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The Effects of X-Irradiation on the Postnatally-Forming Granule Cell Populations in the Olfactory Bulb, Hippocampus, and Cerebellum of the Rat

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Beginning on the second postnatal day, either two (2X group), four (4X group) or six (6X group) daily or alternate daily exposures to low-level X-irradiation (150–200 r) were used to interfere with the acquisition of granule cells in the olfactory bulb, hippocampus, and cerebellum of the rat. At 60 days of age, the relationship between post-irradiation recovery and permanent granule cell loss was assessed with two quantitative techniques. First, the total number of granule cells was determined to estimate the magnitude of permanent loss. Secondly, the number of labeled granule cells were counted on day 60 after a ^{3}H-thymidine injection given on either day 15 or on day 20 to estimate differential rates of cell proliferation during the recovery period.

Permanent loss of granule cells was sustained in all regions by all schedules of irradiation. The time for the most effective exposures was earlier in the hippocampus and olfactory bulb than in the cerebellum. In all regions, both the irradiated groups and the controls showed a decrease in the level of cell proliferation between 15 and 20 days. The number of cells that could be labeled after either the 15 or 20 day injection was below control levels for all groups in the hippocampus, at control levels for all groups in the cerebellum, and either at (2X and 4X) or below (6X) control levels in the olfactory bulb. These results are discussed in the light of the formation time of the granule cells in each region.

INTRODUCTION

The majority of the granule cells in the olfactory bulb (1, 3, 9), hippocampus (1, 8, 9, 15) and cerebellum (1, 2, 4, 9) form after birth in the

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rat brain. The precursors of these cells are killed by exposures to low level X-rays (150–200 r) while differentiating or mature cells remain unharmed (10–12, 15, 17). In the cerebellum, the magnitude of granule cell loss depends on several variables. A single exposure during the first few days of life leads to minimal loss (6) because the surviving precursor cells begin to proliferate, reconstitute the germinal matrix and differentiate after some delay (13, 14). Adding exposures on later days can further delay this recovery (13) or prevent it altogether, presumably because the germinal matrix disappears naturally around 21 days of age (7). Conversely, the later irradiations are started, the smaller the deficit (5) because fewer granule cells remain to be formed. Thus, both the time irradiation is started and the number of exposures greatly influence granule cell deficits.

A recent study established that the peak of cell acquisition occurs much earlier in the hippocampus (15) than in the cerebellum (4). Moreover, permanent loss of hippocampal granule cells can be sustained after early postnatal irradiation (16). These considerations suggested that the same irradiation schedules should produce different losses of granule cells in the olfactory bulb, hippocampus and cerebellum where granule cells are produced at different rates. We examined this in three groups of irradiated animals with two quantitative techniques: total cell counts to estimate the magnitude of granule cell loss and ^{3}H-thymidine autoradiography to estimate differential rates of cell proliferation during the recovery period.

METHODS

Six litters of Purdue-Wistar male rat pups (six to a litter) were exposed to whole head X-irradiation from a Maxitron 300 kv unit either two (2X group), four (4X group) or six (6X group) times; two litters served as controls. The litters in the 6X group were given 200 r on days 2 and 3, followed by 150 r on days 5, 7, 9, and 11. Litters in the 4X group received the first four doses of the 6X schedule; those in the 2X group received the first two doses. On day 15, half the animals from each group received a single injection of ^{3}H-thymidine (New England Nuclear, specific activity, 6.7 Ci/mmole, 10 μCi/g body weight) while the other half was given a single injection on day 20. All animals were killed at 60 days of age by transcardiac perfusion with 10% neutral formalin. The brains were removed and placed in Bouin's fixative for 24 hr; after further fixation in 10% neutral formalin, they were embedded in paraffin. Matched anatomical sections were cut (6 μ) in the sagittal plane. The slides were dipped in Kodak NTB-3 emulsion; exposure time was 12 wk. Labeled and nonlabeled granule cells were counted in ten grids (0.0016 mm^2) from midsagittal sections of the cerebellum (Fig. 3D) and in sections where the internal granular layer occupies a major portion of the interior of the olfactory

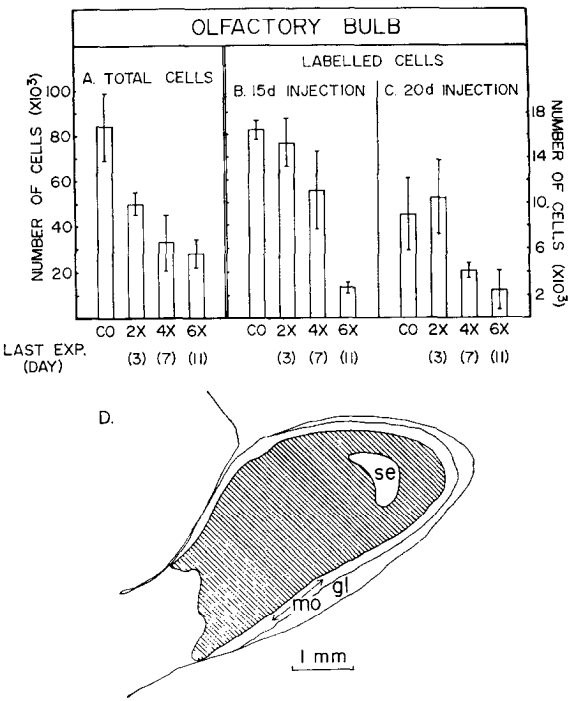

FIG. 1. A. Granule cells in the olfactory bulb at 60 days of age determined by cell counts in unit area grids multiplied by the planimetrically-determined total area. Each mean with standard deviation is based on counts from approximately ten animals. B and C. The number of cells that could be labeled after a single injection of ^{3}H-thymidine on either day 15 (B) or on day 20 (C). Each mean with standard deviation is based on counts from approximately five animals. D. A drawing of the olfactory bulb at the anatomical level used for the cell counts; gl, glomerular layer; mo, molecular layer; se, subependymal layer. Granule cells are located in the shaded area.

bulb (Fig. 1D). Mean unit area counts were multiplied by planimetrically-determined total area to get the total number of cells. All labeled and nonlabeled hippocampal granule cells were counted at level L1490 μ (18) (Fig. 2D).

RESULTS

X-irradiation reduced the total number of cells in all regions (Figs. 1A, 2A and 3A). Since there were no differences in total cell number between the 15 day and 20 day injection groups, the data were pooled. The analysis of variance showed groups differed significantly in the olfactory bulb [Fig. 1A; F (3, 37) = 55.1, $P < 0.001$]. The Scheffé test indicated that the control group was significantly above ($P \leq 0.05$) all irradiated

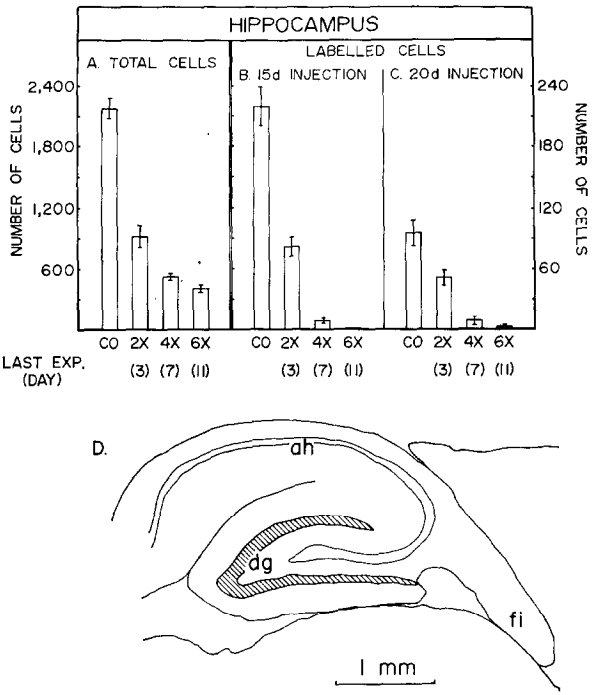

FIG. 2. A. Granule cells in the dentate gyrus of the hippocampus determined by cell counts of the entire granular layer. Each mean with standard deviation is based on counts from approximately ten animals. B and C. The number of cells that could be labeled after a single injection of ^{3}H-thymidine on either day 15 (B) or day 20 (C). Each mean with standard deviation is based on counts from approximately five animals. D. A drawing of the hippocampus at the anatomical level (18) used for the cell counts; ah, Ammon's horn; dg, dentate gyrus; fi, fimbria. Granule cells are located in the shaded area.

groups; the 2X group was above the 4X and 6X groups, which did not differ significantly from each other. There were significant differences between groups also in the hippocampus [Fig. 2A; F (3, 37) = 1106.4, $P < 0.001$] and in the cerebellum [Fig. 3A; F (3, 37) = 170.6; $P < 0.001$]. In both regions, the control group was significantly above ($P \leq 0.05$, Scheffé test) all irradiated groups; the 2X group was above the 4X and 6X groups; the 4X group was above the 6X group.

However, there was a difference between these regions in the magnitude of reduction. Two doses (in the 2X group) were very effective in the hippocampus (58% of the control population was eradicated, 29% per exposure), moderately effective in the olfactory bulb (41% removed, 20.5% per exposure), and least effective in the cerebellum (22.7% removed, 11.3% per exposure). The two additional doses in the 4X group killed fewer cells in the hippocampus (9% per exposure, total reduction 76%) and in the

olfactory bulb (10% per exposure, total reduction 61%). Conversely, the two additional exposures in the cerebellum each removed 17.1% of the population to make the total reduction 56.9%. This trend was also shown after six doses. In both the hippocampus and olfactory bulb, each additional exposure only removed 2.8% of the population to make the total reductions 81.5 and 66.4%, respectively; 9.9% of the population was removed in the cerebellum to make the final reduction 76.8%. Even though the final reduction in the 6X group is similar for both the hippocampus and cerebellum, the most effective doses occurred at different times. The olfactory bulb represents an intermediate situation between the two.

Each granule cell population also behaves differently when only the labeled cells from each group are compared. In the cerebellum (Fig. 3B

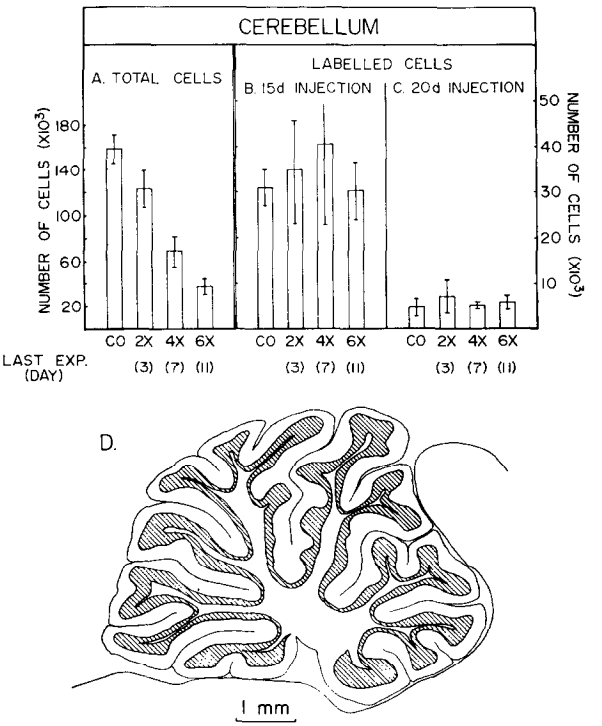

FIG. 3. A. Granule cells in the cerebellum at 60 days of age determined by cell counts in unit area grids multiplied by the planimetrically-determined total area. Each mean with standard deviation is based on counts from approximately ten animals. B and C. The number of cells that could be labeled after a single injection of ^{3}H-thymidine on either day 15 (B) or on day 20 (C). Each mean with standard deviation is based on counts from approximately five animals. D. A drawing of the cerebellum at the anatomical level used for the cell counts. Granule cells are located in the shaded area.

and 3C), there were significant differences between groups [F (7, 33) = 18.6, $P < 0.001$]. All irradiated groups were at the same level as controls after injections on either day 15 or day 20, but there were significantly fewer ($P \leq 0.05$, Scheffé test) labeled cells in each group after the 20 day injection. In the hippocampus (Figs. 2B and 2C) there were significant differences between groups [F (7, 33) = 311.14, $P < 0.001$]. The control group was significantly above ($P \leq 0.05$, Scheffé test) all irradiated groups after injections on either day 15 or day 20; the 2X group was significantly above the 4X and 6X groups. There was also a significant drop in labeled cell number from 15 to 20 days ($P \leq 0.05$, Scheffé test) in the control and 2X groups. The 4X and 6X groups were essentially similar, showing few labeled cells. In the olfactory bulb (Figs. 1B and 1C), there were also significant differences between groups [F (7, 33) = 26.88, $P < 0.001$]. This time only the 6X group was significantly below ($P \leq 0.05$, Scheffé test) controls after injections on either day 15 or day 20. Significantly fewer ($P \leq 0.05$, Scheffé test) cells could be labeled in the control, 2X and 4X groups after injection on day 20; the 6X group showed a low level of labeling throughout.

DISCUSSION

The formation time of the granule cell population in each region can explain the differences between groups in both total cells and labeled cells. In most lobules of the cerebellar vermis, over 50% of the granule cells form after day 13 (2) and proliferation is high during the third week (4). Since few granule cell precursors form during the first few days of life, and the external germinal layer begins to recover four days after the last exposure (13, 14), irradiations on days two and three are ineffective; the 4X and 6X exposure schedules extend into the active formation time and are more damaging to the population. However, recovery of the precursor cells in all irradiated groups occurs when cell formation rate is climbing; therefore, each group reaches the same proliferative level as controls. Granule cell formation has a sharp peak during the first 3 days of life in the hippocampus, begins to drop off before the end of the first week and continues to drop during the second week (15). Exposures on days 2 and 3 occur at the high point and are very effective in reducing the population. Additional exposures remove progressively fewer granule cells since fewer are being formed. Recovery of the precursor cell population in all groups takes place when the rate of granule cell formation is slowing down; this may be an important factor in keeping the proliferative level below that of controls in all groups. Little is known about the formation time of the granule cells in the olfactory bulb, but we have some evidence for a sustained intermediate level of formation up to the first 7–11 days of

life, which gradually drops by the end of the second week and during the third week (Austin and Altman, personal communication). Exposures on days 2 and 3 are somewhat more effective than the additional exposures of the 4X and 6X groups since slightly more granule cells are forming then. The total reduction in olfactory bulb granule cells is less in the 6X group (66.4%) than in any other region (hippocampus, 81.5%; cerebellum, 76.8%). This may be because more granule cells form here after the irradiations and extend their formation time considerably beyond day 20 (see Fig. 1C). Recovery of the precursor pool in the 2X group occurs when granule cell formation is high, placing proliferative levels at those of controls. The 4X and 6X groups show a trend toward lower proliferative levels (significant only in the 6X) and also recover during the time when granule cell formation is gradually slowing. In summary, there appear to be fewer labeled cells in the irradiated animals than in controls if recovery takes place during the time when granule cell formation is slowing down; proliferative levels in the irradiated animals reach those of controls if cell division occurs while granule cell formation is increasing.

Proliferative levels generally tended to be lower on day 20 than on day 15 in the control group and in all irradiated groups with a substantial number of cells labeled on day 15 (2X, all regions; 4X, olfactory bulb and cerebellum; 6X, cerebellum). This suggests that granule cell formation in the irradiated animals was not extended to compensate for losses sustained during the regular formation time. Thus, while under optimal recovery conditions, rates of cell proliferation after irradiation may reach normal levels, the duration of cell formation is "set" and any damage during the formative period is permanent.

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