Behavioral Effects of Neonatal Irradiation of the Cerebellum. I. Qualitative Observations in Infant and Adolescent Rats

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WALLACE, ROBERT B., and ALTMAN, JOSEPH (1969). *Behavioral Effects of Neonatal Irradiation of the Cerebellum. I. Qualitative Observations in Infant and Adolescent Rats.* DEVELOPMENTAL PSYCHOBIOLOGY, 2(4): 257–265. The cerebellum of rats was irradiated from birth with daily doses of 200r for 1, 2, 3, 4, 5, 8, and 10 days. The animals were killed at fixed periods after irradiation and at various ages for histological evaluation of the cerebellum or they were permitted to survive for a prolonged period for behavioral testing. Gross measurement indicated that anteroposterior growth of the cerebellum was retarded as a function of the number of doses delivered except in animals that received a single dose of of 200 r. A number of pre- and post-weaning qualitative behavioral observations (tremor, ataxia, level of activity) correlated with the retardation of cerebellar development produced by the irradiation schedules, though some behavioral recovery was noted in the older animals.

cerebellum X-irradiation neurogenesis motor development ataxia tremor

STUDIES USING thymidine-^{3}H autoradiography in rats have indicated that the bulk of short-axoned neurons that make up the granule cell layers of such brain structures as the cerebellar cortex, the olfactory bulb and the hippocampal dentate gyrus come into existence after birth (Altman & Das, 1965; Altman, 1966; Altman & Das, 1966). Since this population of interneurons is formed and differentiated after birth, the period when behavioral transaction with the external world begins, it is conceivable that this neuronal system is intimately involved in the mediation of environmental influences on the functional or structural organization of the developing brain.

The basket and stellate cells of the molecular layer, and the granule cells of the internal granular layer of the cerebellar cortex of the rat are formed after birth over a period of 21 days, predominantly during the second and third week of life (Altman, 1969). This period of cerebellar neurogenesis coincides with the period during which the adult pattern of locomotion emerges (Bolles & Woods, 1964; Blanck *et al.*, 1967; Gard *et al.*, 1967). To test the hypothesis that these interneurons of the cerebellum are necessary for the mediation of environmental influences (such as proprioceptive feedback) and possibly experience necessary for the coordination of locomotion, we investigated the question of whether or not selective reduction or elimination of these interneurons during infancy will interfere with the development of locomotor capacity.

In our attempt to produce a "selective cellular lesion" in the cerebellar cortex, we took advantage of the circumstance that embryonic (proliferating and

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migrating) cells of the brain, are highly sensitive to ionizing radiation (Hicks & D'Amato, 1966). A pilot study (Altman, *et al.,* 1967) indicated that repeated doses of 200 r X-ray delivered during the first two weeks of life to the skull of kittens destroyed the external granular layer of the cerebellum, which is composed of the precursor cells of the microneurons of the cerebellum, and also greatly reduced the population of granule cells in the internal granular layer. Subsequent studies showed that focal irradiation of the rat cerebellum early in life retarded the development of the cerebellar cortex (Altman *et al.,* 1968) and we have established that reduction in the population of granule, basket, and stellate cells is a function of the number of doses delivered from birth onward (to be published).

A number of investigations involving whole-body prenatal irradiation suggested that such treatment led to increased emotionality and fearfulness (Furchgott & Echols, 1958; Fowler *et al.,* 1962). Studies showing the effects of prenatal X-irradiation on various indices of motor performance in the rat have noted ataxia, tremor, a retardation of the upright and righting responses and an inability to develop appropriate locomotor responses with the hind legs (Werboff *et al.,* 1961). Activity scores also seemed to be affected by prenatal X-irradiation with a generalized depression of activity being observed (Werboff *et al.,* 1962). Tremors, clonic twitching, epileptoid seizures, and paralysis of extremities have been reported with irradiated newborn rats (Yamazaki *et al.,* 1962).

These studies, though indicating a wide range of behavioral consequences of ionizing radiation, have used whole body irradiation and did not make a systematic record of the various concomitants of the treatment over a period of time. Moreover, deficits not apparent on a gross level become obvious when a more detailed, analytical investigation is made (Lipton, 1966). Accordingly, we undertook to investigate some behavioral consequences of focal postnatal irradiation of the rat cerebellum specifically those with a prominent locomotor component, and attempted to correlate these against changes in cerebellar structure.

In the first paper of the series, we report several qualitative parameters of the experimental treatment; the results of quantitative experimental data are described in subsequent publications.

METHOD

ANIMALS

Long-Evans hooded rats inbred in our laboratory for several years were used. The pups of 24 litters were irradiated and animals from 3 additional litters served as non-irradiated controls.

IRRADIATION PROCEDURE

A 2-million volt Van de Graaff generator (High Voltage Engineering Corporation Model AM) was used as the source of X-ray. A target-to-animal treatment distance of 125 cm was used with a dose rate of about 50 R/min. A thermoluminescent dosimetry system (EG and G, Inc. Model TL2B) with CaF:Mn thermoluminescent microdosimeters 0.9×6 mm, with a resolution of 1 mm across the field, were used for dosimetry, which were calibrated against a Victoreen ionizing chamber. Dose distribution studies were carried out every day prior to irradiation of the animals. Lucite blocks 30 cm long, 7 cm wide and 5 cm high served both as absorbers and animal holders. In each block, 10 identical holes were drilled which, to accommodate the growing animals, ranged from about 1.5 to 3.0 cm in diameter. Each block was matched with a set of transparent plastic tubing slit lengthwise and provided with small holes for ventilation, into which the animals were fitted 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 2 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 of the front end of the X-ray beam. No allowances were made for the changing antero-posterior length of the cerebellum in the growing animals. The position of the animals' heads with respect to the line on the lucite block was checked after each irradiation session, and the few animals that had moved their heads during the irradiation session were rejected.

Irradiation of all litters began on the day of birth with a dose of 200 r. These animals were irradiated 1,2,3,4,5,8, or 10 times on successive days. The control animals were also carried to the radiation laboratory or to another site, and were placed in plastic tubes and the lucite block for 10 min, as were the irradiated animals. Further details of the irradiation procedure and technique may be obtained elsewhere (Altman, Anderson, & Wright, 1968).

OBSERVATIONAL PROCEDURES

All litters (Table 1) were observed daily from the conclusion of the irradiation procedure for a period of 21 days. The observations were not done blindly. The pups were removed from the breeding cage and

TABLE 1. *Experimental Design Showing the Number of Animals in Each Condition.*

Control	No. of Litters	Total N
	3	17
1 × 200	3	30
2 × 200	3	30
3 × 200	3	38
4 × 200	3	30
5 × 200	5	63
8 × 200	2	21
10 × 200	5	30

placed in a large tray. Two observers independently noted (as defined below) such factors as response to stimulation, tremor (both intention and at rest), dysmetria, ataxia, general activity, ability to right body and locomotor gait. No attempt was made at this time to identify individual animals.

The following qualitative criteria were employed. Analysis of "response to stimulation" involved picking the animals up, touching them or in other ways disturbing them and noting their response to such stimulation. General activity was measured in a plastic tray with drawn squared divisions, noting how many such squares were entered during the observation period of 1 min. Tremor at rest was measured by noting the magnitude of rhythmic movements involving muscles when the animal was in repose; intention tremor was defined as a slow, coarse tremor of the head and limbs which was intensified upon voluntary movement and which often ceased on rest. Ataxia was defined as in-coordination of muscular action; smoothness and integration of gait were noted as well as positioning of limbs. Any inability to complete a movement pattern, such as evidenced by trying to climb over an obstruction and overshooting in positioning of limbs, was labeled dysmetria. Locomotor gait was to a certain extent noted in the determinations of the degree of ataxia present and the "style" of moving was noted; did the animal walk smoothly or were the components of the behavior involved disrupted and jerky? To test ability to right the body, the animals were simply placed on their backs and it was noted whether they righted themselves or not. Each of the 2 observers rated, on each of the criteria, entire litters in the case of the preweaning observations and individual animals in the case of the postweaning observations. A determination was made as to whether any given factor was present to a slight, to a moderate or to a high degree. If a majority of the preweaning animals in a given condition showed a high degree of the deficit being observed, then the entire group was designated as "high" for that parameter. Data from animals that died during the observation periods has been eliminated from the sample. The same essential procedure was followed for both the pre- and post-weaning observations. In the case of the latter groups, however, the sample size was reduced to 10 animals in each condition randomly selected from the respective litters.

POST-WEANING TREATMENT

Upon weaning the animals were ear punched and transferred to standard rat cages where food and water were available *ad lib.* Because the animals of this colony suffer from a chronic respiratory involvement, at the time of their transfer all animals were injected with penicillin. For 1 month period after transfer, all animals were examined 3 times a week; once again 2 observers made detailed qualitative observations on the various parameters mentioned above. Subsequent to this the animals were placed on a 23-hr deprivation schedule and maintained for a battery of behavioral tests to be discussed in later papers of this series. The behavioral animals were maintained on a standardized diet of Purina chow, cheese and carrots, were weighed weekly and when warranted given a supplementary diet. In addition they were given for 1 week out of every 4, terramycin dissolved in their drinking water.

RESULTS

The effect of the irradiation schedules employed on the length of the cerebellum at 30, 90, and 500 days is shown in Fig. 1. Variability is seen within a given radiation group at different ages but there is a clear trend of decline in cerebellar length with increase in the number of daily doses of 200 r, with no effect in the 1 × 200 r group.

Early behavioral observations indicated that the magnitude of the motor deficits observed was linearly related to the radiation dosage. Generally it was found that in those cases where the observations indicated a parameter to be "high" for only a few of the observation periods, this was noted shortly after the conclusion of the irradiation procedure and there would be a drop to moderate or slight levels by the end of the pre-weaning period.

In Figs 2 to 5 each point represents the percentage of the animals in the litters that showed the specified trait or deficit to a "high" degree. (Since the 2 types of tremor observed tended to co-vary, these categories were combined into one general category of tremor for purposes of graphing in Fig. 4). The percentage of animals that were affected, and the time course of recovery, varied as a function of the number of daily irradiations. The animals that received a higher num-

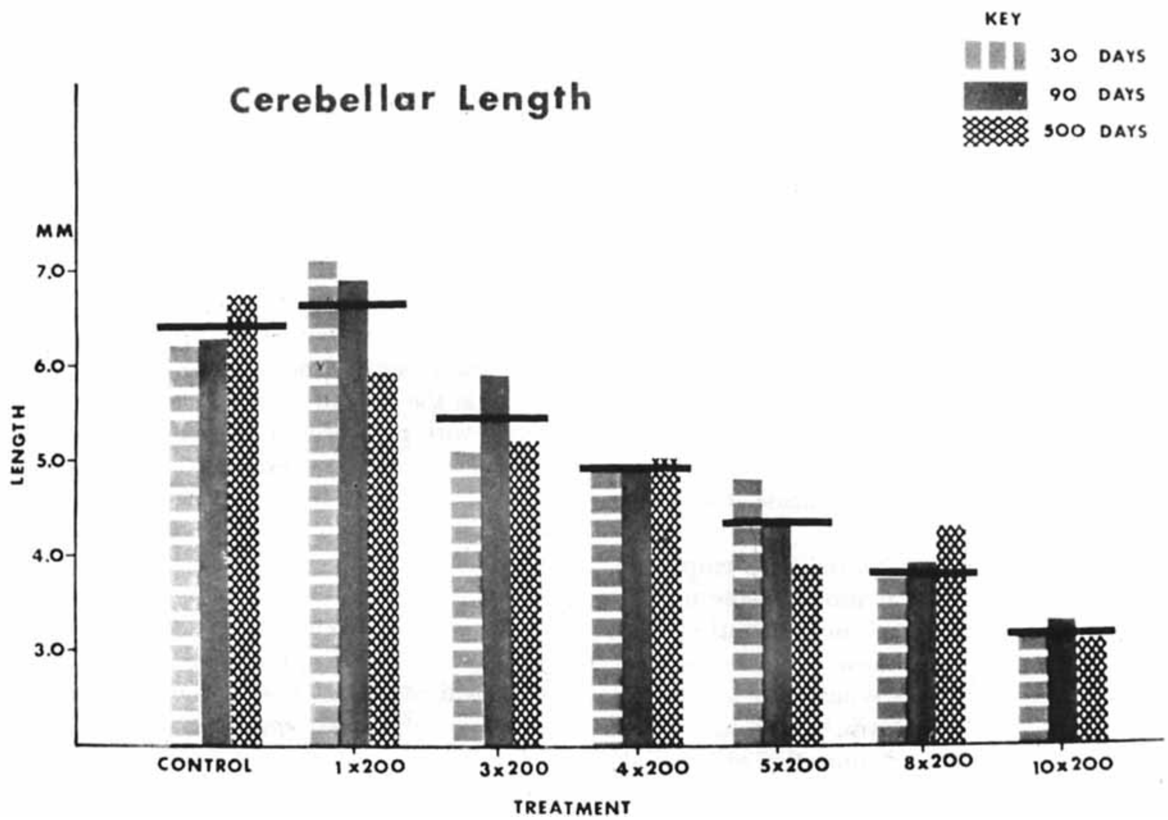

FIG. 1. Measurement of cerebellar length at three different ages as a consequence of treatment.

ber of daily doses of 200 r were more seriously affected, showed less recovery, and also longer delays in recovery. The group that received 10×200 r showed the least amount or no recovery.

Several parameters were noted in addition to those plotted such as the ability of the animals to right themselves, dysmetria (inability to perform skilled movements), general locomotor gait and coordination. In all the cases the data indicated the same trend as that observed in the plotted data.

It might be noted that 2 litters of 4×200 r doses were excluded from the sample due to pronounced abnormalities which in most cases were as severe as any noted in 8 or 10×200 r animals. It is hoped that a histological analysis of the cerebellum of these animals might shed some light on the causes of this eccentricity.

DISCUSSION

The growth of the cerebellum, as determined by gross measurements at 30, 90, and 500 days of age, was retarded in proportion to the number of daily doses of 200 r received focally during early infancy. Paralleling this retardation in cerebellar development, the locomotor deficits that were observed were also directly related to the number of consecutive daily irradiations. These behavioral deficits included reduction in general activity level, and increases in the incidence of severe ataxia and tremor. In general, deficits were not observed in the animals that received 1-2×200 r (irradiations on *day 0* and *1*). This is in line with the result that a single dose of 200 r does not affect cerebellar development, notwithstanding its great destructive effect on the precursor cells of cerebellar microneurons constituting the external granular layer (Altman *et al.*, 1968). This structural recovery can be attributed to the reconstitutive capacity of this germinal matrix during infancy (Altman *et al.*, 1969). The behavioral recovery observed after irradiation in infant rats is conceivably also mediated by this structural recovery; however, it is unlikely that the recovery observed after 21 days of age (when the proliferative external granular layer is dissolved) can be so explained. The degree of behavioral recovery was different for the motor patterns observed. General activity level was normal in all but the rats that received 8-10×200 r 30 days after irradiation, but few of the animals of the latter groups showed any recovery. Tremor also disappeared by this time in all but these two groups, with no recovery evident in the animals that received 10×200 r. In contrast, the persistence of ataxia was pronounced

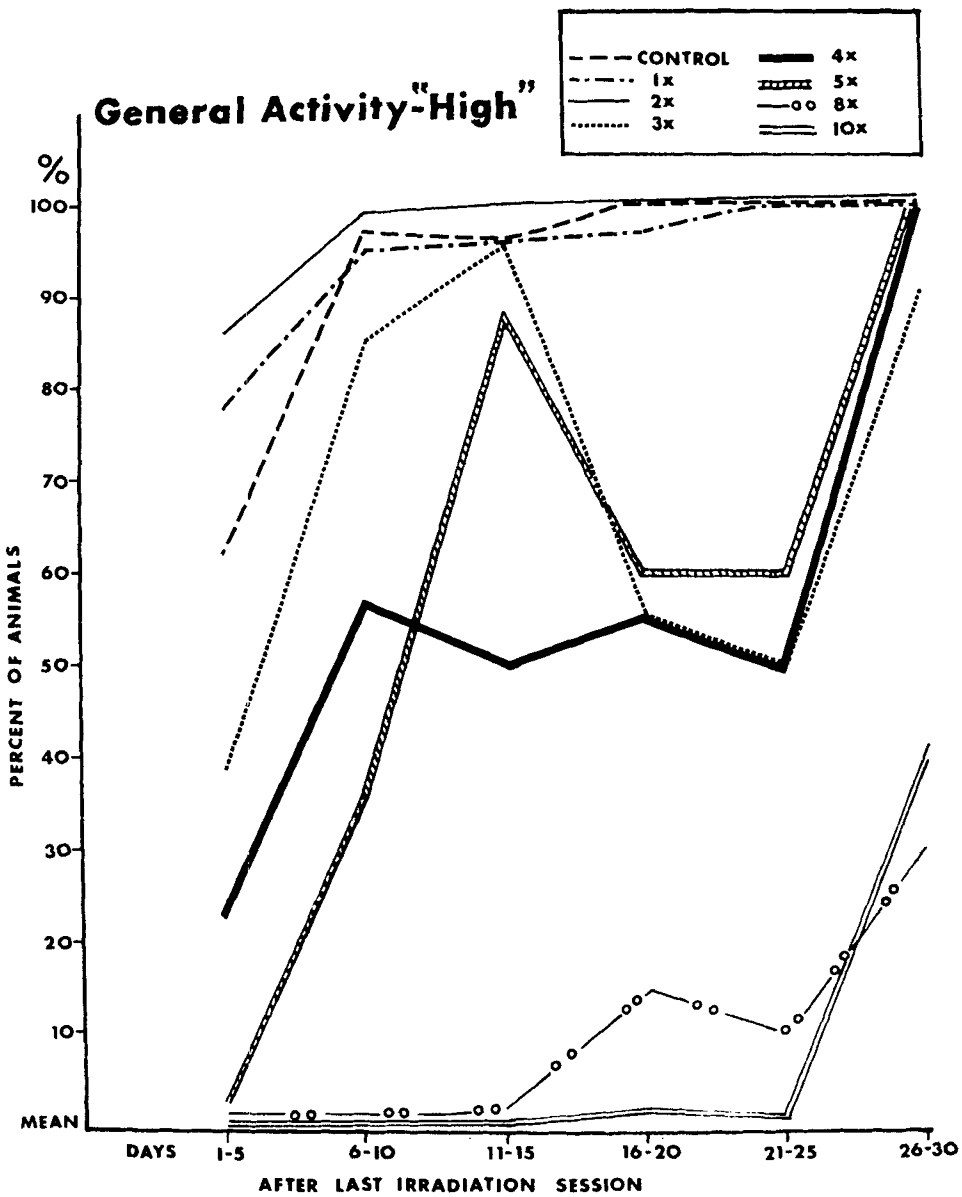

FIG. 2. Percent of animals showing general activity to a high degree on the pre- and post-weaning observation periods.

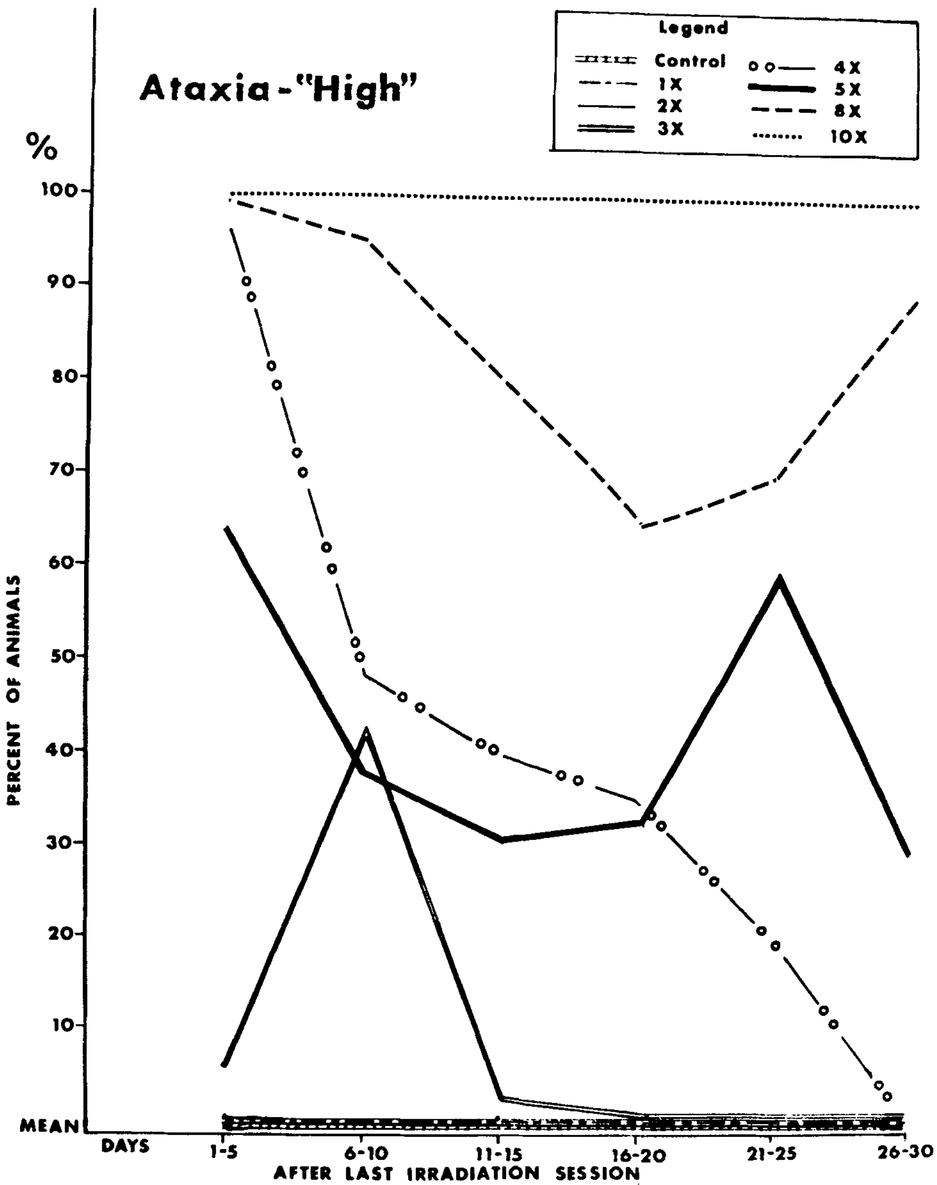

FIG. 3. Percent of animals showing ataxia to a high degree on the pre- and post-weaning observation periods.

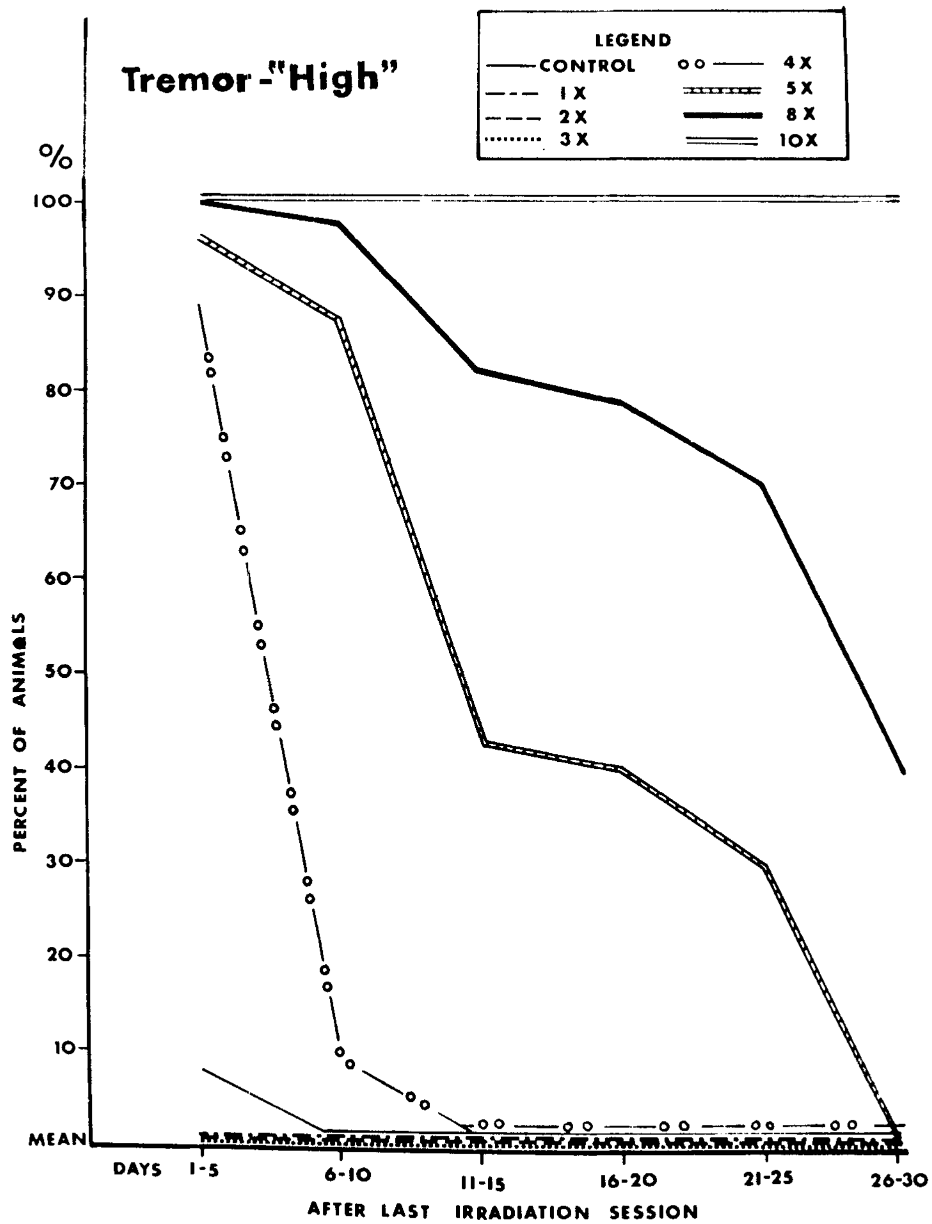

FIG. 4. Percent of animals showing tremor to a high degree on the pre- and post-weaning observation periods.

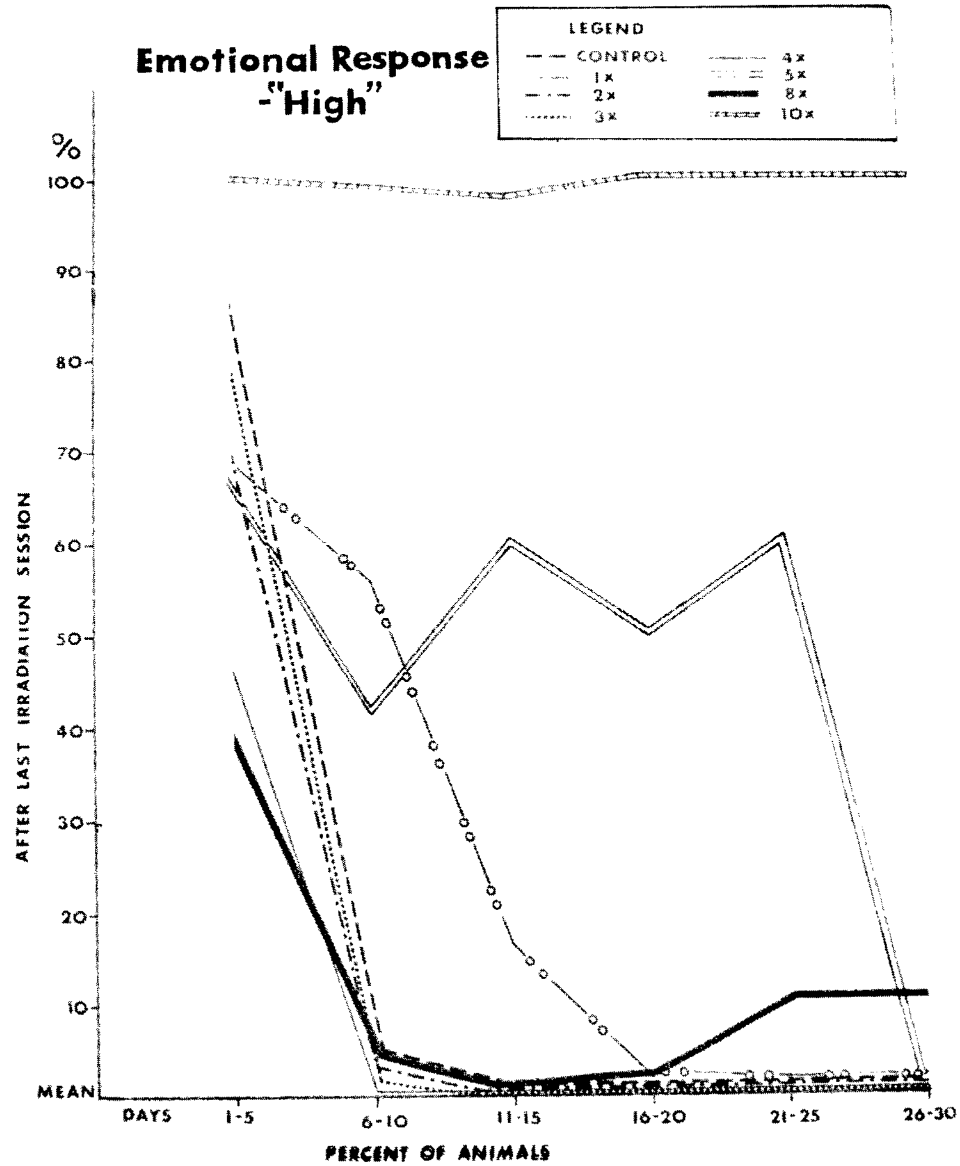

FIG. 5. Percent of animals showing emotional response to a high degree on the pre- and post-weaning observation periods.

up to 30 days in some of the animals that received only 5×200 r.

In addition to these motor deficits, we also observed changes in "emotional responsiveness." All the animals, including those that were not irradiated, were reactive at the beginning of the observation period. All, except those that received 10×200 r habituated after some delay. The nature of this behavioral alteration, which is difficult to attribute to cerebellar damage, remains to be determined. Our earlier study (Altman *et al.,* 1968) showed that the cerebral cortex was not visibly affected by cerebellar irradiations, but the involvement of other brain structures (the underlying medulla and the mid-brain) or the pituitary gland have not been ruled out.

In general, these qualitative observations indicate that radiation-induced retardation of cerebellar development during infancy, which leads to a reduction or elimination of cerebellar microneurons, is associated with motor deficits in infant and adolescent animals. Considerable recovery was seen in the animals in which the cerebellar lesions were moderate, the deficits appeared more persistent in the animals in which retardation of cerebellar development was more severe. Quantitative assessments of motor deficits in young-adult and adult rats are reported in the second paper of this series.

NOTES

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