

Experimental Reorganization of the Cerebellar Cortex

III. REGENERATION OF THE EXTERNAL GERMINAL LAYER AND GRANULE CELL ECTOPIA

JOSEPH ALTMAN

Laboratory of Developmental Neurobiology, Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907

ABSTRACT The heads of Long-Evans hooded rats were irradiated daily from day 3, 4 or 6 onward with schedules of low-level x-ray which differentially delayed the regeneration of the subtotally eradicated external germinal layer or prevented its regeneration altogether. Most schedules that permitted regeneration led to the arrest of many granule cells in the molecular layer where they formed an ectopic zone. The dendrites of ectopic granule cells had synapses with mossy fibers located in their vicinity in the molecular layer. The position of the ectopic zone varied in different lobules depending on several factors: the time when regeneration began, the estimated date of descent of granule cells, regional differences in cortical maturation and in the estimated upward growth rate of mossy fibers. Within the same lobule, the longer regeneration was delayed the higher in the molecular layer the differentiating and descending granule cells were captured by the apparently autonomously ascending mossy fibers. If regeneration started early and allowed the descent of granule cells into the granular layer (early irradiation) or if regeneration of granule cells was prevented altogether (late irradiation) an ectopic zone was not formed. The technique was used for estimating the normal growth pattern of mossy fibers in the different lobules.

We described in the previous two papers of this series (Altman and Anderson, '72, '73) the morphogenetic consequences of exposure of the cerebellum during infancy to different schedules of prolonged x-irradiation that prevent the regeneration of the external granular, or germinal, layer and the formation of basket, stellate and granule cells. This paper deals with the genesis of granule cell ectopia (arrest of granule cells in the molecular layer), a phenomenon seen following certain postnatal exposure schedules that permit regeneration of the external germinal layer.

Granule cell ectopia after irradiation of the cerebellum of rats with 200 r at six days of age was described by Hicks and his collaborators (Hicks and D'Amato, '61, '66; Hicks, D'Amato and Falk, '64). Hicks noted that with the same dose delivered at birth there was no ectopia but a "jumbling" of Purkinje and granule cells. With the procedure used, the ectopic zone was usually occupying the middle of the molecular layer in five or six lobules of the

anterior vermis. He mentioned that ectopia may be formed in other lobules if the dose is delivered on other days after birth. Similar results were reported by Ebels ('70) who irradiated rats with 1000 r on one of the following days: 0, 2, 4, 6, 8 and 12; ectopia was seen only with irradiation on day 6. In the dog, ectopia was produced when 325 or 500 R was delivered on day 2; lower doses did not produce ectopia (Phemister, Shively and Young, '69). With 325 R the ectopic cells were situated in the deeper half of the molecular layer, whereas following exposure to the higher dose they were located in the outer half of the molecular layer (with regional variations in different folia). Ectopia may be produced also by agents other than ionizing radiation, such as viruses (Monjan et al., '71) or chemicals (Nathanson, Cole and Van der Loos, '69; Shimada and Langman, '70a,b).

We shall attempt to show that the arrest of migratory granule cells in the molecular layer after recovery from irradiation

provides information about the normal time-table of the descent of differentiating granule cells through the molecular layer and the growth of mossy fibers into the cerebellar cortex. In a succeeding paper (Altman, '73) another phenomenon associated with the regeneration of the external germinal layer will be described, namely, the spatial reorientation of parallel fibers in the molecular layer.

MATERIALS AND METHODS

The maintenance of the rats and the radiation procedure were described in detail in the first paper of this series (Altman and Anderson, '72). In this study, 38 cere-

TABLE 1

Irradiation schedule of the rats used for establishing the lobular and laminar patterns of granule cell ectopia. All animals received two or more successive daily exposures of 150 r.

Age when irradiated	Number of irradiations	Number of rats
<i>days</i>		
3-4	2×	3
3-6	4×	3
3-8	6×	2
4-5	2×	8
4-7	4×	8
4-9	6×	2
4-11	8×	4
4-13	10×	2
6-7	2×	3
6-9	4×	3

bella were examined in 30 day old rats whose heads were exposed to 150 r x-ray on two or more successive days, beginning on day 3, 4 or 6, when ectopia could be produced (table 1). In order to establish the time course of regeneration of the external germinal layer after different schedules of irradiation, the cerebella of 72 rats were examined whose heads received two or more successive daily exposures of 150 r x-ray from the fourth day onward, and survived for one, two and four days after the last exposure and until 30 days of age (table 2). All these brains were cut sagittally and stained with cresyl violet, hematoxylin-eosin and Bodian's protargol-S method. Four cerebella (pyramis) from rats irradiated on days 4-7 with 150 r and killed at 30 days were prepared for (as described previously) and examined with electron microscopy; ten similar cerebella were impregnated with the Golgi-Cox procedure.

RESULTS

Light microscopic observations

In describing the position of ectopic cells (figs. 1, 6, 7) the following terms will be used: *basal* (situated above but in direct contact with the layer of Purkinje cells), *lower*, *middle*, or *upper strip* (referring to their position in the molecular layer) and *superficial* (in subpial posi-

TABLE 2

Survival schedules for the examination of the regeneration of the external germinal layer following different schedules of irradiation. All animals received two or more successive daily exposures of 150 r from day 4 onward.

Age when irradiated	Number of irradiations	Survival time	Age when killed	Number of rats
<i>days</i>		<i>days</i>	<i>days</i>	
4-5	2×	1	6	5
		2	7	8
		4	9	5
			30	8
4-7	4×	1	8	5
		2	9	3
		4	11	5
			30	11
4-9	6×	1	10	5
		2	11	0
		4	13	2
			30	3
4-11	8×	1	12	3
		2	13	0
		4	15	5
			30	4

tion). These terms denote only relative distances from the layer of Purkinje cells because the thickness of the molecular layer varies depending on the extent of regeneration following irradiation.

Ectopia with irradiations begun on day

3. In rats irradiated with two successive doses on days 3 and 4, little ectopia was seen, and it was restricted to a basal position in the ventral aspect of the nodulus, to part of the lingula and to the depth of the fissura prima (fig. 2, row 1). In some animals a few basal ectopic cells were present in the depth of some other fissures but in no other region of the vermis. Thus with this irradiation schedule ectopia was limited to the earliest maturing regions of the vermis (Altman, '69).

In the rats irradiated with four doses through days 3–6 a pronounced ectopic zone was seen in the lower strip of the molecular layer in the nodulus and part of the uvula, and in the depth of the fissura prima. Elsewhere, except in the tuber vermis, the ectopia was in a basal position (fig. 2, row 4). In the late-maturing tuber vermis (Altman, '69) there was appreciable jumbling of Purkinje cells and regeneration (cells formed after irradiation) but no ectopia. In the rats that received six doses through days 3–8 there was no regeneration of granule cells in the nodulus and ectopia was absent. In general, wherever there were no signs of regeneration, ectopic cells were absent. In the uvula ectopic cells, together with many regenerated cells, were seen in appreciable numbers in the lower or middle strip of the molecular layer. Where regenerated cells were present in the anterior vermis, ectopia was in a basal position.

Ectopia with irradiations begun on day

4. In the rats irradiated with two doses on days 4 and 5 pronounced basal ectopia was present in the nodulus, part of the uvula, the depth of the fissura prima, and in the lingula, but nowhere else (fig. 2, row 2). In the rats that received four doses through days 4–7, the ectopic zone in the nodulus and the uvula, and in the depth of the fissura prima, shifted into the middle strip of the molecular layer (fig. 2, row 5); in the anterior vermis the ectopia was in the lower strip of the molecular layer. In some animals the ectopic zone

was thick, in others thinner; in some it was crisply delineated, in others scattered; in a few animals clusters of cells formed superficial ectopia (fig. 2, row 5).

In the rats that received six doses through days 4–9, there was little regeneration in the nodulus and the few ectopic cells lay in the upper strip of the molecular layer. In the pyramis the ectopia was in the middle strip and in the tuber vermis an ectopic zone was present basally. In the anterior vermis, where regeneration was more widespread, the ectopia tended to be in the lower or middle strip of the molecular layer. In rats that received eight doses through days 4–11 (fig. 2, row 7) the following observations were made: in the nodulus, no regeneration and no ectopia; in the uvula and the pyramis, slight regeneration and upper strip ectopia; in the tuber vermis, more regeneration and upper strip ectopia. That is, after prolonged irradiation (and associated delay in regeneration) the regeneration was more pronounced in the late-maturing lobules and there was an outward shift in ectopia. In animals that received ten doses through days 4–13 there was neither regeneration nor ectopia present in the vermis (fig. 2, row 8).

Ectopia with irradiations begun on day

6. In the rats that received two doses on days 6 and 7, ectopia spread through the entire vermis except parts of the tuber (fig. 2, row 3). In the nodulus, uvula, pyramis and depth of fissura prima the ectopia was faint or diffuse and in the middle strip; in the anterior vermis it was crisper, richer in cells and situated in the lower strip. In the rats that received four doses through days 6–9 there was either no ectopia or a thin ectopic zone in the upper strip in the nodulus and ventral uvula (fig. 2, row 6). A better delineated ectopic zone was seen in the tuber vermis in the lower strip, whereas in the anterior vermis the ectopic cells were located toward the upper strip of the molecular layer.

In general the pattern of ectopia was quite different in the hemispheres than in the vermis, which could be related to the different time table of cortical maturation.

Regeneration of the external germinal layer with irradiations begun on day 4. The foregoing observations indicated the

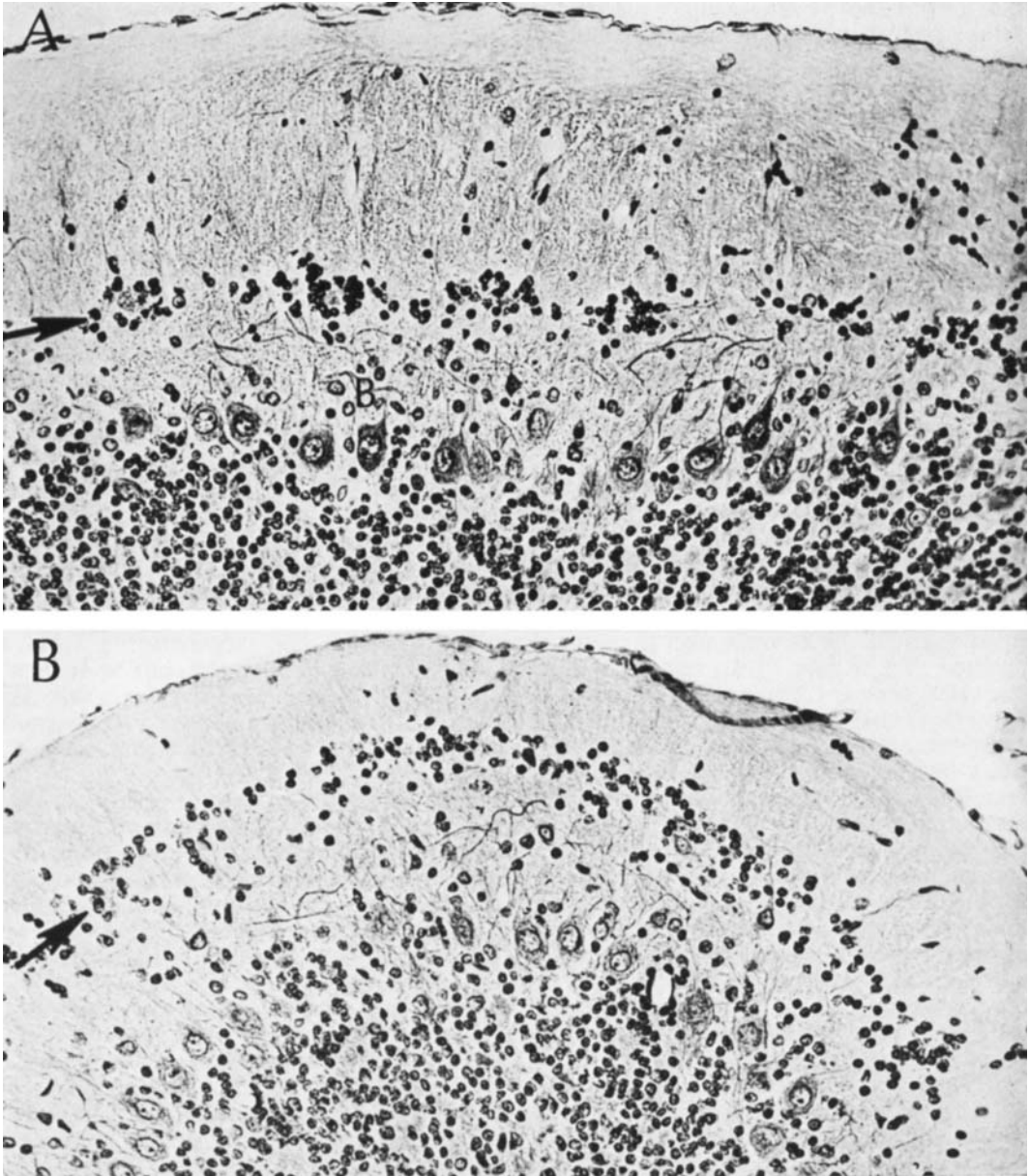


Fig. 1A Ectopic granule cells in the lower to middle strip of the molecular layer (arrow) in the pyramis of a 30-day old rat that was irradiated with successive daily doses of 150 r on days 4, 5, 6 and 7. Scattered ectopic cells are seen below and above the ectopic zone and basket cells (B) below it together with impregnated basket cell axons. Above the ectopic zone the parallel fibers are oriented coronally (note their appearance as dots) but near the surface they are oriented parallel to the sagittal plane of sectioning. Bodian, $\times 256$.

Fig. 1B Ectopic granule cells in the middle to upper strip (arrow) in the pyramis of a rat that was irradiated on days 4, 5, 6, 7, 8 and 9. Note also the decreased total width of the molecular layer due to the reduced time available after the prolonged irradiation for the formation of granule cells. Bodian, $\times 256$.

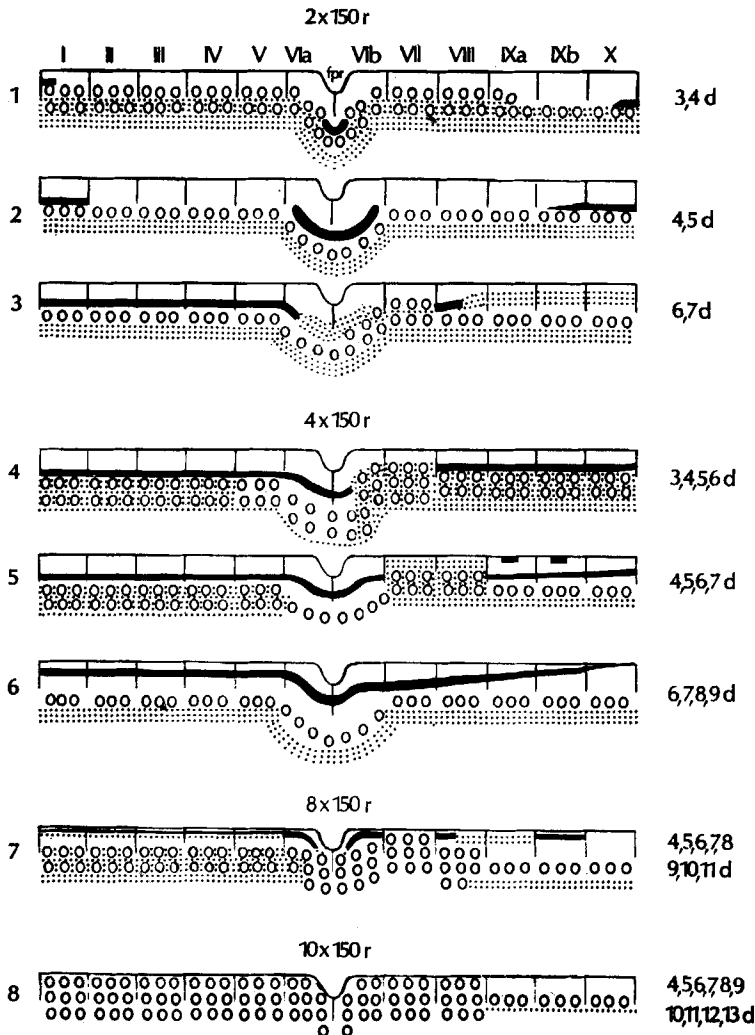


Fig. 2 Schematic survey of the patterns of ectopia (heavy black line) seen in the lobules of vermis (rectangles designated I-X) and in the walls and depth of fissura prima (fpr) in rats irradiated with different schedules, as indicated. The rows (1-8) indicate the typical patterns seen among the many animals examined in the groups illustrated. The reduction in the depth of the molecular layer following prolonged irradiation and the increased crowding of Purkinje cells is schematically indicated. Dots below the Purkinje cells denote the presence of a granular layer; dots in the molecular layer indicate the presence of an indistinct ectopic zone. For details see text.

dependence of ectopia on the production of granule cells after irradiation, that is, on the regeneration of the external germinal layer. When regeneration was absent, as in the animals receiving 10×150 r, ectopia did not occur. Moreover, in the animals in which regeneration was progressively delayed by either prolongation of

the irradiations (compare rows 2, 5 and 7 in fig. 2) or by starting the same schedule at later dates (compare rows 1, 2 and 3) the arrest of the descending granule cells occurred at progressively higher levels within the same lobule. Hence it deemed desirable to determine the time course of cell regeneration in a group of animals

that yielded pronounced ectopia throughout the vermis (table 2).

In the animals irradiated on days 4, 5, 6 and 7 and killed one day after the last irradiation, the external germinal layer was subtotally eradicated (fig. 9). The few scattered radioresistant primitive cells were preferentially located in the depth and walls of the fissures (fig. 11). A row of lightly staining cells in the molecular layer was interpreted as representing radioresistant differentiating elements (fig. 10). The Purkinje cells were lined up regularly two to three cells deep throughout the vermis (figs. 10–11) and only in the nodulus and ventral uvula were enough differentiated granule cells present to form a thin granular layer (fig. 9).

There were no signs of regeneration of the external germinal layer in the rats that lived for two days after the last irradiation. However, in all the rats that lived for four days the regeneration of the external germinal layer began in patches in the depth and wall of fissures (figs. 12–13) where they varied in thickness from five to ten cells; the external germinal layer tended to be much thinner or was altogether absent over the gyri in most lobules. The migratory or differentiating cells of the molecular layer were no longer seen but basket cells were identified. There were no signs of descending, regenerated granule cells nor of ectopic cells. The commencement of regeneration of the external germinal layer four days after the last irradiation is in agreement with previous results with radiation started at birth (Altman, Anderson and Wright, '69).

Observations in Golgi material. Many of the features seen in the cerebellum of 30 day old rats irradiated on days 4–7 were similar to those reported in the previous papers of this series, such as the disorientation and distortion of Purkinje cell dendrites (fig. 3E). Attention was paid in this study to the appearance of granule cells in ectopic zones in the molecular layer (figs. 3A–D) and their features in dystopic regions (figs. 3E, F). The majority of these granule cells were indistinguishable from those seen in the granular layer in normal animals. They had the usual number of dendrites, conspicuous dendritic claws, and were impregnated either singly

(figs. 3A,B) or formed aggregates resembling bunches of grapes (figs. 3C,E). Occasional granule cells had elongated dendrites (fig. 3B); spindle shaped bipolar cells were frequent near the surface (figs. 3C,D,F), suggesting failure in differentiation. Probably these cells belonged to the class of superficial ectopic cells often seen in the Nissl-stained material.

Electron microscopic observations. Ectopic granule cells were numerous in the pyramis of rats irradiated with 150 r on days 4–7 and killed at 30 days of age. Most frequent were rows of ectopic cells in the lower to middle strip of the molecular layer (fig. 14) but scattered granule cells were seen near Purkinje cells (fig. 15) or superficially (figs. 16, 17). In most sites where ectopic granule cells were present there were glomeruli with mossy fiber rosettes and synapses, presumably with granule cell dendrites (figs. 14–17). The ectopic granule cells and associated mossy fiber terminals were indistinguishable from those in the granular layer. The mossy fibers were apparently myelinated in the molecular layer (fig. 18) and the granule cell dendrites formed desmosoid junctions (attachment plaques) with each other, as they do in the granular layer (figs. 16, 17, 19). The only abnormality in the molecular layer was the rotation of parallel fibers from a transverse plane into other directions; this will be considered in detail in the succeeding paper (Altman, '73).

DISCUSSION

We found in agreement with earlier studies (Hicks and D'Amato, '61, '66; Ebels, '70) that granule cell ectopia is produced if the cerebellum is irradiated with low-level x-ray during a specific period after birth. Ectopia was produced in rats by irradiations begun between three to six days; we know from earlier studies (Altman and Anderson, '71; Altman, Anderson and Strop, '71) that it is not produced if irradiation is begun at birth. The cause of ectopia and its dependence on irradiation during a specific period have not been hitherto explained. Hicks suggested that the laterally spreading Purkinje cell dendrites may arrest the descent of granule cells but stated that "We have yet to dis-

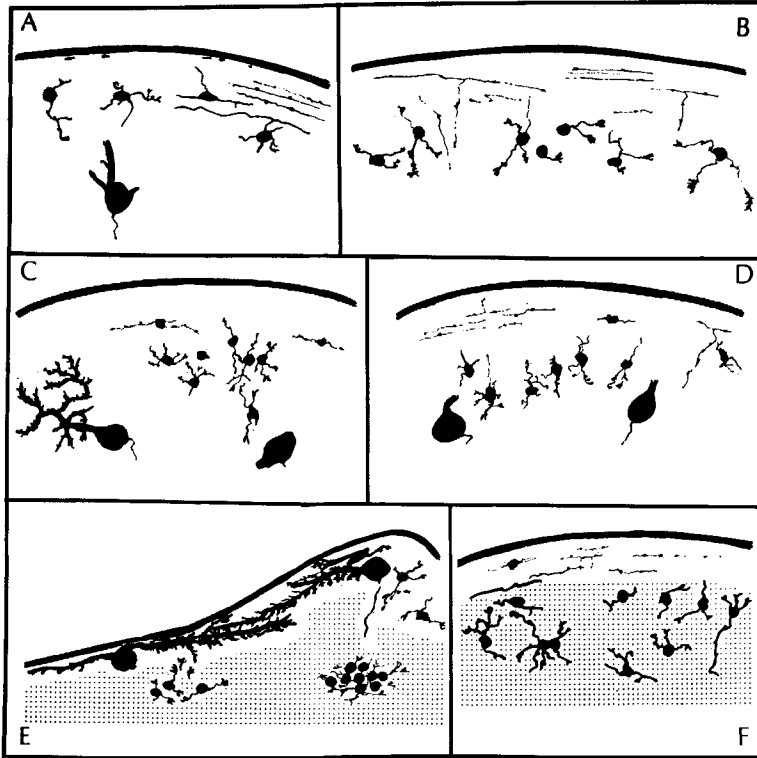


Fig. 3 Impregnated granule cells (with a few parallel fibers and Purkinje cells) in rats irradiated on days 4, 5, 6 and 7 and killed on day 30. A, B, C and D, ectopic granule cells in the molecular layer in uvula. E and F, dystopic granule cells in folium and tuber. Golgi-Cox.

cover what factors normally guide the potential granule cells from the surface to their destination and how they are blocked in the molecular zone following radiation injury" (Hicks, D'Amato and Falk, '64, p. 537).

This study showed that a prerequisite for the formation of ectopia is the regeneration of the external germinal layer. In the animals irradiated with ten doses of 150 r between days 4–13 there was no regeneration or ectopia. In other groups of animals started on the same day but given fewer successive daily doses, granule cells were regenerated and ectopia was produced. This suggested that the date when irradiation is stopped (rather than when it is begun) is important because that determines when the external germinal layer is regenerated. Considering that four days are required for the regeneration of the external germinal layer (Altman, Anderson

and Wright, '69) and another two days for the descent of the newly formed cells through the molecular layer (by the third day labeled cells are accumulated in the granular layer; Altman, '66, '72a), and relating this to the observation that exposure to x-rays on days 3–4 does not produce ectopia in the vermis (except in parts of the early-maturing nodulus), it follows that if the descent of granule cells is not delayed beyond day 10 they are not arrested in the molecular layer. If regeneration is postponed further either by delivering the two doses later (4–5 d; 6–7 d) or by supplementary daily doses (3–6 d; 4–7 d; 6–9 d) the descending granule cells are arrested progressively higher in the molecular layer.

Does irradiation interfere with the descent of granule cells (perhaps by damaging the mechanisms responsible for migration) and later mossy fibers grow outward

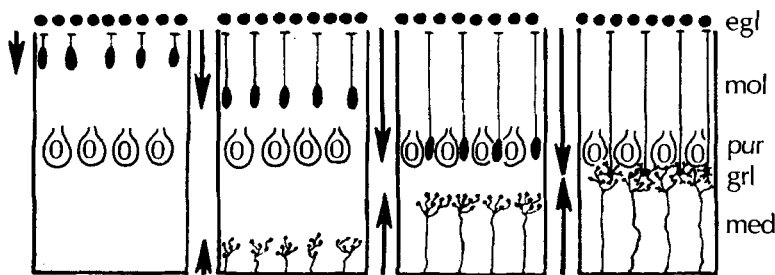


Fig. 4 Schematic illustration of the hypothesis of the synchronized descent of migrating granule cells through the molecular layer (mol) and of the ascent of the growing mossy fiber endings through the medullary layer (med) leading to their concurrent arrival in the prospective granular layer where they meet, cease their movements and form glomerular synapses; egl, external germinal layer; pur, Purkinje cell layer.

into the molecular layer to form synapses with them? Or do the mossy fibers invade the molecular layer if the descent of granule cells is delayed and by forming synapses there arrest the migration of granule cells into the granular layer? The latter hypothesis is supported by the evidence that mossy fibers grow outward and rosettes reach the surface of the cortex in cerebella devoid of granule cells. This was suggested in a histochemical study (Altman and Das, '70; fig. 8b), which showed a dense meshwork of cholinergic mossy fibers in degranulated cerebella above the layer of Purkinje cells, and is supported by electron microscopy (Hámori, '69; Altman and Anderson, '72, '73).

The autonomous growth of mossy fibers suggested the following sequence of events (fig. 4). Under normal conditions, differentiating granule cells descend through the molecular layer according to their regional time schedules and the synchronously ascending mossy fibers grow upward through the medullary layer. The two meet in the granular layer and form glomerular synapses there. According to earlier electron microscopic evidence this occurs in the pyramis at about 12 days of age (Altman, '72b). If the descent of granule cells is temporarily delayed, as by x-irradiation, then the mossy fibers continue their growth past the layer of Purkinje cells into the molecular layer. No ectopia is produced if irradiation is delivered before day 4, because the external germinal layer regenerates in time to permit the descent of granule cells before the growing mossy fibers passed the formative granular layer. Ectopia is produced when the late descending

granule cells meet the ascending mossy fibers in the molecular layer, and the longer regeneration and descent are delayed the higher up their meeting site will be in the molecular layer. The assumption underlying this hypothesis is that whenever granule cells form synapses with mossy fibers they become immobilized.¹

If this interpretation is correct then the location of the ectopic zone in different lobules following various irradiation schedules could be used as a marker for the differential growth pattern of mossy fibers. This was attempted for a few lobules on the basis of the available experimental data (fig. 5). The specifics of the various irradiation schedules were ignored and only the day on which it was stopped was considered. To this were added the four days required for regeneration of the external germinal layer and two days for the onset of differentiation and migration (Altman, '66, '72a). This scheme suggests that in the early maturing nodulus the mossy fibers reach the prospective granular layer on day 9; in the pyramis on day 11 (which is in close agreement with their rare presence at 10 days and more common occurrence at 12 days; Altman, '72b); in the late-maturing folium vermis and the tuber at 12 days. The pattern of ectopia in different lobules corresponds well with the chronology of maturation of the different lobules of the vermis as indicated by thymidine-H³ autoradiography (Altman, '69).

¹ A very similar hypothesis was put forward recently by Ebels (E. J. Ebels, 1972. Studies on ectopic granule cells in the cerebellar cortex, with a hypothesis as to their aetiology and pathogenesis. *Acta Neuropath. (Berlin)*, 21: 117-127).

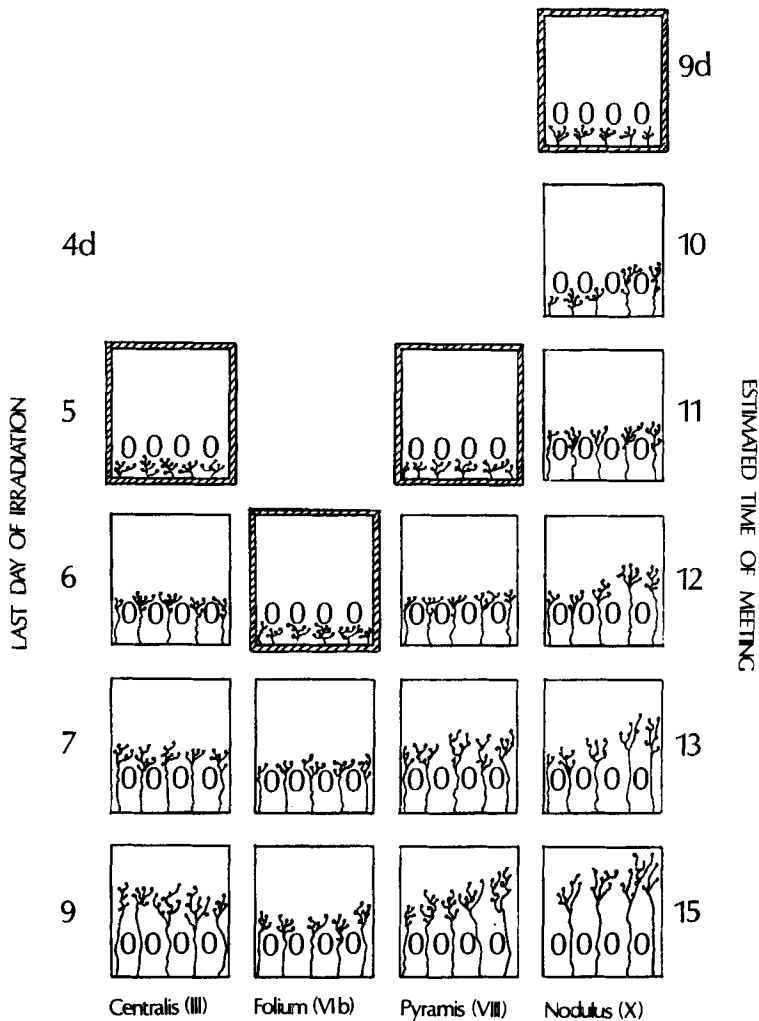


Fig. 5 Inferred location of the upward growing mossy fibers in selected lobules (III, VIb, VIII, X) in some of the irradiated animals; based on the location of the ectopic zone in rats that received their last exposure on the days indicated (left column) and in which granule cells were estimated to have regenerated and descended through the molecular layer six days later (indicated on the right). Mossy fibers were assumed to have reached the prospective granular layer (framed rectangles) one day before ectopia was produced in basal position. The absence of ectopia under conditions where the regenerated cells could descend in time to meet the ascending mossy fibers was confirmed in all instances except for the early-maturing nodulus, estimated at day 9, for which irradiated material was not available.

Among the treatment groups differences were obtained not only in the position of the ectopic zone but also in its width, density, and extent through one or several lobules. While we can account for the position of ectopia in different lobules in terms of the date of onset of regeneration and the chronology of regional maturation,

we cannot at present specify with any certainty the various factors that may affect the configuration of the ectopia. But some hints were obtained. For instance, if we compare the pattern of ectopia in two groups that received their last x-ray exposure on day 7, the ectopia was conspicuous in the nodulus and the uvula if irradiation

tion was started on day 4 (fig. 2, row 5) but indistinct in these lobules if exposure was begun on day 6 (fig. 2, row 3). Presumably in the latter instance some early-differentiating cells were beginning to descend before irradiation was started and were able to reach the ascending mossy fibers in the granular layer. Another phenomenon is the presence of multiple ectopic zones in some lobules (fig. 2, row 5). This may be attributed to arrival of more than one wave of germinal cells at different times from different foci of regeneration. This was suggested by the observation (Altman, '73) that in the molecular layer of these regenerated cerebella there are sublayers in which parallel fibers are oriented in different directions.

ACKNOWLEDGMENTS

I am grateful to William J. Anderson for the irradiation of the animals, to Kunda Das for preparation of the material for electron microscopy, and to Zeynep Kurgun and Donna Whitehurst for the photographic work. This research program is supported by the U. S. Atomic Energy Commission and the National Institute of Mental Health.

LITERATURE CITED

- Altman, J. 1966 Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis in some brain regions. *J. Comp. Neur.*, 128: 431-474.
- 1969 Autoradiographic and histological studies of postnatal neurogenesis. III. Dating the time of production and onset of differentiation of cerebellar microneurons in rats. *J. Comp. Neur.*, 136: 269-294.
- 1972a Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. *J. Comp. Neur.*, 145: 353-397.
- 1972b Postnatal development of the cerebellar cortex in the rat. III. Maturation of the components of the granular layer. *J. Comp. Neur.*, 145: 465-514.
- 1973 Experimental reorganization of the cerebellar cortex. IV. Parallel fiber reorientation following regeneration of the external germinal layer. *J. Comp. Neur.*, 149: 181-192.
- Altman, J., and W. J. Anderson 1971 Irradiation of the cerebellum in infant rats with low-level x-ray: histological and cytological effects during infancy and adulthood. *Exp. Neur.*, 30: 492-509.
- 1972 Experimental reorganization of the cerebellar cortex: I. Morphological effects of elimination of all microneurons with prolonged x-irradiation started at birth. *J. Comp. Neur.*, 146: 355-406.
- 1973 Experimental reorganization of the cerebellar cortex: II. Effects of elimination of most microneurons with prolonged x-irradiation started at four days. *J. Comp. Neur.*, 149: 123-152.
- Altman, J., W. J. Anderson and M. Strop 1971 Retardation of cerebellar and motor development by focal x-irradiation during infancy. *Physiol. Behav.*, 7: 143-150.
- Altman, J., W. J. Anderson and K. A. Wright 1969 Early effects of x-irradiation of the cerebellum in infant rats: decimation and reconstitution of the external granular layer. *Exp. Neur.*, 24: 196-216.
- Altman, J., and G. D. Das 1970 Postnatal changes in the concentration and distribution of cholinesterase in the cerebellar cortex of rats. *Exp. Neur.*, 28: 11-34.
- Ebels, E. J. 1970 The influence of age upon the effect of early postnatal x-irradiation on the development of the cerebellar cortex in rats. *Acta Neuropath. (Berlin)*, 15: 298-307.
- Hámori, J. 1969 Development of synaptic organization in the partially agranular and in the transneuronally atrophied cerebellar cortex. In: *Neurobiology of Cerebellar Evolution and Development*. R. Llinás, ed. American Medical Association, Chicago.
- Hicks, S. P., and C. J. D'Amato 1961 How to design and build abnormal brains using radiation during development. In: *Disorders of the Developing Nervous System*. W. S. Field and M. M. Desmond, eds. Thomas, Springfield, pp. 60-97.
- 1966 Effects of ionizing radiations on mammalian development. In: *Advances in Teratology*. D. H. M. Woollam, ed. Logos Press, London, pp. 195-250.
- Hicks, S. P., C. J. D'Amato and J. L. Falk 1964 Some effects of radiation on structural and behavioral development. *Int. J. Neur.*, 3: 535-548.
- Monjan, A. A., D. H. Gilden, G. A. Cole and N. Nathanson 1971 Cerebellar hypoplasia in neonatal rats caused by lymphocytic choriomeningitis virus. *Science*, 171: 194-196.
- Nathanson, N., G. A. Cole and H. Van der Loos 1969 Heterotopic cerebellar granule cells following administration of cyclophosphamide to suckling rats. *Brain Res.*, 15: 532-536.
- Phemister, R. D., J. N. Shively and S. Young 1969 The effects of gamma irradiation on the postnatally developing canine cerebellar cortex. I. Effects of single sublethal exposure. *J. Neuropath. Exp. Neur.*, 28: 119-127.
- Shimada, M., and J. Langman 1970a Repair of the external granular layer of the hamster cerebellum after prenatal and postnatal administration of methylazoxymethanol. *Teratology*, 3: 119-133.
- 1970b Repair of the external granular layer after postnatal treatment with 5-fluorodeoxyuridine. *Am. J. Anat.*, 129: 247-260.

PLATES

PLATE 1

EXPLANATION OF FIGURES

- 6 Anterior vermis with a wide, middle to lower strip ectopia from a rat that was irradiated with 150 r on days 4, 5, 6 and 7 and killed at 30 days. Hematoxylin-eosin, $\times 101$.
- 7 Thinner middle to lower strip ectopia in the uvula of a rat with similar irradiation history. Note also the supplementary superficial patches of ectopia (arrows). Hematoxylin-eosin, $\times 256$.

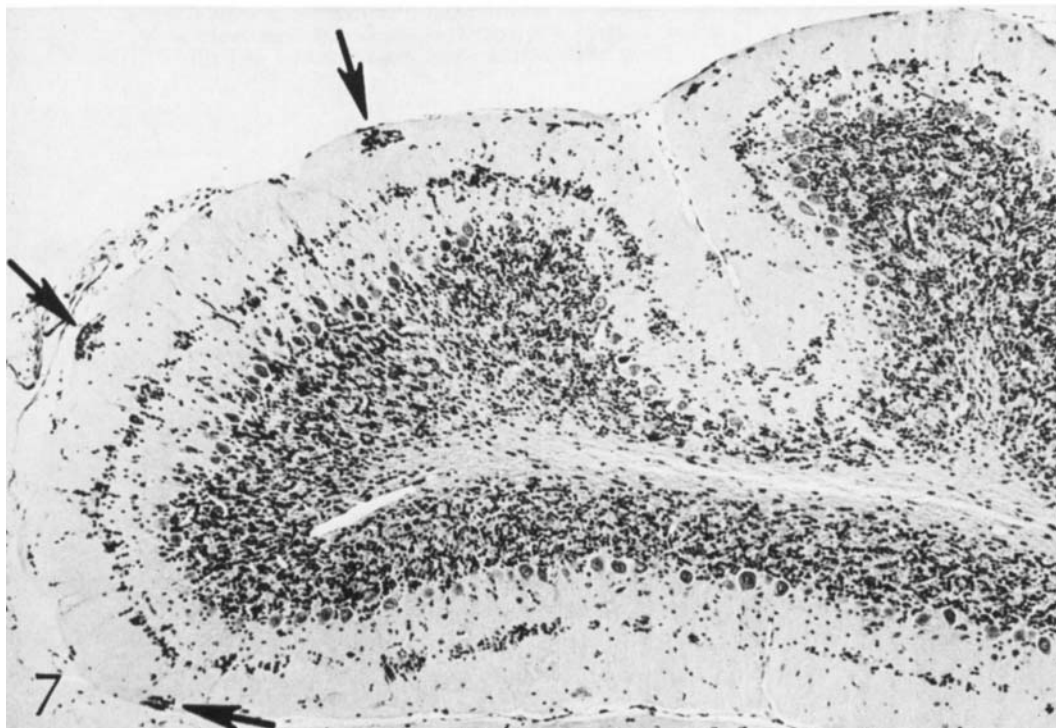


PLATE 2

EXPLANATION OF FIGURES

- 8 Vermis of a normal eight day-old rat. Hematoxylin-eosin, $\times 40$.
- 9 Vermis of an eight day-old rat whose head was irradiated with 150 r on days 4, 5, 6 and 7. Note absence of external germinal layer and the massing of Purkinje cells. At the same magnification as figure 8.

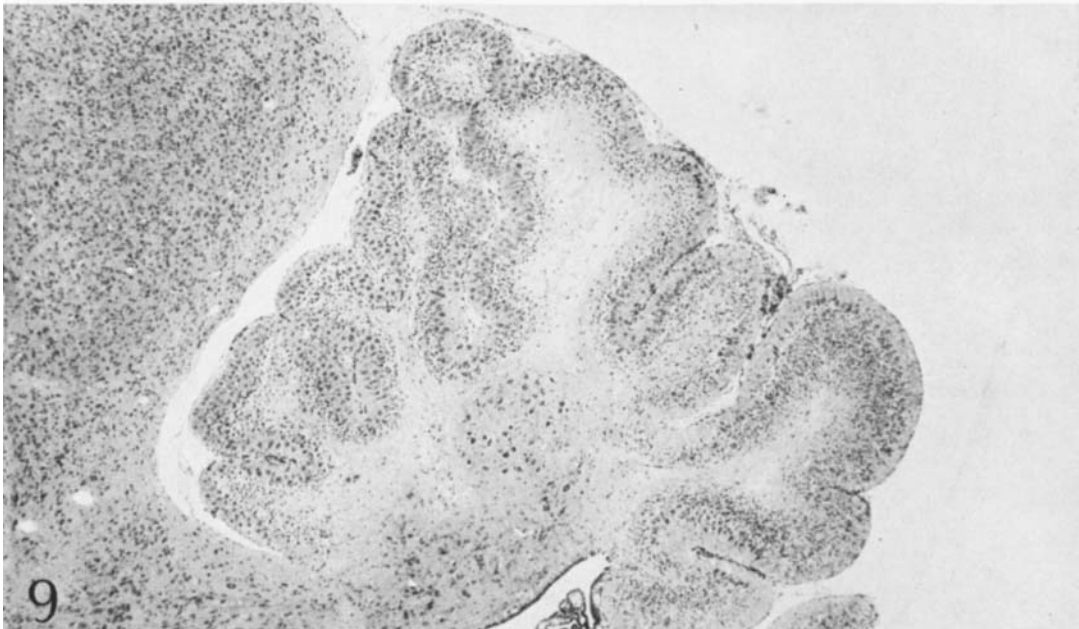
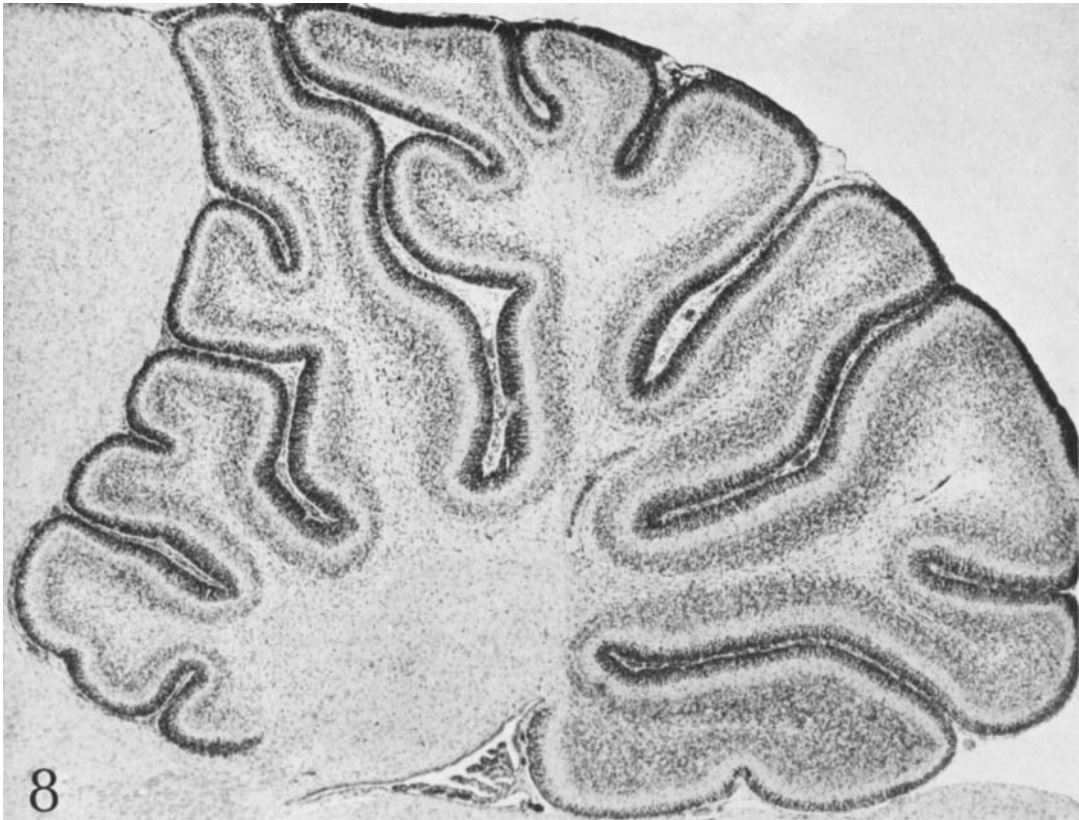


PLATE 3

EXPLANATION OF FIGURES

- 10 Depth of fissura prima in a rat that was irradiated on days 4-7 and killed on day 8. The external germinal layer is eradicated but note the sparing of differentiating cells (arrows) in the thin molecular layer. Hematoxylin-eosin, $\times 256$.
- 11 Depth of fissura posterolateralis showing survival of some radio-resistant cells of the external germinal layer (double arrows). Hematoxylin-eosin, $\times 256$.

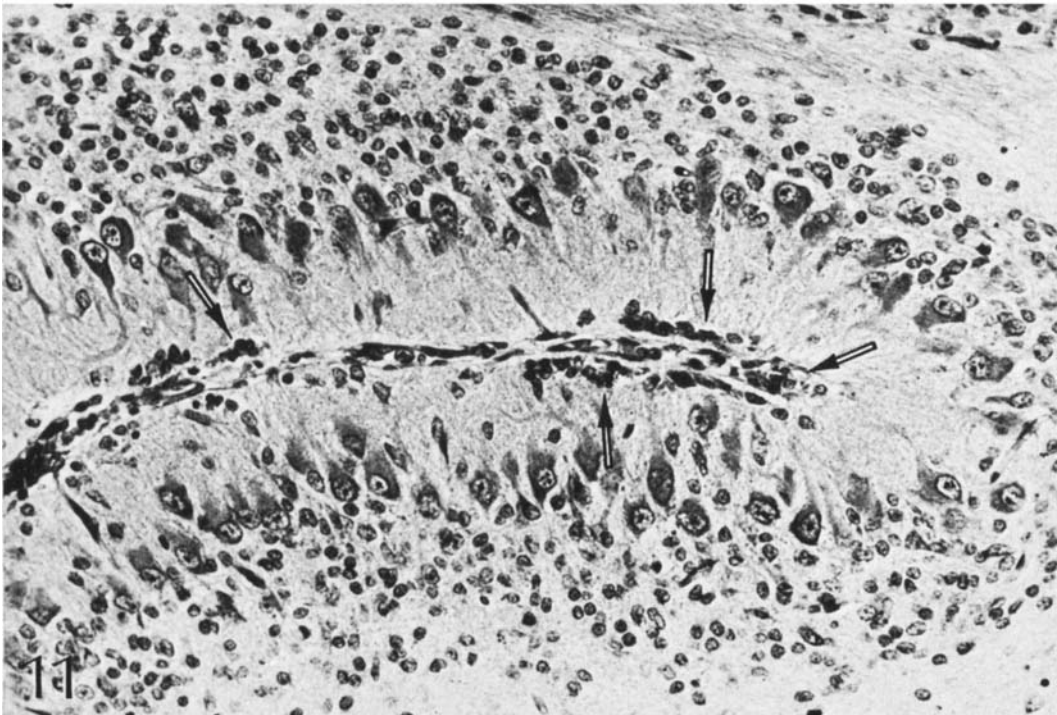
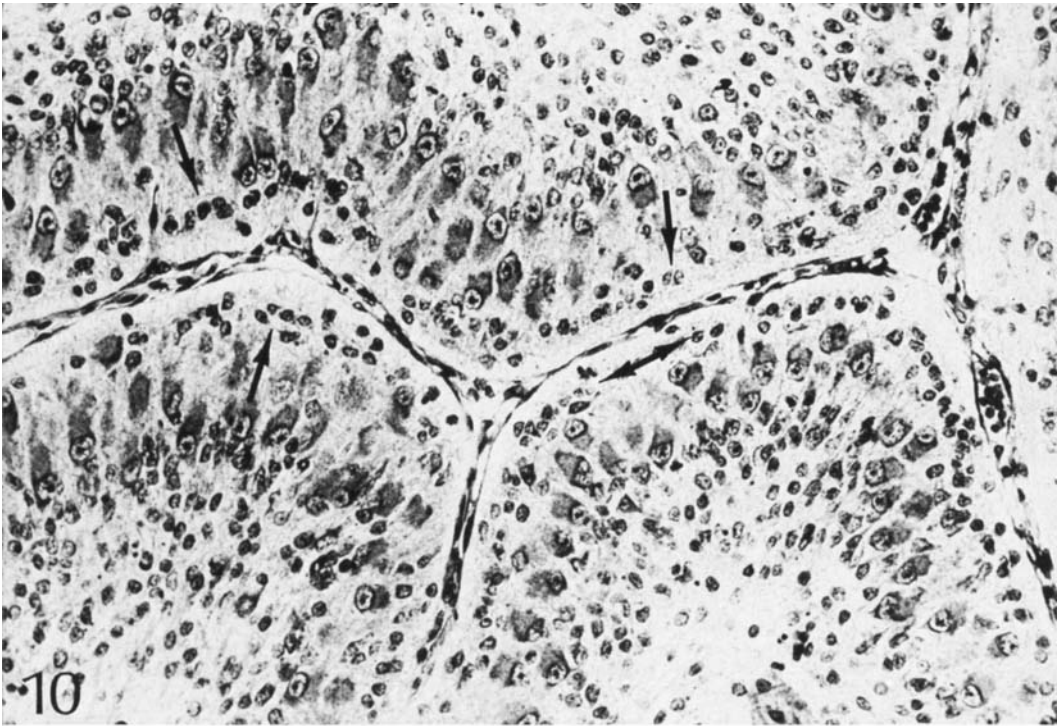


PLATE 4

EXPLANATION OF FIGURES

- 12 Vermis of a rat that was irradiated on days 4, 5, 6 and 7 and killed four days after irradiation at 11 days. Note regeneration of the external germinal layer particularly in the depth and wall of fissures. Hematoxylin-eosin, $\times 40$.
- 13 Fissura prima and adjacent lobules in a rat irradiated on days 4–7 and killed on day 11. There is as yet no evidence of ectopia or migration of the regenerated granule cells. Hematoxylin-eosin, $\times 101$.

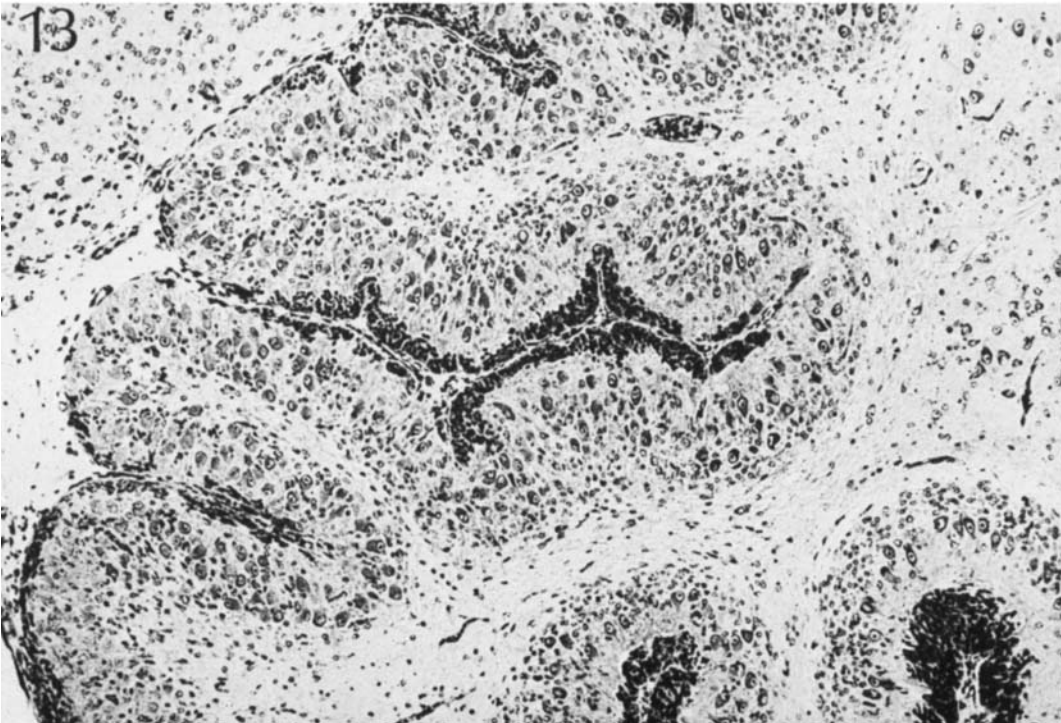


PLATE 5

EXPLANATION OF FIGURE

- 14 Ectopic granule cells in the middle molecular layer of the pyramis of a rat irradiated on days 4-7 and killed at 30 days. A glomerulus is visible on the left with a mossy fiber terminal (MO) and synapses of presumed granule cell dendrites on the terminal and its vicinity (arrows); pf, parallel fibers. $\times 6,384$.

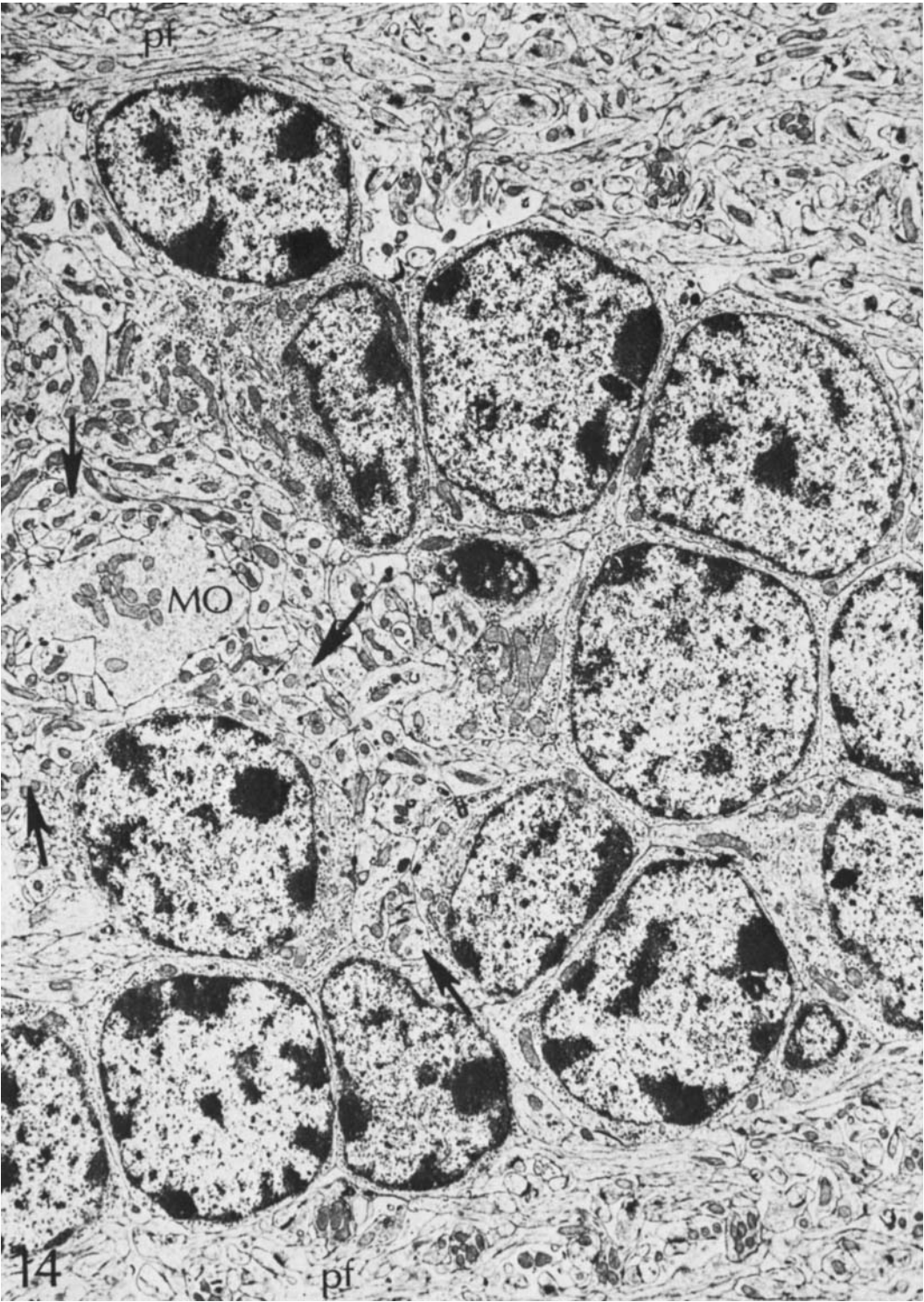
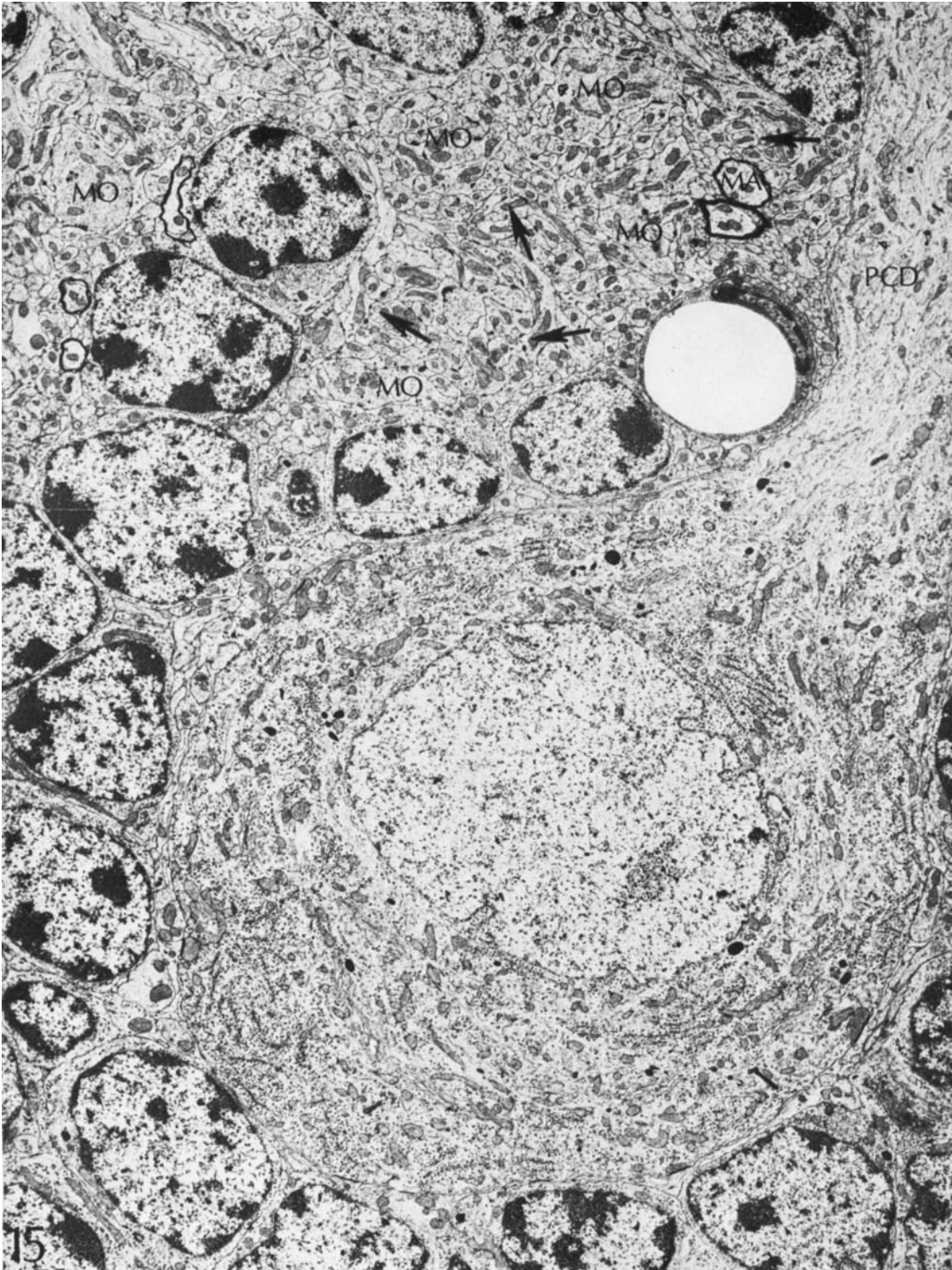


PLATE 6

EXPLANATION OF FIGURE

- 15 Ectopic granule cells in a basal position around the soma and dendrite (PCD) of a Purkinje cell. Mossy fiber terminals (MO), myelinated axons (MA) of presumed mossy fibers, and synapses and desmosoid junctions of presumed granule cell dendrites (arrows) are seen in the lower strip of the molecular layer. $\times 6,202$.



- PLATE 7
- EXPLANATION OF FIGURES
- 16

Ectopic granule cells, mossy fiber terminal (MO), synapses and desmosoid junctions (attachment plaques) of presumed granule cell dendrites (arrows) near the pial surface (PS). BG, Bergmann glial endfoot; pf, parallel fibers. $\times 12,948$.
- 17

Ectopic granule cells, mossy fiber terminal and presumed granule cell dendrites in the upper strip of the molecular layer. $\times 10,944$.

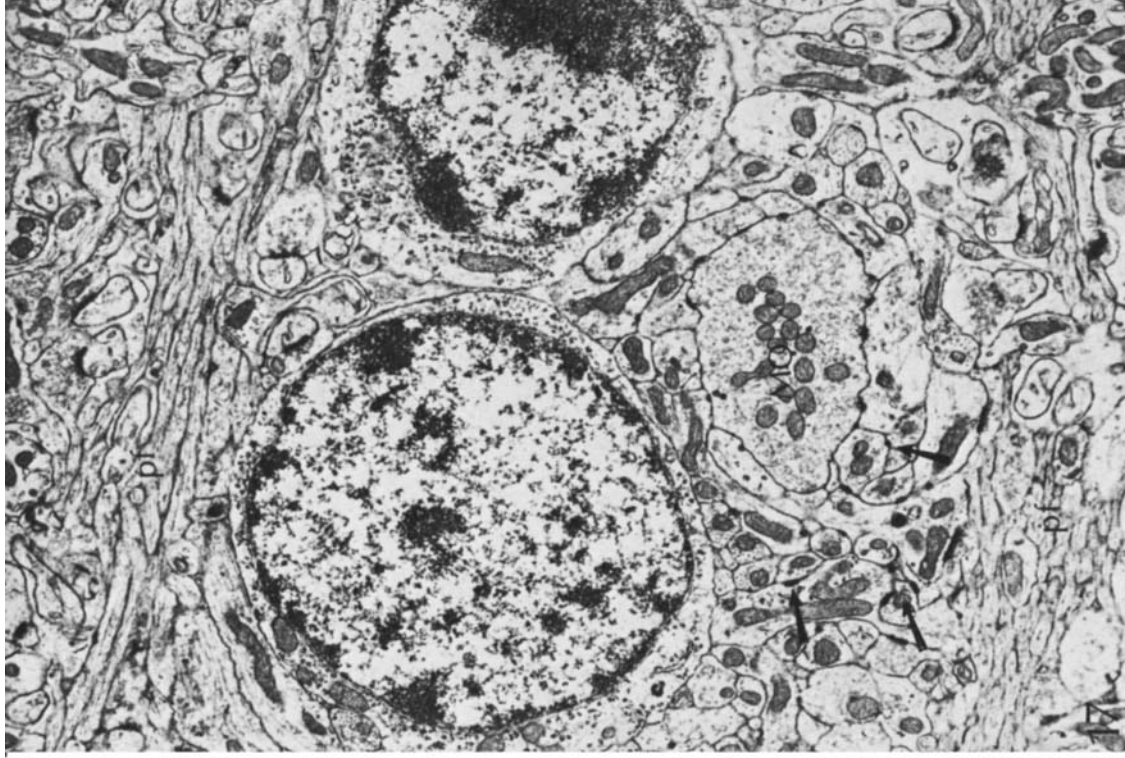
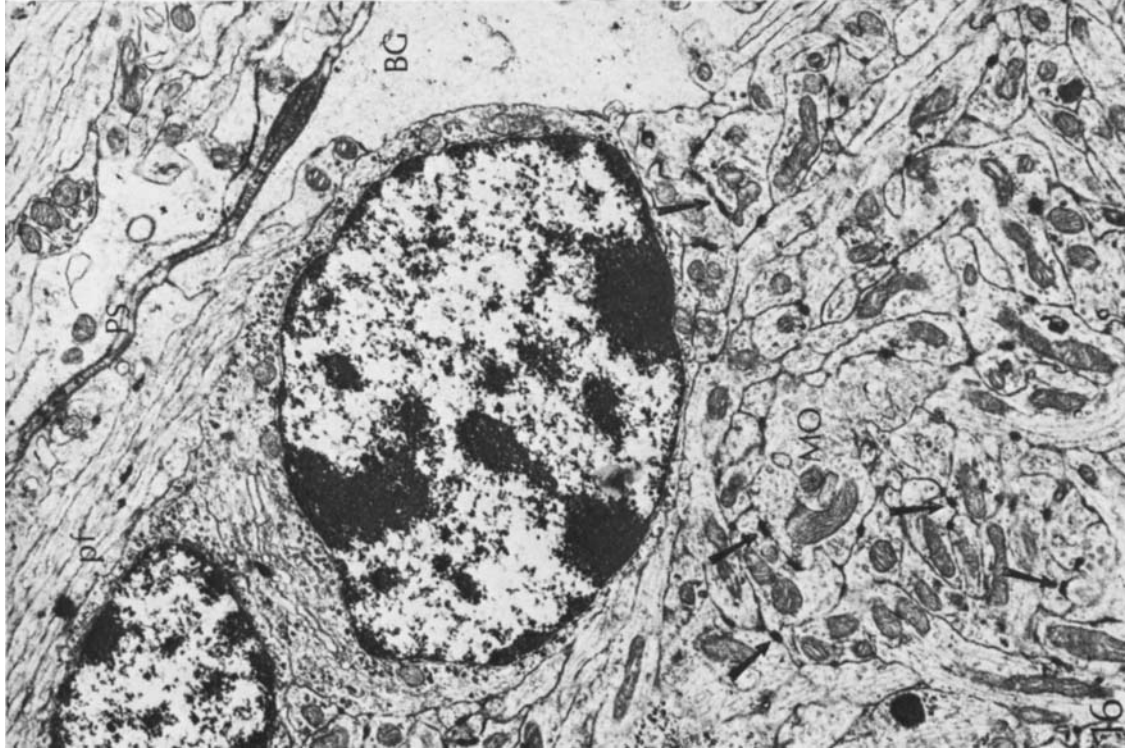


PLATE 8

EXPLANATION OF FIGURES

- 18 Myelinated fibers, presumed mossy fiber axons, in the molecular layer near the pial surface (PS). $\times 11,172$.
- 19 Desmosoid junctions, or attachment plaques (arrows) near a mossy fiber terminal (MO) near an ectopic zone in the middle molecular layer. $\times 41,040$.

