

Experimental Reorganization of the Cerebellar Cortex

V. EFFECTS OF EARLY X-IRRADIATION SCHEDULES THAT ALLOW OR PREVENT THE ACQUISITION OF BASKET CELLS

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ABSTRACT In neonate and infant rats the area of the cerebellum was irradiated with different schedules of single or multiple doses of low-level X-ray. One set of schedules allowed the early recovery of the external germinal layer and the differentiation of all the postnatally-forming cell types while the other selectively prevented the acquisition of basket cells. The first schedule did not interfere with the development of normally oriented and arborizing Purkinje cells. The second schedule led to the growth of twisted and entwined stem dendrites even when, in association with the recovered granule cells, parallel fibers were present in the molecular layer together with Purkinje cell spiny branchlets. Evidence was presented that the alignment of Purkinje cell perikarya in a monolayer does not guarantee the normal growth of Purkinje cell stem dendrites which may be dependent on the presence of basket cells. The problem was discussed whether cell differentiation in the cerebellar cortex is governed by a chronological or sequential principle.

Two previous publications in this series (Altman and Anderson, '72, '73) dealt with the development of the rat cerebellar cortex after irradiation with schedules of low-level (150–200 r) X-rays which prevented the acquisition of basket, stellate and granule cells. Exposure of the cerebellum was started either at birth or on day 4 in order to kill the cells of the external germinal layer (EGL) and it was continued at intervals up to day 13 or 15 to prevent their reconstitution. At 30 days of age the perikarya of Purkinje cells appeared normal in size and ultrastructural organization. However, they were not aligned in a monolayer, the single or multiple stem dendrites were twisted and haphazardly oriented, and typical dendritic arbors, consisting of smooth branches and spiny branchlets in planar orientation, did not develop. It was postulated that the abnormal dendritic growth was not a direct consequence of exposure to X-rays but rather due to the absence of basket, stellate and granule cells which normally enter into intimate relationship with Purkinje cells.

In these continuing publications of the series we describe the effects of several new irradiation schedules on cerebellar development. This paper deals with two

sets of schedules: one that allows early recovery of the EGL and the acquisition of all postnatally-forming cells, including the early-forming basket cells; and another which selectively interferes with the formation of basket cells. The results suggest that in the absence of basket cells, Purkinje cells develop disoriented and twisted stem dendrites. This conclusion is supported by the next study of this series (Altman, '76a) in which X-irradiation was started on day 8, after the acquisition of basket cells but before stellate cells were formed. The treatment led to the development of singularly upright stem dendrites in contiguity with descending basket cell axons. It was postulated that the paucity or lack of smooth branches was due to the absence of stellate cells. When irradiation was started on day 12 after the acquisition of the bulk of stellate cells (Altman, '76b) smooth branches did form. Wherever parallel fibers were present a planar expanse of spiny branchlets developed at a right angle to them.

MATERIALS AND METHODS

As in the previous studies of this series, laboratory-bred, Long-Evans rats were used. The maintenance of the animals and the radiation procedures were described in de-

TABLE 1

X-irradiation schedules and survival after last exposure. Numerals in italics are the number of cerebella examined

Age when irradiated	Survival (in days) after last exposure					Survival (in days) to constant ages				
	1	2	4	6	8	10	15	21	30	
0	5	4	3	3	3	3	3	3	6	4 ¹
0, 1	3	3	3	3	3	3	3	3	6	4 ¹
0, 1, 2	3	3	3	3	3	3	3	3	6	4 ¹
0, 1, 2, 3	3	3	3	3	3	3	3	3	8	4 ¹
4, 5, 6, 7	5	3	6						6	8 ¹

¹ Cerebella impregnated with the Golgi-Cox techniques.

tail before (Altman and Anderson, '72). There were two major experimental groups: in one X-irradiation of the region of the cerebellum was started at birth (day 0), in the other on day 4.

The first group consisted of several subgroups that received a single X-ray dose either on day 0 (0 X), or were exposed on days 0 and 1 (0-1 X), 0, 1 and 2 (0-2 X) and 0, 1, 2 and 3 (0-3 X). Within each of these subgroups three or more animals (table 1) were killed one, two, four, six and eight days after the last exposure in order to assess the extent of the damage done to and the time course of recovery of the EGL, while other rats were killed at the constant ages of 10, 15, 21 and 30 days to determine the long-term effects. Sagittal sections cut at 6 μ were stained with cresyl violet and hematoxylin-eosin. In addition, selected sections in the constant survival group were stained with Bodian's protargol-S method, and four cerebella of 30-day-old rats of each of the four subgroups were prepared with the Golgi-Cox technique. Unirradiated control cerebella were available at all the ages examined.

The second group consisted of rats in which the cerebellar area was exposed to four successive daily doses of 200 r beginning on day 4 (4-7 X). Sagittal cerebellar sections were available from animals killed one, two and four days after the last irradiation and from 30-day-old rats (table 1). Again, control cerebella were used from our normal developmental collection.

RESULTS

A. Effects of X-ray schedules that allow basket cell acquisition

1. Exposure to a single X-ray dose at birth

The destructive effect of exposure of the

cerebellum in newborn rats to a single dose of X-ray (0 X) was examined in sagittal sections of the vermis in animals that were killed one day after irradiation. In three out of five of these 1-day-old rats the EGL was gone as a discrete layer; there were only occasional radio-resistant EGL cells or clumps of cells present, usually in the depth of fissura prima or secunda. In two animals the posterior aspect of the uvula had a one-two cell-thick EGL. This partial sparing in the posterior-most pole of the vermis was due to the narrow X-ray beam used in these experiments; this unintended sparing was noted in several animals that survived for longer periods.

The *time course of recovery* was examined in animals that survived for two, four, six and eight days after irradiation. No change was observed in the first group, except that pyknotic cells were no longer seen. In animals that survived for four days there were clear indications of recovery of the EGL (fig. 1A). The pattern of recovery was highly variable: a one-three cell-thick EGL was present in some regions while in others the surface of the cerebellum was still devoid of these cells. Recovery tended to be more pronounced in the depth of fissures but there were exceptions. This agrees with earlier observations (Altman et al., '69). In the 4-day-old 0 X rats the Purkinje cells were still piled up (fig. 1A), unlike in normal rats of this age in which the Purkinje cells are aligned in a monolayer (fig. 1B) in all lobules except the late-forming folium and tuber. Six to eight days after irradiation the EGL reappeared over the entire vermis (fig. 1C) and in some regions it was supernormal in thickness; the latter phenomenon was often coupled with abnormal orientation of the differentiating bipolar cells. In these

animals the majority of Purkinje cells were aligned in a monolayer with their apical cones oriented, as in normal cerebella, toward the surface; but some exceptions were seen. In the molecular layer translocating granule cells and a few differentiating (presumably basket) cells were present in many regions by eight days; by ten days they were numerous.

The *lasting consequences* of irradiation with a single dose at birth were examined in 30-day-old rats. Well developed molecu-

lar and granular layers were present consistently, the only sign of the early irradiation was a slight or moderate distortion in the foliation pattern of the vermis (fig. 2). Examination of five cerebella of 30-day-old O X rats prepared with Bodian's protargol-S method showed that basket cell axons were present in the lower one-third-one-fourth of the molecular layer in the affected lobules. But in the more distorted lobules the orientation of parallel fibers (normally coronal) and of the horizontal

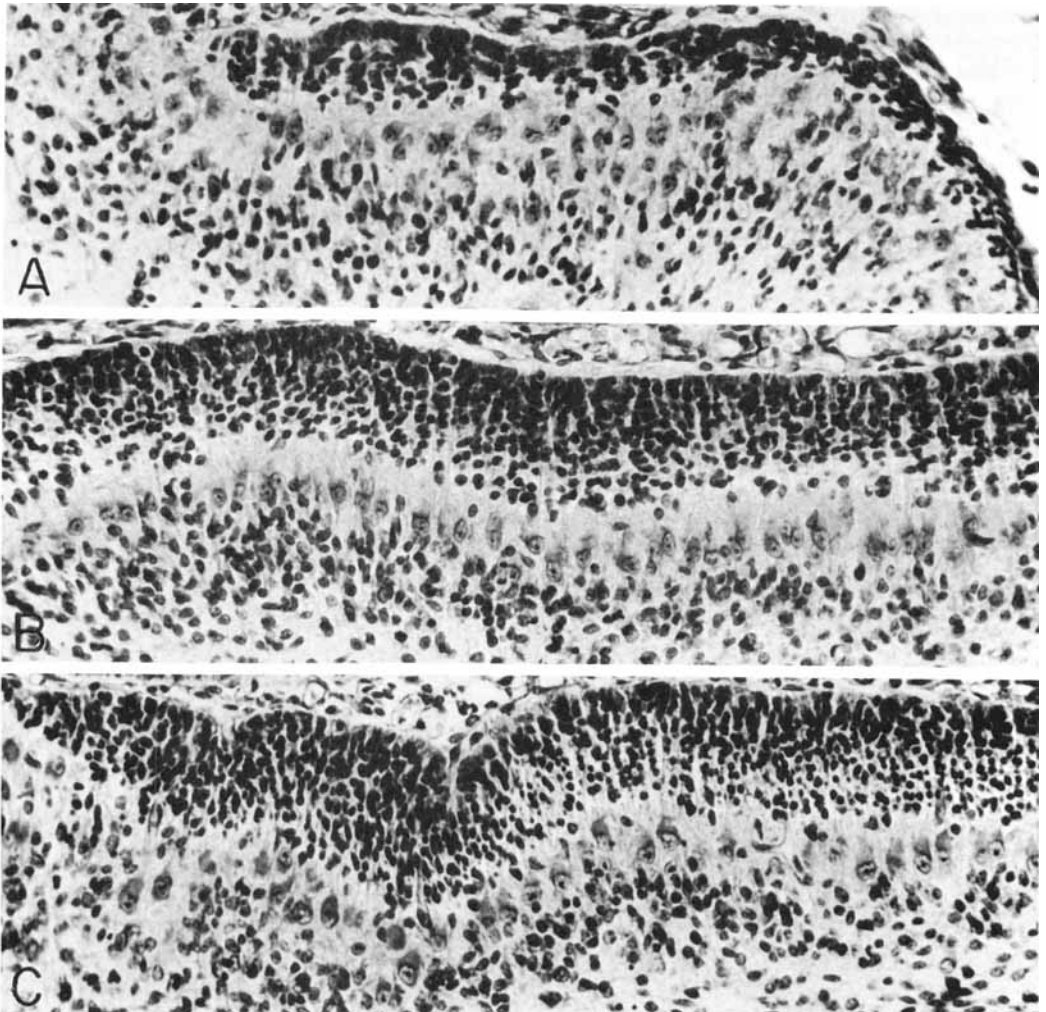


Fig. 1 Bank of fissura prima. A. Recovery of the EGL in a rat irradiated at birth (O X) and killed at four days of age. Note that the Purkinje cells are not aligned in a monolayer. B. Normal rat, four days. Note the thickness of the EGL and the alignment of Purkinje cells in a monolayer. C. Recovery of the EGL in O X rats at six days. Note the delayed alignment of Purkinje cells in a monolayer, the upward orientation of Purkinje cell apical cones, and the presence of some spindle-shaped, translocating cells in the molecular layer. Hematoxylin-eosin, $\times 244.8$.

branches of basket cell axons (normally sagittal) was altered. Impregnated basket axon terminals around the somata of Purkinje cells were also frequent, though the impression was gained that they tended to be less richly ramifying around Purkinje cells in the affected lobules than in control cerebella, perhaps reflecting a reduction in the number of basket cells.

Examination of four sagittally sectioned cerebella impregnated with the Golgi-Cox technique showed that the majority of Purkinje cells were normal in orientation and appearance, with richly arborizing planar dendrites (fig. 3). Basket, stellate, granule, and Bergmann glial cell somata and processes were impregnated in large numbers and were indistinguishable from such cells

in normal animals. The conclusion is, accordingly, justified that exposure of the cerebellum to a single dose of 200 r X-ray at birth, which eradicates subtotally the EGL, does not interfere with the subsequent acquisition of any postnatally-forming cell type of the cerebellar cortex. The only detectable effect is the slight to moderate distortion of some lobules and an associated rotation of parallel fibers and basket cell axons.

2. *Exposure to two successive daily doses beginning at birth*

The destructive effect, course of recovery and lasting consequences of exposure to 200 r X-ray on days 0 and 1 (0-1 X) was examined in a longitudinal series of

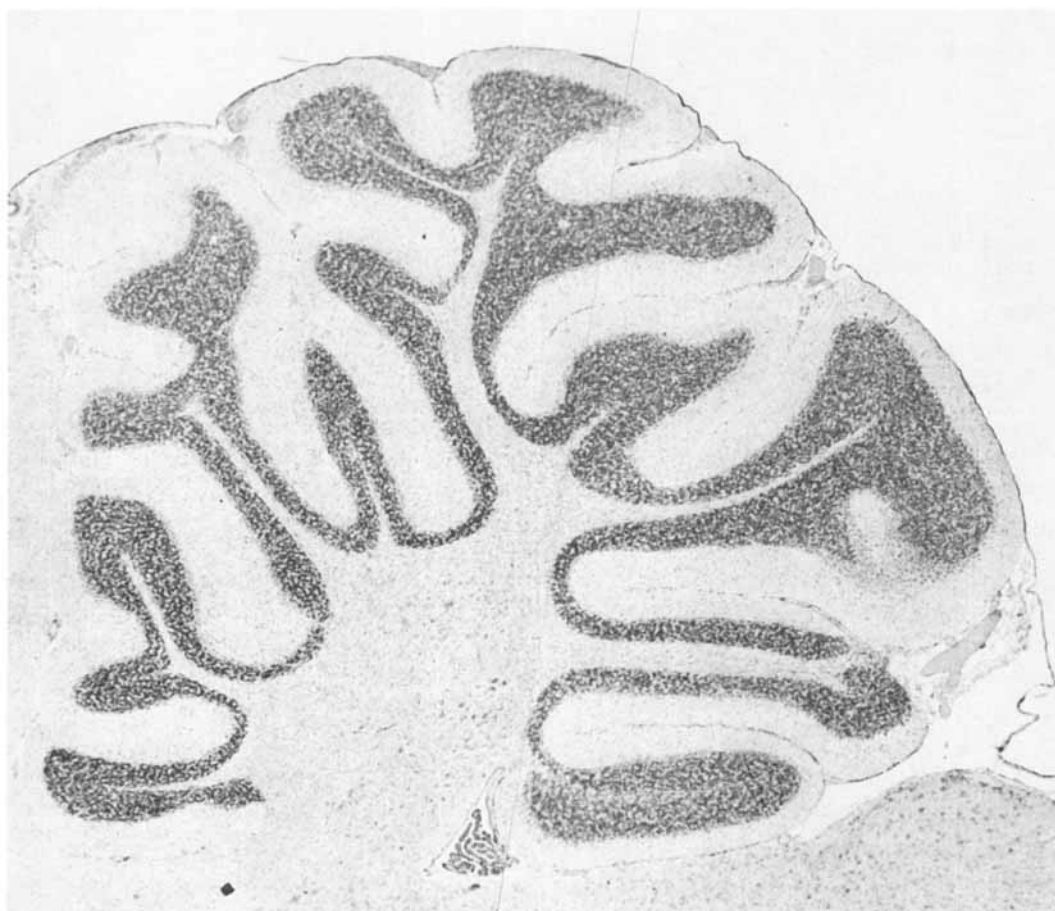


Fig. 2 Sagittal section of the vermis in a 21-day-old, 0 X rat. Molecular and granular layers are well developed but there is moderate lobular distortion. Hematoxylin-eosin, $\times 28$.

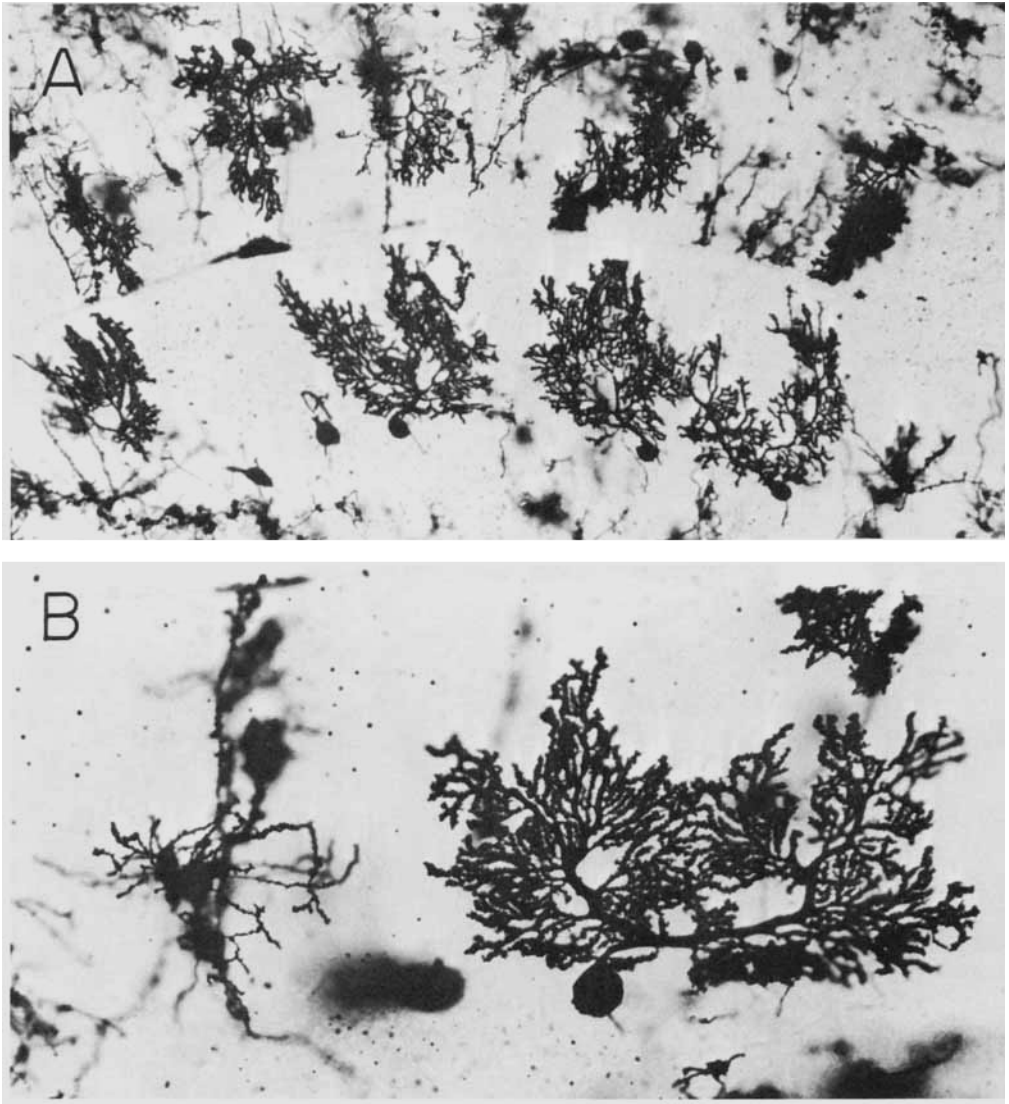


Fig. 3 A. Purkinje cells with normally arborizing, planar dendrites in the fissura prima of a 30-day, 0 X rat. Golgi-Cox, $\times 113$. B. Purkinje cell and basket cell (left) from the same region. $\times 259.2$.

cerebella similar to that described in the previous section. The EGL was gone one day after the last irradiation (2 days of age) except for a few scattered cells or clumps of cells in the depth of some fissures. Recovery was first noted in 5-day-old rats in the form of a thin (1–3 cell-thick) EGL in some lobules, but it was still absent in other regions. The Purkinje cells were not dispersed in a monolayer but the majority had apical cones with nor-

mal orientation. In the 7- and 9-day-old rats the EGL was present throughout the vermis, ranging in thickness from subnormal to supernormal. As yet differentiating cells in the molecular layer were rare or absent. In 10–11-day-old rats the molecular layer, which was often thin, had both differentiating and translocating cells. Lobular and laminar malformation was the general rule, ranging from mild to moderate. Developmental regression in the

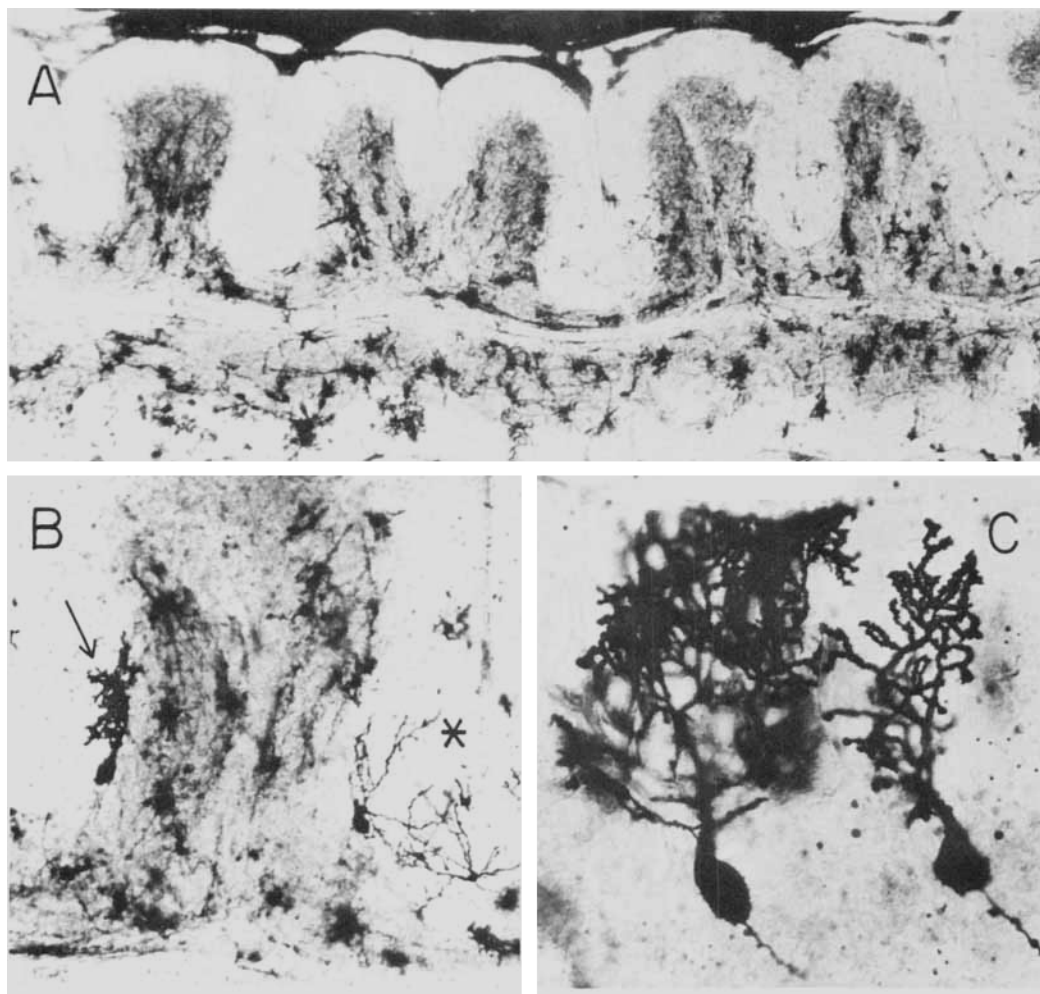


Fig. 4 A. Abnormally foliated uvula in a 30-day-old, 0-2 X rat. Golgi-Cox. $\times 45$. B. Abnormally oriented Purkinje cell (arrow) and stellate or basket cells (star) from another section in above region. $\times 113$. C. Normally arborizing Purkinje cells (one of reduced size) from another section in the same region. $\times 259.2$.

thickness of the EGL was evident in the 15-day-old 0-1 X rats. By this time the molecular and granular layers have grown appreciably. The EGL disappeared, as in normal rats, by 21 days.

The terminal effect in the Nissl-stained cerebella of 30-day-old, 0-1 X rats was a pronounced lobular malformation, some reduction in the size of the vermis, absence of monocellular dispersion of Purkinje cells in various regions with a few disoriented Purkinje cells. But examination of the Bodian-stained material established that even in regions with pro-

nounced lobular malformation there were basket cell axons and terminal baskets present. In these regions the horizontal axons occupied a narrow zone of the molecular layer or were restricted to the interface of the molecular and Purkinje cell layers. Apparently some of the recovered cells differentiated as basket cells but either these were few or their degree of differentiation was limited. Four Golgi-impregnated cerebella from 30-day-old, 0-1 X rats provided a complex picture of the effects of this schedule of irradiation. In all lobules normal granule cells were seen

in abundance together with parallel fibers. The parallel fibers were normally oriented in some lobules, disoriented in others. Bergmann glial processes were relatively rare and some of them looked abnormal (e.g., oblique) in orientation. Stellate cells were frequent throughout but basket cells were recognizable only in minimally affected lobules. In the latter regions, the Purkinje cells that were aligned in a monolayer tended to be normal in orientation and arborization. But where Purkinje cells were not strung out in a monolayer they displayed a variety of abnormal patterns. The most common feature was the disorientation of the stem dendrite, a multiplicity of such dendrites issuing at different points from the perikaryon, and the paucity or absence of spiny branchlets.

3. *Exposure to three successive daily doses beginning at birth*

The destructive effect of exposure to 200 r X-ray on days 0, 1 and 2 (0-2 X) was similar to that seen in the 0-1 X group, but recovery was further delayed and less complete, and the terminal effects were more severe. By eight-ten days

after the last irradiation (10-12-day-old rats) the EGL has recovered throughout the vermis but it was quite thin in most lobules. As a rule, the bipolar cells were disoriented and foliation was quite abnormal. In many regions a molecular layer could not be recognized but where it was present as a thin layer it contained a few differentiating cells and some translocating granule cells. In most regions the Purkinje cells were not strung out in a monolayer but there were some exceptions. Granule cells were accumulating at these ages and became abundant at 15 days. The EGL was gone in the 21-day-old rats.

The terminal effect in 30-day-old, 0-2 X rats was a severe lobular malformation (fig. 4A). This was coupled with pronounced reduction in the area of the cerebellum, attributable to a decrease in the size of the granular and molecular layers. In many regions the molecular layer appeared in the form of transverse streaks or islands and in these regions, as seen in Bodian material, the parallel fibers were haphazardly oriented. Horizontally-oriented basket axons were rarely seen but sparse basket terminals were seen in

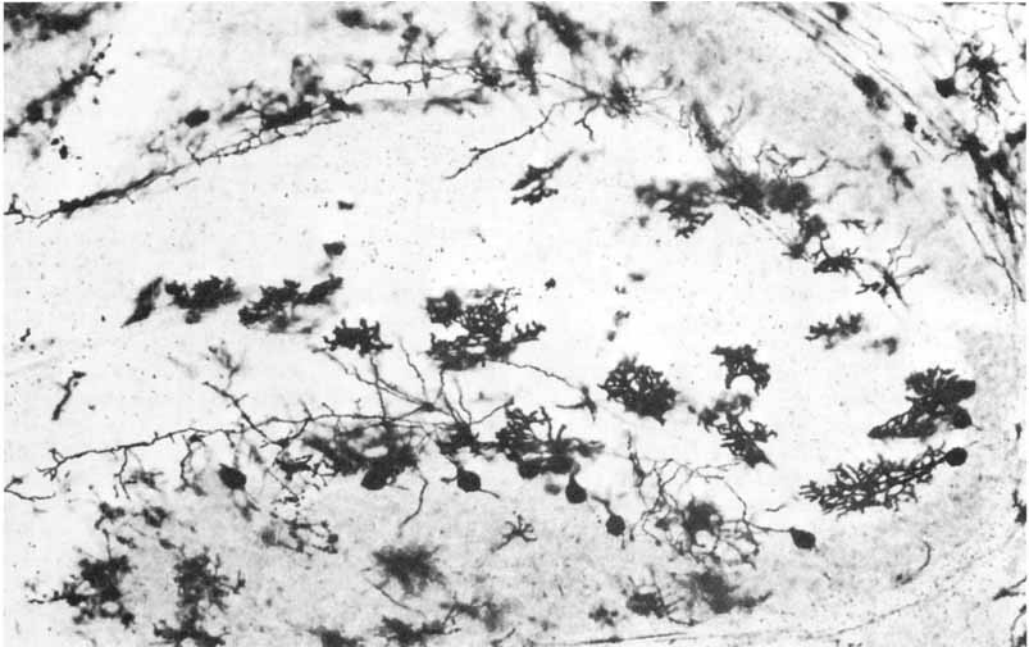


Fig. 5 Portions of dorsal and ventral uvula in a 30-day-old, 0-2 X rat. Note basket cell axons near the interface of the granular and molecular layers. Golgi-Cox, $\times 113$.

those few regions where Purkinje cells formed a monolayer or in association with those Purkinje cells that were superficially situated at the interface of the granular and molecular layers.

In Golgi material the great majority of Purkinje cells appeared abnormal in orientation and arborization (figs. 4B, 5). Nevertheless, a rare normally oriented and normally arborizing Purkinje cell was seen adjacent to many others that were abnormal (fig. 4C). In some regions occasional basket cells could be seen (fig. 5). Granule cells were frequent; stellate and Golgi cells were seen in limited numbers; Bergmann glial processes were extremely rare.

In summary, two–three exposures of the cerebellum to X-ray from birth led to a progressive reduction in the concentration of basket cells, an increase in the number of malformed Purkinje cells, and severe lobular malformation. This was in contrast to the minimal effects seen after exposure to a single dose of X-ray, which resulted in mild lobular malformation and no apparent interference with Purkinje cell dendritic development.

B. Effects of X-ray schedules that prevent basket cell formation

1. Exposure to four successive daily doses beginning at birth

In the animals that were irradiated on days 0, 1, 2 and 3 (0–3 X) there were no signs of EGL recovery, as in the previous groups, four days after the last irradiation. After six days (that is, in 9-day-old rats) recovery was still restricted to the uvula (the region that was occasionally missed by the X-ray beam). The EGL reappeared in fragmentary form in 10-day-old rats and nearly covered the entire vermal surface eight days after the last exposure (11 days of age). In the depth of several fissures the EGL was supernormal in thickness, elsewhere it tended to be subnormal. Where the molecular layer was recognizable it was still devoid of differentiated cells but a few translocating granule cells were present. The Purkinje cells were massed several cells thick and their apical poles were randomly oriented. Differentiating cells in the molecular layer were beginning to appear ten days after the last radiation exposure (13 days of age) and by this time there was an appreciable

accumulation of granule cells. As in all other groups the EGL disappeared at 21 days.

The typical terminal effects seen in the 30-day-old, 0–3 X animals were the following: considerable lobular malformation; thin molecular layer with frequent streaks or islands (this is a result of abnormal and excessive foliation); moderate accumulation of differentiated cells in the molecular layer, including some ectopic granule cells; scattering of Purkinje cells in the granular layer with disoriented apical poles; and, finally, a fair concentration of normally located granule cells. In Bodian-stained sections the molecular layer was devoid of horizontal basket cell axons. Basket terminals with brush-endings around Purkinje cells were either absent or were seen as rare exceptions in a few regions. These observations suggested that the late recovering EGL, which produced ectopic and normally located granule cells, failed to produce basket cells in appreciable numbers.

Normally oriented and normally arborizing Purkinje cells were not seen in Golgi-impregnated cerebella of 30-day-old, 0–3 X rats (figs. 6, 7). The dendrites of the scattered and randomly orienting Purkinje cells took various abnormal shapes. Most frequently a single, randomly pointing stem dendrite gave off several three-dimensionally oriented “smooth” branches. These branches, particularly where they were located in the granular layer (fig. 6) were devoid of spiny branchlets; tufts of spiny branchlets were present on other branches, particularly those ending in the molecular layer (fig. 7A). Other Purkinje cells had two or three stem dendrites issuing from their perikarya (fig. 7B) and, in rarer instances even more. These supernumerary stem dendrites had branches and the latter were occasionally associated with spiny branchlets. As a result of the haphazard orientation of the Purkinje cell dendritic processes they were often entangled or crisscrossing each other.

Granule cells, whether located in the molecular layer or in the granular layer, were normal in appearance. Golgi cells were not infrequent and had richly arborizing axon plexuses. The stellate-type cells seen in the molecular layer had richly arborizing dendrites but basket cell axons

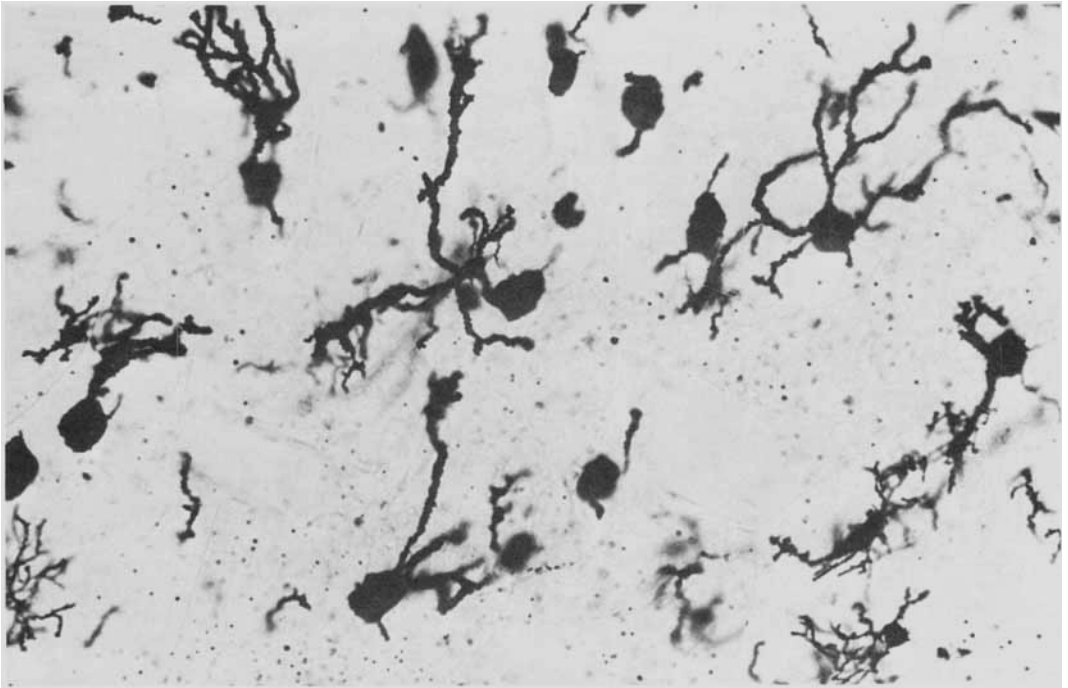


Fig. 6 Purkinje cells with randomly oriented, multiple stem dendrites that are devoid of spiny branchlets. 30-day-old, 0–3 X rat. Golgi-Cox, $\times 230.4$.

and brush terminals were virtually absent, suggesting that independent of their location in the molecular layer these cells were stellate cells. The extremely rare basket terminals encountered were associated with regions where Purkinje cells were aligned in a monolayer, suggesting that these may have been inadequately irradiated sites.

2. *Exposure to four successive daily doses beginning at four days*

The foregoing results established that irradiation of the cerebellum with a single dose of X-ray on the day of birth does not interfere with the development of normally oriented and normally arborizing Purkinje cells while irradiation with four successive daily doses prevents normal development. Two or three successive doses had mixed effects. The development of normal Purkinje cells after a single dose was associated with early recovery of the EGL, alignment of Purkinje cells in a monolayer, and the acquisition of basket, stellate and granule cells in large numbers. The failure of normal Purkinje cell development was associated with delayed

recovery of the EGL, scattering of Purkinje cells, and the absence of basket cells; the later forming stellate and granule cells were present in variable numbers.

In an attempt to establish whether alignment of Purkinje cells in a monolayer or presence of basket cells is the necessary condition of normal Purkinje cell development, the cerebellum was irradiated in a group of rats with four successive daily doses of X-ray from day 4 on (4–7 X), beginning at a time when the Purkinje cells are already aligned in a monolayer in most vermal lobules. It was expected that this irradiation schedule will prevent the acquisition of basket cells which starts around days 4–5 and falls to low levels after days 8–9 (Altman, '72) but will allow the formation of at least a substantial number of stellate and granule cells.

The stage of morphological maturation of the cerebellar vermis was re-examined in three normal 4- and 8-day-old rats, the extent of damage, recovery and terminal effects were studied in three–six animals each that survived for one, two and four days after the last irradiation. Terminal

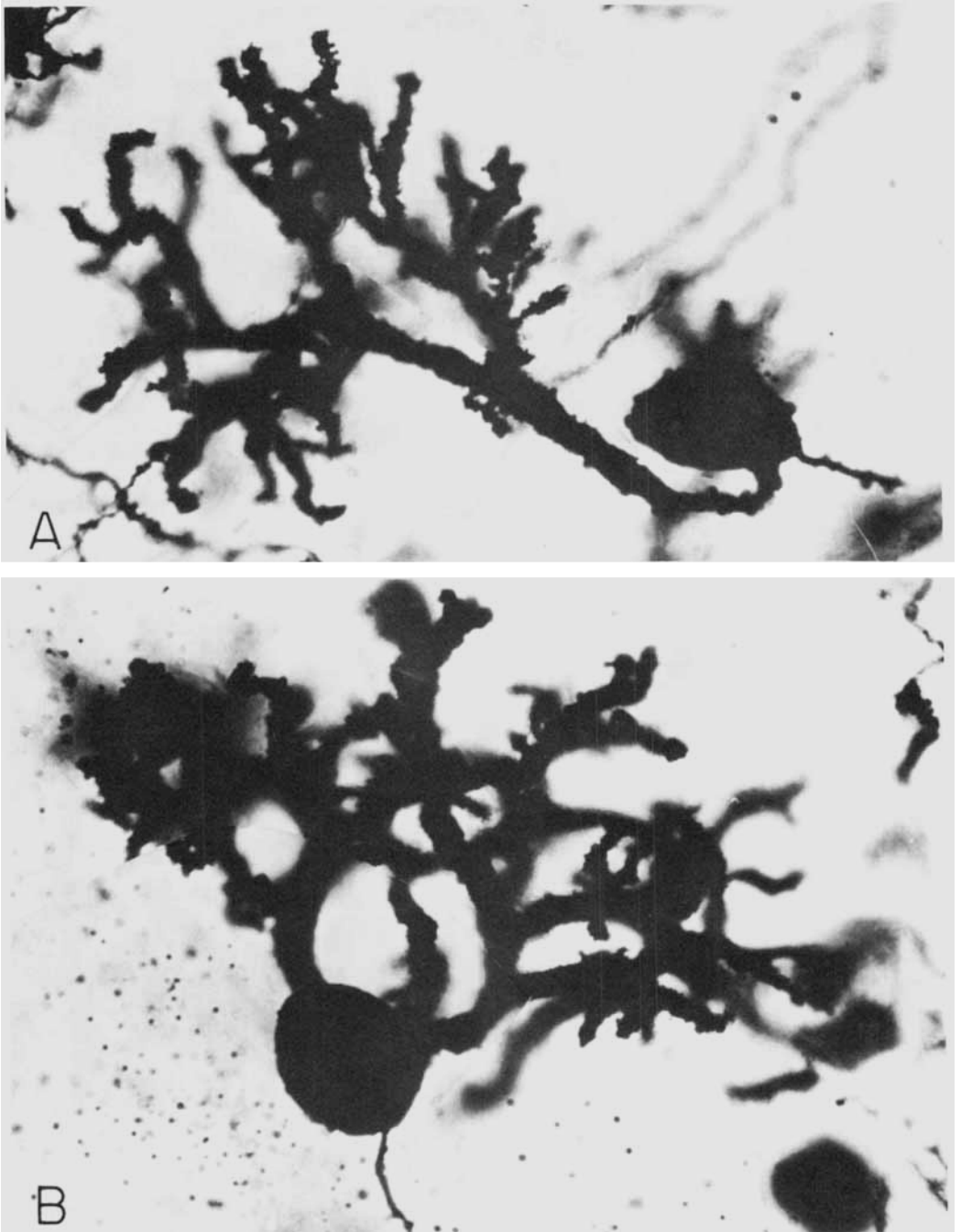


Fig. 7 Purkinje cells in a 30-day-old, 0-3 X rat. A. Cell with a single, abnormally oriented and twisted stem dendrite, several smooth branches and some spiny branchlets. B. Cell with multiple stem dendrites and a few smooth branches. Golgi-Cox, $\times 684$.

effects were assessed in seven cerebella prepared with the Nissl and Bodian techniques, and in eight cerebella impregnated with the Golgi-Cox technique.

In 4-day-old control rats the EGL is about 8 cell-thick in most vermal lobules and the Purkinje cells are aligned in a monolayer, though a few cells may be situated above the row (fig. 1B). In the late maturing lobules (folium and tuber) the Purkinje cells are not yet aligned in a monolayer. The aligned Purkinje cells have large apical cones that are oriented toward the surface and tend to be flat and broad at the top. Only in the earliest maturing lobules (nodulus, ventral uvula, and the depth of fissura prima) are Purkinje cells seen with pointed apical cones, to suggest the onset of stem dendrite development. A thin molecular layer with a few parallel fibers is recognizable in all but the latest maturing lobules, but few differentiating cells are present, with the possible exception of the nodulus and ventral uvula. In summary, the Purkinje cells were mostly aligned in a monolayer but the differentiation of basket cells had hardly begun at the time when irradiations were started.

One day following the last irradiation (8 days, fig. 8B) the EGL was gone with the exception of a few surviving cells in the depth of several fissures. The Purkinje cells were no longer in a monolayer in most regions, but piled up 2-3 cell-thick. In most lobules there was a thin granular layer and molecular layer; the latter contained some normally oriented parallel fibers (seen in Bodian-stained sections) but no cells. The nodulus and ventral uvula were exceptions in some animals, containing Purkinje cells aligned in a monolayer and a few differentiating cells, presumably basket cells. In comparison, in the 8-day-old unirradiated cerebella (fig. 8A) a thick EGL was present, all Purkinje cells were aligned in a monolayer, and the molecular layer contained perpendicularly oriented Purkinje cell stem dendrites and differentiating basket cells two-three cells deep.

Little change was seen in the 4-7 X rats that survived for two days after the last day of irradiation. But in the animals that survived for four days (fig. 8C) EGL recovery began. In all animals a thin, some-

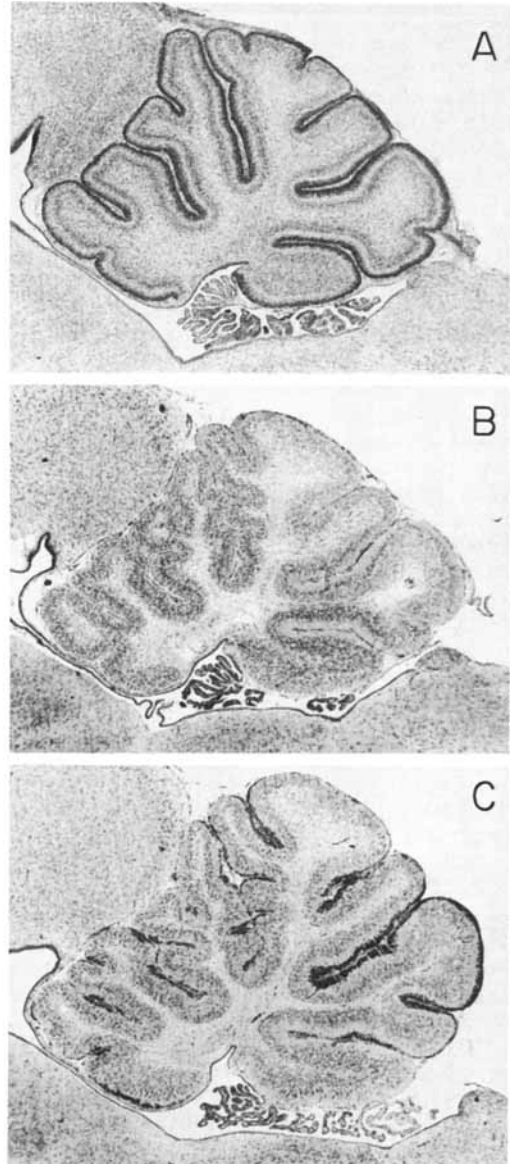


Fig. 8 Sagittal sections of the vermis. A. Normal 8-day-old rat. B. 4-7 X, 8-day-old rat (animal was killed one day after last irradiation). Note absence of EGL. C. 4-7 X, 11-day-old rat (animal was killed four days after last irradiation). Note patchy reappearance of EGL. Hematoxylin-eosin, $\times 18.2$

times patchy EGL was present in the depth and banks of fissures; over the gyral surfaces there was greater variability in recovery of the EGL. In early-maturing lobules (fig. 9A) of these 11-day-old rats there

was a thin molecular layer with differentiating basket cells and Purkinje cells that had normally oriented stem dendrites. It was assumed that these basket cells were formed before irradiation started and were differentiating in the meantime. In intermediate (fig. 9B) and late-maturing (fig. 9C) lobules the molecular layer was thin or virtually absent, basket cells could not be identified, and Purkinje cells had haphazardly oriented stem dendrites. This indicated that cells of the recovered EGL have not yet started to differentiate at 11 days.

In Nissl-stained sections of 30-day-old 4-7 X cerebella ectopic and normally located granule cells were present in variable numbers, ranging from abnormally high concentration to patchy distribution in different lobules and different animals. Likewise, the width of the molecular layer varied among lobules with some streaking and island formation. In Bodian-stained sections impregnated basket axons were not seen in the molecular layer and brush-endings around Purkinje cell perikarya were likewise absent. This suggested that the cells formed in the molecular layer after irradiation were stellate and glial cells not basket cells. In Golgi-impregnated sections granule cells were impregnated in large numbers; some of these were in ectopic position, as previously noted (Altman, '73a), others were situated in the granular layer. Parallel fibers were, likewise, numerous but frequently they were abnormally oriented. Basket cell axons and brush-endings were absent in most regions with the exception of the nodulus and ventral uvula. Cells with richly arborizing dendrites in the molecular layer were seen in variable numbers; it was assumed that these were stellate cells. Many Purkinje cells were normal in orientation and appearance in the nodulus and ventral uvula but everywhere else they had one (fig. 10) or more disoriented and twisted stem dendrite, numerous smooth branches pointing in different directions, and highly variable tufts of spiny branchlets. The most striking characteristic of these cerebella was the intertwining of Purkinje cell arbors as a result of the disoriented and irregular growth of the stem dendrite (fig. 11).

DISCUSSION

Three factors have complicated our at-

tempt to interpret these results. First, there are appreciable differences in the maturational rates of different lobules: Purkinje cells become aligned in a monolayer and basket cells differentiate several days earlier in some regions than in others. This circumstance, by itself, is not difficult to handle because we have some knowledge of the sequence of regional maturation of different lobules (Altman, '69); indeed we could turn it to our advantage by comparing treatment effects in different lobules within the same animal. The second complication is due to the considerable, as yet unaccounted for variability in EGL recovery after X-irradiation. The EGL may be supernormal in thickness in one region while in another, often adjacent region it has hardly begun to reappear. In principle this problem too can be dealt with because the degree of EGL recovery in a particular region can be reconstructed from the terminal size (thickness) of the molecular and granular layers. But in practice this requires statistical treatment and this was not attempted in this study for several reasons, including the small number of brains available within each group. Finally, an additional complication came from the circumstance that we used a narrow, age-adjusted X-ray beam. This was designed to spare structures neighboring the cerebellum but its consequence was, because of the minimal margin for placement error, that in some animals parts of the vermis, particularly the posterior lobules were not always irradiated. Even though unirradiated regions are easy to identify by the absence of lobular and laminar malformation, which is produced by a single exposure at birth, in the case of multiple exposures it is virtually impossible to judge whether the examined region received all the intended irradiations.

Fig. 9 From a sagittal section of the vermis of an 11-day-old, 4-7 X rat (animal was killed four days after last irradiation). A. Early-maturing ventral uvula. In this region the primary dendrites of Purkinje cells are normally oriented (heavy arrows) and there are differentiated basket cells present (light arrows) which were presumably formed before irradiation was started. B. Bank of fissura prima. The apical cones of Purkinje cells in this intermediate region are oriented in different directions (open arrows). There are few if any differentiated basket cells. C. Late-maturing tuber. Molecular layer is hardly recognizable and Purkinje cells display an early developmental phase. Hematoxylin-eosin, $\times 259.2$.

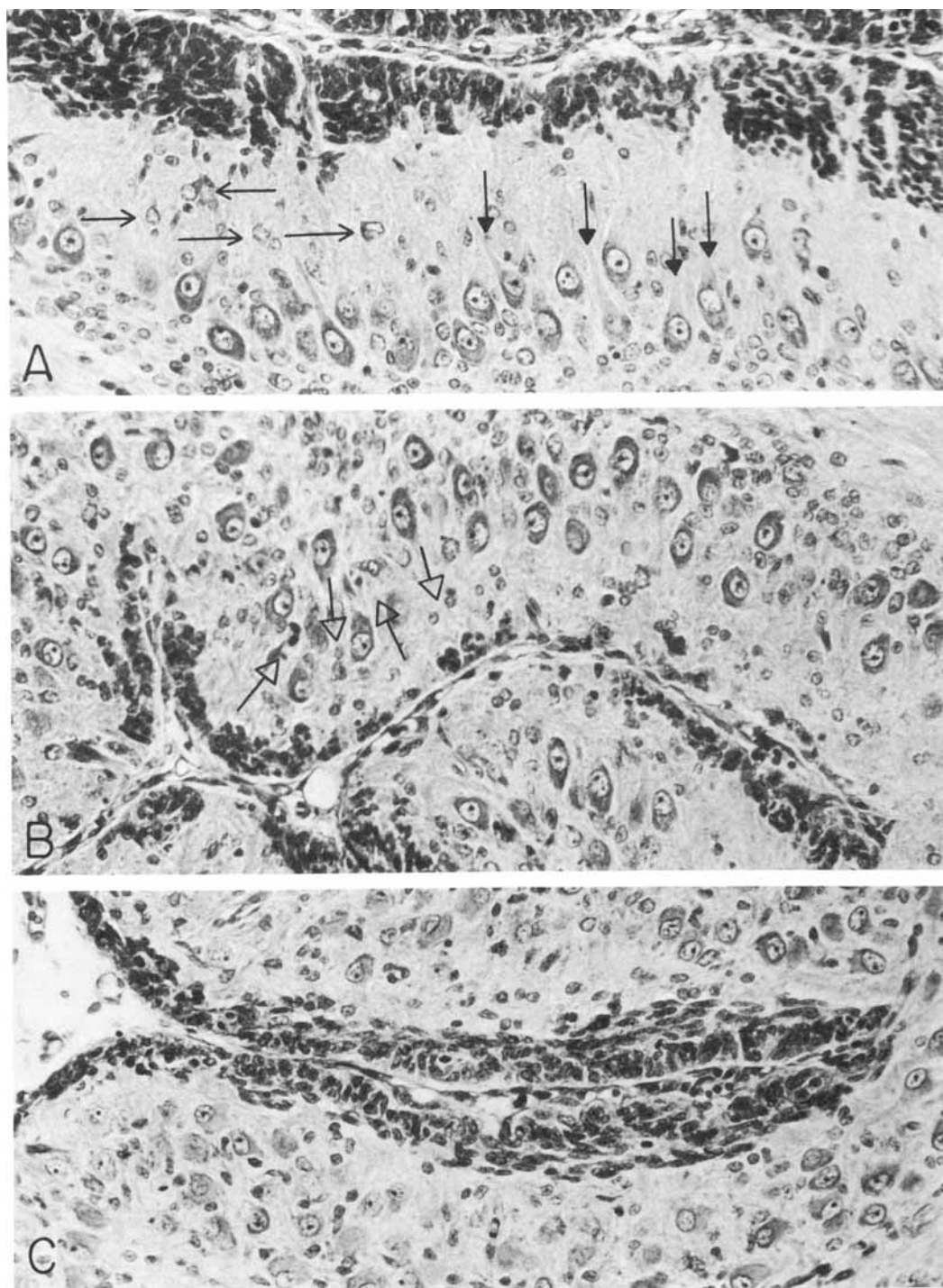


Figure 9



Fig. 10 Purkinje cell from a 30-day-old, 4-7 X rat. Note the single twisted stem dendrite, the paucity of smooth branches and the fair number of spiny branchlets. Golgi-Cox, $\times 612$.

In spite of these complicating factors the results warrant several conclusions and some hypotheses. The first conclusion that we may draw is that X-irradia-

tion *per se* does not interfere with Purkinje cell dendritic development. In cerebella that were adequately irradiated at birth with a single dose of X-ray, as attested by moderate lobular and laminar malformation throughout the vermis, the majority of Purkinje cells had normally oriented stem dendrites, several smooth branches and a rich complement of spiny branchlets. Normally developed Purkinje cells were also seen in regions of the vermis which were exposed to two or three X-ray doses, as verified by the presence of severe lobular malformation, provided that the molecular layer was thick and contained many basket cells, and preferably (though not necessarily) if the Purkinje cells were aligned in a monolayer. Evidently the dendritic system of Purkinje cells develops normally after X-irradiation if there is opportunity for early recovery of the EGL.

Intuitively, the alignment of Purkinje cells in a monolayer could be thought of as an essential condition for the upward growth of their dendritic arbors. But our evidence suggests that it is neither a necessary nor a sufficient condition. When the cerebellum is irradiated with several X-ray doses beginning on day 4, when the perikarya of Purkinje cells are already aligned in a monolayer, the cells become scattered again before the EGL recovers. In the mature cerebellum the stem dendrites are typically twisted and are often entwined around each other in the molecular layer; they are even more abnormal in appearance where the stem dendrites become embedded in the granular layer. This secondary piling up of Purkinje cells was noted previously with a schedule of X-irradiation started on day 4 which prevented recovery of the EGL (Altman and Anderson, '73). The interpretation we offered then was that because of the arrested growth of the cerebellar cortex in the absence of basket, stellate and granule cells (that is, in the absence of a molecular and granular layer) the growing Purkinje cell perikarya could not be sustained in a monolayer. According to our present results, even if the EGL recovers and the spiny branchlets of superficially located Purkinje cells make contacts with parallel fibers in the molecular layer the stem dendrites of these cells are twisted and entwined. Moreover, we noted in animals

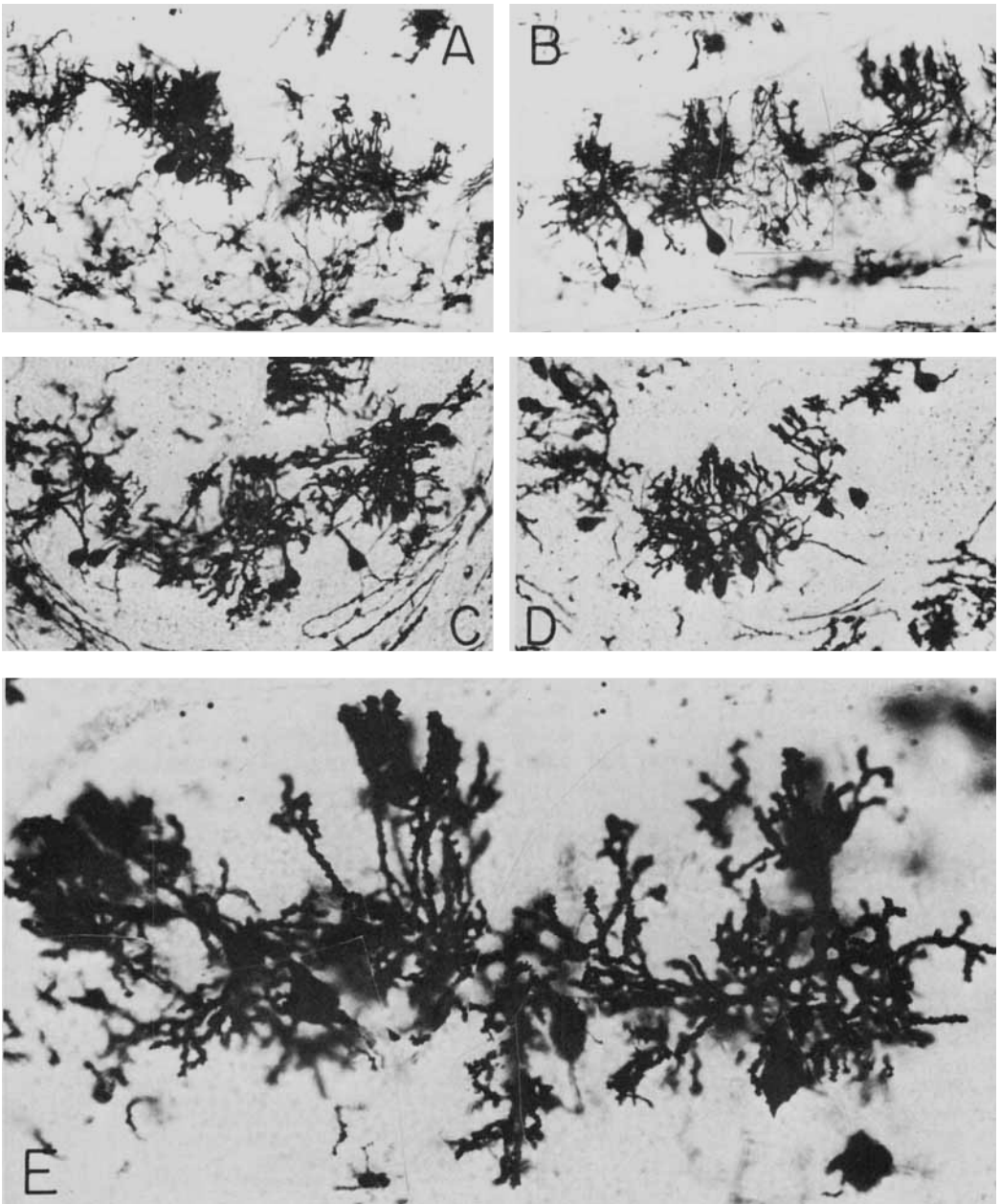


Fig. 11 Purkinje cells in a 30-day-old, 4-7 X rat. A and B. Some normal and intertwined Purkinje cells in the early-maturing uvula. C and D. Entwined Purkinje cells in the intermediate centralis ventralis. E. Extremely intertwined Purkinje cells with disoriented stem dendrites and tufts of spiny branchlets. A-D, $\times 101.7$; E, $\times 259.2$.

irradiated at birth with a single dose of X-ray that even though the alignment of Purkinje cells is delayed by several days, the developing Purkinje cell dendritic sys-

tem assumes a normal appearance. Finally, in animals that received several X-ray doses beginning at birth, which led to a failure of monocellular Purkinje cell align-

ment, nevertheless, those Purkinje cells whose dendrites grew toward the molecular layer were often normal in appearance.

These observations suggest that while monolaminar alignment of Purkinje cells may promote the growth of their dendrites toward the molecular layer it does not by itself guarantee the development of a straight, upward directed stem dendrite. The requirement for this appears to be the presence or recovery of the EGL by about day 6 and the onset of EGL cell differentiation no later than day 8 — the approximate age when in normal animals stem dendrite development begins and the bulk of basket cells have formed (Altman, '72). The hypothesis we offer here is that the necessary condition for the initiation of normal dendritic development (that is, the vertical growth of the stem dendrite) is the presence of basket cell axons. This is compatible with the observation that in animals irradiated with a single or few doses beginning at birth the development of normally oriented and arborizing Purkinje cell dendrites is associated with the presence of basket cells. Conversely, in animals irradiated with three-four doses from birth or with four doses beginning on day 4, which prevent basket cell acquisition, Purkinje cell dendritic development becomes abnormal. More direct evidence for this hypothesis will be presented in the succeeding paper of this series (Altman, '76a) in which we describe the effects of X-ray schedules started on day 8, after the acquisition of the bulk of basket cells. It is shown that, presumably because of the absence of stellate cells, supernormally upright and unbranching stem dendrites grow towards the surface in contiguity with descending branches of basket cell axons.

The hypothesis underlying the X-irradiation schedules used in the second part of this study was that if EGL recovery is delayed beyond the period when basket cells are formed under normal conditions (range: 4–9 days, peak: 6–7 days; Altman, '72) that, in accordance with a strict chronological principle of differentiation, the cells of the molecular layer will differentiate as stellate cells. The alternative possibility was that differentiation is governed by a sequential principle and the first complement of recovered cells would then differentiate as late-forming basket cells. The

evidence we obtained appears to support the original hypothesis but the principle cannot be considered to be definitely established. In both groups (0–4 X, 4–7 X), at least in some animals, an occasional basket cell or basket terminal was encountered. Because the irradiations were occasionally incomplete it is possible that the basket cells that were seen came from spared precursors or that in some regions, because of partial sparing on certain days, recovery of the EGL occurred earlier than assumed. This problem will have to be re-examined in animals in which the extent of irradiations will be better controlled.

We have considered the possibility that the formation of disoriented and twisted stem dendrites may be a direct effect on Purkinje cells when a large number of irradiations are used (3–4 exposures from birth or 4 exposures beginning on day 4) rather than to the absence of basket cells. However, as it will be shown in the subsequent studies, four or more exposures beginning on day 8 (Altman, '76a) or four exposures started on day 12 (Altman, '76b), which do not interfere with basket cell differentiation, do not have such an effect. On the contrary, Purkinje cells develop singularly long and straight stem dendrites.

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