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Postnatal Development of the Hippocampal Dentate Gyrus Under Normal and Experimental Conditions

JOSEPH ALTMAN AND SHIRLEY BAYER

1. Introduction

Recent studies using [^3H]thymidine autoradiography produced convincing evidence that in the development of a particular brain region the small, short-axoned cells come into existence after the larger long-axoned cells. Indeed, in an altricial rodent, the rat, the granular nerve cells of the olfactory bulb, hippocampus, cerebellum, and cochlear nucleus are formed exclusively or predominantly after birth (Altman and Das, 1965a). There are indications that these short-axoned neurons (microneurons) arise from late-forming secondary germinal matrices (like the subependymal layer of the forebrain ventricles and the external germinal layer of the cerebellar cortex), in contrast to the long-axoned neurons (macroneurons) which originate from the periventricular primary matrix, the neuroepithelium (Altman, 1969). We do not as yet have an adequate explanation of the delayed formation of microneurons but the importance of these elements in the maturation of brain functions is indicated by behavioral studies. For instance, interference with the postnatal acquisition of cerebellar granule cells by experimental means produces behavioral deficits comparable to those seen after decerebellation (Wallace and Altman, 1969a,b; Altman *et al.*, 1971; Brunner and Altman, 1973).

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In this chapter, we will attempt to review available information on the postnatal maturation of the dentate gyrus. Autoradiographic studies in mice (Angevine, 1965) and rats (Altman and Das, 1965*b*) have established that a considerable proportion of the dominant neuronal elements, the granule cells, of this hippocampal substructure are of postnatal origin. As yet, we lack an understanding of the role played by the dentate gyrus in hippocampal functioning but it must be a crucial one. This was indicated by recent studies which showed that when the acquisition of the postnatally forming hippocampal granule cells is prevented experimentally, the animals display behavioral symptoms comparable to those seen after surgical destruction of the hippocampus as a whole (Bayer *et al.*, 1973; Haggbloom *et al.*, 1974).

2. *Normal Development of the Dentate Gyrus*

A recent evaluation (Altman, 1975) of the postnatal neurogenesis of the cerebellar cortex suggested that this process can be meaningfully and conveniently subdivided into five distinct, essentially sequential phases. These were referred to as (neuro)cytogenesis, (neuro)morphogenesis, synaptogenesis, gliogenesis, and myelogenesis. In this chapter, we will attempt to describe our knowledge of dentate neurogenesis in these terms.

Cytogenesis is the phase of cell production or acquisition, and its consideration includes such problems as the locus and kinetics of cell proliferation, and the time of origin of neurons of different types. The latter event is usually equated with the period when the precursor cells cease to divide and begin to differentiate. Differentiation consists of several steps. *Morphogenesis* refers to the phase when the postmitotic cells begin to develop their axons and dendrites in accordance with regional characteristics. This is the sculpturing phase of neurogenesis. For some types of cells, this involves migration of the soma and the extrusion of trailing processes, while in the case of others the stationary soma sends out processes that invade close or distant brain regions over simple or tortuous paths. *Synaptogenesis* is the next step, when the cell processes begin to establish contacts with one another. This is the connectivity phase when cell adhesion, membrane specializations, and the maturation of functional synapses result in the establishment of the characteristic regional wiring circuitry. The subsequent phase of *gliogenesis* has many functions, including the segregation of contiguous but independent neuronal elements, the ensheathing of related elements, such as pre- and postsynaptic regions of contact, and the production of materials for the myelination of axons. The last process represents the final phase of regional development, the phase of *myelogenesis*.

It is a reflection of the uneven advances made in the study of the development of different parts of the nervous system that although such a stepwise analysis has proven profitable for cerebellar neurogenesis, in the case of the hippocampus such an attempt merely calls attention to the fragmentary nature of the available information.

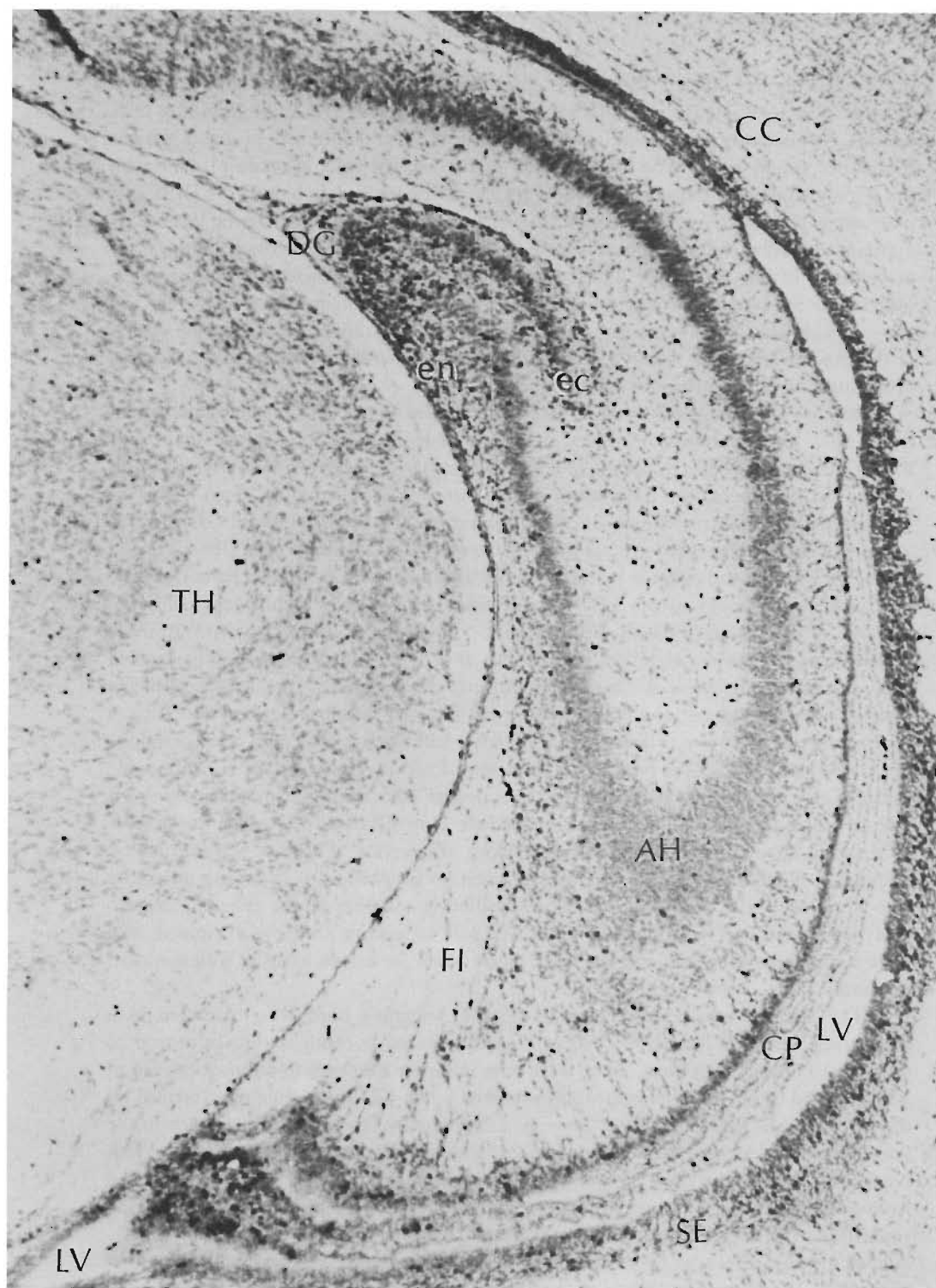
2.1. *Cytogenesis*

The study of neurocytogenesis has been greatly aided in recent years by the use of [³H]thymidine autoradiography. Proliferating cells, but not postmitotic cells, selec-

tively incorporate labeled thymidine into their duplicating chromosome strands (Taylor *et al.*, 1957). Because of the metabolic stability of DNA, the dividing cells become permanently tagged, unless the labeling of DNA molecules becomes diluted by further subdivisions. The visualization of the labeled cells is accomplished with the photographic technique of autoradiography (Messier and Leblond, 1957). If animals are killed shortly after injection, the sites of cell proliferation can be located. If animals are killed following a schedule of graduated survival times, the migratory movements of the originally labeled cells may be reconstructed. If animals are killed after the lapse of several weeks or months, the ultimate fate of the labeled cells can be established with some certainty. Finally, the technique also allows the dating of the time of origin (or "birth date") of different nerve cells, although this task poses several technical and methodological difficulties.

Because of some discrepancies in reports of the time of origin of hippocampal nerve cells, an explanatory technical note is in order here. The usual procedure for dating the time of origin of cells is to count all heavily labeled cells in a selected brain region and plot them as a function of age at injection. For example, if an animal is injected with [^3H]thymidine on day 15 of gestation, the heavily labeled cells seen in any particular brain region are assumed to have differentiated (or been "born") soon thereafter. This assumption is justified by the argument that had the cells in question continued to divide repeatedly after the injection they would be lightly labeled due to dilution of the radiochemical. But there are two major difficulties with this interpretation. One is the technical uncertainty as to what constitutes a heavily labeled cell—the size of the nucleus, the specific activity of the radiochemical, aspects of the photographic procedure, and other factors will have an influence on how much label will be visualized over a cell nucleus. The other difficulty is that high concentrations of isotope need not reflect immediate cessation of cell proliferation. It is conceivable that the cells in question entered a dormant period after the injection and resumed a few divisions at some later date. This procedure evidently would result in the premature dating of the time of origin of the neurons in question. This can be remedied by employing another procedure. In this, the question is asked as to how long injections can be delayed and still label all or a specified proportion of the cells considered. Only when this can no longer be done is it safe to assume that the examined cells have stopped dividing and entered their phase of life as nondividing or differentiated neurons.

The time of origin of pyramidal cells of Ammon's horn was examined in a pioneering study by Angevine (1965). Heavily labeled pyramidal cells appeared as early as 10 days of gestation. They were seen in larger numbers in animals labeled at 12 days, and the highest concentration occurred when [^3H]thymidine was injected on day 14 or 15. By gestational day 17, only a few heavily labeled cells were seen in the late-maturing CA1 and CA3 regions; none was labeled after day 15 in CA2. With regard to the dentate gyrus, intensely labeled granule cells were seen in mice injected from gestational day 13 onward; they were at that time essentially restricted to the superficial zone bordering the molecular layer. The highest concentration of heavily labeled granule cells was present in animals injected on day 18, these cells being distributed in the upper two-thirds of the layer. Because a few heavily labeled granule



cells could be seen at the base of the granular layer in animals injected up to 20 days postnatally, it was concluded that granule cells continue to arise up to that date. Angevine also examined the other layers of dentate gyrus and concluded that "the molecular and hilar layers of area dentata complete their periods of neurons formation by embryonic day 15" (Angevine, 1965, p. 34).

Similar results were obtained recently by Hine and Das (1974) in the rat, except that in this more slowly maturing species heavily labeled pyramidal cells began to appear in Ammon's horn in animals injected on day 16 and they could be seen in appreciable numbers up to day 19. In the dentate gyrus, heavily labeled granule cells began to appear in a superficial position as early as day 15 where they greatly increased in numbers in rats injected on days 20, 21, and 22 prenatally. In the molecular layer of the dentate gyrus, heavily labeled cells were seen as early as day 15 of gestation.

These prenatal studies established that in mice and rats the acquisition of the pyramidal cells of Ammon's horn is completed before birth. However, in view of the previously described limitations for determining the onset of the acquisition of a class of cells by counting heavily labeled cells, the dates set for the commencement of neuron formation in the hippocampus cannot be considered to be adequately established. This reservation is reinforced by other studies (to be described below) in which rats were injected with [^3H]thymidine postnatally. These studies confirmed that the pyramidal cells are of prenatal origin (as none of them was ever labeled), but they also showed that some of the cell types that were assumed to be formed prenatally, as the cells of the molecular layer of the dentate gyrus, are really of postnatal origin. Moreover, these studies revealed that a much larger proportion of granule cells are formed postnatally than would be predicted on the basis of observations of prenatally injected animals.

In the newborn rat, Ammon's horn is clearly delineated by the maturing pyramidal cells, but the dentate gyrus is less distinct because few of its granule cells have started to differentiate in its ectal arm (the arm facing the cerebral hemispheres) and few are recognizable as mature granule cells (defined as round-to-ovoid cells with large, pale nuclei) in the endal arm (Fig. 1). A count made in animals ranging in age from newborn to 300 days of age (Altman and Das, 1965*b*) indicated that less than 20% of all the granule cells present at 60–90 days of age could be identified during the first week of life. The scarcity of identifiable granule cells in the infant does not necessarily imply that the cells are absent, it may merely indicate a lack of differentiation of cells already formed. In infant rats, the hilus of the dentate gyrus and the base of the granular layer (where it was identifiable) are packed with small, darkly staining cells with occasional mitotic figures. The nature and fate of these cells were clarified with short-survival and long-survival autoradiography.

When young rats were injected with [^3H]thymidine and killed several hours af-

← Fig. 1. Low-power photomicrograph of a gallocyanin-chromalum stained autoradiogram of a coronal brain section from a rat injected with [^3H]thymidine at the age of 6 h and killed 24 h later. AH, Ammon's horn; CC, cerebral cortex; CP, choroid plexus; DG, dentate gyrus; ec, ectal arm of DG; en, endal arm of DG; FI, fimbria; LV, lateral ventricle; SE, subependymal layer; TH, thalamus. Slightly modified from Altman and Das (1965*a*).

terward, a large proportion of the small darkly staining cells in both the hilus and the base of the granular layer became labeled (Altman and Das, 1966). This can be dramatically demonstrated with cumulative labeling as shown in Fig. 2. The concentration of these labeled cells was high during the first week, began to decline by the end of the second week (the exact time course of this process has yet to be determined), and in adolescent and young adult rats only a few labeled cells could be seen. In the animals killed at spaced intervals after injection, there was (Fig. 3A,B,C) for up to 6 days an increase in the concentration of labeled cells in the hilus but then the number of these cells declined and there was an accumulation of labeled undifferentiated and differentiated granule cells in the basal aspect of the granular layer by the twelfth day after injection (Fig. 3D). Finally, when animals ranging in age from newborn to 8 months of age were injected with [^3H]thymidine and killed several months later (Altman and Das, 1965*b*; Altman, 1966) it became apparent, in agreement with Angevine's (1965) observations, that the granule cells are acquired in a sequence. The superficially situated cells were formed first, while the later acquired cells were progressively added to the base (Fig. 4).

In a more recent study (Bayer and Altman, 1974), we estimated the proportion of granule cells that is formed prenatally and the proportion that was added subsequently over blocks of 4 days postnatally. The procedure of dated, sequential

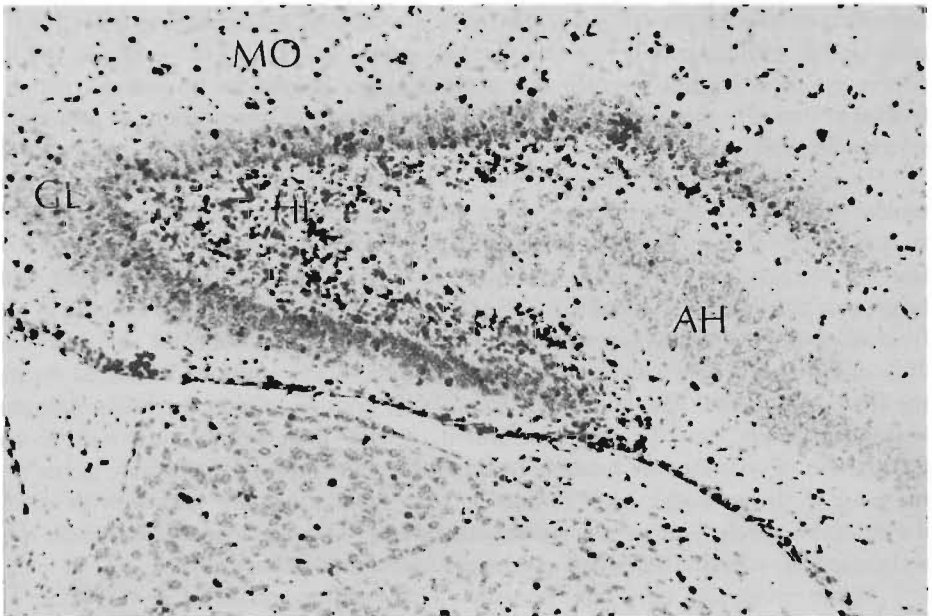


FIG. 2. Low-power photomicrograph of an autoradiogram of the dentate gyrus from a rat injected cumulatively with [^3H]thymidine on days 5, 6, 7, and 8 after birth and killed on day 9. Note the high concentration of labeled cells in the hilus (HI) of the dentate gyrus and also in its molecular layer (MO). The concentration is low in the pyramidal layer of Ammon's horn (AH), and relatively few cells are seen in the granular layer. Slightly modified from Altman (1966).

comprehensive (or cumulative) labeling was used (Fig. 5). The evidence indicated (Fig. 6) that 15% of the granule cells are acquired prenatally, 72% are added between 0 and 16 days, and 13% are formed thereafter. Earlier studies established that granule cells are continued to be formed in adult rats (Altman, 1963) at least up to 8 months of age (Altman and Das, 1965b).

2.2. *Morphogenesis*

Cell proliferation in the dentate gyrus, according to the previously considered evidence, is most pronounced in the hilus and at the base of the granular layer. These are the direct proliferative sites of this system. However, not all the primitive cells at the base of the granular layer, where they form a more or less distinct zone, become labeled. The properties of this zone of the granular layer, which could be the site of the onset of neuronal differentiation, have never been adequately examined. It is interesting to note that this subgranular zone is more conspicuous in the precocial guinea pig (Fig. 7) and the slowly maturing kitten (Fig. 8) than in mice and rats. A previous study (Altman and Das, 1967) showed that in the guinea pig a proportion of these primitive cells become labeled with [^3H]thymidine and differentiate subsequently as granule cells. But there is no information about their proliferative properties in the kitten.

To shed some light on this problem, we have recently tested the sensitivity of these primitive cells to low-level X-irradiation. The rationale of this approach is based on some results on the effects of X-irradiation in the developing cerebellar cortex. The postnatally acquired granule, stellate, and basket cells of the cerebellum arise from the multiplying cells of the superficial zone of the external germinal layer. In the deeper part of this germinal layer, the cells that ceased to divide (and cannot be labeled with [^3H]thymidine) assume a horizontal bipolar shape in the coronal plane. This signals the first step in the differentiation of these cells, the onset of the outgrowth of the horizontal portion of the parallel fibers, the axons of granule cells (Altman, 1972a). When the developing cerebellum is exposed to low-level X-ray (100–200 r), most of the cells in the proliferative zone of the external germinal layer are killed within 3–12 h. This is recognized by the drastic condensation of the affected cells and their rounding up into dark spherules (cell pyknosis). But unlike the multiplying cells of this layer, the bipolar cells are largely spared by the irradiation (Altman and Nicholson, 1971). This suggested that the great radiosensitivity of primitive cells disappears as soon as they begin to differentiate. Applying this technique to the hippocampus (Bayer and Altman, 1974), we found that many of the darkly staining cells at the base of the granular layer became pyknotic (Fig. 9) while others, as well as the maturing or mature granule cells, remained visibly unaffected by the radiation.

On analogy with results in the cerebellar cortex, it is assumed that the radioresistant primitive cells at the base of the granular layer constitute the group that has just started to differentiate, perhaps by sending out axons, the mossy fibers. But tentatively we also have to postulate that the mossy fibers at this stage of their development have not assumed the characteristic biochemical properties of mature

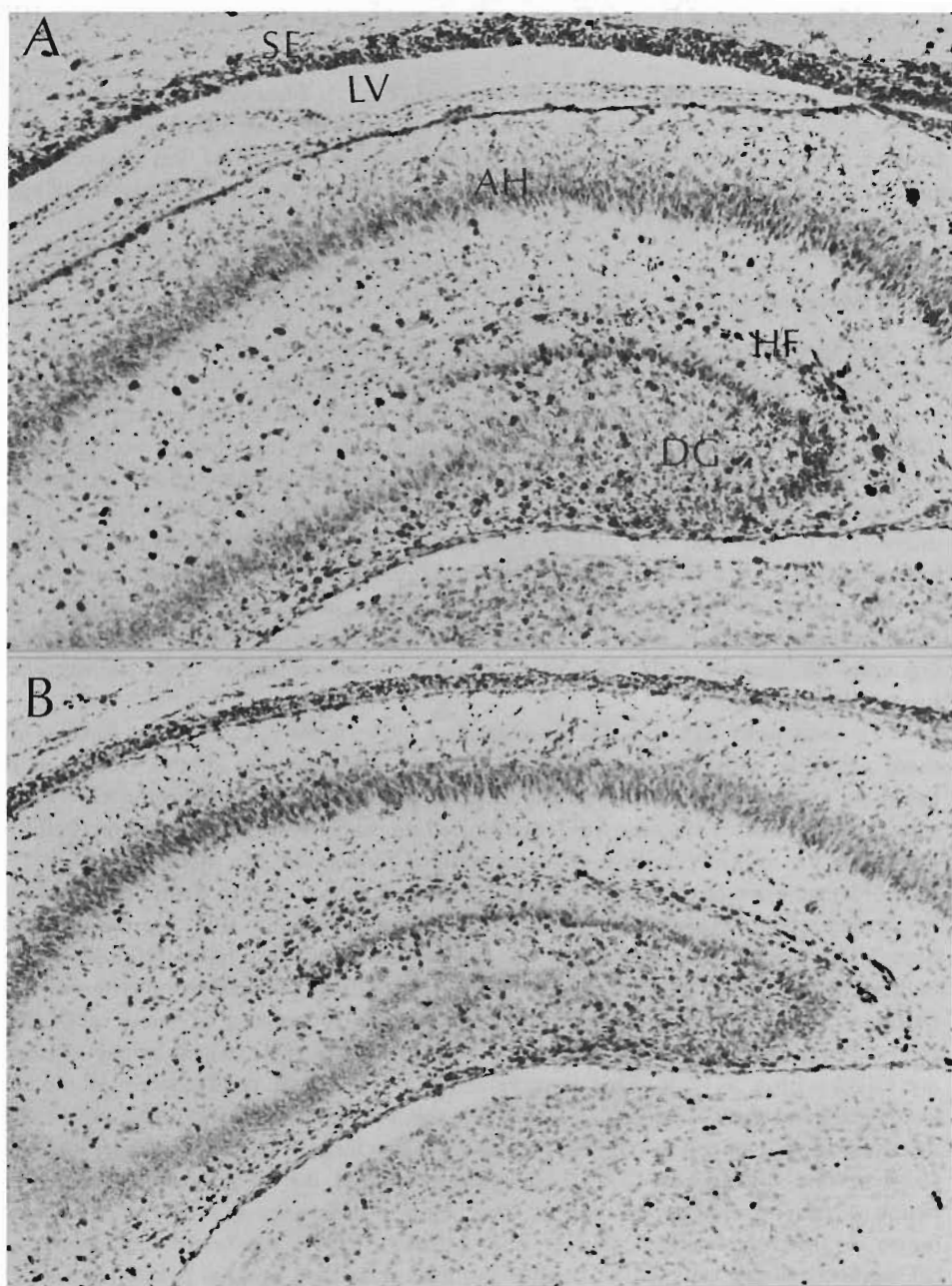
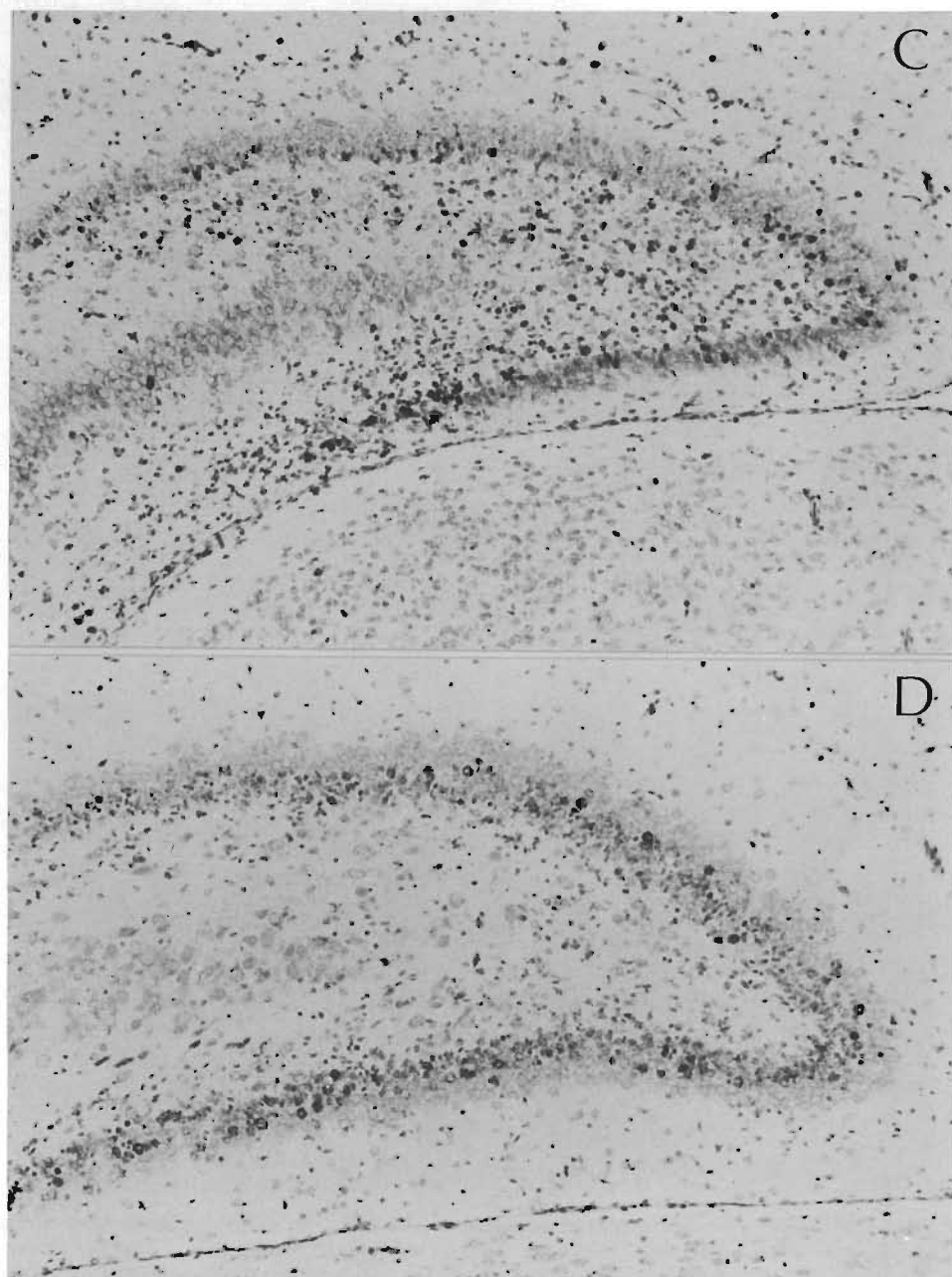


FIG. 3. Photomicrographs of autoradiograms of the dorsal hippocampus illustrating the sequence of events in the proliferation and migration of the precursors of dentate granule cells. All animals were injected with [^3H]thymidine 6 h after birth but were killed at different intervals thereafter. A: From a rat killed 6 h after injection to show the sites of cell proliferation in the hippocampus. Heavily labeled cells abound in the hilus of the dentate gyrus (DG), at the base of the granular layer, and in the region of the unformed extension of the endal arm. Scattered labeled cells are also seen in the various layers of Ammon's horn (AH) and the hippocampal fissure (HF). B: From a rat killed 24 h after injection. Apart from an increase in the number of labeled cells and with an associated label dilution within cells (not discernible at this magnification), there are no major changes at this period.



C: From a rat killed 6 days after injection. Note increase in the number of labeled cells in the greatly expanded endal and ectal arms of the granular layer. But the concentration of labeled cells in the hilus is still high. D: From a rat killed 12 days after injection. Note the reduction of labeled cells in the hilus and the high proportion of labeled granule cells. The heavily labeled granule cells are located in the superficial aspect of the layer, the lightly labeled cells at its base. Apparently a large proportion of the originally labeled precursor cells and their descendants have migrated into the granular layer and have become differentiated. Slightly modified from Altman and Das (1966).

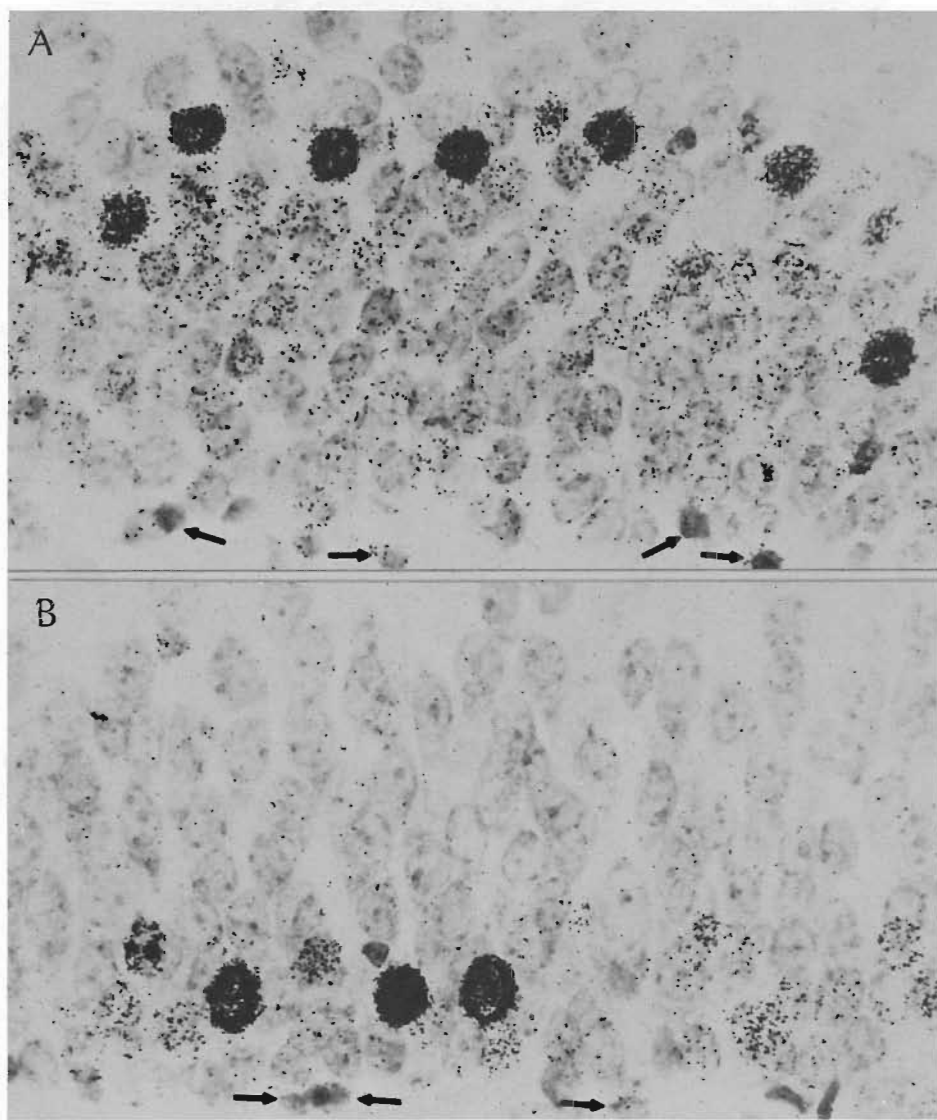


FIG. 4. High-power photomicrographs of autoradiograms of granule cells in the granular layer of rats injected with [³H]thymidine when 2 days old (A) or 13 days old (B). Both animals were killed 60 days after injection. Note that in the animal injected at 2 days of age the heavily labeled granule cells (those that differentiated first) are located in a superficial position while the lightly labeled cells (whose precursors multiplied many times before they began to differentiate) are located more basally. The basally situated cells were heavily labeled in the animal injected at 13 days and all the cells above them are unlabeled because they were formed before the injection. Arrows point to undifferentiated primitive cells. From Altman (1966).

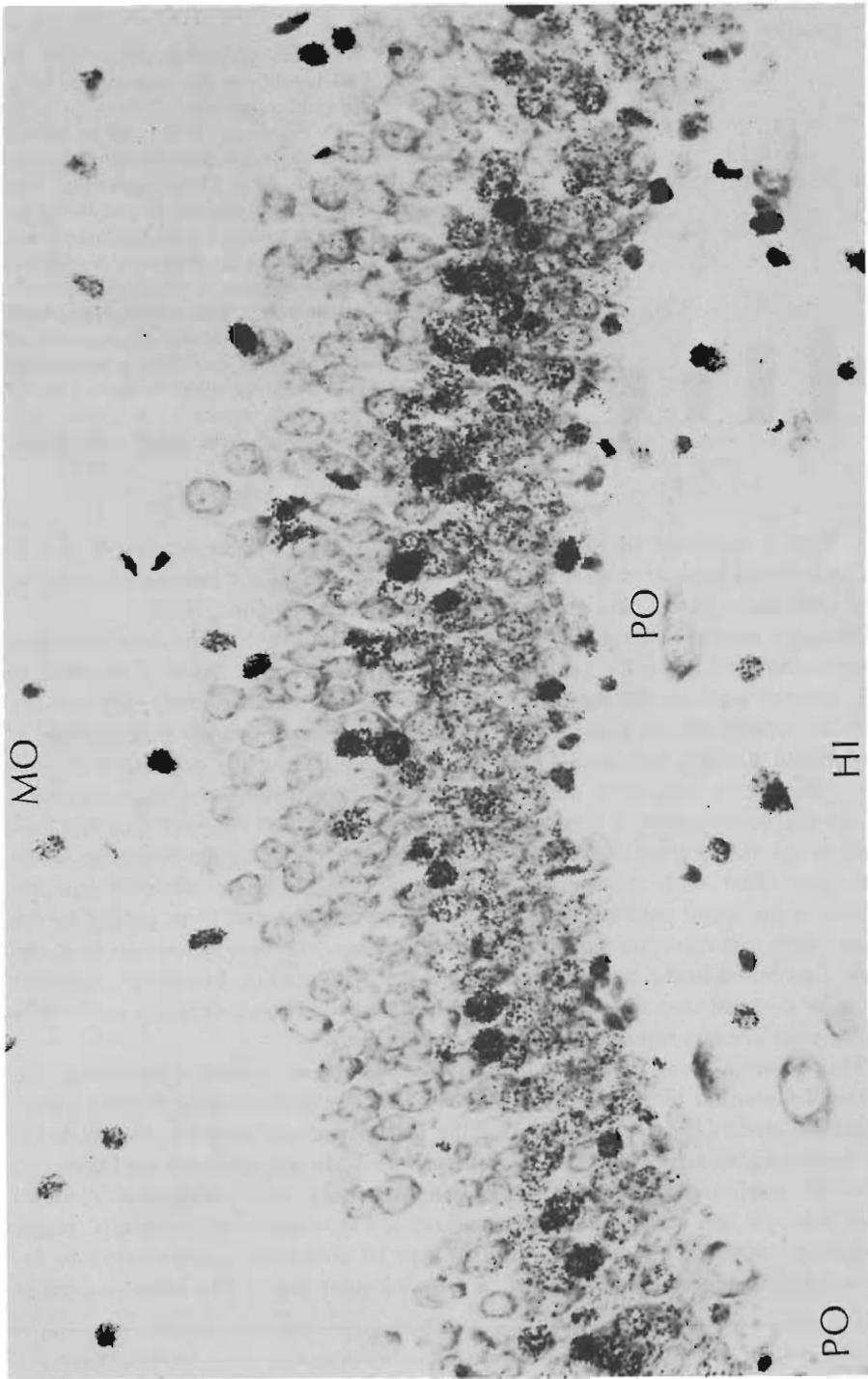


Fig. 5. Photomicrograph of an autoradiogram of the dentate gyrus from a rat injected repeatedly on days 4, 5, 6, and 7 after birth and killed when 60 days old. With this cumulative labeling technique, the great majority of cells formed after day 4 become reliably labeled. In addition to granule cells, many of the cells in the lower half of the molecular layer (MO) and in the hilus (HI) become labeled. The polymorph cells (PO), which are prenatally formed, remain unlabeled. From Bayer and Altman (unpublished data).

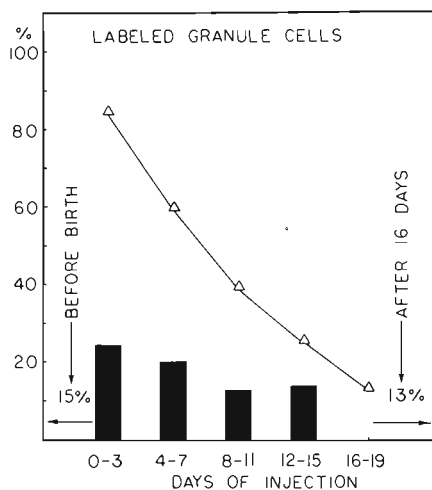


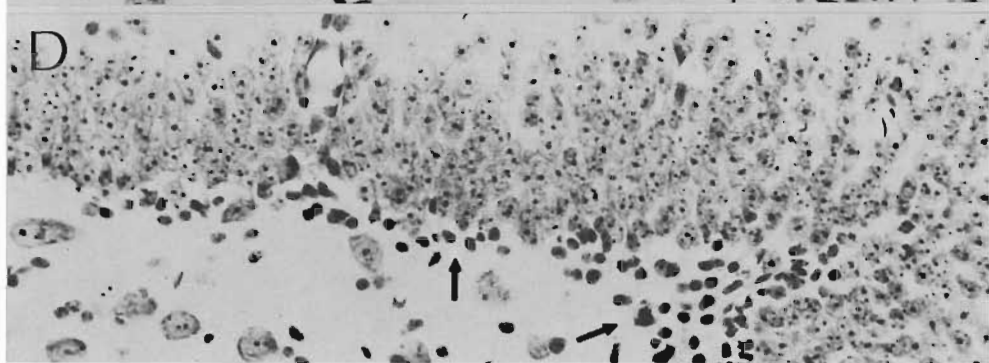
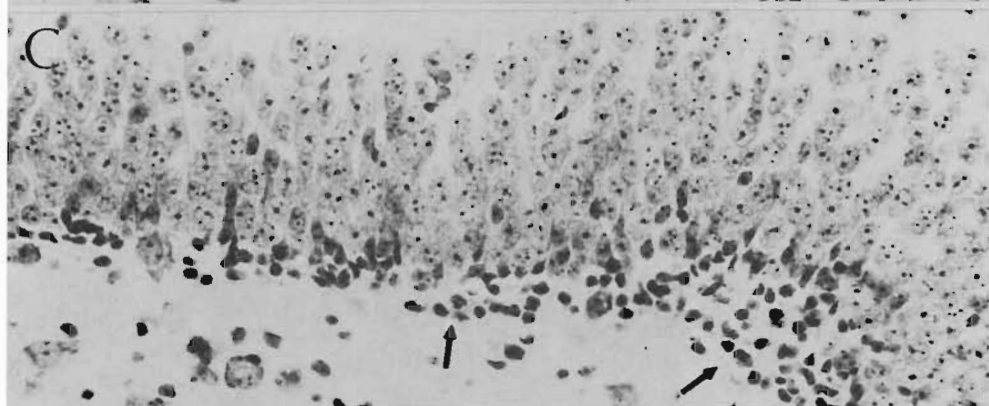
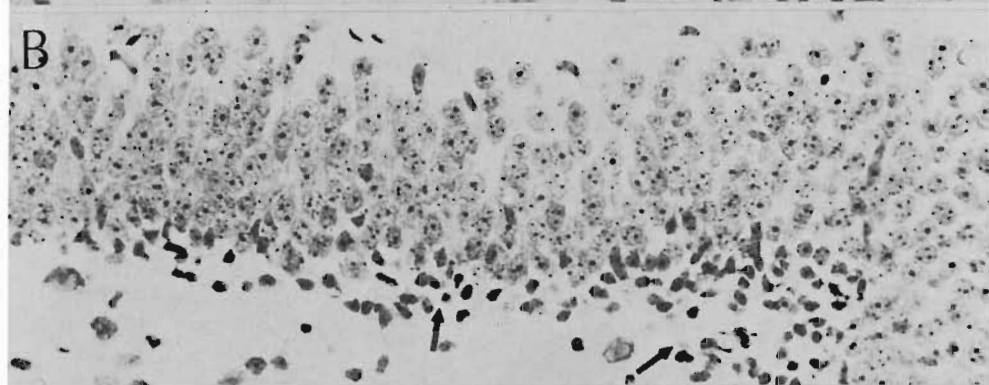
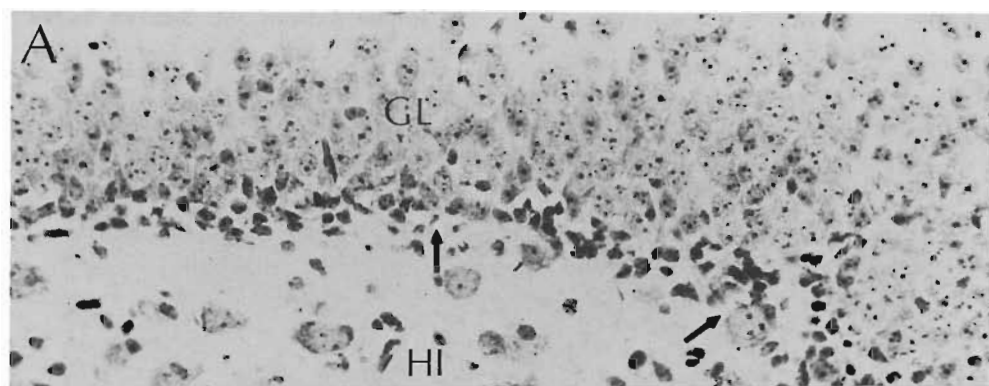
FIG. 6. Proportion of labeled granule cells in groups of 60-day-old rats that were injected daily with [^3H]thymidine on days 0-3, 4-7, 8-11, 12-15, 16-19. Since only 85% could be labeled with injections on days 0-3, it was concluded that 15% of the cells were formed prenatally. The proportion of differentiated cells formed during the blocks of days, as indicated in the histograms, was arrived at by deducting the proportion of cells that could be relabeled during successive periods. Since 13% of the cells were labeled with injections made after 16 days, it is concluded that this proportion of cells is formed after that date. This technique does not permit us to specify either the exact prenatal onset or postnatal cessation of granule cell acquisition. Modified from Bayer and Altman (1974).

axons. This is suggested by the recent report that the high concentration of zinc in the hippocampus associated with mossy fiber terminals does not become apparent in the rat until about 18-22 days postnatally (Crawford and Connor, 1972).

Probably in most central nervous structures the outgrowth of the axon antedates the development of the cell's dendritic system. In the granular layer of the dentate gyrus, situated between the subgranular zone of darkly staining small cells and the superficial zone of mature granule cells, cells may be seen which are intermediate in both size and staining intensity. These typically have a vertically oriented elongated shape. Often these cells have a recognizable apical extension or a thick dendritic shaft. In the development of cerebellar Purkinje cells, it was observed that the outgrowth of the richly arborizing dendritic system is preceded by the formation of an apical cone filled with mitochondria (Altman, 1972b). These mitochondria are produced in an apical position in the vicinity of the nucleus and then, judged by the transient high concentration and shape of the mitochondria, they stream upward and become distributed in the rapidly expanding dendritic branches. In analogy with this event, it is assumed that the pear-shaped cells with apical cones or shafts in the subgranular zone are in a comparable stage of development.

This assumption is supported by histochemical observations on the concentration and distribution of various oxidative enzymes in the developing dentate gyrus. Biochemical studies have established that the majority of oxidative enzymes in brain tissue homogenates are concentrated in mitochondria. In the newborn rat (Meyer *et al.*, 1972), oxidative enzyme (succinic dehydrogenase and cytochrome oxidase) activity was low and restricted to the perikarya of hippocampal granule cells. Staining intensity increased subsequently, but by day 10 perikaryal activity began to decline and the stronger staining shifted to the molecular layer. The adult pattern of

FIG. 7. The granular layer (GL) of the dentate gyrus in guinea pigs aged 6 h (A), 6 days (B), 18 days (C), and 36 days (D). Note the subgranular zone of primitive cells (arrows) at the base of the granular layer and the hilus (HI) of the dentate gyrus. From Altman and Das (unpublished data).



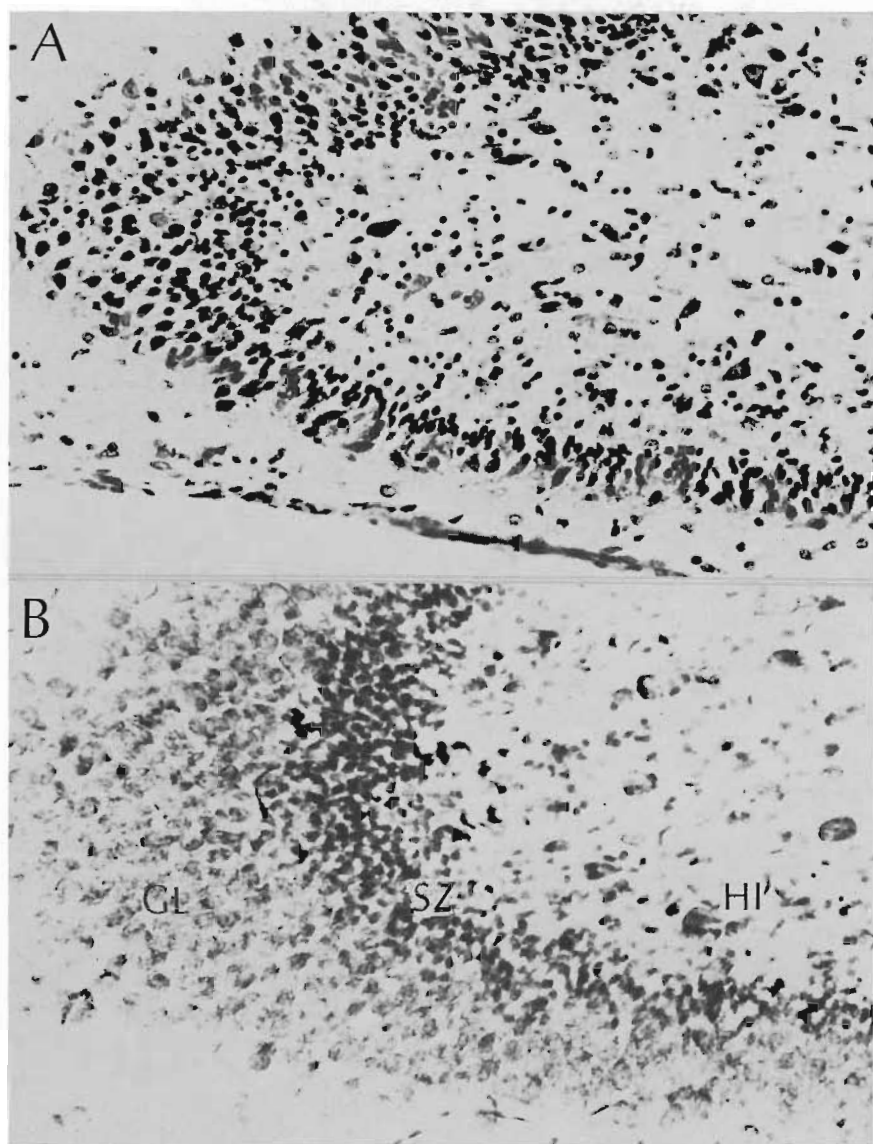


FIG. 8. The granular layer (GL) of the dentate gyrus in kittens aged 9 days (A), 21 days (B), 30 days (C), and 60 days (D). Primitive cells are seen in the hilus (HI) but are most numerous in the subgranular zone (SZ). The presence of pronounced subgranular zone in 60-day-old (postnatal) kittens suggests that hippocampal neurogenesis may be more protracted in the cat than in the rat. Unpublished photomicrographs.

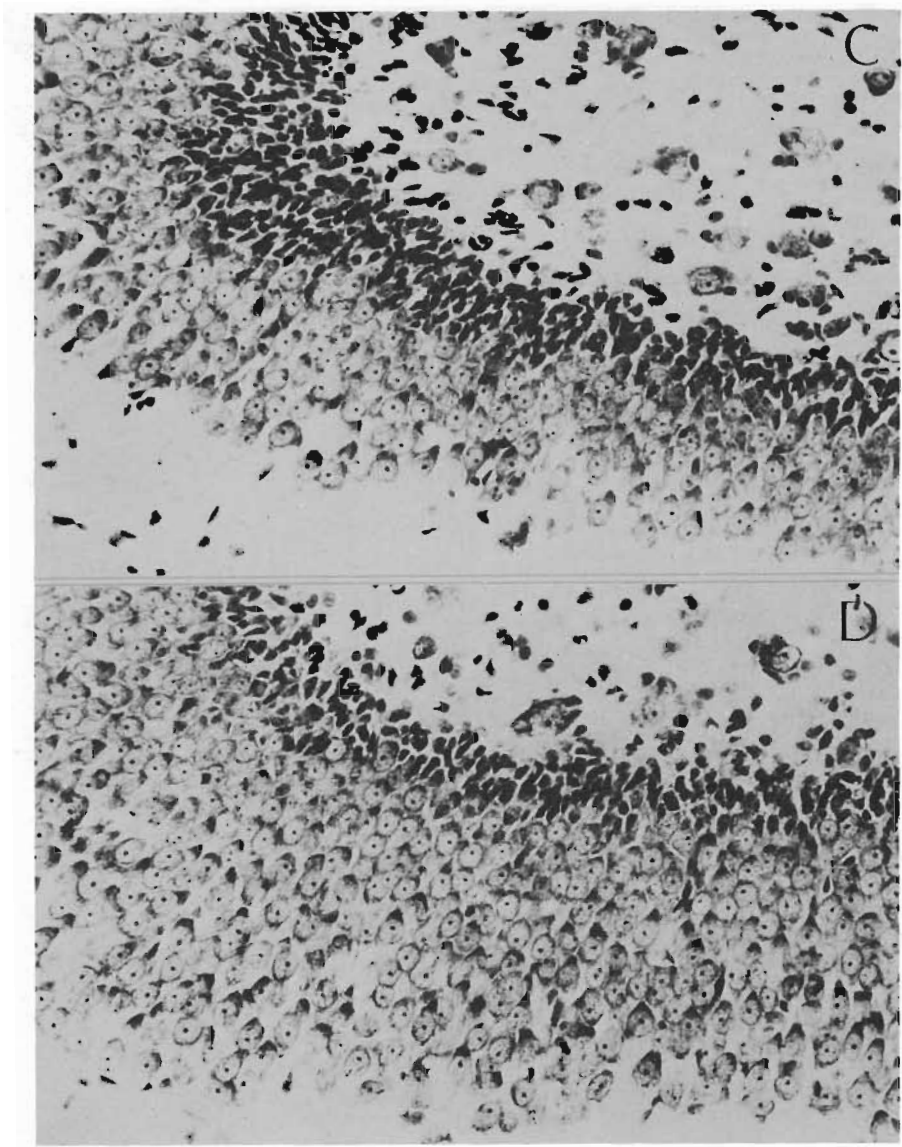
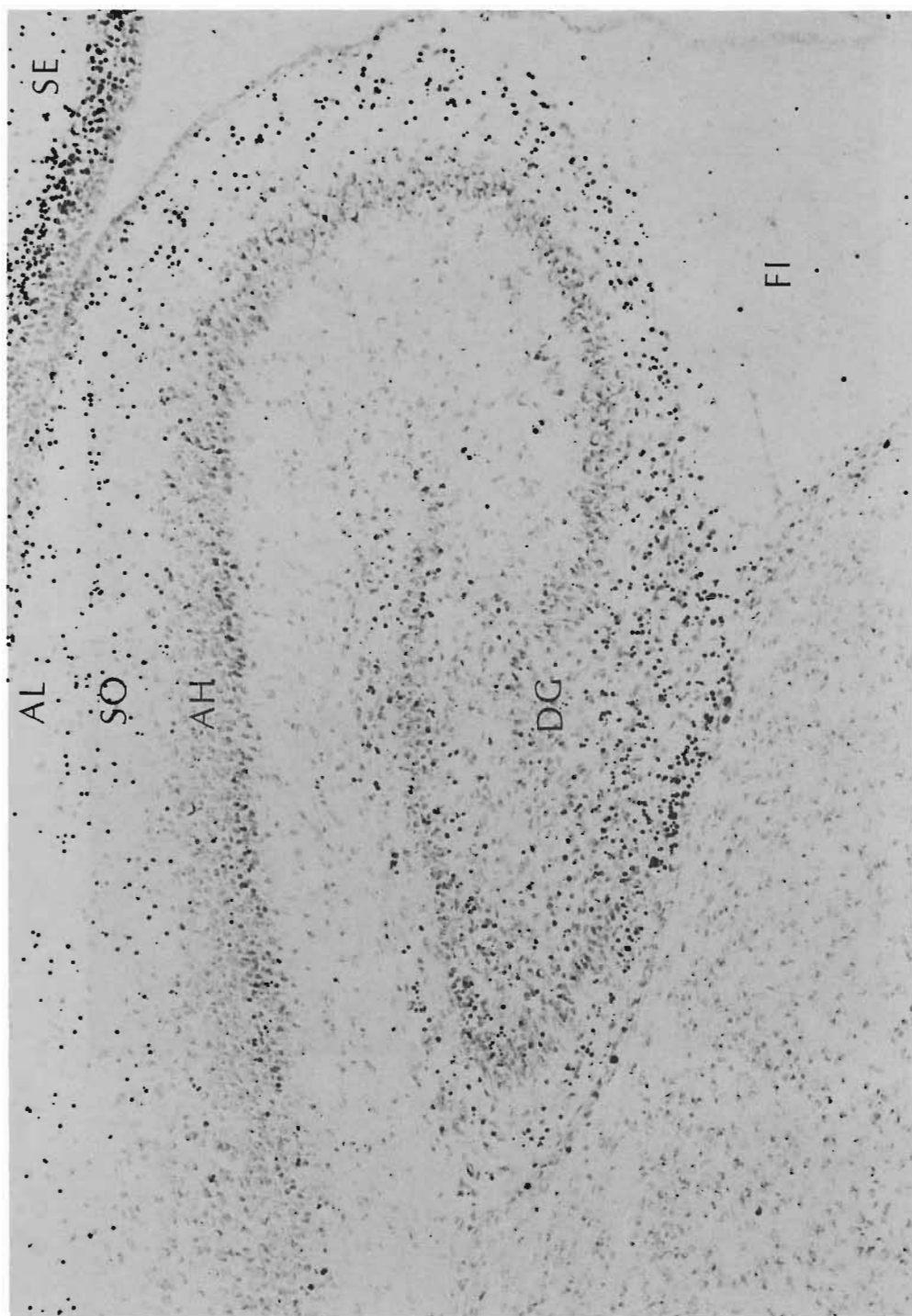


FIG. 8. (Continued)



oxidative enzyme activity was reached by the fourth week of life. These observations agreed with earlier results of Das and Kreutzberg (1967) and were more recently confirmed by Mellgren (1973). They noted during early development a stronger staining of differentiating granule cell perikarya in a superficial position than in the less-differentiated deeper cells, and the small primitive cells of the subgranular zone tended to remain unstained.

In the absence of electron microscopic studies, little more can be said about the morphogenetic phase of dentate development. The fragmentary information that is available suggests that the "outside-in" pattern of cytogenesis is paralleled by a similar gradient of differentiation. The radiosensitive, multiplying cells are situated basally mixed with cells that have become radioresistant and presumably have commenced to differentiate by starting to grow axons. The cells lying above them have started to grow dendrites. As the dendritic system is developing, the cell acquires adult appearance, first in shape and then in size. The developmental course of the differentiation of mature granule cells is summarized in Fig. 10.

2.3. *Synaptogenesis*

The appropriate examination of synaptogenesis requires ultrastructural methods, but unfortunately very few published electron microscopic studies are available that deal with the development of the dentate gyrus. In the pioneering studies of Schwartz *et al.* (1968) and in the associated report by Purpura and Pappas (1968), relatively little attention was paid to the development of the dentate gyrus, and the impression these investigators gained was that the dentate gyrus is quite mature in newborn kittens. Their conclusion on the basis of Golgi observations was that "Nonpyramidal neurons of the neonatal kitten hippocampus, like pyramidal neurons, have a remarkably mature appearance and exhibit little change in overt characteristics in the postnatal period" (Purpura and Pappas, 1968, p. 389). Similarly, they interpreted their electron microscopic observations to indicate that "complex axon terminals resembling typical mossy fiber endings described in adult animals are also well developed in neonatal kitten" (Schwartz *et al.*, 1968, p. 394). In light of other evidence of the late and protracted acquisition of dentate granule cells in the kitten, it must be assumed that these investigators observed the prenatally formed complement of granule cells.

In a more recent study (Crain *et al.*, 1973), synapse counts were made in the molecular layer of the dentate gyrus of rats aged 4, 11, 25, and about 90 days. The number of synapses was very low at 4 days, constituting less than 1% of those seen in adults. Between 4 and 11 days, the extrapolated number of synapses nearly doubled every day. But even by 11 days less than 5% of the adult concentration of synapses

FIG. 9. Photomicrograph of the hippocampus of a 1-day-old rat whose head was irradiated with 200 r X-ray and killed 6 h later. The radiosensitive pyknotic cells appear as opaque dots. They are abundant in the hilus and molecular layer of the dentate gyrus (DG), the subependymal layer (SE) of the cerebral cortex and the alveus (AL) and stratum oriens (SO) of Ammon's horn (AH). Only a few pyknotic cells are seen in the fimbria (FI), where gliogenesis has barely started. From Bayer and Altman (1974).

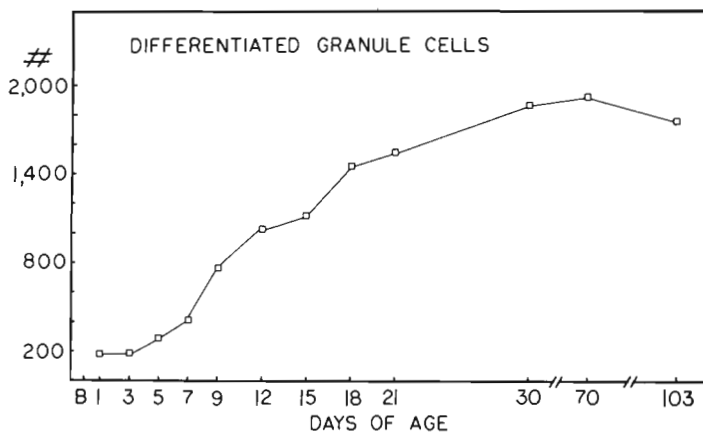


FIG. 10. Histological assessment of postnatal cell acquisition in the granular layer from birth to 103 days of age. Means are based on counts made in six animals at all ages indicated in matched coronal sections of the dorsal hippocampus. Modified from Bayer and Altman (1974).

was obtained in the endal arm of the dentate gyrus. The more than hundredfold increase in synapses was reached by 25 days of age, with no apparent increases thereafter.

Several histochemical and biochemical studies dealing with the maturation of transmitter-related enzymatic activity are relevant to the discussion of synaptogenesis and the development of the circuitry of the dentate gyrus. Evidence is available (Storm-Mathisen and Blackstad, 1964; Shute and Lewis, 1966; Lewis and Shute, 1967; Mellgren and Srebro, 1973) that the histochemically demonstrable acetylcholinesterase activity of the hippocampus is associated with septal afferents. These septohippocampal fibers are distributed in a laminar pattern—in the dentate gyrus in two separate bands, one infragranular and the other supragranular. Ritter *et al.* (1972) reported that acetylcholinesterase reaction is not present in the rat hippocampus until day 3 (however, faint monoamine oxidase reaction was noticeable at birth). Between days 10 and 20, there was a large increase in acetylcholinesterase activity and the adult pattern of distribution and intensity was reached by day 35.

In a more detailed study, Matthews *et al.* (1974) reported that acetylcholinesterase activity was not pronounced in the rat hippocampus at 4 days of age. Staining became more distinct by day 6 in the ectal (lateral) arm of the dentate gyrus; by day 8, the hilus was staining heavily and thereafter there was an increase in staining intensity in all regions of the hippocampus. Staining of the commissural zone was not detectable until day 16 and was not obvious until day 25. Because the staining reaction appeared earlier in the septal (anterior) end of the hippocampus than its more temporal (posterior) portions, it was postulated that the progression of staining marked the growth of septal afferents. Matthews *et al.* (1974) suggested that septal afferents first reach the dentate gyrus by day 4 and are distributed throughout the hippocampus by day 11. In a correlated quantitative histochemical study (Nadler *et al.*, 1974), the attempt was made to obtain some indirect evidence about

“cholinergic synaptogenesis” by determining choline acetyltransferase activity (together with acetylcholinesterase activity) in discrete layers of the dentate gyrus. Choline acetyltransferase activity was low at 11 days, but there was a sharp increase around 16–17 days. It was suggested that the latter dates may mark the onset of rapid synaptogenesis in the dentate gyrus. In view of the uncertain correlations between “cholinergic” afferents and synapses on the one hand and acetylcholinesterase and choline acetyltransferase activity on the other, we must await further information gained with other techniques, especially electron microscopy, for a more definitive assessment of synaptogenesis in the dentate gyrus.

2.4. Gliogenesis and Myelogenesis

The fimbria and fornix are recognizable in the rat by day 18 of gestation (Bayer and Altman, 1974). Insofar as a large proportion of the fornix is made up of efferents of the hippocampus, the outgrowth of axons of pyramidal cells is presumably in progress by this time. But there are few cells in the fimbria and fornix until the end of the first week after birth, suggesting that the glia cells (oligodendroglia) that are responsible for myelination do not appear in appreciable numbers for some time after the formation of axons. Myelination is heralded by a spurt in the proliferation of glia cells, the event is sometimes referred to as “myelination gliosis” (Roback and Scherer, 1935). This was examined in a recent study with the dated cumulative autoradiographic labeling technique. The results (Bayer and Altman, 1975) showed that about 73% of the cells of the fimbria are formed postnatally (Fig. 11). Similar results were obtained with the method of X-ray-produced cell pyknosis (see Fig. 9). There may be a delay of several days between the peak of myelination gliosis and the onset of myelination (Bensted *et al.*, 1957; DeRobertis *et al.*, 1958). The results of Jacobson (1963) indicated that the stainability of the fornix for myelin (Weigert’s technique) is still faint on postnatal day 12 and moderate by day 21. By day 25, the fornix as well as the perforant bundle stain

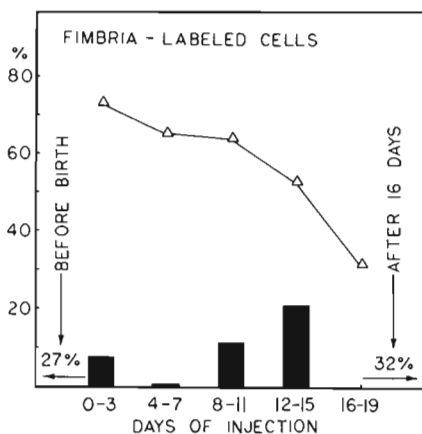


FIG. 11. Proportion of labeled cells in the fimbria in groups of 60-day-old rats that were injected daily with [^3H]thymidine on days 0–3, 4–7, 8–11, 12–15, and 16–19. Method of evaluation the same as in Fig. 6. Modified from Bayer and Altman (1974).

strongly. The late myelination of the fornix was also reported for man. Yakovlev and Lecours (1967) found that the fornix remains unmyelinated until the end of the fourth postnatal month and that the process is a protracted one and may not be completed until the third postnatal year. In none of these studies has the gliogenesis and myelogenesis of the hippocampus been examined in great detail, and on this subject as on so many other aspects of hippocampal neurogenesis there is a great need for further investigations.

3. *Development of the Dentate Gyrus Under Experimental Conditions*

Many lines of evidence suggest that the immature brain has greater powers of recovery after insult than the mature brain. However, in many respects it is the developing nervous system which is the more vulnerable. An example already discussed is the extreme radiosensitivity of multiplying cells. Other treatments that selectively damage the developing brain include under- or malnutrition, drug administration, and hormonal manipulations. Because small neurons and the glia cells of the hippocampus are to a large extent formed postnatally and because exposure to environmental hazards increases greatly after birth, the susceptibility of the developing hippocampus has been the subject of several investigations.

3.1. *Effects of Interference with Cytogenesis*

Since irradiation of the hippocampus leads to the death of a large proportion of the multiplying precursors of granule cells (Fig. 9), it was logical to inquire whether or not this treatment will result in a permanent loss in differentiated granule cells. Earlier studies with the cerebellum showed that the long-term effects of X-irradiation are complicated by the capacity of the subtotally eradicated external germinal layer to regenerate (Altman *et al.*, 1969). To prevent this recovery, the cerebellum was irradiated repeatedly at daily or longer intervals. This approach showed that with the delivery of up to five successive daily doses of 200 r, the speed of regeneration of the external germinal layer was inversely related to the number of exposures. Because the external germinal layer disappears naturally at 21 days of age, the progressive delay in recovery of the germinal matrix resulted in a graded reduction of the postnatally formed cells of the cerebellum (Altman and Anderson, 1971). We have as yet no information about the time course and nature of recovery following irradiation of the hippocampus. However, it has been recently established (Bayer *et al.*, 1973) that irradiation with eight doses of 150–200 r between 2 and 15 days reduced the granule cell population of dentate gyrus to 15–18% of its normal concentration (Figs. 12 and 13A). The treatment presumably prevented the formation of all the postnatally acquired population of granule cells. The prenatally formed granule cells, like the pyramidal cells (Fig. 13B), were spared. A subsequent study (Bayer and Altman, 1975) showed that with two doses of 200 r delivered on days 2 and 3 the granule cell population was halved and the effect of four and six successive doses was similar to that of eight doses, resulting in an asymptotic reduction of

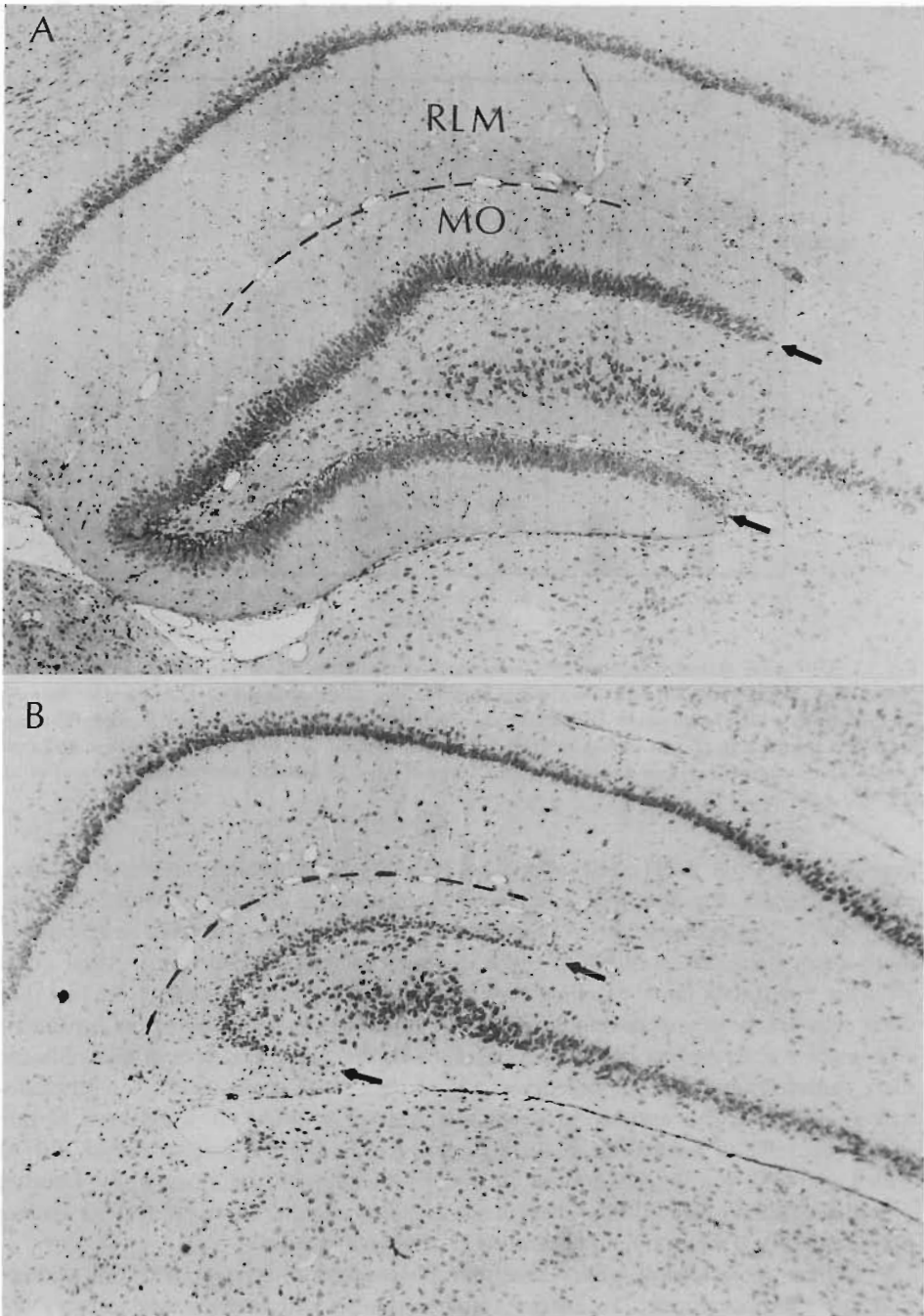


FIG. 12. Photomicrographs of the hippocampus in a control rat (A) and a rat irradiated with eight doses of 150–200 r between days 2 and 15 (B). Broken lines delineate the hippocampal fissure separating the dentate gyrus from Ammon's horn. Note the drastic reduction in the cell thickness of the granular layer (arrows) in the irradiated animal and also in the width of the molecular layer of dentate gyrus (MO). The width of strata radiatum, lacunosum, and moleculare of Ammon's horn (RLM) is not obviously affected. Slightly modified from Bayer *et al.* (1973).

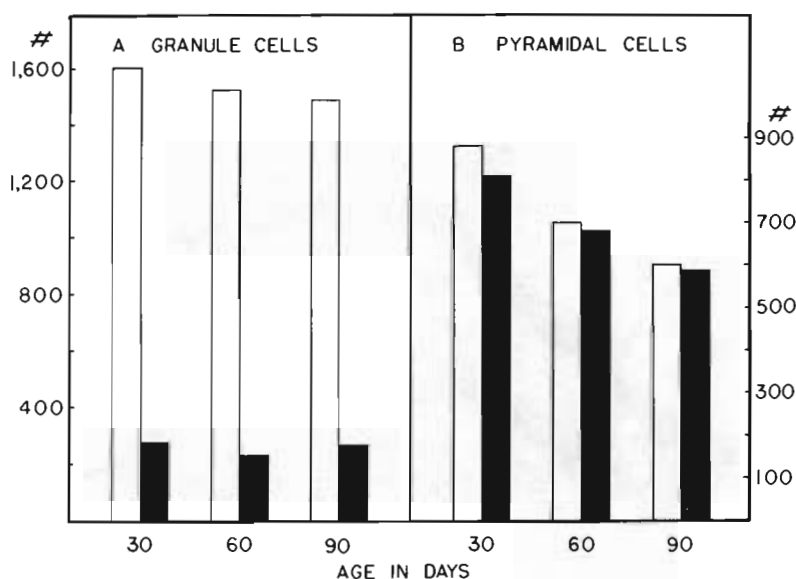


FIG. 13. A: Number of dentate granule cells in matched sections of the dorsal hippocampus in control rats (white bars) and X-irradiated rats (black bars). Each bar represents the mean of data from five animals. The reduction is highly significant. B: Number of pyramidal cells in the same group of animals. The age-dependent reduction in granule cells in control animals and in pyramidal cells in both groups is attributed to the volumetric expansion of the hippocampus between days 30 and 90. Modified from Bayer *et al.* (1973).

granule cells (Fig. 14B). The effects on cells of the molecular layer, which presumably consist of a mixed population of neurons and glia, was somewhat different (Fig. 14C). The delivery of two doses had no obvious effect, and with progressively more doses (four, six, and eight) there was a proportional reduction in these cells, with the magnitude never reaching that seen in granule cells. In view of the fact that these cells are largely of postnatal origin, we have tentatively assumed, in agreement with earlier observations (Altman *et al.*, 1968a), that the precursors of glia cells are more radioresistant than the precursors of neurons. None of the irradiation schedules had an effect on the number of pyramidal cells (Fig. 14A). It is relevant in this context to refer to behavioral studies (Bayer *et al.*, 1973; Haggbloom *et al.*, 1974) which showed that interference with the acquisition of the postnatally forming granule cells and other cellular elements of the hippocampus results in deficits similar to those observed when the hippocampus is destroyed *in toto*.

Do manipulations of milder kind than X-irradiation interfere with cell acquisition in the hippocampus? To answer this question, it will be necessary to carry out investigations of the type that have been done with respect to the cerebellum. It is now well established (*cf.* Altman, 1975) that treatments such as undernutrition or hypo- and hyperthyroidism interfere with cell acquisition in the cerebellum. In one study in which the food intake of infant rats was restricted by increasing litter size, biochemical assessment of DNA concentration indicated a reduction in cell number in

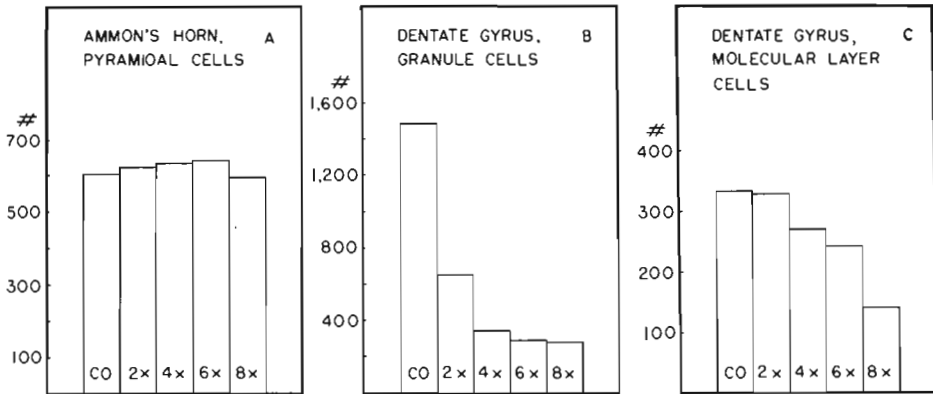


FIG. 14. The effects of two, four, six, and eight successive doses of 150–200 r delivered from day 2 onward on different cell types of the hippocampus. CO, Control. There was no significant effect with any of the radiation schedules used on the pyramidal cells of Ammon's horn (A). There was substantial reduction in granule cells with two doses and near asymptotic reduction was obtained with four doses (B). However, two doses had no effect on cell concentration in the dentate molecular layer and graded reduction was obtained with four, six, and eight doses (C). From Bayer and Altman (unpublished data).

the hippocampus at 17 days of age (Fish and Winick, 1969). Our own results have so far been somewhat ambiguous on this point.

Another approach that requires further investigation is the possible effect of behavioral manipulations in young animals on cell acquisition in the hippocampus. In an exploratory study (Altman *et al.*, 1968b), rats were "handled" daily from day 2 to day 11 after birth. On day 11, these animals and unhandled controls were injected with [^3H]thymidine and killed 6 h or 3 or 30 days thereafter, at which time the brains were processed for autoradiography. The results showed (Fig. 15) a higher concentration of labeled granule cells at all the three ages in the handled animals.

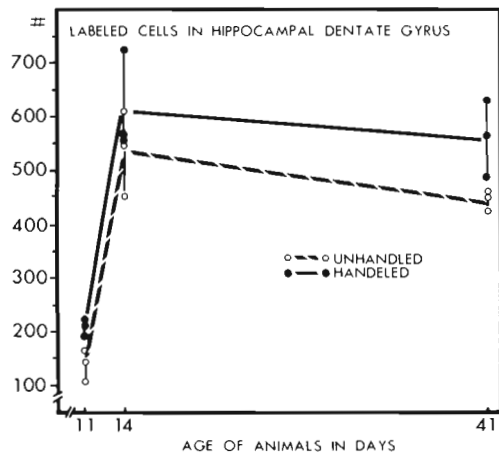


FIG. 15. Number of labeled cells in matched sections of the dorsal hippocampus in five 200- μm -wide strips of the granular layer. From Altman *et al.* (1968b).

Neither the nature of handling nor the meaning of the higher rate of cell labeling is adequately understood at present. For instance, a higher concentration of labeled hippocampal granule cells in the handled animals could be interpreted as a higher rate of cell production or just as a delay in hippocampal maturation, a hypothetical phenomenon that we referred to as "infantilization" (Altman *et al.*, 1968b).

3.2. *Effects of Interference with Morphogenesis*

The concept of neuromorphogenesis includes, on the cytological level, the acquisition by differentiating nerve cells of specific perikaryal, dendritic, and axonal patterns and, on the histological level, the aggregation of cell bodies and of their proximal and distal processes in specific ways. This developmental phase antedates and is a prerequisite to the establishment of the gross and fine circuitry in any brain region.

In one study (Das, 1971), the anterior portion of the dorsal hippocampus was surgically severed in 8- and 15-day-old rabbits so that the precursors of granule cells that migrate to the dentate gyrus could not reach their destination. The morphological organization of the hippocampus was examined when the animals were 40 days old. Das found that the dorsal dentate gyrus remained underdeveloped as a result of the cut but that the proliferating granule cell precursors formed a hypertrophied accessory dentate gyrus, rudiments of which are present in normal rabbits.

A different approach was taken by Lynch, Cotman, and their associates (Lynch *et al.*, 1973a). It is well established that the commissural projection to the molecular layer of the dentate gyrus is confined to a narrow zone above the granular layer. In a group of 11-day-old rats, large lesions were made in the entorhinal cortex to eliminate the entorhinal projection to the hippocampus. When these animals became mature, a second lesion was made to sever the commissural projection; similar lesions were also made in a control group. In the rats with previous entorhinal lesions, the commissural afferents extended over 90% of the width of the molecular layer. The investigators concluded that the developing commissural system will spread out along the granule cell dendrites from its normally restricted domain if the entorhinal afferents that normally innervate the outer dendritic field are eliminated. In addition to this "spreading" effect, Lynch *et al.* (1973b) also observed an increase in the density of commissural terminals in animals whose entorhinal cortex was removed in infancy. Similar effects of lesser magnitude were noted when the entorhinal lesions were made in adulthood. Another effect of entorhinal lesions was the intensification of cholinesterase staining in the outer part of the molecular layer, which could be attributed to the spreading of septal afferents (Cotman *et al.*, 1973). Electron microscopic investigations suggested that this augmentation could be partly attributed to an increase in the number of acetylcholinesterase-rich synaptic endings.

However, a caution is in order against interpreting the latter type of findings as a reflection of the greater "plasticity" of the infant than adult hippocampus and, by implication, a greater potential for "recovery of function." Morphological findings of "spreading" or "sprouting" of axons and axon terminals, even when coupled with electron microscopic demonstration of synaptic junctions formed, cannot be taken as

evidence of the establishment of coordinated functional contacts. This cautionary attitude is the outcome of some recent results regarding the effects of X-irradiation of the cerebellum in infant rats, where we found that numerous indications of structural reorganization were not paralleled by functional recovery (Brunner and Altman, 1974). For instance, when the acquisition of cerebellar granule cells is prevented by X-irradiation during infancy, the mossy fibers, which primarily synapse with granule cells in the granular layer, invade the molecular layer. This was first suggested in a histochemical study dealing with the distribution of acetylcholinesterase (Altman and Das, 1970) and was subsequently confirmed with electron microscopy (Altman and Anderson, 1972). These mossy fibers apparently form synapses with the soma and dendrites of Purkinje cells, thus displaying considerable potential for "reorganization." However, when these animals are tested as adults severe motor deficits are observed (Wallace and Altman, 1969b; Altman *et al.*, 1971) which resemble the effects of decerebellation. Moreover, our studies seem to indicate that schedules of X-irradiation which produce massive reduction in the number of cells lead to less behavioral deficits than schedules which have less effect on the cell population but which result in drastic reorganization of the circuitry of the cerebellar cortex (Altman, 1975).

As yet, we have little information about the nature of the structural changes produced in the hippocampus by the prevention of the recruitment of the postnatally forming granule cells. No gross malformation has been noted in hippocampal morphogenesis, nor is there an evident effect on the number, size, and appearance of pyramidal cells. Tentatively it may be assumed that neither the extrinsic input to Ammon's horn nor its output is seriously interfered with. Nevertheless, as referred to earlier, the irradiated animals display fully all symptoms of surgical destruction of the hippocampus as a whole (Bayer *et al.*, 1973; Haggblom *et al.*, 1974). The conclusion seems inescapable that, as in the case of the cerebellar cortex, the granule cells play a vital role in the integrated functions of the hippocampus.

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