Behavioral Effects of Neonatal Irradiation of the Cerebellum. II. Quantitative Studies in Young-adult and Adult Rats

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WALLACE, ROBERT B., and ALTMAN, JOSEPH (1969). *Behavioral Effects of Neonatal Irradiation of the Cerebellum. II. Quantitative Studies in Young-adult and Adult Rats.* DEVELOPMENTAL PSYCHOBIOLOGY, 2(4): 266–272. Young-adult and adult rats whose cerebellum was focally irradiated with a variable number of daily doses of 200 r X-ray during infancy were tested for spontaneous locomotion, in activity wheels, for muscular strength, in a weight-pulling apparatus, and, for skill in coordination, in a rope-climbing apparatus. Spontaneous activity declined as a function of number of daily irradiations; the interaction of treatment, age, and prior experience was also investigated. Weight pulling capacity, as determined by running time in an alleyway, was not affected by irradiation with 1 to 4 × 200 r with loads equalling the rats' own body weights. Ease of rope climbing was inversely related to the number of daily irradiations in terms of climbing time, number of shocks received, falls off the rope, and other criteria.

cerebellum X-irradiation locomotion muscular strength activity wheel

IN THE first paper of this series (Wallace and Altman, this issue) we reported on a number of qualitatively assessed motor deficits in infant adolescent rats whose cerebellum was irradiated after birth with repeated daily doses of 200 r X-ray. Correlated with retardation of cerebellar development, which was a function of the number of doses delivered on successive days, we observed several locomotor deficits. Among these were reduction in "general activity," tremor, and ataxia. Most of these symptoms were appreciably attenuated or disappeared by the 30th day, except in the animals that received 8 to 10 × 200 r. The experiments described in this paper were carried out on young-adult and adult rats. These tests were undertaken on the assumption that even in rats with fewer doses of X-ray, abnormalities in locomotion might be detected if sensitive quantitative techniques are employed for prolonged periods. These tests included (*a*) "spontaneous" or "volitional" locomotion in activity wheels; (*b*) pulling of weights across a runway for food reward; and (*c*) climbing of vertical ropes in an avoidance situation. Spontaneous activity was tested in all irradiation groups; the other two tests were restricted to animals that received during infancy 1 to 4 × 200 r, focally delivered to the cerebellum.

Activity wheels are commonly used for assessment of the magnitude of spontaneous locomotion. Recently it was reported (Jones *et al.*, 1953; Kimeldorf, 1961) that spontaneous locomotion in normal rats is a function of age and prior experience. For animals of equal experience, activity varies inversely with age, whereas for animals of equal age activity varies directly with experience (though these relationships are not

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linear). Accordingly, age and experience were taken into consideration in the testing of spontaneous locomotion in the irradiated rats.

The test of rope climbing was introduced in an attempt to gauge the coordinative capacity of irradiated rats. However, because in this situation the strength of the animals is a major variable (they have to carry their own weight up vertically), we tested initially the capacity of rats with differential X-irradiation history to carry weights harnessed to their body along a runway.

METHODS

Details of irradiation procedure, maintenance schedule and evaluation of cerebellar deficit were given in the first paper of this series.

SPONTANEOUS ACTIVITY

Animals were run, 11 at a time, in standard activity wheels, calibrated according to the procedure of Lacey (1944) so as to assure equivalent ease of cage operation. All wheels were maintained in a standardized environment; water was available ad lib and food was given for 1 hr out of every 24. Number of revolutions was recorded daily. Groups of animals were selected and introduced into the wheels at 50, 100, and 150 days of age. In addition some animals were taken and rerun in the activity wheels 6 months after their first run. All animals were kept in the units for a period of 1 month.

WEIGHT PULLING

The runway consisted of a starting box and a goal box, each 12 in. in length and 6 in. in width connected by a straight alleyway 48 in. in length. Movable doors separated the starting and goal boxes from the alleyway proper. All surfaces were painted a flat black save for the goal box which was painted flat gray; the top was closed by clear plexiglass doors. Photocells were so positioned as to provide starting time and running time measures (Amsel's *Time 1* and *2*); a Gerbrand's feeder supplied two 45 mg pellets in the goal box. A harness was attached to the rat and to this was affixed, by means of a pulley system, a weight of a particular value. The task of the animal was to run down the alleyway pulling the weight and enter the goal box. Solid state components sequenced the operations and provided a time base; electrically resettable counters recorded starting and running times. For 3 days prior to being run, the animals wore the harness in the home cage for about 15 min per day. The operant was gradually shaped and the weights introduced according to the schedule in Table 1; if on a particular day, any animal had difficulty in running it was shifted to a lower weight for 1 day and then brought up again. Animals were shifted to higher weights when 2 days passed without any appreciable change in either running time or style. Qualitative observations as to style of running were recorded. Nine trials were given per day.

TABLE 1. *Schedule of Introduction of Weights*

25 g	50 g	75 g	100 g
150 g	200 g	250 g	300 g

ROPE CLIMBING

The apparatus consisted of an upright box 12 in. high and square with a hinged clear plexiglass front. All interior surfaces were painted a flat black. The floor of the unit consisted of stainless steel bars across which a scrambled electric current could be pulsed. Three separate ropes of decreasing diameters were suspended from a microswitch so as to provide a climbing time measure; the operant required of the animal was to climb from the starting area to the goal platform by means of the rope. A warning stimulus signalled the initiation of a trial and shock was presented 5 sec later if the animal did not respond by jumping onto the rope. Solid state components sequenced the operations and provided a time base; an electrically resettable counter provided a climbing time measure. The operant was gradually shaped and the heights increased as determined by each animal's behavior. The goal platform could be positioned 10, 20, 30, 40, or 50 in. from the grid floor. The schedule of starting and goal box separations is shown in Table 2. The criterion of change was 2 consecutive days with no appreciable change in climbing time or style. Shock levels were empirically determined and increased as necessary to maintain the behavior; values averaged about 5 milliamperes. In addition to climbing times, qualitative observations were made on mode of climbing and general reactivity to the experimental situation. The technique used was quite similar to that employed earlier to assess the behavioral properties of certain drugs (Winter & Flataker, 1958). Nine trials were given per day. Both studies were run 5 days per week.

TABLE 2. *Schedule of Starting and Goal Box Separations*

Rope 1 (in.)	Rope 2 (in.)	Rope 3 (in.)
20		20
30		30
40	40	40
50	50	50

RESULTS

Figure 1 shows the medium daily revolutions in the activity wheels for the last 3 weeks of testing (the first week was used as a period of adaptation) for the different treatment and age conditions; all of the animals were naive when introduced into the activity wheels. It can be noted that spontaneous activity, as measured by this test, declines both as a function of treatment and age, with two exceptions: (*a*) the animals that received a single dose of 200 r were comparable to normals, whereas (*b*) in the animals that were exposed to 8 and 10 × 200 r locomotor activity has increased at 100 and 150 days of age, suggesting the possibility of functional recovery. Figure 2 shows the median daily revolutions for the last 3 weeks of testing for a group of animals run initially at 50 days of age and then rerun at 230 days of age. Once again the locomotor activity is seen to decrease both as a function of treatment and age. The compensation seen here in the case of the 8 and 10 × 200 r animals, although present, is less pronounced than that shown in the previous figure.

Figure 3 summarizes the results in weight pulling with running time measures (Amsel's *Time 2*). It can be seen that although the functions are displaced upward with increasing weights, relatively there is little difference with respect to irradiation treatment. It

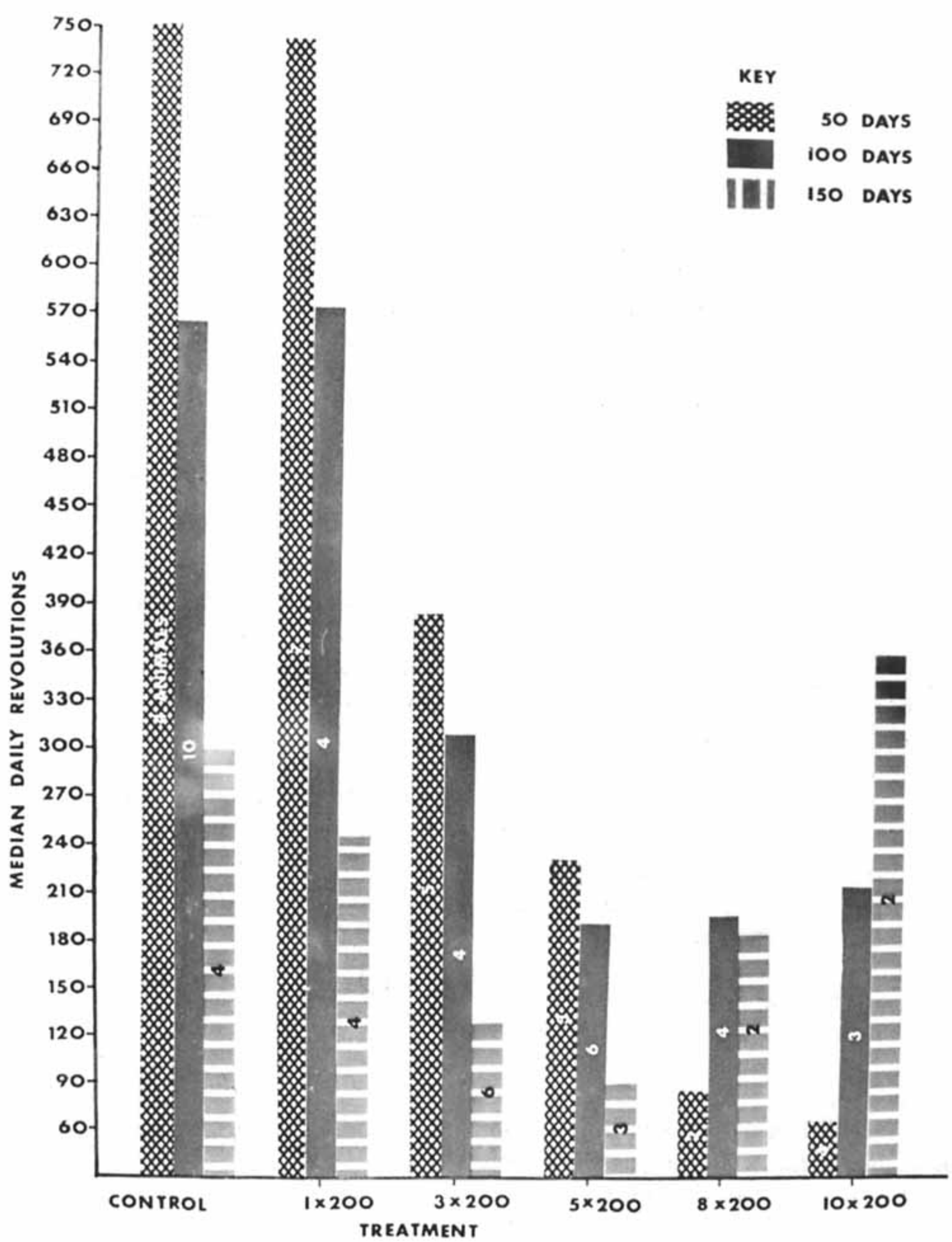

FIG. 1. Median daily revolutions at 3 different ages for animals receiving different treatments.

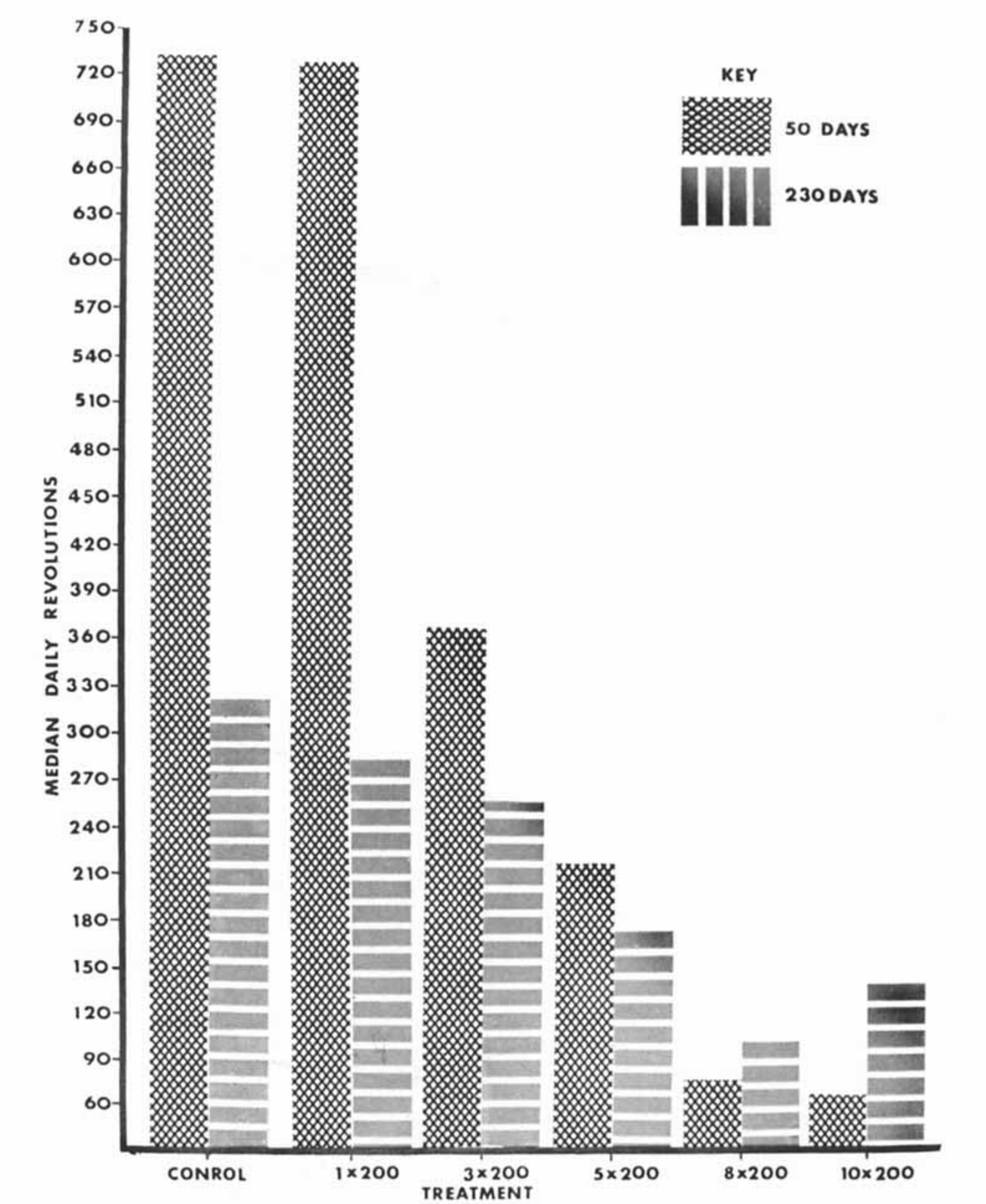

FIG. 2. Median daily revolutions for animals receiving different treatments and introduced into the activity wheels at 2 different ages.

would appear, therefore, that the factor of strength, at least as measured by this test, is not appreciably influenced by magnitude of cerebellar X-irradiation up to 4×200 r. However, several qualitative indices of performance taken during testing, such as smoothness of gait and extent of pausing in the alleyway, tended to suggest some differences across the groups. In these cases the higher the irradiation dosage, the less integrated was the behavior.

Figure 4 shows the plot for rope climbing for the 2nd and 3rd ropes at a height of 50 in. Information from the first rope was essentially meaningless as this was used primarily to shape the response. Each point represents the median of 6 animals. Two factors of interest emerge, first there is clearly greater difficulty with the 3rd rope (small diameter and plastic covered) and secondly there is an almost linear increase in climbing time with increasing number of daily irradiations. Various qualitative indices taken such as number of shocks received, number of falls from rope and

TABLE 3. *Qualitative observations for weight pulling. Daily values for 6 animals for the last week of testing. 300 g weight.*

	Control	1×	2×	3×	4×
Smoothness of gait	Good	Good	Good	Good	Fair
Pausing in runway	None	None	Slight	Slight	Moderate

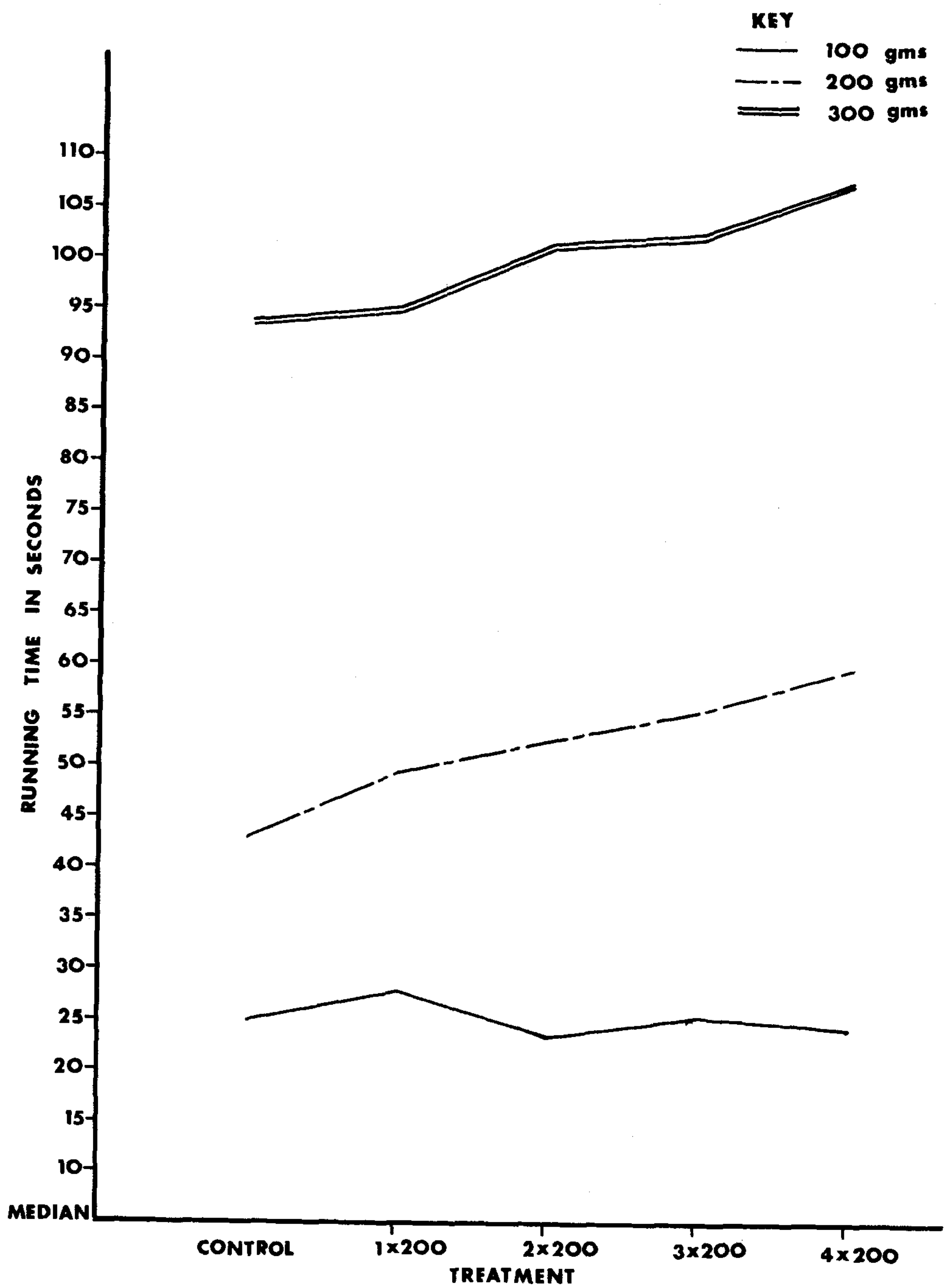

FIG. 3. Running time for animals receiving different treatments.

general style of climbing (coordinated or uncoordinated) also correlate with irradiation dosage (Table 4).

DISCUSSION

In order to assess the behavioral consequences of retardation of cerebellar development (reduction in the population of microneurons produced by focal irradiation during infancy) a battery of tests with loco-

TABLE 4. *Qualitative observations in rope climbing. Mean daily values for 6 animals for the last week of testing. Third rope 50-in. height.*

	Control	1×	2×	3×	4×
No. of shocks received	0	0	0	4	6
No. of falls from rope	0	0	2	7	8
Coordination	Good	Good	Good	Fair	Poor
Transfer to goal platform	Good	Good	Good	Good	Poor

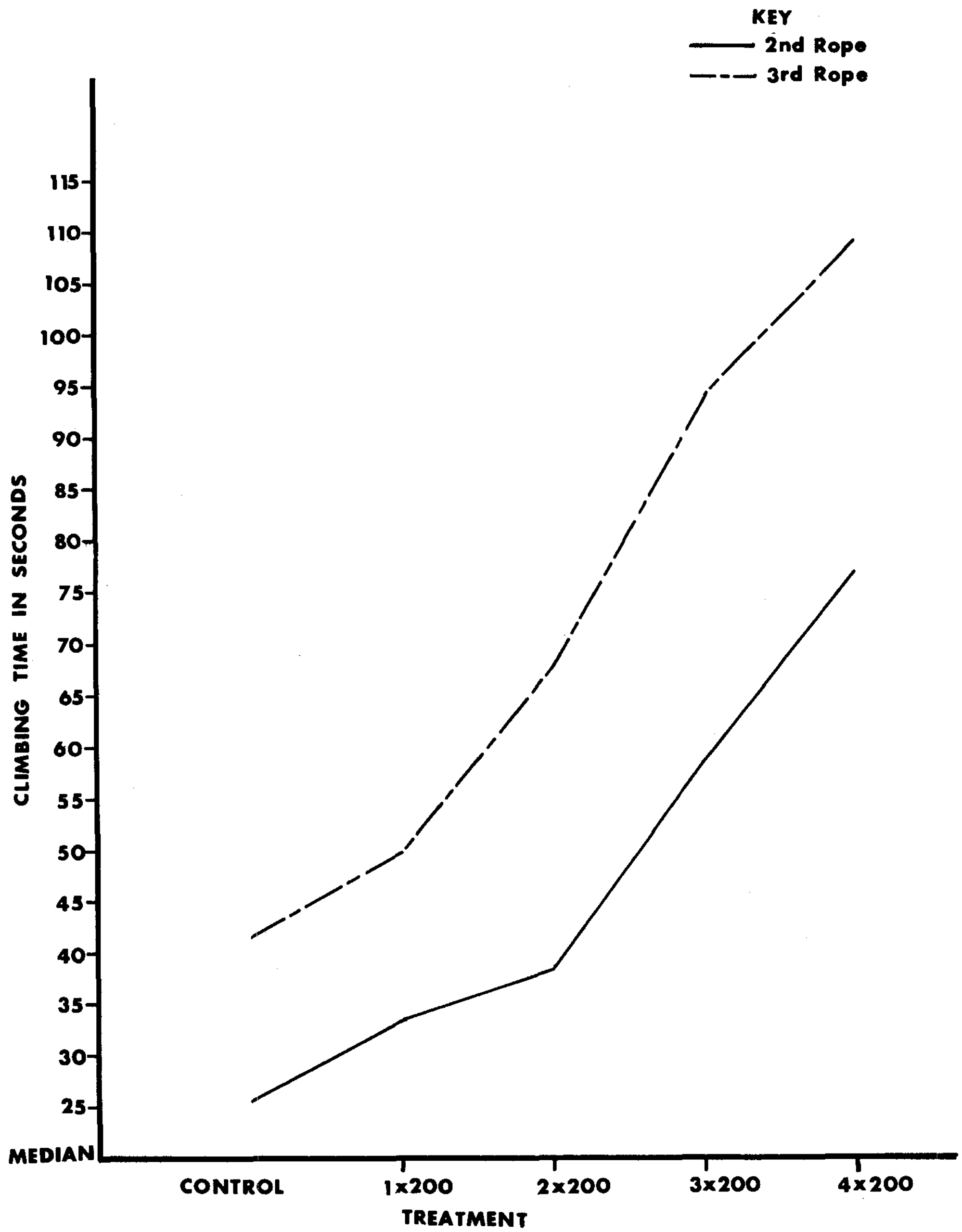

FIG. 4. Climbing time for animals receiving different treatments.

motor components were administered to young-adult and adult rats with different radiation histories. The results indicated that deficits were produced in these animals and the magnitude of the locomotor deficit was proportional to the number of irradiations received after birth. The latter, in turn, correlated with the extent of cerebellar retardation (Altman *et al.*, 1968; Wallace & Altman, *Developmental Psychobiology*—This issue).

Earlier studies showed that whole-body, prenatal irradiation leads to an increase in general activity level (Werboff *et al.*, 1961; Werboff *et al.*, 1962). Our studies, however, showed clearly that locomotor activity decreased within specified periods in irradiated animals with respect to control animals and that it was inversely related to the number of exposures during infancy. The differences in these results may be attributed to differences in time of irradiation (prenatal *vs* postnatal) and the extent of irradiation (whole-body *vs* cerebellar). Localized cellular lesion in the cerebellar cortex achieved with our technique leads to interference with locomotion not only in terms of its coordination and skilled execution but also in terms of the animal's readiness or reluctance to move about. This motivational change may be a direct consequence of reduced coordinative capacity but in this context it is necessary to mention the possibility of pituitary involvement in the irradiations. This would, of course, markedly affect a variable such as locomotor activity. The tentative histological results suggest that there was no pituitary damage, but the possibility cannot at this time be ruled out.

In the weight-pulling test it was established that the irradiated rats ($1\text{-}4 \times 200$ r) were able to pull a load equalling their own body weight at the same speed, though not necessarily with the same style and smoothness. This test of muscular strength had to be applied to rule out this factor in the climbing test. The irradiated animals did not differ significantly in the ease with which they would pull a weight down the alleyway. The study was terminated at the point where they were pulling 300 g, this being an approximation of their body weight. There was a slight tendency for the more highly irradiated animals to show an incoordination in their locomotion but this had no obvious effect on their running times.

With regard to climbing up ropes to avoid electric shock to the feet, we found that there was a dramatic increase in climbing time that correlated with irradiation history. The clearest differentiation of animals as a function of treatment occurred with the third rope; this was perhaps due to the fact that the very small diameter and smooth coating of this rope made it extremely difficult to obtain a secure hold with the digits. In this situation the qualitative parameters observed differentiated them as well as did the climbing time measures. In general, those animals that received higher number of daily irradiations fell off the rope more frequently, often waited to be shocked before climbing, and showed great difficulty in making the transfer from the rope to the goal platform. The particular difficulty observed in the irradiated animals with respect to climbing suggests the possibility of further studies aimed at increasingly difficult coordinative tasks.

In summary, our data seem to indicate that it is the more complex locomotor behavior patterns that are affected by these "cellular lesions" of the cerebellum and support the hypothesis of this study that the microneurons of the cerebellar cortex, which modulate the activities of the neurons of the deep cerebellar nuclei by way of Purkinje cells, are essential for the coordination of late-developing complex locomotor skills. The exact nature of this involvement remains to be elucidated.

NOTES

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