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Gross Morphological Consequences of Irradiation of the Cerebellum in Infant Rats with Repeated Doses of Low-Level X-Ray

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The cerebellum of rats was irradiated with daily doses of 50–200 r hard X-ray, on successive days following birth, total number of exposures ranging from 1 to 10. The purpose was to design a method of selectively eliminating a specifiable proportion of the postnatally proliferating and migrating precursor cells of cerebellar microneurons. In this study the gross morphological consequences of different irradiation schedules were determined. Mortality rate was not raised by localized cerebellar irradiation, except in rats that received ten successive daily doses, and the animals that survived through infancy, including those that received 10 × 200 r, showed normal body growth. Total brain weight was markedly reduced in the animals receiving more than 2 × 100–200 r; the highest reduction was seen in animals that received 10 × 200 r. The bulk of reduction in total brain weight could be attributed to weight loss in the intended area of irradiation. Areal measurements showed that cerebellar irradiation did not affect appreciably the growth with age in the width and length of the cerebrum, but increase in the length and height of the cerebellum was markedly retarded. With single or multiple doses of 200 r cerebellar length was greatly reduced by day 10. Reduction at 30 and 90 days was not evident with 1–2 × 200 r, but with 4–5 × 200 r cerebellar length was reduced to the level of control animals 10 days of age, and with 10 × 200 r to the level of neonates. The cytological bases of the drastic effects produced by radiation remain to be determined.

Introduction

It was established in a series of studies employing thymidine-^{3}H autoradiography in rats (1, 4, 5) that in some brain regions the majority of short-axoned neurons, or microneurons,[2] are formed after birth. Since the event of birth signals the onset of active commerce with the external world, it is conceivable that during this postnatal phase of neurogenesis behavioral influences, by affecting the new neural connections that are

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[2] Defined in reference 3, page 482.

being established, may modify or mold the structural and functional organization of the developing nervous system. To test the hypothesis that these postnatally formed microneurons are the "plastic" elements of the brain (2) we have undertaken two types of experiment. In the first of these we are trying to establish whether different environmental conditions during early or later periods of life have an effect on the rate of proliferation, migration, and mode of differentiation of the precursors of microneurons (6). The purpose of the second approach, which is the major concern of the present series of studies, is to determine (*a*) the effects of decimation or elimination of the precursors of microneurons in infant rats on the structural organization of the brain, and (*b*) the consequences of such cellular lesions on the animal's behavioral plasticity; that is, its capacity to modify old and to acquire new patterns of behavior.

Embryonic cells, unlike differentiated mature cells, are very sensitive to ionizing radiation (7, 17). Irradiation of the brains of infant animals therefore appeared to be a possible method for selective elimination of the precursors of microneurons. In a pilot study (3), the skull of kittens was unilaterally exposed during the first 2 weeks of their lives to multiple doses of 50, 100, 150, 200, 300, and 400 r of X-ray. Particular attention was paid to the fate of the proliferating and migrating cells of the external granular layer of the rapidly growing cerebellum. We found that five repeated doses of 150 r subtotally and 200 r totally destroyed the external granular layer on the irradiated side. These doses greatly reduced the population of the postnatally-forming microneurons of the internal granular layer, but did not visibly affect the prenatally formed Purkinje cells.

For several reasons the cerebellum appeared to us as an ideal structure for the investigation of the problem described above. First, the cerebellum is a very immature region of the brain at birth, which shows intense cell proliferation and considerable postnatal growth in all altricial species (2). It was suspected for some time (11, 20, 23) that the cells of the subpial external granular layer are the precursors of the differentiating granular nerve cells of the internal granular layer, and this hypothesis was recently confirmed in mice and rats with thymidine-^{3}H autoradiography (1, 4, 5, 14, 19, 29). Second, the cerebellum, due to its topographic segregation from other brain structures, is more accessible than many other brain regions to isolated experimental manipulation. Third, the duration of postnatal cerebellar neurogenesis in different species has been correlated with the time required for the development of locomotor skills and the complexity of these skills in individuals of the species (2). These correlations suggest that postnatal cerebellar neurogenesis may have a role in the maturation or acquisition of these skills, which progressively improve as the young animal persists with its playful and serious motor manipulation of

its external environment. Fourth, because the cerebellum mediates motor functions, cerebellar deficits are relatively easy to assess by neurological and behavioral procedures. Finally, evidence is available that severe motor coordination, or ataxia, may result not only from gross cerebellar lesions, but from cerebellar hypoplasia characterized by the paucity of granule cells (18). Indeed, an often described consequence of whole-body or total-head X-irradiation, particularly in young animals, is the destruction of granule cells (or their precursors) in the cerebellum (10, 25, 27, 30, 31) and one of the most common symptoms of X-irradiation is ataxia.

Materials and Methods

Animals. Long-Evans hooded rats, bred in our laboratory for several years and sister-brother mated for 2–4 generations, were used. Forty-five litters (with 388 pups) were irradiated; 8 litters (with 64 pups) served as controls. Irradiation was accomplished in four successive sessions over a period of 9 months. All the pups, irradiated and controls, were removed daily from their home cages and transported to the radiation facilities. To facilitate the acceptance of the young by their mothers, the mothers were first removed from the breeding cages and placed into separate cages where they received supplementary diet. The pups were subsequently removed from the cages and were carried to the radiation facilities. The pups were consistently replaced in the breeding cages before their mothers were returned.

Radiation Source and Dosimetry. A 2-million-volt Van de Graaff generator (High Voltage Engineering Corporation Model AM) was used as the source of X-rays. A target-to-animal treatment distance of 125 cm was used with a dose rate of about 50 r/min. In addition to the inherent defining system of the X-ray generator, additional defining blocks were located 40 cm from the animals in order to minimize the geometric penumbra and thus reduce the volume of tissue irradiated. To obtain a uniform dose over a 6 mm width, a field size of 0.8 × 35 cm was used in all the irradiations.

A thermoluminescent dosimetry system (EG and G, Inc. Model TL2B) was used in the dose-distribution studies and for daily calibration; CaF_2:Mn thermoluminescent microdosimeters 0.9 × 6 mm were calibrated by comparison with a Victoreen ionization chamber. Dose-distribution studies were carried out prior to animal irradiation each day to confirm field position and size, and to provide calibration. Sets of dosimeters placed at 10 and 15 mm depths in the center of the field and at 10 mm depth at the ends of the field provided resolution of the order of 1 mm across the field width. Daily calibration was obtained by averaging 16 readings obtained from the ends and center of the field for the central 6 mm width of

the field. Uniformity of dose across 6 mm width, in depth and over the field length was confirmed by these studies. A typical distribution curve is shown in Fig. 1 for a 1 cm depth. The shape of this distribution curve may be accounted for not only by geometrical penumbra, but also by scattering within the volume of the phantom material.

Radiation Procedure. The pencil-shaped beam made possible the simultaneous irradiation of the area of the cerebellum in an entire litter (up to ten pups) in the following way: Lucite blocks 30 cm long, 7 cm wide and 5 cm high served both as absorbers and animal holders (Fig. 2a, b). In each block ten identical holes were drilled which, to accommodate the rapidly growing animals, ranged in diameter from about 1.5 to 3.0 cm. Each block was matched with a set of transparent plastic tubing (Fig. 2c), slit lengthwise and provided with small holes for ventilation, into which the animals were fitted tightly and immobilized with tape. The approximate position of the area of the cerebellum of each pup in the tube was delimited before irradiation by an erasable black mark drawn transversely on the tube over an imaginary line connecting the frontal border of the two ears. The tubes containing the animals were then so positioned in the lucite block that these marks fell beneath an engraved line on the block which, in turn, allowed the positioning the front end of the pencil-shaped X-ray beam. No allowances were made for the changing anteroposterior length of the cerebellum in the growing animals (about 5 mm at 10 days of age;

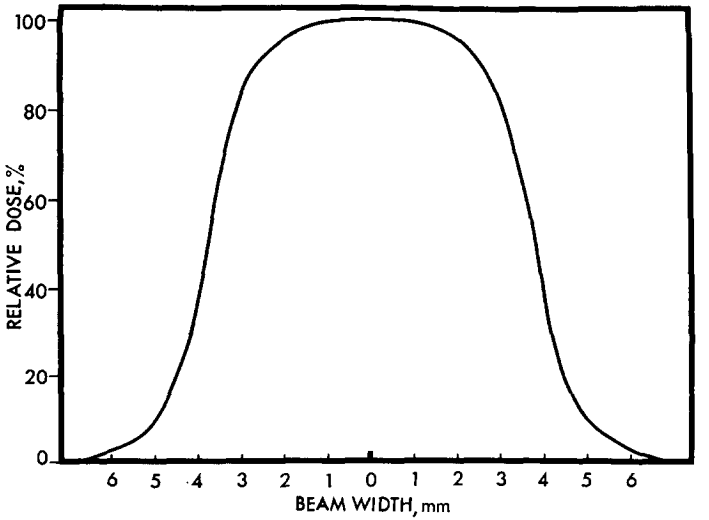

FIG. 1. X-ray dose distribution across the width of an 0.8 × 35 cm field as measured by thermoluminescent microdosimeters. This dose distribution is typical of that used in the irradiation series and shows a uniform dose region of about 6 mm.

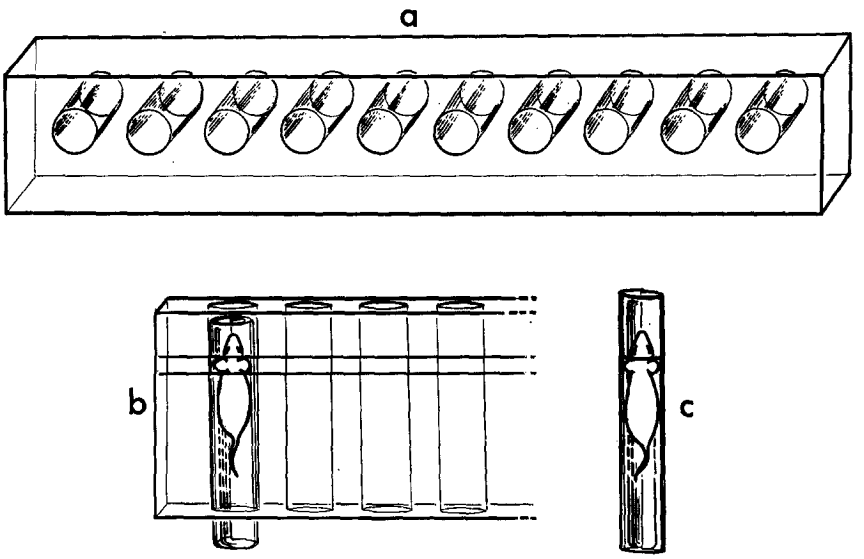

FIG. 2. Schematic drawing of the lucite holders: (*a*) frontal view with ten drilled holes; (*b*) view from above showing demarcation of X-ray beam and rat positioned in plastic tubing; (*c*) plastic tubing with marking delineating the frontal border of ears.

see Fig. 8); the selected width of the beam presumably irradiated the entire cerebellum in even the oldest animals with some margin left for errors in placement. The position of the animals' heads with respect to the line on the lucite block was checked after each irradition session, and the few animals that apparently moved their heads during irradiation were rejected.

Irradiation of all the litters began on the day of birth, with doses of 50, 100, 150, and 200 r. Those exposed to 200 r were irradiated 1, 2, 3, 4, 5, 8, or 10 times on successive days. Animals receiving lower X-ray doses (50, 100, 150 r) were irradiated daily on 5, 8, and 10 occasions. The control animals were also carried to the radiation laboratory or to another site and were placed for 10 min in plastic tubes and the lucite block on 5, 8, or 10 successive days, in the way the irradiated animals were.

Maintenance of Animals. The rats that were destined to live for less than 30 days were kept throughout their survival period with their mothers in breeding cages. Those that were allowed to survive longer were transferred at day 30 to standard rat cages and were given unlimited access to food and water. Of these animals, those that were designated for behavioral studies were transferred, as young adults, to separate quarters. Because the animals of this colony suffer from a chronic respiratory involvement, at the time of their transfer all animals were injected with penicillin, and one

week every month they were provided with terramycin dissolved in their drinking water. These behavioral animals were maintained on a standardized diet of Purina chow, cheese, and carrots, were weighed weekly and when warranted given a supplementary diet.

Killing Schedules. Four groups of animals were used, with subgroups of appropriate survival times following their radiation schedules. The first group was designed for histological and cytological evaluation of the morphological alterations produced by irradiation. These animals were allowed to survive for 2 and 24 hours after the last exposure, and for the constant ages of 10, 30, and 90 days. (Coronal and sagittal brain sections were stained with cresyl violet for cells, Weil's stain for myelinated fibers, or impregnated with Golgi techniques for a comprehensive picture of altered cell structure.) The second group was designed for autoradiography. These animals were injected with thymidine-^{3}H either just before irradiation, to determine the effect of different X-ray doses on cell proliferation in the cerebellum, or just after irradiation, to study the radiosensitivity of newly formed cells. The third group was designed to study the relationship between radiation schedules and reduction in absolute cell numbers in the cerebellum. This was accomplished by means of a maceration technique (to be described), combined with a quantitative study of histologic material. Finally, a large group of animals, which has been permitted to survive for a long time after irradiation, was used to assess, with a variety of procedures, the behavioral deficits produced by irradiation.

Killing. On the designated hour or day the animals to be killed were anesthetized with a specified volume of Nembutal. After determining their body weights, the animals (excepting those that were destined for Golgi or maceration studies, but including neonates) were perfused transcardially with 10% neutral formalin. The brains were then carefully removed and were placed for further fixation in jars containing neutral formalin. Subsequently, the brains were removed, and before subjecting them to histological processing various gross measurements were taken.

Gross Measurements. These included determination of wet-brain weight and areal determination of the length, width, and height of the cerebrum and cerebellum (Fig. 3). All the brains were trimmed in a standardized manner: a transverse, anterior cut was made perpendicular to the plane of a stereotaxic coordinate system (13) through the olfactory bulbs at the border of the anterior cortex; a similar posterior cut was made through the medulla at the posterior border of the cerebellum. The brains were gently wiped with paper towel and then dried a few minutes in air in an air-conditioned room. The total brain weight was then determined on a torsion balance with 1 mg accuracy. (In those brains that were subsequently

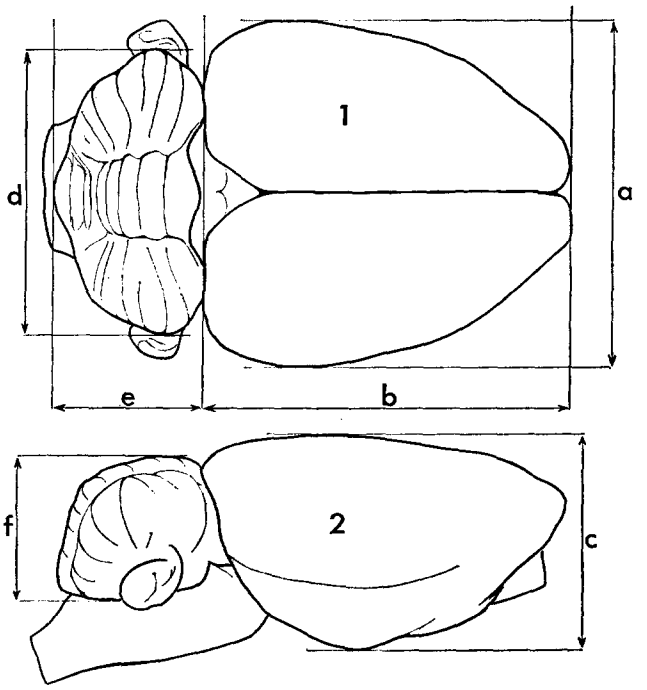

FIG. 3. Schematic outline of rat brain in *1*, dorsal, and *2*, lateral view, with method of measuring (*a*) cerebral width; (*b*) cerebral length; (*c*) cerebral height; (*d*) cerebellar width (flocculi not included); (*e*) cerebellar length; and (*f*) cerebellar height.

cut into transverse block for histological processing, weights of the cerebellar blocks were also taken.) Wet-brain weights were also taken on unprefused brains used for Golgi impregnation and of dissected cerebellums destined for maceration studies. In addition, the height, width, and length of the cerebral cortex and of the cerebellum were determined at × 10 magnification with an operating stereomicroscope with the aid of fine calipers. The accuracy of these measurements was checked in many instances by taking photographs of the brains at constant magnification with a macrophotographic apparatus and making measurements on the photographs.

Results

Mortality During and After Irradiation. Twenty-five out of 388 irradiated rats (Table 1) died before the irradiation sessions were completed; 18 of these were exposed to radiation on 10 successive days, receiving daily doses in the range of 50 to 200 r. In most instances these deaths were attributed to immediate or delayed respiratory failure produced by excessive pressure on the young in the plastic tubes that were used to immobilize

them during irradiation. The fact that fatalities during this period tended to be higher in litters that received a high number of small doses (though low cumulative dose; e.g., 10 × 50 r) than in litters that received a fewer number of large doses (e.g., 8 × 200 r, with higher cumulative dose) suggests that repeated exposure was a major factor contributing to these deaths. However, that radiation may have also contributed to the fatalities is suggested by the fact that there were no fatalities among the control animals that were immobilized in the holder for 10 successive days.

During the period elapsing between cessation of irradiation and prior to weaning, 21 animals died. Of these, 17 belonged, again, to the group irradiated on 10 successive occasions, with daily doses ranging from 50 to 200 r. After weaning, and during an interval extending up to 1 year after irradiation, 18 more animals died. Half of these belonged to two litters that received 10 × 200 r. The other deaths during this period were

TABLE 1

MORTALITY DURING AND AFTER IRRADIATION

Litter No.	Litter size	Rad. dose (r)	Died				Surv. or killed	Total died (%)
			During expos.	Before wean.	After wean.	Total		
A5/f3	9		1	2	—	3	6	
B44/f3	8		—	2	4	6	2	
C2/f1	11	10 × 200	3	1	4	8	3	52
C4/f1	8		—	—	—	—	8	
B17/f2	10		5	1	1	7	3	
B19/f3	9		—	3	1	4	5	
B18/f2	8	10 × 150	1	1	—	2	6	22
B11/f2	10		—	—	—	—	10	
B41/f3	8		—	2	—	2	6	
B4/f2	10	10 × 100	2	—	—	2	8	26
B8/f2	9		2	—	1	3	6	
B14/f2	10	10 × 50	3	5	—	8	2	50
B22/f2	8		1	—	—	1	7	
B56/f3	7		—	—	—	—	7	
B40/f3	8	8 × 200	1	—	—	1	7	6
B6/f1	6		—	—	1	1	5	
B25/f2a	10		—	—	—	—	10	
B20/f3	9	8 × 150	2	1	—	3	6	18
B2/f3	8		—	—	—	—	8	
B3/f2	10	8 × 100	—	1	—	1	9	5
B25/f3b	9		—	—	—	—	9	
B15/f2	6	8 × 50	2	—	—	2	4	33

TABLE 1 (*Continued*)
MORTALITY DURING AND AFTER IRRADIATION

Litter No.	Litter size	Rad. dose (r)	Died				Surv. or killed	Total died (%)
			During expos.	Before wean.	After wean.	Total		
B26/f3	9		—	—	—	—	9	
B10/f1	3		—	—	—	—	3	
B25/f3a	10	5 × 200	—	1	—	1	9	5
B5/f2	9		—	—	—	0	9	
B15/f2	9		—	—	1	1	8	
A10/f3	7		—	—	—	—	7	
B50/f3	9	5 × 150	—	—	—	—	9	4
B25/f3c	10		—	—	1	1	9	
A8/f3	5		2	—	—	2	3	
A2/f3	9	5 × 100	—	—	—	—	9	13
B59/f3	9		—	—	1	1	8	
B3/f2	6		—	—	—	—	6	
B5/f2	10	4 × 200	—	—	1	1	9	5
B60/f3	5		—	—	—	—	5	
B60/f3	4		—	—	—	—	4	
B10/f2	10	3 × 200	—	—	—	—	10	12
B13/f2	11		—	1	2	3	8	
B45/f3	10		—	—	—	—	10	0
B38/f3	10	2 × 200	—	—	—	0	10	
B9/f2	6		—	—	—	0	6	
B57/f3	10		—	—	—	0	10	
B16/f2	9	1 × 200	—	—	—	0	9	0
B2/f2	8		—	—	—	0	8	
B53/f3	9	1 × 100	—	—	—	0	9	0

randomly distributed among the other groups and did not exceed the frequency (two deaths) among the control animals. Therefore, of all the dosage-exposure combinations used in this study only ten daily exposures appeared to contribute to the shortening of the life expectancy of the animals, and this did not hold for all the litters receiving this type of treatment.

Because these fatalities indicated pathological effects that could not be attributed directly to restricted cerebellar irradiation, only the brains of those animals were processed and evaluated in this study which managed to survive and were killed, at the designated survival periods, by cardiac perfusion with formalin. But even in surviving animals, localized cerebellar irradiation, which necessarily affected the underlying medulla (and possibly harmed such vital neighboring structures as the thyroids and pituitary) may

have had direct or indirect systemic effects. Therefore, it became necessary to determine whether, or to what extent, regional irradiation of the cerebellum or the treatments associated with irradiation affected normal body growth and the general health of the animals. The obtained data indicated little difference in body weight at 10, 30, and 90 days of age between control and irradiated animals, or among animals exposed to X-irradiation of different dose-frequency combinations. There was considerable individual variability among the animals, but none that could be clearly attributed to radiation dose and frequency. These data, and subsequent observations on animals surviving for longer periods, indicate that all the irradiated animals (including those receiving 10 × 200 r) have maintained normal body weights under standard laboratory conditions.

Brain Development: Wet-Brain Weight. Wet-brain weight at different ages is plotted in Fig. 4 for the control animals and some groups of irradiated animals. The curve obtained for the normal group suggests three phases in brain development: (*a*) very rapid growth from birth to 10 days, from 0.35 to 1.01 g; (*b*) reduced growth, to 1.63 g, from days 10 to 30; and (*c*) slight growth, from 30 to 90 days, to 1.81 g. (Because

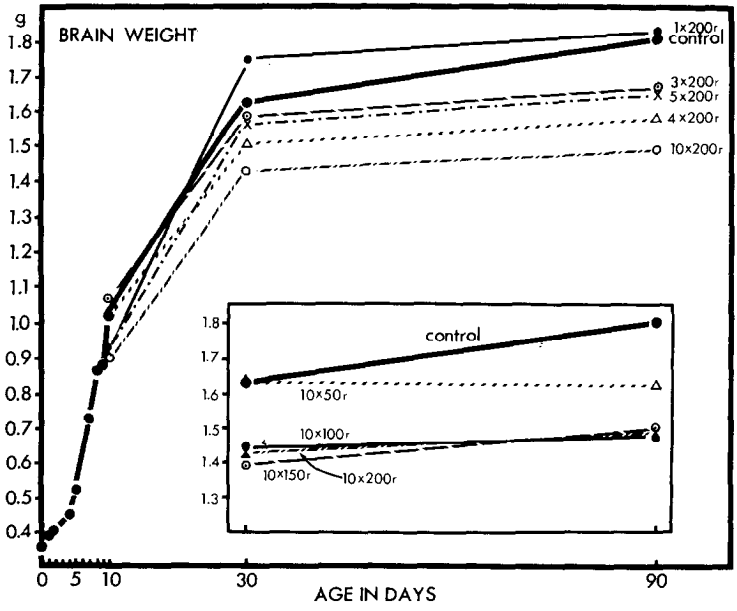

FIG. 4. Growth in wet-brain weight from birth to day 90 in control animals; and at 10, 30 and 90 days in animals whose cerebellum was irradiated daily from birth up to ten occasions with 200 r. Insert summarizes the effect of ten successive daily exposures to doses ranging between 50–200 r.

brains were not available from animals between 10 and 30 days of age, it is possible that the first rapid phase of growth extends beyond 10 days.) In the same figure we also plotted changes in brain development in 10-, 30-, and 90-day-old animals as a function of exposure of the cerebellum to daily doses of 200 r from 1 to 10 days. In addition, in an insert, graphs are presented showing changes in wet brain weight from 30 to 90 days in animals that were exposed to doses of 50–200 r on 10 successive days. At 30 and 90 days the brain weights of animals that received 3, 4, and 5 × 200 r clustered irregularly between the control animals and those receiving 10 × 200 r. The reduction in the latter was above 0.3 g. The effects of 10 × 100, 150, and 200 r were not distinguishable from one another, but 10 × 50 r had less effect than the higher doses.

A cut block was available, from the brains which were prepared for sectioning in the coronal plane, consisting of the cerebellum and underlying medulla. Since this block was delineated by the intended field of irradiation, these brain blocks were weighed to determine to what extent weight loss as observed in the entire brain was attributable to retardation of growth in this directly irradiated block. Measurement of these blocks in control animals showed (Fig. 5b) that, in terms of wet weight, this region begins to increase only at 5 days, with indications of transient decreases from birth to this age. Irradiation reduced the weight of this block in all groups at 10 days, but the effect was irregular with respect to the number of daily irradiations. Such a relation, however, was discernible at 30 and 90 days of age in animals receiving doses of 200 r on 5, 8, and 10 successive days. Exposure to ten daily doses of 200 r reduced the weight of this block from 0.35 g in controls to 0.18 g at 30 days, and from 0.45 g to 0.25 g at 90 days, a reduction which accounts for two-thirds of the whole-brain weight loss. In this figure we also plotted the effect of 10 daily exposures of this brain segment to doses ranging from 50 to 200 r (Fig. 5a). The results suggest that 200 and 150 r were equally effective in retarding development, and that cumulative doses of daily 100 and 50 r also had a substantial, though reduced, effect.

As this brain block consisted of, in addition to the cerebellum, the underlying medulla (which shows little weight gain after birth and was expected to be slightly affected by radiation) it seemed desirable to obtain some direct indication of effect on the development of the cerebellum alone. This was accomplished by dissecting the cerebellum in a few unperfused brains obtained from animals 90 days of age. In the control animals the cerebellum weighed 0.26 g; this was reduced to 0.20 g in an animal that received 200 r on 5 successive days, and to 0.11 g in an animal receiving 200 r on 10 successive days. This drastic reduction (0.15 g) in the latter group suggests that the bulk of the decrease in the irradiated block (0.20

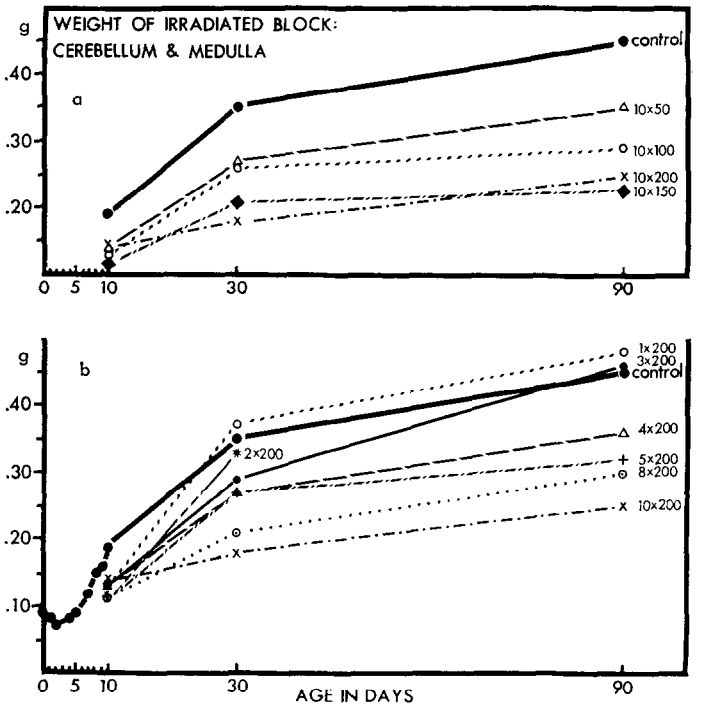

FIG. 5. Wet weight of the irradiated portion of the brain, the cerebellum and underlying medulla; (*a*) in animals that received ten daily exposures to 50–200 r, and (*b*) in animals that received 200 r on 1–10 successive days. Growth curve for normal animals from birth to day 90 also shown in (*b*).

g, as measured in perfused brains) was due to retarded development of the cerebellum.

Development of the Cerebrum: Areal Measurements. Wet-brain weight is a very coarse measure, particularly with brains obtained from formalin-perfused animals which were further hydrated by keeping them for prolonged periods in jars containing formalin. As the next step, a dimensional analysis was made of the unprocessed brains. To determine whether the changes produced were restricted to the cerebellum or also affected other brain regions, the width and length of the cerebrum, in addition to the width, height, and, length of the cerebellum, were measured in all the available animals.

Changes in the width of the cerebrum at 10, 30, and 90 days of age are plotted in Fig. 6. In the control animals there was considerable increase in the lateral dimension of the cerebrum from 10 to 30 days (from 13.8 mm to 15.3 mm), and lesser increase from 30 to 90 days (from 15.3 mm to 15.9 mm). The width of the cerebrum in many of the irradiated animals

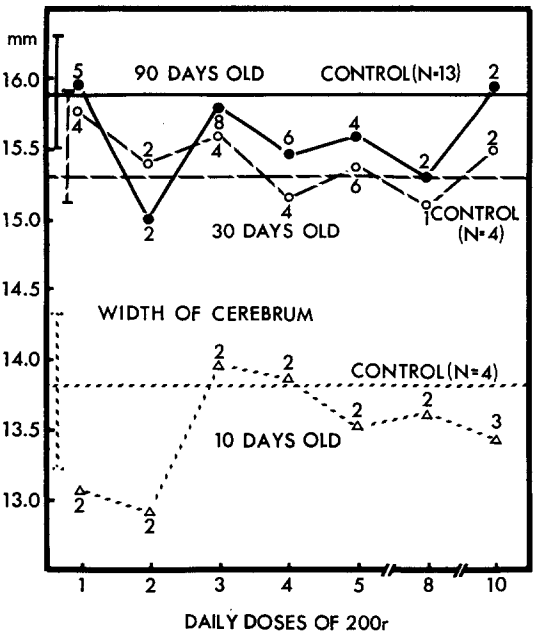

FIG. 6. Mean width of the cerebrum at 10, 30, and 90 days in control animals (straight lines, with vertical lines indicating the range) and in animals whose cerebellum was exposed to 1–10 successive daily doses of 200 r. The number of brains on which the means are based is indicated for each point.

in the different age groups differed considerably from these values, but the variations appeared to be random ones that could not be attributed to the number of daily exposures to X-ray.

Figure 7 summarizes developmental changes in the length of the cerebrum in the three age groups. In the control animals the anteroposterior growth of the cerebrum was considerable; from 12.1 mm at 10 days it increased to 14.3 mm by 30 days, and to 16.2 mm at 90 days. The length of the cerebrum in some of the irradiated animals differed appreciably, though not systematically, from these values, with the possible exception of the 90-day group in which the length of the cerebrum in the majority of the irradiated animals was below the control level. These data indicate that in terms of lateral growth, cerebellar irradiation did not demonstrably affect the development of the cerebrum, and only a slight effect was seen in the anteroposterior dimension in the 90-day-old animals.

Development of the Cerebellum: Areal Measurements. Postnatal irradiation of the cerebellum had a profound effect on its development. Figure 8 summarizes data on changes in cerebellar length as a function of age in control and irradiated animals. In control animals there is considerable

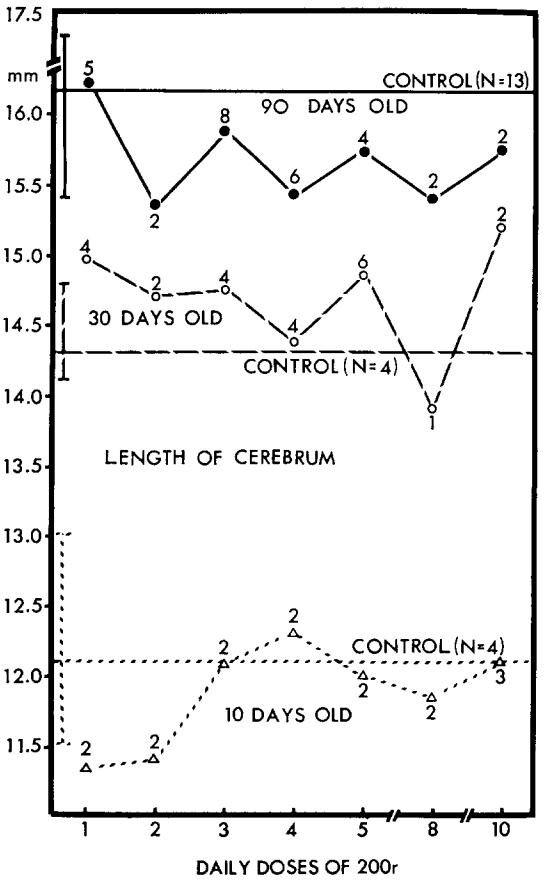

FIG. 7. Length of the cerebrum in control and irradiated animals.

growth between 10 and 30 days, but little, if any, between 30 and 90 days In the irradiated animals there was a considerable drop in cerebellar length at 10 days but with no differences among groups as a function of number of daily doses. The number of daily doses used, however, had a clearly discernible linear effect at 30 and 90 days, except that exposure to 1–2 daily doses had paradoxically enhanced cerebellar length in the 30-day group. With 4–8 daily exposures of the cerebellum to 200 r its growth was retarded to or below the level of the 10-day old control animals; with 10 × 200 r cerebellar length dropped to the level of 10-day old irradiated animals. The height of the cerebellum could not be reliably determined in the 10-day old (or younger) animals; measurements along this dimension were restricted to the 30- and 90-day old (Fig. 9). In the control animals minimal growth was seen from 30 to 90 days, from about 6.0 mm

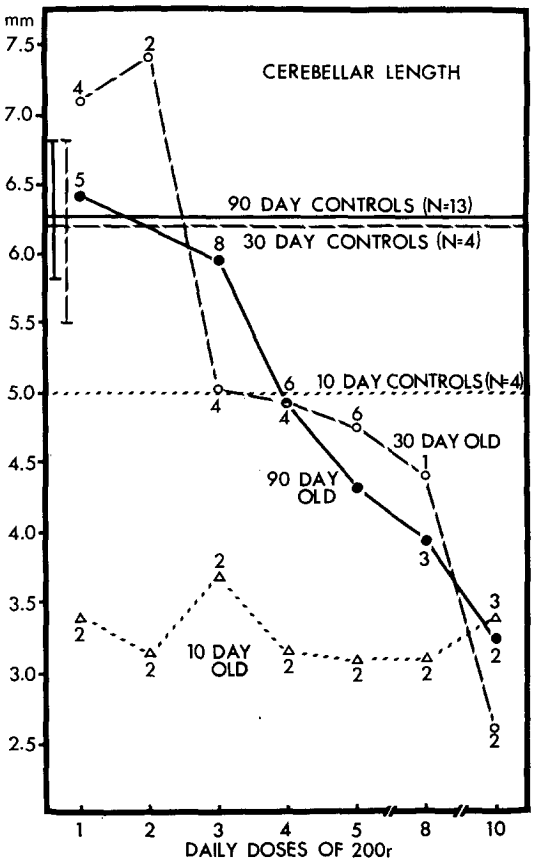

FIG. 8. Length of the cerebellum in control and irradiated animals.

to 6.3 mm. Irradiation retarded growth radically also along this dimension, with cerebellar height reduced linearly as a function of the number of successive daily doses of 200 r. In contrast to cerebellar length and height, the lateral growth of the cerebellum was not appreciably affected by irradiation. Growth in width is considerable in normal animals from 10 to 30 days and appreciable from 30 to 90 days (Fig. 10). Irradiation did not clearly affect this growth pattern. Though all the animals 10 days of age and some of the older ones that received eight or ten daily doses showed some reduction with respect to the control values, the considerable variability seem among animals with fewer daily exposures renders this effect ambiguous.

To summarize these results we plotted in Fig. 11 the effects of different numbers of daily exposures to 200 r on cerebellar growth against growth curves obtained from birth to 90 days in control animals. Cerebellar length

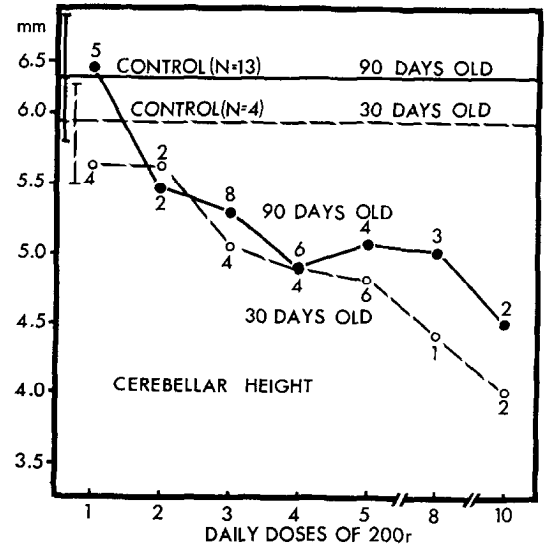

FIG. 9. Height of the cerebellum in control and irradiated animals.

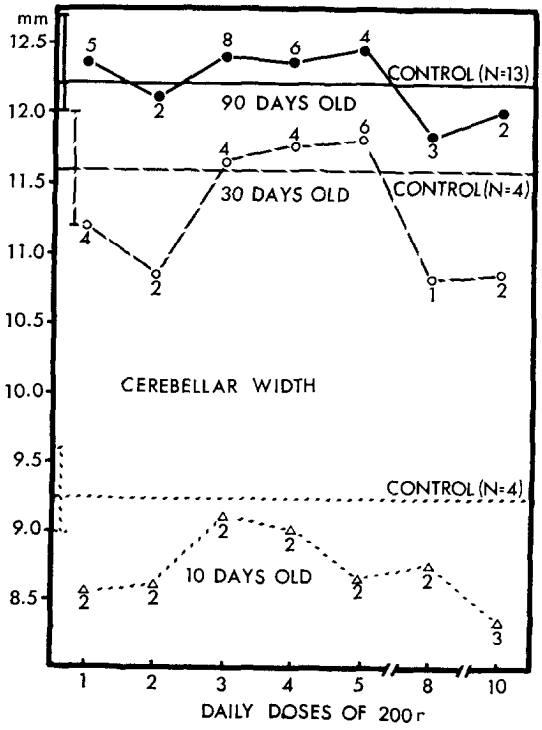

FIG. 10. Width of the cerebellum in control and irradiated animals.

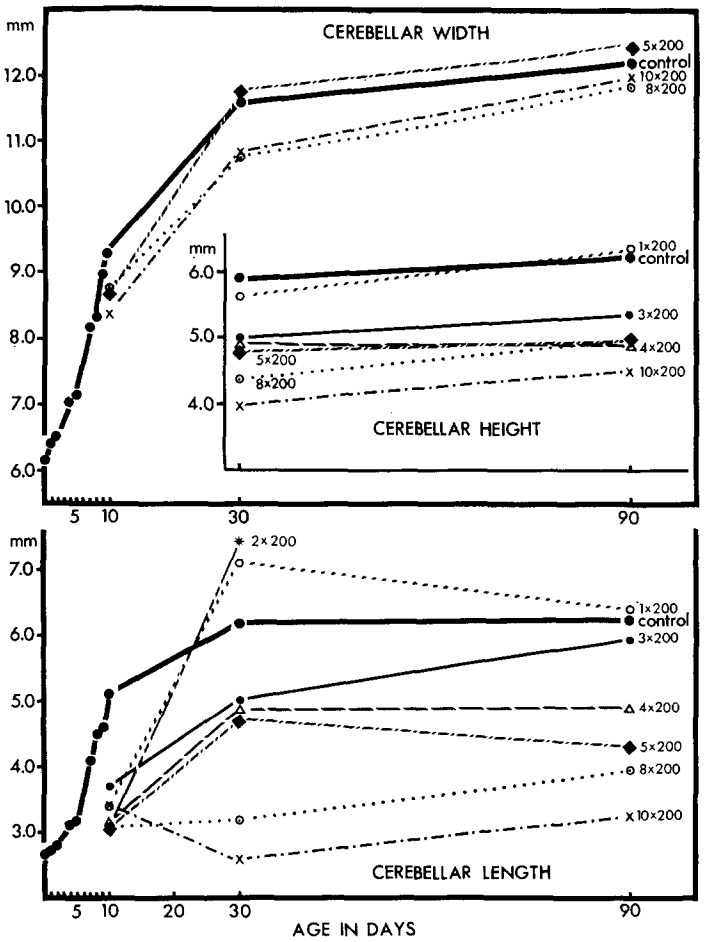

FIG. 11. Width and length of cerebellum in irradiated animals at 10, 30, and 90 days, compared with growth curve in controls from birth to day 90. Changes in the height of the cerebellum (insert) indicated for the 30- and 90-day groups only.

increases from 2.65 mm at birth to 5.1 mm by 10 days; by 30 days it reaches the asymptotic level of about 6.0 mm. Developmental retardation at day 30 and, more clearly, at 90 days, was a function of number of daily exprosures. Cerebellar exposure to 1–2 doses of 200 r appeared to enhance cerebellar growth. With 3–5 exposures to 200 r, cerebellar length in the 30-day group was comparable to the values obtained in 8–10-day-old normal animals; with 8 × 200 r, comparable to 3–4-day-old normal animals; and with 10 × 200 r cerebellar length remained or regressed to the level of normal newborn rats. Cerebellar height was also drastically

affected by irradiation, but due to lack of data about growth along this dimension from birth to 10 days the retardation could not be expressed in terms of developmental age. Although the lateral expansion of the cerebellum was considerable from birth to 10 days, irradiation during this period had minimal effect on growth along this dimension and was restricted to the animals that received 8 or 10 daily doses of 200 r.

In Fig. 12, comparisons were made of the effects of different daily doses of X-ray on the length and height of the cerebellum. Because this effect was irregular with low doses and few daily exposures we have plotted only the differential effects of ten daily doses of 50, 100, 150, and 200 r. The results indicate progressive retardation in cerebellar growth with increasing dose, 10 × 50 r having minimal and 10 × 200 r having maximal effects. For a comparison of the effects of different modes of fractionation a few values with different frequency/dose combinations were inserted for the 90-day group.

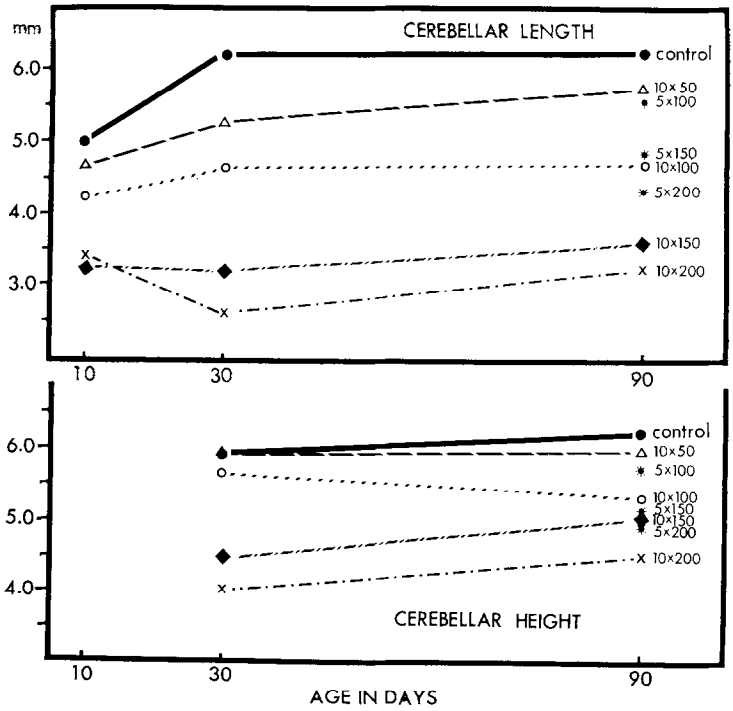

FIG. 12. Cerebellar length and height in control animals and animals that received ten successive daily doses of 50–200 r. Stars indicate the effect of five successive doses of 100–200 r at day 90.

Discussion

The purpose of this series of studies is to develop a radiation procedure which should make possible the selective elimination of a specified proportion of the precursors of the postnatally forming granule, basket and stellate cells of the developing cerebellum. This approach is based on the well-established fact that proliferating and migrating cells, unlike differentiated cells, are destroyed by low-level ionizing radiation (17). If successful, this technique of "selective cellular lesion" will enable us to investigate the consequences of reducing the number of granule, stellate, and basket cells on the structural growth of the cerebellum, and on the development of those behavioral functions which are controlled or influenced by the cerebellum.

The limited objective of this first paper of the series has been to evaluate the effects of irradiation of the cerebellum of infant rats with different doses of X-ray, and different number of daily exposures, on the subsequent development of this structure in terms of such gross criteria as growth in weight and size. In addition to making such measurements on the directly affected cerebellum, comparable measurements were also made in the adjacent cerebrum in order to determine whether the effects of radiation were confined to the cerebellum. The histological and cytological foundations of some of these gross changes will be described in subsequent papers (Altman, Anderson and Wright, unpublished), and we shall deal separately with their behavioral consequences.

The effect of exposure of the developing nervous system to low or intermediate doses of ionizing radiation is most commonly studied by irradiating the entire body or uterus of pregnant mothers, or the whole body of infants (8, 16, 17, 21, 22). This technique yielded a wealth of data about immediate and delayed effects of radiation; but it has an inherent weakness, as it does not allow separation of direct radiation effects on the brain from indirect systemic effects. The technique of confining radiation to the head, or such regions as the forebrain, cerebellum, or spinal cord, is more usually employed in studies using massive doses of X-ray in developing (15, 27) or mature animals (9, 26, 30, 31, 33). Irradiation of the head alone or the spinal cord in developing rats was the technique used by Clemente and his collaborators (12, 32) and their procedure is most closely related to the one used in this study in which irradiation with cumulative doses of low- and intermediate-level X-ray was restricted to a circumscribed brain region.

Fatalities during the period of daily irradiations and before weaning were higher among the animals exposed to ten successive daily doses of 50–200 r than in other groups. Repeated immobilization (with consequent increased likelihood of respiratory complications) was considered the

primary cause of most of these deaths, but the possibility that the high number of daily exposures to radiation was a contributing factor could not be ruled out. The incidence of deaths was low in animals receiving fewer exposures, and those animals that received ten daily doses of 50–200 r which survived past weaning age grew as well as normals. These animals maintained their body weights and were, except for locomotor deficits (to be described elsewhere), in good physical health throughout the period of observation (over 1 year in some instances).

The wet weight of brains is a very coarse measure of altered growth, particularly in view of the observation of Schjeide *et al.* (24) that X-irradiation with 750 r slightly elevated the water content of the brain in some regions. Nevertheless, the procedure yielded useful data about the postnatal growth of the brain and its retardation with certain irradiation schedules. The rapid increase in the weight of the brains of rats after birth is well known. Sugita (28) reported an increase in brain weight in albino rats from about 0.24 g at birth to about 1.78 g at 90 days, values which are comparable, though not identical, with the measures obtained by us in hooded rats. Sugita also reported an increase in cerebellar weight during this period from 0.008 to 0.244 g (we obtained 0.26 g), a phenomenal 30-fold increase. Irradiation with doses of 200 r on 3–10 successive days led to a reduction of brain weight, the reduction being 0.3 g in animals receiving ten doses of 200 r on successive days. Of this reduction in total brain weight, 0.2 g could be attributed to weight loss in the directly irradiated segment of the brain (the block including the cerebellum and medulla), and within this region 0.15 g apparently was due to retarded growth of the cerebellum itself (as determined on an unperfused brain with cerebellum dissected and weighed).

Measurements of wet-weight changes, though indicative of gross alterations, are not suitable for the finer analysis of regional alterations produced by radiation. More useful data were obtained with areal measurements. Determinations of the length and width of the cerebrum indicated, first, that postnatal growth in the control animals was much more pronounced lengthwise than laterally, which agrees with similar observations made by Sugita (28). The increment in the length of the cerebrum exceeds three times the increment in width between 30 and 90 days. The significance of this prolonged growth in the length of the cerebrum remains to be investigated. Little effect of cerebellar irradiation could be demonstrated on cerebral growth, except for a slight reduction in length at 90 days of age. This finding suggested that though cerebral growth is considerable after birth the regional irradiation of the cerebellum did not appreciably affect this neighboring structure. In contrast, the effect of radiation on cerebellar growth, in terms of similar measurements, was considerable.

The anteroposterior growth of the cerebellum, the main axis of growth of the arborizing dendrites of Purkinje cells, was considerable from birth to 30 days in the control animals, but there was little discernible increment in this direction between 30 and 90 days. Retardation of growth in the length of the cerebellum as a consequence of irradiation was appreciable by 30 and 90 days. At 90 days, this retardation was a function of the number of successive daily doses of 200 r with the exception of the animals receiving a single dose of 200 r on the day of birth. Similarly, a linear function was indicated in the effectiveness of ten daily exposures with doses of increasing magnitude from 50 to 200 r. The net effect of ten doses of 200 r was a retardation in cerebellar length by 90 days comparable to the level of normal neonates. Similar effects were seen in the growth of the cerebellum along the vertical dimension, the other axis of growth of the arborizing dendrites of Purkinje cells. In contrast, radiation had no discernible influence on the lateral growth of the cerebellum (even though the postnatal growth in the width of the cerebellum is also considerable) except for a small effect in animals that received from eight to ten successive daily doses of 200 r.

These results suggest that the procedure of cerebellar irradiation that we used in this study was successful in retarding cerebellar growth. The technique of repeated daily irradiations was designed to destroy successive waves of migratory cells (16, 17) moving from the site of proliferation in the external granular layer into the molecular and internal granular layers, without harming the prenatally formed Purkinje cells.

Quantitative histological and cytological evaluation of this material (in preparation) indicates that the developmental retardation accomplished is attributable to reduction in the number and, with 8 and 10 × 200 r, subtotal elimination of the granule, basket, and stellate neurons of the cerebellar cortex. The prenatally produced Purkinje cells were not destroyed by this procedure, though their structural properties were changed.

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