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Radiation-induced Interference with Postnatal Hippocampal Cytogenesis in Rats and Its Long-term Effects on the Acquisition of Neurons and Glia

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ABSTRACT The long term consequences of graduated interference with the acquisition of hippocampal neurons and glia during early infancy were examined with quantitative histology and ^{3}H-thymidine autoradiography. The head region containing the hippocampus was irradiated from day two on with either two (2X), four (4X), six (6X) or eight (8X) doses of 150–200r X-rays. The animals were killed at 30, 60, 90 and 120 days of age.

The morphology of the hippocampus was normal in all irradiated groups with the characteristic interlocking folds of the pyramidal and granular layers. While the number of pyramidal cells of Ammon's horn was unaffected, the number of granule cells of the dentate gyrus was progressively and permanently reduced from control levels by the different dosage schedules (2X, 59% reduction; 4X, 77%; 6X, 83%; 8X, 84%). Incidental observations in control animals indicated a 20% increase in granule cells between 30 and 120 days of age in agreement with earlier observations of granule cell labelling after ^{3}H-thymidine injections in adult rats.

The time of origin of the approximately 15–16% of the granule cells surviving irradiation in the 8X group was determined by injecting either pregnant females (gestation days 19–20) or pups (days 0 and 1) with two successive doses of ^{3}H-thymidine; the animals were irradiated from day two on with eight X-ray doses. The granule cells surviving in the postnatally injected group were all unlabelled and comparable in number to the unlabelled cells in control animals that were given five successive postnatal injections of ^{3}H-thymidine. This established that the radioresistant complement of granule cells is formed prenatally. In the prenatally injected group, over half the surviving cells were labelled; it was assumed that those not labelled were formed before gestation day 19.

In contrast to the permanent reduction in the number of granule cells, there was some reestablishment of the number of cells in the dentate molecular layer and the Ammonic stratum oriens; in the fimbria, recovery in cell number was complete by 60 days. In a supplementary autoradiographic experiment, cell proliferation in the granular layer and in the fimbria was determined at 60 days of age after a single postnatal injection of ^{3}H-thymidine on either day 15 or day 20 in the control, 2X, 4X and 6X groups. The number of labelled cells in the irradiated groups was always well below control levels in the granular layer, but it was either above or at the same level as controls in the fimbria. Tentative interpretations were offered for the differential long-term effects of variable X-ray schedules on the neuronal and glial populations of the hippocampus.

The rat hippocampus contains postnatally forming cell populations, such as the granule cells of the dentate gyrus (Altman and Das, '65, '66; Altman, '66; Bayer and Altman, '74), the glial cells of the fimbria and others (Bayer and Altman, '74). The proliferating and migrating precursors of these cells are extremely radiosensitive and are killed by a dose of X-ray (150–200r) which does not affect differentiating or mature cells (Hicks and D'Amato, '66; Altman et al., '68). Thus, by irradiating the rat hippocampus during the early postnatal period, one should selectively reduce

the postnatally forming cell populations. Such a technique has been successfully used in the cerebellum (reviewed in Altman, '75). Using an experimental design based on the cerebellar studies, we found in a pilot study (Bayer et al., '73) that exposure of the rat hippocampus to eight successive doses of low-level X-ray during the early postnatal period removed 83–85% of the dentate granule cells. It was postulated that the spared granule cells are the prenatally forming elements (Bayer and Altman, '74).

Since the biological significance of postnatal (or late) cytogenesis is not known, a technique allowing selective elimination of various cell populations can be useful in testing the role of these elements in bringing about adult brain morphology. The effects of X-irradiation are repeatable and can be easily applied to large numbers of animals at various times during the early postnatal period. In this report we examine the anatomical effects of hippocampal irradiation beginning on postnatal day 2 and extending for various periods into the first and second weeks of life. Particular attention is paid to the sparing of the prenatally-forming elements, including a specific proportion of granule cells. The differential effects of X-irradiation on the postnatally-forming elements, especially the neuronal population of the dentate granular layer and the glial population of the fimbria, gives insight into the recovery mechanisms available to the immature nervous system after subtotal destruction of its formative cell populations.

MATERIALS AND METHODS

Purdue-Wistar male rats were cross-fostered and raised six to a litter. Before irradiation the experimental pups were immobilized in lucite holders and placed under two protective lead sheets separated by a narrow slit so that only that portion of the head containing the hippocampus was exposed. The location of the hippocampus from 2 to 18 days of age had been previously determined in sagittal slices of the head by measuring the length from the snout to the anterior and posterior borders of the dorsal hippocampus (Bayer et al., '73). The X-ray source was a Maxitron 300 kV unit. Irradiation target measurements for each age were verified by killing a group of animals six hours after a single exposure to 200r and checking for cells killed by the X-ray (pyknosis) in the hippocampus and other brain regions.

The four groups of pups to be irradiated received either 2, 4, 6 or 8 exposures to X-rays beginning on day 2. For the pups in the 8X group, 200r was delivered on days 2 and 3 followed by 150r on days 5, 7, 9, 11, 13 and 15. The remaining groups of irradiated animals received only the initial parts of the 8X schedule. The pups in the 6X group received their last exposure on day 11, the 4X group on day 7 and the 2X group on day 3.

Between 10–20 animals from the control and each X-irradiated group were killed by transcardiac perfusion with 10% neutral formalin at 30, 60, 90 and 120 days of age. The brains were carefully removed, placed in Bouin's fixative for 24 hours, further fixed in 10% neutral formalin and embedded in paraffin. Serial sections (6 μ) of the hippocampal region were cut in either the sagittal or coronal planes. The pyramidal cells of Ammon's horn and the cells of the stratum oriens (endothelial cells excluded) were counted at level A3.8 (De Groot, '59). Granule cells in the ectal limb (facing the cerebral hemispheres) and endal limb (facing the thalamus) of the dentate molecular layer (excluding endothelial cells) were counted at level A3.0 (De Groot, '59). Cells within equal areas of the fimbria were counted at levels ranging from A4.2 to A3.4 (De Groot, '59). In addition, granule cells and pyramidal cells in the dorsal part of the hippocampal region were counted in the sagittal plane (L1490 μ; König and Klippel, '63); the area of the hippocampus was determined planimetrically at the same level (fig. 1D).

In order to more fully characterize the effect of X-irradiation on cell populations in the hippocampus, two supplementary experiments were done involving the use of ^{3}H-thymidine injections (New England Nuclear, specific activity, 6.7 Ci/mmole; 10 μCi/gbw). The first experiment investigated the effects of X-irradiation on the pre- and postnatally forming granule cells. In one group, pregnant females were given subcutaneous injections of ^{3}H-thymidine on days 19 and 20 of gestation. In the other group, pups were given two successive subcutaneous injections of ^{3}H-thymidine on days 0

(the day of birth) and 1. The pups in both groups were irradiated according to the 8X schedule. One litter of control pups was injected with ^{3}H-thymidine on days 0, 3, 6, 9 and 12. All animals were killed at 30 days of age. The second experiment investigated radiation recovery. Two litters each were assigned to control, 2X, 4X and 6X groups and treated as previously described. Half the pups from each group were given a single injection of ^{3}H-thymidine on day 15, while the other half were injected on day 20. All animals were killed at 60 days of age and the brains were processed as described above. Sections (6 μ) of the hippocampus were cut in either the coronal or sagittal planes and were processed for autoradiography. The slides were dipped in Kodak NTB-3 emulsion; exposure time was ten weeks. Labelled cells were counted in the coronal plane at level A3.0 (De Groot, '59) and in the sagittal plane at L1490 μ (König and Klippel, '63).

RESULTS

Morphological effects of variable X-irradiation exposures

The appearance of Ammon's horn in the irradiated animals (fig. 16 shows a typical example) does not differ from that of controls (fig. 15). The number of granule cells decreases and their packing density becomes more sparse as the exposures increase from two to six doses (figs. 17–20); eight doses are similar to six doses. However, the remaining granule cells are normally arranged. Camera lucida drawings of Golgi-impregnated material from the control and 8X groups show no obvious differences in granule cell morphology (Bayer, unpublished observations).

The analysis of variance showed a significant overall change (fig. 1A) in the *total area of the hippocampus* in the sagittal plane (L1490 μ; König and Klippel, '63) between groups ($F = 30.2$, $df = 11/94$, $p \leq 0.001$). The Scheffé test was used to determine the differences between group means. There were significant differences ($p \leq 0.05$) between the control group and the 4X and 8X groups at 30 days and between the control and 8X groups at 60 days. There were no differences between groups at 120 days. Each group showed a significant ($p \leq 0.05$, Scheffé test) increase with age.

The analysis of variance indicated that the *area of Ammon's horn* (fig. 1B) also increased significantly with age in each group ($F = 21.6$, $df = 11/94$, $p \leq 0.001$), but here X-irradiation had no effect. The *area of the dentate gyrus* (fig. 1C) likewise increased with age in each group but there was an irradiation dosage effect ($F = 142.8$, $df = 11/94$, $p \leq 0.001$). The Scheffé test indicated significant ($p \leq 0.05$) differences between the control group and each X-irradiated group, between the 2X and 4X, and between the 2X and 8X groups; there was no significant difference between the 4X and 8X groups.

Differential effects of variable X-irradiation exposures on neuronal, glial and mixed cell populations of the hippocampus

Dentate gyrus

Granule cell loss appeared uniform throughout the entire dentate gyrus and was assessed quantitatively at one coronal (A3.0; De Groot, '59) and one sagittal level (L1490 μ; König and Klippel, '63; figs. 2A,B). The analysis of variance showed significant differences between treatment groups in the coronal plane (fig. 2A; $F = 5420.8$, $df = 14/130$, $p \leq 0.001$). There were significant differences ($p \leq 0.05$, Scheffé test) at 30, 60 and 90 days between the control group and each X-irradiated group and between 2X and 4X, 4X and 6X and 4X and 8X groups. There was no significant difference between the 6X and 8X groups. Since granule cell number in the coronal plane remains constant in all groups with age, the data were pooled to estimate the percentage of permanent granule cell reduction in the irradiated animals: 2X-59% below control levels, 4X-77%, 6X-83%, 8X-84%.

In the sagittal plane (fig. 2B) the analysis of variance again showed significant differences between groups ($F = 613.0$, $df = 11/94$, $p \leq 0.001$). The test for least significant difference indicated significant differences at all ages between the control group and each X-irradiated group, between the 2X and 4X, and between the 4X and 8X groups. Since the 6X and 8X groups were similar in the coronal plane, the 6X group was omitted in the sagittal plane. As in the coronal sections, no irradiated group showed any significant increase with

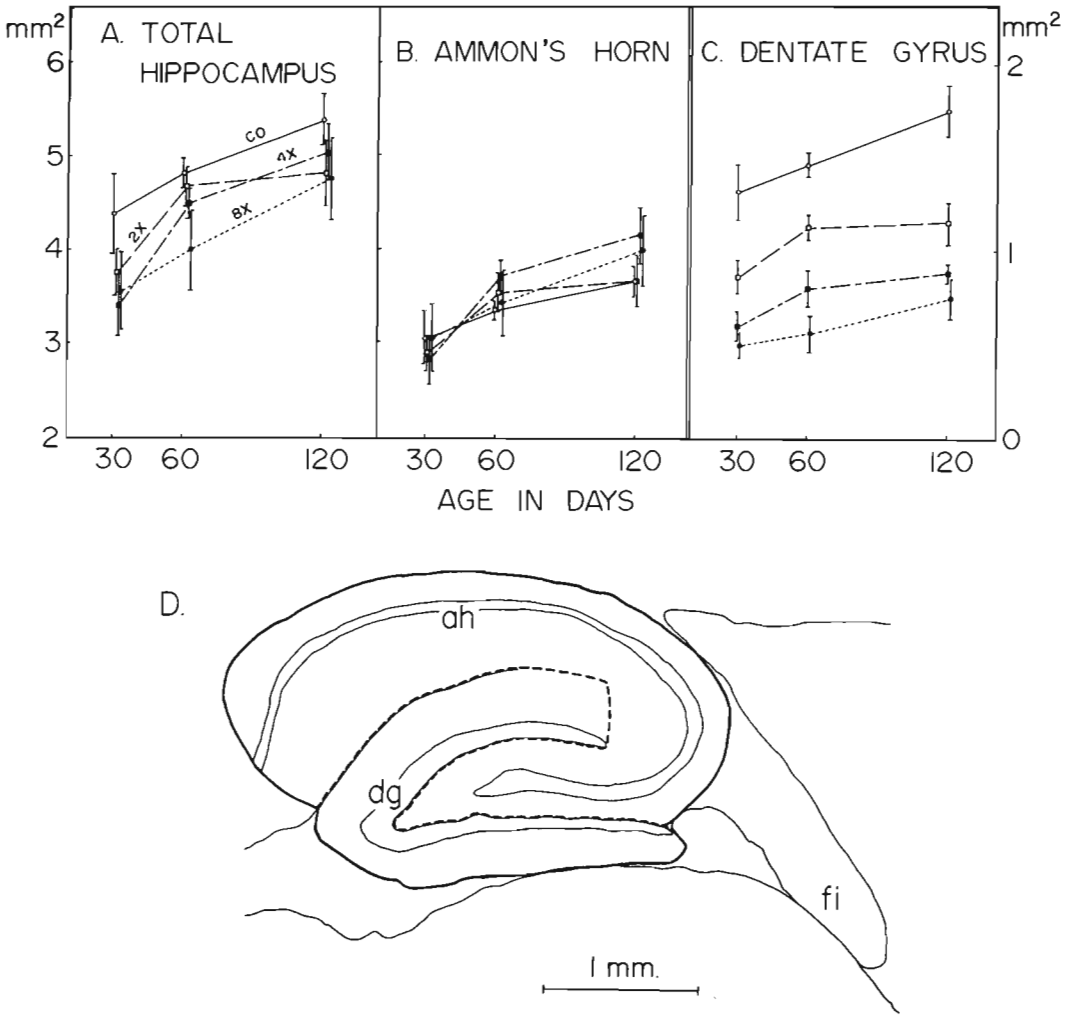


Fig. 1 Area of the hippocampus at level L1490 μ (König and Klippel, '63) from one to four months of age. Each mean with standard deviation is based on counts from approximately ten animals. A, total hippocampus; B, Ammon's horn; C, dentate gyrus; D, drawing of the hippocampus at the level studied with the areas of each part indicated. The heavy line encloses the total area; the dotted line separates the area allotted to Ammon's horn (ah) from that allotted to the dentate gyrus (dg). All groups show areal increase with age. The radiation-induced areal reduction in the total hippocampus (A) is attributable to an effect on the dentate gyrus (C), with no significant difference between groups in Ammon's horn. Note that the scale for the dentate area (C) has been enlarged. fi, fimbria.

age, indicating that cell reduction is permanent. However, the control group showed a significant (20.94%; $p \leq 0.05$, least significant difference test) increase in granule cell number from 30 to 120 days. This supports the claim of Altman and Das ('65) that granule cells are formed in adults. The increase in cell number is in line with the areal expansion of the dentate gyrus in the sagittal plane (fig. 1C). The growth of the dentate gyrus in controls includes both the molecular and granular layers, while the molecular layer is mainly responsible for the expansion in the irradiated animals.

The results in figure 3 are based on total counts of neurons and glia (endothelial cells excluded) in the molecular layer ad-

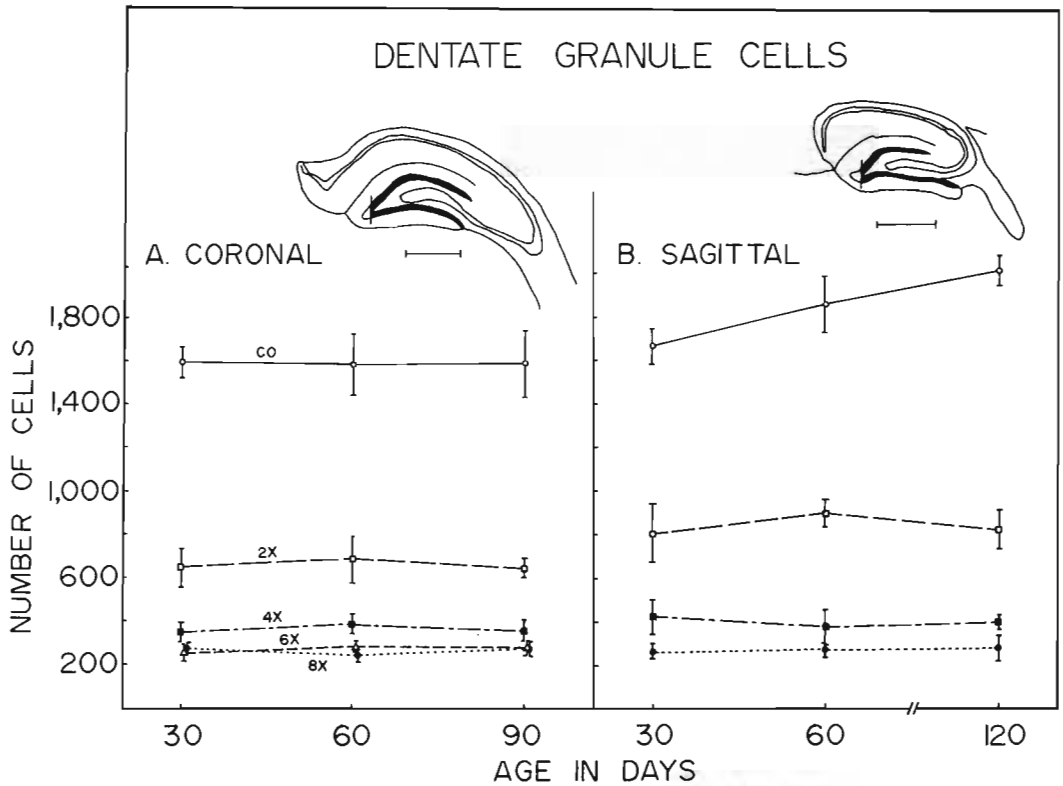


Fig. 2 Dentate granule cells in the ectal and endal limbs of the dorsal dentate granular layer. Each mean with standard deviation is based on counts from approximately ten animals. A. At coronal level A3.0 (De Groot, '59), as illustrated in the drawing; scale: 1 mm. The darkened areas of the granular layer indicate the regions where cells were counted. The number of granule cells remains constant with increasing age in all groups. B. At sagittal level L1490 μ (König and Klippel, '63), as illustrated in the drawing; scale: 1 mm. The number of granule cells in controls increases significantly with increasing age. The irradiated animals show the same reduction as in A with no increase in cell number from 30 to 120 days of age.

jacent to the ectal and endal limbs of the granular layer at level A3.0 (De Groot, '59). Two doses of 200r on days 2 and 3 led to a loss of about 11% of the molecular layer cells at 30 days; 4, 6 and 8 doses led to a loss of 38%, 50% and 55%, respectively. The percentages of reduction were similar at 90 days. The analysis of variance showed significant differences between treatments for each of the observed ages (for example, 30 days: $F = 148.8$, $df = 4/42$, $p \leq 0.001$). At 30 days there were significant differences ($p \leq 0.05$, Scheffé test) between control and 2X, 2X and 4X, 4X and 6X or 8X. As in the granule cell population, there was no significant difference between the 6X and 8X groups. By 60 or 90 days, only the 4X, 6X and 8X groups were significantly below the controls. The analysis of variance showed each group changed with age (for example, controls: $F = 13.1$, $df = 2/27$, $p \leq 0.001$). The increase in cell number was significant ($p \leq 0.05$, Scheffé test) between 30 and 90 days in each group. These results showed that the effects of X-irradiation were less severe in the molecular layer than in the granular layer and that there was some net cell gain after 30 days.

Ammon's horn

Total counts of the dorsal hippocampal pyramidal cells in the coronal plane (A3.8; De Groot, '59; fig. 4A) and sagittal plane (L1490 μ; König and Klippel, '63; fig. 4B) from one to four months of age showed that, in contrast to the dramatic reduction of

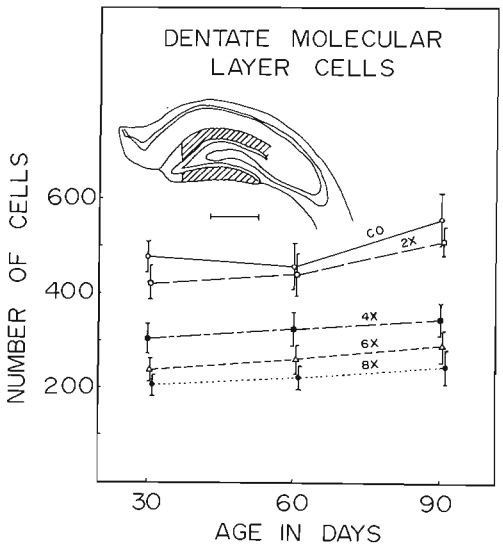


Fig. 3 Dentate molecular layer cells (neurons and glia; endothelial cells excluded) in the area adjacent to the ectal and endal limbs of the dorsal granular layer. Drawing at level A3.0 (De Groot, '59); shaded area indicates regions where cells were counted; scale: 1 mm. Each mean with standard deviation is based on approximately ten animals. X-irradiation reduces cell number, but not nearly so effectively as in the granular layer (figs. 2A,B). All groups show some increase in cell number from one to three months of age, indicating that X-ray insult did not completely retard cell proliferation in the various dosage groups.

granule cells, X-irradiation does not change pyramidal cell number in any group. However, the analysis of variance indicated significant age changes in the coronal plane ($F = 155.3$, $df = 14/130$, $p \leq 0.001$). The Newman-Keuls test showed that significant differences ($p \leq 0.01$) occurred between the 30 and 90 day age groups for the controls and each X-irradiated group. The reduction in pyramidal cell number with age does not represent a cell loss, but a decreased packing density in the coronal plane. In the sagittal plane, there is no reduction in cell number. This may be accounted for by the increase in the area of Ammon's horn in the sagittal plane (fig. 1B). The areal increase was correlated with an anterior-posterior lengthening of Ammon's horn from 2.7 mm at 30 days to 3.1 mm at 120 days ($p \leq 0.05$, Scheffé test) in the control group. Ammon's horn also increased significantly with age in the anterior-posterior dimension in the irradiated animals.

Figure 5 summarizes the numbers of neurons and glia (endothelial cells excluded) in the dorsal hippocampal stratum oriens (A3.8; De Groot, '59) from 30 to 90 days. Irradiation with 200r on days 2 and 3 produces a loss of only 9% of the population at 30 days; 4, 6 and 8 doses lead to 16%, 28% and 36% losses, respectively. At 90 days the losses for each X-irradiated group are smaller (2X, 0.2%; 4X, 4.6%; 6X, 14.5%; 8X, 22.5%) showing that cell proliferation can occur. The analysis of variance showed that cell reduction was significant at all ages (for example, 30 days: $F = 39.3$, df 4/42, $p \leq 0.001$). But, according to the Scheffé test, the 2X group never differed significantly from controls, the 4X group only at day 30, while the 6X and 8X groups were sifnificantly below ($p \leq 0.05$) controls at all ages. Only the control group showed any significant change between 30 and 60 days ($p \leq 0.05$, Scheffé test) which may be due to the rapid expansion of the hippocampus in the sagittal plane (fig. 1B).

Fimbria

Figure 6 gives the results of glial cell counts in 0.1 mm^2 areas of the fimbria (A4.2 to A3.4; De Groot, '59) for the control and each X-irradiated group at 30, 60 and 90 days. At 30 days, two doses produced a 3% loss in the population; four doses, 25%; six doses, 46%; eight doses, 55%. The analysis of variance indicated significant differences between these groups ($F = 42.9$, $df = 4/42$, $p \leq 0.001$). The Scheffé test showed these differences ($p \leq 0.05$) occurred between controls and the 4X, 6X and 8X groups. However, by 60 and 90 days the effects of the various doses of X-irradiation have disappeared with no significant difference between treatment groups (for example, 90 days: $F = 1.4$, $df = 4/45$, $p \leq 0.26$). There were no significant changes ($p \leq 0.05$, Scheffé test) with age in either the control or 2X groups; the 4X, 6X and 8X groups had a significant increase from 30 to 60 days; the 8X group also showed a significant increase from 60 to 90 days. The glial nuclei (interfascicular oligodendroglia) are similar in number at both ages in controls (figs. 7, 9), while the 8X group shows a marked increase from 30 days (fig. 8) to reach the control level by 90 days (fig. 10). The nuclei in both groups are larger at 30

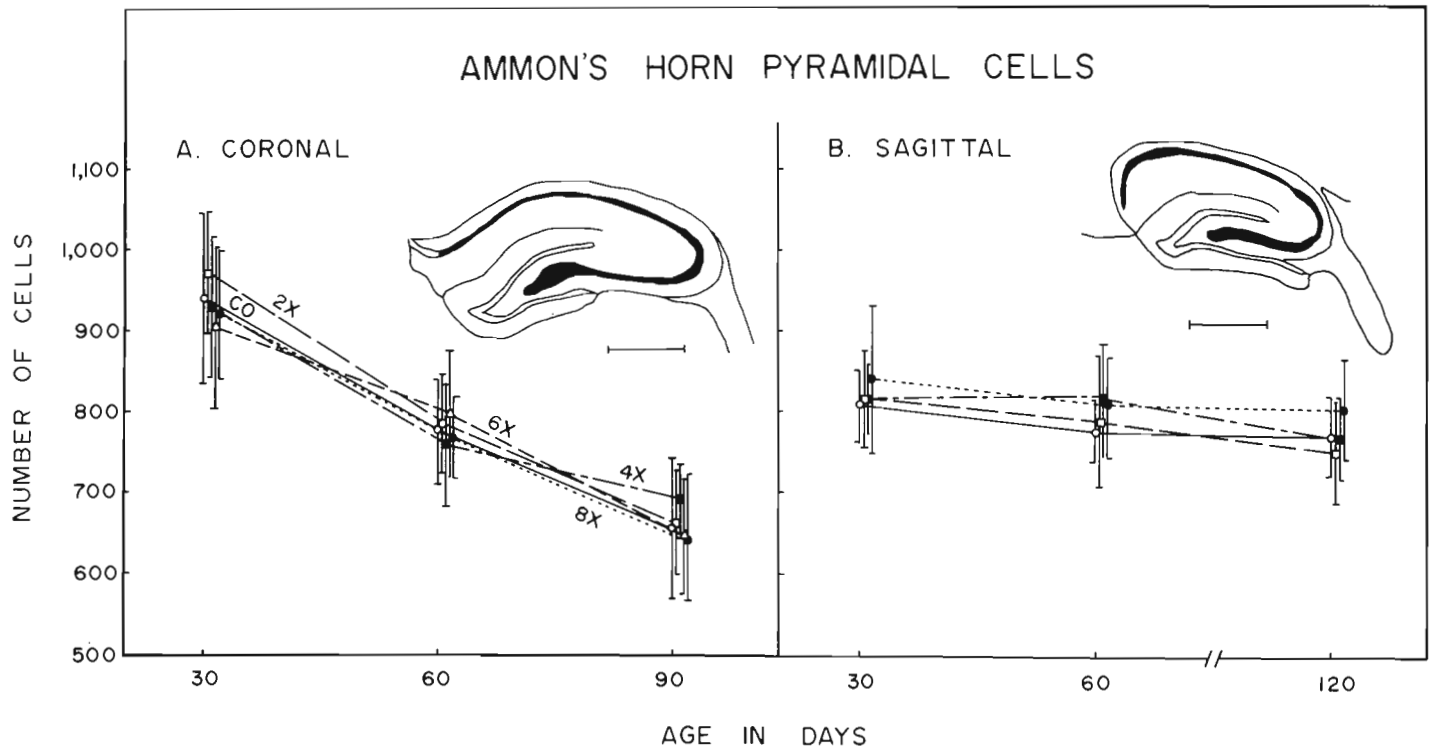


Fig. 4 A. Ammon's horn pyramidal cells of the dorsal hippocampus in the coronal plane. Drawing is at level A3.8 (De Groot, '59); darkened area: region where cells were counted; scale: 1 mm. X-irradiation has no effect on cell number. Each group shows a decrease in cell packing density from one to three months of age due to a volumetric expansion of the hippocampus during this time period (fig. 1B). B. Pyramidal cells of the dorsal hippocampus in the sagittal plane. Drawing at L1490 μ (König and Klippel, '63); darkened area: region where cells were counted; scale: 1 mm. The number of pyramidal cells remains constant with age in all groups. Again, X-irradiation has no effect on cell number. Each mean with standard deviation is based on approximately ten animals.

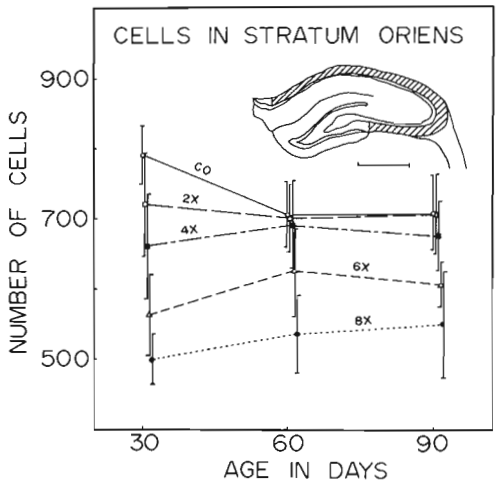


Fig. 5 Numbers of neurons and glia (endothelial cells excluded) in the stratum oriens of the dorsal hippocampus. The drawing is at level A3.8 (De Groot, '59); shaded area: the region where cells were counted; scale: 1 mm. At 30 days the magnitude of cell reduction correlates with the number of irradiations. At 60 and 90 days, reduction is apparent only in the 6X and 8X groups. The decrease in cell number between 30 and 60 days in the control group is synchronous with the rapid increase in the area of Ammon's horn (fig. 1B). Each mean with standard deviation is based on counts from approximately ten animals.

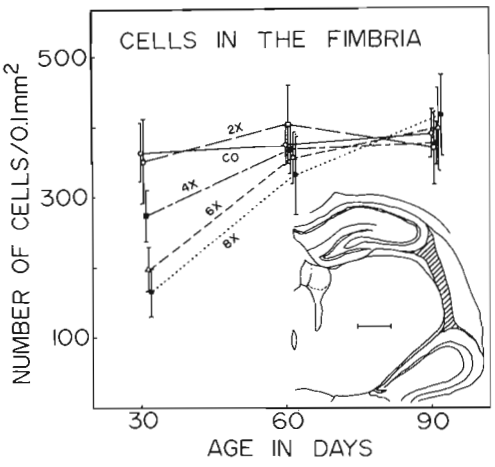


Fig. 6 Glia cells per 0.1 mm² in the fimbria. The shaded area in the drawing indicates a typical region selected for the unit area counts; scale: 1 mm. The effects of X-irradiation are only evident at 30 days. All groups reach control levels by 60 days. The control population is at a steady state from 30 to 90 days. Each mean with standard deviation is based on counts from approximately ten animals.

days than at 90 days. Oligodendroglia nuclei were reported to have a larger diameter during myelination (Mickel and Gilles, '70), a process that may be occurring in both control and 8X groups at 30 days. By 90 days, the oligodendroglia nuclei are much smaller and are interspersed with an occasional astrocyte. The parallel rows are a characteristic arrangement of glial nuclei in longitudinally sectioned fiber tracts after the active period of myelination (Glees, '55; Penfield, '65; Matthews and Duncan, '71). Thus, unlike the other hippocampal components studied, the glial population of the fimbria recovers completely from X-ray insult.

At this point we wish to insert a cautionary note regarding the method used to analyze the changes in the glial population of the fimbria. The cell counts are all based on equal area samples rather than on counts of the total area. Fimbrial cells are evenly distributed throughout and lend themselves well to this type of analysis. Calculating total area in coronal sections of the fimbria is very difficult due to large changes in area with slight shifts in anatomical levels among the sections to be compared. Consequently, there may be a total area reduction in the irradiated animals resulting in some net cell loss that we could not assess.

Autoradiographic identification of cells retained or lost after postnatal irradiation of the hippocampus

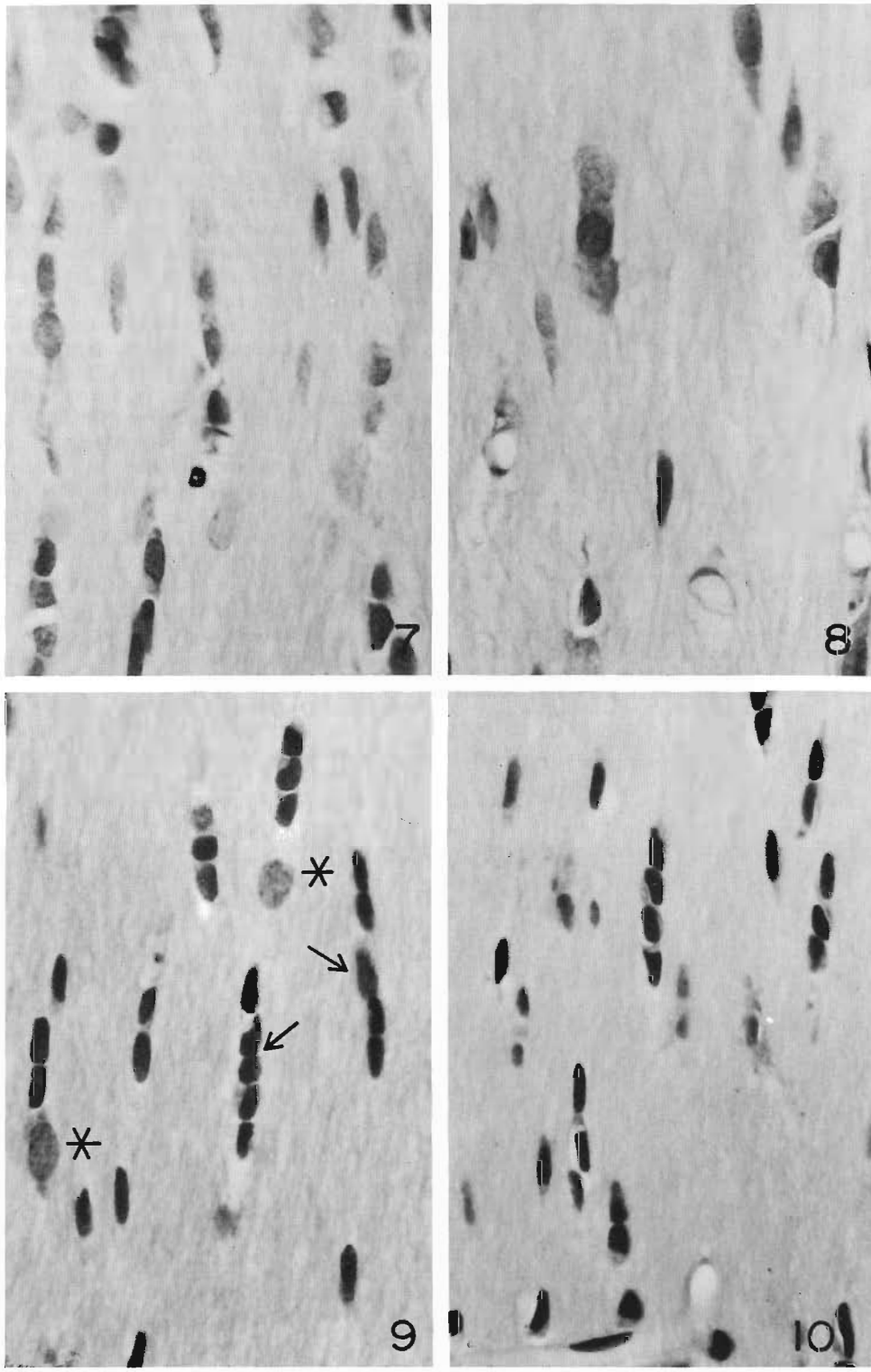
The estimate obtained in this study and in our previous report (Bayer et al., '73) of the reduction in granule cells in the 8X group (84%) corresponds closely to the

Fig. 7 Glial cells of the fimbria in a control animal at 30 days of age. Note the pale nuclear staining. Hematoxylin and eosin. × 720.

Fig. 8 Glial cells of the fimbria in an 8X animal at 30 days of age. The nuclei are larger than controls at this age and much fewer in number. Hematoxylin and eosin. × 720.

Fig. 9 Glial cells of the fimbria in a control animal at 90 days of age. The oligodendroglia nuclei (arrows) are small, densely stained and are arranged in rows parallel to the fimbrial fibers. Astrocytic nuclei (*) are larger and less densely stained. Hematoxylin and eosin. × 720.

Fig. 10 Glial cells of the fimbria in a 90-day old 8X animal. Note the similar appearance to controls at this same age, indicating complete recovery from X-ray insult. Hematoxylin and eosin. × 720.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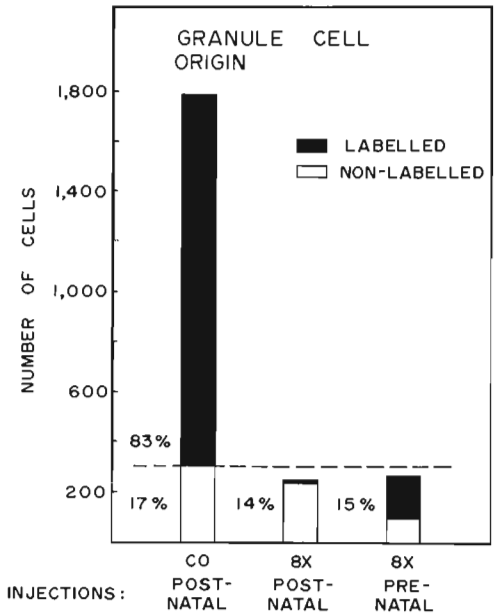

Fig. 11 Labelled and nonlabelled granule cells in the ectal and endal limbs of the dorsal hippocampal granular layer at level A3.0 (De Groot, '59). Each mean is based on counts from six animals. Control animals: injections on postnatal days 0, 3, 6, 9 and 12; 8X animals: two groups injected either on postnatal days 0 and 1 or on gestation days 19 and 20. All animals survived until 30 days of age.

autoradiographic estimate (85%) of postnatally forming granule cells (Bayer and Altman, '74). Accordingly, we undertook to test directly whether or not the granule cells remaining in the 8X group were the prenatally formed component of the total population. ^{3}H-thymidine was administered to a group of pregnant females on gestation days 19 and 20 to label the prenatally forming cells and to a group of pups on day 0 and the first postnatal day to label the postnatally forming cells. Both groups were irradiated according to the 8X schedule from day 2 on. There were many labelled cells in the prenatal injection group situated basally to some unlabelled granule cells (fig. 21), presumably formed before gestation day 19; few granule cells were labelled in the postnatal injection group (fig. 22). Figure 11 gives the results of counts of unlabelled and labelled dorsal hippocampal granule cells in anatomically matched sections (A3.0; De Groot, '59). The total number of granule cells in the two X-irradiated groups is slightly below the number of unlabelled cells (those prenatally formed) in the control animals. This indicated that beginning irradiation on postnatal day 2 allowed most of the prenatally formed granule cells to survive, but killed

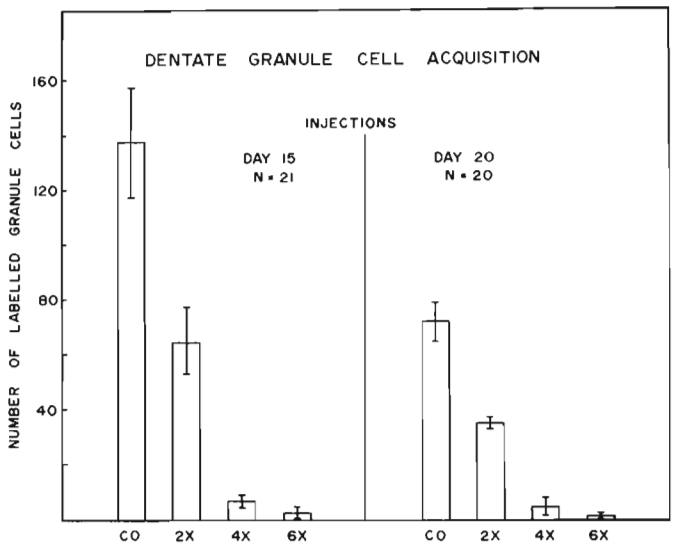

Fig. 12 Labelled dorsal hippocampal granule cells at 60 days of age in matched sagittal (L1490 μ; König and Klippel, '63) sections in control, 2X, 4X and 6X animals after a single injection of ^{3}H-thymidine on day 15 or day 20. Each mean with standard deviation is based on counts from approximately five animals. All irradiated groups are well below control levels on both days.

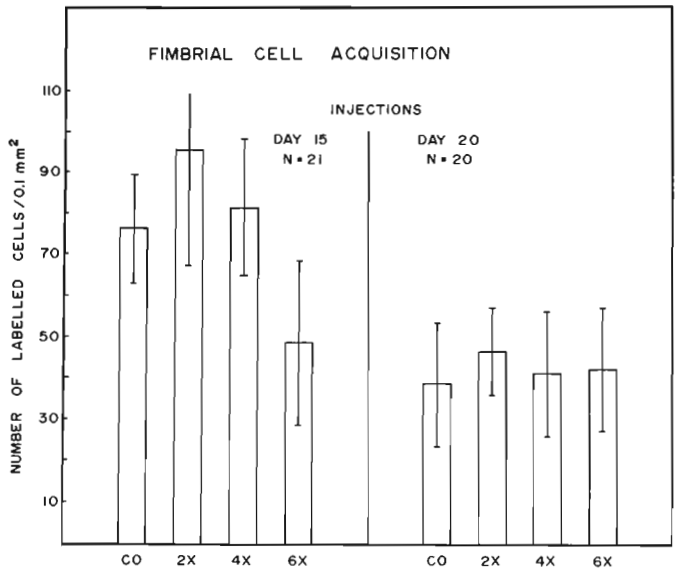

Fig. 13 Labelled cells per 0.1 mm² at 60 days of age in the fimbria of controls, 2X, 4X and 6X animals after a single injection of ^{3}H-thymidine on either day 15 or day 20. Each mean with standard deviation is based on counts from approximately five animals. In contrast to the labelling in the dentate granular layer, the irradiated animals are either above or at the same level as controls.

both the proliferating granule cell precursors and the migrating granule cells that were formed on or before postnatal day 1.

Quantitative studies described above (figs. 2A,B) indicated no recovery in granule cell number between 30 and 120 days. In a supplementary autoradiographic study we investigated the effects of different X-irradiation schedules on granule cell and glial cell acquisition after day 15 and day 20 (fig. 12). The labelled granule cells were counted in matched sagittal sections (L1490 μ; König and Klippel, '63) in normal and 2X, 4X and 6X groups two months after a single injection of ^{3}H-thymidine. In all groups the number of labelled cells was higher when injections were made on day 15 than when injections were made on day 20. In both the 15 and 20 day injection groups there was a progressive decline in the number and proportion of labelled granule cells with more irradiation exposures. The analysis of variance showed that the treatment groups differed significantly ($F = 135.19$, $df = 7/33$, $p \leq 0.001$). The 2X, 4X and 6X groups were significantly below controls ($p \leq 0.05$, Scheffé test) on both day 15 and day 20. The number of labelled cells dropped significantly on day 20 in the control and 2X groups. These results indicated that the precursors of granule cells are not capable of compensatory proliferation shortly after X-irradiation.

We also examined recovery of cell density in the fimbria, since glial cells in all irradiated groups reach control levels by 60 days of age. Figure 13 shows the number of labelled cells (none were excluded) within equal areas of the fimbria (L1490 μ; König and Klippel, '63) after a single injection of ^{3}H-thymidine on either day 15 or day 20. In contrast to the granule cell population, labelled cells in the X-irradiated groups are either above or at the same level as controls. The analysis of variance showed groups differed significantly ($F = 8.89$, $df = 7/33$, $p \leq 0.001$). At 15 days the 2X group was significantly above controls ($p \leq 0.05$, least significant difference test), while the 4X and 6X groups were at the same level. There was a significant drop in the number of labelled cells from 15 to 20 days in the controls, 2X and 4X groups, but there were no longer any differences between groups.

DISCUSSION

The effects of X-irradiation on neuronal populations in the hippocampus

Dentate granule cells

Prolongation of exposure of the hippocampal region from two successive daily doses of low-level X-ray to four and to six doses led to an irreversible reduction in the dentate granule cell population by approximately 59, 77 and 83 percent, respectively; the addition of two exposures (8X group) had no extra effect. This progressive reduction in the number of granule cells is best explained by a consideration of the chronology of normal granule cell acquisition and a hypothetical reconstruction of acquisition following variable X-ray treatments, as illustrated in figure 14.

The area under the curve for the control group (fig. 14A) represents the entire granule cell population with respect to time of formation. The shape of the curve is based on the autoradiographic data described fully in our previous report (Bayer and Altman, '74; fig. 5) which stated that the granule cell population accumulated according to the following schedule: 15%, prenatal; 25%, days 0–3; 20%, days 4–7; 13%, days 8–11; 14%, days 12–15; 13% after day 16 and into the adult period. There is additional autoradiographic evidence for a peak on day 2 (Bayer and Altman, '74) and for the initiation of granule cell formation on gestation day 16 (Hine and Das, '74).

The area under the curve for each irradiated group (figs. 14B,C,D) also represents the time span for formation of their respective granule cell populations. Based on the autoradiographic data in this report (figs. 11, 21, 22), all irradiated groups and controls have the same prenatally formed complement of granule cells and differ only in the number of postnatally formed cells (2X, 26% of control levels; 4X, 8%; 6X, 2%). There are two effects of irradiation. The first is to retard proliferation in the granule cell precursor pool; the longer the exposure to irradiation, the greater the delay. Recovery of precursor cell proliferation in the cerebellum occurs four to six days after the last irradiation exposure (Altman et al., '69). If we assume a similar recovery in the hippocampus, granule cells are formed

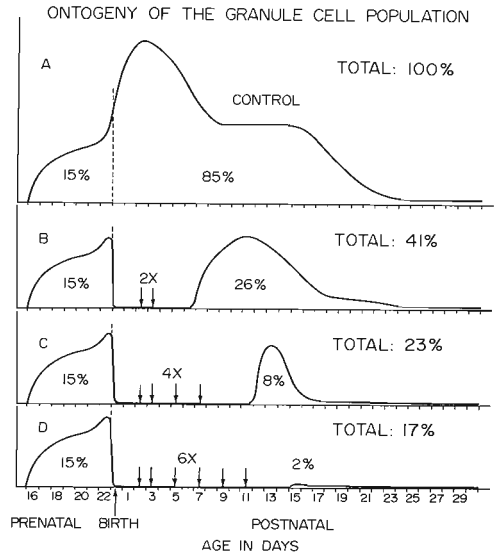

Fig. 14 Formation time of the granule cell population in the control animals (A), and in the 2X (B), 4X (C) and 6X (D) irradiated groups. The shape of the control population is based on the autoradiographic data presented in our previous report (Bayer and Altman, '74).

sometime after day 7 in the 2X group, after day 12 in the 4X group and after day 15 in the 6X group. The second effect of irradiation is to reduce the size of the granule cell precursor pool; the longer the exposure to irradiation, the greater the reduction. A total of 40% of the granule cells form after day 7 in normal animals; only 26% is acquired in the 2X animals. A total of 27% of the granule cells form after day 12 in normal animals; only 8% forms in the 4X group. A little over 13% of the granule cells form after day 15 in normal animals; less than 2% forms in the 6X (or 8X) group. The reduced number of labelled cells observed in the irradiated animals after ^{3}H-thymidine is injected on either day 15 or day 20 (fig. 12: 2X, 50% of normals for both 15 and 20 days; 4X, 5% of normals; 6X, less than 2% of normals) gives evidence for the reduction of the precursor pool. The effects of irradiation would not be permanent if the reduced precursor pool could compensate for the loss by producing granule cells for a longer time. However, the drop in granule cell labelling from 15 to 20 days occurs not only in normal animals, but also in those irradiated (fig. 12). Apparently, the production time for hippocampal

granule cells is set and is independent of manipulations of the population.

The incidental observation of a 20% increase in granule cells from 30 to 120 days in normal animals sheds some light on the significance of labelling of hippocampal granule cells after ^{3}H-thymidine injections in adults (Altman and Das, '65). If this can be confirmed in future studies, it would represent the first known structure in the mammalian central nervous system where a neuronal population increases in the adult period.

Ammon's horn pyramidal cells

Ammon's horn pyramidal cells are formed entirely prenatally (Angevine, '65; Hine and Das, '74). At the time of X-irradiation used in this study, the pyramidal cells were differentiating. Such populations are resistant to low levels of X-irradiation (Hicks and D'Amato, '66; Altman et al., '67; Altman and Nicholson, '71; Bayer and Altman, '74). Consequently, irradiation does not eliminate pyramidal cells or, presumably, any of the other prenatally-forming hippocampal neurons, such as the polymorph cells. Furthermore, irradiation neither curtails the growth of Ammon's horn in the sagittal plane, nor changes its total area from that of controls (fig. 1B).

The effects of X-irradiation on a glial cell population — the fimbria

In contrast to the irradiation-induced permanent granule cell loss, the reduction of the glial cell population in the fimbria is both less marked and transient. This may be due to all or some of the following possibilities. The time course of cytogenesis in the fimbria is different from that of the dentate granular layer in that few cells are formed during the first week; most of them are formed either within or after the second week (Bayer and Altman, '74). In the animals irradiated on days 2 and 3, negligible reduction was seen in the glial population of the fimbria at 30 days (fig. 6) either because the dormant glial precursors are less radiosensitive or because the decimated pool had sufficient time to recover. By extending the irradiation exposures to the end of the first week and into the second week, proportionally more glial cells were missing from the population at 30 days. By 60 days, fimbrial glial cells in all irradiated groups returned to control levels. The fact that a cellular deficit of over 50% at 30 days (8X group) can totally disappear by 60 days provides support for the generally held view that glial populations are more plastic than neuronal populations in both their proliferation times and rates.

The effects of X-irradiation on mixed neuron and glia populations

Cytogenesis of the mixed neuron and glia populations in the dentate molecular layer and in the Ammonic stratum oriens is pronounced during the second week of life (Bayer and Altman, '74). There is little damage to these cell populations when the exposures are limited to the first week; more damage results when exposures extend into the second week. As in the fimbria, the level of reduction is less than in the granular layer. But unlike the fimbria, the 4X, 6X and 8X groups have permanent losses in either one or both regions. It is tempting to postulate that this intermediate situation is due to a permanent reduction of neurons and a recovery of glial elements. However, we do not know to what extent neurogenesis extends into the postnatal period in either region. Quantitative analyses using electron microscopy and autoradiography are needed to determine the identity of the postnatally formed cells and the differential long-term consequences of low-level X-irradiation in these hippocampal sites.

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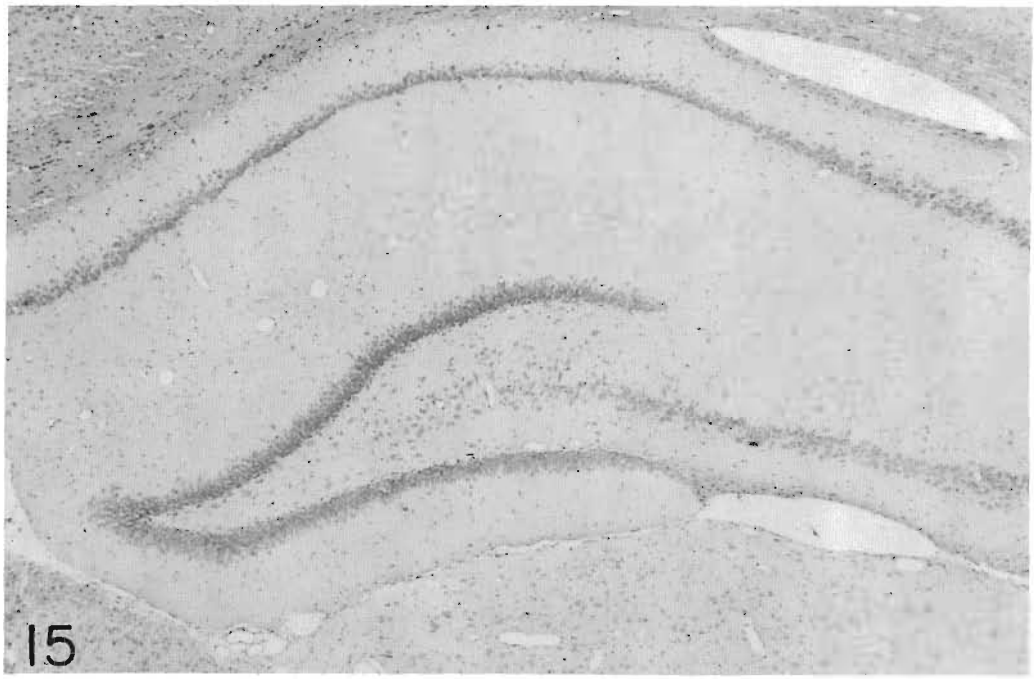
PLATE 1

EXPLANATION OF FIGURES

15 Dorsal hippocampus of a control animal at 60 days of age. Hematoxylin and eosin. × 40.5.

16 Dorsal hippocampus of a 6X animal at 60 days of age. Note the normal morphological appearance of the hippocampus and the appreciable reduction in the cell population of the dentate gyrus. Hematoxylin and eosin. × 40.5.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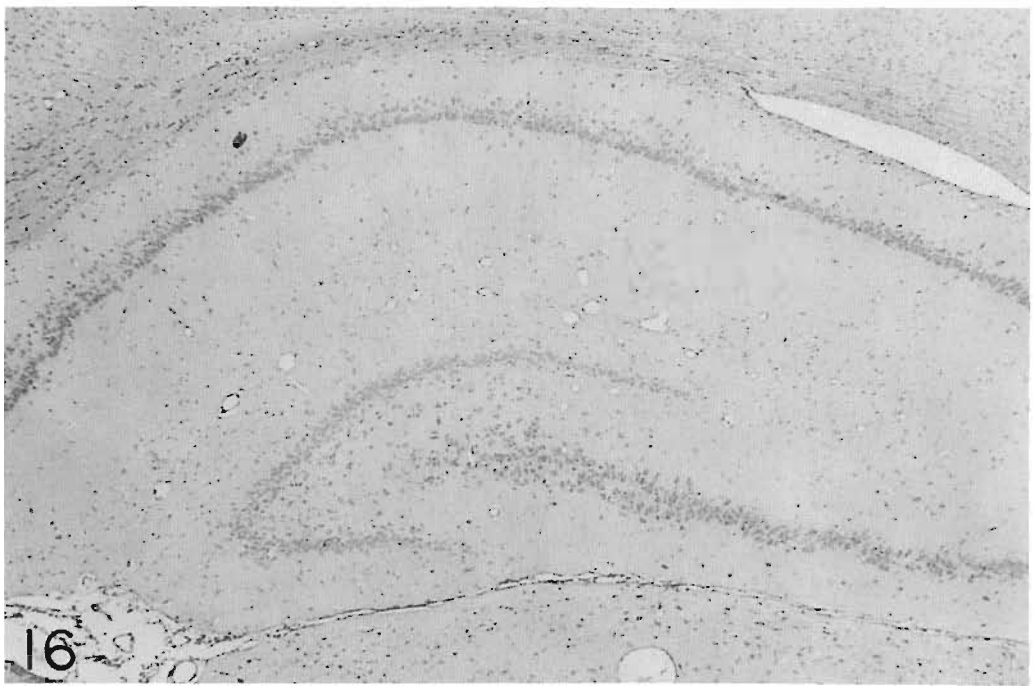
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PLATE 2

EXPLANATION OF FIGURES

Granule cells in the ectal arm of the dentate granular layer from the control and irradiated animals at 60 days of age. Note the progressive decrease in granule cell number and packing density with increasing dose. Hematoxylin and eosin. × 648.

17 Control.
18 2X.
19 4X.
20 6X.

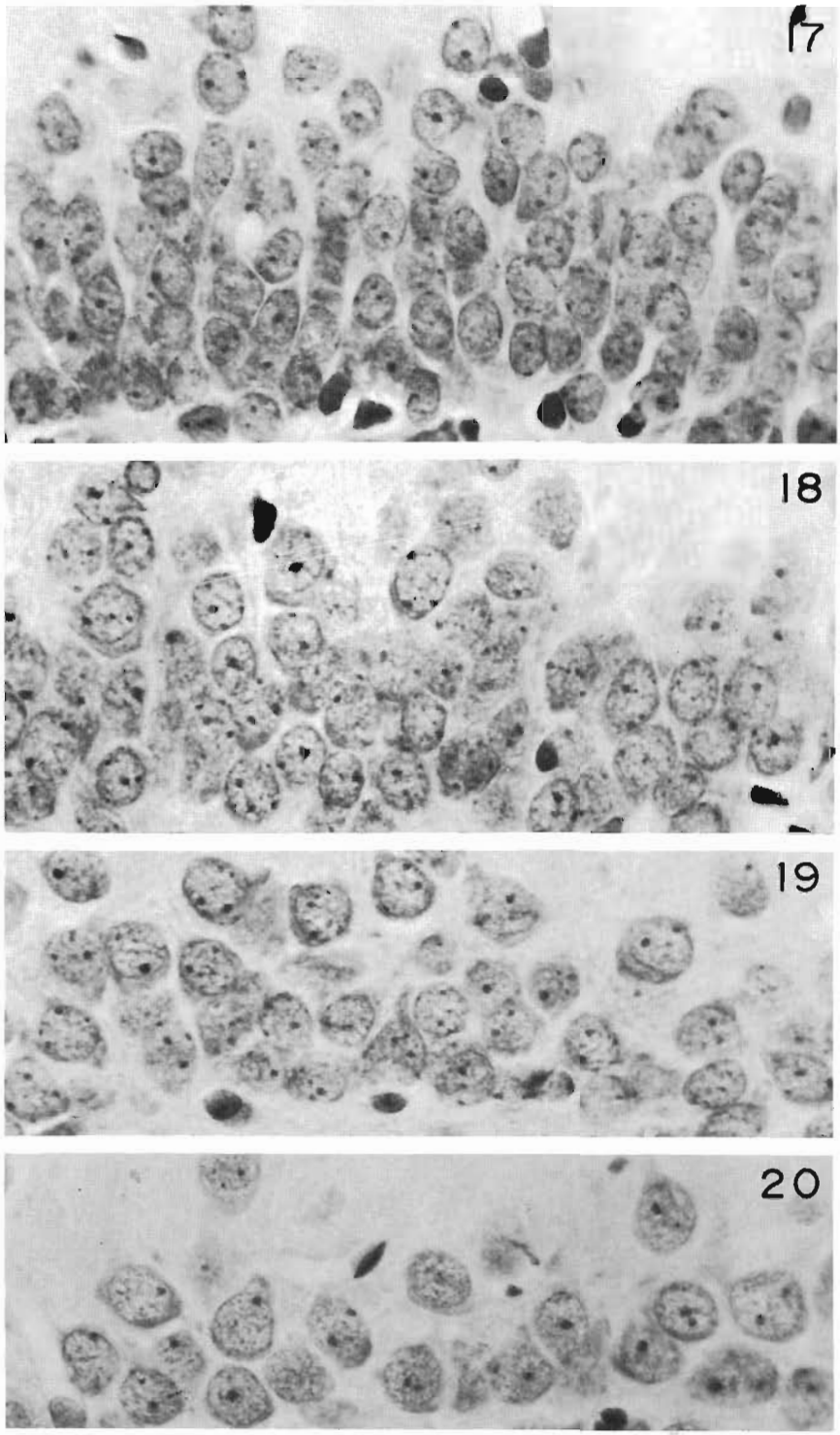
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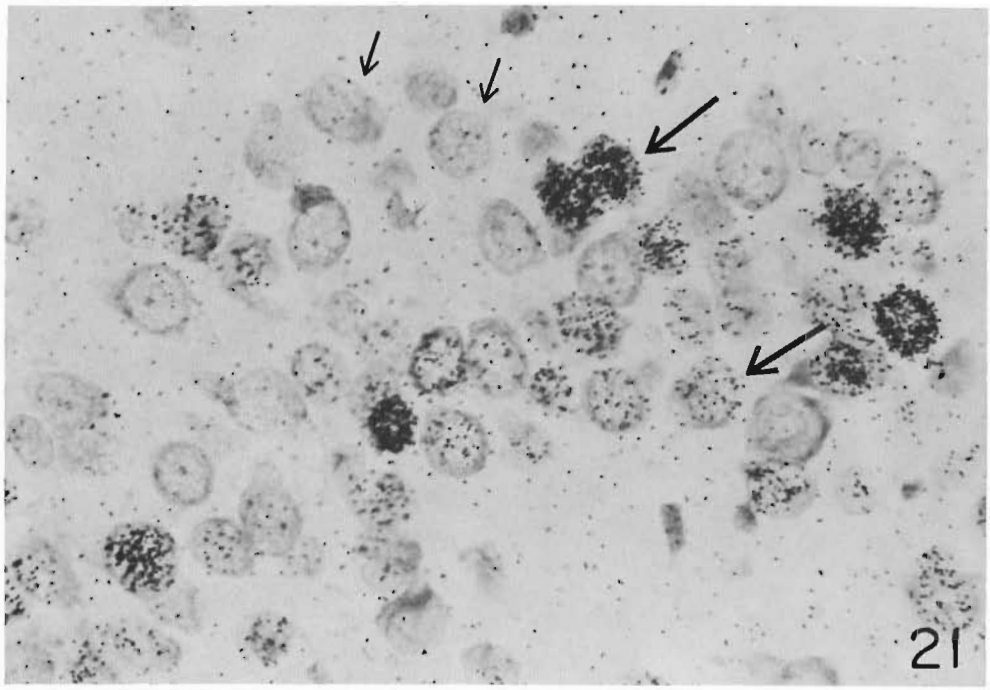
PLATE 3

EXPLANATION OF FIGURES

21 Dentate granular layer in an 8X animal injected with ^{3}H-thymidine on gestation days 19 and 20 and killed on postnatal day 30. The numerous labelled granule cells (large arrows), many of which are lightly labelled, are located basal to the unlabelled cells (small arrows). Presumably, the unlabelled granule cells were formed before gestation day 19. Gallocyanin chromalum. × 576.

22 The same region in an 8X animal injected on postnatal days 0 and 1. Granule cells are not labelled. The few labelled cells in the dentate granular layer are not granule cells but cells with smaller nuclei (neuroglia?). Gallocyanin chromalum. × 576.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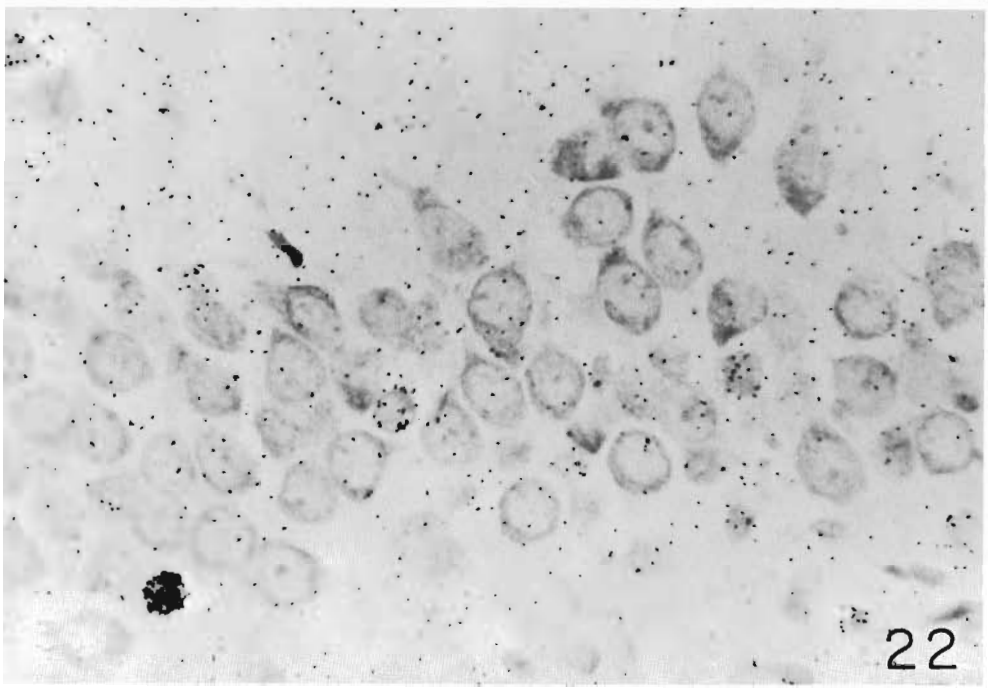
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