Postnatal Development of the Cerebellar Cortex in the Rat

I. THE EXTERNAL GERMINAL LAYER AND THE TRANSITIONAL MOLECULAR LAYER

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ABSTRACT The multiplication of cells in the proliferative zone of the external germinal layer and the early steps in the differentiation of basket, stellate and granule cells were studied in the cerebellar cortex of rats aged 0, 3, 5, 7, 10, 12, 15, 21 and 30 days with histological, histochemical, autoradiographic and electron microscopic techniques. Between 0–9 days the proliferative zone has a constant depth of four to five cells; the bipolar cells in the underlying premigratory zone increase in depth during this period from 0–6 cells. Thereafter, there is a decline in the cell depth of both zones. In the premigratory zone there is a gradient in the length of the extruded processes of the bipolar cells (concentration of such profiles), the future parallel fibers. Presumably when the latter reach their final length, the cell body migrates downward and the parallel fiber becomes part of the upper zone of the molecular layer.

Thus, parallel fibers are progressively formed on the surface of others from the bottom upward by a stacking process and the external germinal layer, as a consequence, is continually pushed upward. This design makes possible the assembly of a matrix of very long, thin and straight beams of horizontally oriented parallel fibers which pile up vertically according to their age. When synaptogenesis starts during the second week in the molecular layer the enlarging junctional processes produce a spurt in the growth of this layer.

Basket cells which are formed in the pyramis on days 6–7 are arrested in the formative molecular layer because their processes are oriented at a right angle to the underlying bed of parallel fibers. Therefore, there is also a stacking of the cells of the molecular layer from the bottom upward as a function of time of onset of their differentiation. Parallel fiber synapses may be seen on differentiating basket cells as early as the seventh day, forming connections with these inhibitory interneurons before they synapse with spines of Purkinje cells.

Looking broadly at the history of the study of the development of the cerebellar cortex, two peaks become apparent. The first, which is more clearly discernible because of the available historical perspective, started with the identification of the different cellular and laminar constituents of the developing cerebellar cortex (Obersteiner, 1880; Schwalbe, 1881; Vignal, 1888; Lahousse, 1888; Schaper, 1894; Lugaro, 1894; Popoff, 1896; Athias, 1897; and others) and culminated in the works of Ramon y Cajal ('11, '60) who gradually succeeded in unraveling the major steps in the migration and transformations of the different cellular constituents of the cerebellar cortex, basing his conclusions and inferences mainly on material prepared with the Golgi technique. For about 50 years thereafter relatively little was added to our understanding of the development of the cerebellar cortex, perhaps because no new techniques were added to its examination. The recent application of two new procedures, one permitting the analysis of the dynamics of cell transformations and movements during cerebellar neurogenesis, the other the ultrastructural

examination of synaptogenesis, have seemingly started another spurt in productive activity in this field. The analysis of the dynamics of cerebellar neurogenesis is based on the application of the technique of thymidine-H^3 autoradiography (Uzman, '60; Miale and Sidman, '61; Fujita et al., '66; Fujita, '67; Altman, '66, '69; Altman and Das, '66) which permits the tagging of newly-formed cells and the tracing of their movements and transformations. The electron microscopic examination of synaptogenesis in the developing cerebellar cortex (Glees and Sheppard, '64; Mugnaini and Forstrønen, '67; Mugnaini, '69; Kornguth et al., '67, '68; Larramendi, '69) was made possible by the considerable progress made in the ultrastructural examination of the mature cerebellum (Gray, '61; Palay, '61, '64; Herndon, '63; Fox et al., '64, '67; Hámori and Szentágothai, '65, '66; Eccles et al., '67; Uchizono, '67; Larramendi and Victor, '67; Lemkey-Johnston and Larramendi, '68; and others).

This series of investigations consists of two separate but related studies; one normative, the other experimental. The normative portion is based on the concurrent examination of the development of the cerebellar cortex in rats from birth to adolescence with several research methods (various histological procedures, including the Golgi technique, histochemistry, autoradiography and electron microscopy). Because of practical considerations this normative study, which is essentially concerned with the establishment of some of the discernible phases of postnatal cerebellar neurogenesis, is divided into three parts. The first part (this paper) is concerned with the dynamic and ultrastructural properties of the subpial germinal matrix of the cerebellar cortex, the first steps in the differentiation of the cells arising from it, and the initial growth of the molecular layer. The second paper (Altman, '72a) deals with the various phases in the maturation of Purkinje cells. The third paper (Altman, '72b) is concerned with the maturation of the constituents of the granular layer. The attempt was to provide a tentative unified picture of the major steps in postnatal cerebellar neurogenesis to the limited extent that this can be accomplished with the descriptive (nonexperimental) techniques used in this study.

These descriptive studies complement available reports about the development of the cerebellar cortex that were mostly carried out in chicks (Mugnaini and Forstrønen, '67; Mugnaini, '69), mice (Larramendi, '69; Meller and Glees, '69) and monkeys (Kornguth et al., '68; Rakic, '71). But the major reason for its undertaking was to obtain normative data for a comparison of the changes produced in the organization of the developing cerebellar cortex of the rat by focal irradiation with low-level x-ray (Altman et al., '68, '69; Altman and Anderson, '71). With this experimental technique it has now become possible (Altman and Anderson, '72) to interfere systematically with the maturation of the cerebellar cortex at any stage of its development and selectively eliminate all or any one type of postnatally formed cerebellar interneurons. This provides a tool for checking some of the inferences made about the identity of the different types of synapses. But it has also allowed us to examine the morphogenetic properties of different cell constituents by studying the re-organization of cerebellar "fine-wiring" following selective elimination of different cell types.

MATERIALS AND METHODS

Several hundred, laboratory-bred Long-Evans hooded rats of different ages were prepared for this study and the cerebella were examined in detail in 330 (tables 1–7). These included material prepared for light microscopic histology and cytology, thymidine-H^3 autoradiography, various histochemical procedures, Golgi impregnation, and electron microscopy.

Histology and cytology. At birth the pups delivered by the mothers were pooled and eight pups were randomly assigned to each mother. At daily intervals between 0–21 days, and on day 30, an entire litter was anesthetized with ether, bled, and the removed brains were immersed in Bouins' fluid. The fixed brains were embedded in Paraplast, sectioned sagittally at 6 μ and stained alternately with cresyl violet and hematoxylin-eosin. The number of cerebella examined is summarized in table 1. In addition to this material Epon-Araldite

TABLE 1

Number of Nissl stained (cresyl violet and hematoxylin-eosin) cerebella examined

Age	No. of rats	Age	No. of rats	Age	No. of rats
0	8	8	4	16	2
1	4	9	4	17	4
2	5	10	7	18	4
3	7	11	5	19	2
4	4	12	4	20	4
5	7	13	4	21	4
6	3	14	4	30	6
7	7	15	3	Total	106

embedded and azure-B stained "thick" sections that were cut on an ultramicrotome (see below) were also available for light microscopic examination.

Autoradiography. Three procedures were used. In the first, rats were injected intraperitoneally with thymidine-H^3 (specific activity 6.7 C/mM; 10 μC/gm body weight) at 0, 2, 6 and 13 days of age, and pairs of animals were killed 1, 6, 24 and 72 hours after injection and at 20, 60, 120 and 180 days of age (table 2). In the second experiment pairs of rats were injected with two successive daily doses of thymidine-H^3 (5 μC/gm body weight) over the entire period between 0–19 days and were killed at 60 days (table 3). In the third experiment pairs of animals were injected in a similar manner with four successive daily doses over the same time span and were also killed at 60 days (table 4). All the brains were embedded in Paraplast and cut sagittally or coronally at 6 μ. The sections were stained with gallocyanin chromalum or hematoxylin-eosin, coated with Kodak NTB-3 nuclear emulsion, exposed at 5°C for three months and developed. Various qualitative observations and quantitative data gained from the first experiment were reported earlier (Altman, '66; Altman and Das, '66). In the present study specific attention was paid to various aspects of the development of the vermis (the pyramis in particular) in the light of problems that were raised by the other techniques employed.

Histochemistry. Rats from constant-size litters (8 per mother) were killed by bleeding at 0, 3, 7, 10, 15, 21 and 30 days of age. The brains were rapidly removed, quenched in isopentane and acetone at about −55°C, and stored at −40°C. In most cases within one week after removal of the brains, parasagittal sections were cut in a cryostat at 12 μ and an attempt was made to prepare some sections from each animal for all the histochemical procedures used. Modifications of the nitrophenyl ditetrazolium technique (Nachlas et al., '57, '58a,b; Thompson, '66) were used in staining for succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), NAD — and NADP — diaphorase. For cytochrome oxidase (CYO) Burstone's ('59) technique was used. Incubation time at 37°C was 60 or 90 minutes for SDH, LDH, CYO and NAD-diaphorase; 40 or 50 minutes for NADP-diaphorase. Staining for acetylcholinesterase (AChE) was done according to Gomori's modification of Koelle's acetylthiocholine method (Thompson, '66); incubation time was 15 hours. In all instances, except CYO, some of the sections were counterstained with neutral red in order to allow a better localization of the enzyme staining reactions and, in addition, control sections from each animal were stained with cresyl violet. The number of cerebella of different ages that were examined is summarized in table 5.

Golgi impregnations. The Golgi-Cox and rapid Golgi techniques were used and

TABLE 2

Number of cerebella examined in rats injected with a single dose of thymidine-H^3 (total of 64)

Age at injection	Short survival Time after injection (hours)				Long survival Age when killed (days)			
	1	6	24	72	20	60	120	180
days								
0	2	2	2	2	2	2	2	2
2	2	2	2	2	2	2	2	2
6	2	2	2	2	2	2	2	2
13	2	2	2	2	2	2	2	2

TABLE 3

Number of cerebella examined in rats injected with two successive daily doses of thymidine-H³. All animals were killed at 60 days of age

Ages	No. of rats	Ages	No. of rats	Ages	No. of rats
0–1	2	8–9	2	16–17	2
2–3	2	10–11	2	18–19	2
4–5	2	12–13	2		
6–7	2	14–15	2	Total:	20

TABLE 4

Number of cerebella examined in rats injected with four successive daily doses of thymidine-H³. All animals were killed at 60 days of age

Ages	No. of rats	Ages	No. of rats
0–3	2	12–15	2
4–7	2	16–19	2
8–11	2	Total:	10

TABLE 5

Number of histochemically prepared cerebella examined (total of 53)

Treatment	Age								Total
	0	3	5	7	10	15	21	30	
AChE	6	5	10	7	3	5	7	9	52
SDH	6	6	11	8	3	5	8	6	53
LDH	5	4	10	8	3	5	7	6	48
CYO	4	5	10	7	3	5	6	7	47
NAD	6	5	8	8	3	6	7	7	50
NADP	6	5	10	7	3	6	7	6	50

TABLE 6

Number of Golgi-impregnated cerebella examined (total of 28)

Age	No. of rats	Age	No. of rats
0	4	7	3
3	4	10	3
4	1	15	3
5	1	21	3
6	1	30	5

the sections were cut at 50 and 100 μ in sagittal and coronal planes (table 6).

Electron microscopy. Rats from entire constant-size litters were used at each of the following ages: 0, 3, 5, 7, 10, 12, 15, 21 and 30 days. The animals were anesthetized with ether and were killed by cardiac perfusion with buffered 6% glutaraldehyde. For each age, perfusion height and the gauge of needle used was established empirically by using a combination which produced instantaneous discoloration of the nose but did not break the capillaries to produce oozing of the fluid. After further fixation for two hours in buffered glutaraldehyde the cerebellum was resected, embedded in agar and cut sagittally or coronally in 235 μ thick blocks on a Sorval TC-2 chopping microtome. These blocks were processed simultaneously in a specially designed tissue carrier and were postfixed in 1% osmium for one hour, then embedded in Epon-Araldite. The blocks were trimmed to one lobule, the pyramis of the vermis, in an attempt to eliminate variability due to the different rate of maturation of the different lobules (Altman, '69). Thick and thin serial sections were cut with diamond knives on a Porter-Blum MT-2 ultramicrotome. The thick sections were stained with azure B for light microscopy. The thin sections were stained with lead citrate and uranyl acetate and viewed and photographed with a Philips 300 electron microscope. The majority of the cerebella were of good quality and the number that were examined in detail is summarized in table 7.

RESULTS

1. *The proliferative zone of the external germinal Layer:[1] structural and dynamic properties*

Light microscopic observations. In the posterior vermis of newborn rats the external germinal layer is about four to five cells deep. Most of these cells are roundish in shape, stain darkly, and several of them show mitotic activity throughout the entire depth of the layer (fig. 8). Within several days the depth of the layer increases to six to eight cells through the addition to the cells of the *proliferative zone*, a zone of another cell type. These cells appear to be smaller than the cells of the proliferative zone in sagittal sections (fig. 9), and are elongated, spindle-

[1] The term "external germinal layer" is proposed here in place of the commonly accepted term of "external granular layer." There are several difficulties with the latter term. (a) It does not reflect the fact that it is a transient germinal matrix. (b) It suggests unique relation to granule cells, when granule cells are only one of the several cell types to which it gives rise. (c) It necessitates the use of the term "internal granular layer" for the layer that is usually referred to simply as the "granular layer." The proposed term makes reference to the general property of this stratum as a germinal zone and also to its unique position on the surface of the cerebellar cortex.

TABLE 7

Number of cerebella examined with electron microscopy

Age	No. of rats	Age	No. of rats
0	7	12	6
3	5	15	5
5	4	21	5
7	5	30	6
10	6	Total:	49

shaped in coronal sections. There are no mitotic cells among these spindle-shaped cells which form the *premigratory zone* [2] of the external germinal layer.

In the pyramis of one-week old rats the external germinal layer increases in depth up to ten cells (fig. 10). Of these the upper four to five rows are composed of larger, roundish cells, the lower four to five rows of spindle-shaped cells. This indicates that the increase in the thickness of the germinal layer is due to the progressive accumulation of premigratory cells during this period, the population of stem cells in terms of depth does not increase (fig. 1). A further increase is seen in the depth of the entire layer up to nine to ten days which is due to further accumulation of premigratory cells (fig. 11). From the eleventh day onward there is a sharp decline in the depth of the proliferative zone and this is followed by a more sluggish decline in the cell depth of the premigratory zone. By days 16–19 the width of the layer is reduced to three to four cells and the proliferative zone no longer forms a continuous row of cells, although many individual proliferative cells are seen scattered in a superficial position.

Electron microscopic observations. The cells of the proliferative zone of the germinal layer in newborn rats and throughout the later stages of development show similar characteristics. The most prominent component is the polymorphous nucleus which varies in size from 4–6 μ and contains dispersed electron dense granules or granules coalesced into randomly situated clumps (figs. 12, 13). The cytoplasm varies in size from a thin band around the nucleus to irregular protrusions of several micra. It is rich in free ribosomes, contains mitochondria and Golgi apparatus, but is devoid of granular endoplasmic reticulum and microtubules. Basal bodies and cilia are often seen, coated vesicles and multivesicular bodies rarely. Cells in all phases of mitosis are common. The cells are either contiguous with one another or are separated by smaller or larger intercellular spaces. Sometimes short desmosomal junctions are seen between these

[2] The use of this term is justified in the next section.

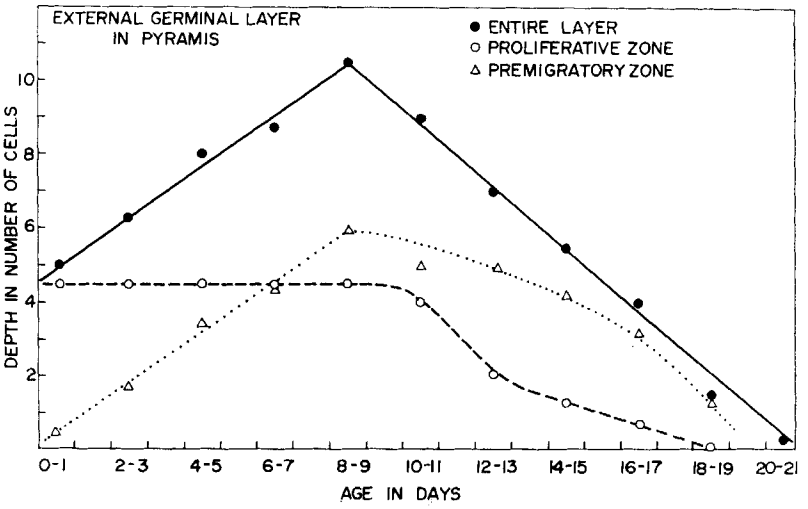

Fig. 1 Changes in the cell-depth of the external germinal layer, and its two zones, as a function of age. Because of variability the means were computed for blocks of two days (N = 106). Note the constancy of the depth of the proliferative zone between 0–9 days.

cells, but these are not frequent. Synapses are never seen in the external germinal layer.

In addition to the cells and processes of the external germinal layer, other cellular processes are also seen in the external germinal layer from birth onward with increasing frequency and these are distinguished by their light staining quality. In the five day old and older animals several contiguous, slender and vertically oriented processes may form a single column that terminate with bulbous endfeet on the surface of the cortex (fig. 14). On the basis of Golgi observations, these processes are identified as those of Bergmann glia cells. These endfeet together with the cytoplasm or processes of germinal cells form the surface of the cerebellar cortex and they are jointly coated with the basal lamina.

Only a few changes were noted in the external germinal layer as a function of age. In coronal sections in five-day old and older animals, horizontally oriented, spindle-shaped cells are seen with increasing frequency below the polymorphous cells, representing the premigratory zone (fig. 12). The space occupied by the endfeet of Bergmann glia cells tends to increase with age (fig. 14). From day 15 onward these endfeet contain concentric (up to 8–10) double-membraned lamellae that are studded with glycogen granules (fig. 15). In the 21-day old animals, only scattered germinal cells remain in the pyramis and most of these are no longer contiguous with the subpial surface, as the glial endfeet form an almost continuous sheet over the cortex. A rare mitotic cell is still seen and this is contiguous with the pia. In the 30-day old animals, germinal cells are no longer present on the surface of the cerebellar cortex.

Autoradiographic results. In newborn or two-day old animals that were injected with a single dose of thymidine-H^3 and killed one hour later, a large proportion of the cells in the upper three-fourths of the external germinal layer were labeled. In the animals that were injected with a single dose of thymidine-H^3 at six days of age and killed one hour later, in the upper five rows composed of round cells, the majority of the cells were labeled; the two to three rows of spindle-shaped cells situated below them were not labeled.

In the animals that were injected with a single dose at six days and survived for six hours after injection, the pattern of cell labeling was similar to the previous group except that in these, about one-half of the premigratory spindle-shaped cells were also labeled, preferentially those contiguous with the round germinal cells. Cells in the molecular layer were not labeled. In the animals of this group that survived for 24 hours after injection, considerable label dilution was seen in the germinal zone and all the spindle-shaped cells were either intensely or lightly labeled. In addition, in these animals virtually all the migratory cells in the molecular layer were also labeled. These results (fig. 2) indicate that in the six-day old animals, within 6 hours after labeling of the newly synthesized DNA, many of those cells destined to leave the stem cell population have been displaced or dipped into the premigratory zone. Within 24 hours all the unlabeled cells in the premigratory zone were replaced by the newly-formed cells and many of these were in the process of migration through the molecular layer. Because at this age there is local cell proliferation in the (internal) granular layer, it could not be determined whether or not some of the cells formed in the germinal layer have reached their destination in the granular layer.

In the animals injected with thymidine-H^3 at 13 days of age and killed one hour afterwards, the round, proliferative cells composing the upper two to three rows of cells of the external germinal layer were intensely labeled, the four to six cell-thick lower row of spindle-shaped cells was not labeled. Virtually no migratory or other type of cell was labeled in the molecular layer. A few intensely labeled cells were seen in the granular layer and several of them in the medullary layer. Essentially the same pattern of labeling was observed in the animals that survived for six hours after injection. In these animals, unlike in those injected at six days of age with six hour survival, the spindle-shaped cells of the premigratory zone were not labeled, indicating a delay with age in the transit from the proliferative into the premigra-

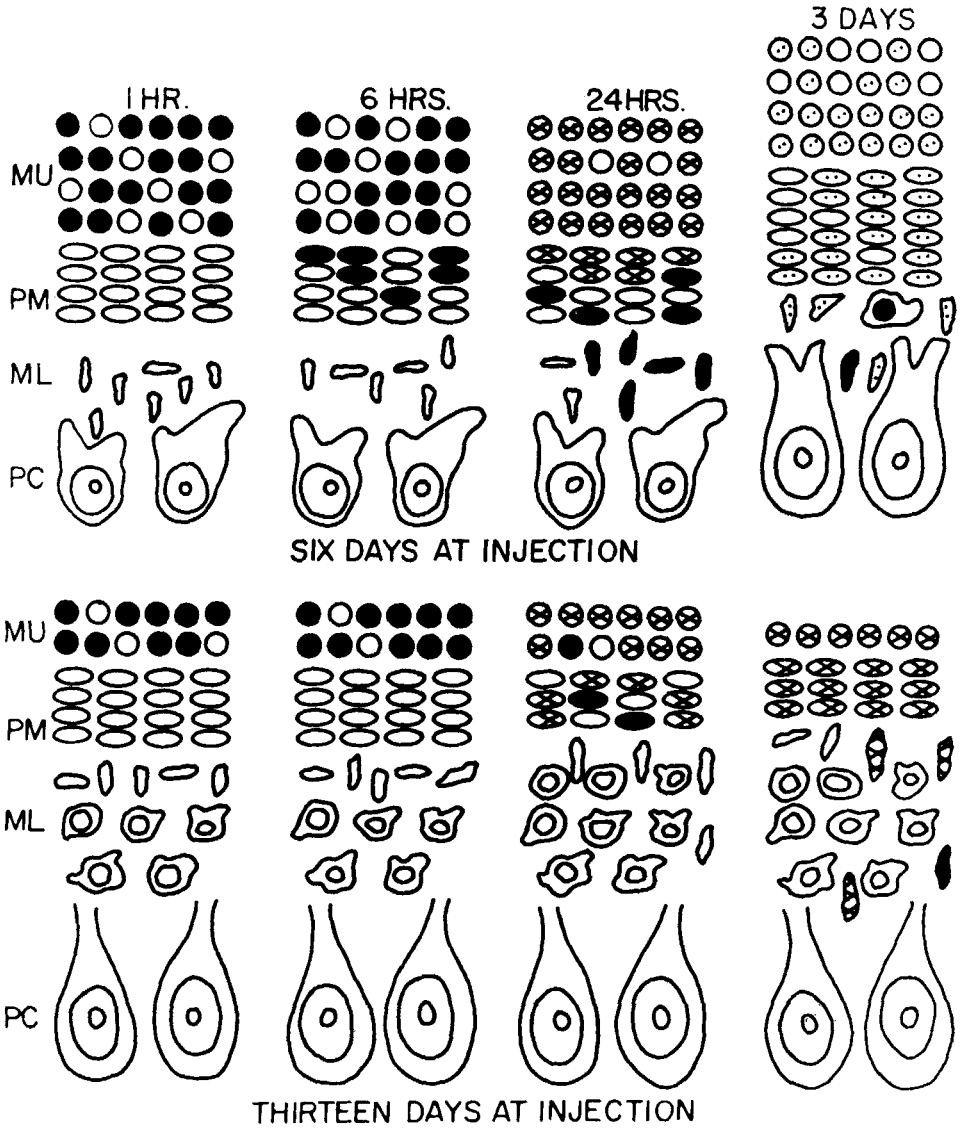

Fig. 2 Diagrammatic presentation of the autoradiographic evidence of the pattern of movement of cells formed in the proliferative zone of the external germinal layer (MU), into the premigratory zone (PM), and through the migratory channels of the molecular layer (ML) toward the granular layer. Compare changes in transit and differentiation times in one-week old rats (injection with thymidine-H^3 at 6 days) with two-week old rats (injection at 13 days). Primary labeled cells fully blackened; crosses and dots indicate different degrees of label dilution due to remultilplication prior to migration and differentiation; empty figures unlabeled cells that were formed before injection.

tory zone (fig. 2). In the 13-day old animals that were killed 24 hours after injection, all the cells of the external germinal layer (proliferative and premigratory) showed intermediate labeling, with an occasional intensely-labeled cell being situated in the bottom rows of the premigratory zone. In these animals, the horizontally or vertically oriented spindle-shaped migratory cells of the molecular layer were not labeled. This indicated that in these older animals the transit of cells from the

premigratory zone of the external germinal layer into the migratory compartment of the molecular layer was delayed with respect to the six-day old animals that were killed 24 hours after injection. In the animals that were injected at 13 days and killed three days later, there was a weak labeling of cells in the external germinal layer, indicating dilution due to many subdivisions. In the molecular layer some of the spindle-shaped migratory cells were lightly labeled. In the granular layer, many of the granule cells were labeled; some of these showed intense labeling, others medium or light labeling. These results indicated that in the two-week old animals, migration through the molecular layer occurred some time between 24 hours and three days after the cells were formed; by the third day, most of the migratory cells settled in the granular layer.

Conclusions. The foregoing results indicate that the proliferative zone of the external germinal layer is composed of stem cells which are distributed from birth onward, four to five cells deep below the pial membrane. There is rapid cell proliferation in the proliferative zone from birth, but this never leads to an increase in its depth. Initially, this increase leads to an enlargement of the stem cell population through expansion over the rapidly growing surface of the cerebellar cortex (Altman, '69). During this early period, relatively few cells leave this fixed-width compartment to form the premigratory layer. But there is a gradual stacking up (see below) of the postmitotic cells in the premigratory zone where optimal width is reached about nine to ten days of age. Thereafter, there is a rapid fall in the stem cell population, notwithstanding the circumstance, as indicated by autoradiography, that transfer from the proliferative into the premigratory compartment is slowed down and so is also the movement from the latter into the migratory compartment of the molecular layer.

2. *The premigratory zone of the external germinal layer: stacking of parallel fibers in the formative molecular layer*

Light microscopic and electron microscopic observations. The gradual accumulation during the first few days of life of laterally-oriented, spindle-shaped cells underneath the proliferative zone was described earlier. In agreement with the morphological data, according to which mitotic cells are absent from this zone, the autoradiographic results showed that new cells appeared here some time after they became labeled with thymidine-H^3 in the proliferative zone (within 6 hours in the 1-week old rats, within 24 hours in the 2-week old ones). The shape of the majority of these cells (roundish and small in sagittal sections, horizontally-oriented and spindle-shaped in coronal sections) indicated that when these cells were transferred from the proliferative zone into the premigratory zone, they became elongated laterally in the direction of the long axis of the folium. Electron microscopic examination of these cells indicate that this change in the shape of cells is partly due to a comparable alteration in the shape of the nucleus, but more importantly, to the accumulation of darkly staining cytoplasm at the two lateral poles of the cell. The nucleoplasm of these cells is similar to that of cells in the proliferative zone and there is little obvious change in the cytoplasm which remains particularly rich in free ribosomes and has few or no microtubules. However, an examination of this zone in sagittal sections indicates that intermingled with the soma of these cells, two types of profiles are abundant in this zone: larger profiles with an ultrastructure similar to the perikarya of cells, and smaller profiles with a few microtubules which are identified as parallel fibers. In the upper portion of this zone, the majority of the profiles are of the first kind, with a small admixture of parallel fibers. In the lower portion of this zone, the proportion of parallel fibers greatly increases, and the bottom row of premigratory cells are embedded in a rich matrix of parallel fibers (fig. 13). In a few fortuitous sections, parallel fibers were seen issuing from one or both cytoplasmic poles of these cells.

Considering the shape of the lower germinal zone cells, several investigators including ourselves (see DISCUSSION SECTION) have assumed that these are migratory elements and, accordingly, this zone has been designated as the migratory zone. The evidence presented earlier, together with logical considerations, leads us to the conclusion that while situated in this zone,

the laterally-oriented, spindle-shaped cells are not migrating, but are undergoing the first step in their differentiation into granular neurons. The shape of these cells is not attributed to a migration parallel to the surface, but to the accumulation of growth cytoplasm at opposite poles where the parallel fibers grow out laterally by an extrusion process. It is postulated that the cell body remains in the plane in which the parallel fibers grow over the surface of already-formed parallel fibers, until the total length of the fibers is achieved (fig. 3). (Then presumably the cell body makes a right angle turn, the vertical portion of the granule cell begins to form, and the granule cell soma migrates to the granular layer.) In the newly-forming, or upper zone, of the molecular layer the bulk of the tissue is made up of thin parallel fibers. Parallel fiber enlargements and dendritic processes with which they will form synapses are rare (see the succeeding paper), indicating that the stacking of parallel fibers antedates synaptogenic activity.

Conclusions. The profile gradient observed in electron micrographs of the premigratory zone of the external germinal layer, namely, a high concentration of cytoplasmic profiles and low concentration of parallel fibers in the upper part of the zone, and a reversal of the pattern and final transition into the parallel fiber-rich molecular layer, suggests that the lower cells are more advanced in their differentiation (have longer bipolar outgrowths, or parallel fibers-in-the-making) than the upper cells of the premigratory zone. This is schematically illustrated in figure 3 which also shows that when the future granule cell descends, the parallel fiber becomes part of the molecular layer. That is, parallel fibers are formed on the horizontal surface of already present parallel fibers by a stacking process, the earlier-formed parallel fibers being situated underneath the later-forming ones. The consequence of this is that the cells of the external germinal layer are continuously pushed upward as the depth of the molecular layer increases through the stacking of newly-formed parallel fibers. Parallel fiber enlargements and dendritic profiles are rare in the newly-formed zone of the molecular layer.

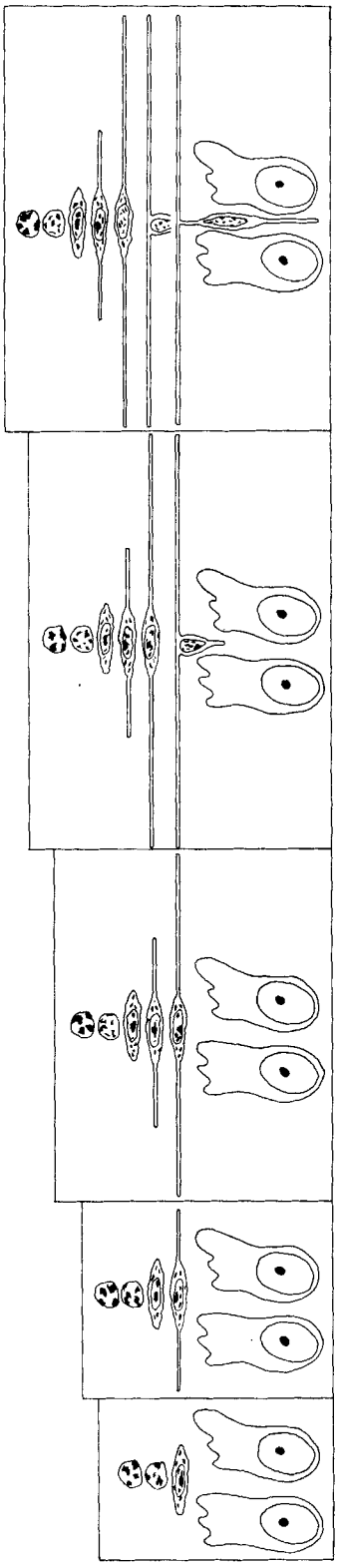

Fig. 3 Diagrammatic illustration of the transformation of proliferative round cells into spindle-shaped premigratory, differentiating cells which first produce by extrusion the horizontal portion, then the vertical portion of the parallel fibers, as first described by Ramon y Cajal ('60). It also illustrates the principle of the progressive stacking of parallel fibers from the bottom of the molecular layer upward and the continued upward displacement thereby of the external germinal layer.

3. *The nonmigratory cells of the transitional molecular layer: stacking of basket and stellate cells in the molecular layer*

Light microscopic observations. Below the external germinal layer, a "spongy" transitional zone is recognizable, forming the differentiating superficial part of the molecular layer (figs. 8, 9). Towards the end of the first week, darkly-staining, horizontally and sagittally oriented, spindle-shaped or polymorphous cells begin to increase in number in this zone (figs. 9, 10). Differentiating and differentiated cells in the molecular layer (cells that have lightly-staining nuclei, surrounded by visible cytoplasm) are not seen during this period. In the pyramis of eight-day old rats, a few differentiated cells begin to appear and these increase in number from the tenth day onward (fig. 11). Thus, in the ventral half of the pyramis, differentiated cells are present one to two cells deep at nine to ten days, three to four cells deep by the twelfth day, and up to eight to ten cells deep by the fourteenth day. This rapid increase of differentiating cells of the molecular layer is concurrent with the onset of the rapid growth in the depth of this layer (fig. 4) to which contributions are made by the commencement of synaptogenic activity (see succeeding paper). But throughout this period there remains a spongy zone below the external germinal layer in which darkly-staining undifferentiated, horizontally-oriented cells are seen in considerable concentration.

In the seven-day old rats, a few Golgi-impregnated cells are seen that resemble basket cells. These cells tend to have rudimentary terminals (not true baskets) near the upper part of the soma of Purkinje cells and their thickly impregnated dendrites reach the surface of the molecular layer. In ten-day old rats, several cells were seen which had horizontally spread processes from which descending branches issued (fig. 5A). In a few of these cells,

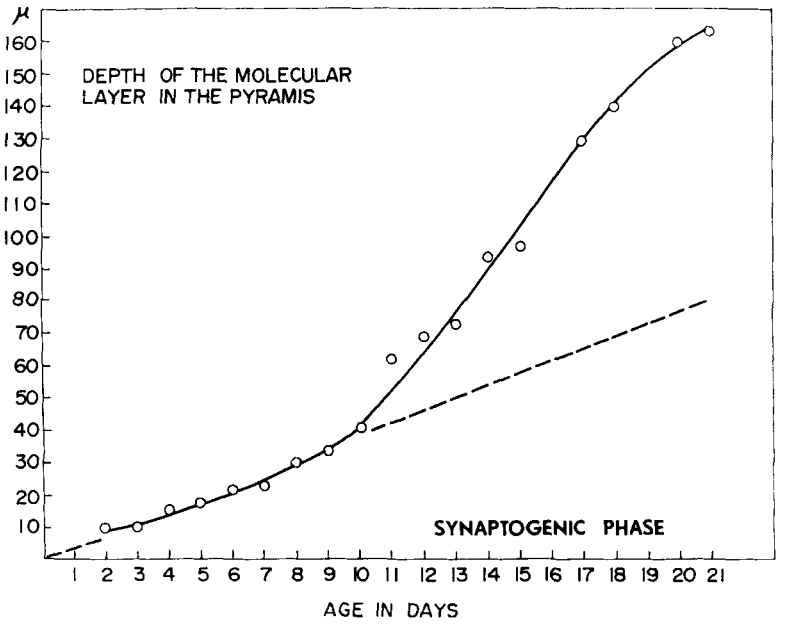

Fig. 4 Increase in the depth of the molecular layer in the pyramis between 2–21 days of age. A slow growth rate is indicated for the first ten days; a fast one during the second ten days. (Broken line is extrapolated growth curve based on the slow phase.) The first, slower phase is attributed to the formation of parallel fibers (with some contributions made by basket and stellate cells). The faster phase is attributed to the commencement of synaptogenesis, which involves the enlargement of the junctional processes of parallel fibers and the penetration of the dendritic processes of Purkinje cells (Altman, '72a, figs. 45–47). (N = 87).

the terminals of the branches surrounded the soma of Purkinje cells and these were identified as maturing basket cells. Basket cells are quite common in the 15-day old rats (fig. 5B). They often have extensive dendritic arborizations and dense pericellular baskets around the soma of Purkinje cells. At this age, a few stellate cells with thickly-impregnated short dendrites, are also seen (fig. 5C). Mature stellate cells become more frequently impregnated in the 21-day and older rats (fig. 5D).

Autoradiographic results. In a previous study (Altman, '69), evidence was obtained that the cells of the lower molecular layer (where the basket cells are located) differentiate before the cells of the upper molecular layer (which include the stellate cells) and that the time of differentiation of the granule cells overlaps with the other two. The conclusion was based on counts of the number of intensely-labeled cells in autoradiograms of the cerebellum of adult rats that were injected with thymidine-H^3 at 0, 2, 6, and 13 days of age. Maximal number of intensely-labeled "basket cells" were found in the animals injected at six days of age, with very few at 2 or 13 days; intensely-labeled "stellate cells" were seen in highest number at 13 days. In the (internal) granular layer, the concentration of heavily tagged cells was highest at 13 days, but there was also a moderate concentration of these at six days.

In the present study, in which virtually the entire period of postnatal cerebellar neurogenesis was covered in two multiple-

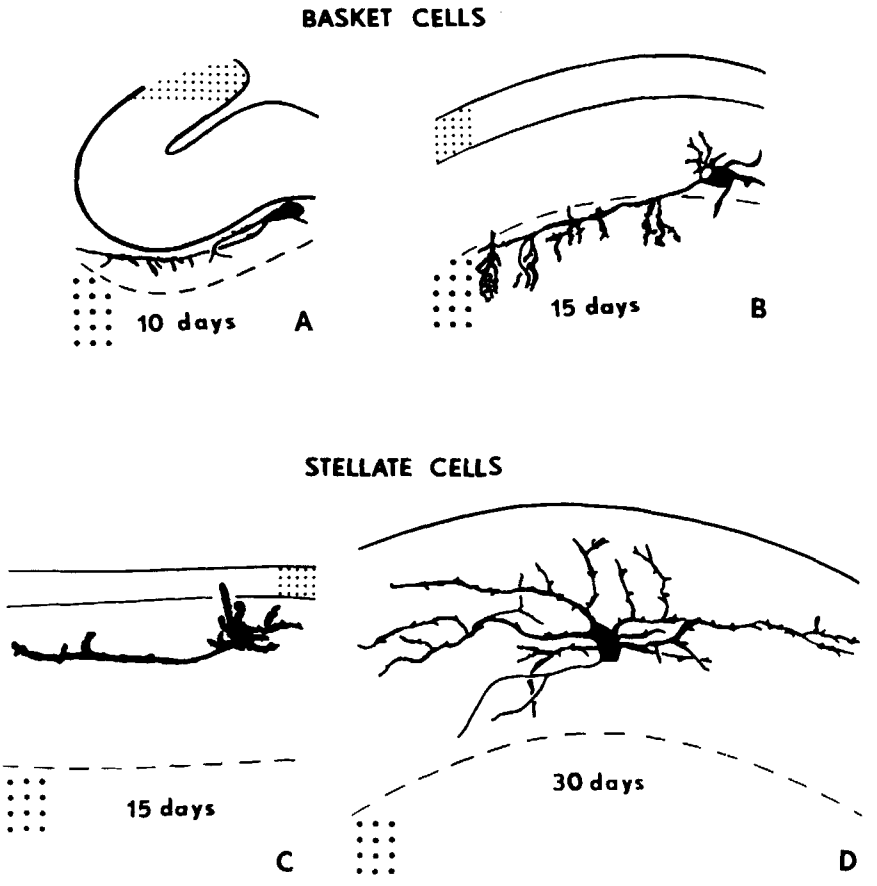

Fig. 5 Drawings of basket and stellate cells from Golgi-impregnated sections in rats of different ages. External germinal layer is indicated by fine dots in a corner, the granular layer by coarse dots.

injection groups of rats (this method was adopted for reasons of economy) the highest concentration of intensely-labeled "basket cells" was seen (fig. 6) in the animals injected at four to seven days in the group receiving four successive daily injections, and at six to seven days in the group that received two injections. The fact that nearly as many intensely-labeled cells were seen in the latter group as in the former indicates that the injections on days 4–5 contributed few cells to the "basket cell" population in the group receiving four injections (this is indicated directly for these days in the double-injection group). Therefore, it is concluded that the bulk of the "basket cells" in the pyramis are formed on days 6–7.

In the group that received four successive daily injections, the highest concentration of "stellate cells" was seen in the animals injected at 8–11 days (fig. 6). In the group injected with two successive daily doses, comparable concentrations of intensely-labeled cells were obtained in both the animals injected at 8–9 days and at 10–11 days. However, in these animals, the number of such cells was less than half of that counted in those that received four successive injections on days 8–11. This difference permits several interpretations. First, the "stellate cells" are formed over the protracted period of four days. Second, the "stellate cells" are formed over a two-day period on days 9–10 with injection on day 8 in the 8–9 day group and on day 11 in the 10–11 day group contributing few intensely-labeled cells. The third possibility is that the "stellate cells" are composed of two populations and those in the lower aspect of the upper molecular layer are formed earlier (8–9 days) than those in the upper aspect (10–11 days). This possibility was supported by the observation of a gradient in the molecular layer, with the cells situated near the surface being among the last labeled.

Electron microscopic observations. The molecular layer is a thin band in the newborn and three-day old animals, with a few dark cells embedded in a "spongy" tissue filled with extracellular spaces and scattered parallel fibers (fig. 16). In view of the virtual absence of identifiable, differentiated granule cells at these ages, the

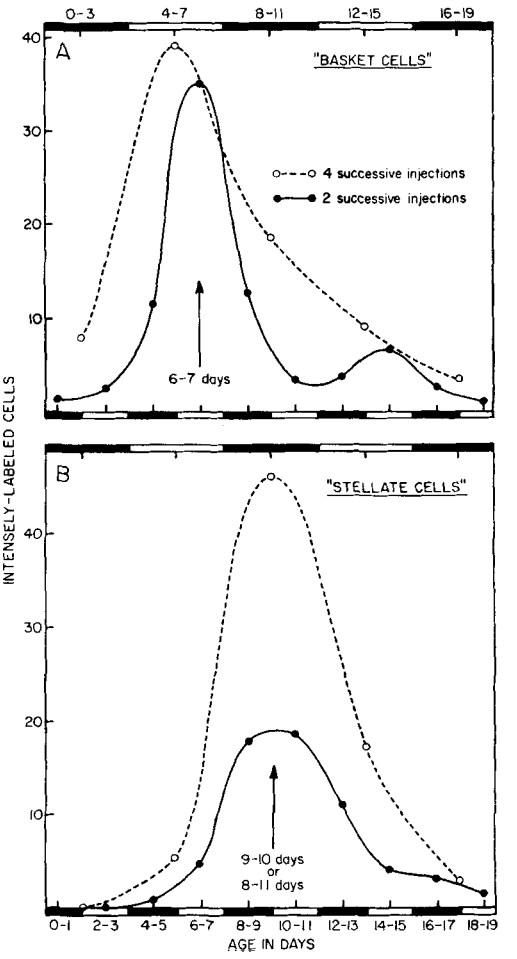

Fig. 6 Concentration of intensely-labeled cells in the lower half of the molecular layer ("basket cells") and its upper half ("stellate cells") in adult rats that were injected with two or four successive daily doses of thymidine-H^3 between 0–19 days.

relatively high concentration of parallel fibers in the lower aspect of the external granular layer, in the thin molecular layer, and among the Purkinje cells, supports the conclusion that the parallel fibers are formed before the granule cells descend. These parallel fibers are loosely packed and are either of the mature type with few microtubules, or are thicker and occasionally have as many as a dozen microtubules in cross section. Parallel fiber enlargements with vesicles are not seen and mature synapses are absent. Occasionally,

parallel fibers are seen with junctional dense membranes and open coated vesicles (see below).

In the five-day old rats, darkly staining, sagittally-oriented, spindle-shaped cells are occasionally seen below the external germinal layer which, on the basis of some further transformations seen in the seven to ten day old animals, are identified as presumptive basket cells. In the latter groups, the typical cell in this position has a dark nucleus surrounded by bipolar or multipolar cytoplasm from which long sagittally-oriented processes emerge. The perinuclear region, particularly where the processes emerge, has a "reticular organization" which could be followed into the processes. This organization is characterized by a rich concentration of Golgi apparatus, cisterns of the agranular reticulum, mitochondria, and occasional coated vesicles. In the processes there is a variable concentration of microtubules, in the periphery of the soma clusters of free ribosomes are present in high concentration. In many of these cells, open coated vesicles are seen on the soma or on the processes (occasionally at a considerable distance from the soma), generally opposite parallel fibers. In similar position, symmetrical, conspicuous dense membranes are present in many cells or mature synapses of the asymmetrical type which were interpreted as presumptive or mature parallel fiber synapses (fig. 17).

Two types of immature cells abound in the transitional molecular layer: spindle-shaped cells that are oriented horizontally and similarly shaped cells that are oriented vertically, or at a right angle to the surface (fig. 18). But whereas in the former, synaptogenic activity and mature synapses are often seen on the soma of processes (figs. 17, 19), the vertically oriented cells show no synaptogenic activity at this or any later age in the molecular layer even though the soma and bipolar processes are directly contiguous with parallel fibers and other processes (fig. 20). Accordingly, the sagittally oriented cells are distinguished from the precursors of granule cells by two obvious criteria, namely their plane of orientation and their synaptic responsiveness. As illustrated in figure 7, by virtue of their orientation at a right angle to the bed of parallel fibers, the sagittally expanding cells are mechanically immobilized immediately after their transfer from the external germinal layer. It is not known whether this rotation occurs in the lower zone of the external germinal layer where the majority of the cells are oriented laterally, or whether this occurs in the superficial, formative surface of the molecular layer, but the cells thus rotated perforce become stationary elements and, according to the evidence presented, begin to form synapses immediately or within a few days. As the parallel fibers are continued to be stacked in the developing molecular layer, and the external germinal layer which gives rise to other basket and stellate cells is raised upward, the differentiating interneurons of the molecular layer become stacked themselves, the early-forming ones remaining permanently situated below the later-forming ones.

In the ten-day old rats, differentiating cells are seen about two cells deep in the molecular layer. These, as described earlier, have open coated vesicles, symmetrical junctional membranes without vesicles, and occasional developed asymmetrical synapses on their soma and dendrites with parallel fibers. In the 12-day old animals, well differentiated cells are present both in the lower and middle portion of the molecular layer. In general, the perikarya of the cells situated in the lower molecular layer tend to have larger nuclei and more cytoplasm, but there are no other criteria to aid in distinguishing presumed basket cells from stellate cells in electron micrographs. In both cell types, the cytoplasm stains lightly with a moderate concentration of mitochondria, Golgi apparatus, and agranular reticulum; granular endoplasmic reticulum is scarce. The initial portion of the dendrite and many of the distal ones, tend to be thick and stain lightly with occasional patches of free ribosomes and scattered microtubules. Both on the soma and the dendrites there is a high concentration of small boutons with conspicuous, asymmetrical synapses which are identified as parallel fiber synapses (fig. 21). In addition, elongated boutons are seen in lower concentration which have inconspicuous symmetrical synapses (fig. 21). The identity of these is not known for cer-

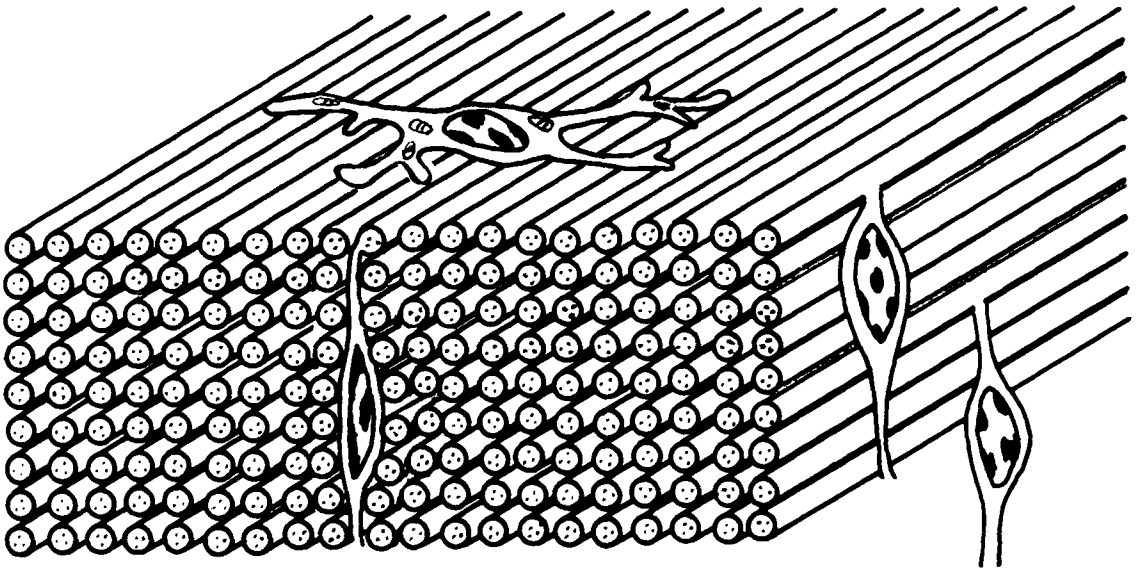

Fig. 7 Schematic illustration of the principle that because of their orientation at a right angle to the underlying bed of parallel fibers, the differentiating basket cells are rendered immobile from the moment that their processes begin to be formed. In contrast, the cell bodies of the differentiating granule cells, leaving behind their horizontal processes (parallel fibers) can move downward between sheaves of parallel fibers by slightly displacing them. This migration is made easier by the flattening of the vertically-oriented, spindle-shaped cell bodies of the granule cells in the longitudinal plane of the folium (Mugnaini and Forstrønen, '67; Rakic, '71), parallel to the orientation of the parallel fibers.

tain, but they resemble basket or stellate cell synapses and are presumably those of the latter. In a few instances basket cells were encountered with an axon issuing from the soma (fig. 22). The basket cell axon has the typical undercoating of other types of neurons. In one instance (figs. 23, 24) a basket cell was identified in which a downward directed axon issued from a horizontally oriented dendrite which had several synapses.

Cells with synapses in the upper molecular layer are seen in the 15-day old and older animals. The typical stellate cell (fig. 25) has a few conspicuous, asymmetrical synapses on its soma and dendrites. The number of these tends to be much lower than in basket cells and, unlike in the former, inconspicuous symmetrical synapses are rarely if ever seen. A striking characteristic of both basket and stellate cells, even in older animals, is the intimate relation between the membranes of their soma and those of parallel fibers (fig. 26), that is, the absence of a glial barrier.

Conclusions. In view of the available Golgi evidence that the majority of neurons situated in the molecular layer in the proximity of the soma of Purkinje cells are basket cells, and considering the autoradiographic evidence that the majority of the cells situated in the lower aspect of the molecular layer are formed on days 6–7, the conclusion is justified that the first group of differentiating cells in the molecular layer, which become arrested on the surface of the parallel fibers that are piling up, are basket cells. (The appearance of basket cell terminals on the soma of Purkinje cells is described in the succeeding paper). The non-migrating basket cells, and later the stellate cells, become stacked from the bottom of the molecular layer upward as a function of their time of origin. The differentiation of stellate cells is a protracted process and may not be completed when cerebellar neurogenesis is terminating. The major input to the basket and stellate cells is from the parallel fibers with characteristic conspicuous, asymmetrical synapses, but inconspicuous, sym-

metrical synapses are also present on their somata and dendrites.

DISCUSSION

The proliferative zone. The presence of a "superficial granular zone" (Ramon y Cajal, '60) in the developing cerebellar cortex was first described by Obersteiner (1880) and was confirmed immediately thereafter by many investigators. The nature and significance of this transient, subpial layer was initially not understood. For instance, Vignal (1888) suggested that they were migrating leucocytes, and Obersteiner (1880) and Schwalbe (1881) that they were elements of the reticulo-endothelial system. Later, Obersteiner (1883) proposed that they gave rise to glia cells, whereas Lahousse (1888), Schaper (1894) and Popoff (1896) maintained that they were undifferentiated cells which could differentiate into both glia and neurons. Popoff (1896) suggested that the neurons that these cells gave rise to were those located in the molecular layer and it was for some time thought that the majority of the granule cells arose from the germinal layer of the ventricle with some contributions made by the cells of the external germinal layer (Schaper, 1894; Lugaro, 1894).

Ramon y Cajal ('60, p. 266) initially believed that the cells of the external germinal layer produced the stellate type cells of the molecular layer, but he soon became convinced that the granule cells of the granular layer also arise from this stratum. He distinguished two subzones within this germinal layer: a superficial zone of proliferative "epitheloid" cells and a deeper zone of "bipolar horizontal" cells. Ramon y Cajal noted that the protoplasmic processes of the latter extended over long distances parallel to the long axis of the folia, and that the deeper ones of these cells had descending processes. When he discovered that the cell bodies associated with the bifurcating T-shaped processes could be scattered between the superficial and internal granule cell layers, he became convinced that he was witnessing the differentiation and migration of granule cells.

Definite proof of the transformations and migrations of the cells of the external germinal layer had to await the introduction of a reliable tagging technique for multiplying cells. Curiously, the first results with this technique did not contribute to our understanding of the ongoing events, as Uzman ('60) erroneously concluded that the external germinal layer gives rise to granule cells only during the first phase of cerebellar development, whereas during the next stage this process ceases and cells then migrate from the internal granular layer into the molecular layer. In a subsequent study, Miale and Sidman ('61) were able to show that in mice injected on the tenth and fifteenth day of age, some scattered labeled cells appeared within five days of injection in the molecular layer and large numbers in the granular layer, even though very few were seen in these positions in mice killed two hours after injection. Similarly we have shown (Altman, '66) that in rats injected at 13 days of age, there were essentially no labeled cells in the granular layer at 1, 6 or 24 hours after the injection, but by the third day 20% and by the sixth day 45% of the cells of the granular layer were labeled. The conclusion that the steady accumulation of cells in the latter position was due to migration was inescapable, and their speed of migration was determined. The same study also showed that whereas 70–80% of the cells of the molecular layer were labeled in animals injected at birth or two days of age, only 20% were labeled in the rats injected at 13 days. From this it was concluded that the neurogenesis of the molecular layer antedates that of the granular layer (p. 455).

Comparable results were obtained in mice by Fujita ('67) using a cumulative labeling technique. Up to ten hours after repeated injections with thymidine-H^3, labeled cells were restricted to the outer half of the external germinal layer. But as the cumulative labeling proceeded beyond ten hours, the region of labeled cells spread row by row inward, and by the twenty eighth hour the entire external germinal layer was labeled. Then between 28 and 31 hours, also the vertically oriented spindle-shaped cells, the migrating granule cells become labeled and by this time there was a marked increase in the number of labeled cells in the granular layer. Fujita also showed that starting

cumulative labeling from birth, all the cells of the cerebellar cortex, except Purkinje and Golgi cells, became labeled. If the cumulative labeling was started after the third or fourth day, cells in the lower portion of the molecular layer (presumed basket cells) were often unlabeled and by the seventh day, few labeled stellate cells could be seen. From this, Fujita concluded that stellate cells as a whole were formed before the granule cells, as 95% of the granule cells came into existence between postnatal days 4 and 15 in mice. In a subsequent study in rats we showed (Altman, '69) that "basket cells" (cells in the lower half of the molecular layer) were formed before "stellate cells" (upper half of the molecular layer) and that in different regions of the vermis from 25–75% of the granule cells are formed during the third week of life, after all the nerve cells of the molecular layer had come into existence.

Ramon y Cajal remarked ('60, p. 274) that the two zones of the external germinal layer are easier to distinguish in rats, cats or dogs that are several days old than in neonates because the younger the animal the thicker the zone of "epitheloid" (proliferative) cells with respect to the zone of bipolar (premigratory) cells. This observation was confirmed by Addison ('11). He found that in rats in which there are six to eight rows of cells in the external germinal layer at birth and eight to ten rows by ten days, the outer (proliferative) zone had only one to three rows at birth which increased to five to seven rows by five days. There is some quantitative discrepancy between Addison's results and ours since our evidence indicates virtually no premigratory zone at birth. This discrepancy may be partly due to the sampling of different regions and perhaps more importantly, to an improvement in histological procedure which aids in distinguishing the cells of these two layers.

Our results indicated that after birth there is no increase in the depth of the proliferative zone of the external germinal layer which remains, until a decline sets in, at about four to five rows of cells. This does not imply that there is no increase in the net population of stem cells, because during this period there is a phenomenal extension of the surface area of the cortex (Altman, '69). What it does indicate is that about four to five cells represent the optimal depth of the proliferative zone and that the cells produced in excess of that number (perhaps because they are too far removed from the pia-arachnoid space or for other reasons) lose their mitotic potency and form the premigratory zone where they begin to differentiate. The gradual increase during the first ten days in the cell depth of the later region may, in turn, represent the imbalance between the rate that the cells enter the premigratory zone and the time required here to complete their first step in neuronal differentiation into either future granule cells and basket or stellate cells.

The premigratory zone. The largest proportion of the cells of the external germinal layer become granule cells. In the premigratory zone two characteristic transformations mark the onset of the differentiation of granule cells: the stretching out of the cells into a spindle shape in the coronal plane (parallel to the long axis of the folium) and the outgrowth of horizontal processes in the same direction. That the change in the shape of these cells is due to "stretching" is suggested by the observation that whereas in coronal sections these cells tend to be elongated, in the sagittal section they are round and much smaller in diameter than the proliferative cells. The force that produces their regular orientation is unknown, although evidence is available that the direction of the orientation of these cells can be rotated when the external germinal layer regenerates after exposure to x-irradiation (in preparation). The assumption of the spindle shape and the outgrowth of processes at the two poles are probably associated events. The accumulation of "growth" cytoplasm at the poles of the cells must be the metabolic requirement for the production of the material constituting the parallel fibers which grow, as if by an extrusion process, in the direction set up by the orientation of the poles. Presumably, as long as the growth of the horizontal portion of the parallel fibers continues, the cell body retains its horizontal spindle shape and remains stationary.

The question has often been asked why the development of the cerebellar cortex requires a unique, subpial secondary germinal matrix. Lahousse (1888) and Schaper (1894) suggested that this may be necessary because the myelination of the fibers of the medullary layer becomes an obstacle in the supply of cells to the cortex from the ventricular wall. Uzman ('60) pointed out that this cannot be entirely valid, because myelination does not begin until the end of the period of rapid cell proliferation. More recently an attractive hypothesis was put forward by Miale and Sidman ('61). They suggested that the differentiating granule cells first migrate side by side along the surface of the cortex in the transverse plane, then inward along the dendritic tree of the Purkinje cell into the granular layer, and during this migration synaptic contacts are established. They admitted that the side by side migration in the homogeneous germinal layer would be difficult to demonstrate with autoradiography and whether or not synapses are established by horizontal fibers before the granule cell has descended could not easily be tested at that time. The thrust of the hypothesis was that the function of the migration of granule cells through the cerebellar cortex is the establishment of synaptic relations. The available electron microscopic evidence (dealt with in the subsequent paper) indicates clearly that synapses are not formed by parallel fibers in the upper zone of the molecular layer and it is simpler to assume that the bipolar cells while in the external germinal layer, are stationary.

The data presented in this paper suggests that the function of the unique superficial position of the external germinal layer is to make possible the assembly of great masses of very thin, long and densely packed beams of parallel fibers, all stacked regularly in straight horizontal rows and oriented in the same direction. This is easily accomplished by the stacking process described, whereby the newly forming parallel fibers at the base of the external germinal layer are laid down on the surface or "grooves" of already present parallel fibers, assuring their straight growth in the two horizontal planes. This assembly process makes possible the regular stacking of parallel fibers from the bottom upward, as the external germinal layer is pushed upward and the bottom set of its cells produce new rows of parallel fibers. This mode of accretion of parallel fibers suggests that the basic organization of the cerebellar cortex requires an orderly lattice of parallel fibers and that their position from bottom to top (which is related to their time of origin) has particular functional significance. This will be further discussed in the succeeding paper where it will be shown that the different stacks of parallel fibers are related to different domains of other elements in the molecular layer. (Other aspects of the migration and differentiation of granule cells will be dealt with in the third paper of this series.)

In addition to the differentiating granule cells, the precursors of basket and stellate cells are also identifiable soon after the commencement of their differentiation. They are distinguishable from future granule cells by several criteria. One, they are oriented transversely to the long axis of the folium (sagittally) at a right angle to the orientation of the horizontal bipolar cells. This was referred to by Ramon y Cajal ('60, p. 298) as the phase of "horizontal bipolarity." It is not known whether or not this orientation occurs in the external germinal layer; it is easily observed in the transitional molecular layer. Two, from the beginning, the cells show presumed synaptogenic activity, in particular with parallel fibers, manifested in the formation of open coated vesicles and desmosoid contacts (Altman, '71) or actual synapses with them. The somata or processes of bipolar cells do not show synaptogenic activity while the granule cell is in the horizontal position and the soma is devoid of such contacts even when it is descending. Three, the differentiating basket cells may have multiple horizontal processes, against the two of granule cells, and these show even more intense synaptogenic activity than the soma. Finally, it is assumed that unlike the granule cells, the basket and stellate cells do not migrate but are "frozen" into their position by virtue of the orientation of their soma and processes at a right angle to the massive bed of parallel fibers.

Ramon y Cajal ('60, p. 299) spoke of the "sinking" or "decent" of basket cells under the pressure of newly-forming elements. That the basket cells are stationary elements was indicated by the autoradiographic evidence, which showed an orderly acquisition of cells in the molecular layer from the bottom upward. It was also supported by the consideration that the basket cell processes grow at a right angle to the underlying bed of parallel fibers very early during development (when they begin to form synapses) which perforce arrests them in their position. This, of course, does not imply complete immobilization. The commencement of synaptogenesis in the lower molecular layer during the second week (Altman, '72a), which was suggested by the growth spurt in the width of the molecular layer, must lead to dislocations of the originally neatly stacked parallel fibers and with them to dislocation of the basket cells.

The electron microscopic evidence indicates that parallel fibers form synapses with basket cells soon after the latter are formed, several days before they form synapses with Purkinje cells (Altman, '72a). Similarly basket cells form synapses with Purkinje cells quite early (Altman, '72a). If it is further considered that both basket and stellate cells are formed and begin their differentiation before the bulk of the granule cells have come into existence, and that they have to descend first before they can become functional, then the conclusion seems justified that the earliest maturing granule cells tend to form synapses with basket and stellate cells, which have an inhibitory influence on Purkinje cells (Eccles et al., '67) before they establish direct contact with the Purkinje cell dendritic spines to exert an excitatory influence.

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Abbreviations

(plates 1–2)

BAd, differentiating basket cells; BAm, mature basket cells; BG, Bergmann glia cell; BGp, Bergmann glia process; BP, bipolar cell in cross section; DGR, descending granule cell; GM, mitotic glia cell; GO, Golgi cell; GR, granule cell; MOL, molecular layer; MU, proliferative zone of the external germinal layer; PM, premigratory zone of the external germinal layer; PU, Purkinje cells; PUL, Purkinje cell layer.

PLATE 1

EXPLANATION OF FIGURES

Epon-embedded semi-thin sections of the cerebellar cortex cut in the sagittal plane. Stained with azure B, oil immersion, × 1600.

8 Newborn rat.

9 Five-day old rat.

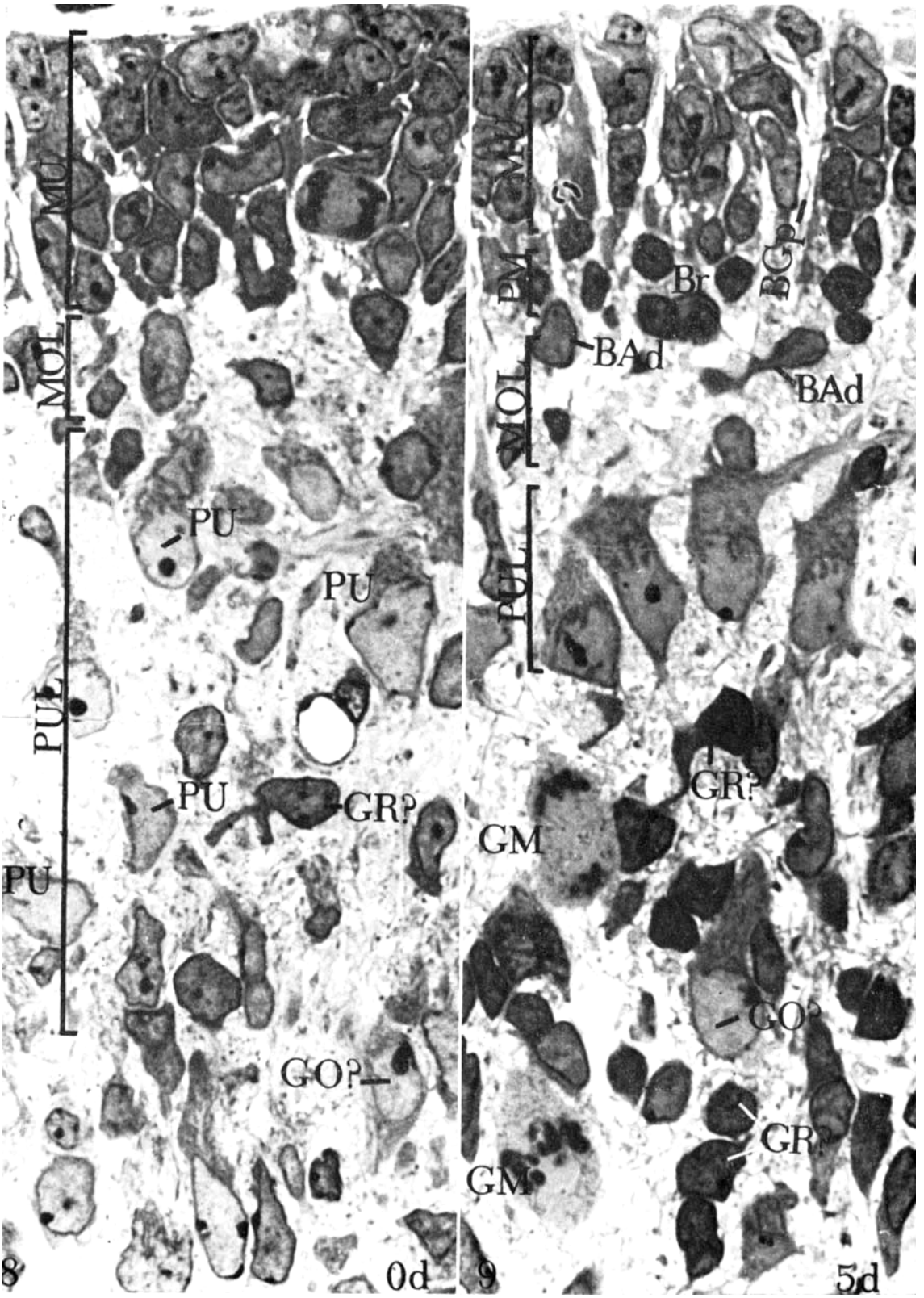

MOL
MOL
PU
PU
PUL
PU
PU
GR?
PU
GO?
8
0d
PM
Br
BG?
BAd
BAd
MOL
PUL
GR?
GM
GO?
GR?
GM
9
5d

PLATE 2

EXPLANATION OF FIGURES

Epon-embedded semi-thin sections of the cerebellar cortex cut in the sagittal plane. Stained with azure B, oil immersion, × 1600. Abbreviations as in plate 1.

10 Seven-day old rat.

11 Ten-day old rat.

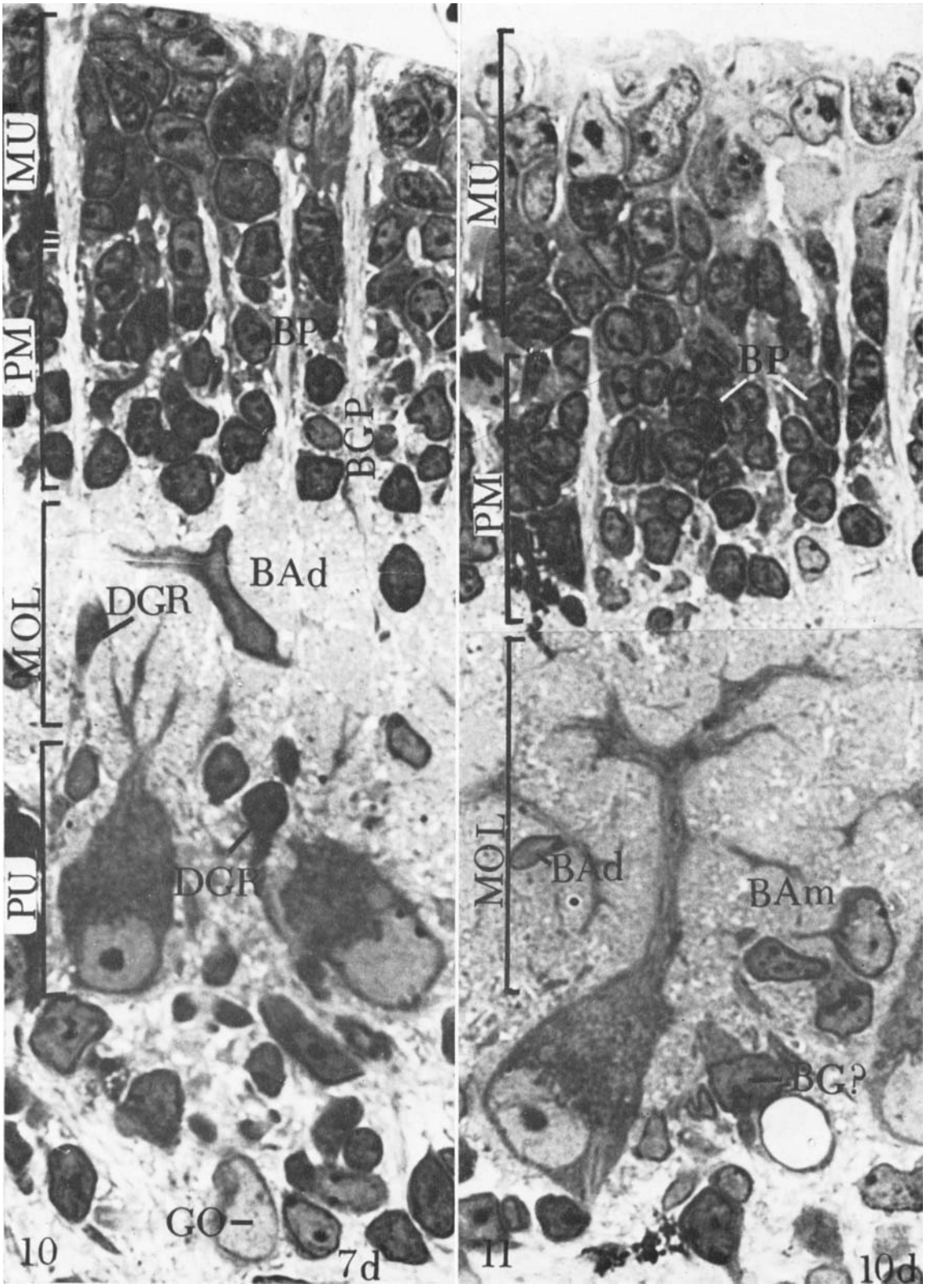

MU
PM
BP
BGP
MOL
BAd
DGR
PU
DGR
GO
10
7d
MU
BP
PM
MOL
BAd
BAm
BG?
11
10d

PLATE 3

EXPLANATION OF FIGURES

12 The external germinal layer of the pyramis of a seven-day old rat in coronal section. BC, bipolar cells cut parallel; MI, mitotic cell; PF, parallel fibers.

13 The external germinal layer of the pyramis of a seven-day old rat in sagittal section. BC, bipolar cells cut in cross section; DB, differentiating basket cell.

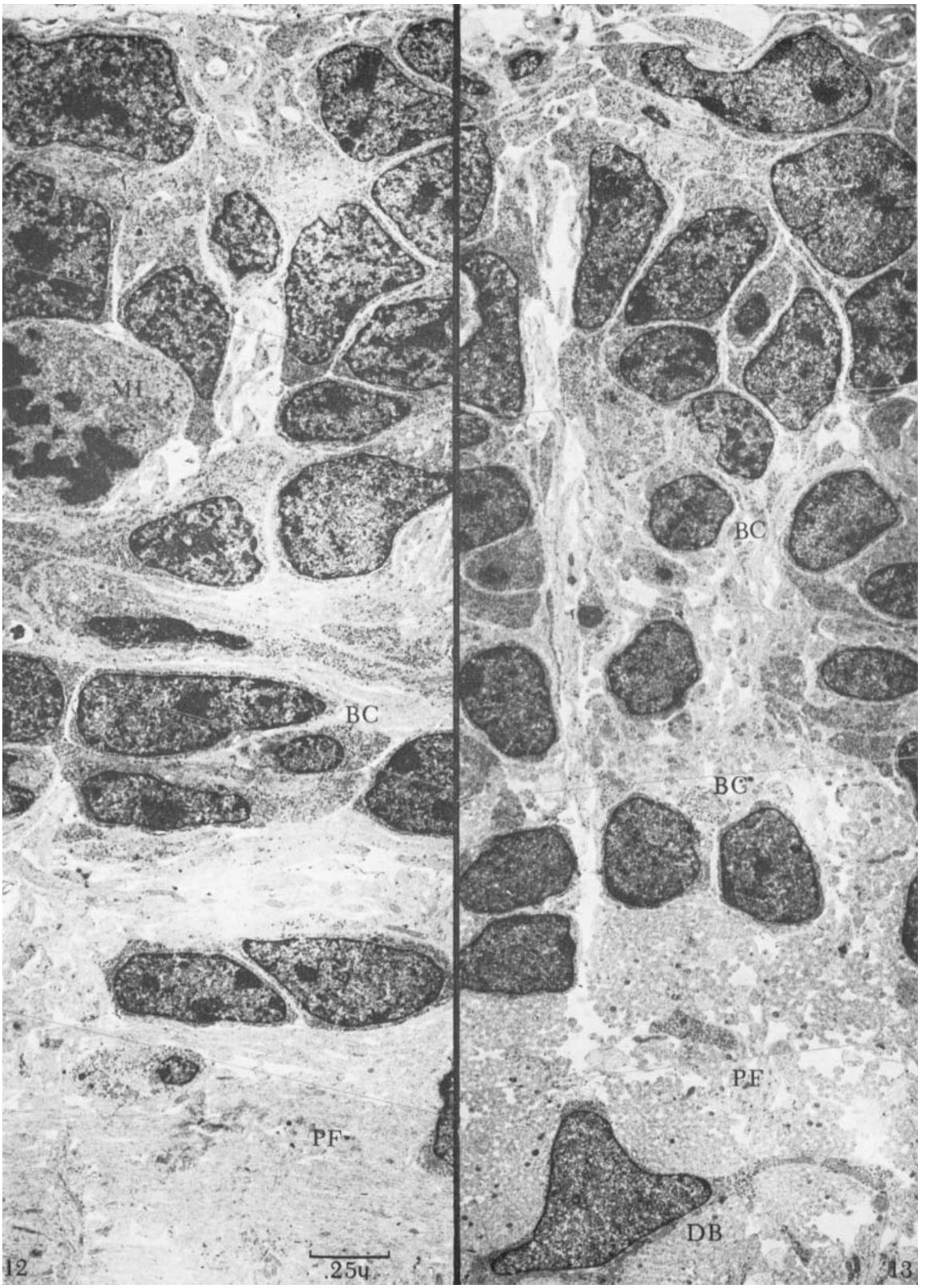

ML
BC
PF
.25u
12
BC
BC
PF
DB
13

PLATE 4

EXPLANATION OF FIGURES

14 Scattered cells of the external germinal layer (EGC) which are no longer continuous with the pial membrane (P) from which they are separated by the endfeet (BGe) of Bergmann glial processes (BGp). Parallel fibers (PF) are abundant. Pyramis, 21 days.

15 Concentric rings of the Bergmann glial endfeet. Pyramis, 15 days.

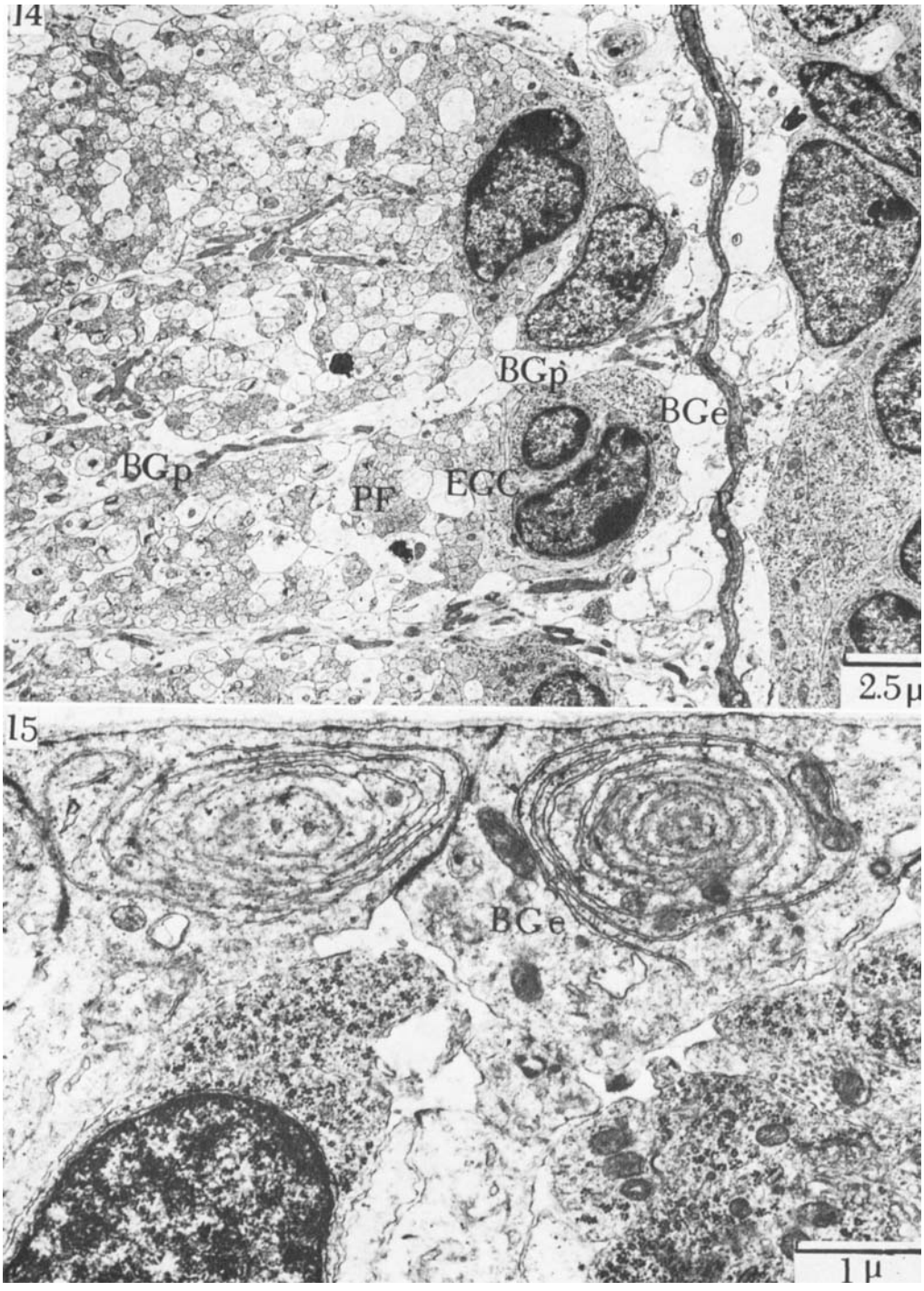

14
BGp
BGe
BGp
PF
ECC
2.5μ
15
BGe
1μ

PLATE 5

EXPLANATION OF FIGURE

16 Cells of the external germinal layer and the thin band of molecular layer composed of loosely packed parallel fibers. Posterior vermis, newborn rat.

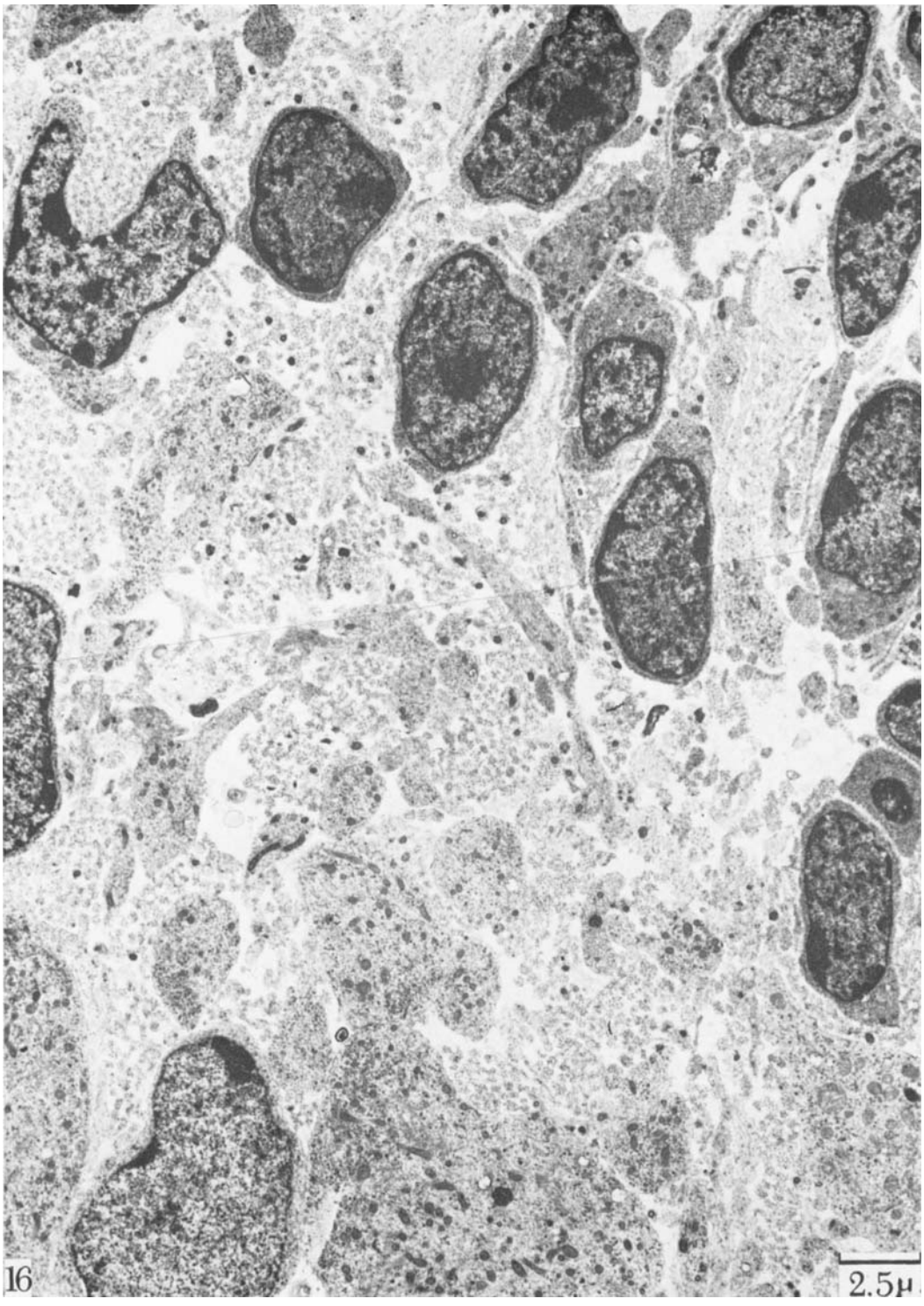

16
2.5μ

PLATE 6

EXPLANATION OF FIGURE

17 A horizontally oriented cell below the cells of the external germinal layer in the formative molecular layer. The cell is oriented, with its presumed dendritic process (DP) at a right angle to the direction of the parallel fibers (PF). A conspicuous, asymmetrical synapse of a parallel fiber (inset) is present on the soma. This is rare at this age on presumed basket cells. Pyramis, seven days.

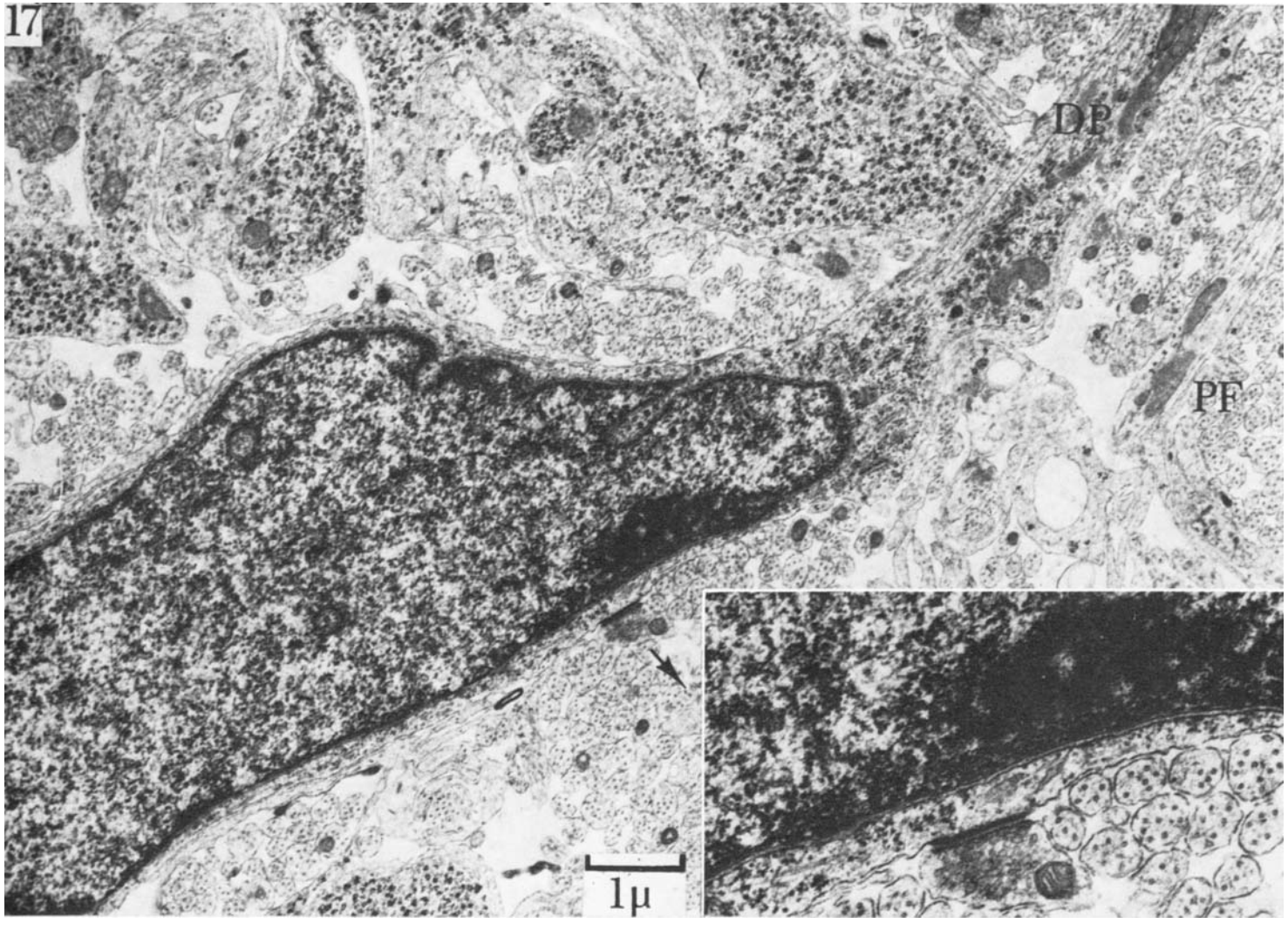

17
DP
PF
1μ

PLATE 7

EXPLANATION OF FIGURE

18 A horizontally oriented cell, presumably differentiating and arrested basket cell (BCd), and vertically oriented, descending granule cells (DGC) in the transitional, or formative, molecular layer densely packed with parallel fibers (PF). In the bed of parallel fibers, synapses (arrow) are extremely rare at this level. Pyramis, 12 days.

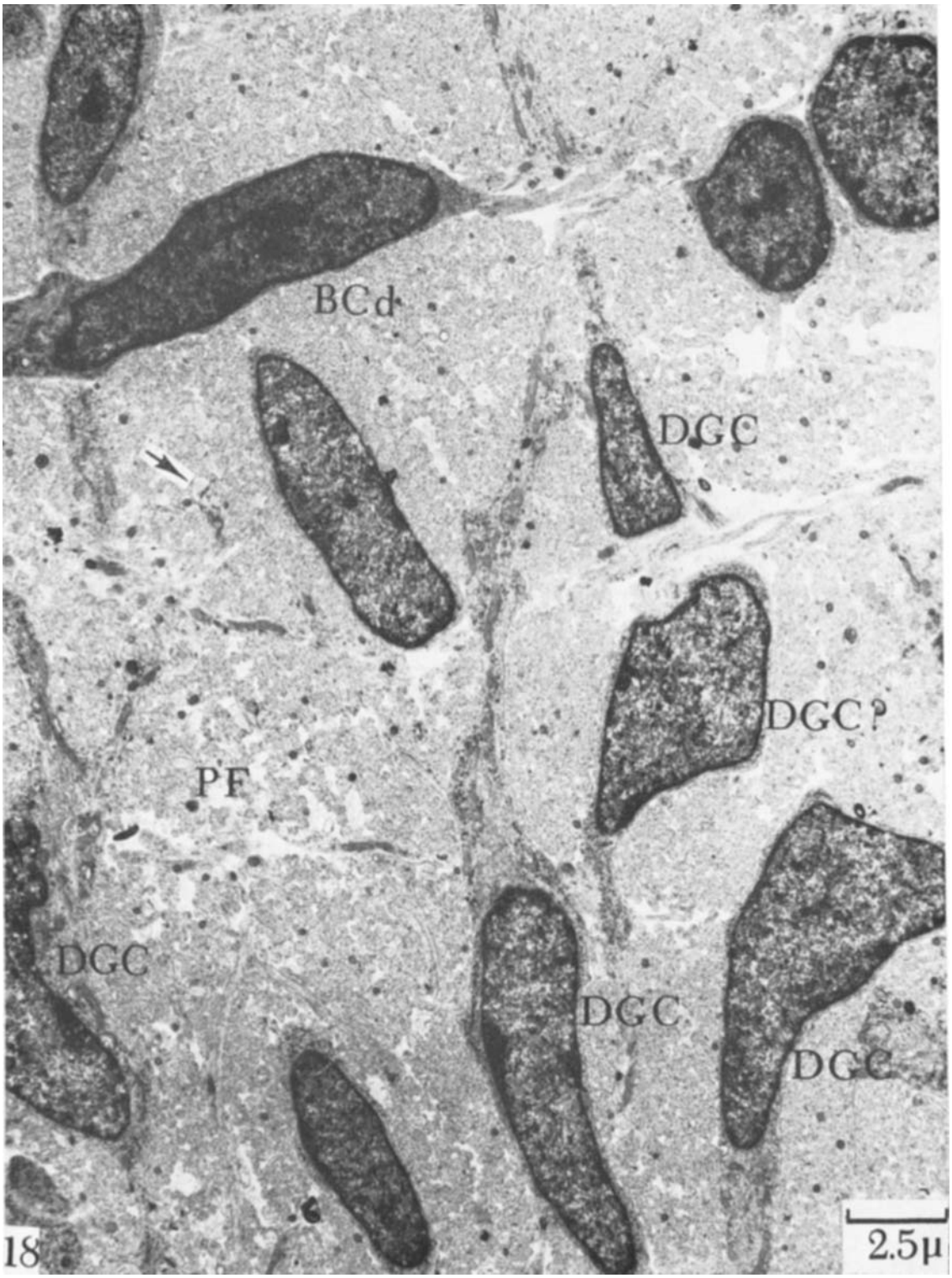

BCd
DGC
DGC?
PF
DGC
DGC
DGC
18
2.5μ

PLATE 8

EXPLANATION OF FIGURES

19 A horizontally oriented cell in the transitional molecular layer with dendritic processes and parallel fiber synapses (arrows) on them. Pyramis, 12 days.

20 A vertically oriented cell between two horizontally oriented cells from the same region. Such cells are lacking in open coated vesicles, dense membranes, or mature synapses, opposite contiguous parallel fibers, indicating that these migratory cells show no synaptogenic activity in the molecular layer. Pyramis, 12 days.

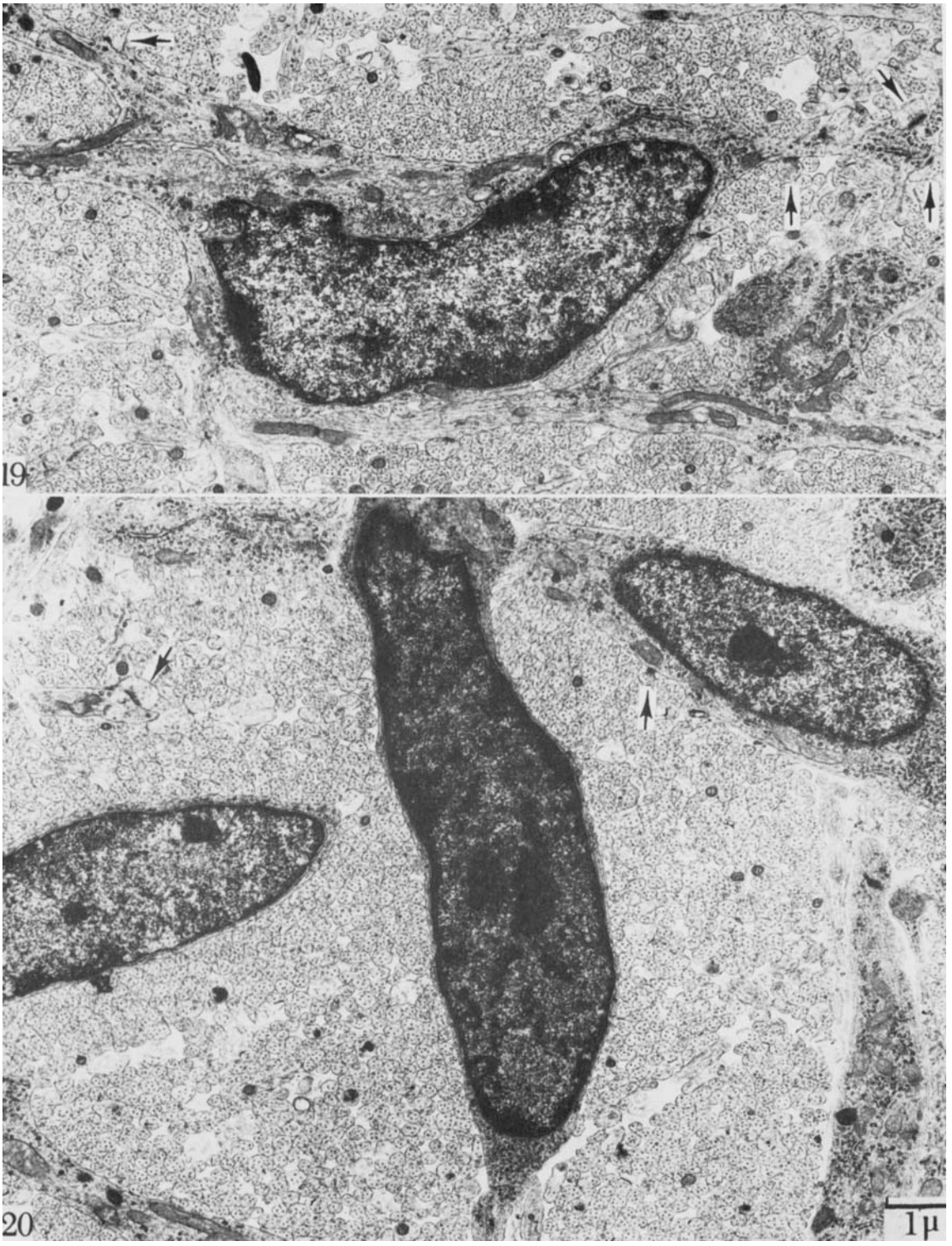

19
20
1μ

PLATE 9

EXPLANATION OF FIGURE

21 Two types of synapses on the soma of a basket cell. Upper inset shows a typical conspicuous, asymmetrical synapse (Gray, type I), presumably that of a parallel fiber. The lower inset shows two inconspicuous, symmetrical synapses (Gray, type II), presumably those of stellate cells. Differences in the size or shape of vesicles are not very obvious. Pyramis, 30 days.

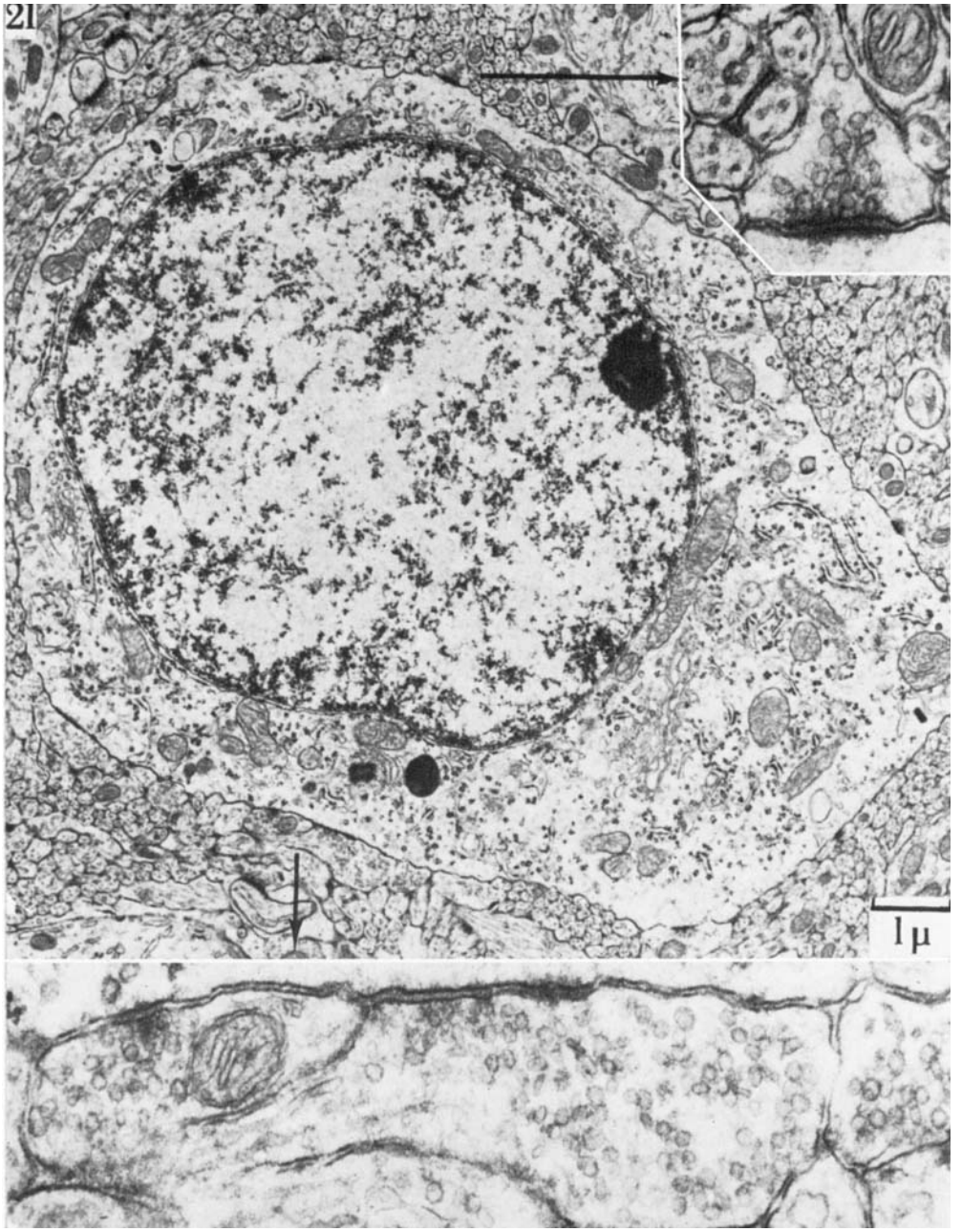

21
1μ

PLATE 19

EXPLANATION OF FIGURE

22 A basket cell soma with a dendrite (D) and an axon (A). Axon is shown at higher magnification in inset with its undercoating. Pyramis, 15 days.

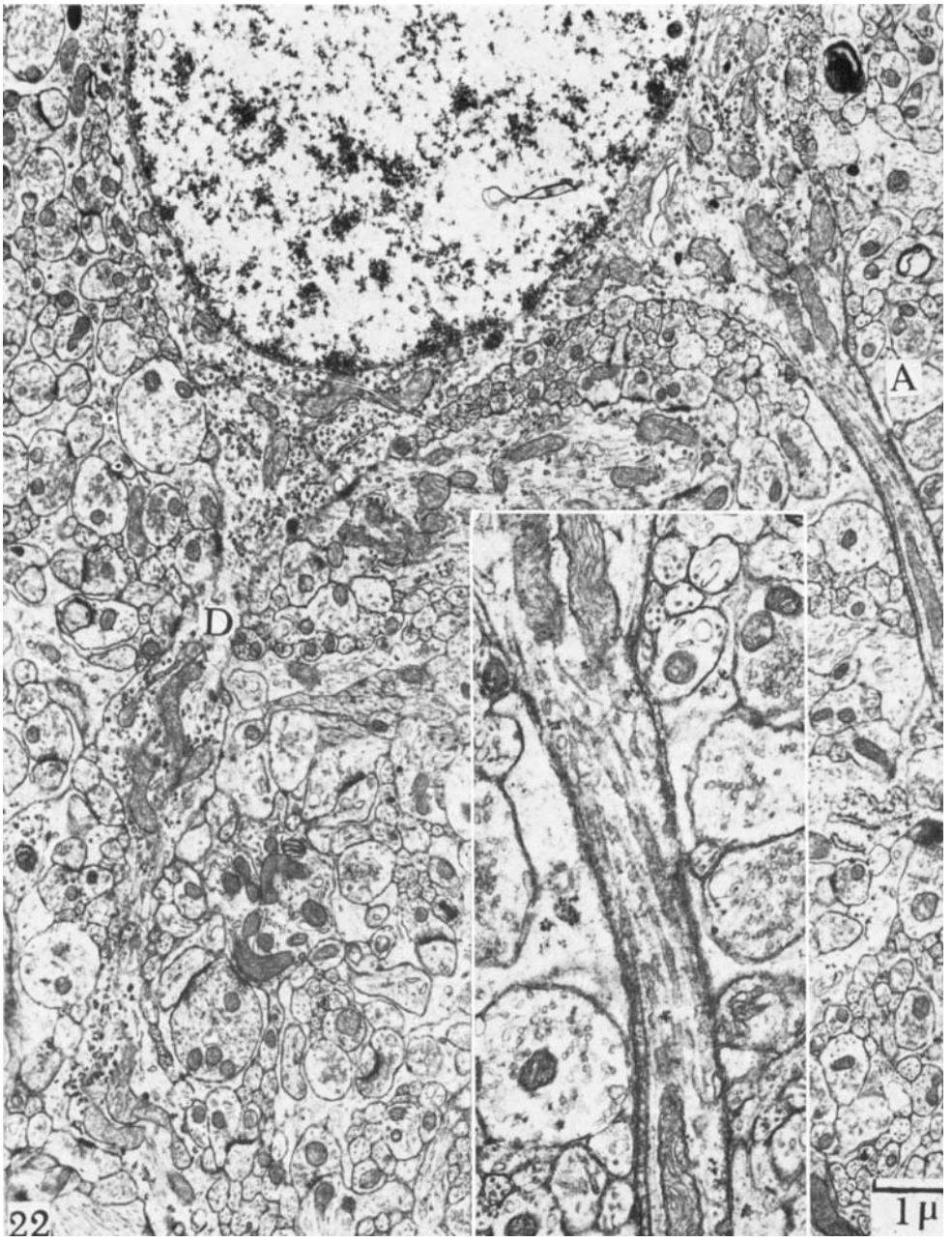

A
D
22
1 μ

PLATE 11

EXPLANATION OF FIGURES

23 Basket cell with a horizontally oriented dendrite from which an axon issues with characteristic undercoating. The soma of the basket cell is directly contiguous with parallel fibers and has parallel fiber synapses on it (arrows). There is also a synapse on the dendrite where the axon issues. PD, branching Purkinje cell dendrite. Pyramis, 30 days.

24 The portion of the dendrite where the axon originates at higher magnification. The synapse appears to be conspicuous but symmetrical. The axon has the typical undercoating.

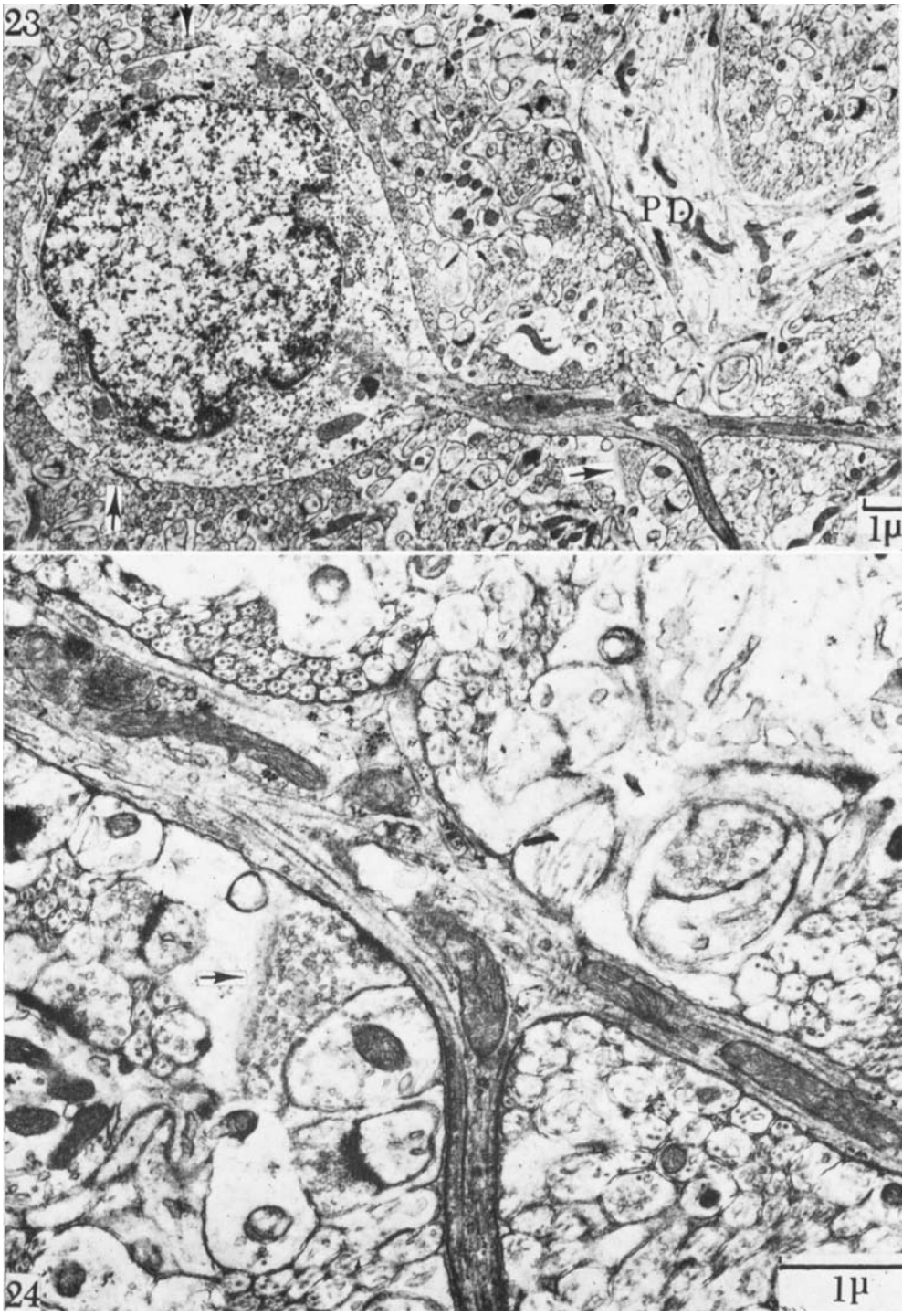

23
PD
1μ
24
1μ

PLATE 12

EXPLANATION OF FIGURE

25 Differentiating stellate cell with a stout dendrite and a few parallel fiber synapses (inset) on it. Pyramis, 15 days.

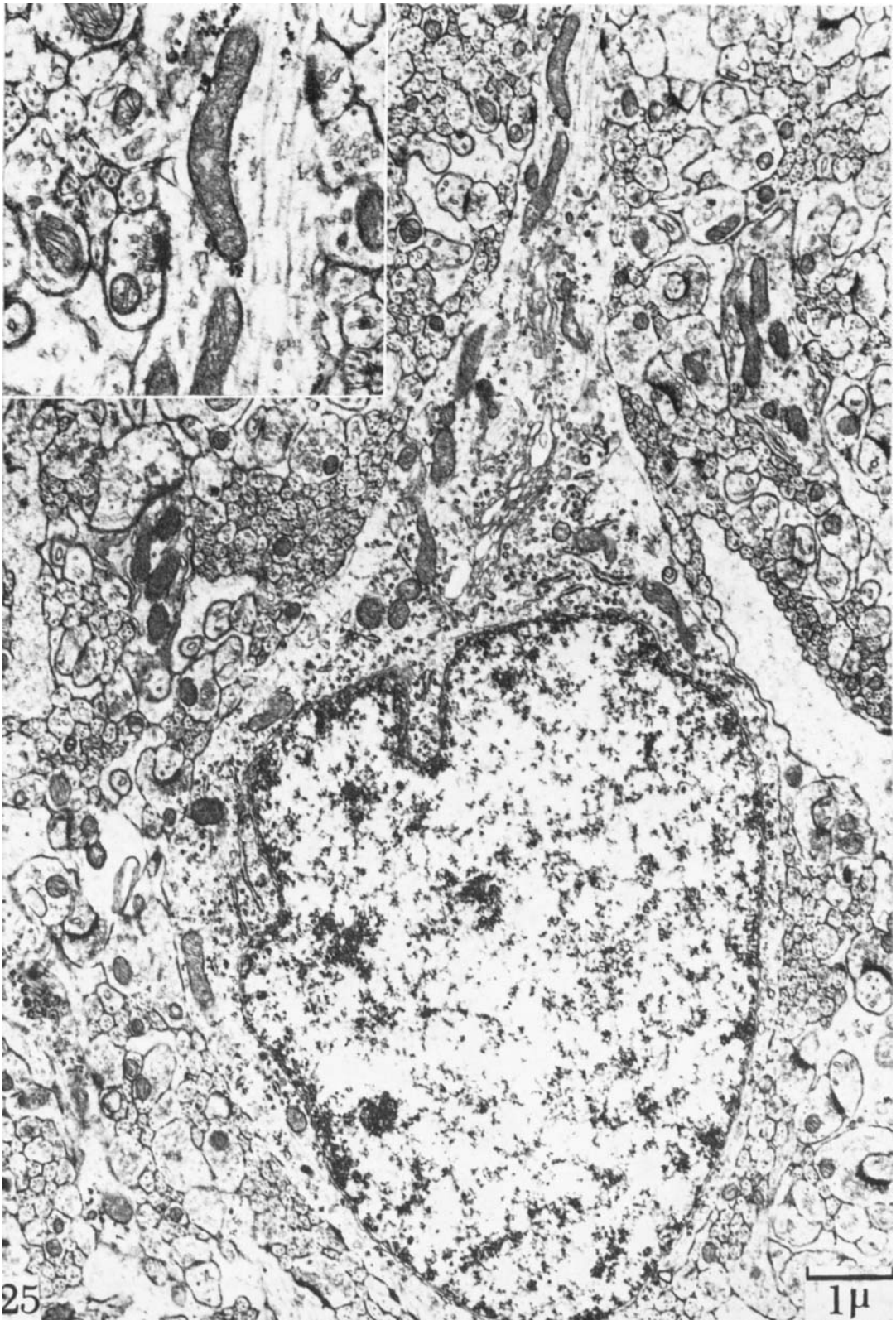
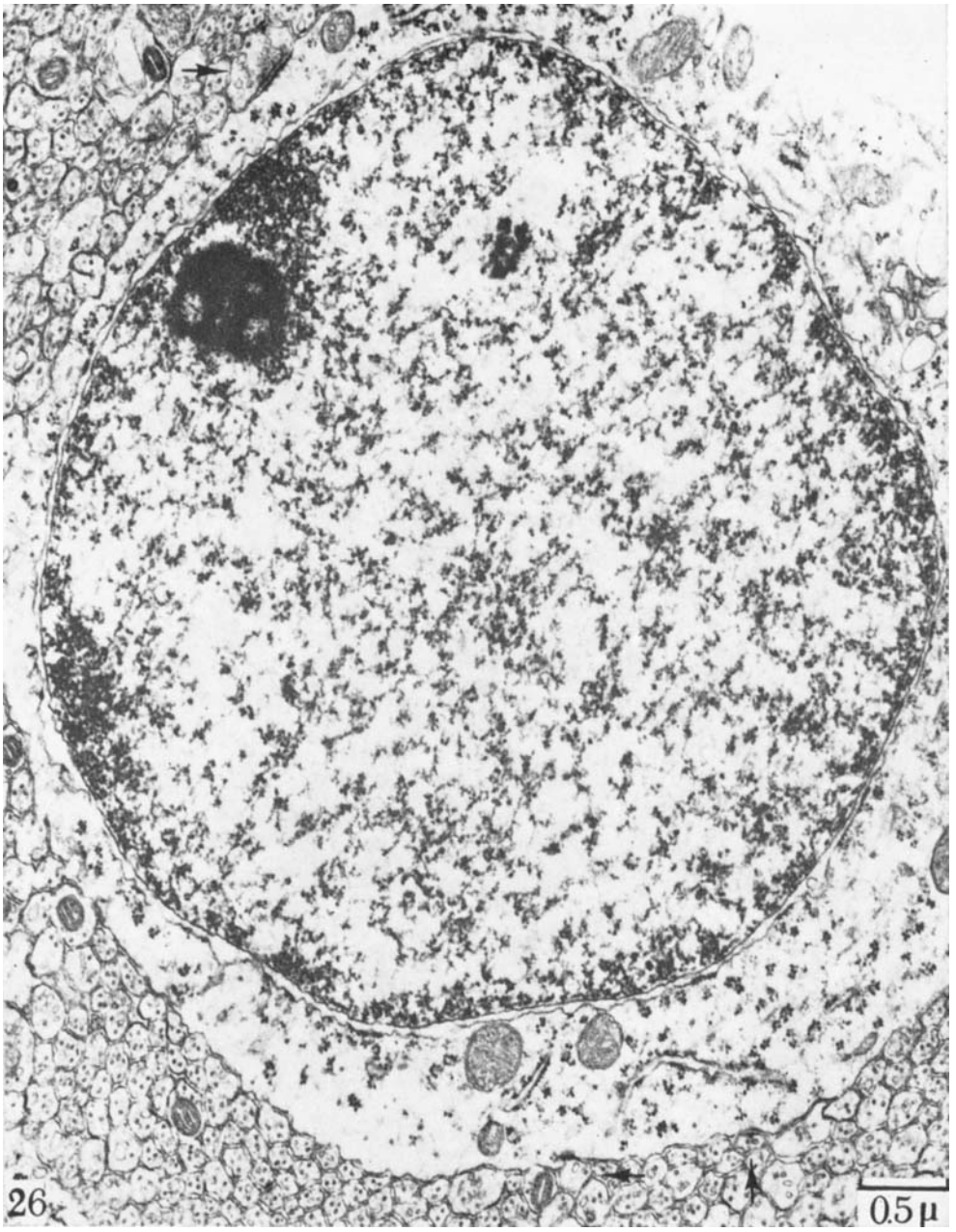

25
1μ

PLATE 13

EXPLANATION OF FIGURE

26 The soma of a stellate cell. The direct contiguity between its entire surface and the parallel fibers is obvious. A parallel fiber synapse is seen in left top; some synaptogenic activity is indicated at the bottom of the cells (arrows). Pyramis, 30 days.


26
0.5 μ