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Autoradiographic and Histological Evidence of Postnatal Hippocampal Neurogenesis in Rats [1]

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ABSTRACT In the autoradiograms of young rats injected with thymidine-H^3 many of the granule cells of the dentate gyrus were found labeled. The number of labeled cells declined rapidly with increased age at the time of injection. Histological studies showed the presence in young rats of a large germinal matrix of mitotic cells in the ependymal and subependymal layers of the third and lateral ventricles. The areal extent and cell population of this germinal pool declined rapidly from birth on, with a transient rise with a peak at about 15 days. During this latter period the number of "undifferentiated" cells near the granular layer of the dentate gyrus showed a rapid rise with a subsequent decline. The decline in the number of "undifferentiated" cells was accompanied by a rise in the number of differentiated granule cells. Cell counts in homologous parts of the dentate gyrus indicated a six-fold increase in the number of differentiated granule cells from birth to three months. We postulated that undifferentiated cells migrate postnatally from the forebrain ventricles to the hippocampus where they become differentiated. The possible functional significance of delayed hippocampal neurogenesis is discussed with reference to our finding of incorporation of testosterone-H^3 by cells of the hippocampus, implicating that they may function as receptors of gonadal hormones.

It is commonly held that neurons in the central nervous system of higher vertebrates are formed during embryonic development and that neurogenesis does not occur postnatally. This belief is based on the absence of neurons with mitotic figures in the brains of adult birds and mammals, in general, and the absence of signs of regenerative neuronal proliferation following brain lesions or trauma in particular. This conclusion has not been seriously questioned until recently, even though some investigators argued for the existance in the mature brain of "indifferent cells" (Schaper, 1897) or "medulloblasts" (Bailey and Cushing, '25) which can differentiate into neurons (for a history, see Globus and Kuhlenbeck, '44; Jones, '32; Kershman, '38), while others claimed to have observed mitotic neurons in young mammals (for a history, see Kjellgren, '44).

In pilot studies employing fine-resolution autoradiography, we have recently observed (Altman, '62b) that following intracranial injection of thymidine-H^3 into young adult rats there was an accumulation of reduced silver grains over the nuclei of a few neurons in the neocortex and, more commonly and consistently, over the granule cells of the hippocampus. This finding was subsequently confirmed in normal adult rats and adult cats after intraperitioneal or intraventricular injection of thymidine-H^3 (Altman, '63a). Since there is good evidence that thymidine, a specific precursor of chromosomal DNA, is utilized exclusively by the nuclei of cells that are preparing for multiplication (Hughes, '59; Hughes, et al., '58; Leblond, et al., '59; Taylor, et al., '57), these results suggested the possibility of neurogenesis in some forebrain structures in adult mammals. That the autoradiographic "labeling" of cell nuclei in the brain following injection of thymidine-H^3 is associated with cellular proliferation was supported by our observation of the labeling of a good proportion of those glia cells that were induced to multiply in regions of experimental brain lesions or in areas structurally and functionally connected with the traumatized sites (Altman, '62a). The same conclusion could also be drawn from the finding that neuroglia and microglia cells

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tended to be labeled at loci of "spontaneous" gliosis in "normal" animals (Altman, '63).

We have recently undertaken a series of studies in an effort to clarify the problem of postnatal neurogenesis and to throw light on the nature and significance of postnatal cell multiplication in the brains of rats: (a) To obtain information about the longitudinal course of cell labeling during development and maturation, rats of different ages were injected with thymidine-H^3 and killed after a constant survival period following injection. (b) To assess the role of pathological processes in cell mutiplication of the brain, thymidine-H^3 was injected in another group of young rats following the production of small cortical lesions. (c) In order to obtain data about the possible transformation of labeled cells over time, their migratory behavior and life span, adult rats were injected with thymidine-H^3 and their brains were removed after different survival periods. Finally, (d) to gather independent evidence of cellular multiplication, we have undertaken the histological evaluation of a large series of brains obtained from non-injected rats of different ages. In this paper we shall deal, under separate headings, with our findings regarding the uptake of thymidine-H^3 by cells in the hippocampus and with the histological evidence corroborating postnatal hippocampal neurogenesis.

I. The autoradiographic evidence

MATERIALS AND METHODS

Three groups of Long-Evans hooded rats were injected intraperitoneally with thymidine-H^3. The first group, in which survival after injection was held constant and age at the time of injection varied, consisted of pairs of animals aged one, four, six and eight months. These animals were injected on successive days in two equal doses with a total of 10 μc/gm body weight of thymidine-H^3 (specific activity 6.7 C/mM, the radiochemical dissolved in isotonic saline) and killed after a survival period of two weeks. A second group of rats was injected with thymidine-H^3 in an identical manner at the age of four months, and the brains were removed in one animal after four days, and in pairs of animals after survival periods of two weeks, two, four, six and eight months. In a third group of young rats, small, unilateral striate cortex lesions were produced at the ages of 10, 20 and 40 days. These animals were injected with thymidine-H^3, 36 μc/gm body weight, on two successive days following the operation, and the animals were permitted to survive after the injection for two months. All the animals were killed by cardiac perfusion with 10% neutral formalin. The removed brains were further fixed in formalin and were then embedded in paraffin. Serial sections were cut at 6 μ and three out of every 30 sections were preserved. The preserved sections were stained with Einarson's gallocyanin chromalum, cresyl violet, and Bodian's protargol method. The gallocyanin chromalum stained sections were coated in the dark by the dipping technique with Kodak NTB-3 nuclear emulsion, exposed in light-proof boxes containing Drierite at 5°C for 13 weeks, and were then developed and processed in the usual manner (Altman, '64a; Kopriwa and Leblond, '62).

RESULTS

In all the animals injected with thymidine-H^3, and in all the sections studied, differentiated granule cells and smaller cells with dark nuclei could be seen with overlying silver grains in the dentate gyrus of the hippocampus. Most of these were found (fig. 1) in the basal region of the granular layer of the dentate gyrus. The number of labeled cells was highest in the animals injected at ten days of age, with a decline in the frequency of labeled cells in the animals injected at older ages. To obtain a quantitative measure of this decline, the cells with labeled nuclei were counted in homologous areas of the granular layer of the dorsal dentate gyrus in circular areas 220 μ in diameter. In all animals 20 adjacent circular areas were sampled on each side at 800 times magnification in the external and internal arms of granular layer of the dentate gyrus. The mean number of labeled cells found within the specified area in the different age groups is plotted in figure 2. In the animals injected at ten days of age the mean number of labeled cells in the specified area was 35, which declined to about five labeled cells

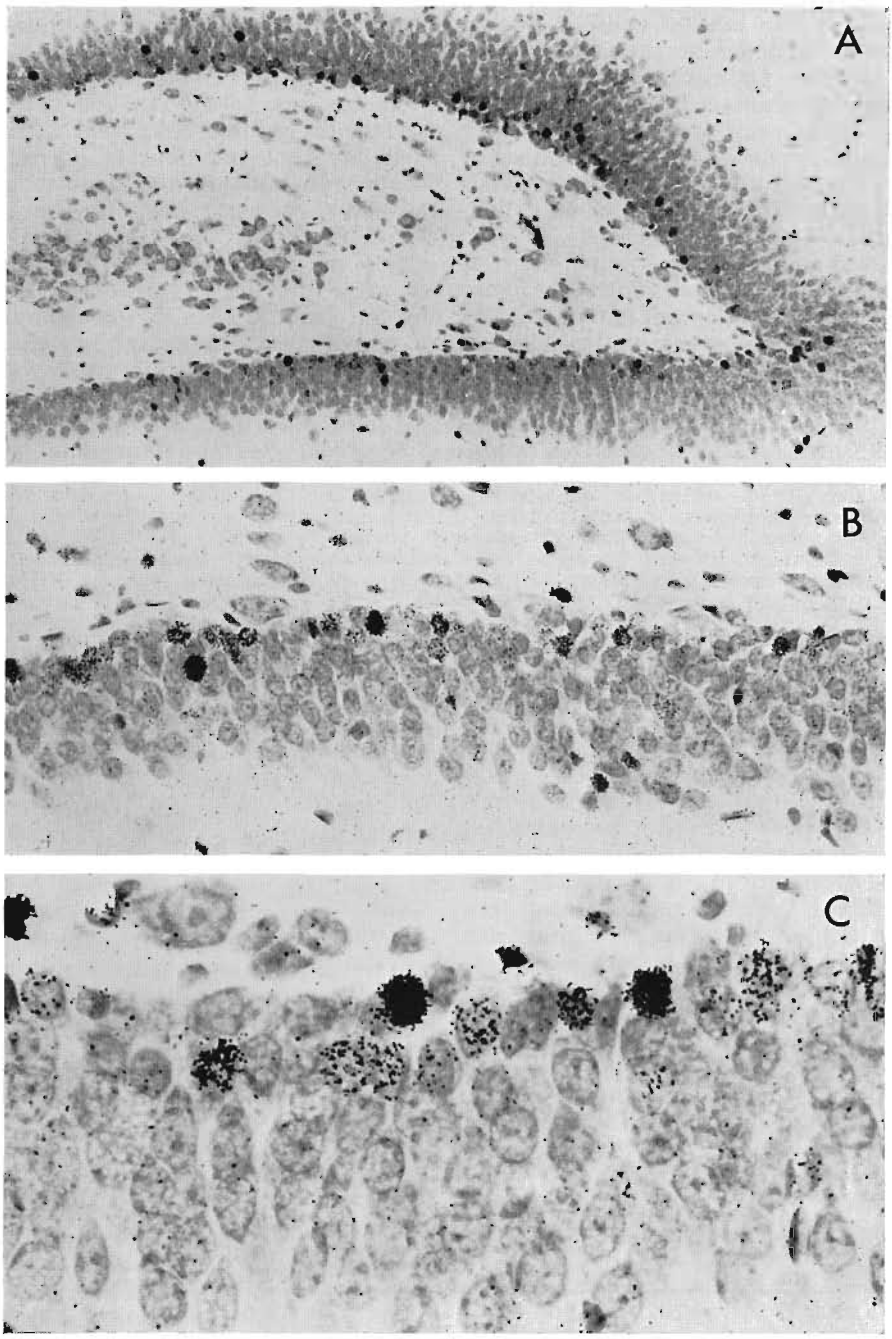

Fig. 1 Low and high power microphotographs of autoradiograms from the area of the dentate gyrus of the hippocampus in a rat injected with thymidine-H^3 at the age of ten days and killed two months after the injection. Note labeling of granule cells, predominantly in the internal border (basal surface) of the granular layer. A, 100 ×; B, 256 ×; C, 640 ×.

in the animals injected at 30 to 40 days, and to about two labeled cells or less in the animals injected at the age of four months or older (adults). Comparing the two groups of animals (one injected with 36 μc/gm body weight, and the other with 10 μc/gm), a somewhat larger number of labeled cells is indicated for the animals injected with a larger dose of thymidine (compare 30 and 40 day groups), but the trends are similar in both groups. Similar curves were obtained, as indicated in figure 2, by plotting the percentage of labeled cells in the dentate gyrus in the different age groups. A total of 2,000 dentate cells (1,000 on each side) was counted in the regions previously specified, and of these about 19% was found labeled in the animals injected at ten days of age, which declined to about 3% in the 40 day group, and to values below 1% in the adult animals.

In contrast to the considerable number of labeled granule cells seen in the granular layer of the dentate gyrus, labeled polymorph cells in the dentate gyrus or labeled pyramidal cells in the adjacent Ammon's horn were not encountered in any of the animals studied. This suggested that the marked utilization of thymidine by the granule cells of the dentate gyrus, in contrast to neurons in other parts of the brain, was not due simply to a greater availability of this substance in the hippocampus as a whole. To test further the possibility of preferential availability, rather

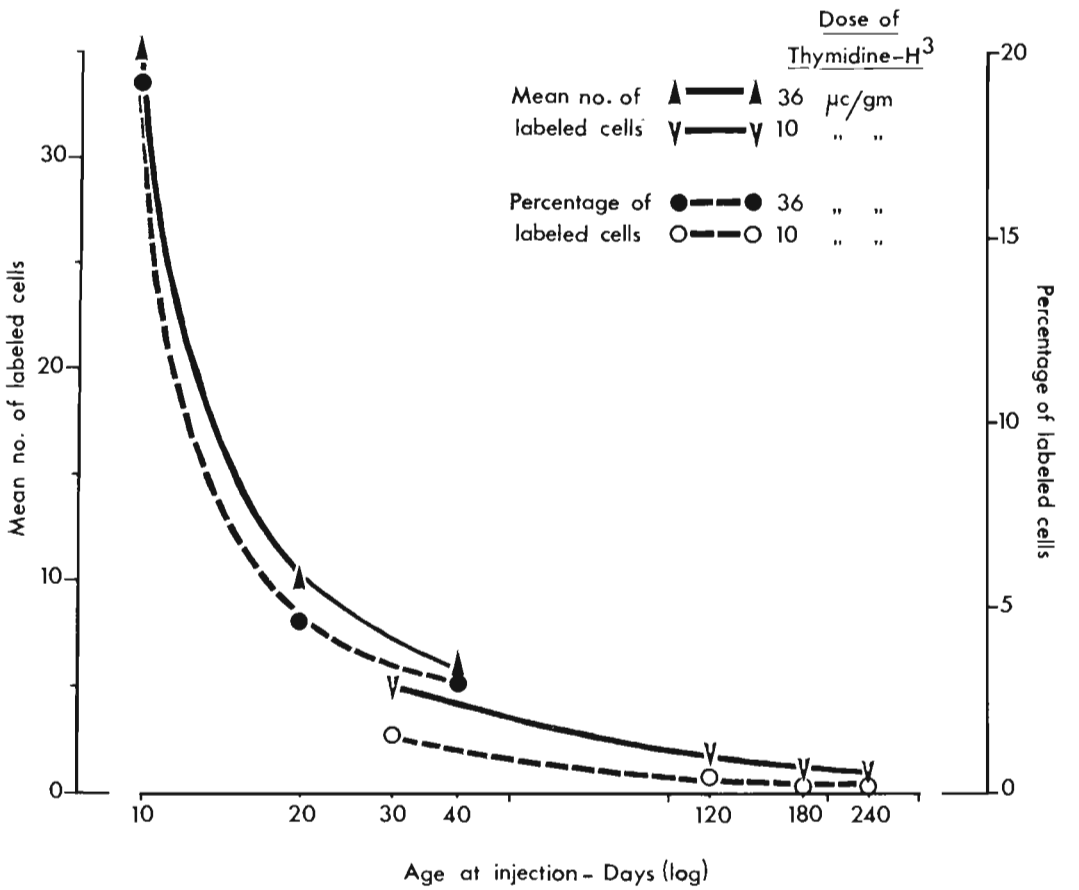

Fig. 2 Graphs showing decline in number and percentage of labeled cells in the granular layer of the dentate gyrus as a function of age at the time of injection. Data from two groups of animals are given.

than differential rate of utilization of thymidine by the granule cells of the hippocampus, we have compared in the group of animals with unilateral striate lesions the number of labeled cells in the dentate gyrus on the normal side with the side in which the overlying cortex was removed and thus the "blood-brain barrier" presumably interfered with. The results are summarized in figure 3, which shows that, with the exception of one animal (40 days), the number of labeled cells was not necessarily higher on the side where the hippocampus was partially exposed than on the normal side. The ablation procedure in this group of animals also permitted us to determine whether the declining number of labeled cells in the brain as a whole, with increasing age at the time of injection, is due to decreased demand or, instead, to decreasing availability of thymidine as a consequence of the changing properties of the "blood-brain barrier" as the animals grow older. We have compared in the different age groups the number of labeled cells (mostly neuroglia and microglia cells) in the immediate surround of the lesion. Figure 4 indicates that there was no systematic difference in the mean number of labeled cells in the different age groups in the region surrounding the lesion where cell proliferation was experimentally provoked by brain damage. That is, at sites where proliferation was in progress, thymidine-H^3 was taken up as readily in older as in younger animals. These findings, then, suggest that the decrease in the number of labeled granule cells as thymidine-H^3 is injected into progressively older animals is due to decreased utilization of this DNA precursor, presumably as a consequence of a decreasing rate of cell proliferation.

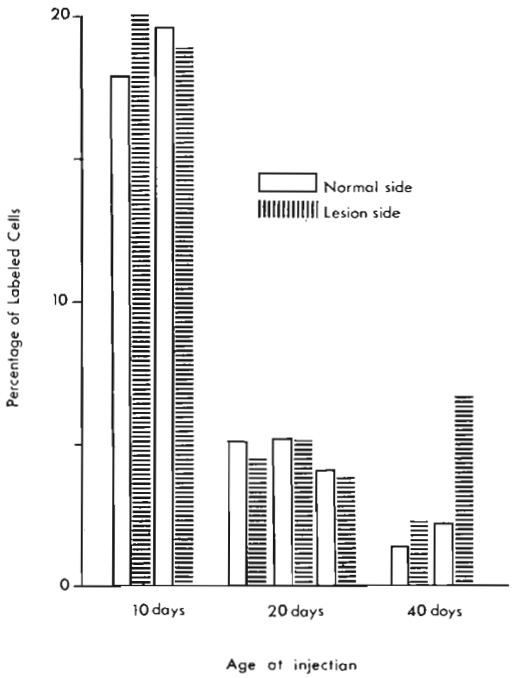

Fig. 3 Percentage of labeled cells in the granular layer of the dentate gyrus on the normal and lesioned side (parts of overlying cortex removed). Pairs of bars represent the two sides in single animals.

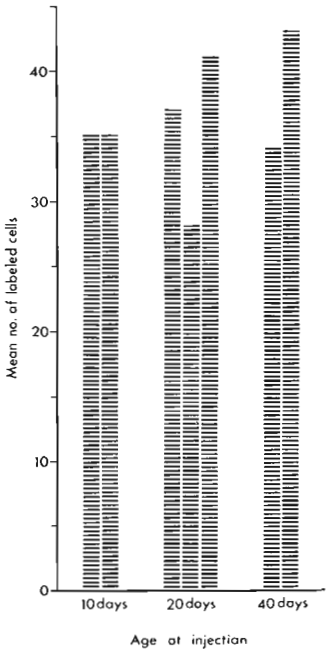

Fig. 4 Mean number of labeled (mostly glia) cells in the cortex in the vicinity of the lesion. Each bar represents one animal.

Our interpretion of these results, namely that the granule cells of the dentate gyrus multiply at a high rate in young animals, a process which declines rapidly but does not cease altogether in the mature animals, is difficult to reconcile with the absence of signs of mitotic activity in differentiated granule cells. We have, accordingly (un-

dertaken to investigate the possibility that undifferentiated precursor cells take up thymidine, multiply, and subsequently differentiate into granule cells. This possibility was suggested to us by the presence of small cells with darkly staining nuclei in the inner border or base of the granular layer, a region where most of the labeled cells were encountered. There is little information in the literature about the properties of these small cells with dark nuclei, presumably because they are considered to be glia cells (they are sometimes described as astrocytes). However, in mature animals neuroglia cells are seldom encountered in the dentate gyrus, whereas in young animals, as we shall describe in detail later, these cells with small dark nuclei are quite abundant. We have, therefore, postulated that these are undifferentiated cells which can take up thymidine and multiply, and may subsequently differentiate into granule cells. If this hypothesis were correct, then we should find a larger proportion of labeled small cells with dark nuclei in animals killed soon after injection with thymidine-H^3, whereas in animals surviving for longer periods after injection the labeled small cells should decrease and the labeled differentiated cells should increase at the expense of the former. We could investigate this hypothesis in the group of animals injected at the age of four months and killed at varying intervals from four days to eight months after the injection. The results are summarized in figure 5, indicating that a large proportion of the labeled cells are of the small type with dark nuclei in the animals killed four days after injection, the proportion declines to less than half in the animals that survived for two weeks, and (excepting in one animal of the four-month survival group) only differentiated granule cells are labeled in the animals that survived for two months or longer after the injection. Also of great importance is the finding in this experiment that the total number of labeled cells in the dentate gyrus did not decline with prolonged survival within the periods tested, attesting to the long life span of granule cells and the metabolic stability of thymidine-H^3 incorporated into these cells.

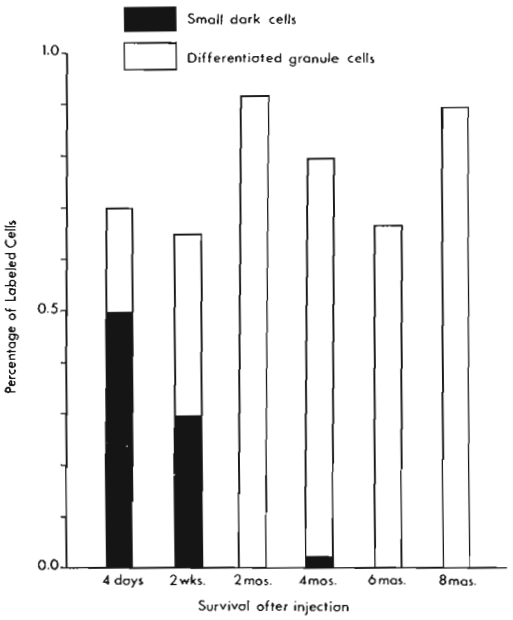

Fig. 5 Types of cells labeled in the granular layer in rats injected as adults (4 months), following which they survived for the periods indicated.

DISCUSSION

The results obtained in these autoradiographic experiments suggested an active process of neurogenesis in the dentate gyrus of infant and young rats, a process which continues at a low rate into adulthood, at least up to eight months of age. The results also suggested that neurogenesis in the dentate gyrus might be attributable to the multiplication of small cells with dark nuclei which occur commonly, particularly in young animals, at the base of the granular layer of the dentate gyrus, and which would subsequently differentiate into granule cells.

However, utilization of thymidine by neurons or their presumed precursors represents, strictly speaking, only indirect evidence of cell multiplication, since it is conceivable that the DNA turnover of neurons is not associated with chromosomal duplication and cell proliferation as it is in the case of other cells of the brain and body. Indeed, if DNA metabolism in the brain does imply cell multiplication, and if cell multiplication is as prevalent in the dentate gyrus of the hippocampus in young

rats as suggested by the extent of its thymidine uptake, then it should be possible to obtain independent histological evidence of this proliferative process, and thus confirm and throw additional light on the nature of postnatal neurogenesis in the hippocampus. Accordingly, we have undertaken to study by histological methods the brains of a large series of noninjected rats, ranging from neonates to mature adults.

II. The histological evidence

MATERIALS AND METHODS

The brains were removed from pairs of normal, laboratory-bred, noninjected rats at the age of about 6 hours; 2, 5, 8, 12 and 15 days; 1, 2, 3, 5, 7 and 9 months. The fixed brains were embedded in paraffin and cut serially at 6 μ. Of these, three sections out of 20 were preserved and stained with Einarson's gallocyanin chromalum and cresyl violet for cells, and Bodian's protargol method for fibers. Microscopic evaluation of this material is described below.

RESULTS

As illustrated in figure 9, there is in young rats an extensive pool of undifferentiated cells along the lateral ventricles, which is largest in extent in the six-hour old animals and which becomes progressively smaller (with a transient expansion at 15 days) as the animals grow older (fig. 6). This ventricular germinal pool or matrix is composed of an ependymal and a subependymal layer of cells on either side of the ventricular lumen, where the cells of the external subependymal layer, the region adjacent to the cerebral hemispheres, and the cells of the internal subependymal layer, the region adjacent to the hippocampus and diencephalon, are morphologically clearly distinguishable from one another. Many cells in both the ependymal and subependymal layers were found in various phases of mitotic activity (figs. 10, 11), and mitotic cells were also seen in various structures near the margin of the subependymal layers. Accordingly, the ependymal and subependymal layers (and to a lesser extent the "marginal layer") of the lateral ventricle represent an actively proliferating germinal pool in the young animals. Portions of this ventricular wall, particularly in the anterior part of the forebrain, were found in a proliferative condition, with many darkly staining cell nuclei and occasional mitotic ones, at least up to one month of age and, according to the autoradiographic evidence to be described later, late into maturity (at least up to eight months of age).

A similar germinal matrix could be recognized in the young animals around the wall of the third ventricle. The ependymal wall in the middle portion of the third ventricle (the region surrounded by various thalamic structures) was found to assume, with its reduced cell thickness and lightly staining nuclei, the resting condition by the fifth day postnatally, whereas the wall of the ventral floor of the third ventricle (surrounded by the hypothalamus) remained in a germinal condition much longer, at least up to 15 days of age. The dorsal roof of the third ventricle, where the dorsal hippocampus reaches the midline, remained in an active proliferative state past one month of age.

To determine quantitatively the changes in the size and cell population of the germinal pool of the lateral ventricle, three brain sections with maximal extent of the pool were selected in single animals from each age group, and with the aid of a Leitz microprojector drawings were made of the germinal areas at 40 times magnification on graph paper with a 1 mm^2 grid. The mean cell density of the 1 mm^2 grid unit (25 μ^2 of tissue area) was determined separately for each section by counting the number of cells in 20 samples of corresponding tissue size at 800 times magnification through the eyepiece grid of a microscope. The unilateral mean area of the germinal pool for a given animal was established by determining the number of grid squares occupied by projections of the ventricular germinal pools. An estimate of the mean cell population on one side in a single section was arrived at by multiplying the mean number of squares occupied by the pool by the mean number of cells in a single square in the particular animal. The results are summarized in figure 7A which shows a rapid decline in the size of the cell population of the ventricular germinal pool from six hours of age to 5–8 days, a transient ascent reaching its

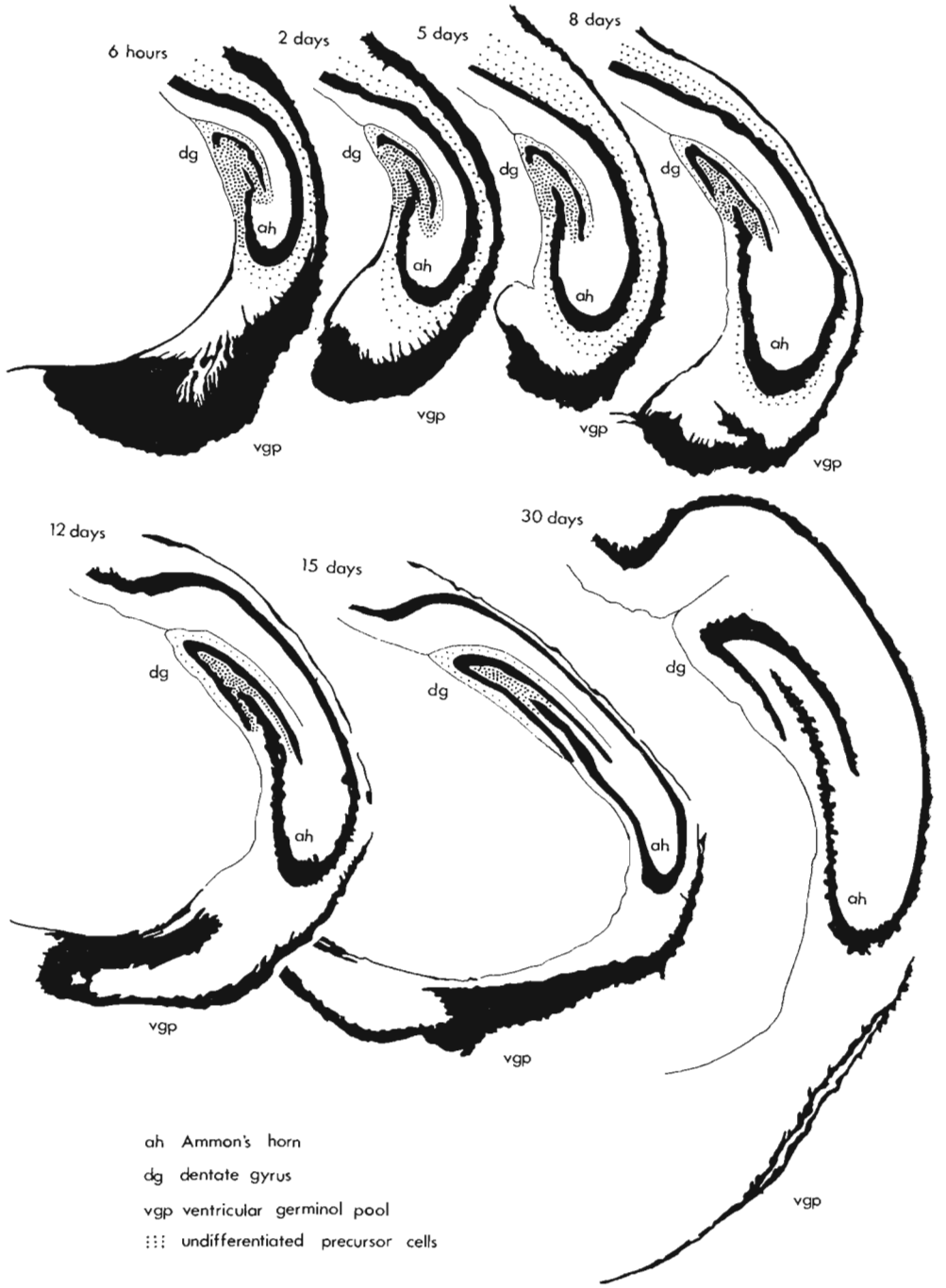

Fig. 6 Tracings of the germinal pool of the lateral ventricle (vgp), dentate gyrus (dg) and Ammon's horn (ah) from the brains of animals of different ages. Outlines of the ventricular pool are drawn accurately, the dots representing the undifferentiated cells of the hippocampus are schematic.

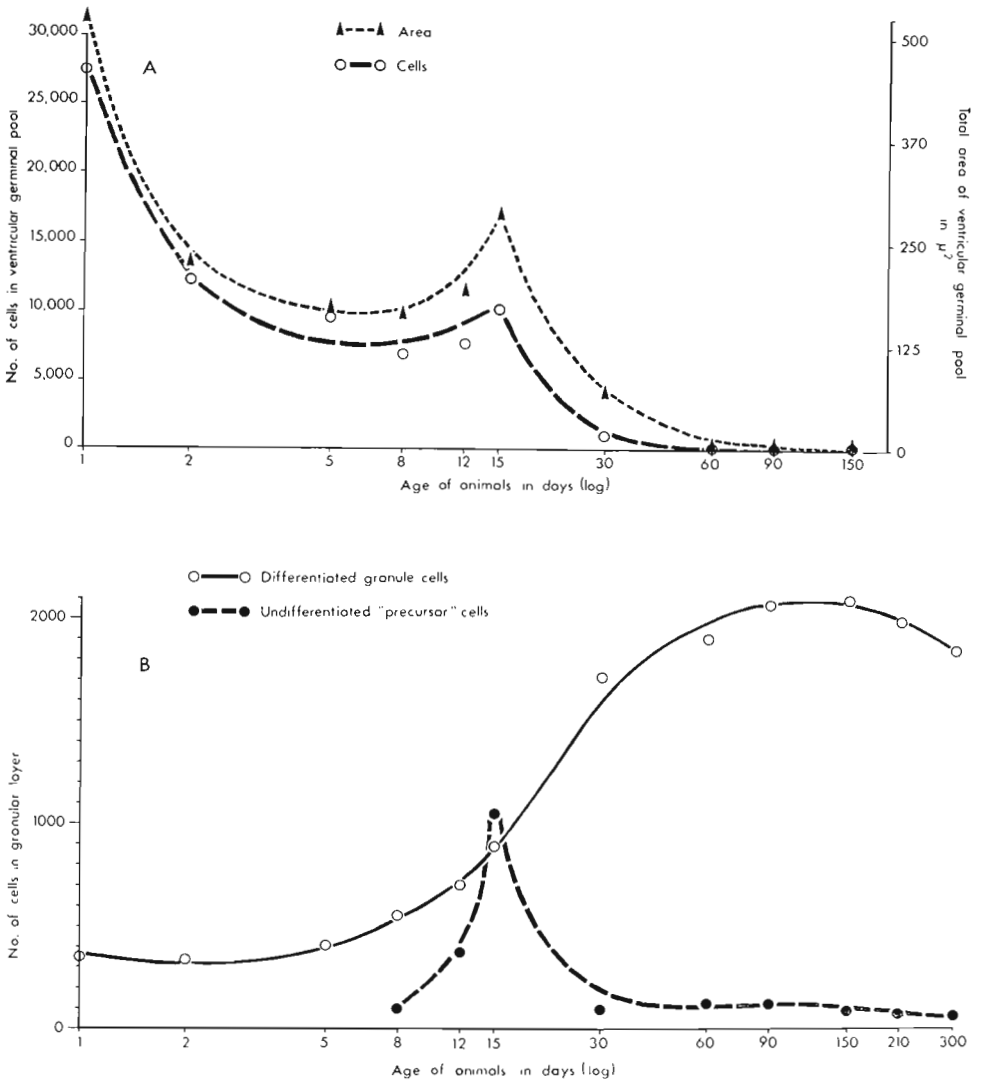

Fig. 7 A. Graphs showing changes in cell population and in area occupied by ventricular germinal pool in animals of different ages. B. Mean number of undifferentiated "precursor" cells and differentiated granule cells in the granular layer of the dentate gyrus in animals of different ages.

peak at 15 days, and a permanent decline from that age onward.

A histological study of the hippocampus in this group of animals revealed radical alterations in the animals of different ages in the size and cell population of Ammon's horn and dentate gyrus. The gross changes in the size of the hippocampus are indicated in the tracings in figure 6, which show a reciprocal trend with respect to the ventricular germinal pool: as the size of the ventricular germinal pool decreases, the size of the hippocampus increases. Microscopic study of the same material showed that in the six-hour old animals, small, undifferentiated cells with dark nuclei were present in large number in Ammon's horn, a few of them with signs of mitotic activity, together with many differentiated pyramidal cells. At eight days of age few undifferentiated cells were seen in Ammon's horn and mitotic cells

became very scarce (a single mitotic pyramidal cell was seen in a 12-day old animal). In contrast, in the dentate gyrus differentiated granule cells were present in the six-hour old animals only in the external arm of the granular layer (the arm facing the cerebral hemispheres) and in the hilus of the gyrus, but not in its internal arm. Indeed, the internal arm of the granular layer has not yet become differentiated at this age and only a diffuse mass of undifferentiated cells were present in this region, presumably migrating here from the adjacent portion of the ventricular germinal pool. Our material indicates that the differentiation of granule cells takes place first at the external or apical surface of the granular layer and new undifferentiated cells are added to it largely, though not exclusively, from its internal or basal surface. Thus, in the younger animals, these undifferentiated small cells with dark nuclei are usually found lined up in varying numbers at the base of the granular layer, and an occasional undifferentiated cell in this region can always be found even in mature animals. In the younger animals mitotic cells are also quite common in the molecular layer between Ammon's horn and the dentate gyrus in the basal border region of the granular layer (figs. 12, 13, 14). Mitotic cells in these regions may be encountered at least up to one month of age.

Quantitative assessment of changes in the cell population of the granular layer of the dentate gyrus was accomplished by counting the total number of cells in sample sections in the granular layer of the dorsal hippocampus. An attempt was made to obtain homologous hippocampal regions by selecting pairs of adjacent sections from each animal at a level where the right and left hippocampi are juxtaposed near the midline, and where certain diencephalic structures (medial habenular nucleus, lateral geniculate body) showed comparable appearance. In these pairs of sections the total cell population of the granular layer was counted at 800 times magnification on both the right and left side, from which the mean cell population of the granular layer on one side was estimated. In counting the cells of the granular layer a distinction was made between differentiated granule cells (defined as large, round-to-ovoid cells with pale nuclei) and undifferentiated cells (small cells with darkly staining nuclei). The results are summarized in figure 7B, showing a six-fold increase in the number of differentiated granule cells from six hours to three months of age, with an indication of gradual decline in the mature animals from the age of seven months onward. We were unable to determine the total number of undifferentiated cells in the granular layer in the youngest animals (6 hours to 5 days), since the internal arm of the granular layer is largely absent or in its formative stage, and the entire bed between the presumptive internal arm of the granular layer and Ammon's horn appears as a diffuse mass composed of small, darkly staining undifferentiated cells. From the ages of 5 to 8 days there was a sudden increase in the total number of undifferentiated cells in the granular layer, mostly at the basal surface of the layer, which reached its peak at 15 days. There was a corresponding upward swing in the total number of differentiated granule cells, but this growth did not reach its peak until three months of age. By one month the total number of undifferentiated cells declined sharply in the granular layer. The falling curve of the undifferentiated cells is an approximate mirror image of the rising curve of the differentiated granule cells, suggesting that the increase of the latter is due to gradual transformation of "indifferent cells" into neurons.

DISCUSSION

The presence in neonates and young animals of a large pool of germinal cells in the wall of the third and lateral ventricles near the hippocampus; the decline in the cell population of this germinal pool with age; the presence of numerous "undifferentiated" cells within and in the regions surrounding the granular layer; and, finally, the decline with age in the number of undifferentiated cells in or near the granular layer with corresponding increase in the total number of differentiated granule cells, suggest a process of postnatal neurogenesis that resembles prenatal neurogenesis insofar as the latter is dependent on cell multiplication in the epen-

dymal and mantle layers around the lumen of the neural tube. However, postnatal neurogenesis is not restricted entirely to the ventricular germinal pool, since small mitotic cells were commonly seen in the dentate gyrus in the younger animals and occasionally, even differentiated granule cells were seen in various phases of mitosis. We should note at this point that the germinal pool of the forebrain ventricles need not supply cells exclusively to the dentate gyrus. A comparison of the two sets of curves in figure 7, which summarize the changes in cell population in the germinal pool of the lateral ventricle and the granular layer of the dentate gyrus, suggests that the initial steep decline in the area and population of the germinal pool between six hours and five days of age cannot be due to migration of the undifferentiated cells to the dentate gyrus, since the dentate gyrus shows but a sluggish growth during this period. Since Ammon's horn of the hippocampus, and possibly also the neocortex, are still undergoing neurogenesis, these structures may be the primary recipients of the multiplying undifferentiated cells of the ventricular pool during this early period of postnatal neurogenesis. The sudden increase in undifferentiated and differentiated cells in the granular layer of the hippocampus at about the age of eight days is correlated, instead, with the transient upsurge in the cell population of the germinal pool of the lateral ventricle at the same age, which reaches its peak at 15 days, and then declines rapidly.

We should also add here that whereas the ventricular germinal pool in the posterior portion of the forebrain is in a process of dissolution by one month of age, cells with darkly staining nuclei, and occasional mitotic ones, are abundant even in mature animals in the dorsal roof of the anterior lateral ventricle. This was recognized in the past by various investigators using untreated animals (Allen, '12; Globus and Kuhlenbeck, '44; Jones, '32) and confirmed more recently in colchicine-treated animals (Bryans, '59; Kjellgren, '44) and in animals injected with thymidine-H^3 (Altman, '63; Altman, '64b; Messier et al., '58; Smart, '61). The present study showed that in rats injected with thymidine-H^3 at the age of four months as many as 51% of the ependymal and subependymal cells were labeled in this region after a survival time of four days. The rate of labeling declined rapidly to 7% after two weeks survival, and to less than 1% after survival of two months. In the animals with two weeks survival the labeled cells tended to be some distance away from the roof of the ventricle below the subcallosal fasciculus. These facts would suggest a high rate of cell proliferation in adult rats in this region and the migration of labeled cells to as yet undetermined brain regions.

Finally, we would like to deal with the question of the possible functional significance of the relatively late, postnatal maturation in the rat of the hippocampus in general and the dentate gyrus in particular. Since the hippocampus is considered to be a phylogenetically ancient structure, on basis of genetic considerations one would expect it to mature before the phylogenetically younger neocortex. Accordingly, a genetic explanation cannot be invoked to account for the late maturation of the hippocampus. Another possible explanation is a functional one, namely, that whatever physiological or behavioral processes are controlled by the hippocampus are not yet mature or in a functional state in young animals. One example of such a function is sexual behavior and, indeed, it has been previously postulated on the basis of electrophysiological studies (MacLean and Ploog, '62) that the hippocampus has a role in sexual processes. That is, paralleling the relatively late maturation of sexual mechanisms and behavior, the hippocampus (either as a cause or an effect) is also delayed in its maturation. This hypothesis has gained tentative support from ongoing research in our laboratory with labeled sex hormones.[2]

Male rats were castrated at maturity and two weeks after the operation they were injected systemically with 2 mc of testosterone-1,2-H^3 (specific activity 36 C/mM; radiochemical dissolved in ethanol). Pairs of animals were killed two and eight hours after administration of the labeled sex hormone. The fixed brains were embedded in water-soluble carbowax, and the cut and stained sections were coated with nuclear

[2] These experiments are carried out in collaboration with Donald Pfaff and Elizabeth Altman.

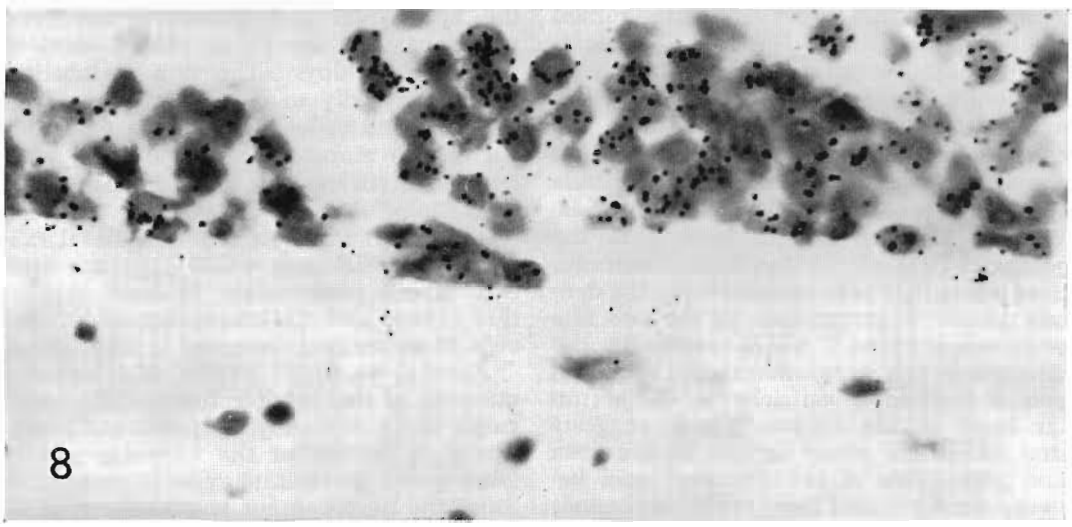

Fig. 8 Microphotograph of an autoradiogram of the granular layer of the dentate gyrus from a mature animal injected with testosterone-H^3. Carbowax embedding, 808 ×.

emulsion in the usual manner. Preliminary evaluation of an incomplete series of autoradiograms indicates binding of the radiochemical exclusively by the granule cells of the dentate gyrus and the pyramidal cells of Ammon's horn, with reduced grains also to be found in the vicinity of these cells presumably due to leaching (fig. 8). These results suggest that the neurons of the hippocampus may serve as receptors for gonadal hormones. We must add, however, that we have not ruled out the possibility that a few other structures (such as some hypothalamic nuclei) may also bind testosterone, nor have we tested the possibility of binding by hippocampal neurons of other hormones which are not directly implicated in sexual processes.

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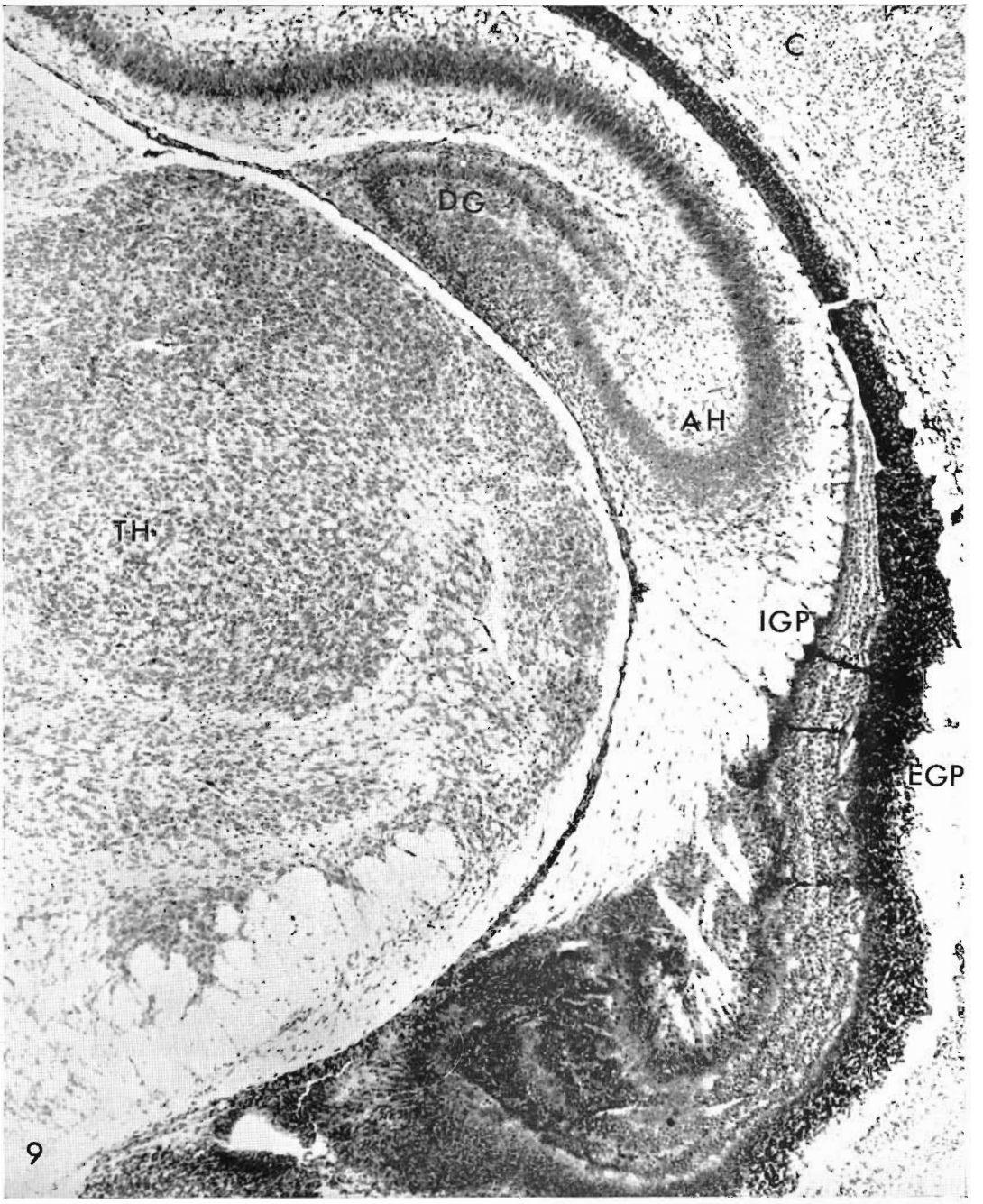
ADDENDUM

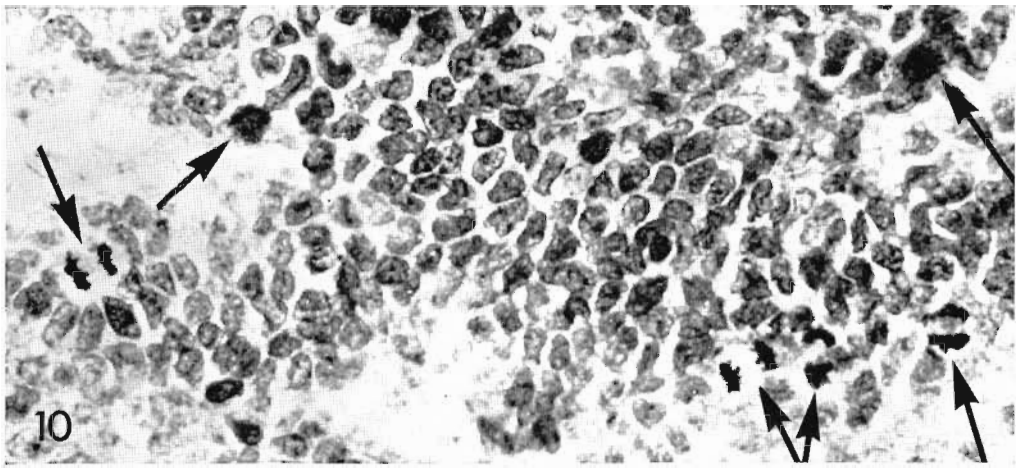
Recent studies in our laboratory have confirmed the hypothesis presented in this paper of the postnatal origin of a large proportion of the granule cells of the dentate gyrus of the hippocampus. Moreover, these studies have shown that a large proportion of those granule cells that compose granular layers in other brain regions, as in the olfactory bulb, cerebellum and ventral cochlear nucleus, are also formed after birth in the rat.

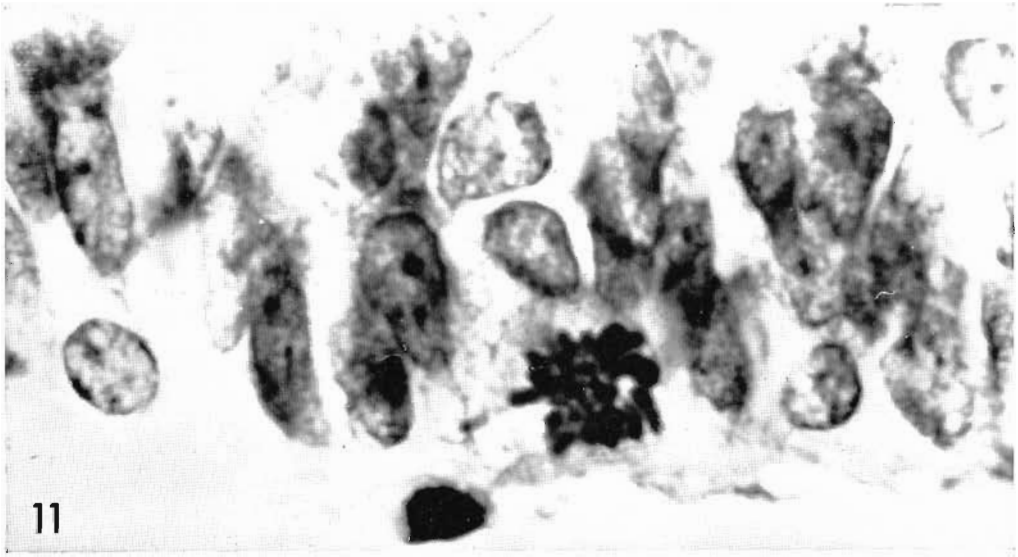
PLATE 1

EXPLANATION OF FIGURES

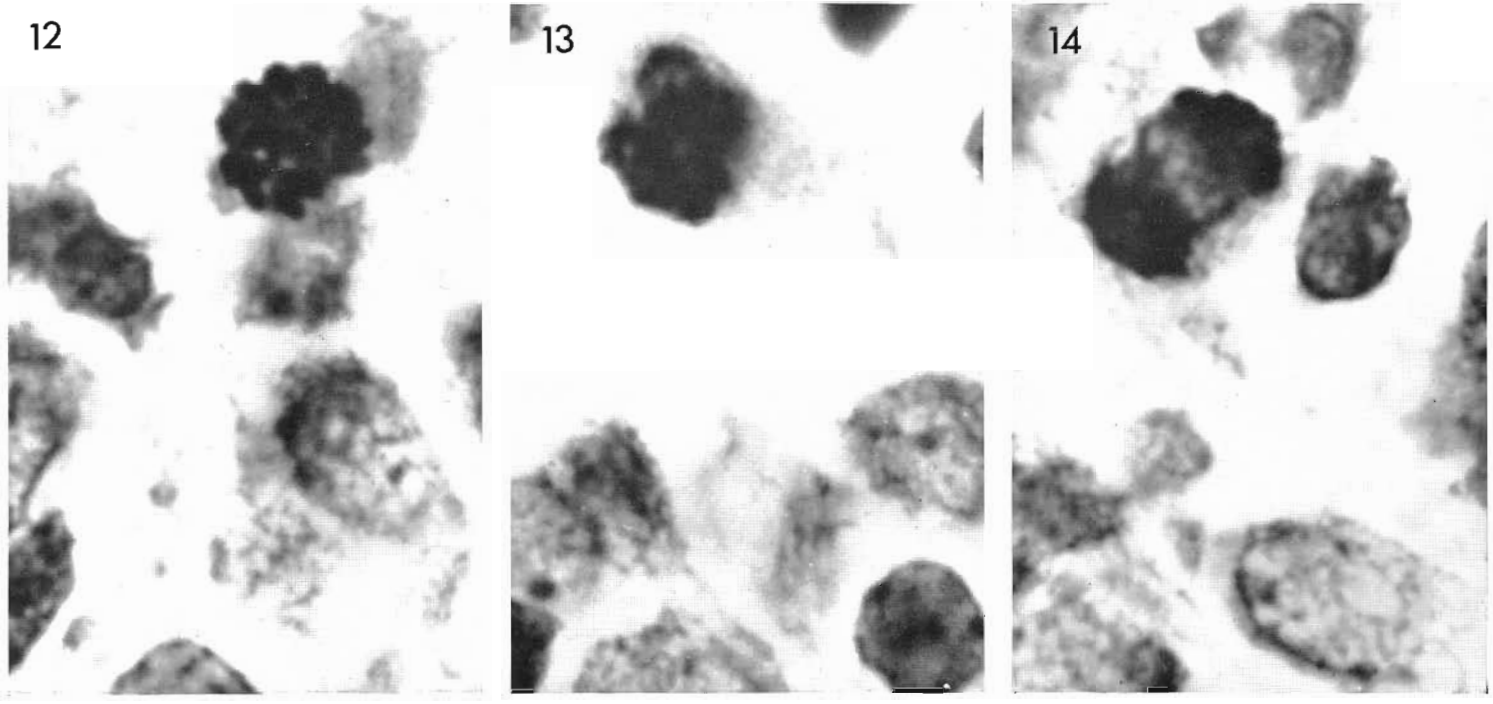
9 Low power microphotograph (80 ×) from the region of the dorsal hippocampus in a six-hour old rat. Note the large germinal pool surrounding the lateral ventricle. AH, Ammon's horn; C, cortex; DG, dentate gyrus; EGP, external germinal pool; IGP, internal germinal pool; TH, thalamus.


C
DG
AH
TH
IGP
EGP
9





High power microphotographs from the region of the ventricular germinal layer with mitotic cells. Cresyl violet. 10 — 240 ×. 11 — 1,200 ×.



12, 13, 14 Cells in various phases of mitosis near the granular layer of the dentate gyrus in a six-hour-old animal. Oil immersion microphotographs, 1,600 ×.