

# Experimental Reorganization of the Cerebellar Cortex

## IV. PARALLEL FIBER REORIENTATION FOLLOWING REGENERATION OF THE EXTERNAL GERMINAL LAYER

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 with a schedule of low-level x-ray which allowed regeneration of the external germinal layer. In the subsequently regenerated molecular layer, parallel fibers were often reoriented. In some cerebella this was restricted to a single lobule, frequently several lobules were affected, rarely almost the entire vermis. It was postulated that the reorientation of parallel fibers was determined by the invasion route of multiplying cells from spared foci of the external germinal layer. Where parallel fibers were rotated transversely, the axons of basket cells and the arborizing dendrites of Purkinje cells became oriented longitudinally. This, and other considerations indicated that parallel fibers exert a guiding influence on the pattern of growth of the spiny branchlets of Purkinje cells.

A prominent feature of the cerebellar cortex is its relatively precise geometric organization. The parallel fibers are oriented parallel to the surface of the cortex along the longitudinal axis of the folium (in the vermis largely in the coronal plane); the basket cell axons and the arborizing planar, Purkinje cell dendrites are oriented transversely (largely in the sagittal plane). This architectonic organization must be of paramount functional significance (Eccles et al., '67) but it is not known what morphogenetic factors determine it. Some observations are available which suggest that the orientation of the Purkinje cell dendrites may be secondary to and dependent on an earlier established coordinate system. Thus Ramon y Cajal ('11) noted that in infant mammals the transient lateral dendrites (perisomatic processes) of Purkinje cells grow in three dimensions, and our observations showed that the growing apical cones of Purkinje cells are still three dimensional at seven days of age in rats (Altman, '69, figs. 19-20). In contrast, from the beginnings of postnatal life the earliest-forming parallel fibers are oriented in the coronal plane

(Altman, '72a) and so are the spindle-shaped, or bipolar, cells of the external germinal layer. Since the basket cells do not come into existence until the beginning of the second week (Altman, '69, '72a) the orientation of their axons could, likewise, be determined by an already established coordinate system.

In the previous paper of this series (Altman, '73) reference was made to the incidental observation that if the external germinal layer is destroyed by x-irradiation the parallel fibers of the regenerating molecular layer are not oriented consistently in the coronal plane, as in normal animals, but become reoriented and are often aligned in the sagittal plane. In this study the nature and possible causes of this reorientation were examined together with the effects of this reorientation on the architectonic organization of the rest of the cerebellar cortex.

### MATERIALS AND METHODS

In this investigation use was made of part of the material that was analyzed in the previous study of this series (Altman, '73). The examined cerebella were ob-

tained from rats whose heads were irradiated on days 4, 5, 6 and 7 with 150 r, a schedule that permitted the regeneration of the external germinal layer. The animals were killed at 30 days of age and their cerebella were prepared for light microscopy (cresyl violet, hematoxylin-eosin, Bodian's protargol-S and the Golgi-Cox method) and electron microscopy. Particular attention was paid to material in which the parallel fibers were reoriented in the molecular layer.

### RESULTS

The sagittally cut sections stained with Bodian's protargol-S method were suitable for visualizing on the light-microscopic level the orientation of parallel fibers in the molecular layer. The parallel fibers are not truly impregnated but acquire a pinkish tone which reveals whether the fibers are cut in cross section or parallel. (Obliquely sectioned parallel fibers were difficult to identify because of the inadequate resolution.)

In parasagittal sections of the vermis in

normal animals, parallel fibers are consistently oriented in the coronal plane (parallel to the long axis of the folium). In cerebella in which the external germinal layer regenerated after radiation, that is, where there was evidence of a post-irradiation increase in the width of the molecular layer (Altman, '72a, fig. 4) regions could be identified where parallel fibers were oriented in other than the coronal plane. In some, the reorientation was restricted to a single lobule, more frequently several lobules were affected, rarely almost the entire vermis.

Figure 1 illustrates a frequent situation where in one lobule the parallel fibers were oriented normally, whereas in an adjacent lobule they became rotated in the transverse plane (sagittally). Figure 2 shows another situation where in two adjacent lobules the parallel fibers are oriented normally in the lower half of the molecular layer but are rotated in the upper half. Finally, figure 3 illustrates an instance where the rotated parallel fibers are crisscrossing the field.

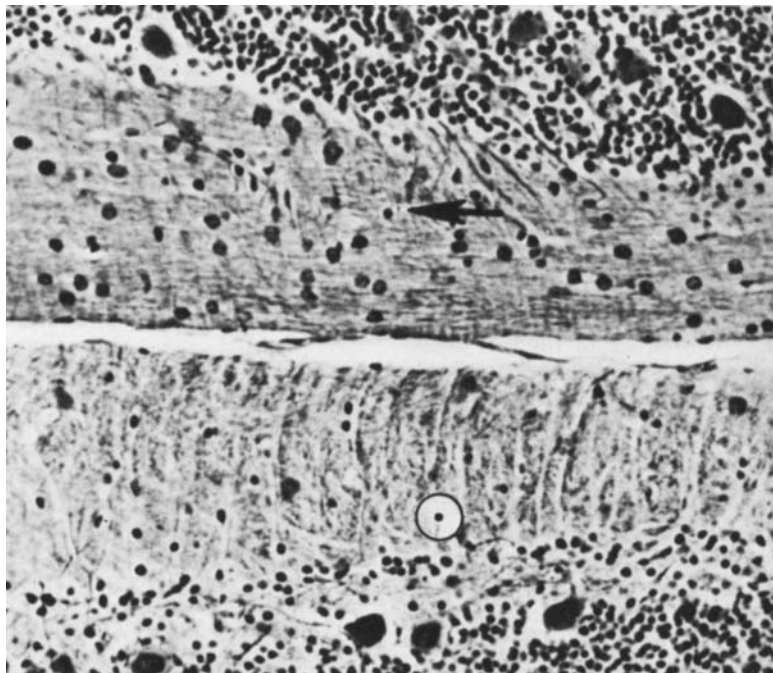


Fig. 1 In the bottom lobule (uvula) the regenerated parallel fibers are cut in cross section ( $\odot$ ) indicating normal longitudinal orientation. In the upper lobule (pyramis) the parallel fibers are rotated (arrow) parallel to the sagittal plane of sectioning. Bodian,  $\times 256$ .



Fig. 2 In two adjacent lobules parallel fibers are cut in cross section (normal longitudinal orientation) in the lower molecular layer and parallel (rotation in transverse direction) in the upper molecular layer. Bodian,  $\times 256$ .

Fig. 3 Rotated parallel fibers which are horizontally oriented in the upper molecular layer; vertically or obliquely in the deeper regions. Bodian,  $\times 256$ .

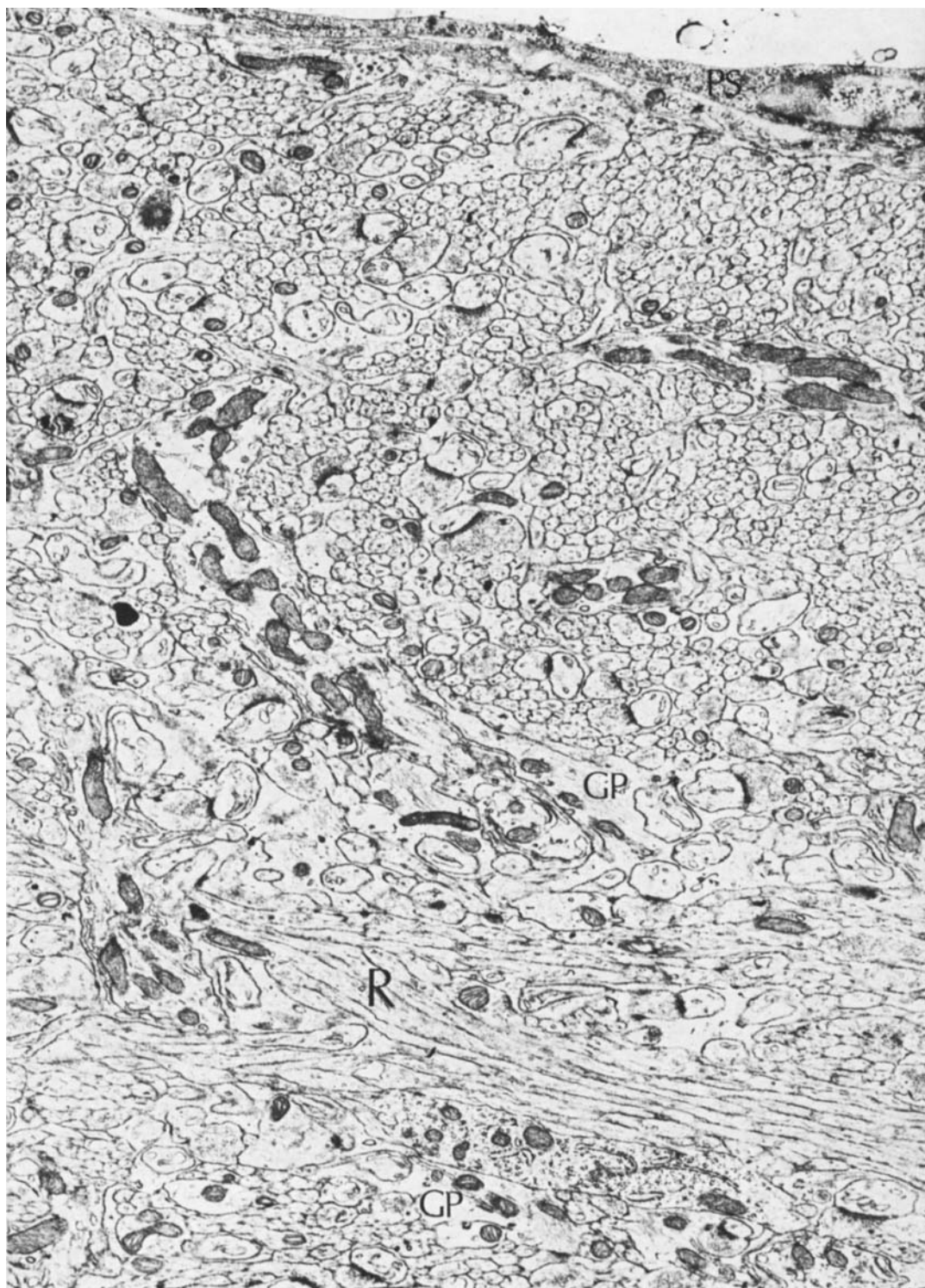
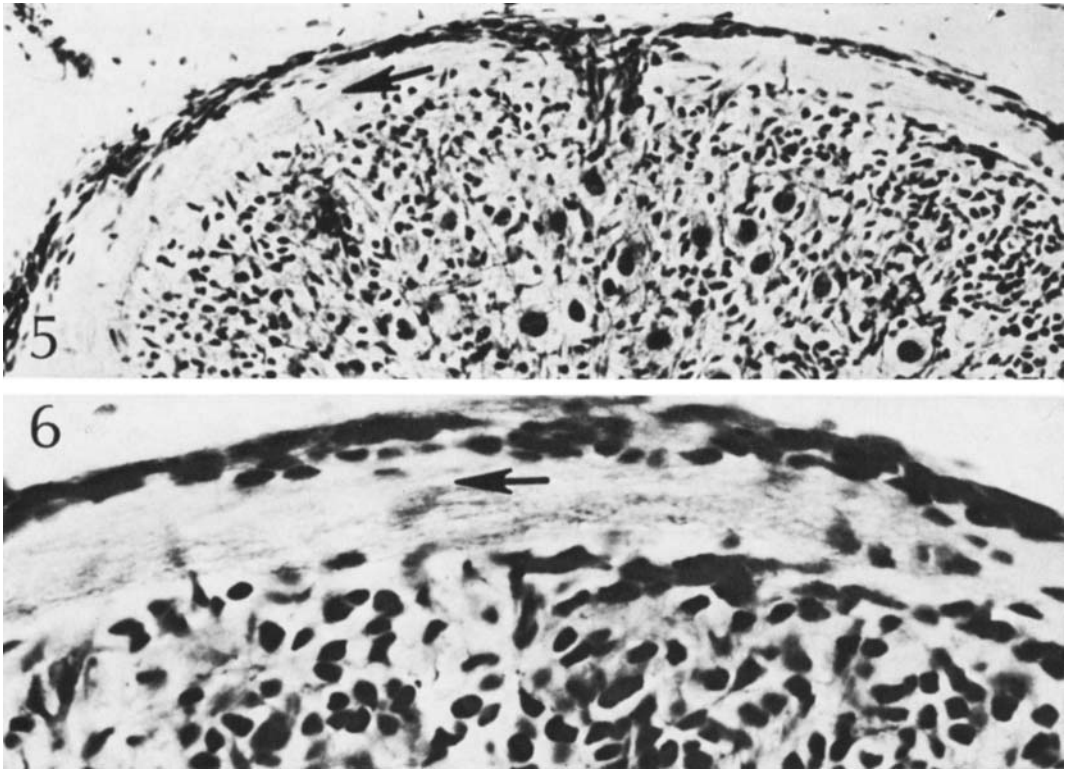


Fig. 4 In this sagittally sectioned electron micrograph of the regenerated molecular layer the parallel fibers are normally oriented (cut in cross section) in the upper half and bottom of the field but are rotated (R) between. Glial processes (GP) partly delineate the interface region. PS, pial surface. Pyramis,  $\times 13,680$ .



Figs. 5-6 Sagittally-oriented, spindle shaped cells (migratory or bipolar cells) of the external germinal layer situated above the rotated parallel fibers. Thirty day-old rat, declive. Bodian,  $\times 256$  and  $\times 640$ .

The rotation of parallel fibers in the irradiated vermis was confirmed with electron microscopy. Normally oriented parallel fibers are seen in figure 4 below the pia and deeper in the field, but interdigitated is a strip of parallel fibers which is sagittally oriented. A more frequently observed situation is illustrated in figure 9 where in the lower half of the molecular layer the parallel fibers are normally oriented, whereas in the upper half they are rotated. In rare instances endfeet of Bergmann glia cells were present at the interface of differently oriented beds of parallel fibers (fig. 10) suggesting that at some phase during development this region represented the external surface of the cerebellar cortex and the reoriented parallel fibers were added on later. Synaptic profiles are less frequent in the molecular layer of these 30 day old rats than in normal animals (Altman, '72b) indicating that the delay

in the regeneration of the external germinal layer led to a retardation of synaptogenesis.

In the 30 day old rats the external germinal layer was occasionally still extant in some lobules, unlike in normal animals where it disappears at 21 days. Where this was seen, the reorientation of parallel fibers was associated with a corresponding orientation of the spindle-shaped (migratory or bipolar) cells of the external germinal layer (figs. 5, 6). Moreover where parallel fibers become rotated sagittally in the lower molecular layer, the Bodian-impregnated axons of basket cells were rotated at a right angle to them in the coronal plane (fig. 7). This raised the possibility that the reorientation of the parallel fibers also caused rotation of the Purkinje cell dendrites away from the normal sagittal orientation.

Purkinje cells with a variety of abnormal



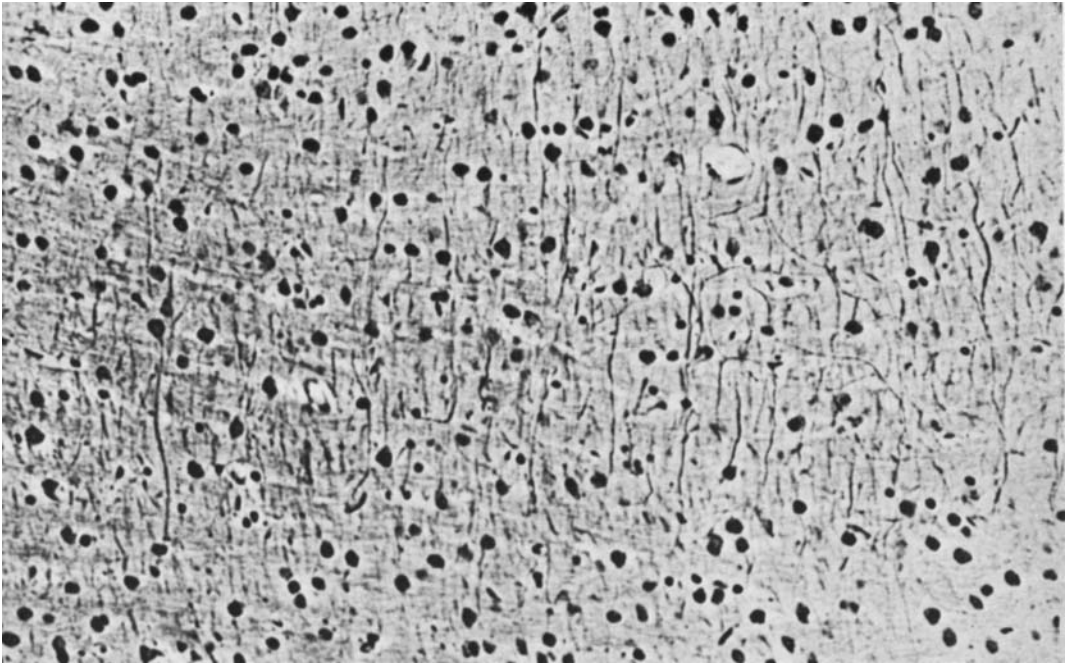


Fig. 7 The impregnated axons of basket cells are oriented at a right angle to the rotated parallel fibers. This nominally sagittal section is presumably cut parallel to the surface. Bodian,  $\times 256$ .

shapes were impregnated with the Golgi technique in the rats irradiated on days 4–7. These included cells with three-dimensionally oriented dendrites or dendrites penetrating the medullary layer. Attention was paid to Purkinje cells whose dendrites grew in the normal, upward direction. Figure 8A illustrates the situation where normally oriented dendrites in the lower molecular layer failed to invade the rotated upper portion of the molecular layer. What appears to be late, secondary expansion and sprouting of a primary dendrite, without rotation, is shown in figure 8B. Sprouting of spiny branchlets in two planes is illustrated in figures 8C,D. Of greatest interest are the Purkinje cells (figs. 8E,F,G) which have several discrete branches in the upper molecular layer rotated obliquely or at a right angle to the normally-oriented, lower branches, revealing a guiding influence exerted by the orientation of parallel fibers on the growth of the spiny branchlets of Purkinje cells.

#### DISCUSSION

The reorientation of parallel fibers fol-

lowing irradiation or other treatment during development has, to our knowledge, not been previously reported. The pattern of reorientation did not appear to be systematic; in different lobules the parallel fibers of the regenerated molecular layer were oriented in various horizontal planes or had differing orientations within the same lobule. Particular attention was paid to rotation at right angle to the long axis of the folium because this was easiest to examine.

Our earlier study (Altman, '73) of the regeneration of the external germinal layer after irradiation suggested that radio-resistant cells are preferentially located in the walls and depth of fissures and the regeneration starts at these foci (Altman, '73, figs. 11–13). Accordingly, it is presumed that from such foci waves of multiplying cells spread over the surface of the lobules and that the differently oriented strata of parallel fibers originate from separate waves of invading cells. An extension of this hypothesis is that the original deployment route of the proliferative cells determines the ultimate orientation of

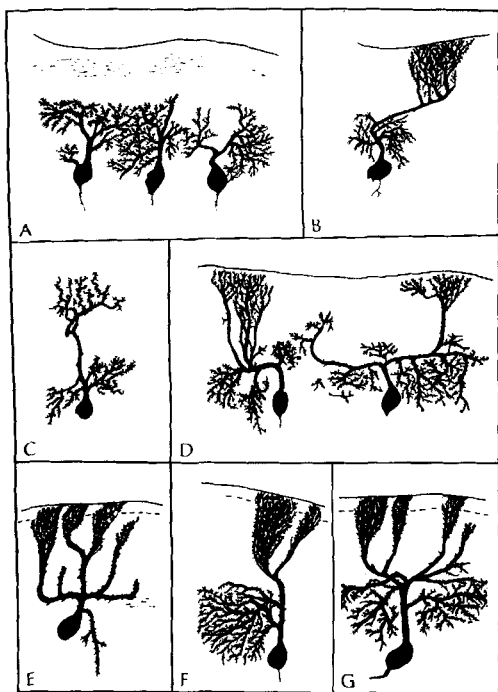


Fig. 8 Drawings of Purkinje cells from regions with multiple strata in the molecular layer. The Purkinje cells in A failed to invade the upper molecular layer with rotated parallel fibers; in B secondary sprouting of spiny branchlets is indicated; in C and D the orientation is normal in both the early and late formed zones of molecular layer (with some indication of weeping willow type growth in the lower zone); in E, F and G the dendritic branchlets are rotated in the coronal plane in the upper molecular layer. Golgi-Cox.

bipolar cells and parallel fibers. That is, if in a particular lobule the regenerating cells originate from a lateral focus, because the invasion route is parallel to the long axis of the folium the differentiating bipolar cells are oriented correspondingly; if the regeneration foci are anteriorly or posteriorly placed the multiplying cells spread through the transverse plane and the bipolar cells and parallel fibers become rotated.

This hypothesis attributes the normal longitudinal orientation of parallel fibers to the generally held view that during the late stages of embryonic development (in the rat) the cells of the external granular layer derive from the neuroepithelium of the laterally placed rhombic lip (what later

becomes the lateral recess of the fourth ventricle). This proposed mechanism can be tested experimentally by sparing during irradiation fragments of the external germinal layer either laterally (portion of hemispheres) or else anteriorly or posteriorly (parts of vermis).

It was interesting to observe that where parallel fibers became rotated, the axons of basket cells and the arborizing dendrites of Purkinje cells were oriented at a right angle to them. If it is considered that where parallel fibers are absent or scarce, spiny branchlets are not formed and the thorny massive branches are oriented in all planes (Altman and Anderson, '72, '73) then it is reasonable to assume that it is the presence of parallel fibers which induces the growth of spiny branchlets, and orients them orthogonally to the bed of parallel fibers. If these assumptions are correct, it follows that the autonomy of Purkinje cell dendritic development is restricted to the outgrowth of massive dendrites with postsynaptic membrane thickenings on amorphous thorns (Altman and Anderson, '72, '73) whereas the sprouting of spiny branchlets in appropriate orientation depends on the presence of parallel fibers.

#### 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, 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. 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: 353-398.
- 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 the cerebellar cortex. III. Regeneration of the

- external germinal layer and granule cell ectopia. *J. Comp. Neur.*, 149: 153-180.
- 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.
- Eccles, J. C., M. Ito and J. Szentágothai 1967 *The Cerebellum as a Neuronal Machine*. Springer, New York.
- Ramon y Cajal, S. 1911 (1955) *Histologie du Système Nerveux de l'Homme et des Vertébrés*. Instituto Ramon y Cajal, Madrid.

## PLATE 1

## EXPLANATION OF FIGURE

- 9 (A) In this sagittal section through the pyramis the regenerated parallel fibers in the upper molecular layer are rotated, whereas in the lower molecular layer (B) they are oriented normally. Synaptic profiles are relatively sparse.  $\times 13,680$ .



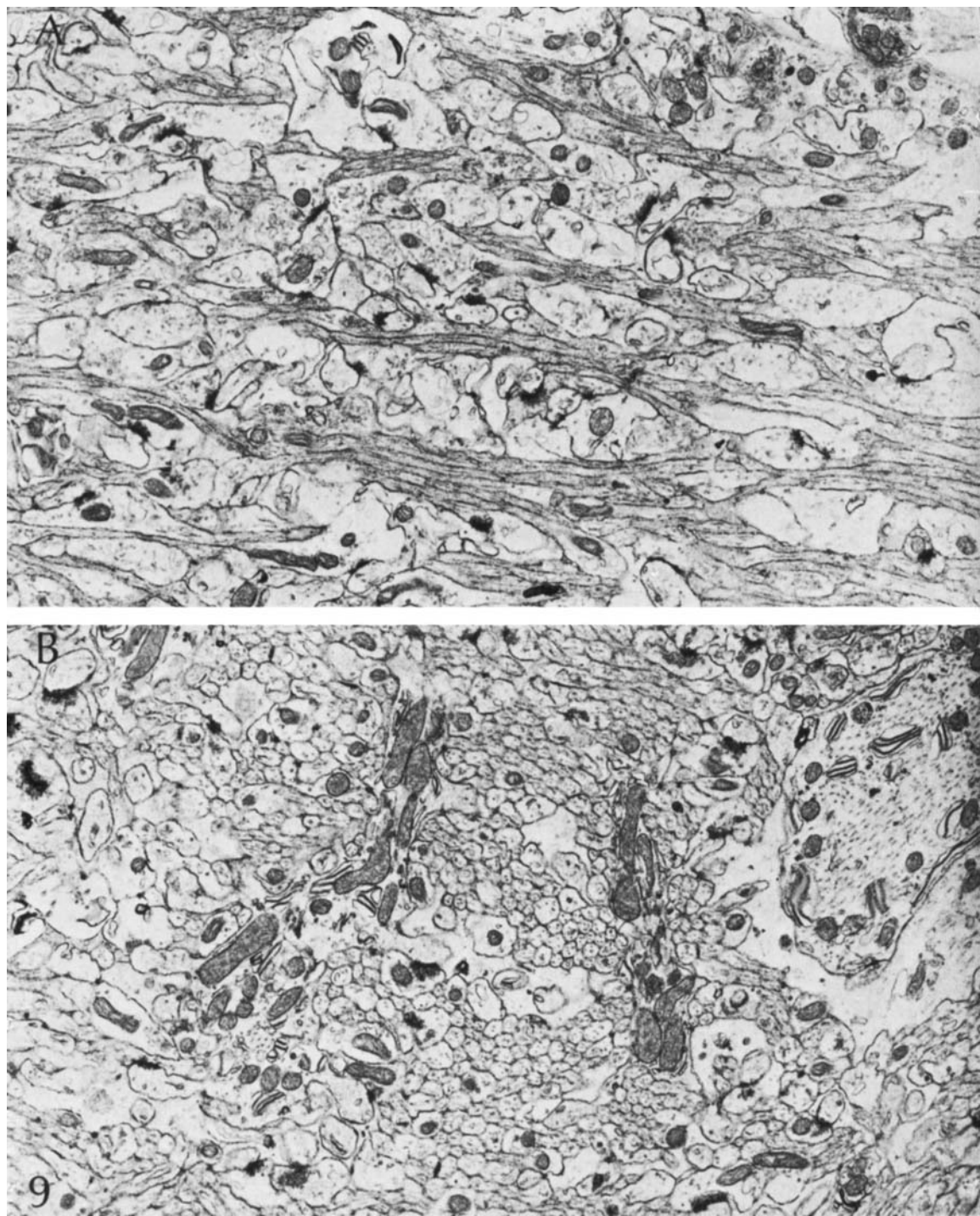


PLATE 2

EXPLANATION OF FIGURE

- 10 In the interface region of parallel fibers with different orientation, glial processes (GP) resembling the endfeet of Bergmann cells are seen. In the center the bifurcation point of a parallel fiber (arrow).  
× 16,168.

