Experimental Reorganization of the Cerebellar Cortex

VI. EFFECTS OF X-IRRADIATION SCHEDULES THAT ALLOW OR PREVENT CELL ACQUISITION AFTER BASKET CELLS ARE FORMED

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ABSTRACT In Long-Evans rats the area of the cerebellum was X-irradiated with two schedules beginning on day 8 by which time the bulk of basket cells were formed. The shorter schedule of four successive daily doses of 200 r between 8–11 days was designed to allow some cell recovery, the longer schedule between 8–15 days was expected to prevent it. Neither of the schedules interfered with the differentiation of basket cells. Purkinje cells remained aligned in a monocellular layer and formed singularly long, upright stem dendrites which were surrounded by the descending collaterals of basket cell axons. This supported the hypothesis that the directed growth of Purkinje cell stem dendrite is promoted by morphogenic interaction with basket cells. The upright stem dendrites had few or no smooth branches where cell recovery was prevented or had few such branches where recovery occurred. It was postulated that the outgrowth of smooth branches is dependent on interaction with stellate cells which form after the acquisition of basket cells. The absence or scarcity of smooth branches did not prevent the formation of spiny branchlets which grew downward to establish synaptic contacts with the spared parallel fibers of granule cells formed before the start of irradiation. In the group with some granule cell recovery, spiny branchlets grew to a limited extent upward into the pile of parallel fibers formed after the irradiation.

The preceding study (Altman, '76a) showed that if the rat cerebellum is irradiated from birth with one or two doses of 200 r X-ray, which results in decimation of the external germinal layer (EGL) followed by its recovery and the differentiation of all cortical cell types, that the normal growth of Purkinje cell dendrites is not interfered with. But longer schedules of X-irradiation started at birth or four days of age, which disturb the alignment of Purkinje cell perikarya in a monolayer and prevent selectively the acquisition of basket cells, results in the growth of abnormally oriented and shaped Purkinje cell dendrites. It was hypothesized that the presence of basket cells is necessary to ensure the outgrowth of vertically-oriented stem dendrites. Accordingly, in this study we examined the effects of X-irradiation started on day 8, after the acquisition of the bulk of basket cells (Altman, '72a). Two schedules were used, one that prevented and another that allowed some cell acquisition after the irradiations.

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 detail before (Altman and Anderson, '72). In the first experimental group, the area of the cerebellum was irradiated with 200 r, beginning on day 8, on four successive days (8–11 X). In the second group 200 r was delivered on days 8 and 9, and supplementary doses of 150 r were given on days 11, 13 and 15 to prevent recovery of the EGL (8–15 X). All rats were killed at 30 days. In the first, limited irradiation group four brains were stained with cresyl violet, hematoxylin-eosin and Bodian's protargol-S method and eight brains were impregnated with the Golgi-Cox technique. In the second, prolonged irradiation group eight

brains were stained with the previous procedures, two brains were impregnated with the Golgi-Cox technique and four brains were used for electron microscopy. Control cerebella were used from our normal developmental collection.

RESULTS

A. *X-irradiation schedule that prevents cell acquisition after basket cells are formed*

The stage of morphological development of the cerebellar vermis in 8-day-old rats was described earlier (Altman, '76a). Briefly, the EGL is 8–12 cells deep; Purkinje cells are aligned in a monolayer (except in portions of the latest maturing folium and tuber); the molecular layer contains differentiating, presumed basket cells in growing numbers; the molecular layer also contains vertically oriented, translocating granule cells and perpendicularly oriented stem dendrites of Purkinje cells; finally, a granular layer of variable thickness is present throughout the vermis. If X-irradiation is started on day 8 and sustained until day 15 to prevent regeneration (8–15 X) the cerebellum at 30 days is normally laminated and Purkinje cells (with the exceptions noted) remain aligned in a monolayer. As there is minimal increase in the area of the molecular and granular layers, the cerebellum appears normal in shape but is miniature in size.

However, at higher magnification several peculiarities become apparent. The first of these is the shape of the stem dendrites of the tightly packed Purkinje cell perikarya; they tend to be unusually straight and are lacking in secondary branches. This was most striking in material stained with Bodian's technique (figs. 1, 12–15). The paucity of smooth branches was confirmed in the comparison of Golgi-impregnated Purkinje cells in normal (figs. 2A–C) and 8–15 X rats (figs. 2D–I). In the experimental group the vertical stem dendrite bends or branches near the surface of the cortex and numerous spiny branchlets issue from it either directly or from the few smooth branches which tend to follow a downward course and may reach as far as the depth of the molecular layer. The Purkinje cells assume, as a result, the

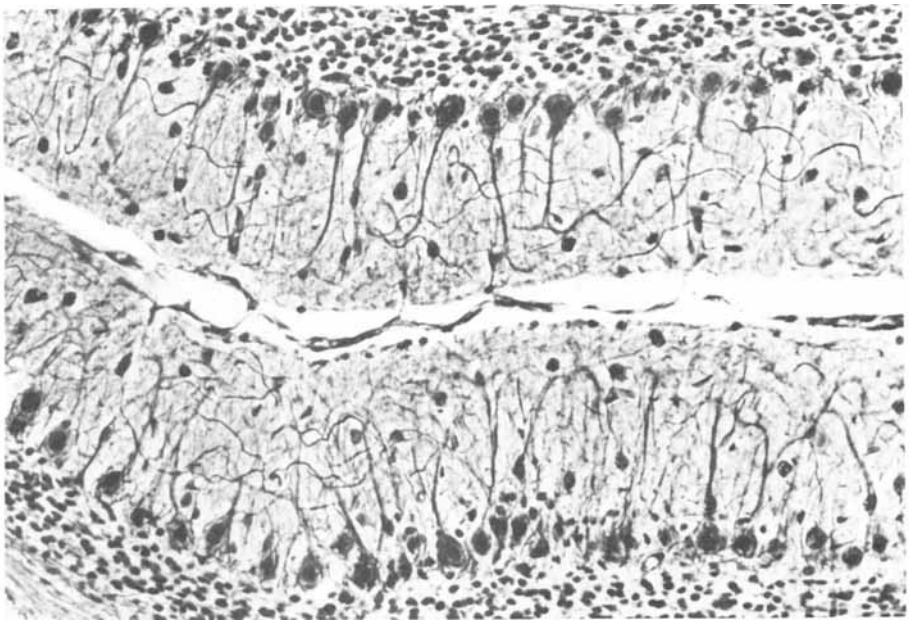

Fig. 1 Pyramis and tuber in a 30-day-old, 8–15 X rat. Note the presence of horizontally oriented impregnated basket cell axons through the entire width of the thin molecular layer. Bodian's protargol-S, × 244.8.

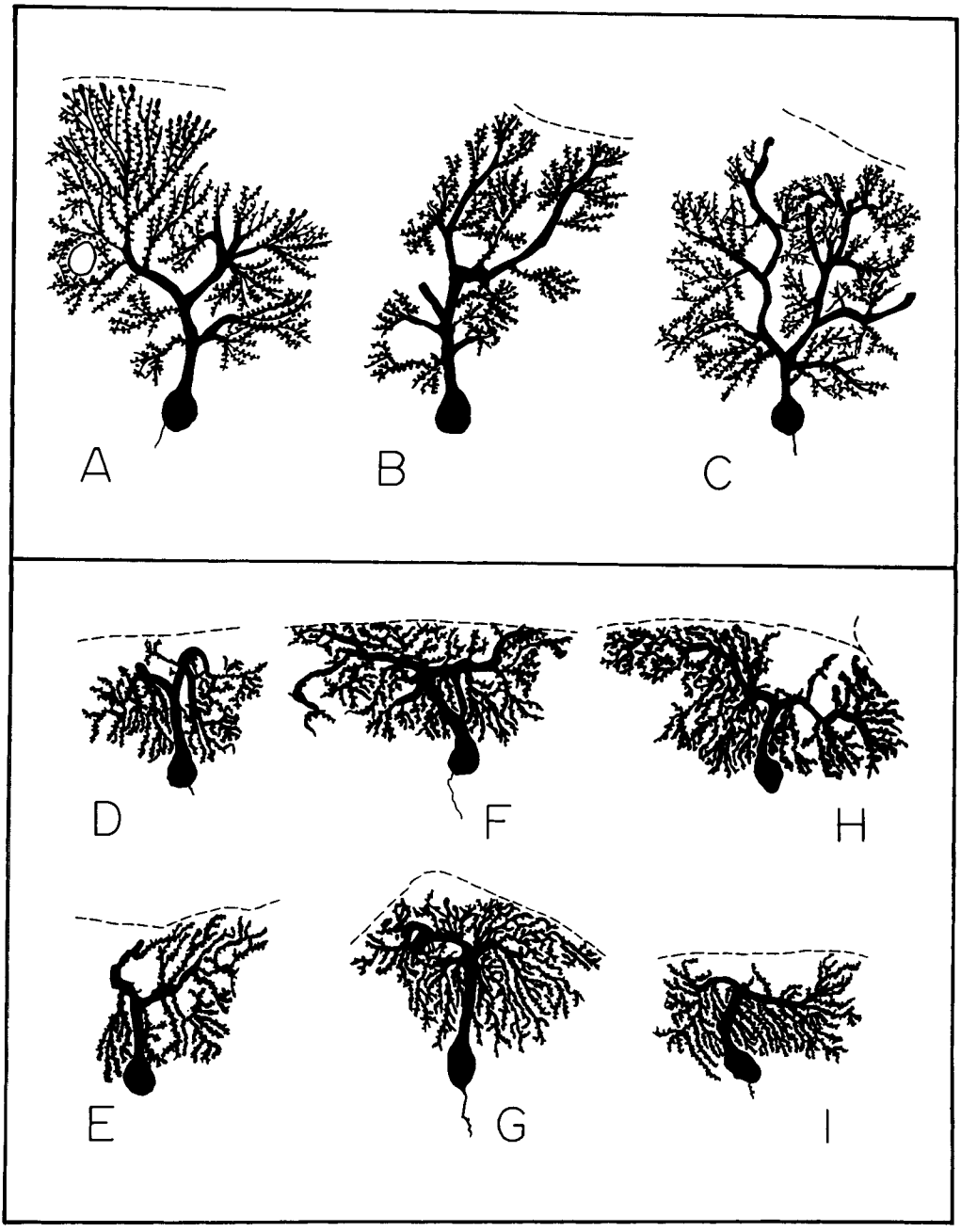

Fig. 2 A,B,C. Drawings of Purkinje cells in the cerebellum of a 30-day-old normal rat. In spite of great variability in dendritic patterns, all cells have one (and rarely more than two) stem dendrite, several smooth branches, and a variable number of spiny branchlets. D–I. Purkinje cells in the cerebellum of a 30-day-old, 8–15 X rat. Most cells have a tall stem dendrite which bifurcates midway or near the surface of the molecular layer. There are few smooth branches instead the spiny branchlets issue directly from the stem dendrite and tend to follow a downward course. Compare with figure 11. Golgi-Cox.

shape of weeping willows. In this material Bergmann glial palisades (Altman, '75) were not seen.

Purkinje cells with upright stem dendrites were frequently encountered with electron microscopy (fig. 3). Inconspicuous, symmetrical synapses, typical of basket cells were seen on the perikarya of Purkinje cells (fig. 4A) on the initial portions of their stem dendrites and along the slender stem dendrites (fig. 4B). Intermediate type of conspicuous, asymmetrical synapses were also associated with the stem dendrite (fig. 4C), presumably representing the contacts made by climbing fibers, while conspicuous, asymmetrical synapses of the parallel fiber type made contacts with spines issuing from the stem dendrites (fig. 5). Throughout the depth of the molecular layer parallel fiber synapses were present (fig. 6) presumably with the downward oriented spiny branchlets of Purkinje cells. The paucity of glial processes was noted in the molecular layer and glial endfeet at the surface were scarce.

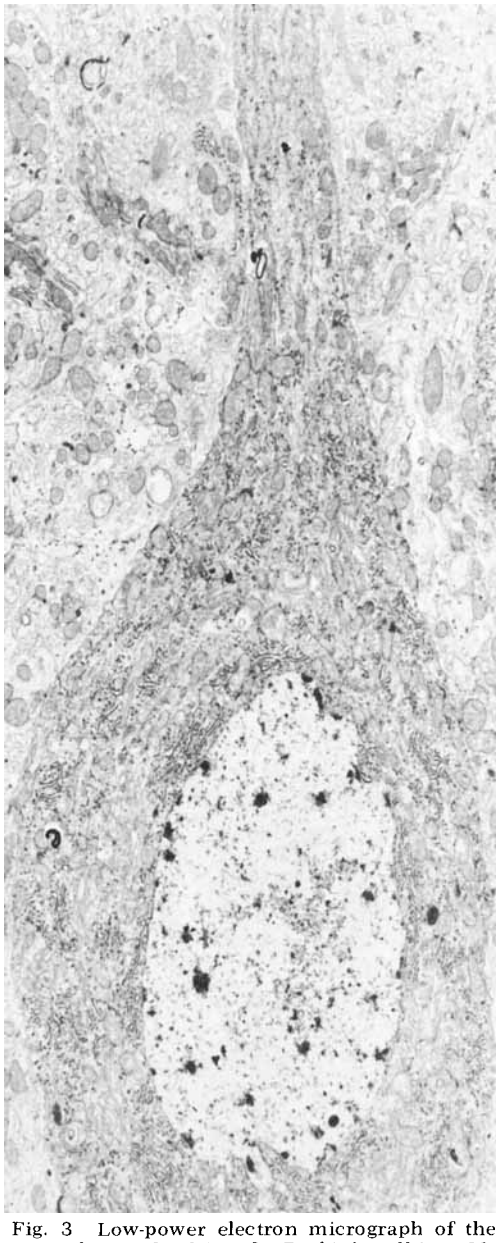

Fig. 3 Low-power electron micrograph of the soma and stem dendrite of a Purkinje cell in a 30-day-old, 8–15 X rat. × 4,788.

With respect to basket cells, the material prepared with the Bodian technique showed that basket cell axons are impregnated virtually throughout the entire molecular layer (fig. 1). This contrasts with the distribution of basket cell axons in normal cerebella, where they are restricted to the lower one-third to one-fourth of the thick molecular layer (Altman and Anderson, '72: fig. 7). Evidently the thin molecular layer in the 8–15 X rats represents the domain of the early-forming basket cells (Altman, '72b). Examination of the Bodian material also revealed that as a rule the upright and long Purkinje cell stem dendrites were contiguous with several descending branches of the impregnated basket cell axons (figs. 7, 12–15). The intimate relationship between the two is further attested by the observation that where the Purkinje cell stem dendrite bends, usually near the truncated surface of the molecular layer, the associated basket cell axons follow the same course (fig. 8).

In Golgi material basket cells were seen in abundance throughout the molecular layer. The dendrites often reached the surface while the axon terminals formed baskets around the perikarya of Purkinje cells. Some basket cell axons were seen apposed to straight stem dendrites of Purkinje cells with apparent contacts. In material prepared for electron microscopy, the perikarya of basket cells (fig. 9A) had both symmetrical (fig. 9D) and asymmetrical synapses (fig. 9B); typical parallel fiber synapses

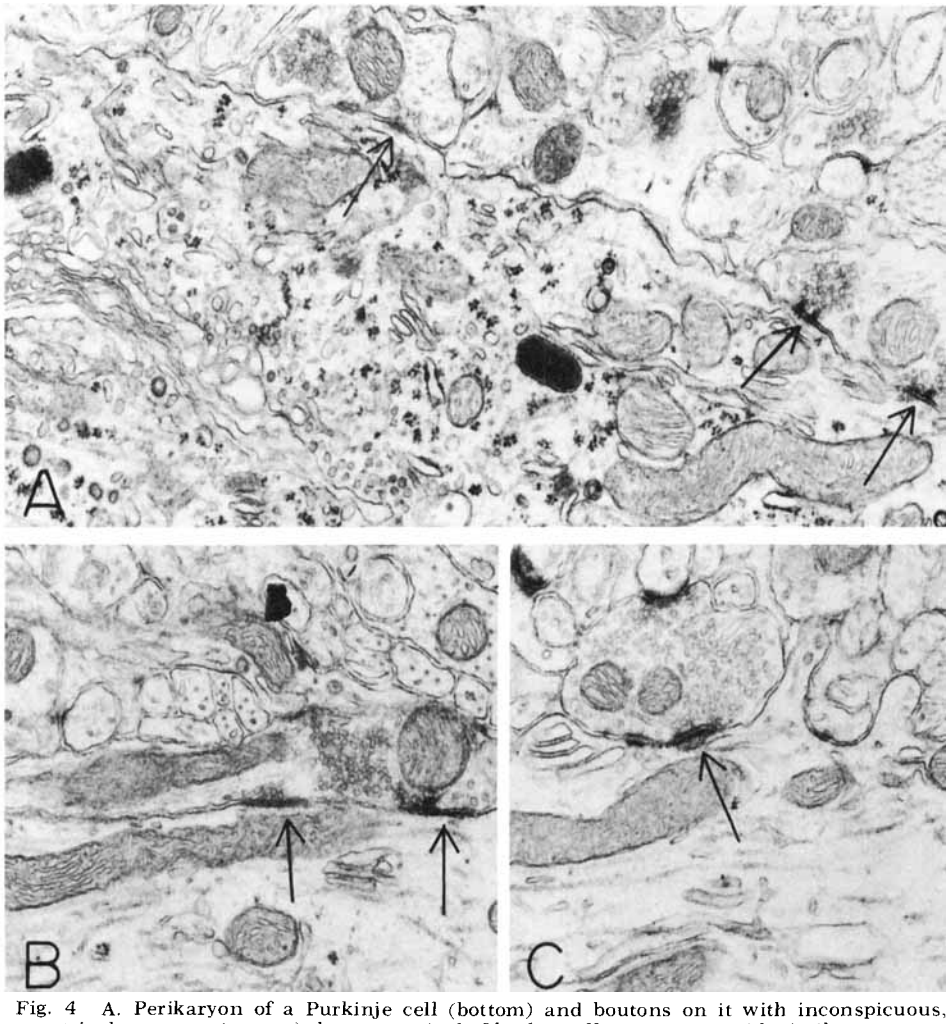

Fig. 4 A. Perikaryon of a Purkinje cell (bottom) and boutons on it with inconspicuous, symmetrical synapses (arrows) that are typical of basket cells. B. Bouton with similar synapses (arrows) on the straight stem dendrite. C. Intermediate type of conspicuous, asymmetrical synapse (arrow) on a stem dendrite, possibly belonging to a climbing fiber. Thirty-day-old, 8–15 X rat. × 22,572.

were frequent on their dendrites (fig. 9C). Mossy fiber rosettes were seen in large numbers in the granular layer but they tended to have few synapses (fig. 10A) presumably because of the paucity of granule cells. Single claw endings of granule cell dendrites made multiple contacts with mossy fiber rosettes (fig. 10B). Perhaps because of the low concentration of granule cells, terminals that were believed to be those of mossy fibers penetrated the molecular layer as far as its surface. Ectopic granule cells were not seen in these cerebella in which recovery of the EGL was prevented by the extended radiation schedule.

B. *X-irradiation schedule that allows cell acquisition after basket cells are formed*

This group consisted of rats in which the cerebellum was irradiated with four successive daily doses of 200 r from day 8 (8–11 X) with the expectation that due to

recovery of the EGL by about day 15 cells would be acquired until day 21. The effects of this irradiation schedule were evaluated at 30 days in four cerebella stained with Nissl and Bodian techniques and in eight cerebella impregnated with the Golgi-Cox technique.

There was no lobular malformation in the 8–11 X group but the miniaturization was less pronounced than in the previous group. Purkinje cells were aligned in a monolayer including the late-maturing folium and tuber. Mild granule cell ectopia was seen in a few lobules in some animals but, in general, the recovered cells followed a normal course of differentiation, evidently enlarging the cell population and territory of the molecular and granular layers.

In Bodian-stained sections the regularly aligned, straight and upright Purkinje cells were prominent in many lobules. But some of them seemed to have thick branches in the upper one-third to one-fourth of the molecular layer. Impregnated basket axons were distributed in most regions throughout the molecular layer and basket end-

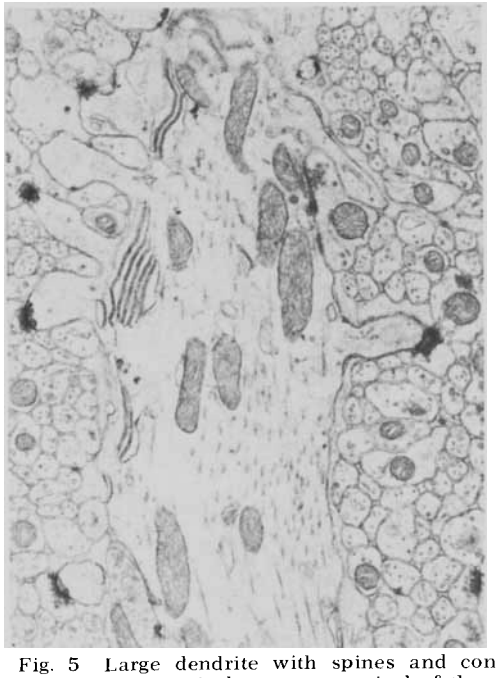

Fig. 5 Large dendrite with spines and conspicuous, asymmetrical synapses typical of those with parallel fibers. Thirty-day-old, 8–15 X rat. × 12,141.

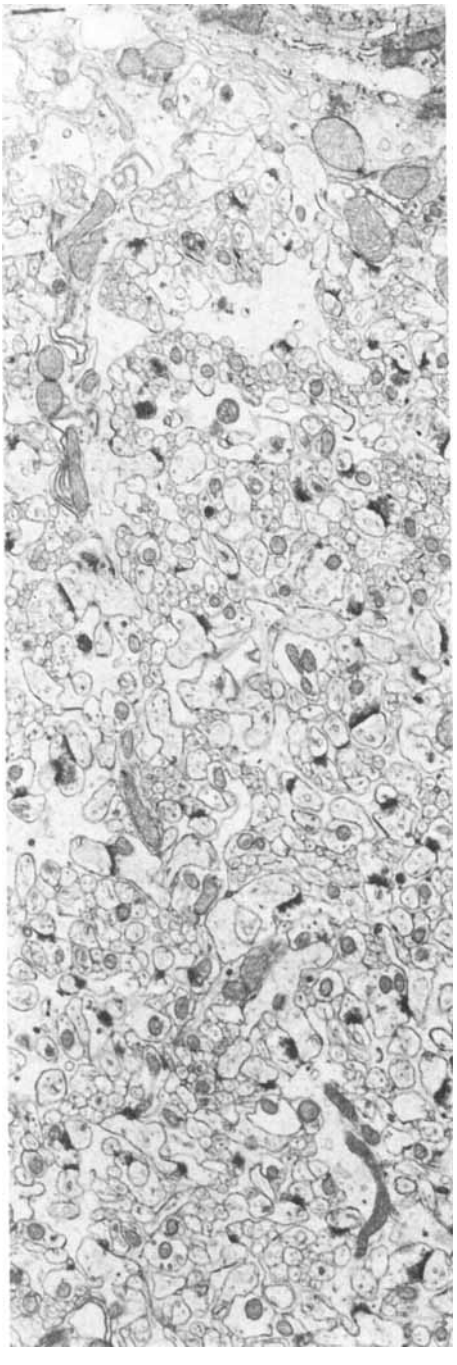

Fig. 6 Upper aspect of the molecular layer with parallel fiber profiles and many conspicuous asymmetrical synapses. Thirty-day-old, 8–15 X rat. × 7,752.

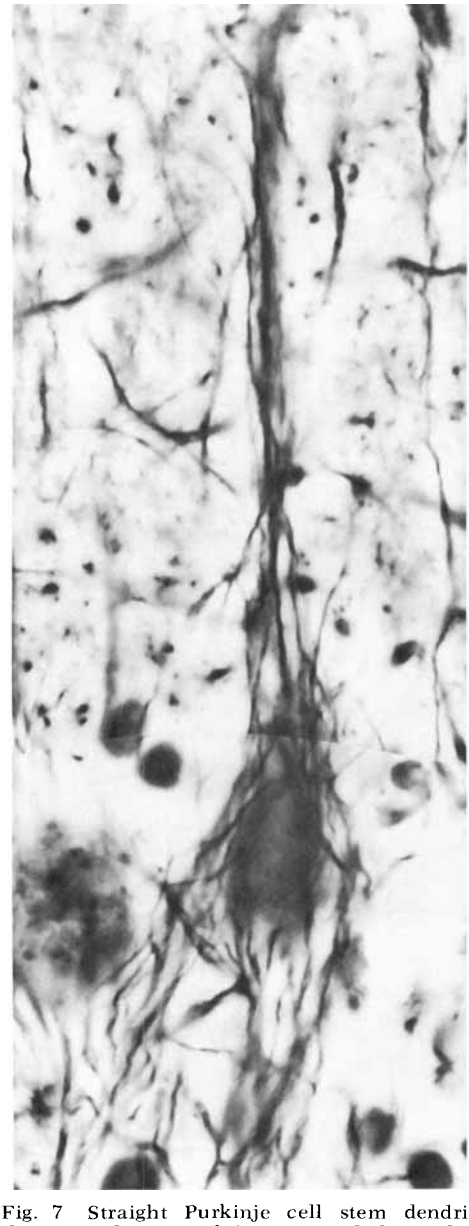

Fig. 7 Straight Purkinje cell stem dendrite with intimately apposed, impregnated, descending basket axon collaterals. Note also the rich basket plexus. Thirty-day-old, 8–15 X rat. Bodian, oil immersion, × 1,350.

ings were regularly seen around the perikarya of Purkinje cells. In Golgi material basket cells and Purkinje cells were impregnated in large numbers. Many of the latter resembled weeping willows (fig. 11A)

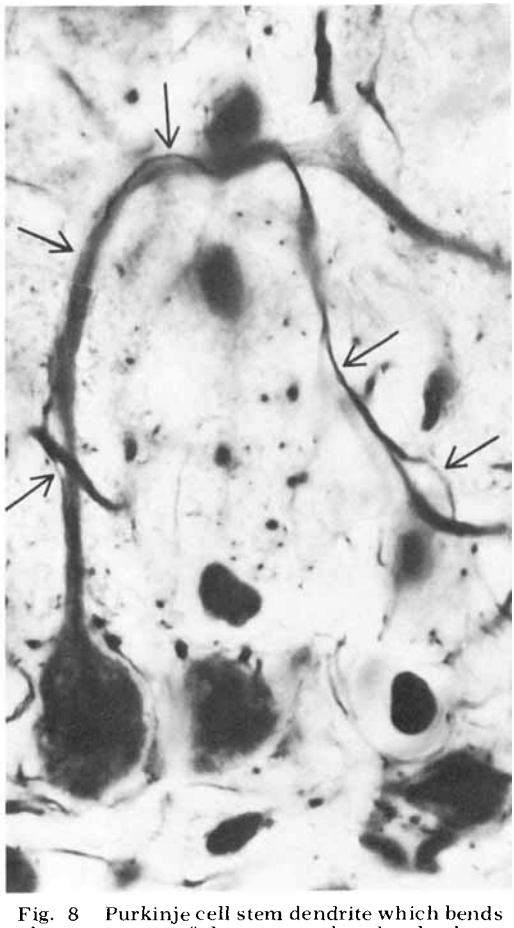

Fig. 8 Purkinje cell stem dendrite which bends in the upper part of the truncated molecular layer with intimately apposed basket cell axon (arrows). Thirty-day-old, 8–15 X rat. Bodian, oil immersion, × 1,350.

the type so frequent in the previous group but generally with longer stem dendrites. Others had, in addition to the downward directed spiny branchlets, upward directed branchlets occupying the upper one-fourth to one-fifth of the molecular layer (figs. 11C,D). Still others had numerous smooth branches issuing from the upper portion of the stem dendrite (figs. 11E,F) and occasionally from other regions of the Purkinje cell.

DISCUSSION

The morphogenic effects produced by four daily successive doses of 200 r delivered from day 8 on (8–11 X) contrast sharply with similar treatments (Altman, '76a)

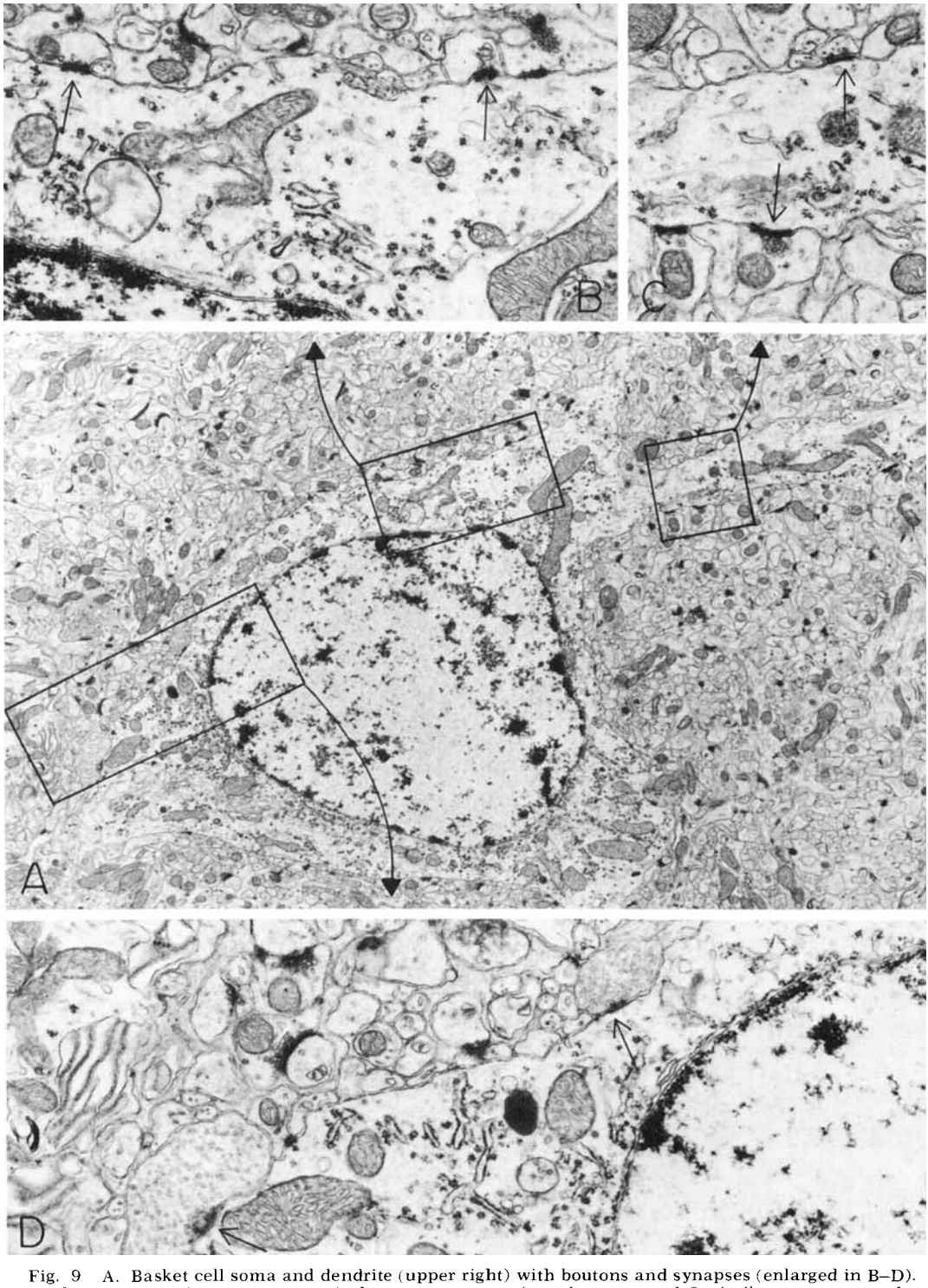

Fig. 9 A. Basket cell soma and dendrite (upper right) with boutons and synapses (enlarged in B–D). × 5,426.4. B. Conspicuous asymmetrical synapses (arrows) on the soma and C, similar synapses on the dendrite. These are probably parallel fiber synapses. D. Boutons of a different type with inconspicuous, symmetrical synapses on the soma. Thirty-day-old, 8–15 X rat. B–D, × 17,556.

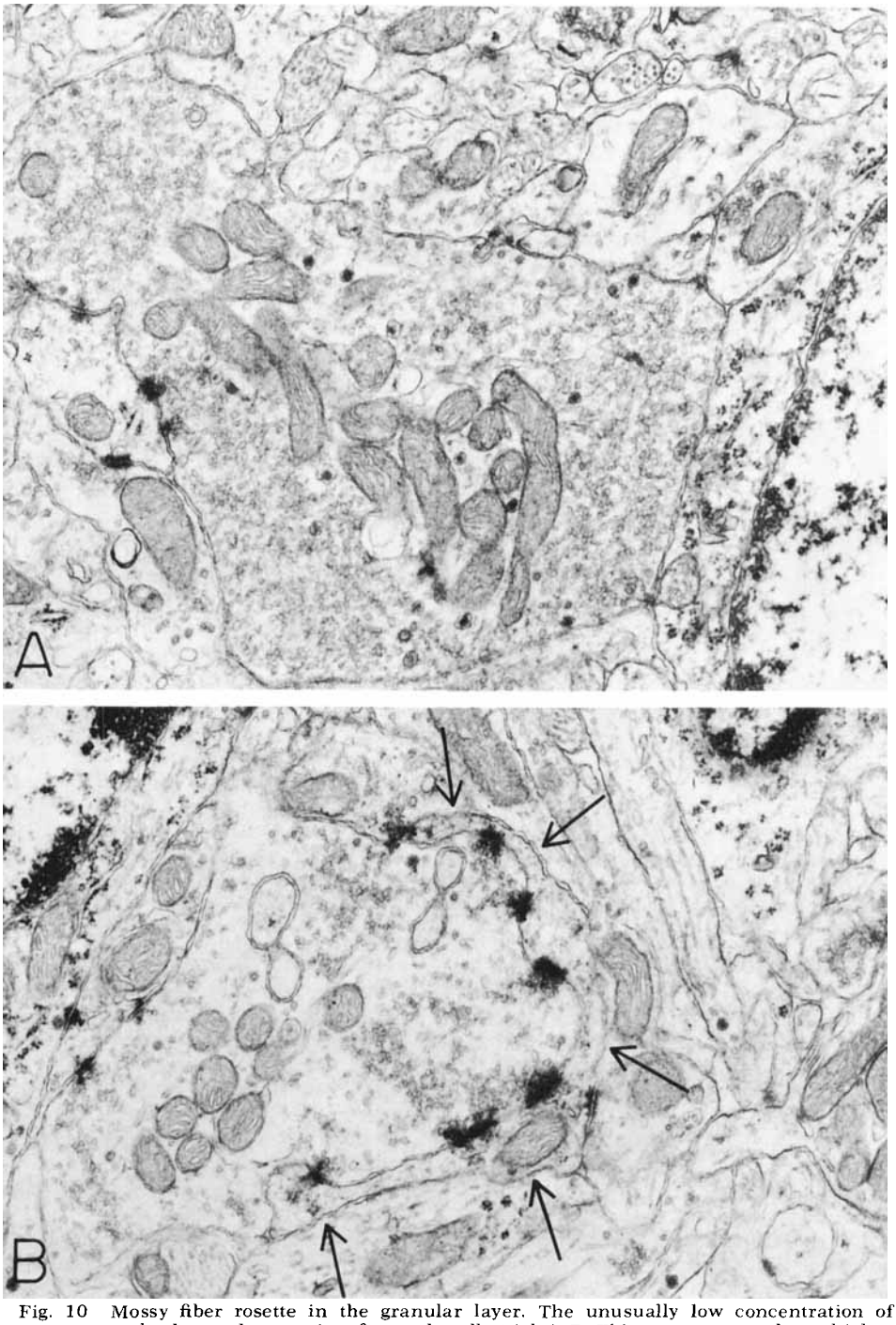

Fig. 10 Mossy fiber rosette in the granular layer. The unusually low concentration of synapses must be due to the scarcity of granule cells (right). B. This mossy rosette has a higher concentration of synapses but most of them are with a single claw ending (arrows) of a granule cell dendrite. Thirty-day-old, 8–15 X rat. × 22,572.

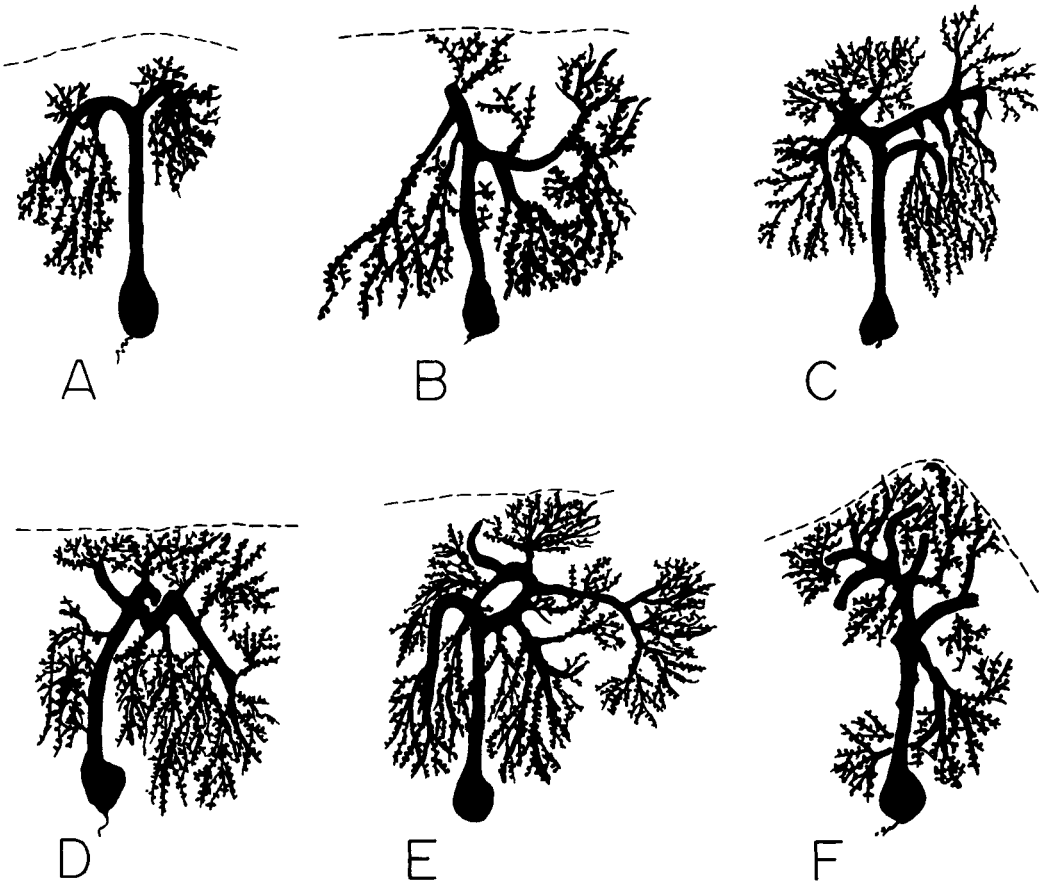

Fig. 11 Purkinje cells with different dendritic patterns in 30-day-old, 8–11 X rats. Some (A) are the weeping willow type, others (C and D) also have upward directed spiny branchlets, still others (E and F) have a few smooth branches issuing from the initial straight stem dendrite some distance from the soma. Golgi-Cox.

started at birth (0–3 X) or four days (4–7 X). Unlike in the 4–7 X group, the disturbance of monocellular alignment of Purkinje cell perikarya is not seen in this or the 8–15 X group, except in the late-maturing folium and tuber where basket cells are still not present at eight days. This preservation of monocellular alignment could be due to a number of factors, among them the directed vertical growth of Purkinje cell stem dendrites which, according to our hypothesis, is assured by the presence of basket cells. Interestingly the results of this study show that the presence of basket cells does not only ensure the vertical growth of Purkinje cell stem dendrites but that if X-irradiation is started after acquisition of basket cells, the stem dendrites become singularly erect and tend to have few or no secondary branches. The structural association between Purkinje cell stem dendrites and basket cells was best seen in Bodian material. As a rule, the upright stem dendrite was surrounded by descending branches of basket cell axons and where the stem dendrite became deflected, usually near the surface, it appeared to be associated with horizontal branches of basket cell axons.

Since Purkinje cell stem dendrites do not have a strong intrinsic disposition to grow perpendicularly to the surface (Altman and Anderson, '72, '73; Altman, '76a) we will assume tentatively that the branches of the horizontal axons of basket cells do have such a tendency, at least in

the presence of Purkinje cell dendrites beneath them. According to previous studies, the downward growth of basket cell axon collaterals begins about ten days (Altman, '72a: fig. 5) coinciding with the outgrowth of the Purkinje cell stem dendrite (Altman, '72a: fig. 11; Altman, '72b: fig. 5). Presumably, the interaction of the two is responsible for the directed perpendicular growth of the latter. The nature of this interaction is not known but it is not likely to be a synaptic one since synapse formation of basket cell axons with Purkinje cell stem dendrite and soma does not start until day 12 (Altman, '72b: figs. 28, 37).

The question raised by this study is why, after the upward growth of the Purkinje cell stem dendrite is induced by the presence of descending basket cell axons, the stem dendrite becomes supernormally erect. This unbranched pattern is rarely seen in normal animals in which the stem dendrite begins to divide a short distance from the perikaryon. Since this branching must occur some time about ten days, we offer the hypotheses that the presence of a cell type formed after day 8 is responsible for the production of secondary smooth branches. That granule cells are not likely to be these cells is suggested by the observation that the stem dendrites remain unbranched not only in the 8–15 X group, which has spared granule cells, but also in the 8–11 X group, at least in the lower half of the molecular layer, which also has recovered granule cells. The cells that might be responsible are the stellate cells which form between 6–15 days with a peak either between 9–10 or 8–11 days (Altman, '72a: fig. 6). The acquisition of stellate cells is near-totally prevented by the 8–15 X schedule. Branching of Purkinje cell smooth dendrites in the upper molecular layer was seen in the 8–11 X group, which might be attributed to some recovered stellate cells. The hypothesis of the role of stellate cells in the formation of Purkinje cell smooth branches will be considered in the next paper of the series where we examine the effects of irradiation schedules started on day 12, after the acquisition of stellate cells (Altman, '76b).

A previous study (Altman, '73) indicated that the outgrowth of Purkinje cell spiny branchlets is induced by parallel fibers, which determine their orientation in one plane at a right angle to the direction of parallel fibers. Two observations made in this study reinforce the hypothesized role of parallel fibers. The first is that after the stem dendrite reaches the surface spiny branchlets issue from it in great abundance, taking a downward direction where the spared parallel fibers (those formed up to day 8) are located. The resulting weeping willow type of Purkinje cells were observed previously (Shofer et al., '64; Hámori, '69) after X-irradiation. The electron microscopic evidence is that there is rich synaptic relation between these spiny branchlets and parallel fibers. Synaptogenesis presumably starts at the same time (about day 15) in the irradiated animals as it does in normals (Altman, '72b); it seems completed by day 30 (fig. 6). The second observation is that in the 8–11 X animals, in which there is recovery of granule cells, there are also upward directed spiny branchlets, probably establishing relationship with parallel fibers of recovered granule cells. These observations strengthen the hypothesis that Purkinje cell spiny branchlet formation is induced by parallel fibers. Finally, it is worth noting that the low-level X-irradiation used in these experiments, which destroy a large proportion of the undifferentiated cells of the external germinal layer, have no obvious deleterious effect on synapse formation with the just-differentiated basket cells and the maturing Purkinje cells.

ACKNOWLEDGMENTS

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LITERATURE CITED

Altman, J. 1972a Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. J. Comp. Neur., *145*: 353–398.

——— 1972b Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. J. Comp. Neur., *145*: 399–464.

——— 1973 Experimental reorganization of there cerebellar cortex. IV. Parallel fiber reorien-

tation following regeneration of the external germinal layer. J. Comp. Neur., *149*: 181–192.

——— 1975 Postnatal development of the cerebellar cortex in the rat. IV. Spatial organization of bipolar cells, parallel fibers and glial palisades. J. Comp. Neur., *163*: 427–448.

——— 1976a Experimental reorganization of the cerebellar cortex. V. Effects of early X-irradiation schedules that allow or prevent the acquisition of basket cells. J. Comp. Neur. *165*: 31–48.

——— 1976b Experimental reorganization of the cerebellar cortex. VII. Effects of late X-irradiation schedules that interfere with cell acquisition after stellate cells are formed. J. Comp. Neur. *165*: 65–76.

Altman, J., and W. J. Anderson 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.

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. Chicago, Ill. Amer. Med. Assoc., pp. 845–858.

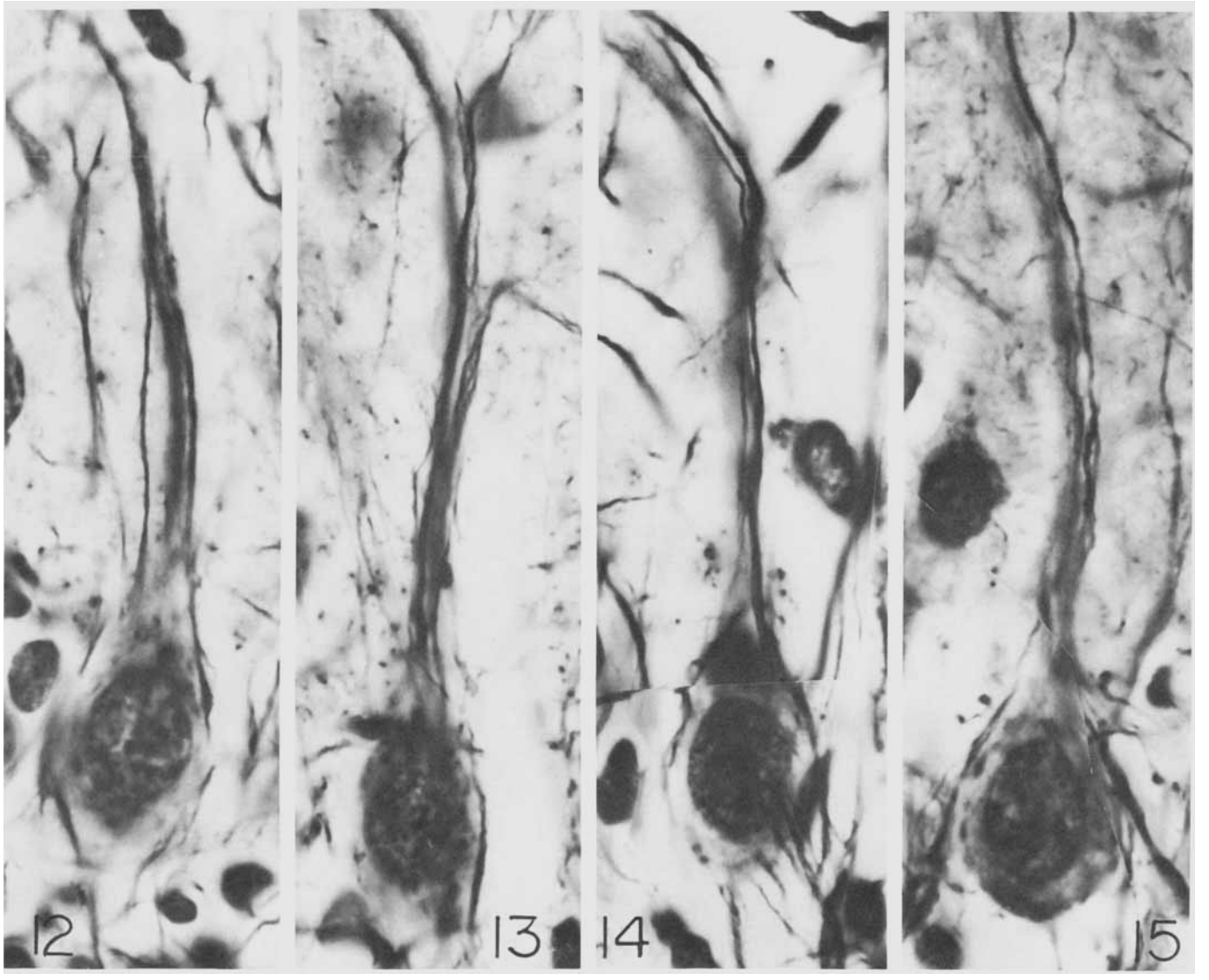
Shofer, R. J., G. D. Pappas and D. P. Purpura 1964 Radiation-induced changes in morphological and physiological properties of immature cerebellar cortex. In: Response of the Nervous System to Ionizing Radiation. T. J. Haley and R. S. Snider, eds. Boston, Little, Brown, pp. 476–508.

PLATES

PLATE 1

EXPLANATION OF FIGURES

12–15 Purkinje cells with undividing straight stem dendrites and intimately apposed descending basket axon collaterals. Thirty-day-old, 8–15 X rat. Bodian, oil immersion, × 1,620.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