrus in cats aged 30 day-old (*top*) and 60 day-old (*bottom*) cats. Postnatal hippocampal neurogenesis may be more prominent in carnivores than in rodents. Reproduction of Fig 8C-D in Altman and Bayer (1975)



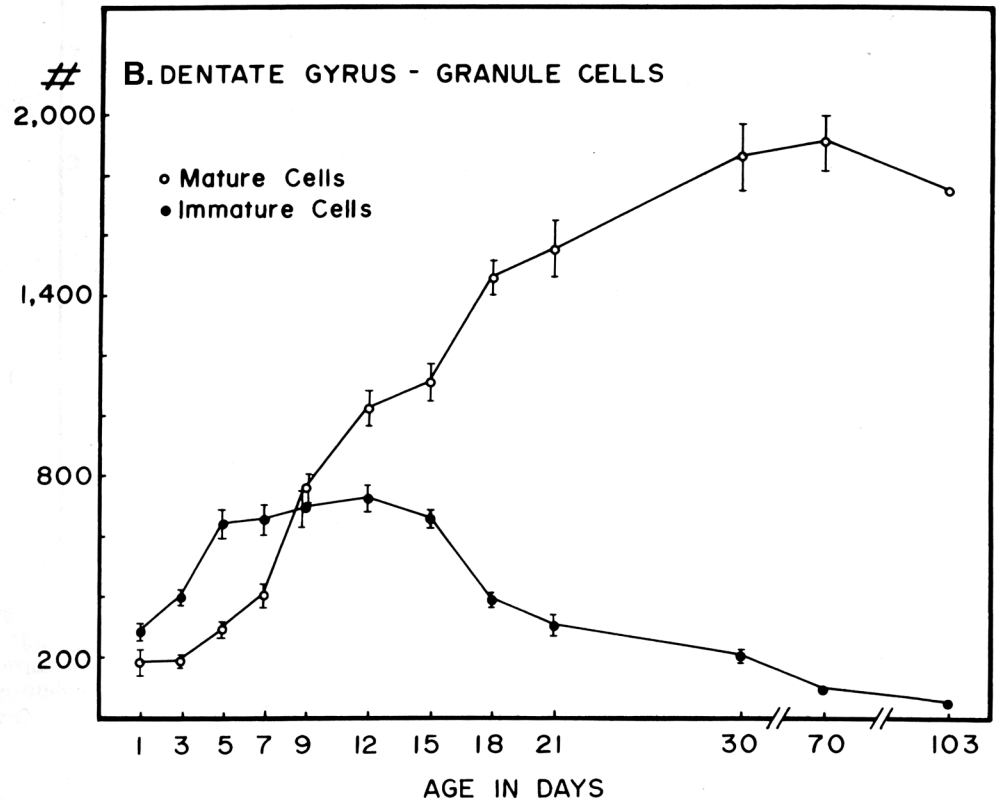


Figure 11. *Top*. Estimate of the percentage of dentate granule cells formed in 4 day-blocks, using the "progressively delayed cumulative labeling technique" (see text). The results indicate that, as judged at 2 months of age, 85 percent of the granule cells in the rat hippocampus are generated postnatally, 15 percent prenatally. *Bottom*. Estimate of the number of immature and mature dentate granule cells in matched sections of the dorsal hippocampus. Each point, up to day 70, represents the mean from 6 rats. There is close to a tenfold increase in the number of mature granule cells between postnatal days 3 and 70. Reproduction of Figs 9A and 13A in Bayer and Altman (1974)

Shirley Bayer joined our laboratory in 1970. As the first step in preparation for her scientific career, she undertook a quantitative assessment of the proportion of dentate granule cells acquired during the postnatal period (Bayer and Altman, 1974, 1975). Using a procedure that we called the "progressively delayed cumulative labeling technique," we administered ^{3}H-thymidine daily to large groups of rats on postnatal days 0-3, 4-7, 8-11, 12-15 and 16-19, and sacrificed the animals at 60 days. In this procedure, which labels virtually all the proliferating cells of the developing brain, instead of counting the labeled dentate granule cells, she counted the number of unlabeled cells as a function of advancing postnatal age, on the assumption that the unlabeled cells are those that differentiated (became postmitotic) before the injection. The results indicated that, as judged by the labeling pattern at 60 days, 15 percent of the dentate granule cells are generated prenatally, while 85 percent are generated postnatally (**Fig. 11**, top). The postnatal acquisition of dentate granule cells is highest during the first week, declines somewhat by the second week, and 16 percent of the granule cells are generated after postnatal day 16. Counting all mature granule cells in matched sections of the dorsal hippocampus in a large number of rats established that the population increased tenfold from 3 days to 70 days. Do the dentate granule cells acquired during adulthood replace dying neurons or do they expand that neuronal population? Shirley Bayer examined this question several years later in a computerized volumetric study (Bayer et al 1982; Bayer 1982). The results indicated a linear increase in the total number of dentate granule cells in the right hippocampus of rats aged 30, 120, 200 and 365 days of age (**Fig 12**). The estimated increase between 1 month and 1 year of age was in the range of 35-43 percent.

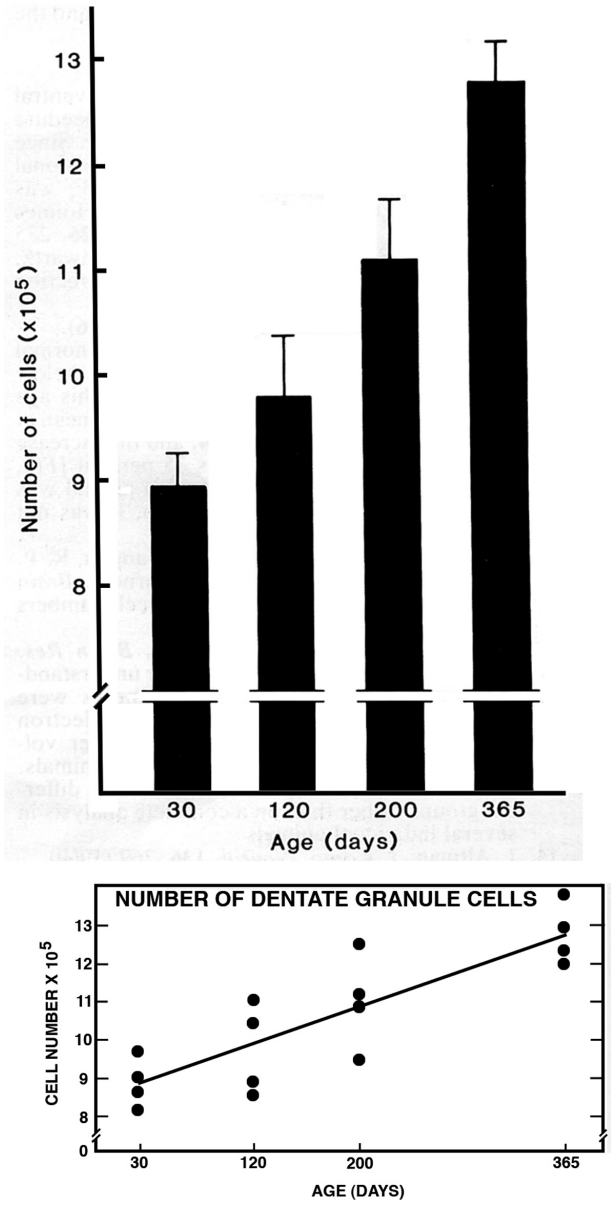


Figure 12. *Top*. Estimate of the total number of dentate granule cells in the right hippocampus of rats aged 30, 120, 200 and 365 days. Each bar represents the mean from 4 rats. Reproduction of Fig 2A in Bayer et al (1982). *Bottom*. Range in the number of granule cells in individual rats as a function of age. Reproduction of Fig 2B in Bayer (1982)

1.2.2. HIPPOCAMPAL GRANULE CELL HYPOPLASIA PRODUCED BY X-IRRADIATION.

Our next step was to substantiate our autoradiographic results with other experimental procedures. In a series of earlier studies dealing with the postnatal development of the cerebellar cortex, we found that exposure of the cerebellum to a single dose of 150-200 R X-ray, decimated its subpial proliferative matrix, the external germinal layer (EGL). Evidently, the EGL composed of the precursors of cerebellar microneurons is extremely radiosensitive. However, we also found that within a few days after irradiation, the EGL regenerated and produced a near-normal complement of granule, basket, and stellate cells (Altman et al 1969a, b; Altman and Anderson 1971, 1972). Because we wanted to study the behavioral role of microneurons, we prevented this regeneration by exposing the cerebellum to repeated daily doses of X-ray from birth until about days 10-15. This procedure produced a hypoplastic cerebellum that contained the full complement of prenatally generated deep neurons and Purkinje cells but was virtually devoid of granule, basket, and stellate cells. In a series of behavioral studies we then showed that by preventing the acquisition of cerebellar microneurons, the rats displayed many of the symptoms previously described following ablation of the cerebellum (e.g., Altman et al 1971; Brunner and Altman, 1973; Pellegrino and Altman, 1979). Performing the same procedure by exposing the hippocampus to successive daily doses of low-level X-ray we obtained similar results (**Fig 13**). Repeated counts showed that with the right number of daily exposures of the hippocampus we could consistently produce rats in which the dentate gyrus contained only the 15 percent of the normal complement of granule cells (e.g., Bayer et al 1973; Bayer and Altman 1974, 1975; Altman and Bayer 1975). That is, we obtained a perfect match between the histological and autoradiographic estimates of the proportion of granule cells generated postnatally and that obtained with X-irradiation.

1.2.3. BEHAVIORAL EFFECTS OF HIPPOCAMPAL GRANULE CELL HYPOPLASIA.

In an early exploratory study, we found differences in the number of ^{3}H-thymidine labeled cells in the dentate granular layer (we made no distinction between undifferentiated and differentiated cells) in rats that were "handled" daily from 2 to 11 days after birth and "unhandled" rats (Altman et al 1968). These rats were flash-labeled with the radiochemical on day 11 and were killed 3 hours, or 3 or 30 days thereafter. At all ages, the concentration of labeled granular layer cells was somewhat higher in the handled rats than in the unhandled rats, suggesting influence by that treatment on hippocampal cell proliferation (**Fig. 14**). Several years later we carried out a series of behavioral studies in adult rats, comparing irradiated rats with hippocampal hypoplasia (which involved handling them daily in preparation for the irradiation) with normal rats (Bayer et al, 1973). Using a variety of tests, these studies consistently showed that granule cell hypoplasia had the same effect on the behavior of adult rats as was previously reported after extensive hippocampal lesions. In the first of these studies we used two control groups: one that was wrapped in the same way ("handled," "stressed") as the experimental group but was not irradiated, and another that was left undisturbed ("unhandled"). Among the behavioral changes we found the following. Rats with granule cell hypoplasia traversed more squares and reared more often in open field tests than did the two control groups (**Fig 15a, b**). This suggested hyperactivity. To a much lesser extent, the wrapped

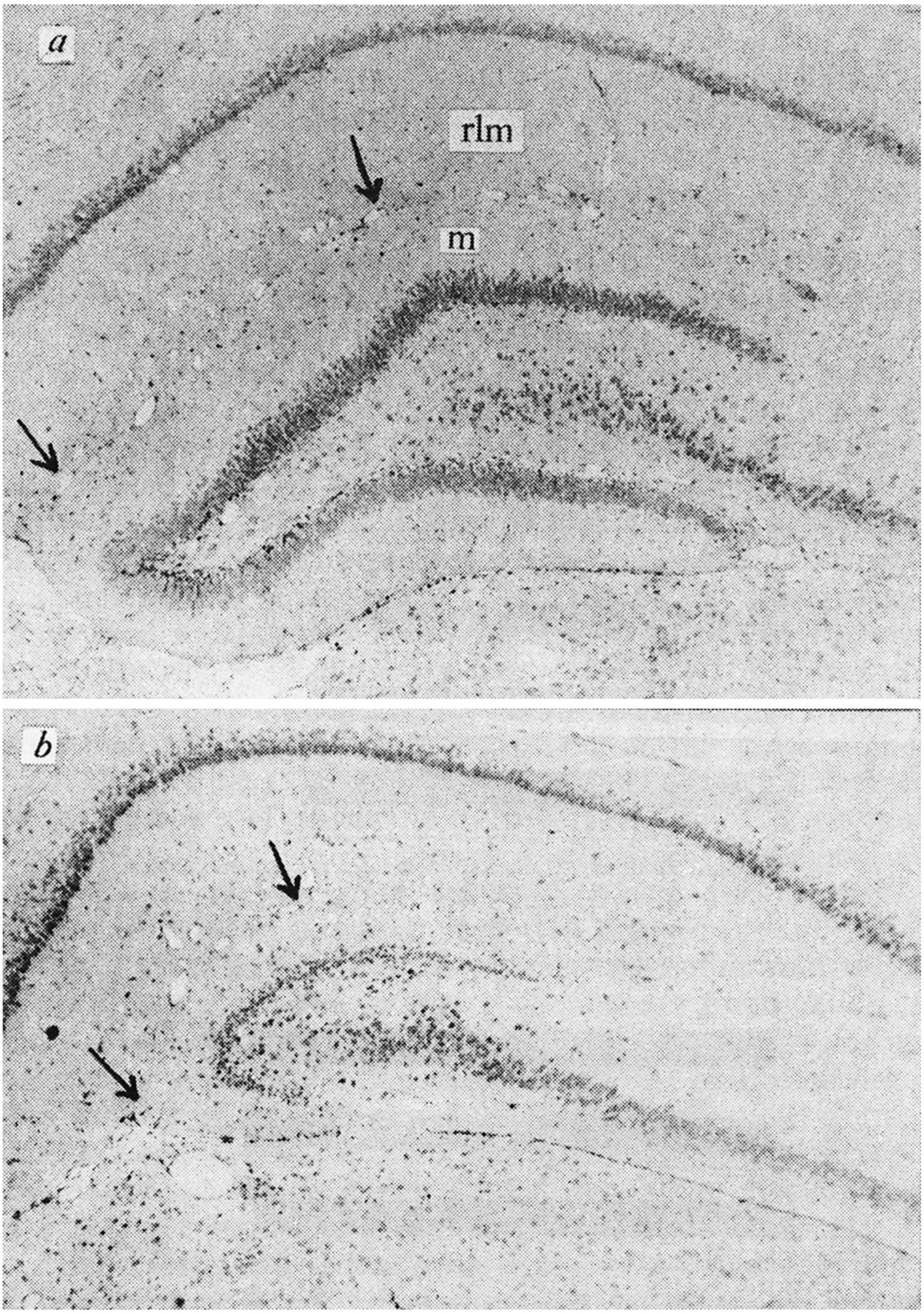


Figure 13. The dentate gyrus in a normal rat (*top*) and a rat irradiated with 8 doses of low-level X-ray between postnatal days 2 and 15 (*bottom*). Note the selective reduction in the population of granule cells in the irradiated rat. Reproduction of Fig 2 in Bayer et al (1973)

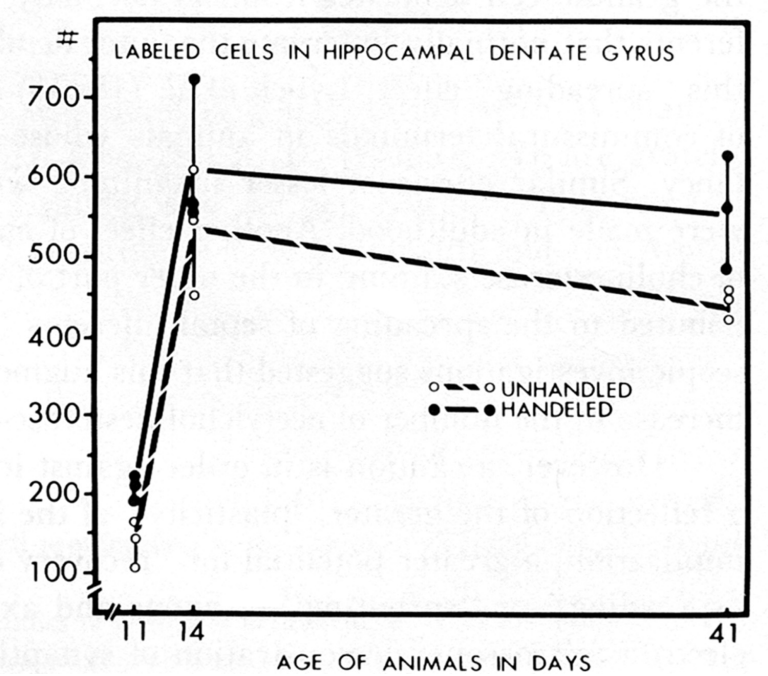


Figure 14.
Results of an exploratory study of the number of labeled cells in the dentate gyrus of the dorsal hippocampus in unhandled rats and rats that were handled daily from day 2 to day 11 and survived for 3 hours, 3 days and 30 days thereafter. No distinction was made in this study between small precursors cells and differentiated granule cells. Reproduced from Altman et al (1968)

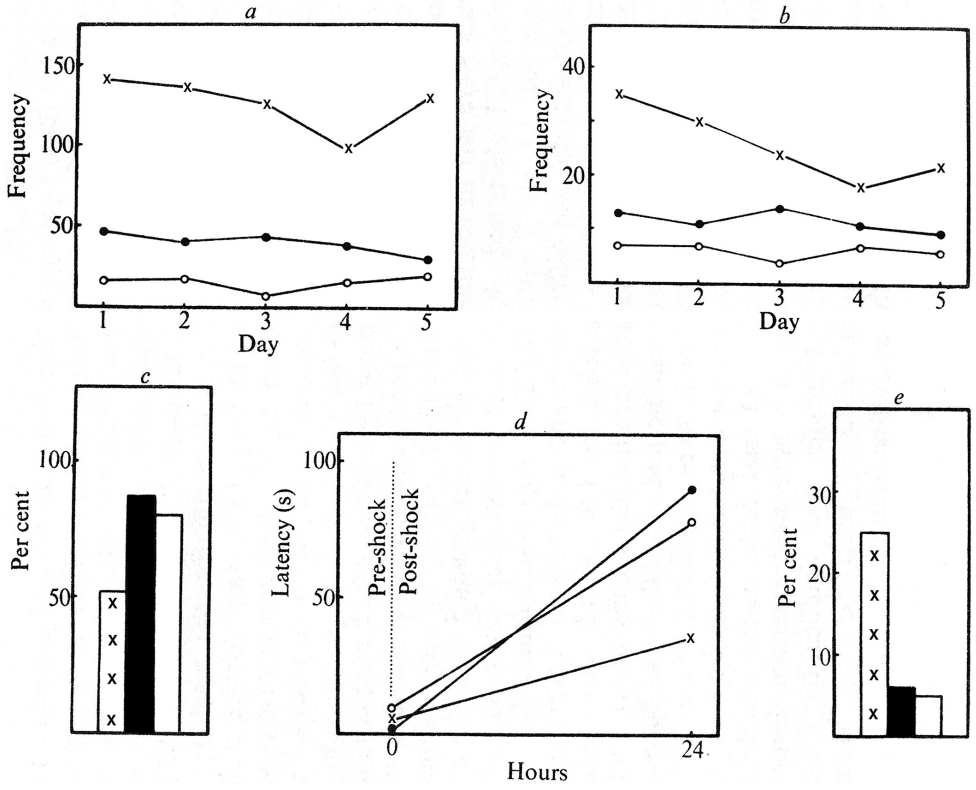


Figure 15. Behavioral effects of hippocampal granule cell hypoplasia in the irradiated rats (*x* in graphs or bars) on several behavioral tests in comparison with two groups of controls (*opaque* versus *open* circle or bar). See text for details. (a) Increase in the number of squares traversed in an open field. (b) Increase in the number of rearing responses in the open field. (c) Random alternation in a spontaneous alternation test in a T-maze. (d) Reduced latency in approaching a food cup after being shocked. (e) Reduced avoidance response in a two-way avoidance task. Reproduction of Fig 3 in Bayer et al (1973)

rats were also hyperactive. In the alternation test in a T-maze, normal rats tend to randomly explore one or the other arm on their first trial but will explore on their second trial, with a high degree of probability, the opposite arm. This behavior is not seen after hippocampal lesions. Correspondingly, we found in rats with granule cell hypoplasia (unlike the two control groups) they randomly chose either arm of the maze on their second trial (**Fig. 15c**). This suggested short-term memory deficit for what arm the animal has chosen on its first trial. The same deficit was suggested by the failure of the irradiated rats in a passive avoidance task, which involves the withholding of a natural response (feeding from a cup) that leads to punishment (**Fig 15d**), and their performance in a two-way active avoidance apparatus (shuttle box), in which the irradiated rats (unlike normal rats) readily return to the compartment where they were previously shocked (**Fig 15e**). Similar abnormalities were obtained with other avoidance learning and extinction tasks (Brunner et al 1974; Haggbloom et al 1974). In another study, we examined the effects of hippocampal granule cell hypoplasia on the initial learning and the subsequent reversal of a series of tactile and visual discrimination tasks graded in term of their difficulty (Gazzara and Altman 1975). The results indicated that the irradiated rats were not handicapped on easy sensory discrimination tasks, irrespective of the modality used, but were handicapped in difficult tasks in which the intensity or detectability of the stimuli was low. We suggested that the rats with hippocampal hypoplasia might have been inattentive because of their hyperactivity. These behavioral studies, in combination with the results obtained after cerebellar irradiation alluded to above, led to our formulation of the concept of a type of attentional deficit disorder due to microneuronal hypoplasia (Altman 1986, 1987). While there have been some supportive follow up studies (e.g., Diaz-Granados et al 1994; Highfield et al 1998), this hypothesis deserves further investigation.

1.2.4. POSTNATAL AND ADULT NEUROGENESIS IN THE NEOCORTEX AND THE OLFACTORY BULB.

In an early application of the autoradiographic technique, Smart and Leblond discovered that cells of the subependymal layer of the mouse cerebrum are labeled with ^{3}H-thymidine (Smart 1961; Smart and Leblond 1961). Confirming that in the rat (Altman 1963c), I also found that after prolonged survival there was a rapid decline in the percentage of intensely labeled subependymal cells and an increase in the percentage of lightly labeled cells (**Fig 16**, top). This was in sharp contrast to the locally multiplying glia in the white matter and gray matter of the cortex where, after an initial spurt, the concentration of labeled cell remained relatively constant (**Fig 16**, bottom). Evidently, the cortical subependymal layer is a proliferative germinal matrix. What types of cells does this proliferative matrix generate? At least a partial answer to this question came when I discovered that an extension of the anterior cortical subependymal layer is a source of a large stream of spindle shaped, darkly staining cells that migrate to the olfactory bulb. I called this system, the rostral migratory stream (RMS, Altman 1969a). Analysis of sequential autoradiograms in rats that were flash-labeled with ^{3}H-thymidine on day 30 indicated that the RMS contains cells that migrate into the olfactory bulb and settle there as the granule cells of the granular and periglomerular layers (**Fig 17**). Quantification of the labeling pattern in an adult rat indicated the following pattern (**Fig 18**). One hour after injection, the percentage of labeled cells is highest in the subependymal layer near the fountain of the RMS, is lower in the vertical limb of the RMS, and is negligible in the horizontal limb and the olfactory bulb.

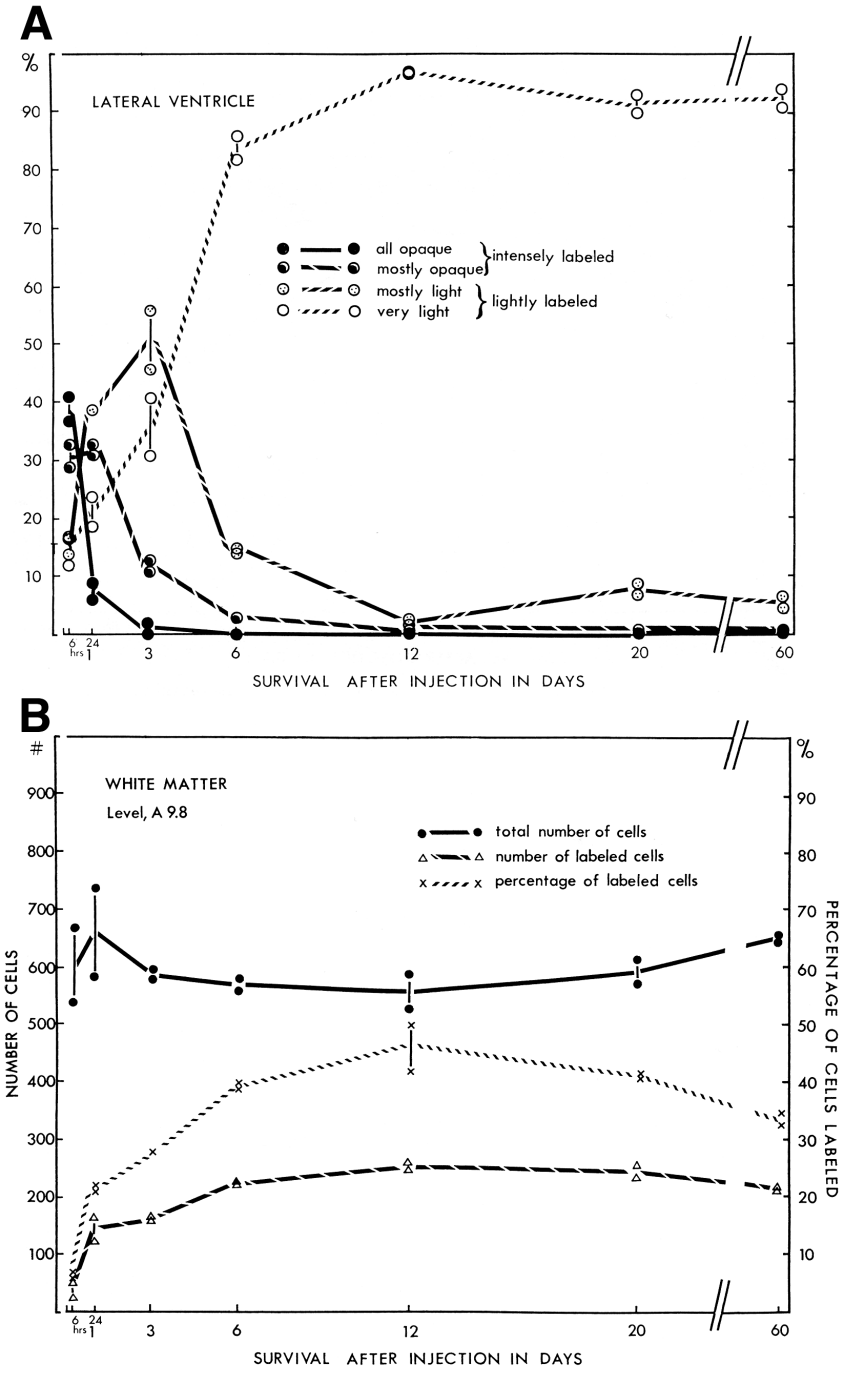


Figure 16. *Top*. Percentage of labeled cells in the subependymal layer of the lateral ventricle of rats that flash-labeled with ^{3}H-thymidine on postnatal day 13 and killed at different hourly and daily intervals thereafter. Note the rapid decline in precursor cells with high levels of the radiochemical (all opaque, mostly opaque) and increase of cells in which the radiochemical became diluted (mostly light, very light) due to continuous cell divisions. *Bottom*. Percentage of labeled glia (most of them heavily labeled) after survival ranging from 6 hours to 60 days, and the total number of glia in unit areas in matched sections of the cortical white matter. The initial increase in the number and percentage of labeled glia cells was followed by relatively little change over time. This indicates a much lower rate of cell proliferation at this site than in the subependymal layer. Reproduction of Figs 3 and 7 in Altman (1966c)

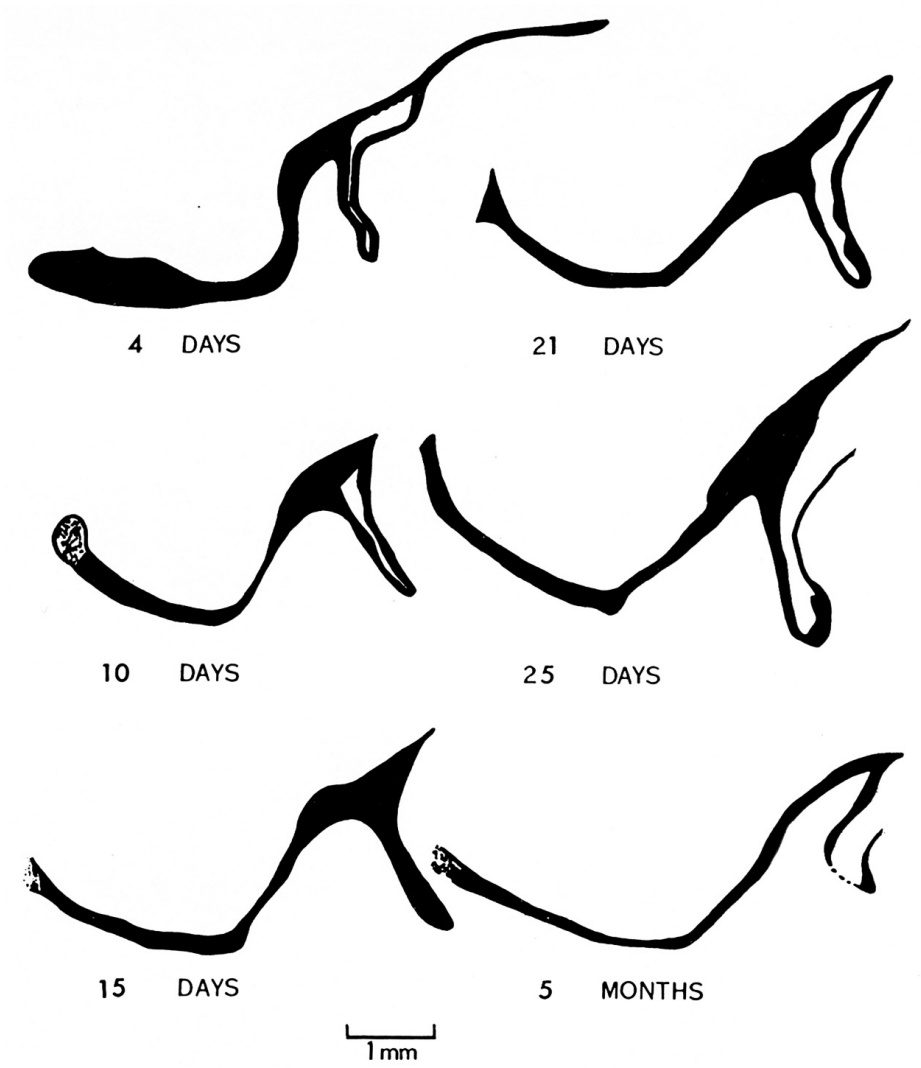


Figure 17. Tracings of the rostral migratory stream (RMS) in sagittal sections of the forebrain in rats ranging in age from postnatal day 4 to 5 months. The olfactory bulb portion of the RMS is not shown in the older animals. Reproduction of Fig 2 in Altman (1969a)

Three days after the injection, the percentage of labeled cells becomes high in the vertical limb of the RMS but is still low in the olfactory bulb. Six days after the injection, the percentage of labeled RMS cells becomes low near the lateral ventricle, is higher in the vertical limb, and is highest in the olfactory bulb. Twenty days after injection there were no longer any labeled cells in the RMS but the olfactory bulb had a high concentration of labeled cells (as seen in **Fig. 18**). I concluded that the RMS cells migrated into the olfactory bulb and settled there some time between 3 and 20 days after administration of the radioactive thymidine. This study also established that, although diminishing in size, the rostral migratory stream is still prominent in adult rats (**Fig 19**). A later investigation (Rosselli-Austin and Altman 1979) showed that there is a steep increase in the ratio between the postnatally generated granule cells and the prenatally generated mitral cells in the rat olfactory bulb between the second week of life and 90 days of age (**Fig 20**, top). Linda Rosselli-Austin's study indicated that 89 percent of the olfactory bulb granule cells are acquired postnatally, and that the labeled granule cells survive up to 1 year of age (**Fig 20**, bottom).

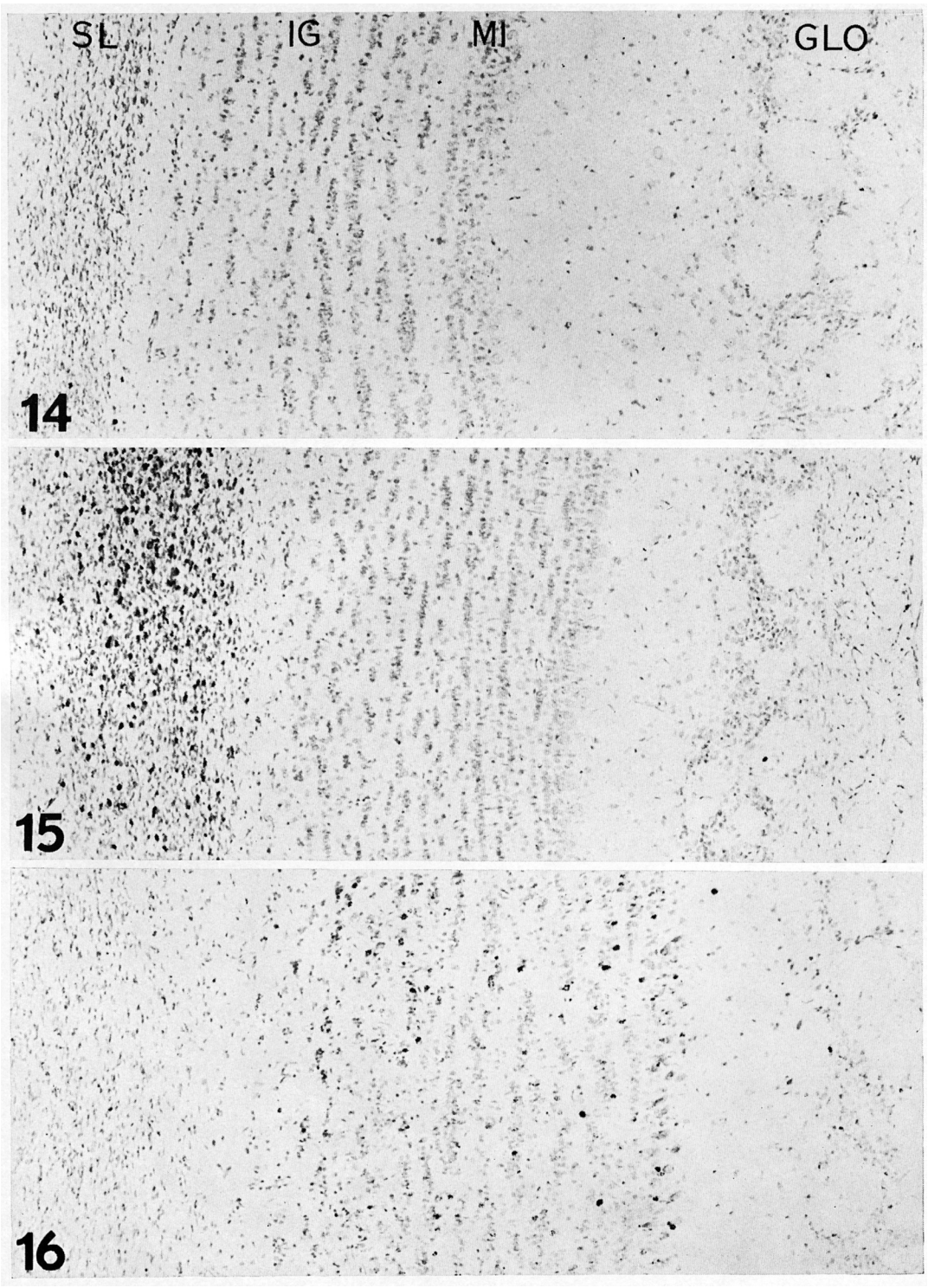


Figure 18. Pattern of cell labeling in the olfactory bulb of rats that were flash-labeled with ^{3}H-thymidine on postnatal 30 and killed 1 hour (top), 3 days (middle), and 20 days (bottom) after the injections. There are few labeled cells in any of the layers of the olfactory bulb after 1-hour survival, suggesting minimal local multiplication. Labeled cells abound in the RMS after 3-day survival, and thy have apparently migrated into the olfactory bulb after 20-day survival. Abbreviations: GLO, glomerular layer; IG, internal granular layer; MI, mitral cell layer; SL, RMS of the subependymal layer. Reproduction of Figs 14-16 in Altman (1969)

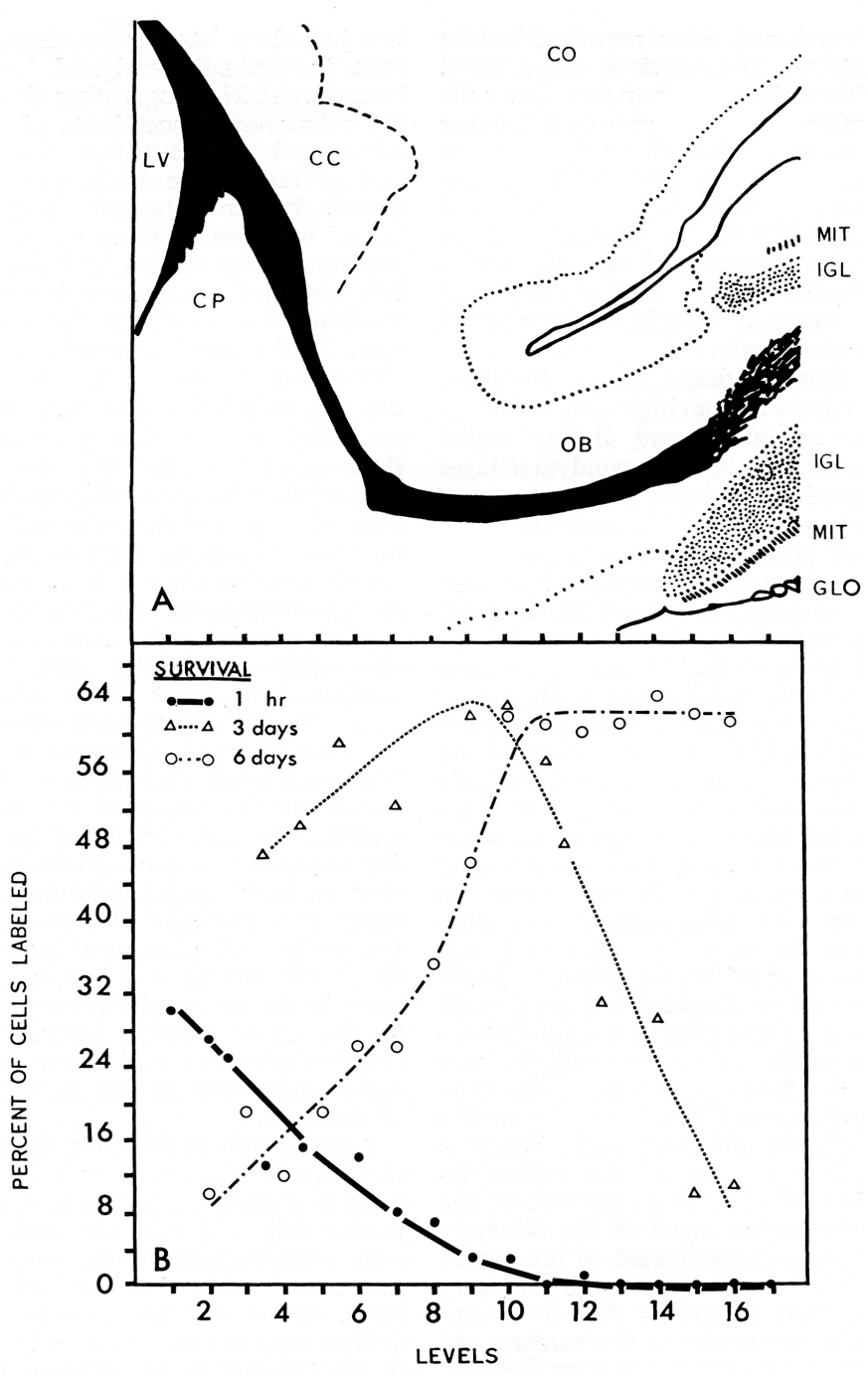


Figure 19. *Top*. Sagittal view of the RMS (black) in a young-adult rat. Abbreviations: CC, corpus callosum; CO, cerebral cortex; CP, caudate-putamen; GLO, glomerular layer; IGL, internal granular layer; LV, lateral ventricle; MIT, mitral cell layer; OB, olfactory bulb. *Bottom*. Percentage of labeled cells at different levels of the RMS, from caudal (near the lateral ventricle) to rostral (olfactory bulb) in young-adult rats that survived for 1 hour, 3 days and 6 days after flash labeling with ^{3}H-thymidine. One hour after injection the concentration of labeled cells was highest near the subependymal layer and the vertical limb of the RMS but was negligible in the olfactory bulb. Six days after injection the concentration of labeled cells decreased caudally and was highest rostrally in the horizontal limb of the RMS and in the olfactory bulb. These findings demonstrated that the RMS contains a high proportion of cells that migrate into the olfactory bulb. Reproduction of Fig 5 in Altman (1969a)

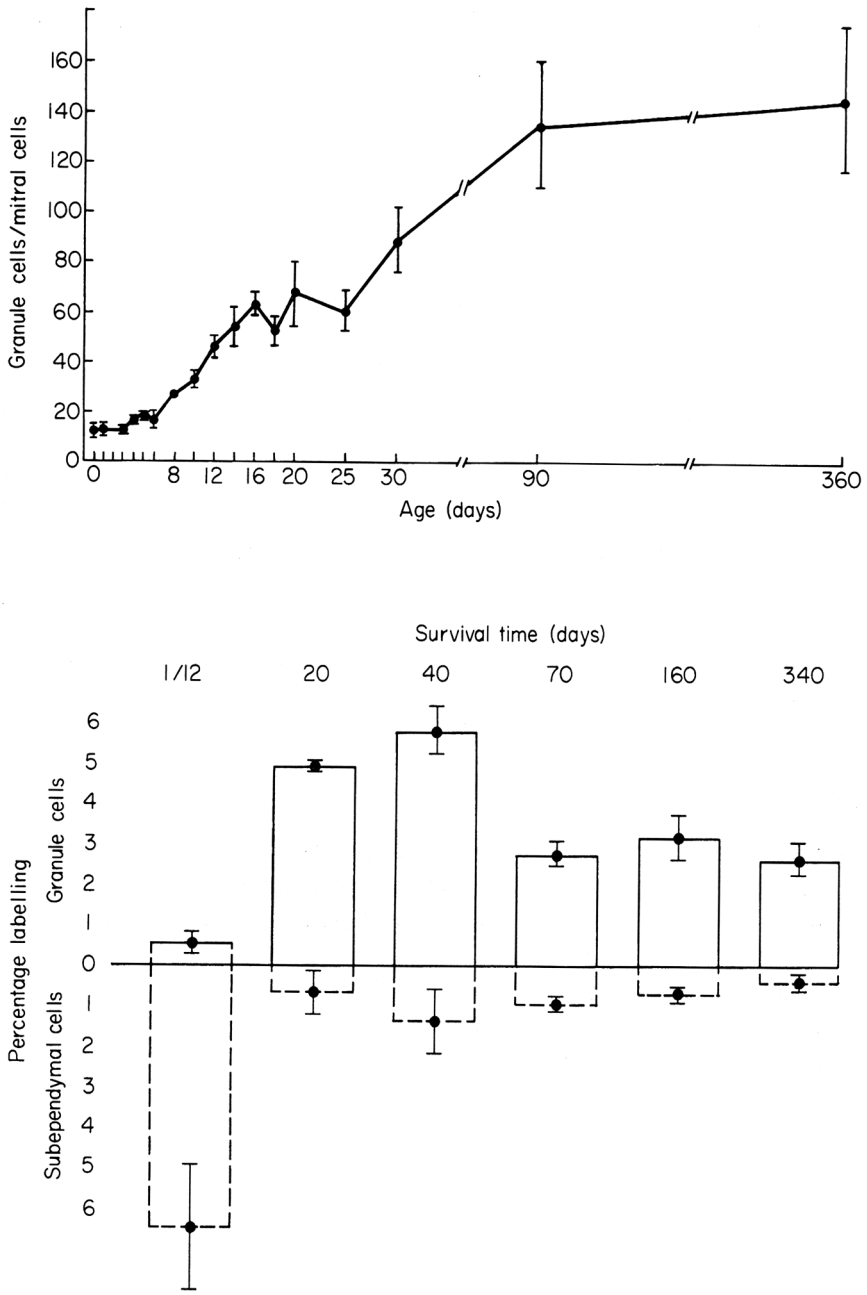


Figure 20. *Top*. Age changes in the ratio of the postnatally generated granule cells of the olfactory bulb and the prenatally generated mitral cells from birth to 1-year of age. *Bottom*. Percentage of labeled subependymal (RMS) cells and granule cells as a function of survival in rats that were flash-labeled with ^{3}H-thymidine at 30-days of age and survived up to 340 days thereafter. Reproduction of Figs 8-9 in Rosselli-Austin and Altman (1979)

In summary, our studies carried out in the 1960s and 1970s established definitively that a high proportion of the cerebellar, hippocampal, and olfactory bulb microneurons are generated postnatally. We also showed that whereas the generation of cerebellar microneurons is limited to the infantile period (postnatal day 21 in the rat), the production of hippocampal and olfactory bulb microneurons continues, albeit at a reduced rate, through adulthood.

1.2.5. THE RELATIONSHIP BETWEEN PRENATAL AND POSTNATAL NEUROGENESIS.

From the outset, our approach to adult neurogenesis has been a developmental one. We conceptualized central nervous system (CNS) neurogenesis as a prolonged process, one that begins during early embryonic development and proceeds through late embryonic, early fetal, perinatal, infantile, juvenile, and adult periods with distinctive age-related features and properties. It was with that in mind that we have switched by the late 1970s from the confined analysis of neurogenesis in selected brain regions to a comprehensive histological and autoradiographic analysis of the prenatal and postnatal development of the entire CNS. We started this longitudinal project with rats as subjects (e.g. Bayer and Altman, 1991; Altman and Bayer, 1997) and then, in the early 1990s, we turned to humans. The latter resulted in a comprehensive account of the prenatal development of the human central nervous system (Bayer and Altman, 1991; Altman and Bayer 2002; Bayer and Altman 2002-2007). This investigation remains to be extended to the postnatal period.

An example of how this longitudinal approach has shed some light on adult hippocampal neurogenesis is the study in which we tracked the development of the rat hippocampus from the earliest stages of CNS development, i.e., when the future forebrain consists only of a proliferative "stockbuilding" neuroepithelium (Altman and Bayer 1990a, 1990b, 1990c). As we conceive of it, the neuroepithelium (NEP) is the primary germinal matrix of the CNS that is composed of a population of pluripotent neural stem cells, as well as precursors of neurons and neuroglia with progressively reduced fate potentials. Initially, the future hippocampal NEP can be delineated in histological and thymidine-autoradiographic sections from the rest of the cortical NEP only by its medial position. But after a few days, three morphologically different components become discernible in this medial portion of the cortical NEP: the ammonic NEP, the dentate NEP, and the fimbrial GEP (glioepithelium) (**Fig 21**). Analysis of sequential autoradiograms indicates that the cell-dense and intensely-labeled ammonic NEP is the source of the early-generated large pyramidal cells (hippocampal macroneurons) that will form Ammon's horn. The pyramidal cells sojourn for several days in the intermediate zone (a simpler field than the "stratified transitional field" found in the neocortex; Altman and Bayer, 2002) before settling in the stratum pyramidale. The dentate NEP around a ventricular indentation, the dentate notch, initially contains fewer cells and has a different labeling pattern than the ammonic NEP. But soon a stream of proliferating and migrating spindle-shaped cells leave the dentate NEP to form the dentate gyrus. By the time of birth, early-settling granule cells form the thin external leaf of the dentate stratum granulosum but the bulk of these precursors cells sojourn in the hylar subgranular zone. The subgranular zone is most prominent and mitotically active during the juvenile period but persists as a secondary neurogenic matrix into adulthood. Finally, the fimbrial GEP is the primary germinal source of the oligodendrocytes of the progressively expanding hippocampal fiber system, the alveus and the fornix.

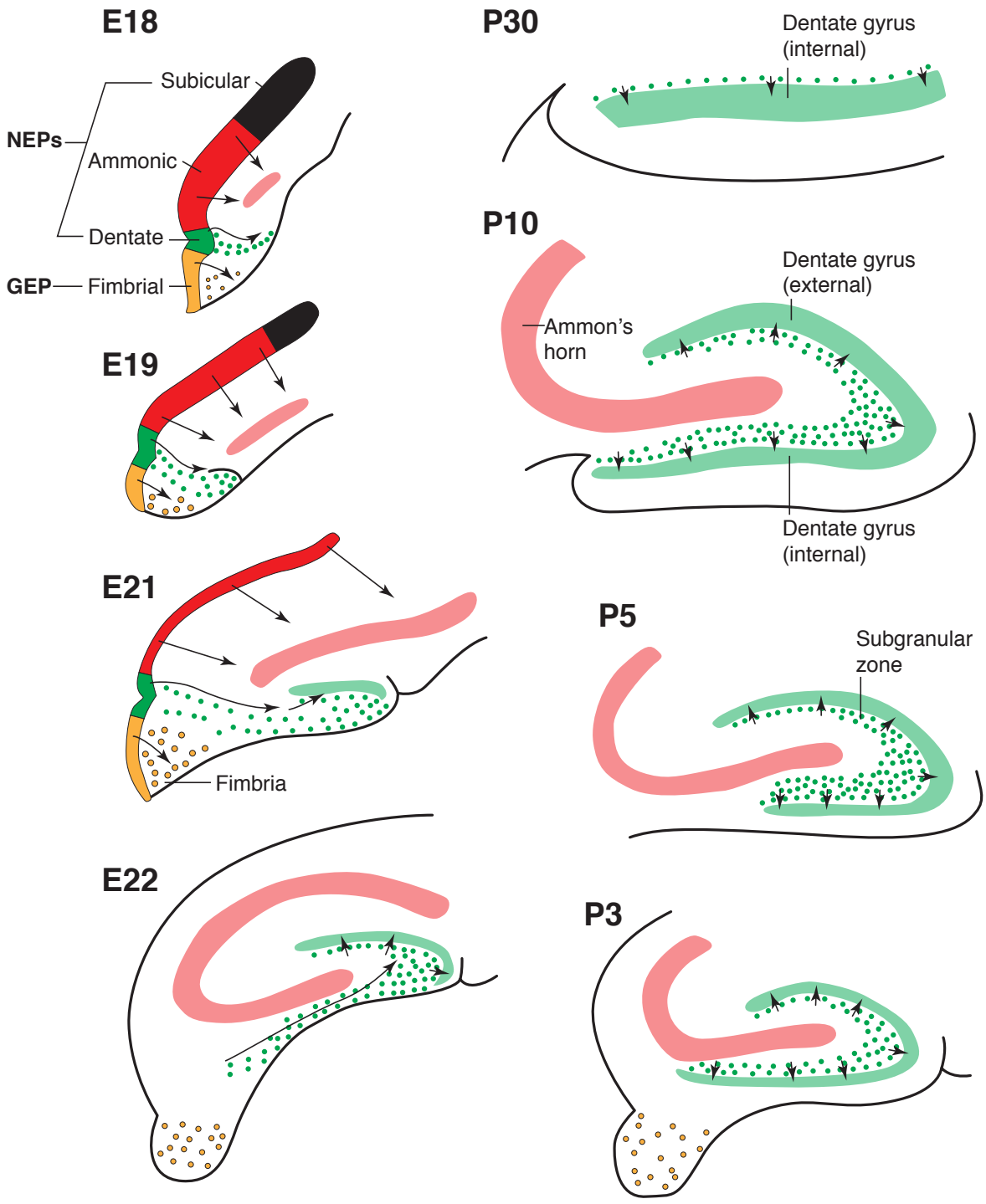


Figure 21. Schematic summary diagram of the primary and secondary germinal matrices of the hippocampus. The primary germinal matrix consists of the subicular, Ammonic and dentate NEPs, and the fimbrial GEP (glioepithelium). These persist until the perinatal period. The dentate NEP is the source of migrating precursor cells that form the subgranular zone, a secondary germinal matrix that is the source of the (mostly) postnatally differentiating granule cells, one that persists as a neurogenic zone through adulthood. Modified after Figs 16 in Altman and Bayer (1990b), Fig 13 in Altman and Bayer (1990c), and Fig 11 in Altman and Bayer (1993)

1.3. PEER REACTIONS: INITIAL EXCITEMENT, FOLLOWED BY MARGINALIZATION AND SUBSEQUENT DISQUALIFICATION

1.3.1. A SHORT PERIOD OF EXCITEMENT.

Charles Gross deserves credit to be among the first neuroscientists who recently tried to reconstruct the history of the discovery of postnatal neurogenesis in the mammalian brain (Gross 2000). As a colleague of mine at MIT in the 1960s, he had personal knowledge of some of the events that transpired when I began to publish papers on this subject. Regrettably, his interpretation of the nature of my original demonstration is not quite accurate, and his reconstruction of peer reaction to it differs substantially from what I can recollect and can at least partially document. For instance, he states in reference to our work, "the available techniques were not really adequate for an unambiguous demonstration that the adult-generated cells were neurons rather than glia" (Gross 2000, p. 68). As I see it, no neuroanatomist looking at our early illustrations, unless blinded by prejudice, should have failed to see that the reduced silver grains in our autoradiograms were unambiguously over dentate granule cells (see, Figs 2 and 5). Neuroanatomists familiar with the cytology of the hippocampus know that the stratum granulosum is a distinctive neuronal layer that is densely packed with granule cells distinguished by pale nuclei, a layer that is virtually devoid of smaller and more darkly staining neuroglia cells. And what more direct and unambiguous way is there to demonstrate nuclear DNA duplication and cell proliferation than using ^{3}H-thymidine, a selective marker that can be tracked over several generations as it is diluted with successive cell divisions (Fig 5)? In his review of the discovery of neurogenesis in adult birds, Fernando Nottebohm graciously states a few years later that "Altman's observations and suggestions on postnatal neurogenesis were original and his wording was cautious … Yet at the time, his claims met stiff resistance." Nottebohm justifies this peer reaction by saying, "It has been often said that the standard of proof is proportional to the importance and novelty of a claim" (Nottebohm, 2002, pp. 737-738), and he lists, again, among the several possible objections that the critics may have entertained that the labeled cells were not neurons.

More recently, Gerd Kempermann (2006) reproduced my earliest illustration of ^{3}H-thymidine-labeled dentate granule cells (Altman 1963), and refers to them as "the first known depiction of adult neurogenesis" (Kempermann 2000, p. 37). Kempermann continued, with reference to a subsequent paper I published with the late Gopal Das (Altman and Das, 1965): "One strength of this study was that it sought evidence of neuronal development and did not deliver a mere snapshot in time." Another strength was, Kempermann wrote, the demonstration with a second method "that postnatal and adult hippocampal neurogenesis caused a sixfold increase in the number of granule cells between 6 days after birth and the age of 3 months" (Kempermann 2000, p. 38). What Kempermann might have added is that, in fact, we used extensively an additional methodology to support postnatal hippocampal neurogenesis, as well as a series of studies exploring its possible functional importance. As I mentioned earlier, we used focal irradiation of the hippocampus with low-level x-ray to destroy the radiosensitive precursors of dentate neurons, a procedure that consistently produces a hippocampus with only about 15 percent of the normal complement of granule cells, the same percentage that we were estimating to form prenatally with quantitative histological and autoradiographic techniques.

With regard to the behavioral significance of the 85 percent of granule cells that are acquired postnatally, our research showed that the prevention of this development produces behavioral abnormalities that mimic partial or total destruction of the hippocampus.

My recollection of my standing in the scientific community during the 1960s also differs from Gross's account. Gross says, referring to me: "he was a self-taught postdoctoral fellow working on his own in a Psychology Department …" (Gross, 2000, p. 68), and, referring to the dogma of 'no-new-neurons in the adult brain,' speculates about "the difficulty that unknown and junior scientists have in challenging such traditions" (Gross 2000, p. 72). As a matter of fact, I did have the proper academic credentials; I was known in the neuroscientific community; and most of the scientists who tried to stop my work were younger and many of them were less well established than I was. To begin with, on the strength of my preceding training in neurophysiology and neuroanatomy (which I alluded to above), and possibly in recognition of my accomplishments, I was appointed to the faculty of MIT in 1962, with the rank of Associate Professor. The position involved the usual academic obligations as well teaching several courses in behavioral neurobiology. To support the latter task, I used a mimeographed textbook (entitled, *The Biology of Behavior*), which was later published and became widely distributed nationally and internationally (Altman 1966b).

Charles Gross leaves the impression in his historic review that because I was an unknown scientist my early publications could be ignored by the neurobiological community. However, my recollection is that they were not ignored at all but created considerable publicity. The discovery of postnatal neurogenesis was widely reported in the press at the time and it created considerable interest, if not excitement, among many researchers for years thereafter. For instance, I still have a copy of an article in the *Washington Post* about the possible importance of the discovery of adult neurogenesis in mammals, written by Joshua Lederberg (pioneer molecular biologist, later president of the Rockefeller University). Referring to one of my papers (Altman 1967), Lederberg wrote: "In a nutshell, Dr. Altman has obtained good evidence for the continued multiplication of neurons in young rats or kittens, especially in a region of the brain called the hippocampus" (Lederberg 1968, pA15). [To be precise, I actually argued for the multiplication of the "precursors" of neurons.] And in reference to another paper that appeared in *Developmental Psychobiology* (Altman et al 1968), Lederberg wrote approvingly about the possibility of "stretching out the period of the brain's development" by "intentionally handling or playing with young rats for just 15 minutes daily during their first 11 days of life." "The main point we have to ponder," he wrote, "is the complicated interplay between the environmental experience of the rat (or human infant?) and the development of the actual structure of the brain" (Lederberg, 1968, p. A15).

As I remember it, I got too much rather than too little attention from the scientific community during the 1960s. There were endless invitations to give lectures at different universities throughout the United States. Perhaps after repeating the same lectures over and over again my performance became lackluster. After all, what I wanted was not publicity but to get back to the laboratory and continue the exciting but time-consuming research we were engaged in. Nor were we isolated once back in the laboratory. I recall visits, some lengthy ones, from many scientists from the USA as well as abroad. Among the visitors I recall

John Eccles from Australia, Otto Creutzfeldt from Germany, Jean Piaget from Switzerland, Jerzy Konorski from Poland, Michel Jouvet from France, and several others. I also recall William Windle (then the editor of *Experimental Neurology*) coming by, asking questions and examining our histological slides, and encouraging me to send our forthcoming papers on neuronal regeneration to his journal. I recall Maxwell Cowan (who was to become the editor of the *Journal of Comparative Neurology*) visiting the laboratory and assuring me that, unlike the retiring editor, he will see to it that our papers will be promptly put through the review process. I also recall an invitation from Dominic Purpura (then editor of *Brain Research*) to submit papers to his journal. Moreover, I was invited by the outgoing editor of the *Journal of Comparative and Physiological Psychology* to take over the editorship of the journal (which I politely declined) and from the editors of *Experimental Brain Research* to become the editor of the developmental neurobiology section of that journal (which I accepted). I was grateful for this initial expression of support from the scientific community, and with the generous financial support that we were receiving from three government agencies we were able to pursue our research goals and disseminate the data we gathered.

But then things started to change in the late 1960s, although it took me several more years to realize that something was amiss. The first wake-up call came when I was supposed to be granted tenure at MIT and my promotion was denied. According to the letter I received, some outside referees did not consider my promotion justified. That was no problem. By that time I intended to leave the Boston area for personal reasons and had received expressions of interest as well as several firm and attractive offers from a number of universities to join their faculty. I accepted the offer from the Department of Biological Sciences of Purdue University and joined it as a tenured professor in 1968. I selected that Department not only because of the generous support I was offered but also because I thought that moving to a rural milieu will give me and members of my laboratory the peace and quiet that we needed to pursue our research. Indeed, we succeeded in establishing a productive laboratory, one that remained in operation at Purdue University (West Lafayette) and later at Indiana/Purdue University (Indianapolis) for over three decades. We continued to receive generous public funding for many years and had little difficulty in publishing the papers that we were submitting to various peer-reviewed journals. Inded, the papers must have found an audience. According to a note in *Current Contents* in 1981, analysis of the *Science Citation Index* database for 1965-1978 indicated that I was among the 1000 most cited authors in all the sciences (Garfield, 1981). The list contained only 24 individuals in the neurosciences under the categories: “neurology”, “neurobiology,” and “neuropharmacology.” I would not argue that the number of citations could in any sense be construed to reflect scientific merit or significance. I merely want to point out that our work was widely read and quoted by our peers during that period.

1.3.2. MARGINALIZATION.

I now know that there was a concerted attempt by some influential members of the neuroscientific community to marginalize us, but at the time I did not pay much attention to it. Reaching for some textbooks on my bookshelf published during the 1970s on developmental neurobiology, I find the flat statement by Marcus Jacobson: “there is no convincing evidence of neuron production in the brain of adult mammals …” (Jacobson 1970, p. 33). Jacobson

modifies this dogmatic statement somewhat in the second edition of his book: "There is no evidence showing that neurons are formed in the subventricular zone in adult mammals, although granule cells continue to be formed in various other germinal zones for a relatively short period after birth" (Jacobson 1978, pp. 72-73). While he refers to several of our papers on adult neurogenesis, he misquotes them to the effect that "granule cells of the fascia dentate originate from day 10 of gestation to postnatal day 20 …" (Jacobson 1978, p. 73). That position that was advocated by Angevine (1965) not by us. Another introductory text on brain development (Lund 1978), makes no reference at all to adult neurogenesis and quotes none of our papers on that subject.

This neglect of our work continued during the 1980s. As I continue checking my bookshelf, I find no reference to adult neurogenesis in the textbook on developmental neurobiology by Purves and Lichtman (1985) or in the chapter dealing with the development of the CNS in Eric Kandel's first edition of the *Principles of Neural Science* (Schacher, 1981). In the third edition of that textbook, published in 1991, Jessell states: "Neurogenesis ceases early in the development of the mammalian brain, but persists into adulthood in some vertebrates, such as fish and birds. For example, Fernando Nottebohm and his colleagues found that the number of neurons in certain nuclei in the brains of adult songbirds changes cyclically on a seasonal basis." Denying postnatal mammalian neurogenesis, Jesssell qualifies the preceding statement by saying that "…neurons can be generated from undifferentiated progenitor cells in the brain of some adult nonmammalian vertebrates. The underlying mechanisms of such differentiation are not understood well enough to determine whether cells in the adult mammalian central nervous system have a similar potential" (Jessell, 1991, p. 268). Reference to our published evidence of postnatal neurogenesis was omitted not only by these widely circulated introductory textbooks (of course, I find textbooks that did refer to our work) but also in some more advanced publications. For instance, a book of reprints in developmental neurobiology, with 47 journal articles from the 1960s and 1970s, contains none of our papers (Patterson and Purves1982). The book contains three reprints authored or co-authored by Pasko Rakic but there is no reference in any of them to our work (although he was fully familiar with our demonstrations). There was obviously a movement afoot to marginalize us.

1.3.3. DISQUALIFICATION

Open criticism of a scientific claim that runs counter to an established dogma is fully justified; indeed, such a claim must be subjected to a thorough scrutiny before the established paradigm is prematurely abandoned. However, I am not aware of any public criticism or rebuttal of the data we have presented. Moreover, the dismissal of our claim for postnatal neurogenesis has increased, rather than decreased, as we gathered more and more evidence in its favor, and linked the phenomenon of postnatal neurogenesis to its prenatal antecedents. Instead of open criticism, there appears to have been a clandestine effort by a group of influential neuroscientists to suppress the evidence we have presented and, later on, to silence us altogether by closing down our laboratory. I make this accusation for the following reasons: (a) by the early 1980s we were starting to have difficulties in getting our grant applications approved; (b) by the mid-1980s we lost *all* our grant support; and (c) by the early 1990s we had several of our submitted papers outright rejected. [The latter included a series of three papers in which we

reported the discovery of the "stratified transitional field" in the developing rat cerebral cortex. The papers were cited as "Altman J, Bayer SA, submitted, 1991a, 1991b, 1991c" in Bayer and Altman 1991.] In fact, we have never since been able to get our grant applications approved, not even when the topic of our research shifted from adult neurogenesis to the comprehensive embryological study of CNS development in rats (Bayer and Altman 1991; Altman and Bayer, 1995, 1997) and, later, in humans (Altman and Bayer 2001, 2002; Bayer and Altman, 2002-2008).

What might have possessed a group of influential scientists to seek to destroy a dedicated and productive laboratory? I do not have a factual answer to these questions because I have never been informed of the reasons why we were disqualified. What I know unfortunately that the laboratory lost all its public support by the mid-1980s, at about the time when Pasko Rakic published his widely quoted paper in which he denied neurogenesis in the brains of adult primates (Rakic 1985). We were not the only targets of Rakic's public disapproval of adult neurogenesis. I recall a meeting organized in 1984 in New York City by Fernando Nottebohm, called *Hope for a New Neurology*, with a focus on adult neurogenesis. (I was not invited to speak at that meeting but my wife, Shirley Bayer, was and I have accompanied her). At that occasion Rakic berated Michael Kaplan who reported his work on postnatal neurogenesis in rats. Kaplan, an enthusiastic young scientist at the time, later described that experience as the "death of a dogma and a research career" (Kaplan, 2001). In the late 1970s Kaplan presented his combined electron microscopic and autoradiographic evidence that the radioactively labeled, newly formed hippocampal and olfactory bulb granule cells of adult rats had axons, dendrites and synapses and, hence, met all the ultrastructural criteria of neurons (Kaplan and Hinds 1977). Instead of being congratulated for presenting this new evidence, Kaplan was severely castigated. Having rejected the evidence for postnatal neurogenesis in rodents in the 1970s, Rakic later admitted the possibility of that phenomenon in lower mammals but, as noted, denied that in primates. He stated categorically: "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" (Rakic 1985, p. 1054). But Rakic was wrong again. Several reports were published by the late 1990s of postnatal neurogenesis in the monkey hippocampus (e.g., Gould et al 1999) and also in humans (Eriksson et al 1999). As the evidence mounted, Rakic joined these authors (now using BrdU rather than ^{3}H-thymidine) and reported that there is "continuous generation of neurons, oligodendrocytes, and astrocytes in the hippocampal dentate gyrus of adult macaque monkeys" (Kornack and Rakic, 1999, p. 5768). Why did Rakic fail earlier to obtain autoradiographic evidence for postnatal neurogenesis in primates? He owed the scientific community an explanation but offered none. Instead, he gave the historical record a new twist by stating that "Unlike cells in most tissues … most neurons of the mammalian brain are entirely generated during early development … One exception, which was first suspected in rodents 30 years ago [quoting Altman and Das 1965] and later substantiated [quoting Kaplan and Hinds 1977] is the granule neurons of the dentate gyrus of the hippocampus" (Kornack and Rakic, 1999, p. 5768). Note the disingenuous term "suspected" to characterize

our multipronged demonstrations. Kaplan is now "in" (after all, he is no longer a competitor) but Altman is still "out."

How could a coterie of scientists succeed in making a productive laboratory terminally lose its public funding? Were we blacklisted? I consider that unlikely. We have never been accused of doing anything wrong and have remained productive to this day. The criteria for granting government research awards, as I heard it over and over again when I served on grant-awarding panels, are "scientific merit" and "past performance." And what might have justified professional administrators, serving as trustees of public funds, to try to close down a productive laboratory without an open public inquiry? I suspect that both sociological and psychological factors contributed to our disqualification. The sociological factors are easy to reconstruct; the psychological factors are more difficult and speculative.

To begin with the sociology. Neurobiology was still a Small Science when I entered the field in the early 1960s. There were relatively few neuroscientists around, the expense of doing neurobiological research was far more modest than it is today, and any capable and hard working researcher could accomplish a lot with the assistance of a few collaborators and technicians. However, within a few decades neurobiology turned into a Big Science, with many more applicants seeking public funding than the granting agencies could possibly support. Universities admitted ever more graduate students to be trained in the neurosciences, research laboratories increased in number and size, and consequently there arose a fierce competition for research and training grants by the 1980s. As this transformation took place, many neuroscientists who started out as bona fide researchers turned into administrators and public relation managers. Instead of sitting at the workbench, the job of the "principal investigator" became to coordinate what went on in his or her laboratory, edit papers written by associates who did the research that he or she no longer had the time (and sometimes the training) to perform, and spend endless hours politicking and paper work to keep the laboratory financially solvent. The leader of a research group had to engage in public relations work at home to drum up interest in the work the laboratory was pursuing; write progress reports and write re-write grant applications; spend days traveling from one place to another to attend meetings and conferences to find outside supporters and confederates; and, above all, establish good relationship with the administrators of public funds to obtain preferential treatment. While initially it did not much matter whom you knew but what you knew, increasingly it became more and important whom you knew, and how many of them you knew, to get funded.

Perhaps the idea of training so many neuroscientists, all of whom could not possibly be supported by available funds, was that those with the greatest ability and perseverance will prevail and those less well qualified will leave the field and pursue some other career. Unfortunately, instead of the scientifically best qualified, faculty positions were increasingly occupied by those with an administrative bent and great political skills; people who could attracts large sums of money and support their institution. As a consequence of this selection process, the panels of granting agencies and the editorial boards of journals – which became flooded with grant applications and manuscripts – increasingly became filled with a new breed of scientists, individuals skilled in forming alliances to support one another's projects and getting ahead of their competitors. I have personally witnessed that just a few derogatory

remarks made by one or two members of a panel judging a grant application meant that the unfortunate applicant received a "priority" rating that was officially "approved" but was not funded. The same may happen when a biased editor sends a submitted manuscript to a reviewer known to be hostile to the author or his group.

Let me now turn to two possible psychological factors contributing to our disqualification. First, we bucked the trend by practicing Small Science in an environment that increasingly favored Big Science. By spending endless hours in the laboratory and doing very little public relations work inevitably led to our isolation. Having failed to spend the necessary time and effort in the market place, we failed to recruit a cadre of confederates and supporters. Students and postdoctoral fellows quickly learned while they listened to popular speakers making their rounds, and dominating endless symposia and conferences, as to who was "in" and who was "out"; whom to quote or not quote in your bibliography to make it more likely that it will be reviewed by a peer sympathetic to your approach or findings; and what line of research to pursue in light of what is favored or not favored by the granting agencies at any given time. Again, in my personal experience, I watched how most of my former students and even associates, realizing that we were out of favor, stopped working on problems related to postnatal neurogenesis (which they received their training for) and found other projects to commit themselves to or abandoned their research career altogether. But there may have been another psychological factor that has actually contributed to our becoming outcasts. Big Science needs administrators. In addition to those who turn to administration with the selfish motive of exercising power and influence, there are undoubtedly those who sacrifice their research career for the public good. But that sacrifice may have unfortunate consequences. What scientist would not rather make a great discovery himself or herself than be the cheerleader of the group that makes such a discovery? The researcher turned administrator may console himself or herself that in an age of Big Science that is no longer possible. But what if that is contradicted by an individual or a small group of researchers who come up with a new discovery? They become the envy of the advocates of Big Science. They are liable to be distrusted and their claim discounted as unlikely to be true.

1.1.4 PROSPERING UNDER ADVERSE CONDITIONS AND SWITCHING TO THE COMPREHENSIVE STUDY OF CNS DEVELOPMENT

How did our laboratory survive, indeed prosper, without research grants and how could we remain productive while facing peer indifference and outright hostility? And why did we turn by the early 1980s from focusing on postnatal and adult neurogenesis to the study of the embryonic development of the CNS? To answer the first question I have to divulge some personal matters that I have never before aired in public. To the second question I have a more objective answer.

Our laboratory survived and prospered, notwithstanding peer hostility and the withdrawal of all public funding, because several circumstances worked in our favor. The most important of these was that by the time we could no longer had public funds we have amassed a large

collection of fully-processed embryos and brains. Financing the work ourselves, we were in a position to analyze the available materials and, instead of submitting papers to mainstream journals (a costly and painful enterprise) we started to summarize our results in books that also yielded some royalty. But there was also a psychological factor; namely, that I was well prepared by my earlier life experiences not to be discouraged by public indifference and hostility. When I committed myself to the study of brain-behavior relationships as a teenager (and wrote my first programmatic essay on the subject) I was barred from finishing my formal education in quasi-fascist Hungary because I belonged to a disenfranchised religious/ethnic minority. When the Wehrmacht occupied Hungary, I was incarcerated in a forced-labor camp and worked on a railroad gang. After some time there, I escaped and lived clandestinely in Budapest, which very soon came under siege. My survival was aided, I tend to believe, by a syndrome that I developed, what I call "aparanoia." Blissfully neglecting the fact that they were after my kind, I did not hide but walked with head erect through the streets, past bayoneted soldiers and gendarmes looking for Jews and deserters. I convinced myself that I was going to survive no matter what the Nazis' intentions were. When the Red Army liberated us in 1945, I resumed my formal education. However, I could not tolerate the rising Communist dictatorship and fled the country in 1946. I became a stateless, displaced person in West Germany, waiting there for many years before I could secure the requisite documents to migrate to Australia. While that sounds like a misfortune, those were very good years. I was able to attend lectures and seminars at a prominent German university and spent endless hours in its library. I continued informally with my education in Australia, where I worked through the first half of the 1950s as a college librarian. In my spare hours there, I wrote the bulk of the manuscript that was later published as a textbook in behavioral neurobiology (Altman 1966).

When I migrated to the United States in 1955, I embarked on a research career in neurobiology by getting formal predoctoral and postdoctoral training at different facilities in New York City (described above). In 1959 I organized a small laboratory at the New York University Medical School and worked there with Elizabeth Altman and some student assistants. In the early 1960s we moved to the Massachusetts Institute of Technology (also described above) and after working there for many years, the greatly expanded laboratory and staff was transferred to Purdue University in 1968. At Purdue I had the great fortune that Shirley Bayer joined the laboratory as a graduate student in 1970. Although we came from a very different background, we soon established a close personal and professional partnership. For many years we both received generous public grants, and the laboratory became very productive. Then abruptly we lost all our funding and had to face all the consequences that lack of financial support produces in the academic world. How did we react to that misfortune? We thought of fighting our disqualification and did some protesting. But, not being able to accomplish anything, we decided that it made better sense to finance our research ourselves rather than waste our time and energy in writing and re-writing grant applications and battling our adversaries. This is probably not the course of action that many scientists might have taken in a similar situation but I was adequately prepared to work in isolation.

To turn to second question: Why did Shirley Bayer and I turn by the mid-1980s from the study of postnatal and adult neurogenesis to the embryonic development of the CNS in rats and humans? Perhaps peer indifference contributed to our reduced enthusiasm for that

work. But a more important consideration was our conviction that adult neurogenesis must be studied as a facet of CNS development. We maintained all along that adult neurogenesis is the end phase in the continuum of CNS development. Accordingly, we embarked in the mid-1970s on a long-term project to re-examine the whole course of CNS development by combining traditional histological techniques with ^{3}H-thymidine autoradiography. By the end of the 1980s we had prepared close to 2000 histologically and autoradiographically processed prenatal and postnatal rat embryos and dissected brains. This enabled us to carry out a detailed investigation of the prenatal and postnatal development of the rodent cerebellum, spinal cord, brainstem, diencephalon, hippocampus and basal ganglia, as well as some components of the cerebral allocortex and neocortex (references to these studies are listed in Altman and Bayer 1982; Bayer and Altman 1991, 2004; Altman and Bayer, 1995, 1997). We then turned in the mid-1990s to the study of human CNS development. To accomplish that, we worked for months at the National Museum of Health and Medicine in Washington, DC, which houses the Carnegie, Minot, and Yakovlev Collections of prenatal and postnatal human brains. (We did seek grant support for this effort but got none; except that the high daily fee to use the facility was graciously waived.) We took over 10,000 low- and higher-power photomicrographs there in preparation for a 5-volume *Atlas of Human Central Nervous System Development*. That task was completed recently (Bayer and Altman, 2002-2007).

Our developmental approach, we believe, has shed some light on the nature and significance of postnatal and adult neurogenesis (Altman and Bayer 2007). It brought support for our concept of two neurogenic production sites in the developing CNS: the primary neuroepithelium (NEP) and the secondary germinal matrices (SGM). The NEP is composed of stem cells and progenitor cells of the macroneurons that form the core structures and gross circuitry of the CNS. It originates early during embryonic development as the open neural plate. After the neural plate folds and fuses dorsally, the lumen of the cephalic vesicles become filled with cerebrospinal fluid to form the narrow protoventricles. The protoventricles then expand to form the superventricles of the telencephalon, diencephalon, mesencephalon, and rhombencephalon. Because the stem cells and progenitor cells of the NEP have to shuttle to the ventricular lumen to undergo mitosis, the size and configuration (eminences, protuberances, invaginations) of the variegated shorelines of the superventricles are major determinants of the number of neurons generated for different brain structures at different NEP compartments (mosaics). We provided some evidence that the NEP is initially composed of stockbuilding precursor cells that expand and form the region-specific compartments, and postulated that their proliferation and progressive fate-restriction are dependent on two important regulatory mechanisms: hypothetical trophic factors contained in the cerebrospinal fluid system, and reciprocal signaling between the NEP and the concurrently developing peripheral sense organs and motor systems. In the next stage of CNS development, the stockbuilding NEP cells start to generate exiting daughter cells of two types, postmitotic cells that differentiate as neurons and neuroglia, and distinctive precursor cells that retain their proliferative capacities. The postmitotic young projection and relay neurons migrate radially, tangentially and, at some sites, follow tortuous routes and sojourn in intermediate zones before they settle down. These early-generated neurons form the brain parenchyma by filling the expansion spaces of a unique, hitherto unrecognized tissue, the initially expanding then shrinking superarachnoid reticulum. In mammals, the periventricular NEP is principally active during the prenatal period.

The NEP, as noted, is also the source of progenitor cells that form the SGMs. Unlike the NEP, the SGMs persist for varying periods postnatally and generate the locally-arborizing, short-axoned microneurons that become important constituents of the fine circuitry of certain brain structures. Among the SGMs of the developing mammalian brain are the following. (a) The bulk of the subependymal layers of the forebrain that generate the microneurons of the cerebral cortex and the neostriatum during the late embryonic and perinatal periods. (b) The subpial external germinal layer of the cerebellum that generates the microneurons of the cerebellar cortex during the infantile and juvenile periods. (c) The interstitial subgranular zone of the hippocampus that generates the microneurons of the hippocampal dentate gyrus throughout life. (d) The circumscribed subependymal layer of the anterior cerebrum that, likewise, generates the microneurons of the olfactory bulb throughout the entire life span of all mammals. While the macroneurons that are generated in mammals during gestation are minimally affected by extrauterine events, the postnatally generated microneurons of the cerebellum, olfactory bulb, and hippocampus are subject to considerable environmental influences. That led to our earlier suggestion that they may play a major role in responding to environmental factors, perhaps by mediating certain types of learning processes (Altman, 1967, 1970).

1.1.5 THE CHANGED PARADIGM IN THE STUDY OF ADULT NEUROGENESIS

Scientific theories – unlike political attitudes, religious faiths or aesthetic judgments – are not matters of personal or group preferences. A person's or a group's political views are understandably guided by economic and social interests, and idiosyncratic or conventional preferences. Religious beliefs are based on social tradition and indoctrination, and a faith in a supernatural world inaccessible to the senses. Aesthetic choices may be a matter of personal taste or of changing fads, fashions or artistic trends. But scientific theories cannot be sustained by appeal to such subjective values. Scientific theories must comply with the objective facts ascertained by empirical observations and research findings; they stand or fall as new data gathered confirm them or refute them. Of course, science is pursued by human beings with different interests, backgrounds and tastes, and therefore personal and social factors necessarily play a role what a scientist studies and what theories he or she will favor. But sooner or later the facts will prevail and no matter how powerful or prestigious the supporters of a refuted theory, the theory will eventually be abandoned. In the past, with few scientists around, false scientific theories, such as the Ptolemaic system of the structure of the universe, could be upheld for some time after it has been shown to be inadequate. Nicolas Copernicus' *De revolutionibus orbium coelestium* was published in 1543, providing good astronomical evidence that the earth rotates around the sun, but his theory was not widely accepted until 1687, when Isaac Newton, using Johann Kepler's additional observations and calculations, developed his theory of gravitation in his *Philosophiae naturalis principia mathematica*. In the meantime the ecclesiastical authorities censured and put under house arrest Galileo Galilei, and burned Giordano Bruno at stake, because thy dared to argue in favor of the heliocentric theory. As science has become such a large enterprise with so many practitioners, untenable theories fall by the wayside much faster nowadays than they did in the past. The theory of adult neurogenesis, of course, does not have either the same global significance as the theory of gravitation nor does it challenge the biblical account of the Earth's place in the solar system. But the theory of adult neurogenesis

does have important theoretical and practical implications. It supports an emerging new view of the CNS, one with greater dynamic properties and regenerative potentials than was hitherto contemplated. Because of the rapid increase in the pace of scientific progress, it took only about 20-30 years that an old dogma of "no postnatal neurogenesis" was abandoned by all but a few diehard scientists.

The study of adult vertebrate neurogenesis became a respectable research endeavor in the 1980s when Fernando Nottebohm and his collaborators reported their findings about the seasonal turnover of neurons in the forebrain of songbirds (Goldman and Nottebohm 1983; Alvarez-Buylla and Nottebohm 1988; see the next Chapter for details). And the study of mammalian neurogenesis became a truly popular subject soon thereafter when two new techniques were introduced. One was the increased use of bromodeoxyuridine (BrdU), instead of ^{3}H-thymidine, to label the proliferative precursors of neurons. The other was the introduction of various molecular markers for the identification of the proliferating, differentiating and maturing neurons. These two techniques were very important innovations. The BrdU procedure is much simpler and faster than ^{3}H-thymidine autoradiography. Indeed, I suspect that one of the reasons why more investigators have not studied adult neurogenesis in the 1970s and 1980s was that they were discouraged by the difficulty of effectively using the liquid emulsion technique for high-resolution ^{3}H-thymidine autoradiography. We spent several years to develop a reliable and consistent methodology. This included the use of ^{3}H-thymidine of the right specific activity; injection of the optimal dose of the radiochemical in relation to the animal's body weight; proper and consistent dilution of the nuclear emulsion; slow drying of the emulsion in the dark before packaging and refrigeration to avoid mechanical artifacts; very long exposure period (we consistently used 3 months) to get optimal results; and, last but not least, use of counterstaining techniques that do not remove part or all of the emulsion. Significantly, I am not aware of reports of false positive results with ^{3}H-thymidine but only of false negatives (e.g., Rakic 1985). And I must also note that with all its advantages, the BrdU technique has its pitfalls. Most importantly, BrdU is a toxic substance that produces morphological and behavioral abnormalities, triggers cell death, and leads to the formation of teratomas (Kolb et al 1999; Sekerkova et al 2004; Taupin 2006). In contrast, with survival almost as long as 1 year after the administration of our standardized dose of ^{3}H-thymidine, we have observed no deleterious somatic effect in our experimental animals and little or no reduction in the number or proportion radioactively tagged granule cells (see, for instance, Fig 20). Hence the comparative validity and reliability of these two techniques warrants further investigation.

The molecular markers that have been added to the armamentarium of the traditional techniques for the identification of neurons and neuroglia have also been very useful in the study of adult neurogenesis. Validated molecular markers allow investigators to distinguish small neurons from different types of neuroglia, and identify stages in the differentiation of young neurons. However, there are again pitfalls because molecular markers are only *indirect* indicators whether or not a cell is a true neuron. *Direct* evidence requires the application of traditional anatomical, physiological and behavioral techniques. By definition, a cell is not a bona fide neuron unless it has dendrites and an axon; is capable of producing generator potentials and action potentials; has synapses that allow information transmission from sense

organs to neurons, from one neuron to the next, and from motor neurons to muscles or other effectors; and serves as a link in the coordination of behavior. Since differentiating, migrating and maturing young neurons may lack some of these features and properties, the cell identified with a neuronal marker may be a potential neuron, a neuron in the process of differentiation, or a cell that has some neuronal features but may never become a true functional neuron. Let me illustrate the pitfalls of reliance on markers in the categorization of developing CNS cells with reference to one of the earliest and most commonly used molecular marker in neuroscience, i.e., glial fibrillary acidic protein (Bignami and Dahl 1974). This protein (GFAP) is widely claimed to be a definitive marker for astrocytes. However, it has been known for some time that GFAP is present in germinal matrices where precursor cells generate both neurons and neuroglia (Levitt et al 1981), and the possibility has been raised that a marker that specifically reacts in the mature brain with glial filaments may also react with transient filamentous elements in progenitor cells destined to become neurons (Bennet 1987). For example, the precursors of granule cells in the hippocampal subgranular zone are GFAP-positive (Seri et al 2001; Namba et al, 2005). The assumption that GFAP is a definitive marker of astrocytes led some investigators to conclude that the hippocampal granule cells are progeny of astrocytes (e.g., Seri et al, 2001). However, a more reasonable conclusion would be that CNS progenitor cells, irrespective whether they will produce neurons or neuroglia, share the property of expressing GFAP (e.g., Steiner et al, 2004). That would invalidate the currently popular theory that "radial glia" are progenitors of neurons (e.g., Anthony et al, 2004; Englund et al, 2005). The currently available evidence supports the view that both neurons and neuroglia derive from NEP and SGM precursor cells that share many molecular properties.

It will require the work of a professional science historian to reconstruct how attitudes have changed over the period when the study of postnatal and adult neurogenesis was not considered a topic deserving public support and the time when it became a popular subject matter studied by hundreds of investigators. Typing the term "hippocampal neurogenesis" into the search engine of Google Scholar (the date is 01-15-08) the query retrieves 5,150 references; "subgranular zone" retrieves 2,750 references; and "rostral migratory stream" retrieves 2,330 references. Having identified these phenomena (and coined the terms) in the decade between 1965 and 1975, we feel gratified that by 2008 they have captured the attention of so many investigators. (Never mind, that so many of the articles consulted do not credit us for these discoveries but attribute them to others. Why get involved in old controversy and risk unfavorable treatment by a reviewer in the opposing camp? Quoting an article recently published in mainstream journals is a safer bet. After all that passed the Imprimatur of a semi-official Establishment journal.) We also feel gratified that, judging by the increasing number of references to them, some of our later empirical and conceptual contributions are also becoming accepted. For instance, we have advocated for decades the replacement of the narrowly conceived term "ventricular zone" with the broader term "neuroepithelium." We did that to stress the fact that the NEP is not just a component of the maturing brain but the source of all its neural elements, one that forms and expands greatly before the brain has any differentiated neurons. Likewise, we have advocated the replacement of the term "external granular layer" of the cerebellum with "external germinal layer" to stress two facts. First, that this subpial tissue is not composed of "granule" cells, which is a universally accepted term for such differentiated neurons as the granule cells of the cerebellum, hippocampus and olfactory

bulb. Second, the term "germinal" reminds us that this transient proliferative matrix is an important component of a late-generated neurogenic system of the developing cerebellum.

Much of the foregoing is about the past. As we are entering a new period in neuroscience, what Nottebohm called the New Neurology, it is more rewarding to look forward and contemplate what the future may bring. The substantive chapters that follow this personal memoir, will acquaint the reader with some of the recent advances in the study of adult neurogenesis. Adult neurogenesis has been identified by now not only in all vertebrates, fishes, reptiles, birds, and mammals. Important investigations are being carried out in various laboratories about the physiological properties of the postnatally acquired hippocampal and olfactory bulb granule cells, their migration and integration into the circuitry of these structures, and their role in how animals adapt to their environment and learn. Also of great promise are the exploratory studies concerned with the possible role of adult neurogenesis in stress disorders and depression, and such brain abnormalities and pathologies as schizophrenia, Parkinsonism, stroke and ischemia. There is no way to predict what these ongoing studies will bring to light but the full examination of the dynamic properties and remedial potentials of the adult brain is certainly a well-justified scientific enterprise.

Acknowledgments: I thank Dr Tatsunori Seki for the invitation to present this personal memoir, and dedicate this contribution to Dr Shirley Bayer who shares credit for all that we have accomplished together in the last 3-4 decades.

REFERENCES

Altman J (1962a) Autoradiographic study of degenerative and regenerative proliferation of neuroglia cells with tritiated thymidine. Exp Neurol 5:302-318

Altman J (1962b) Are new neurons formed in the brains of adult mammals? Science 135:1127-1128

Altman J (1963a) Regional utilization of leucine-H^3 by normal rat brain: Microdensitometric evaluation of autoradiograms. J Histochem Cytochem 11:741-750

Altman J (1963b) Differences in the utilization of tritiated leucine by single neurones in normal and exercised rats: An autoradiographic investigation with microdensitometry. Nature 199:777-780

Altman J (1963c) Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anat Rec 145:573-591

Altman J (1966a) Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis in some brain regions. J Comp Neur 128:431-474

Altman (1966b) Organic Foundations of Animal Behavior. New York: Holt, Rinehart and Winston

Altman J (1966c) Proliferation and migration of undifferentiated precursor cell in the rat during postnatal gliogenesis. Exp Neur 16:263-278

Altman J (1967) Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory. In, Quarton G, Melnechuk T, Schmitt FO (eds), The Neurosciences: A Study Program, pp 723-743. New York: Rockefeller Univ Press

Altman J (1969a) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neur 137:433-458

Altman J (1969b) DNA metabolism and cell proliferation. In. Lajtha A (ed), Handbook of Neurochemistry, vol. 2, pp 137-182. New York: Plenum Press

Altman, J (1970) Postnatal neurogenesis and the problem of neural plasticity. In, Himwich WA (ed) Developmental Neurobiology, pp 95-197-237. Springfield IL: Thomas

Altman J (1986) An animal model of minimal brain dysfunction. In, Lewis M (ed) Learning Disabilities and Prenatal Risk, pp 241-304. Urbana: Univ Ilinois Press

Altman J (1987) Morphological and behavioral markers of environmentally induced retardation of brain development: An animal model. Environ Health Perspect 74:153-168

Altman J, Altman, E (1962) Increased utilization of an amino acid and cellular proliferation demonstrated autoradiographically in the optic pathways of pigeons. Exp Neurol 6:142-151

Altman J, Anderson WJ (1971) Irradiation of the cerebellum in infant rats with low-level X-ray: Histological and cytological effects during infancy and adulthood. Exp Neur 30:492-509

Altman J, Anderson WJ (1972) Experimental reorganization of the cerebellar cortex. I. Morphological effects of elimination of all microneurons with prolonged X-irradiation started at birth. J Comp Neur 146:355-406

Altman J, Anderson WJ, M Strop (1971) Retardation of cerebellar and motor development by focal X-irradiation during infancy. Physiol Behav, 7:143-150

Altman J, Anderson WJ, Wright KA (1969a) Early effects of X-irradiation of the cerebellum in infant rats: Decimation and reconstitution of the external granular layer. Exp Neur 24:196-216

Altman J, Anderson WJ, Wright KA (1969b) Reconstitution of the external granular layer of the cerebellar cortex in infant rats after low-level X-irradiation. Anat Rec 163:453-472

Altman J, Bayer SA (1975) Postnatal development of the hippocampal dentate gyrus under normal and experimental conditions. In, Isaacson RL and Pribram KH (eds) The Hippocampus, vol. 1, pp. 95-122. New York: Plenum Press

Altman J, Bayer SA (1982) Morphological development of the rat cerebellum and some of its mechanisms. Exp Brain Res, Suppl 6:8-46

Altman J, Bayer SA (1990a) Mosaic organization of the hippocampal neuroepithelium and the multiple germinal sources of dentate granule cells. J Comp Neur 301:325-342

Altman J, Bayer SA (1990b) Prolonged sojourn of developing pyramidal cells in the intermediate zone of the hippocampus and their settling in the stratum pryramidale. J Comp Neur 301:343-364

Altman J, Bayer SA (1990c) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. J Comp Neur, 301:365-381

Altman J, Bayer SA (1993) "Are new neurons formed in the brains of adult mammals?" A progress report, 1962-1992. In, Cuello AC (ed), Neuronal Cell Death and Repair, pp. 203-225. Amsterdam: Elsevier (Restorative Neurology, vol. 6)

Altman J, Bayer SA (1995) Atlas of Prenatal Rat Brain Development. Boca Raton, FL: CRC Press

Altman J, Bayer SA (1997) Development of the Cerebellar System in Relation to its Evolution, Structure and Functions. Boca Raton, FL: CRC Press

Altman, J, Bayer SA (2001) Development of the Human Spinal Cord: An Interpretation Based on Experimental Studies in Animals. New York: Oxford Univ Press

Altman J, Bayer SA (2002) Regional differences in the stratified transitional field and the honeycomb matrix of the developing human cerebral cortex. J Neurocytol 31:613-632

Altman J, Bayer SA (2007) Concluding essay. In, Bayer SA, Altman J, The Human Brain During the Early First Trimester, pp 426-489. Boca Raton, FL: CRC Press (Atlas of Human Central Nervous System Development, Vol 5)

Altman J, Das, GD (1964a) Autoradiographic and histological investigation of changes in the visual system of rats after unilateral enucleation. Anat Rec 148:535-545

Altman J, Das, GD (1964b) Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. Nature 204:1161-1163

Altman J, Das, GD (1965a) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neur, 124:319-336

Altman J, Das, GD (1965b) Postnatal origin of microneurones in the rat brain. Nature 207:953-956

Altman J, Das, GD (1966) Autoradiographic and histological studies of postnatal neurogenesis. J Comp Neur 126:337-390

Altman J, Das GD and Anderson WJ (1968) Effects of infantile handling on morphological development of the rat brain: An exploratory study. Dev Psychobiol 1:10-20

Alvarez-Buylla A, Nottebohm F (1988) Migration of young neurons in adult avian brain. Nature, 335:353-354

Angevine JB (1965) Time of neuron origin in the hippocampal region: An autoradiographic study in the mouse. Exp Neur, Suppl 2: 1-70

Anthony TE, Klein C, Fishell G, Heintz N (2004) Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 41:881-890

Bayer, SA (1982) Changes in the total number of dentate granule cells in juvenile and adult rats: A correlated volumetric and ^{3}H-thymidine autoradiographic study. Exp Brain Res 46:315-323

Bayer SA, Altman J (1974) Hippocampal development in the rat: Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. J Comp Neur 158:55-80

Bayer SA, Altman J (1975) Radiation-induced interference with postnatal hippocampal cytogenesis in rats and its long-term effects on the acquisition of neurons and glia. J Comp Neur 163:1-20

Bayer SA, Altman J (1991) Neocortical Development. New York: Raven Press

Bayer SA, Altman J (2004) Development of the telencephalon: Neural stem cells, neurogenesis, and neuronal migration. In, Paxinos G (ed) The Rat Nervous System, 3rd ed, pp. 27-73. New York: Elsevier

Bayer SA, Altman J (2002-2007) Atlas of Human Central Nervous System Development. (5 volumes) Boca Raton, FL: CRC Press (Taylor & Francis Group)

Bayer SA, Brunner RL, Hine R and Altman J (1973) Behavioural effects of interference with the postnatal acquisition of hippocampal granule cells. Nature New Biol 242:222-224

Bayer SA, Yackel JW and Puri PS (1982) Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life . Science 216:890-892

Bennett G (1987) Changes in intermediate filament composition during neurogenesis. Curr Topics Dev Biol 21:151-182

Bignami A, Dahl D (1974) Astrocyte-specific protein and neuroglial differentiation: An immunofluorescence study with antibodies to the glial fibrillary acidic protein. J Comp Neur 153:27-38

Brunner RL, Altman J (1973) Locomotor deficits in adult rats with moderate to massive retardation of cerebellar development during infancy. Behav Biol 9:169-188

Brunner RL, Hagbloom SJ, Gazarra RA (1974) Effects of hippocampal X-irradiation-produced granule-cell agenesis on instrumental runway performance in rats. Physiol Behav, 13:485-494

Diaz-Granados JL, Greene PL, Amsel A (1994) Selective activity enhancement and persistence in weanling rats after hippocampal x-irradiation in infancy. Behav Neur Biol 61:251-259

Englund C, Fink A, Lau C et al (2005) Pax6, Tbr 2, and TBR1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci 25:247-251

Eriksson PS, Perfilieva E, Bjork-Eriksson T et al (1998) Neurogenesis in the adult human hippocampus. Nat Med, 4:1313-1317.

Garfield E (1981) The 1,000 contemporary scientists most-cited 1965-1978. Part I. The basic list and introduction. Current Contents 41:5-14

Gazzara RA, Altman J (1981) Early postnatal X-irradiation of the hippocampus and discrimination learning in adult rats. J Comp Physiol Psychol 95:484-495

Gross CG (2000) Neurogenesis in the adult brain: Death of a dogma. Nature Rev Neurosci 1: 67-73

Gould E, Reeves AJ, Graziano MSA et al (1999) Neurogenesis in the neocortex of adult primates. Science, 286:548-552

Haggbloom, SJ, Brunner RL, Bayer SA (1974) Effects of hippocampal granule-cell agenesis on acquisition of escape from fear and one-way active-avoidance responses. J Comp Physiol Psychol 95: 86:447-457

Highfield DA, Hu D, Amsel A (1998) Alleviation of x-irradiation-based deficit in memory-based learning by D-amphetamine: Suggestions for attention deficit-hyperactivity disorder. Proc Natl Acad Sci 95:5785-5788

Jacobson M (1970) Developmental Neurobiology. New York: Holt, Rinehart and Winston

Jacobson M (1978) Developmental Neurobiology. Second ed. New York: Plenum Press

Jessell TM (1991) Reactions of neurons to injury. In, Kandel E et al, Principles of Neural Science (Third ed), pp. 258-269. New York: Elsevier.

Kaplan MS, Hinds JW (1977) Neurogenesis in the adult rat: Electron microscopic analysis of light radioautographs. Science 197:1092-1094

Kaplan MS (2001) Environment complexity stimulates visual cortex neurogenesis: Death of a dogma and a research career. Trends Neurosci, 24:617-620

Kempermann G (2006) Adult Neurogenesis: Stem Cells and Neuronal Development in the Adult Brain. New York: Oxford UP

Kolb B, Pedersen B, Ballermann M et al (1999) Embryonic and postnatal injections of bromodeoxyuridine produce age-dependent morphological and behavioral abnormalities. J Neurosci 19:2337-2346

Kornack DR, Rakic P (1999) Continuation of neurogenesis in the hippocampus of the adult macaque monkey. Proc Natl Acad Sci USA, 96:5768-5773

Lederberg J (1968) Stretching out the period of the brain's development. Washington Post, October 26, p. A15

Levitt P, Cooper ML, P Rakic (1981) Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: An ultrastructural immunoperoxidase analysis. J Neurosci 1:27-39

Lund RD (1978) Development and Plasticity of the Brain: An Introduction. New York: Oxford UP

Goldman SA, Nottebohm F (1983) Neuronal production, migration and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci USA, 80:2390-2394

Namba T, Mochizuki H, Onodera M et al (2005) The fate of neural progenitor cells expressing astrocytic and radial glial markers in the postnatal rat dentate gyrus. Eur J Neurosci 22:1928-1941

Nottebohm F (2002) Neuronal replacement in adult brain. Brain Res Bull, 57:737-749

Patterson PH, Purves D (1982) Readings in Developmental Neurobiology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory

Pellegrino LJ, Altman J (1979) Effects of differential interference with postnatal cerebellar neurogenesis on motor performance, activity level, and maze learning of rats. Comp Physiol Psychol 93:1-33

Purves D, Lichtman JW (1985) Principles of Neural Development. Sunderland, MA: Sinauer Associates.

Rakic P (1985) Limits of neurogenesis in primates. Science 227:1054-1056

Rosselli-Austin L, Altman J (1979) The postnatal development of the main olfactory bulb of the rat. J Dev Physiol 1:295-313

Schacher S (1981) Determination and differentiation in the development of the nervous system, In, Kandel ER, Schwartz JH (eds) Principles of Neural Science, pp. 503-520. New York: Elsevier

Sekerkova G, Ilijic E, Mugnaini E (2004) Bromodeoxyuridine administered during neurogenesis of the projection neurons causes cerebellar defects in the rats. J Comp Neur 470:221-239

Seri B, García-Verdugo JM, McEwen BS et al (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci 21:7153-7160

Smart I (1961) The subependymal layer of the mouse brain and its cell production as shown by radioautography after thymidine-H^3 injection. J Comp Neur 116:325-347

Smart I, Leblond CP (1961) Evidence for division and transformations of neuroglia cells in the mouse brain, as derived from radioautography after injection of thymidine-H^3. J Comp Neur 116:349-367

Steiner B, Kronenberg G, Jessberger S et al (2004) Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46:41-52

Taupin P (2006) BrdU immunohistochemistry of studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. Brain Res Rev 53:198-214