Effects of Differential Interference With Postnatal Cerebellar Neurogenesis on Motor Performance, Activity Level, and Maze Learning of Rats: A Developmental Study

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The region of the cerebellum was X-irradiated in infant rats with selected exposure schedules designed to produce animals in which the cerebellar cortex was (a) essentially normal except for agenesis of late forming granule cells with axons situated in the uppermost molecular layer (12–15X), (b) lacking in stellate cells, with a severe reduction in granule cells with axons in the upper molecular layer (8–15X), (c) morphologically disorganized but had only intermediate cell agenesis (4–5X), or (d) disorganized and devoid of practically all postnatally forming interneurons (4–15X). In the first two experiments young adults had to traverse rotating rods that differed in texture and types of obstacles. The 8–15X animals showed no deficits on any of the rods tested. The third study dealt with spontaneous motor performance in the open field at three ages. The 4–5X and 4–15X animals were hypoactive as infants and young adults; this was attributed to their motor deficits. The 8–15X and 12–15X animals were hyperactive in the open field as young adults. The fourth experiment examined intra- and/or intersession habituation. No group differences were found in habituation patterns. In the fifth experiment, using activity wheels, the 4–15X group was hypoactive as young adults. The fifth and sixth experiment young adults were tested for learning performance in a multiple-unit water maze. The 4–15X group was deficient on single alternation; the 4–5X and 12–15X groups on double alternation. The seventh experiment shed some light on the single alternation deficit of the 4–15X group; only these animals failed to alternate spontaneously in a nonaversive situation. In conclusion, these behavioral results, combined with those of recent morphological investigations, suggest that the cerebellar cortex is hierarchically organized: The basal domain of Purkinje cells and the lower molecular layer are implicated in the coordination of movements; the apical domain of Purkinje cells and the upper molecular layer, in the coordination of actions.

The cerebellar cortex has an unusually precise geometric organization (Eccles, Ito, & Szentágothai, 1967). This is paralleled by the precise chronology of the time of origin not only of neurons of the cerebellar cortex (Altman, 1969; 1972a, 1972b, 1972c) but of the Purkinje cells and neurons of the cerebellar deep nuclei (Altman & Bayer, 1978a).
and also of the neurons of the brain stem precerebellar nuclei (Altman & Bayer, 1978b) that are sources of afferents to the cerebellum. The sequential origin of neurons of the cerebellar system (Figure 1) and the geometric organization of the cerebellar cortex have made it possible to manipulate systematically with X-irradiation (Altman, 1975) the cellular composition and laminar organization of the cerebellar cortex (Figure 2). Results described in this article suggest that the morphogenetic and anatomical stratification of the cerebellum are associated with a hierarchical functional organization.

In the rat, the neurons of the inferior olive (which are the major, if not exclusive, source of climbing fibers) form on Embryonic Days 13–14 (E13-14), and the neurons of the lateral reticular nucleus (LRN), the nucleus reticularis tegmenti pontis (NRTP), and pons (which are sources of mossy fibers) form sequentially on Days E13–15, E15–17, and E16–19, respectively (Altman & Bayer, 1978b). The neurons of the cerebellar deep nuclei form on Days E13–14 (Altman & Bayer, 1978a), the Purkinje cells on Days E14–15 (Altman & Bayer, 1978a; Das & Nornes, 1972; Schultze, Nowak, & Maurer, 1974), and the Golgi cells from Days E19 to the perinatal period (Altman & Bayer, 1978a). Of the postnatally forming interneurons of the cerebellar cortex, the basket cells form predominantly on Days P6–7, the stellate cells on Days P8–11, and the granule cells throughout this period until Day P21 (Altman, 1969, 1972a). In association with this regularity in neurogenesis, the parallel fibers (axons of the granule cells) are laid down in a strict chronological order from the bottom of the molecular layer upward (Altman, 1972b), and the mossy fibers begin to form synapses with the granule cells that descend gradually from the external germinal layer (EGL) to the granular layer, from Days P10–12 onward (Altman, 1972c).

Since the multiplying precursors of neurons are extremely radiosensitive (Altman, Anderson, & Wright, 1967; Hicks & D'Amato, 1966), focal X-irradiation of the cerebellar region with a single dose of 150–200R kills a large proportion of the cells of the EGL (Altman, Anderson, & Wright, 1969). The surviving cells reconstitute the EGL in 4 days, but this can be prolonged or altogether prevented if the number of successive exposures is sufficient to delay recovery of

![Figure 1](image-url). The embryonic (E) and postnatal (P) time of origin (bracketed numbers) of components of the cerebellar system. (Included are the following precerebellar nuclei [PCB]: the pontine gray [pons], the nucleus reticularis tegmenti pontis [NRTP], the lateral reticular nucleus [LRN], and olivary nuclei [olive]; neurons of the deep nuclei; and the following neuronal elements of the cerebellar cortex: the Purkinje cells [PC], the stellate cells [SC], and the basket cells [BC]. The time of origin of the descending granule cells is not shown, but that of their axons, the parallel fibers [PF], is indicated from the bottom of the molecular layer upward in the upper left panel of the illustration. The formation of glomerular synapses [GL] in the granular layer [GRL] extends from P10 to P25 and beyond. The indicated selective connections between the mossy fibers [MF] of the early and late forming precerebellar nuclei, and the early and late descending granule cells, are hypothetical. The implication is a hierarchical organization of spinal and reticular versus neocortical input to the cerebellar cortex. CF, climbing fibers.)
the EGL until it naturally disappears by the end of the third week (Altman, Anderson, & Wright, 1969). Thus by increasing the number of exposures to X-ray, the size of the developing cerebellum can be reduced in a graduated manner (Altman, Anderson, & Wright, 1968), with a correlated reduction in the postnatally forming interneurons without a reduction in the prenatally forming Purkinje cells (Altman & Anderson, 1971).

With seven focal cerebellar exposures between birth and Day 13 (0–13X) or between Day 4 and Day 15 (4–15X), the formation of practically all basket, stellate, and granule cells can be prevented (Altman & Anderson, 1972, 1973). With these irradiation schedules the Purkinje cells in adults are not aligned in a monolayer, and the foliation pattern of the cerebellar cortex tends to be abnormal. Golgi and electron microscopic studies indicated that these anomalies were associated with abnormal dendritic growth and synaptic organization of Purkinje cells (Altman & Anderson, 1972, 1973). Evidence was adduced later that the disorganization of Purkinje cell development is due not to direct radiation effects but to morphogenetic changes produced by the altered cellular composition of the cerebellar cortex. Basket cells appear to be essential for the directed upward growth of the Purkinje cell stem dendrite (Altman, 1976a, 1976c). If irradiation is started on Day P8 (8–15X), after the basket cells have formed, the Purkinje cells are normally aligned in a monolayer, but their erect stem dendrites have few smooth branches (which was adduced to develop in interaction with stellate cells) and the spiny branchlets grow downward into the spared portion of the molecular layer consisting of axons of the granule cells that formed before the irradiation (Altman, 1976b). If irradiation is delayed until Day P12 (12–15X) to spare both basket and stellate cells, only granule cells are missing from the miniature cerebellar cortex (Altman, 1976c). Accordingly, by varying the irradiation schedules in terms of number of exposures (which determines whether or not the EGL can recover) and the time of onset of the first exposure, it is possible to prepare in a routine manner healthy animals with cerebella characterized by (a) structural disorganization coupled with the virtual
absence of cerebellar interneurons (4-15X),
(b) structural disorganization with extensive
cell reduction (4-5X), (c) drastic cell re-
duction (mostly stellate and granule cells)
without structural disorganization (8-15X),
and (d) selective but moderate reduction in
granule cells (12-15X).

Rats irradiated at an early age with long
schedules producing cerebellar disorgani-
zation coupled with drastic cell reduction
display profound and lasting motor deficits,
such as tremor, ataxia, dragging of the
hindfeet, and falling during locomotion
(Altman, Anderson, & Strop, 1971; Anderson
& Altman, 1972; Wallace & Altman, 1969a,
1969b). Rats irradiated early with short
schedules producing disorganization but
moderate cell reduction do not display gross
motor deficits but are handicapped in
climbing a rope for food reward (Wallace &
Altman, 1969b), traversing a rotating rod
(Brunner & Altman, 1973), or rearing (Alt-
man et al., 1971). Pilot studies showed that
if irradiation is started relatively late (Day
8 or Day 12), there are no apparent motor
deficits. But the possibility remained that
by increasing task difficulty (such as placing
hurdles on the rotating rods), handicaps may
emerge. Accordingly, in the first study we
examined the performance of normal and
experimental rats rewarded with food to
traverse rods graded in difficulty in terms of
texture (rough or smooth surface) and ob-
stacles (low, medium, or high hurdles; hurdles varied in spacing, height, or both) at
different speeds of rotation. The experi-
mental groups consisted of the following:
4-15X (disorganization with drastic cell re-
duction), 4-5X (disorganization with ex-
tensive cell reduction), and 8-15X (massive
cell reduction without disorganization).
Because of a lack of demonstrable motor
deficit in the 8-15X group, the less affected
12-15X group was not examined on these
tasks.

Experiment 1:
Rotating Rods With Regularly Spaced
Hurdles

Method
Subjects. Litters of laboratory-bred Long-Evans
hooded rats were reduced to six males on the day after
birth. The litters were weaned at 21 days of age and
maintained in large colony cages on ad lib rat chow and
water until 60 days of age when experimental food de-
privation was begun. All rats used were experimentally
naive.

Irradiation. A detailed description of the X-irra-
diation procedure used in this study has been published
elsewhere (Bayer & Peters, 1977). X-rays were deliv-
ered from a General Electric Maxitron 300 KV x-ray
unit at a rate of 46R/min, with the added filtration of
1.5 mm of copper. Prior to X-ray exposure the pups
were placed in specially designed lead-shielded Lucite
holders which kept the animals immobile during ex-
posure. A slit in the lead shield allowed only the por-
tion of the head containing the cerebellum to be directly
exposed to X-rays. Control subjects (n = 18) were
immobilized in the same manner as irradiated subjects
but were not exposed to X-rays. The 4-15X group (n
= 11) was exposed to 200R X-ray on Days 4 and 5 and
to 150R on Days 7, 9, 11, 13, and 15. The 4-5X group
(n = 17) received 200R on Days 4 and 5. The 8-15X
group (n = 17) received 200R on Days 8 and 9 and 150R
on Days 11, 13, and 15.

Apparatus. The rotating rod (Figure 3) was housed
in an enclosed plywood chamber (315 × 33 × 100 cm)
the front of which contained a 15 × 245 cm Plexiglas
observation window. The rod was suspended 75 cm
from the floor on two pivots at either end of the cham-
ber. One pivot was attached to a variable speed Bodine
motor (Model HSN-34RH) located outside the cham-

Figure 3. Schematic diagram of rotating rod appara-
tus illustrated with a typical rod with hurdles in
place.
CEREBELLAR REORGANIZATION AND BEHAVIOR

The speed of rod rotation was monitored on a tachometer and was controlled by the experimenter through a rheostat connected to the motor.

The rods were constructed from 12 × 275 cm smooth plastic pipe which had been painted flat black. Rough surface rods were made by evenly spreading sand on the surface of the rod while the paint was still damp. A final coat of paint over the sand permanently secured the sand to the rod. Three different-height, circular hurdles (low: diameter 12 mm; medium: 25 mm; high: 50 mm) were painted white and glued to the rods at 46-cm intervals. All the hurdles on a given rod were of uniform height. The 16 × 32 cm start platform was located 2.5 cm above the rod at the left end of the chamber. The 24 × 32 cm goal platform was located 2.5 cm above the rod at the right end of the chamber and contained a dish with the chocolate milk reward. Illumination was provided by five evenly spaced 7.5-W light bulbs located on the back wall of the chamber above the rod. The floor of the chamber was padded with 5-cm foam rubber so that a fall from the rod would not cause injury to the animal. Animals that fell from the rod were removed through trap doors located on the lower front wall of the chamber.

Procedure. At 60 days of age each subject was introduced to the food deprivation schedule by gradually reducing its food allotment over a 10-day period until its body weight stabilized at 85% of ad lib weight. Adjustments in the food allotment were made daily to maintain the animals at this weight throughout the experiment. During this adaptation period the animals were fed once daily at the approximate time of day they would be tested, and they usually consumed all the food within the first 2 hr. During the actual experiment each animal was returned to its home cage after testing and fed 30 min later.

Preliminary training was done on a stationary rough rod with no hurdles. A movable goal platform containing a dish of chocolate milk was first placed directly against the start platform and on successive days was moved toward the goal platform by 30-cm increments. Initial shaping was done in groups of three littermates. Experience indicated that this was more effective than training single animals. When all animals were reliably exploring the entire length of the rod, individual testing began. Reward for reaching the goal platform consisted of a 5-sec-duration drink of chocolate milk. The criterion for completion of the stationary rough rod test was three consecutive trials in which the subject ran the full length of the rod in 10 sec or less. All animals were given six trials/day up to 3 days to meet this criterion, and animals that could not meet it were not tested further. On the day following completion of the stationary rod test, rotating rod testing was begun.

At the beginning of a test session, each subject was "baited" by placing it on the goal platform and allowing it a 10-sec drink of chocolate milk. The subject was then placed on the start platform and was given up to six trials at each speed of rotation (beginning with 2.5 rpm) to successfully cross the rod twice with a maximum running time of 10 sec per trial. After the subject met the criterion at a given speed, the speed of rotation was increased by increments of 2.5 rpm up to a limit of 20 rpm on the first day of testing. On the second day of testing, the subject was allowed one trial at each previously successful speed starting with 2.5 rpm and increasing by increments of 2.5 rpm. When the subject reached the highest successful speed of the previous day, the criterion of two trials at 10 sec or less running time (with a limit of six trials at any single speed) was re instituted until the animal reached the limit of its capabilities or 30 rpm. Any combination of three successive

Figure 4. Photomicrographs illustrating the cerebella of normal and irradiated rats at the sagittal level used for histological evaluation (Figures 10 and 11) at the same magnification (horizontal bar). (Arrows point to the pyramis [vermal lobule VIII], shown at higher magnification in Figures 5 and 6.)
Figure 5. Pyramis of normal and irradiated rats. (In the 4-5X animal, laminar disorganization is shown by ectopic granule cells in the molecular layer [arrows] and Purkinje cells embedded in the granular layer. Abbreviations: Co, control; GR, granular layer; ME, medullary layer; MO, molecular layer; PU, Purkinje cell layer.)

Figure 6. Lateral aspect of the pyramis shown in Figure 5. (Arrow points to the ectopic granule cells in the 4-5X animal. Note the concentration of Purkinje cells per unit area in relation to extent of degranulation.)
(a) falls from the rod, (b) returns to the start platform, or (c) refusals to run in less than 180 sec was scored as a failure on that rod, and testing was terminated for the day. If an animal failed at a given speed on two successive days, it was terminated on that rod, and its score for that rod was the previous successful speed achieved. As long as a subject's performance on a rod continued to improve over successive days, testing continued on that rod until the subject reached the limits of its capabilities or 30 rpm.

Each animal was tested on the following sequence of eight rods: (a) rough, no hurdles; (b) smooth, no hurdles; (c) rough, low hurdles; (d) smooth, low hurdles; (e) rough, medium hurdles; (f) smooth, medium hurdles; (g) rough, high hurdles; (h) smooth, high hurdles. A massed-trial procedure was employed in which the subject was returned to the start platform immediately after either a 5-sec reward or a fall from the rod.

Several measures of performance were recorded on each trial: (a) latency to leave the start platform, (b) running time required to reach the goal platform, (c) whether the subject performed "tested" the speed of rod rotation with its forepaws before leaving the start platform. Finally, (d) a daily record was kept of each subject's highest successful speed of rod rotation.

**Histology.** After completion of behavioral testing, each subject was anesthetized with sodium pentobarbital (75 mg/kg) and perfused intracardially with 10% buffered formalin, and its brain was removed. Following 24 hr of fixation in Bouin's solution, the coronal and sagittal dimensions of the cerebral hemispheres and cerebellum were determined with a micrometer (Altman et al., 1968). The brains were then embedded in paraffin, and the 6-μm sagittal sections of the cerebellum were stained with hematoxylin and eosin for later histological evaluation.

Figure 4 illustrates the overall changes produced in the cerebellum by the different irradiation schedules in animals used in this study (including the 12-15X group utilized in subsequent experiments). Laminar changes in the cerebellar cortex are illustrated in the uvula (vermal lobule VIII) of the same animals at higher magnification in Figure 5. Note the disorganized molecular layer, with ectopic granule cells, in the 4-5X animal. Finally, the cellular effects of the different irradiation schedules are illustrated in Figure 6. The latter shows a lack of apparent effects, as revealed with Nissl staining, in the prenatally forming Purkinje cell perikarya.

**Results and Discussion**

**Anatomical.** The sagittal (length) and coronal (width) dimensions of the cerebral hemispheres and cerebella of control and irradiated rats are summarized in Table 1. Significant reductions were obtained in all irradiated groups in cerebellar length, and the magnitude of reduction was largest for the 4–15X group. Cerebellar width was significantly reduced only in two experimental groups, and the magnitude of reduction was small. These differential effects are comparable with earlier findings (Altman et al., 1969) and are attributed to a reduction in the sagittally oriented dendritic arbors of Purkinje cells in the experimental animals (Altman, 1976c, Figure 5) and a reduction in the number of parallel fibers (as correlates of reduction in granule cells) but relatively little change in the length of parallel fibers, which are oriented coronally in the vermis, of those granule cells that have formed. The width of the shielded cerebral hemispheres was not affected in any experimental group, but the length increased. The latter paradoxical effect is attributed to expansion of the cerebral hemispheres into the space vacated by the shortened cerebellum. Quantitative histological and cytological data of comparably treated cerebella in infant, young adult, and adult rats are presented in Experiment 3.

**Behavioral.** Three-way analyses of variance were performed on the following measures of rotating rod performance: (a) latency to leave the start platform, (b) running time required to reach the goal platform, (c) whether the subject performed "tested" the speed of rod rotation with its forepaws before leaving the start platform, and (d) a daily record was kept of each subject's highest successful speed of rod rotation.

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cerebral hemispheres</th>
<th>Cerebellum</th>
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<tbody>
<tr>
<td></td>
<td>Coronal</td>
<td>Sagittal</td>
</tr>
<tr>
<td>Control</td>
<td>15.22 ± .48</td>
<td>14.74 ± .60</td>
</tr>
<tr>
<td>8–15X</td>
<td>14.94 ± .28</td>
<td>15.30 ± .46*</td>
</tr>
<tr>
<td>4–5X</td>
<td>15.19 ± .50</td>
<td>15.21 ± .39*</td>
</tr>
<tr>
<td>4–15X</td>
<td>15.36 ± .30</td>
<td>16.25 ± .44**</td>
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* = p < .05.
** = p < .01.

Note. Significance levels (Newman-Keuls) are with respect to controls.
highest successful speed of rotation on each of the rods, and (c) running time to reach the goal platform. Additional one-way analyses of variance were done to determine whether there were any X-ray treatment effects in (a) latency to leave the start platform on the trial immediately following a fall from the rod and (b) the percentage of trials in which the animals “tested” the speed of rod rotation with their forepaws before leaving the start platform.

The 4-15X treatment group with severe motor deficits was not included in any of these statistical analyses. Only 3 of the 11 animals in this group succeeded in negotiating the rough rod without hurdles at the lowest rotation speed, and an additional 4 animals failed to negotiate the rod at all, even while it was stationary (0 rpm). Their performance on the smooth rod without hurdles was even worse. Eight of the 11 animals failed to successfully negotiate the rod while it was stationary. A similar pattern emerged on the rough rod with low hurdles, the last rod on which these animals were tested.

Latencies. Statistical analysis of this measure revealed no significant X-ray treatment effects. Significant hurdle height, $F(3, 147) = 37.85, p < .001$, and rod surface, $F(1, 49) = 33.87, p < .001$, main effects were found. There were no significant Treatment × Hurdle Height or X Rod Surface interactions. Further analysis of the hurdle height

![Figure 7. Top rotation speed reached (A) and time to traverse the rods (B) in the four groups on rough rods with regular hurdles. (Single asterisk indicates that not all the animals in the group could perform the task; double asterisk indicates that none qualified on the task. Vertical lines above bar, standard error of the mean. Bars are displayed in order for control, 8-15X, 4-5X, and 4-15X groups.)](image-url)
effect indicated that with increasing rotating rod experience (starting with no hurdles and progressing to high hurdles) latencies became significantly shorter. Latencies were significantly longer for the smooth rods than for the rough rods.

Top rotation speeds. Statistical analysis revealed significant X-ray treatment, \( F(2, 49) = 4.10, p < .03 \), hurdle, \( F(3, 147) = 55.52, p < .001 \), and rod surface, \( F(1, 49) = 38.54, p < .001 \), effects (Figures 7A and 8A). The Treatment \( \times \) Hurdle Height and Treatment \( \times \) Surface interactions were not significant. Comparison of treatment means by a Newman-Keuls test indicated that the performance of the 4–5X group was significantly \( (p < .05) \) below that of the control and 8–15X groups but that the control and 8–15X groups were not significantly different. The hurdle height and rod surface main effects revealed that as hurdle height increased or as rod surface changed from rough to smooth, the task became more difficult and performance declined. The lack of significant Treatment \( \times \) Hurdle Height or Treatment \( \times \) Rod Surface interactions indicated that as the task became more difficult, it did so equally for all treatment groups.

Running times. Statistical analyses revealed significant treatment, \( F(2, 49) = 5.38, p < .01 \), hurdle height, \( F(3, 147) = 5.92, p < .002 \), and rod surface, \( F(1, 49) = 12.42, p < .002 \), main effects (Figures 7B and 8B). The Treatment \( \times \) Hurdle Height and \( \times \) Rod Surface interactions were not significant. Further analysis of the treatment means by a Newman-Keuls test indicated that the 4–5X group had significantly longer running times than the control group \( (p < .01) \) and the 8–15X group \( (p < .05) \). The 8–15X animals did not differ from controls on this measure.

Analysis of the hurdle height effect revealed that the running times on the rods with the high hurdles were longer \( (p < .01, \) Newman-Keuls) than those on rods with either low or medium hurdles. The running times on rods with no hurdles were not significantly different from those on rods with
Experiment 2: Rotating Rods With Irregular Hurdles

In Experiment 1, the 8-15X group was not handicapped in traversing rods with regularly spaced hurdles of uniform height. This experiment was designed to determine whether a deficit could be demonstrated in this group (in addition to an increased handicap in the 4-5X group) by increasing task difficulty.

Method

Subjects. Litters of inbred male Long-Evans hooded rats were reduced to six pups prior to exposure to X-irradiation. The X-ray schedules and procedures were identical to those employed in Experiment 1. The treatment groups consisted of controls (n = 15), 8-15X (n = 8), and 4-5X (n = 14). An additional four animals in the 8-15X group had to be discarded because they refused to run on the rod. All animals were experimentally naive at the beginning of this experiment.

Apparatus. The rotating rod apparatus was the same as in the previous experiment except the height and arrangement of the hurdles were modified. All rods except the initial training rod have smooth surfaces. Three different-height, circular hurdles were used: medium (25 mm), high (50 mm), and very high (62 mm). The hurdles were constructed in a manner that permitted the experimenter to secure them in any location on the rod so that hurdle height and spacing could be varied systematically. The first six rods (Patterns A, B, C; medium and high) in the testing sequence were designed to have hurdles with constant height and variable spacing. In the next three rods (Patterns D, E, F) the spacing between the hurdles was kept constant while the heights of the hurdles were varied. In the final three rods in the sequence (Patterns G, H, I) both hurdle height and spacing were varied randomly (Figure 3).

Procedure. The procedures used in this experiment were identical to those used previously, including all training and testing methods and the performance criteria. At the completion of the behavioral test, all the brains were measured, then prepared for histological examination, as described previously.

Results and Discussion

Anatomical. The effects of cerebellar X-irradiation with the 8-15X and 4-5X schedules on the dimensions of the cerebellum and cerebral hemispheres are summarized in Table 2. The alterations obtained in this experiment were similar to those of the previous study (Table 1).

Behavioral. Two-way analyses of variance were carried out on the following mea-
Table 2

Brain Measurements (in mm) of Young Adult Rats Tested in Experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Cerebral hemispheres</th>
<th>Cerebellum</th>
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<tbody>
<tr>
<td></td>
<td>Coronal</td>
<td>Sagittal</td>
</tr>
<tr>
<td>Control</td>
<td>15.41 ± .42</td>
<td>14.99 ± .27</td>
</tr>
<tr>
<td>8-15X</td>
<td>14.93 ± .41*</td>
<td>15.05 ± .40</td>
</tr>
<tr>
<td>4-5X</td>
<td>15.30 ± .24</td>
<td>15.09 ± .35</td>
</tr>
</tbody>
</table>

Note. Significance levels (Newman-Keuls) are with respect to controls.
* p < .05.
** p < .01.

Measures of performance: (a) latency to leave the start platform, (b) highest successful speed of rotation on each of the rods, (c) running time to reach the goal platform, and (d) percentage of trials in which the animals "tested" the speed of rod rotation with the forepaws. Latencies on the trial following a fall from the rod were analyzed by one-way analysis of variance.

Although the 4–5X group tended to be inferior and the 8–15X group superior to the controls in terms of top successful speed of rotation (Figure 9A), the differences were not significant, F(2,34) = 2.10. The statistical analyses indicated no significant differences between the treatment groups in latency to leave the start platform, F(2,34) = 3.08, running times, F(2,34) = .66 (Figure 9B), or in the latency to leave the start platform on the trial immediately following a fall from the rod, F(2,34) = .70.

These results indicate that the attempt to increase locomotor task difficulty by spacing the hurdles irregularly, by using hurdles of different heights, or by both failed to demonstrate handicaps in the experimental groups. Indeed, the inferiority of the 4–5X group obtained in Experiment 1 did not

Figure 9. Performance on smooth rods with irregular hurdles, as indicated. (Notations as in Figure 7. Bars are displayed in order for control, 8–15X, and 4–5X groups.)
reach a significant level in this experiment.

General Discussion of Experiments 1 and 2

The 4–15X animals, with a cerebellar cortex characterized by morphological disorganization and agenesis of all the postnatally forming interneurons, displayed severe motor deficits. Only a few animals could traverse the rough or smooth rods at the lowest rotation speeds, and many were unable to negotiate the full length of the rod for the food reward even on the stationary rod. The animals that were successful needed over twice as much time to traverse the rod as did the controls.

The 4–5X animals, in which the cerebellar cortex is disorganized but is not drastically reduced either in size or granule cell number, displayed mild locomotor deficits. With regularly spaced hurdles or hurdles of uniform height (Experiment 1), they could reach lower top rotation speeds, coupled with longer running times than could controls. But the magnitude of their handicap was small. Moreover, the deficits obtained were not dependent on task difficulty. Going from no hurdles to hurdles of increasing heights, which resulted in progressively lower top rotation speeds in the control rats (Figures 7A and 8A), did not increase the handicap of this experimental group. (Incidentally, running time was not affected by task difficulty in this experiment [Figures 7B and 8B] perhaps because the animals tended to run at an optimal speed to avoid being thrown off the rotating rod.) Further increase in task difficulty in Experiment 2, by spacing the hurdles irregularly, mixing hurdle heights, or both, did not increase the handicap of the 4–5X group; in fact, their deficit was not statistically significant.

The 8–15X animals, in which the cerebellar cortex appears histologically normal but the granule cell population is greatly reduced (~78%; Experiment 3), displayed no locomotor deficits in any of the tasks used. Indeed, in 9 of 12 tests in the two experiments, they were superior to the controls, but the difference was not significant. Accordingly, we conclude that drastic granule cell agenesis per se does not produce demonstrable motor deficits in the induced motor tasks employed. The apparent superiority of the 8–15X animals might have been due to their hyperactivity, which was suggested for this and the 12–15X group in pilot studies. In three succeeding studies we, therefore, examined the level of activity of experimental groups and control animals in an open field and in activity wheels. Because spontaneous motor activity varies greatly with age and because of an interest in the possibility of recovery of function after brain insult delivered during early infancy, three age groups (infant, young adult, and adult) were compared in several of the tests used. The results brought evidence that the younger 8–15X and 12–15X animals are hyperactive.

Experiment 3: Performance in the Open Field

Method

Subjects. Litters of inbred Long-Evans hooded rats, which had been culled to six males 24 hr after birth, were used. A total of 413 experimentally naive animals consisting of three age groups were tested (infants, 17–21 days; young adults, 70–74 days; adults, 180–184 days). Each age group contained five treatment groups (infants: controls, n = 27; 4–15X, n = 33; 8–15X, n = 24; 4–5X, n = 28; 4–15X, n = 28; 15X, n = 27; young adults: controls, n = 23; 4–15X, n = 31; 8–15X, n = 28; 4–5X, n = 33; 4–15X, n = 28; 15X, n = 25). Irradiation. The X-ray procedures for the 4–15X, 4–5X, and 8–15X groups are identical to those employed in the previous experiments. The added 12–15X group received 200R on Days 12 and 13 and 150R on Day 15.

Apparatus. A 54 × 54 × 28 cm open field was constructed from unpainted wood on three walls and clear Plexiglas on the front wall. The black Plexiglas floor was divided into twenty-five 10-cm squares. Illumination consisted of a 40-W bulb suspended 60 cm above the center square of the open field. The subjects were observed in a sound-attenuating cubicle from behind an 85 × 75 cm one-way mirror.

Procedure. Each subject was placed in the center of the open field for a 3-min test session on five successive days. The following responses were recorded over the 3-min test session: (a) number of squares crossed with all four paws, (b) rearing responses with support (i.e., leaning on one of the walls with either the forepaws or the back), (c) rearing responses without support, (d) falls on back or side while walking or attempting to rear, and (e) boluses. The open field was washed with a sponge
and dried with paper towels after each subject was tested.

After behavioral testing, all animals were anesthetized with sodium pentobarbital (75 mg/kg) and perfused intracardially with 10% buffered formalin. The brains were removed, placed in Bouin’s fixative for 24 hr, and then measured (length and width of both cerebrum and cerebellum) with a micrometer. After paraffin embedding, the brains were sectioned sagittally at 6 μm and stained with hematoxylin and eosin. For quantitative measurement of the area of the vermis, the area of the granular layer, and of Purkinje cell and granule cell counts, matched sections (approximate lateral coordinate, 950 μm) were evaluated from 10 randomly chosen animals in each group. A semiautomatic projector-computer system was used for this purpose, consisting of a Zeiss macro-microprojector, a mirror system, an electromagnetic cursor, a Summagraphics digitizer, and a Wang 2200 computer. The image of the cerebellum was projected at 44.25 magnification onto the surface of the digitizer. The boundaries of the cerebellar cortex and of the granular layer were traced twice with the cursor, and the information was fed into the computer and the areas were calculated. At higher magnification (457X) granule cells were counted in square grids (9.228 × 10^-4 sq. mm) in each of the 10 lobules of the vermis, and from these and the areal data the estimated total number of granule cells in each section was computed. All Purkinje cells with nucleoli were counted microscopically (X450).

**Anatomical Results**

**Brain measurements.** The cerebellar measures of control and experimental infants were below those of the older groups in both the coronal and the sagittal planes (Table 3). As in the previous experiments, the magnitude of reductions in the experimental groups was larger in the sagittal than the coronal plane. In the sagittal plane the rank order of reductions was the same at all ages (control, 12–15X, 4–5X, 8–15X, 4–15X). There was a comparable increase in the dimensions of the cerebrum from infancy to young adulthood, but there were no systematic treatment effects in the shielded hemispheres (some experimental groups were significantly below or above controls in a few measures; Table 3).

**Areal measurements.** As in the larger sample of cerebellar length measures, there was an X-ray treatment effect, F(4, 135) = 400.21, p < .001, an age effect, F(2, 135) = 116.01, p < .001, and a Treatment × Age interaction, F(8, 135) = 13.17, p < .001, in the histologically assessed area of the cerebellum in the sagittal plane (Figure 10A). In the control and 8–15X groups, total cerebellar area increased from infants to young adults (p < .01, Newman-Keuls) and again from young adults to adults (p < .01, New-
man-Keuls). In the 12–15X group, the areas of the young adults and adults were larger than those of the infants (p < .01, Newman-Keuls). In the 4–5X group, adults' areas were larger than infants' (p < .05, Newman-Keuls). Finally, in the 4–15X group, the area of the adults was larger than the area of either the infants or the young adults (p < .01, Newman-Keuls). In all age groups total cerebellar area in each of the X-irradiated groups was reduced significantly (p < .01, Newman-Keuls) when compared with that of controls. In addition, with only one exception, each of the X-irradiated groups was significantly different (p < .05, Newman-Keuls) from all other irradiated groups within an age group (the difference between the 12–15X and the 4–5X infant animals was not significant), and, with the exception noted, the rank order of sagittal areaal reduction was the same (control, 12–15X, 4–5X, 8–15X, 4–15X) as on the larger sample of gross measurements.

A significant X-ray treatment effect, $F(4, 135) = 407.08$, p < .001, age effect, $F(2, 135) = 47.80$, p < .001, and Treatment X Age interaction, $F(8, 135) = 9.07$, p < .001, were found in the area of the granular layer. The area of the granular layer (Figure 10B) was significantly larger in adults than in infants or young adults in the control, 8–15X, and 4–15X groups (p < .01, Newman-Keuls). In the 12–15X and 8–15X groups, the granular area was smaller in infants than in either young adults or adults (p < .05, Newman-Keuls). The only treatment that showed no age effect on this measure was the 4–5X group. At all ages the area of the granular layer...
layer of the irradiated animals was reduced significantly from that of controls \( (p < .05, \text{Newman-Keuls}) \). Also, most irradiated groups were significantly different \( (p < .05, \text{Newman-Keuls}) \) from each other except the 4–5X group, which did not differ from the 12–15X infants and from the 8–15X young adults and adults.

Cell counts. Because granule cells are small (4–5 \( \mu m \) in diameter), estimates of their concentration in a given section are affected by the exact thickness of the section and the intensity of cell staining (for instance, in a thinner section or a lightly stained section, peripherally transected cells may not be identified and counted). In the material utilized, which was nominally cut at the same thickness (6 \( \mu m \)) and stained in a standardized manner, the necessary uniformity has not been achieved. This is reflected in the apparent increase in estimated number of granule cells as a function of age (Figure 11B), which cannot be true since granule cell production comes to an end in the rat at the age of 21 days (Altman, 1969, 1972c), before the infants were killed. With these considerations in mind, the data in Figure 11 are presented without statistics to convey two important facts: (a) that the reduction in cerebellar gross measures in the treatment groups cannot be attributed to a reduction in the number of Purkinje cells and (b) that the rank order of morphological effects obtained in the larger sample of unprocessed cerebella (Table 3) and areal measurements (Figure 10) is paralleled by

\[<Figure 11.>\text{Counts of Purkinje cells (A) and of granule cells (B) in the cerebellar cortex. (Bars are displayed in order for control, 12–15X, 8–15X, 4–5X, and 4–15X groups.)}\]
the changes in estimated granule cell populations (Figure 11B).

The number of Purkinje cells (Figure 11A) was comparable at all ages for the treatment groups except for the 4–15X animals, which tended to have a higher concentration of Purkinje cells than the others. This was probably due to the tighter packing of these cells in the greatly reduced volume of the cerebellum (Figure 6). These results indicate, in agreement with previous results (Altman & Anderson, 1971), that the X-irradiation doses used in these studies do not reduce the Purkinje cell population. The agenesis of granule cells resulting from the different treatments (Figure 11B) was of the same order at all ages, and the pooled reduction was as follows: 12–15X, 40%; 4–5X, 62%; 8–15X, 78%; and 4–15X, 96%.

Behavioral Results

Infants. Analysis of variance revealed significant X-ray treatment effects on the number of squares crossed, $F(4, 137) = 17.86$, $p < .001$; rears with support, $F(4, 137) = 13.17$, $p < .001$; rears without support, $F(4, 137) = 9.19$, $p < .001$; falls, $F(4, 137) = 14.81$, $p < .001$; and defecation scores $F(4, 137) = 5.62$, $p < .001$. The 4–15X treatment group

![Figure 12. Falls (A) and ambulatory scores (B) of the five treatment groups at three age levels in the open field. (Bars are displayed in order for control, 12–15X, 8–15X, 4–5X, and 4–15X groups.)](image-url)
fell more frequently in the open field (Figure 12A; \( p < .001 \), Newman-Keuls); crossed fewer squares (Figure 12B; \( p < .001 \), Newman-Keuls); reared with support less often (Figure 13A; \( p < .001 \), Newman-Keuls); and defecated more frequently (\( p < .05 \), Newman-Keuls) than all other treatment groups. The 4–15X group also reared without support less frequently than the control, 12–15X, and 8–15X groups (Figure 13B; \( p < .05 \), Newman-Keuls). The 4–5X group crossed fewer squares and reared both with and without support less often than either the control or the 12–15X group (Figures 12 and 13; \( p < .05 \), Newman-Keuls). No other significant treatment differences were found in the infants.

**Young adults.** Significant X-ray treatment effects were found on the following measures: squares crossed, \( F(4, 120) = 19.75, p < .001 \); rears with support, \( F(4, 120) = 31.18, p < .001 \); rears without support, \( F(4, 120) = 36.36, p < .001 \); falls, \( F(4, 120) = 29.75, p < .001 \); and defecation scores, \( F(4, 120) = 3.46, p < .02 \). The 4–15X group crossed fewer squares (Figure 12B; \( p < .001 \), Newman-Keuls) than the control, 12–15X, and 8–15X groups, reared both with and without support less often, and fell more frequently than all other treatment groups (Figures 12 and 13; \( p < .001 \), Newman-Keuls). The 4–5X group crossed fewer squares than control, 12–15X, and 8–15X groups (Figure 12B; \( p < .01 \), Newman-Keuls), reared with support less often than the 12–15X group (Figure 13A; \( p < .01 \), Newman-Keuls), and reared without support less frequently than the control, 12–15X, and 8–15X groups (Figure 13B; \( p < .01 \), Newman-Keuls). The 8–15X rats reared without support less often than the controls (Figure 13B; \( p < .001 \), Newman-Keuls). The 12–15X group crossed more squares and reared with support more frequently than

![Figure 13. Rears with support (A) or without support (B) in the open field. (Bars are displayed in order for control, 12–15X, 8–15X, 4–5X, and 4–15X groups.)](image-url)
control animals (Figures 12 and 13; \( p < .05 \), Newman-Keuls). However, the 12–15X group reared without support less often than controls (Figure 13B; \( p < .001 \), Newman-Keuls). Finally, only the 12–15X and control groups were different in their defecation scores (\( p < .01 \), Newman-Keuls).

Adults. Significant X-ray treatment effects were found on the following measures: squares crossed, \( F(4, 141) = 5.46, p < .001 \); rears with support, \( F(4, 141) = 18.73, p < .001 \); rears without support, \( F(4, 141) = 13.30, p < .001 \); falls, \( F(4, 141) = 59.29, p < .001 \); and defecation scores, \( F(4, 141) = 2.58, p < .05 \). The 4–15X group crossed fewer squares than either the 12–15X or the 8–15X group (Figure 12B; \( p < .05 \), Newman-Keuls), reared less frequently both with and without support than control, 12–15X, and 8–15X groups (Figure 13; \( p < .001 \), Newman-Keuls), and fell more frequently than all other groups (Figure 12A; \( p < .001 \), Newman-Keuls). The 4–5X group crossed fewer squares and reared with support less frequently than the 12–15X group (Figures 12 and 13; \( p < .01 \), Newman-Keuls) but did not differ from controls on these two measures. However, the 4–5X group did rear without support less frequently than controls (Figure 13B; \( p < .01 \), Newman-Keuls) and also defecated more frequently than controls (\( p < .05 \), Newman-Keuls). In contrast, both the 12–15X and 8–15X groups reared with support more frequently than controls (Figure 13A; \( p < .01 \), Newman-Keuls).

Discussion

The four major responses examined may be conceived of as measuring motor abnormality (falls), activity level changes (squares traversed; rearing with support), and the interaction of activity level with a difficult motor performance (rearing without support). In these terms, this study confirmed the results of Experiments 1 and 2 that only the 4–15X group suffered gross ambulatory deficits. Instead of abating with age, falls became more frequent in young adults and adults of this group. This group was characterized by hypoactivity, with fewer squares traversed in infants and young adults, and a low incidence of rearing with or without support at all ages. This hypoactivity may be related to the profound motor deficits in this group. The only indication of partial recovery was seen in ambulation level in adults, reflecting perhaps the most general of motor performances observed.

The 4–5X group shares with the 4–15X group the structural disorganization of the cerebellar cortex but differs from it in the extent of cell reduction. Falls were rare, but there was hypoactivity with respect to either controls or the late-irradiated groups at most ages in square crossings and in rearing with support. The hypoactivity of this early-irradiated group may reflect the presence of mild motor deficits, which were seen in some induced motor tasks used in Experiments 1 and 2. It remains to be determined whether the early irradiates would be normal or even hyperactive in tasks in which they are not handicapped (swimming).

In contrast to the hypoactivity of the early irradiates, hyperactivity was indicated for the 8–15X group, which in most instances was not significant with respect to the controls but was significant at all ages when compared with the 4–15X group. The hyperactivity was most pronounced in the 12–15X rats in terms of squares crossed by young adults and rearing with support both by young adults and adults. The only performance in which there was hypoactivity in the 12–15X group was in rearing without support. It is suggested that the inclination toward hyperactivity was counteracted in this group (and in the 8–15X animals) by deficits in displaying this difficult and late maturing (Altman & Sudarshan, 1975) motor act.

Experiment 4:

Habituation in the Open Field

The demonstration of hyperactivity in the open field in the late-irradiated groups at some ages led us to reexamine these results with a design that permitted an answer to the question whether the heightened activity level was due to reduced intra- and/or intersession habituation.

Method

Subjects. Litters of inbred Long-Evans hooded rats, which had been culled to six males 24 hr after birth,
were used. A total of 190 experimentally naive animals in three age groups were tested (infants, 19–21 days; young adults, 70–72 days; adults, 180–182 days). Each age group contained three treatment groups (infants: controls, n = 22; 12–15X, n = 25; 8–15X, n = 23; young adults: controls, n = 19; 12–15X, n = 21; 8–15X, n = 19; adults: controls, n = 18; 12–15X, n = 19; 8–15X, n = 23). The irradiation procedure and testing apparatus were identical to those used in the previous experiment.

Procedure. Two modifications were made in the open-field testing procedures. First, all animals were tested in three consecutive daily test sessions for 5 min per session. Second, all responses (except bolus counts) were cumulated over 1-min intervals. The infants used in this study were subsequently utilized in a study of spontaneous alternation (Experiment 7). These were killed as young adults. The animals in the other two age groups were perfused immediately after completion of testing in the open field; the brains were measured and embedded in paraffin as previously described.

Results

Anatomical. Cerebellar sagittal measures in the two groups killed as young adults and in the adults were comparable in magnitude (Table 4) to those obtained in the corresponding late-irradiates in the previous experiment (Table 3). There was variability (increases or decreases with respect to controls) in the dimensions of the shielded cerebral hemispheres, but no systematic treatment effects were obtained.

Behavioral. Infants. Analyses of variance revealed significant X-ray treatment effects on the following measures: squares crossed, $F(2, 65) = 5.68$, $p < .01$; rears with support, $F(2, 65) = 8.79$, $p < .001$; and rears without support, $F(2, 65) = 3.70$, $p < .03$. Both the 12–15X and 8–15X groups were more active than controls as measured by the number of squares crossed (Figure 14A; $p < .01$, Newman-Keuls) and rears with support ($p < .01$, Newman-Keuls). Neither of the two X-ray groups differed from controls in the rearing without support measure, but the 12–15X animals did display this response more frequently than the 8–15X group ($p < .05$, Newman-Keuls).

There was marked intrasession habituation, $F(4, 260) = 73.70$, $p < .001$, beginning with Minute 1 in the number of squares crossed (Figure 14A) and Minute 2 in the number of rears with support. The number of rears without support peaked in Minute 3 and declined thereafter. Intersession habituation was found only in the number of squares crossed, $F(2, 130) = 12.00$, $p < .001$. Since no significant Treatment X Minute, $F(8, 260) = .97$, or Treatment X Day, $F(4, 130) = 1.44$, interactions were found, it is concluded that both intra- and intersession habituation rates are equal in all treatment groups.

Young adults. Significant X-ray treatment effects were found on the following measures: squares crossed, $F(2, 57) = 4.03$, $p < .03$; rears with support, $F(2, 57) = 7.39$, $p < .002$; and rears without support, $F(2, 57)$

<table>
<thead>
<tr>
<th>Table 4</th>
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<tr>
<td>Brain Measurements (in mm) of Rats Tested in Experiment 4</td>
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<tr>
<td>Group</td>
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<td>Infants</td>
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<td>Young adults</td>
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<td>Control</td>
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<td>12–15X</td>
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<td>8–15X</td>
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</tbody>
</table>

Note. Infants were killed as young adults. Significance levels (Newman-Keuls) are with respect to controls.
* $p < .05$.
** $p < .01$. 

$F(2, 57) = 4.03$, $p < .03$; rears with support, $F(2, 57) = 7.39$, $p < .002$; and rears without support, $F(2, 57)$
Figure 14. Intrasession and intersession habituation in ambulation in the open field in three treatment groups at three ages.
= 6.40, \( p < .002 \). The 12–15X group was more active than controls in terms of both the number of squares crossed (Figure 14B; \( p < .05 \), Newman-Keuls) and rears with support (\( p < .01 \), Newman-Keuls). When compared with controls, a deficit in the number of rears without support was found in the 8–15X group (\( p < .01 \), Newman-Keuls) but not in the 12–15X group. The number of rears both with and without support was significantly lower in the 8–15X group than in the 12–15X group (\( p < .05 \), Newman-Keuls).

There was marked intrasession habituation beginning with Minute 1, in both the squares crossed (Figure 14B), \( F(4, 228) = 252.91, \ p < .001 \), and rears with support measures, \( F(4, 228) = 116.83, \ p < .001 \). Significant intersession habituation was found in all three measures: squares crossed, \( F(2, 114) = 13.80, \ p < .001 \); rears with support, \( F(2, 114) = 30.64, \ p < .001 \); rears without support, \( F(2, 114) = 20.05, \ p < .001 \). There were no significant Treatment \( \times \) Minute interactions, and the only significant Treatment \( \times \) Day interaction, \( F(4, 114) = 3.28, \ p < .01 \), occurred in the number of rears without support. These results indicate that with the one exception noted, intra- and intersession habituation rates were not different among the treatment groups. Further analysis revealed that this one exception was due to a more rapid habituation in the control group from Day 1 to Day 2 in the number of rears without support.

Adults. No X-ray treatment effects were found in the number of squares crossed (Figure 14C), \( F(2, 59) = 1.94 \), or the number of rears with support, \( F(2, 59) = .47 \). However, a significant X-ray treatment effect was found in the number of rears without support, \( F(2, 59) = 8.17, \ p < .002 \), and the 8–15X group was deficient on this measure when compared with either the control (\( p < .001 \), Newman-Keuls) or the 12–15X group (\( p < .05 \), Newman-Keuls). Intrasession habituation was found in both the square crossing, \( F(4, 236) = 180.58, \ p < .001 \), and rearing with support, \( F(4, 236) = 74.19, \ p < .001 \), measures with no significant Treatment \( \times \) Minute interactions. Rears without support peaked at Minute 3 and declined thereafter. A significant Treatment \( \times \) Minute interaction, \( F(8, 236) = 4.86, \ p < .001 \), was found in this measure. The 8–15X group peaked in Minute 2, and the controls and 12–15X animals peaked in Minute 3. Intersession habituation occurred in both rearing with support, \( F(2, 118) = 5.15, \ p < .01 \), and without support, \( F(2, 118) = 15.03, \ p < .001 \), but not in the number of squares crossed, \( F(2, 118) = 1.29 \). No significant Treatment \( \times \) Day interactions were found in either measure of rearing.

Discussion

The results of this experiment replicate and extend the main findings of Experiment 3. The 12–15X and 8–15X infants and the 12–15X young adults were again found to be hyperactive with respect to the controls. These effects again disappeared by 180 days of age. Also, a deficit in rearing without support was found in the 8–15X young adults which showed no recovery of function by 180 days of age.

When the activity data were analyzed for habituation effects, the results indicated no intra- or intersession habituation deficits in either the 12–15X or the 8–15X treatment group. We conclude that the hyperactivity found in these two groups in both experiments cannot be attributed to a failure to habituate to the test situation.

Experiment 5: Running in Activity Wheels

This experiment was designed to investigate with another procedure the differential hypo- and hyperactivity produced by different schedules of cerebellar X-irradiation in the open field.

Method

Subjects. Litters of inbred Long-Evans hooded rats, which had been culled to six males within 24 hr after birth, were used. A total of 189 experimentally naive animals in two age groups were tested (young adults: 60–90 days, \( n = 121 \); adults: 180–210 days, \( n = 50 \)). The young adults consisted of five treatment groups (control, \( n = 20 \); 12–15X, \( n = 25 \); 8–15X, \( n = 33 \); 4–5X, \( n = 22 \); 4–15X, \( n = 21 \)), the adults of four treatment groups (control, \( n = 17 \); 12–15X, \( n = 16 \); 8–15X, \( n = 17 \); 4–15X, \( n = 18 \)).

Apparatus. Wahmann activity wheels (Model
LC-34), which had been modified with new axles and ball bearings for more reliable operation, were used.

**Procedure.** Each subject was placed in an activity wheel at 60 days or 180 days of age and remained in that wheel for 30 days. Ad lib water and food in the form of ground Wayne Lab Blox plus one Lab Blox pellet per day were made available to the animals. The wheels were housed in an air-conditioned room with a 12:12 hr light/dark cycle. Data were collected, and the wheels were checked for proper operation once daily shortly after the lights came on in the morning (7:00 a.m.). After removal from the wheels all animals were perfused; the brains were measured and then embedded in paraffin as described previously.

**Results**

**Anatomical.** Cerebellar sagittal measures (Table 5) in all the treatment groups at the two ages studied were similar to those obtained in Experiment 1 (Table 1) and Experiment 2 (Table 2). As in the previous experiments, there were no systematic treatment effects in the shielded cerebral hemispheres.

**Behavioral.** Young adults. An analysis of variance revealed a significant X-ray treatment effect (Figure 15A), $F(4, 116) = 15.91, p < .001$, day effect, $F(29, 3364) = 40.36, p < .001$, and Treatment X Day interaction, $F(116, 3364) = 3.98, p < .001$. In comparison with the controls, the 12-15X and 8-15X animals were hyperactive ($p < .001$, Newman-Keuls), the 4-15X group was hypoactive ($p < .001$, Newman-Keuls), and the 4-5X animals did not differ significantly from controls. However, the 4-5X group appeared to contain two populations, one of which was hyperactive (i.e., >2,000 revolutions in a single day; $n = 10$) and one of which was not ($n = 12$). Comparisons of the gross cerebellar measurements of these two populations revealed differences in cerebellar size which approached but did not reach statistical significance, $t(20) = 1.66, p < .10$. A more accurate quantitative analysis of the cerebellar anatomy in these two populations may reveal differences that were masked by the error inherent in the gross measurements.

Adults. No significant differences were found between any of the treatment groups (Figure 15B), $F(3, 64) = 1.87$.

**Discussion**

The results of this experiment add to and reinforce the earlier open-field observations that the cerebellar late-irradiates without morphological disorganization (12-15X and 8-15X) are hyperactive whereas the early-irradiates with disorganized cerebella (4-15X) are hypoactive. As in the open-field experiment, these activity changes tend to disappear by the time the animals reach 180 days of age, and general activity level diminishes in the controls.

<table>
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<th>Group</th>
<th>Cerebellum Coronal</th>
<th>Cerebellum Sagittal</th>
<th>Cerebral hemispheres Coronal</th>
<th>Cerebral hemispheres Sagittal</th>
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<td>15.60 ± .33*</td>
<td>11.41 ± .32**</td>
<td>3.21 ± .24**</td>
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<td>4-5X</td>
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<td>15.70 ± .44**</td>
<td>10.72 ± .51**</td>
<td>2.56 ± .23**</td>
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</table>

Note: Significance levels (Newman-Keuls) are with respect to controls.

* $p < .05$.

** $p < .01$. 

Table 5

Brain Measurements (in mm) of Rats Tested in Experiment 5
General Discussion of Experiments 3–5

The rats with cerebellar disorganization and agenesis of all the postnatally forming neurons (4–15X) had low rearing scores in the open field, and low ambulation scores both in the open field and in the activity wheels. This hypoactivity was undoubtedly due to their motor handicap, which was revealed in Experiment 1 by their inability to traverse rotating rods and in Experiment 3 by the high incidence of falls. The falls and reduced rearing persisted into adulthood. The rats with cerebellar disorganization but less severe cellular agenesis (4–5X) tended, likewise, to be hypoactive in terms of crossings and rearing without support in the open field. However, the activity-wheel study suggested that this group is composed of two subpopulations, one hypoactive and the other hyperactive. An explanation for this may lie in the variability (unpublished observations, Altman, 1968) in the extent of recovery of the external germinal layer that occurs after a few X-ray exposures in the neonate, the factor that determines the degree of cellular agenesis in this preparation.

Although the rotating rod experiment failed to reveal any motor handicap in the 8–15X group, the present study showed a subtle deficit in this group and in the 12–15X animals in the form of lower incidence of rearing without support. Presumably, balancing on the hindlimbs is a complex task which is affected by minimal cerebellar abnormality. The more surprising finding of the study was the hyperactivity of the late-irradiated groups. This appeared initially in the increased ambulation scores of infants and young adults in the open field. We obtained evidence that the hyperactivity of these two groups was not attributable to altered intra- or intersession habituation in the open field. The genuine hyperactivity of the 8–15X and 12–15X animals was further shown by the consistently higher running levels in the activity wheels over a 30-day
period. What is important is that the hyperactivity of these experimental rats was no longer evident in adulthood when all animals had low running scores.

These experiments suggest that the cerebellar cortex is involved in at least two functions, the coordination of movements and the control of response emission. Moreover, these two functions may be hierarchically organized. Gross motor disturbance obtains only if X-irradiation is begun early and there is interference with input to the soma of the Purkinje cell (basket cells) and its lower dendritic domain. If radiation prevents the formation of parallel fibers through the middle (8–15X) or in the uppermost (12–15X) portion of the molecular layer, the major effect is hyperactivity. Presumably, the released cerebellar response inhibition is mediated by the later forming parallel fibers that synapse directly with the distal portion of the Purkinje cell dendritic system. Such an inhibitory function of the cerebellar cortex is reconcilable with the known physiological properties of Purkinje cells which, when activated, inhibit the cerebellar deep neurons (Eccles et al., 1967). An implication of this hypothesis is that all forms of interference with cerebellar organization should produce hyperactivity. The evident hypoactivity of the 4–15X animals, and to a lesser extent of the 4–5X animals, would then have to be attributed to a masking by their motor deficits. The prediction follows that if these animals were tested under conditions in which they are not handicapped (e.g., swimming; Experiment 6), they would be likewise hyperactive.

The hyperactivity of the cerebellar-irradiated animals in which there is no morphological disorganization but substantial granule cell agenesis is reminiscent of the hyperactivity of the similarly affected hippocampal-irradiated rats (Bayer, Brunner, Hine, & Altman, 1973). It was suggested in a theoretical review (Altman, Brunner, & Bayer, 1973) that the late developing hippocampal dentate gyrus (which is essentially a granule cell system) has a developmental function by “braking” response emission in “exuberant” infants and young adults. Such a function might also be attributed to the late developing granule cell system of the cerebellum insofar as the hyperactivity of the irradiates is evident only in infants and young adults.

Experiment 6:
Learning in a Multiple-Unit Water Maze

The preceding experiments indicated that X-ray-produced interference with the development of the upper parts of the cerebellar molecular layer has only minimal effects on muscular coordination, and none that involves locomotion, but that it seriously affects the level of response emission in the open field or an activity wheel. The possibility that the cerebellar cortex is hierarchically organized and its later forming components are involved in the control of molar action strategies rather than the execution of movements was explored by testing the four groups of X-irradiated animals (12–15X, 8–15X, 4–5X, and 4–15X) in a series of maze learning tasks. A previous study (Altman & Bulut, 1976; Bulut, 1976) showed that cerebellar X-irradiation affects the performance of infant and young adult rats in a T-maze. In this study we used a multiple-unit water maze and examined the animals’ performance on tasks involving repeated turns, single alternation, and double alternation.

Method

Subjects. Litters of inbred Long-Evans hooded rats, which had been culled to six males within 24 hr of birth, were used. Eighty-four experimentally naive animals consisting of five treatment groups were tested (control, n = 17; 12–15X, n = 20; 8–15X, n = 19; 4–5X, n = 17; 4–15X, n = 11). All animals were 60 days old at the start of the experiment.

Apparatus. The swimming maze (Figure 16) was contained in a large galvanized tank (240 × 140 × 46 cm) which was painted gray. The changeable maze partitions were constructed from Plexiglas boxes (12 × 12 × 43 cm) which were open at both ends and fitted over evenly spaced Plexiglas pedestals on the bottom of the tank. When clipped together along the top edges, the boxes formed solid, gap-free walls with alleys of 12-cm width between them. Maze patterns could be changed by removing the clips, moving the boxes, and then reinstalling the clips. A ramp located in the goal box was constructed from galvanized hardware cloth and permitted the animal to escape from the cold water to a dry, solid surface. The water in the tank was changed daily and kept at a depth of 25 cm during testing. (Water temperature was gradually reduced by .5 °C steps every fourth day during testing, from 19 °C to 15

period. What is important is that the hyperactivity of these experimental rats was no longer evident in adulthood when all animals had low running scores.

These experiments suggest that the cerebellar cortex is involved in at least two functions, the coordination of movements and the control of response emission. Moreover, these two functions may be hierarchically organized. Gross motor disturbance obtains only if X-irradiation is begun early and there is interference with input to the soma of the Purkinje cell (basket cells) and its lower dendritic domain. If radiation prevents the formation of parallel fibers through the middle (8–15X) or in the uppermost (12–15X) portion of the molecular layer, the major effect is hyperactivity. Presumably, the released cerebellar response inhibition is mediated by the later forming parallel fibers that synapse directly with the distal portion of the Purkinje cell dendritic system. Such an inhibitory function of the cerebellar cortex is reconcilable with the known physiological properties of Purkinje cells which, when activated, inhibit the cerebellar deep neurons (Eccles et al., 1967). An implication of this hypothesis is that all forms of interference with cerebellar organization should produce hyperactivity. The evident hypoactivity of the 4–15X animals, and to a lesser extent of the 4–5X animals, would then have to be attributed to a masking by their motor deficits. The prediction follows that if these animals were tested under conditions in which they are not handicapped (e.g., swimming; Experiment 6), they would be likewise hyperactive.

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Experiment 6:
Learning in a Multiple-Unit Water Maze

The preceding experiments indicated that X-ray-produced interference with the development of the upper parts of the cerebellar molecular layer has only minimal effects on muscular coordination, and none that involves locomotion, but that it seriously affects the level of response emission in the open field or an activity wheel. The possibility that the cerebellar cortex is hierarchically organized and its later forming components are involved in the control of molar action strategies rather than the execution of movements was explored by testing the four groups of X-irradiated animals (12–15X, 8–15X, 4–5X, and 4–15X) in a series of maze learning tasks. A previous study (Altman & Bulut, 1976; Bulut, 1976) showed that cerebellar X-irradiation affects the performance of infant and young adult rats in a T-maze. In this study we used a multiple-unit water maze and examined the animals’ performance on tasks involving repeated turns, single alternation, and double alternation.

Method

Subjects. Litters of inbred Long-Evans hooded rats, which had been culled to six males within 24 hr of birth, were used. Eighty-four experimentally naive animals consisting of five treatment groups were tested (control, n = 17; 12–15X, n = 20; 8–15X, n = 19; 4–5X, n = 17; 4–15X, n = 11). All animals were 60 days old at the start of the experiment.

Apparatus. The swimming maze (Figure 16) was contained in a large galvanized tank (240 × 140 × 46 cm) which was painted gray. The changeable maze partitions were constructed from Plexiglas boxes (12 × 12 × 43 cm) which were open at both ends and fitted over evenly spaced Plexiglas pedestals on the bottom of the tank. When clipped together along the top edges, the boxes formed solid, gap-free walls with alleys of 12-cm width between them. Maze patterns could be changed by removing the clips, moving the boxes, and then reinstalling the clips. A ramp located in the goal box was constructed from galvanized hardware cloth and permitted the animal to escape from the cold water to a dry, solid surface. The water in the tank was changed daily and kept at a depth of 25 cm during testing. (Water temperature was gradually reduced by .5 °C steps every fourth day during testing, from 19 °C to 15
Table 6  
Brain Measurements (in mm) of Young Adult Rats Tested in Experiment 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Cerebral hemispheres Coronal</th>
<th>Cerebral hemispheres Sagittal</th>
<th>Cerebellum Coronal</th>
<th>Cerebellum Sagittal</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>14.80 ± .31</td>
<td>14.15 ± .46</td>
<td>11.60 ± .29</td>
<td>5.38 ± 18</td>
</tr>
<tr>
<td>12-15X</td>
<td>15.44 ± .22**</td>
<td>14.92 ± .29*</td>
<td>11.34 ± .26*</td>
<td>4.32 ± .25**</td>
</tr>
<tr>
<td>8-15X</td>
<td>15.36 ± .29**</td>
<td>15.19 ± .38**</td>
<td>10.56 ± .26**</td>
<td>3.30 ± .16**</td>
</tr>
<tr>
<td>4-5X</td>
<td>15.07 ± .40*</td>
<td>15.18 ± .37**</td>
<td>11.49 ± .34</td>
<td>3.67 ± .29**</td>
</tr>
<tr>
<td>4-15X</td>
<td>14.41 ± .50*</td>
<td>15.18 ± .39**</td>
<td>9.99 ± .31**</td>
<td>2.25 ± .22**</td>
</tr>
</tbody>
</table>

Note. Significance levels (Newman-Keuls) are with respect to controls.  
* p < .05.  
** p < .01.

°C.) Figure 16 illustrates the training alleys and maze patterns used.  

Procedure. For the entire duration of the experiment, all animals had oxytetracycline in their drinking water to reduce the possibility of respiratory infection.

In all alley and maze tests, subjects were given five trials per day and were run in squads of five or six animals with an intertrial interval of about 5 min. During the intertrial interval each subject was towel dried and then placed in a holding cage. No animal was permitted to swim for more than 180 sec on a single trial in any of the alley or maze problems. Latency to leave the start area and swimming time to the goal platform were recorded for each trial.

Initial training began in Alley 1, with the goal at G1. On the second day the goal was moved to G2 and then on the third day to G3. Training criterion for Alley 1 was a mean swimming time of 15 sec or less for the five trials in a single session.

Subjects were then tested in Alley 2 for the two five-trial sessions to determine whether they had a position preference and, if so, whether it was consistent over the 2 days. Alley 3 was an extension of position preference testing with the goal at G1, G2, and G3 on Days 1, 2, and 3, respectively. At the end of testing in Alley 3, all animals were divided into those that had a right or left turning preference (defined as more than 66% of the choices in a given direction) and those that showed no consistent preference. Each animal was then assigned to a position-habit test opposite the preference observed in Alley 3 (Mazes 4A or 4B). Those subjects that showed no preference in Alley 3 were assigned randomly to Mazes 4A and 4B in equal numbers within each treatment group wherever possible.

Each position-habit test consisted of learning to make three successive turns to the right (4A) or left (4B). Learning criterion was four out of five errorless trials in one session. Each animal was permitted up to 25 trials to reach criterion. All animals except four achieved the criterion in 25 trials or less. In addition to latency and swimming-time measures, the number and location of blind alley entries were recorded on each trial in this maze and all subsequent mazes. Upon reaching the acquisition criterion, subjects were then tested in a position-habit reversal problem (Mazes 5A and 5B) to the same criterion of four out of five errorless trials. No cutoff was employed in this or any subsequent maze tests. The four subjects that did not reach the acquisition criterion in 25 trials were not tested on the reversal problem but were tested on single and double alternation.

Pilot studies indicated that the single alternation problem (Maze 6) had to be run in stages. On Day 1,
the goal was located at $G_1$ and each subject was given five trials. On Day 2, subjects were given one warm-up trial with the goal at $G_1$ and then five trials with the goal at $G_2$. A similar warm-up procedure was used on Day 3, with the goal at $G_2$ and $G_3$, respectively. Learning criterion was once again four out of five errorless trials in a single session, with no cutoff employed.

The double alternation test (Maze 7) was also run using a shaping procedure. On Day 1, the goal was at $G_1$, and on Day 2 subjects were given one warm-up trial with the goal at $G_1$ followed by five trials with the goal at $G_2$. In all subsequent test sessions the goal remained at $G_2$. Learning criterion was again four out of five errorless trials, with no cutoff.

Following completion of the double alternation test, all subjects were perfused with 10% formalin; brains were removed, measured, and embedded in paraffin for later histological examination.

**Results**

**Anatomical.** Sagittal measures of the cerebellum in the five groups of young adult rats utilized in this experiment (Table 6) were comparable with those obtained in the same age group in the previous experiments.

As in Experiments 1–5, reductions in the

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**Figure 17.** Swimming times (with standard errors) in the shaping alleys (A) and test mazes (B). (Bars are displayed in order for control, 12–15X, 8–15X, 4–5X, and 4–15X groups.)
coronal plane were of smaller magnitude, and changes in the shielded cerebral hemispheres were not systematic, presumably because of variable expansion of the cortex into space vacated in the skull by the reduced cerebellum.

Preference in the shaping alleys. The control and irradiated animals exhibited a strong tendency to turn consistently to the right or left in both shaping Alley 2 (83%) and shaping Alley 3 (78%) in their attempt to escape from the cold water. There were no significant differences between the treatment groups in either alley.

Swimming times. Analyses of variance of the swimming times in the training alleys and test mazes (Figure 17) showed that there were no significant differences between the treatment groups. These results are important because they demonstrate that the

![Maze Learning Graph](image_url)

**Figure 18.** Number of trials (A), and errors (B) to criterion, and latency in the start area (C) on four tasks. (Bars are displayed in order for control, 12–15X, 8–15X, 4–5X, and 4–15X groups.)
irradiated animals (including the 4–15X rats) can swim as efficiently as controls.

**Learning measures.** No significant differences between the treatment groups were found in either position habit acquisition or habit reversal in trials to criterion (Figure 18A) or errors (Figure 18B). In the single alternation test, X-ray treatment effects were found in both trials to criterion, $F(4, 83) = 13.31, p < .001$, and errors to criterion, $F(4, 83) = 26.39, p < .001$. The only animals that were found to have a deficit when compared with controls were the 4–15X group ($p < .001$, Newman-Keuls). In the double alternation test, X-ray treatment effects were again found in both trials to criterion, $F(4, 82) = 17.91, p < .001$, and errors to criterion, $F(4, 82) = 10.92, p < .001$. Further analysis revealed that both the 12–15X and 4–5X groups were deficient as measured by both trials to criterion (Figure 18A; $p < .001$, Newman-Keuls) and errors to criterion (Figure 18B; $p < .001$, Newman-Keuls).

**Latencies.** Analyses of the latencies to leave the start box revealed two general trends. First, the 4–15X group had consistently shorter latencies than the other treatment groups (Figure 18C). This was presumably due to the fact that these animals were incapable of using their hindlimbs to support themselves against the walls of the start box and in attempts to climb out of the water instead of swimming. Second, there was a trend toward longer than normal latencies in the 4–5X group, but only in the position habit reversal problem was this trend significant, $F(4, 77) = 5.18, p < .001$.

**Discussion**

In agreement with the classic observation of Luciani (cited in Dow & Moruzzi, 1958) that cerebellar damage that produces severe locomotor symptoms in dogs does not affect their swimming ability, the present study showed that cerebellar irradiation does not affect swimming speed even in the 4–15X group which, as previously shown (Experiment 1), cannot traverse a rotating rod for a food reward. It is usually assumed that the cerebellum plays an essential role not in the regulation of motor rhythmicity but in the maintenance of postural balance during movement, which in the buoyant swimming animal may be a relatively easy task. Accordingly, the performance changes in the irradiated animals (except the short latency of the 4–15X group, as previously noted) cannot be attributed to motor handicaps.

The control animals displayed a strong position preference in the shaping alley. This is in contrast to the spontaneous alternation of rats in a nonaversive T-maze (Douglas, 1966). The irradiated rats did not differ significantly from the controls in this respect. There was no treatment effect in the acquisition of the position habit (which represented a reversal of the preference displayed in the shaping alleys) or in the reversal of the trained habit. The insensitivity of these two tests to the irradiation treatments may have been due to the ease with which they were mastered, in about 10 trials by all groups. However, in the more difficult single and double alternation tests, differential handicaps were displayed by the 4–15X, 12–15X, and 4–5X animals, but there were no deficits in the 8–15X group. The 4–15X rats showed deficits in learning single alternation but not double alternation, whereas the 12–15X and 4–5X rats were handicapped on double alternation but not single alternation. These differential effects could not be attributed to motor deficits, as there were no differences in swimming time. Nor could the activity level differences noted in the open field and activity wheels (Experiments 3–5) be invoked because both the 12–15X and 8–15X groups were hyperactive but only the former had a deficit in double alternation. There were latency differences among the groups (decreased latency in the 4–15X group and a trend toward an increase in the 4–5X group), but these differences were not systematically related to the learning deficits. The fact, however, that there were latency differences suggested that the experimental groups may have differed in their response strategies in the maze. Reduced tendency to spontaneously alternate, for instance, would retard animals in mastering a single alternation task but enhance their learning a double alternation. This possibility was examined in a dry T-maze in which normal animals display a tendency to alternate (Douglas, 1966).
Experiment 7: Spontaneous Alternation

Method

Subjects. Litters of inbred male Long-Evans hooded rats were reduced to six pups prior to exposure to X-irradiation. The X-ray schedules and procedures were identical to those employed in Experiment 1. The treatment groups consisted of controls \((n = 77)\), 12-15X \((n = 86)\), 8-15X \((n = 72)\), 4-5X \((n = 24)\), and 4-15X \((n = 22)\). All subjects were 60-70 days of age at the beginning of the experiment. Some subjects in the control, 12-15X, and 8-15X groups had been previously tested in an open-field habituation experiment (Experiment 4) as infants.

Apparatus. The T-maze was constructed from plywood, with alleys that were 11.5 cm wide \(\times\) 11.5 cm high and painted flat gray. The main alley was 45 cm long, and the left and right alleys were 34 cm long. A guillotine door was located 25 cm from the end of the main alley and formed the end of the start box. Guillotine doors were also located 3.5 cm to the left and right of the choice point, which enabled the experimenter to confine the subject in the goal box and prevent retracing. The floor and roof of the maze were constructed from clear Plexiglas. Illumination was provided by a 7.5-W bulb located 45 cm above the choice point. Testing was done in a sound-attenuating cubicle.

Procedure. Each subject was placed in the start box of the T-maze, and the guillotine door was raised 5 sec later. After the subject turned either left or right at the choice point, it was confined in the goal box area for 15 sec by lowering the guillotine door behind the subject. Immediately following the period of confinement, the subject was placed back in the start box for the second trial. Each subject was given two trials per day for 3 days. Trials in which the subject did not respond in less than 180 sec were scored as no choice and were not included in the data analyses. This occurred on only 1.9% of the trials. The T-maze was thoroughly washed and dried after each subject was tested.

Results and Discussion

Anatomical. Coronal and sagittal measures of the cerebral hemispheres and cerebellum were available for three of the five treatment groups and were comparable with those obtained in previous experiments (Table 7). The animals in the 4-5X and 4-15X groups were scheduled for additional testing in other experiments, and, therefore, brain measurements are not yet available.

Behavioral. Control subjects and three of the four X-irradiated treatment groups (12-15X, 8-15X, and 4-5X) spontaneously alternated at rates significantly above chance levels on all 3 days (Table 8). Only the 4-15X treatment group failed to alternate above a chance level in any of the three test sessions (Table 8). The 4-15X group also was the only X-irradiated treatment group that differed significantly from controls in its rate of spontaneous alternation \((z = 7.5, p < .001)\).

These results clearly demonstrate that the 4-15X animals do not have the normal tendency to spontaneously alternate in a T-maze which is reliably found in rats (Douglas, 1966). Although spontaneous alternation in a T-maze such as this one may not be directly comparable with alternation behavior in a swimming maze, where the animal is motivated to escape from cold water and is rewarded for selection of the correct pathway (e.g., Experiment 6, Maze 6), these results are reconcilable with the deficit of the 4-15X group in single alternation and their lack of deficit in double alternation.

General Discussion

In an often quoted study, Lashley and McCarthy (1926) examined the role of the cerebellum in maze learning. They antici-

Table 7
Brain Measurements (in mm) of Young Adult Rats Tested in Experiment 7

<table>
<thead>
<tr>
<th>Group</th>
<th>Cerebral hemispheres</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coronal</td>
<td>Sagittal</td>
</tr>
<tr>
<td>Control</td>
<td>15.43 ± .39</td>
<td>14.82 ± .45</td>
</tr>
<tr>
<td>12-15X</td>
<td>15.27 ± .39</td>
<td>15.04 ± .49*</td>
</tr>
<tr>
<td>8-15X</td>
<td>15.36 ± .56</td>
<td>15.43 ± .64**</td>
</tr>
</tbody>
</table>

Note. Significance levels (Newman-Keuls) are with respect to controls.
* \(p < .05\).
** \(p < .01\).
pated that because of the close anatomical relation of the cerebellum to the proprioceptive and vestibular systems, cerebellar lesions might interfere with maze learning. "Adult" rats were trained in a rectangular maze with eight blind alleys and were retested for retention after extensive cerebellar removals. Lashley and McCarthy found no deficits. Moreover, they reported that "one animal with the cerebellum completely destroyed learned to run the maze without error" (p. 432) and concluded that "there is no evidence that the cerebellum plays any part in the performance of the maze habit" (p. 432). These negative findings apparently discouraged physiological psychologists from any further examination of the involvement of the cerebellum in maze learning. In contrast, neurophysiologists, using different experimental methodologies or strictly on the basis of theoretical considerations, have suggested that the cerebellar cortex may be involved in motor learning (Eccles, 1977; Gilbert, 1974; Gilbert & Thach, 1977; Ito, Shidea, Yagi, & Yamamoto, 1974; Marr, 1969). Our experiments do not bear on the latter studies as we have not investigated the acquisition of motor skills, but they are at variance with the negative conclusion of Lashley and McCarthy.

Previous studies from our laboratory, using a different cerebellar X-irradiation procedure (2-15X), indicated acquisition and reversal deficits in a T-maze in infant (Altman & Bulut, 1976) and young adult experimental rats (Bulut, 1976) but not in 8-mo-old rats (Bulut, 1976). In the present study, using not only different X-irradiation schedules but different testing procedures (e.g., allowing error correction), we obtained no effects on the acquisition or reversal of an easily mastered position habit but deficits in either single or double alternation in all but the 8-15X group. It would not be rewarding to compare our results with those of Lashley and McCarthy; they used a different maze task and a different cerebellar manipulation, and their animals may have been older than ours. But our results are at variance with

### Table 8

**Percentage of Spontaneously Alternating Subjects**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77</td>
<td>86**</td>
<td>84**</td>
<td>87**</td>
</tr>
<tr>
<td>12-15X</td>
<td>86</td>
<td>85**</td>
<td>80**</td>
<td>86**</td>
</tr>
<tr>
<td>8-15X</td>
<td>72</td>
<td>85**</td>
<td>81**</td>
<td>78**</td>
</tr>
<tr>
<td>4-5X</td>
<td>24</td>
<td>75*</td>
<td>79*</td>
<td>78*</td>
</tr>
<tr>
<td>4-15X</td>
<td>22</td>
<td>50</td>
<td>67</td>
<td>59</td>
</tr>
</tbody>
</table>

**Note.** Significance levels (chi-square comparison) are with respect to chance levels of performance.

* p < .01.
** p < .001.

### Table 9

**Behavioral Changes in Young Adult Irradiated Groups in Comparison with Controls**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>12-15X</th>
<th>8-15X</th>
<th>4-5X</th>
<th>4-15X</th>
</tr>
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<tbody>
<tr>
<td>On dry surface</td>
<td>*</td>
<td>(†)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Rotating rods, regular hurdles</td>
<td>*</td>
<td>(†)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Rotating rods, irregular hurdles</td>
<td>*</td>
<td>(†)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Ambulation in open field</td>
<td>↑</td>
<td>(†)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Running in activity wheels</td>
<td>↑</td>
<td>(†)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Rears with support</td>
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<td>(†)</td>
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<td>Rears without support</td>
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<tr>
<td>Spontaneous alternation</td>
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</table>

**In water medium**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>12-15X</th>
<th>8-15X</th>
<th>4-5X</th>
<th>4-15X</th>
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</thead>
<tbody>
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<td>Swimming speed (shaping alleys)</td>
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<td>Position habit, reversal</td>
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<tr>
<td>Single alternation</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>⊳</td>
</tr>
<tr>
<td>Double alternation</td>
<td>↓</td>
<td>=</td>
<td>=</td>
<td>⊳</td>
</tr>
</tbody>
</table>

**Note.** Meaning of symbols: * Group not tested; (†) increased nonsignificantly; ↓ reduced significantly; ⊳ reduced severely; (↑) reduced nonsignificantly; ↑ increased significantly; ⊳↑ increased severely; = not different.
their unqualified negative conclusion since most cerebellar treatments examined by us have had a deleterious effect on some form of maze learning in at least infant and young adult rats.

We cannot at present specify the nature of the deficits or explain the dissimilar effects produced by the different cerebellar treatments. The learning tasks used in this study and the experimental treatments are very complex. Single and double alternation seem to be "spatial" tasks, but we cannot specify what extramaze cues or strategies the animals were using (Restle, 1957). It is conceivable that the treatments affected cue utilization or the strategies employed by the animals. We have presented evidence that one group (4–15X) did not display spontaneous alternation in a nonaversive situation and suggested that this may have accounted for its inferiority in learning single alternation but not double alternation. We also noted group differences in start latencies in the maze but could not relate them to the learning deficits. We cannot offer an explanation why the 4–5X and 12–15X groups were handicapped in double alternation but not the 8–15X group; the only shared characteristic of the former groups was a smaller reduction in granule cells than in the 8–15X group. To shed light on the nature of the learning deficits produced in relation to the induced morphogenetic reorganization of the cerebellum, we will have to use behavioral procedures that will permit us to specify better the cues relied on and the strategies used by the animals.

To summarize the findings of the present series of studies, our most important finding was the dissociation of effects between the early-irradiates (4–15X and 4–5X) and the late-irradiates (8–15X and 12–15X). Only in the early-irradiates did we obtain locomotor deficits. This is the group in which there was structural disorganization of the cerebellar cortex and a loss of basket cells. The 4–15X group was severely affected both in induced tasks (rotating rods) and spontaneous response emission (open field and activity wheels), and the 4–5X group showed mild motor deficits. In the late-irradiates, we could not demonstrate locomotor deficits but rather an increase in activity level in the open field and in activity wheels, and some learning deficits, as previously discussed. This is the group in which there were no gross structural abnormalities but there was a graded loss in those granule cells that synapse with the upper domain of the Purkinje cell dendrites. We suggest as a working hypothesis that early irradiation interferes with both motor execution and response strategies but late irradiation interferes only with the latter.

The implication of this is that the cerebellar cortex is hierarchically organized and that the early forming lower molecular layer is implicated in motor coordination while the late forming upper molecular layer is involved in action coordination. In this context we may recall our morphogenetic evidence (Altman & Bayer, 1978a, 1978b) that in the mossy fiber afferent system to the cerebellum, the prerecerebellar structures relaying spinal input (spinal cord and lateral reticular nucleus) form before the structure relaying neocortical input (the pontine nuclei). This allows for the possibility that the earlier forming parallel fiber system of the lower molecular layer (whose elimination produces motor deficits) is the stratum that receives peripheral proprioceptive input from the spinal cord whereas the late forming parallel fiber system of the upper molecular layer (whose elimination produces only changes in action strategies) is the stratum that receives input from the neocortex.

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