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The Recurrent Collaterals of Purkinje Cell Axons: A Correlated Study of the Rat's Cerebellar Cortex with Electron Microscopy and the Golgi Method*

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Summary. Each Purkinje cell axon with its recurrent collaterals occupies a roughly triangular space in the folium, apex pointed towards the white matter and base against the Purkinje cell layer. The axon is smooth initially but develops distensions that become more obvious at twists and turns and at points where collaterals originate. These thin, finely beaded collaterals make characteristic acute angles with the axon from which they issue. The collaterals bifurcate further, their terminal branches becoming more varicose, intertwining with each other to form plexuses in the molecular and granular layers. These fiber plexuses are found in three locations: (1) the recurrent collateral plexus in the granular layer which synapses with dendrites and somata of deep Golgi II neurons; (2) the profuse infraganglionic plexus, boutons of which terminate in relation with the somata and dendrites of Purkinje cells and Lugaro cells, in addition to participating in other complex synaptic arrangements in the neuropil; (3) the sparse supraganglionic plexus which forms synapses with dendrites of Purkinje cells and occasionally with basket cells.

In electron micrographs, terminals belonging to recurrent collaterals contain a mixture of neurofilaments, microtubules, and slender mitochondria with a loose array of flat, elliptical, and round synaptic vesicles embedded in a dark filamentous matrix. It is usual to find a cluster of boutons on the postsynaptic surface. Each synapse consists of several separate macular junctional complexes. The synaptic cleft is widened and contains a dense fibrous material while both pre- and postsynaptic components have very shallow, symmetrical filamentous densities adherent to the cytoplasmic surfaces of the membranes.

It is suggested that recurrent collaterals from axons of Purkinje cells may provide a rapid monosynaptic feed-back mechanism for inhibitory control of Purkinje cell responses. These collaterals may also participate in a slower positive feed-forward circuit or resetting mechanism involving at least two synapses. The existence of this circuit is indicated by synapses on deep Golgi II neurons. The inhibition of Golgi II cells may depress their inhibitory activity on surrounding granule cells, thus resetting the mechanism for the subsequent responses to excitatory afferent input. Recurrent collateral inhibition also may aid in the disinhibition of Purkinje cells through the depression of basket cell activity.

Key-Words: Cerebellar cortex — Purkinje cell — Axon — Recurrent collateral — History — Synapses.

Introduction

Almost a hundred years ago in an address before the *Istituto Lombardo di Scienze e Lettere* Camillo Golgi (1874, reprinted in Golgi, 1903) presented the first evidence for the existence of axonal collaterals in the brain. Through the applica-

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tion of his method on human cerebellar cortex, Golgi discovered the recurrent collaterals that issued from the Purkinje cell axons. These collaterals either branched into smaller fibers in the granular layer or entered the molecular layer as an important contribution to that neuropil. Ramón y Cajal (1888, 1889, 1890; Cajal and Illera, 1907, summarized in 1911) confirmed the presence of these recurrent collaterals in the cerebella of a variety of birds and mammals. He extended Golgi's original description, and reported myelinated and retrograde collaterals that described a graceful curve in their course through the granular layer to ascend to the zone of Purkinje cells. Here their subsequent branches were seen to become entangled in a supraganglionic plexus above the cells and in an infraganglionic plexus below them. In 1912 Cajal reported a pericellular plexus of recurrent collaterals surrounding certain fusiform cells situated beneath the Purkinje cells. Since then, recurrent collaterals of Purkinje cells have also been observed in Golgi preparations of the monkey (Fox *et al.*, 1967), several other mammalian and avian species (O'Leary *et al.*, 1968), and the mormyrid (Niewenhuys and Nicholson, 1969). In addition, Larramendi and Victor (1967), Lemkey-Johnston and Larramendi (1968), and Larramendi and Lemkey-Johnston (1968) have identified terminals of recurrent collaterals of Purkinje cells in the mouse cerebellar cortex using the electron microscope.

The present investigation provides an account of the extensive *intrafolial* network of Purkinje cell recurrent collaterals in Golgi preparations of the cerebellar cortex in the young adult rat. The general disposition of the plexus as well as the detailed three-dimensional arrangement of their terminals will be described. These light microscopic observations are correlated with an electron microscope study of the terminations of these axons and their synaptic relationships with other cerebellar neurons.

Materials and Methods

Rapid Golgi preparations were made from the brains of young adult rats 180–200 g in weight, and also from animals at least 90 g in weight and more than four weeks old. Slight modifications on the method of Valverde (1970) were used to obtain successful rapid Golgi impregnations. Most of the brains from the animals were fixed by immersion after the administration of a lethal dose of chloral hydrate. Slices of brain, 5 mm thick, cut in the three cardinal planes were immersed in an osmium dichromate solution (2.33% w/w potassium dichromate and 0.19% w/w osmium tetroxide in aqueous solution) for at least a week (7 to 10 days) followed by a short immersion in 3/4% silver nitrate (12–24 h). Most blocks underwent a double impregnation; rarely was a third necessary. Impregnations subsequent to the first necessitated a shorter immersion in the osmium dichromate solution used in the first impregnation (5–7 days) followed by a period in silver nitrate (24–48 h). The success of impregnations in these blocks was judged in test sections obtained by hand-sectioning with a single-edged razor. If the impregnations were satisfactory, the blocks were dehydrated, embedded in celloidin, and cut at 100–150 μ on a sliding microtome. The observations on the recurrent collaterals of Purkinje cell axons were gathered from this collection, and drawings from two folia in the ventral paraflocculus (lobule IX, Larsell, 1952) and other selected fields were made with the camera lucida.

The electron microscopic observations presented in this paper were made from an extensive collection of electron micrographs and montages of specific areas in the rat's cerebellar cortex. The regions examined were the bases of lobules IV, V, IX, and X in the vermis, parts of lobule IX in the ventral paraflocculus, lobule X in the flocculus, lobule VII in crus I and crus II, and lobule VIII in the copula pyramidis (Larsell, 1952). These tissues came from the

cerebella of a total of twenty young adult rats (Sprague-Dawley, 200 g) prepared for electron microscopy according to procedures described in a previous paper (Chan-Palay and Palay, 1970).

Results

Light Microscopy

The Purkinje Cell Axon. Figs. 1–4 are camera lucida drawings from rapid Golgi preparations showing parasagittal views of the intrafolial course and the terminal branches of recurrent collaterals of Purkinje cell axons. Figs. 3 and 4 are composite drawings derived from camera lucida drawings of rapid Golgi preparations in lobule IX, the ventral paraflocculus. In the former, two deep Golgi type II cells and two Lugaro cells have been superimposed upon recurrent collaterals belonging to several Purkinje cells in the folium in order to show the relationships amongst these neuronal elements that have been seen in electron micrographs. In the latter, part of the recurrent collateral network including terminals of a single Purkinje cell has been juxtaposed with an intermediate cell of Lugaro to show details of some of these relationships.

The network of recurrent collaterals belonging to Purkinje cells, like the dendritic arborizations of these cells, is best displayed in the parasagittal plane. The axon emerges from the inferior pole or the side of the Purkinje cell and runs directly through the granular layer toward the white matter. At various points along its length collaterals emerge which fan out, to return towards the molecular layer. The arrangement of each individual axon with its constellation of recurrent collaterals brings to mind the stems in a head of Queen Anne's lace in late autumn, bare of petals, and pressed between the pages of a book. Each axon with its recurrent collaterals occupies a roughly triangular space in the folium with its apex pointed towards the white matter and its base against the Purkinje cell layer. The largest expansion of the axonal plexus is in the parasagittal plane; however, the collaterals can be traced for a very limited extent on either side of this plane. The main axon of the Purkinje cell is smooth initially but soon develops slight distensions along its length which become more obvious at twists and turns, particularly at points where the collaterals originate. At such bifurcations, which occur at numerous sites along the length of the fiber throughout the granular layer, the axon enlarges to form a triangular, gnarled distension, one arm giving rise to a thin, very finely beaded collateral. The beads are small, about 0.4–0.5 μ in diameter, and the connecting thread is exceedingly tenuous. Often a thin and very finely beaded collateral can be traced as it issues from the main axon, bifurcating and forming terminal branches that become increasingly varicose as one follows them into various plexuses in granular and molecular layers. The recurrent collaterals, after their exit from the main axon, may ramify and terminate in the granular layer, or may ascend to terminate in the vicinity of the Purkinje cells. These retrograde collaterals are distinctive because they generally make an acute angle with the axon in returning upwards through the granular layer.

The Plexus of the Granular Layer. As is shown in Fig. 3, collaterals that originate deep in the granular layer generally divide several times, thus giving rise to secondary and tertiary branches, which form a plexus in this layer. Such a plexus of collaterals is to be distinguished from the supra- and infraganglionic

plexus found above and below the layer of Purkinje cells. The secondary and tertiary branches that make up this plexus of recurrent collaterals consist of numerous fairly large varicosities (about 0.8–1 μ) connected by a thread. Thus, the terminal branches of the recurrent collaterals appear to be fleshier than they are at their points of origin. In addition, they terminate as irregularly shaped, large, clavate bulbs, about 1.5 μ in diameter, approximately twice as large as the small beads found along the more proximal portions of the collaterals. Electron micrographs suggest that these enlargements, both terminal and *en passant*, are the sites of synapses with dendrites and somata of Golgi II neurons and possibly with the dendrites of granule cells.

The Infraganglionic Plexus. Collaterals of Purkinje cell axons that emerge from bifurcations in the middle or upper thirds of the granular layer generally ascend to the zone of the Purkinje cells (Figs. 1–4). Here their subsequent branchings contribute to the formation of two main plexuses, the supra- and the infraganglionic plexus situated above and below the level of the Purkinje cell body, respectively. The main contributions to the plexus in the infraganglionic region are made by beaded collateral branches that arrive in the vicinity of the Purkinje cell somata. At a level of about 8–10 μ below the inferior pole of the Purkinje cells these axons bend to travel a somewhat tortuous horizontal course in the parasagittal plane of the folium. These horizontal collaterals may terminate after a short path but more often continue for a distance of about 150–250 μ across the territories of several successive Purkinje cells. Generally such branches bifurcate several times, giving rise to shorter, varicose secondary and tertiary axons that weave in and out amongst neurons in the area and end in a large bouton or a small, rounded nodule in the infraganglionic region. Electron micrographs of this area show that collaterals terminating in the infraganglionic plexus end in relation with the somata and initial segments of dentrites belonging to Purkinje cells and to Lugaro cells.

As is illustrated in Figs. 3 and 4, the intermediate cells of Lugaro appear in rapid Golgi preparations as small, fusiform neurons (with cell bodies about 12 μ in diameter) amongst and beneath the Purkinje cell somata. The Lugaro cells bear few dendrites and they are long with almost no thorns. On leaving the cell body they radiate horizontally into the molecular layer, spreading out in the parasagittal plane. Sometimes short, slender twigs issue from these processes. The teardrop-shaped cell body may have, in addition, an apical dendrite that after a short, perpendicular traverse in the molecular layer bifurcates to course horizontally in the folium. It is usual to find the axon of the Lugaro cell emerging from an arm of one of these dendrites and traversing the molecular layer parallel to the folium. From the axon side branches ascend perpendicularly into the molecular layer as short (15 μ), finely beaded fibers.

The Supraganglionic Plexus. The horizontal limbs of recurrent collaterals give rise to a few vertical, varicose tertiary branches that do not end in the infraganglionic region but instead bend away at right angles and ascend vertically to the apical pole of the Purkinje cell. A very few short terminal segments emerge from these vertical branches ending either in a large, clavate or smaller, round bouton. The supraganglionic plexus in the young adult rat is extremely sparse and poorly developed. Profiles of terminations belonging to this plexus are

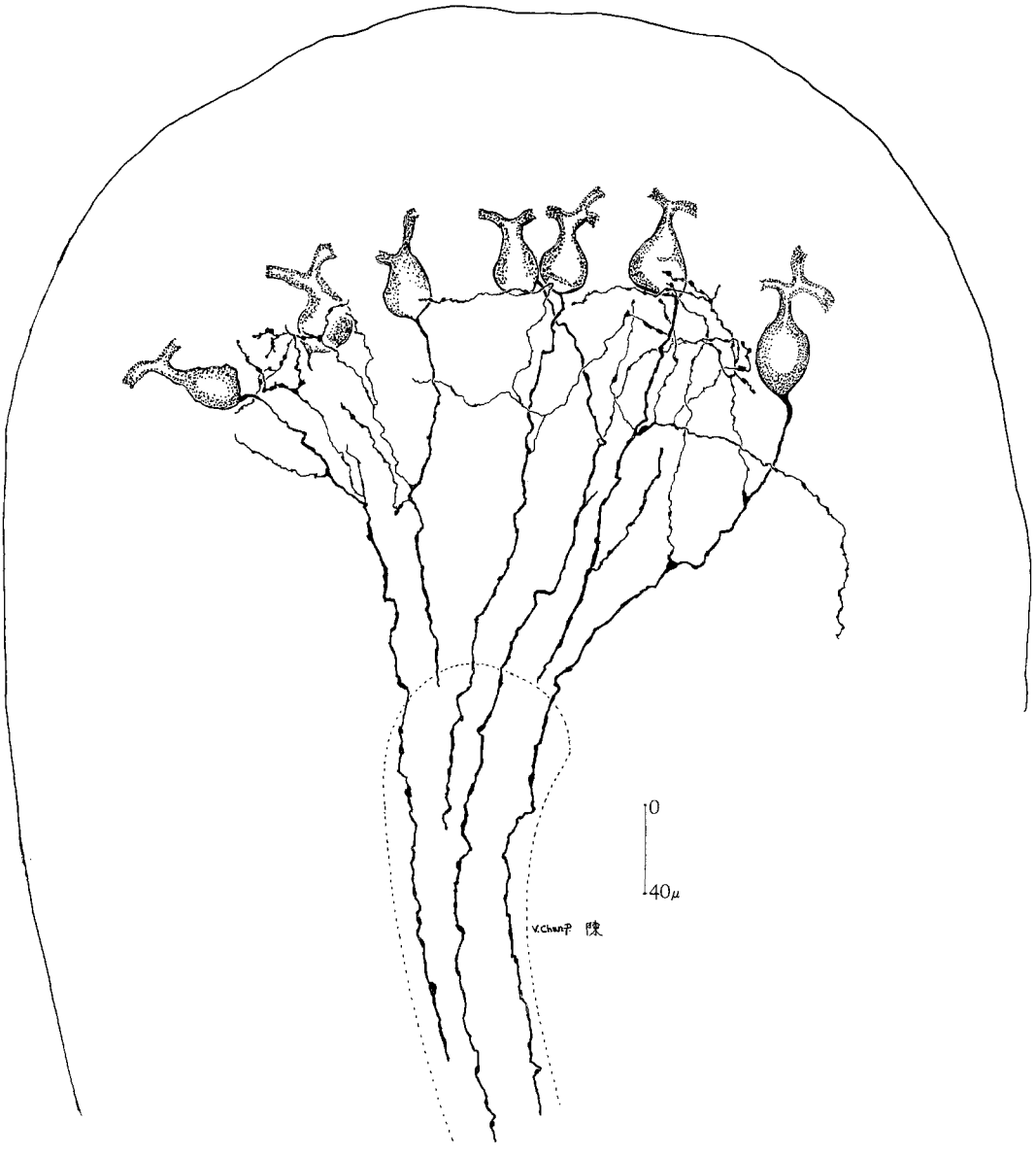


Fig. 1. The recurrent collateral plexuses of Purkinje cell axons. Camera lucida drawing of a Golgi preparation made from a single section of lobule IX in the ventral paraflocculus. Each axon emerges from the inferior pole of the Purkinje cell and runs through the granular layer toward the white matter. At various points along its length collaterals emerge which fan out to return towards the molecular layer. Each axon together with its recurrent collaterals occupies a roughly triangular space in the folium with the apex pointed towards the white matter and the base against the Purkinje cell layer. Three fiber plexuses are formed by branches of these recurrent collaterals, one in the granular layer, a second profuse plexus in the infraganglionic region, and a sparse supraganglionic plexus in the molecular layer. The recurrent collateral plexuses in this illustration should be compared with strikingly similar though more exuberant recurrent collateral networks drawn by Retzius (1892: Tafel VIII, Fig. 1) from Golgi preparations of the cerebellum of an infant (7 day) rabbit. The axons and recurrent collaterals of the first and third Purkinje cells on the left are reproduced in larger detail in the next figure. Parasagittal section, 120 μ, $4^1/_2$ week old rat, 90 g in weight

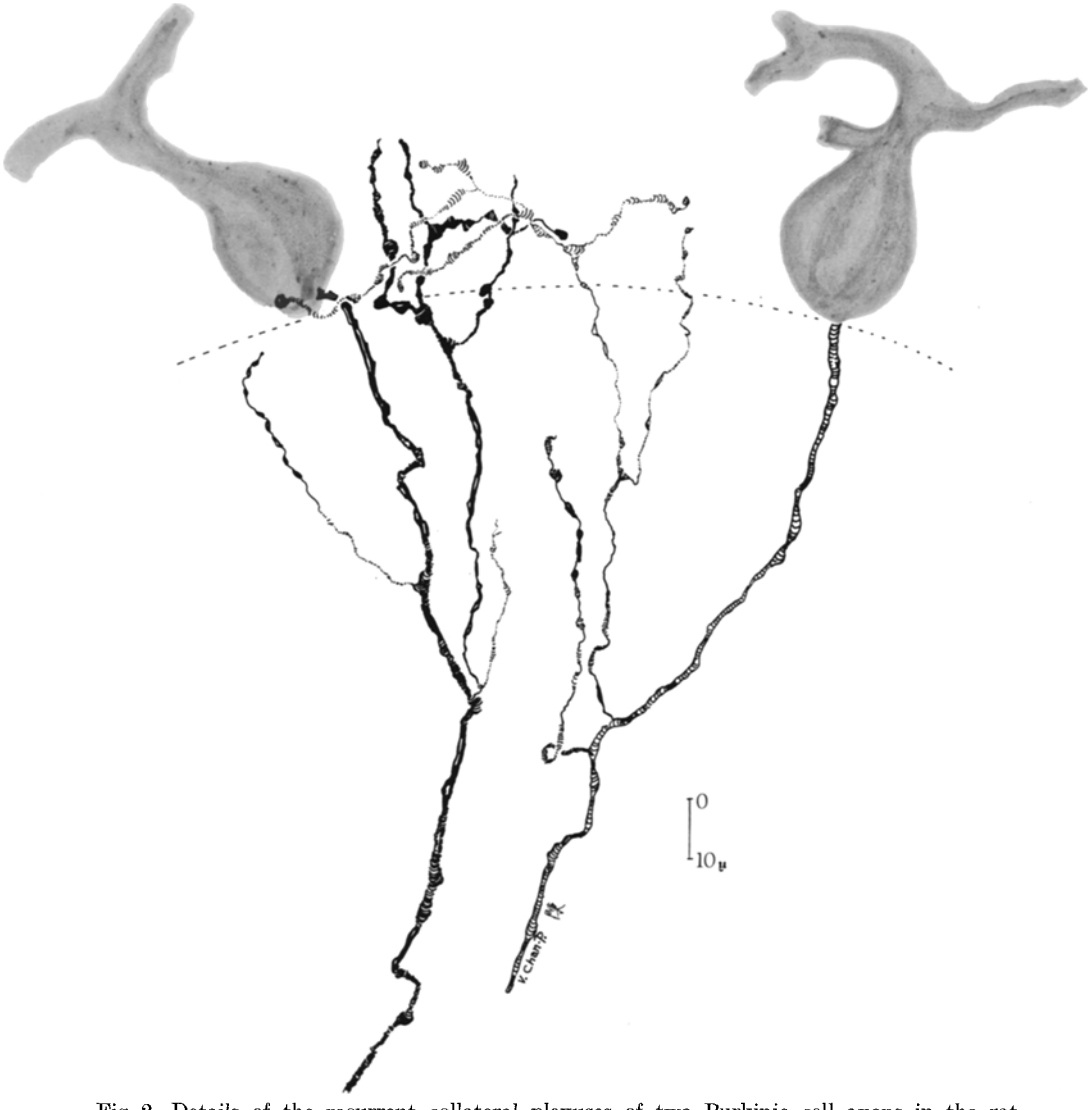


Fig. 2. Details of the recurrent collateral plexuses of two Purkinje cell axons in the rat cerebellar cortex. The main axon of the Purkinje cell is smooth initially but soon develops slight distensions along its length which are more obvious at twists and at the bifurcations of collaterals. Here the axon enlarges to form a triangular, gnarled distension, one arm giving rise to a thin, very finely beaded collateral. These collaterals may ramify and terminate in the granular layer, or they may ascend to terminate in the vicinity of the Purkinje cell. The infraganglionic plexus is the most profuse plexus, and the main contribution is made by branches with large varicosities that ramify and intertwine in the region of the Purkinje cell somata. Some branches do not end in the infraganglionic region but bend away at right angles and ascend vertically to the apical pole of the Purkinje cell, where a very sparse supraganglionic plexus is found. Parasagittal section, 120 μ thick, rat $4^1/_2$ weeks old, 90 g

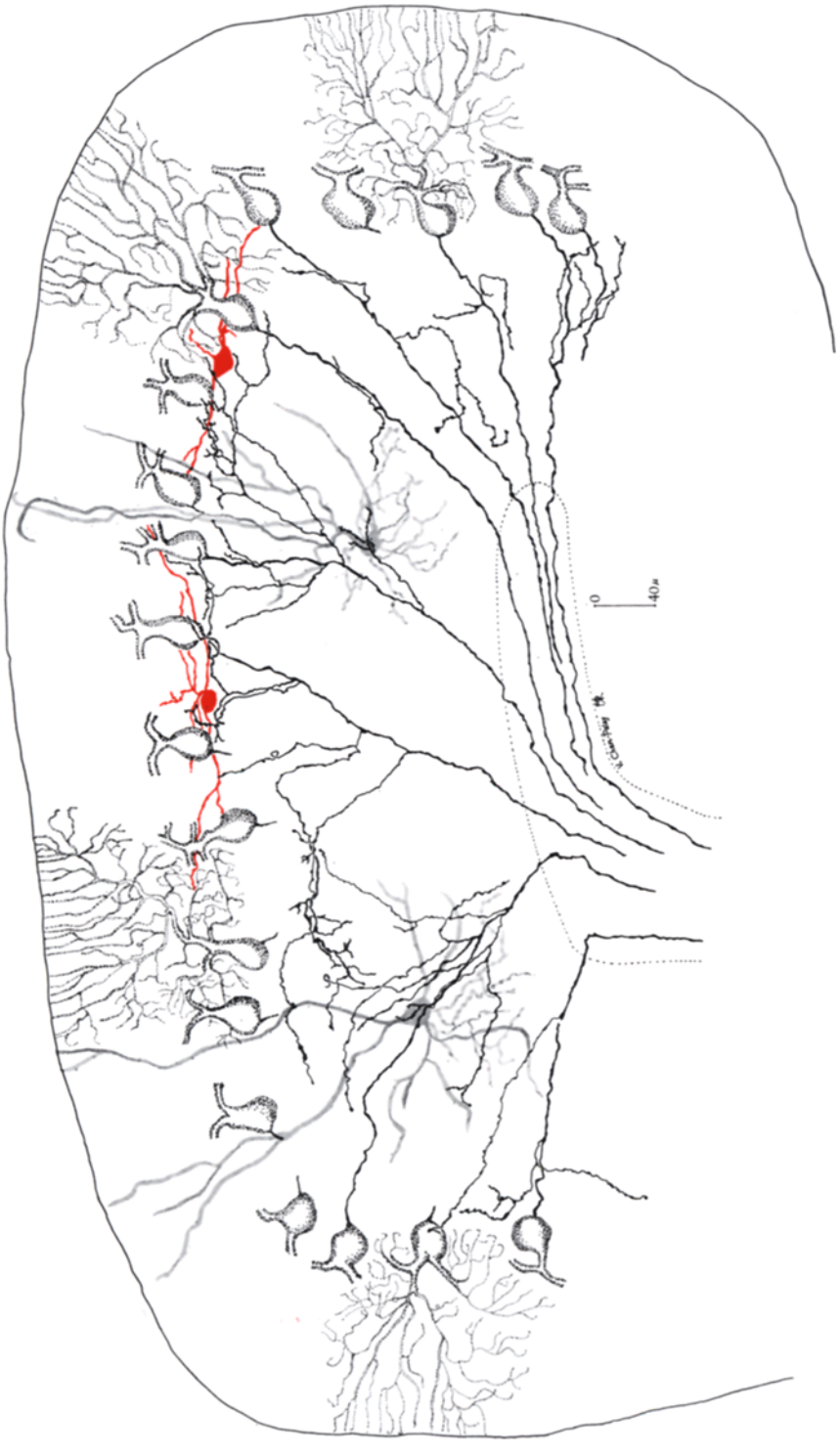


Fig. 3

uncommon in electron micrographs of the molecular layer, but when found are in synapse with primary dendrites, smaller dendrites, and, occasionally, thorns of Purkinje cells. Rarely, synapses are also found on the dendrites and somata of basket cells.

The secondary and tertiary branches of recurrent collaterals that contribute to the infra- and supraganglionic plexus are each composed of a series of large, globular varicosities (about 0.8–1 μ) connected by a thinner thread. These varicosities are fleshier and more irregular in shape than the fine beads found on the ascending portions of recurrent collaterals that emerge from the main axon. This is well illustrated in Figs. 2 and 4, where several examples of such varicose branches intertwine in the vicinity of Purkinje cells. By far the majority of the recurrent collateral branches seen in our Golgi preparations are involved in plexuses beneath the Purkinje cell body in the infraganglionic zone.

As is shown in Fig. 3, rapid Golgi preparations display the remarkable organization of the dendritic and axonal arborization of each Purkinje cell. Each cell has a dendritic pattern that is defined by the position of the cell in the folium. At the summit of the folium the dendritic tree is fully expanded parasagittally with branches more or less symmetrically arranged on either side of an imaginary line running through the cell body. On the slopes of the folium the dendritic arborization is widest nearer the summit but trimmed and slanted as the sulcus approaches. At the depth of a sulcus the dendritic expansion is flattened out onto either side of the sulcus and is quite circumscribed. In a like manner the course taken by the main axon of each Purkinje cell and the arrangement of its recurrent collaterals are singular. The axon of a Purkinje cell located at the summit of a folium arrives at the white matter by the most direct route, usually describing a gentle arc through the granular layer. The recurrent collateral network fans out like an equilateral triangle with its apex pointed towards the white

Fig. 3. Diagram showing the interrelations between Purkinje cell axons and recurrent collaterals with deep Golgi II neurons and Lugaro cells. Camera lucida drawings of recurrent collateral arborizations of several Purkinje cells in a folium (lobule IX ventral paraflocculus; stipple and black), two deep Golgi II cells (gray), and two Lugaro cells (red) were superimposed to produce this diagram. The interrelations of the processes of these cells have been observed in the electron micrographs, and are reproduced in this figure in order to provide the light microscope equivalent. Recurrent collaterals that originate deep in the granular layer generally divide several times, giving rise to varicose terminal branches that form a plexus in this layer. The varicosities or enlargements of these terminal branches are the sites of synapses with dendrites and somata of deep Golgi II neurons. Recurrent collaterals that emerge from the main axon in the middle or upper thirds of the granular layer generally ascend to the zone of the Purkinje cells. Here their subsequent branchings contribute to the formation of two plexuses, the infra- and the supraganglionic plexuses, situated below and above the level of the Purkinje cell body respectively. The infraganglionic plexus consists of varicose branches of collaterals, some of which bend to travel a tortuous horizontal course in the parasagittal plane of the folium. The varicosities end in relation with the somata and dendrites of Purkinje cells and Lugaro cells and also in complex synaptic arrangements in the surrounding neuropil. The supraganglionic plexus is very sparse and consists of occasional varicose branches that ascend into the low molecular layer to end on dendrites of Purkinje cells and rarely on basket cells. The terminal arborization of one of these recurrent collaterals (fifth from right) is reproduced in detail in the next figure. Parasagittal section, 150 μ thick, $4^1/_2$ week old rat

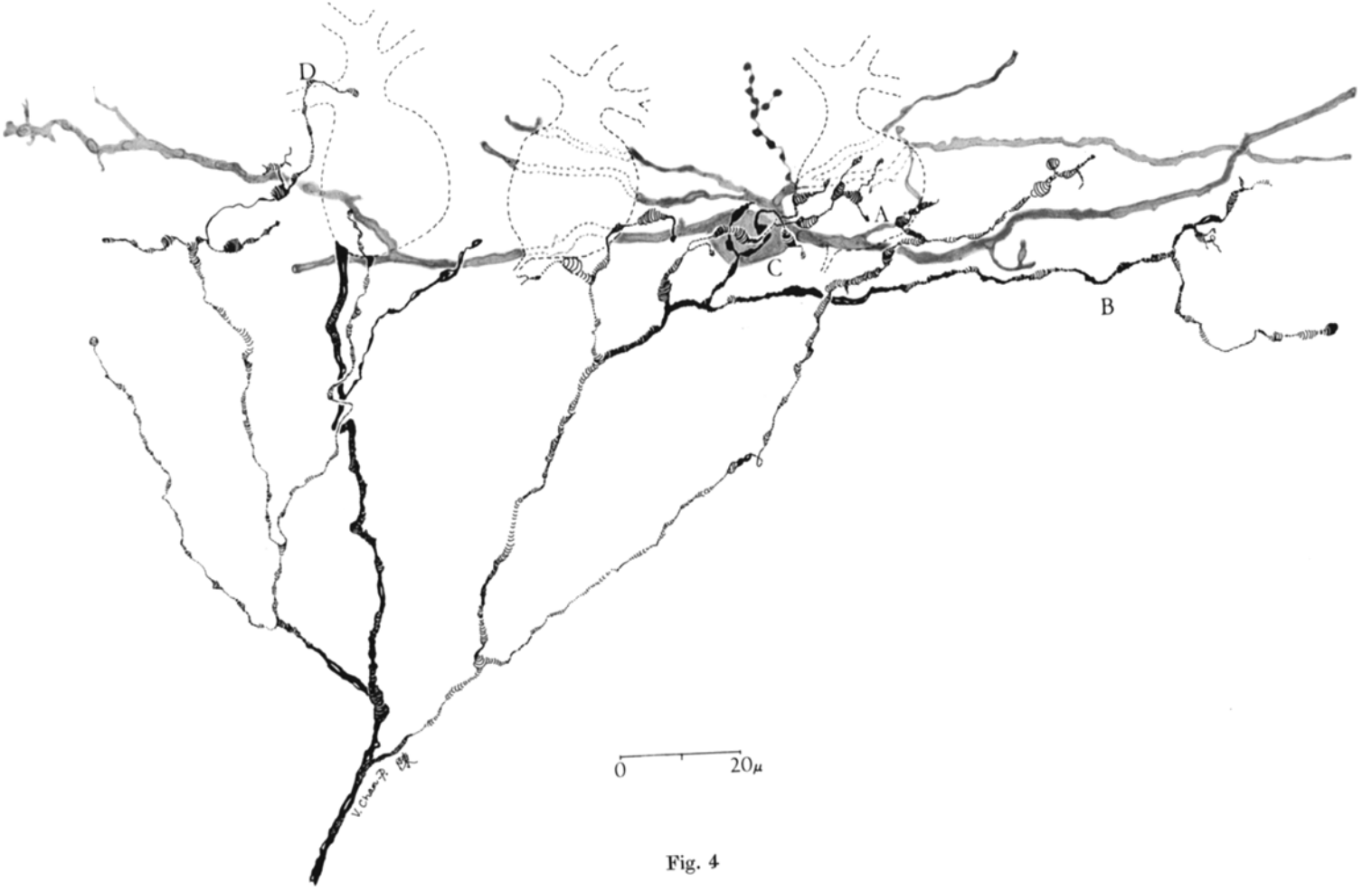


Fig. 4

matter and its base against the Purkinje cell layer. Axons of Purkinje cells situated on the shoulders of a folium approach the white matter in a direct diagonal traverse; their recurrent collaterals usually occupy a tall, acute-angled triangle with a narrow base. Other cells in the depth of the sulcus have axons that enter the white matter only after a right-angled bend and show a constricted, narrow recurrent collateral network.

Electron Microscopy

The proper electron microscopic identification of terminals belonging to any one neuronal type in the cerebellar cortex depends, in the first instance, upon an understanding of the three-dimensional form of the cells, dendrites, and axons that make up the tissue. Of no less importance is the knowledge of the disposition of the neuronal processes in relation to the cells in the successive cortical layers. This type of information can be successfully gathered from the examination of Golgi preparations. As we have described in the previous section, the entire intrafolial network of Purkinje axon recurrent collaterals can be found in these preparations, including the terminations of tertiary branches in the plexuses. However, since the Golgi material cannot disclose the nature of contacts between neuronal elements, we turn to electron micrographs for specific information on the synaptic relationships of recurrent collaterals. Ultimately, one can pool together with information from the Golgi preparations the clues garnered from the electron micrographs on shapes, sizes, and the differential distribution of cytological elements such as synaptic vesicles, microtubules, neurofilaments, etc., thus achieving a coherent understanding of the tissue at both optical and fine structural levels. Montages that we have assembled from electron micrographs of large areas of the cerebellar cortex provide us with detailed maps of strips of the entire tissue, stretching from the edge of the white matter to the surface of the brain over the molecular layer. In this way all profiles belonging to an axonal system such as the recurrent collaterals can be picked out and the details of synaptic patterns studied.

Fig. 4 A–D. Drawing showing the interrelations between the infra- and supraganglionic plexuses of the recurrent collateral network of a Purkinje cell axon with other Purkinje cells and a Lugaro cell. Camera lucida drawings of a part of the recurrent collateral network of a single Purkinje cell, the cell bodies of two other Purkinje cells, and a Lugaro cell (gray) were superimposed to produce this diagram. The interrelations between neurons shown in this diagram have been observed in electron micrographs, and have been reproduced here to show the light microscopic equivalent. A The varicose terminal branches in the infraganglionic region form a complex and intertwined plexus in the vicinity of the Purkinje cell somata. These terminal branches may end on the soma of the same Purkinje cell or on a neighboring Purkinje cell. B Often, at a level of about 8–10 μ below the inferior pole of the Purkinje cell, some collaterals may travel horizontally in the parasagittal plane of the folium. Such a horizontal collateral may terminate after a short path, but more often continues for longer distances across the territories of several successive Purkinje cells. C The varicose terminal branches of collaterals also form an intertwined cluster around the cell body and dendrites of Lugaro cells that are found amongst and beneath the Purkinje cell somata. D A very few short, varicose branches of recurrent collaterals ascend vertically to the apical pole of the Purkinje cell where their boutons end in relation to dendrites of Purkinje cells or on basket cells, thus forming the sparse supraganglionic plexus. Parasagittal section, 150 μ thick, $4^1/_2$ week old rat

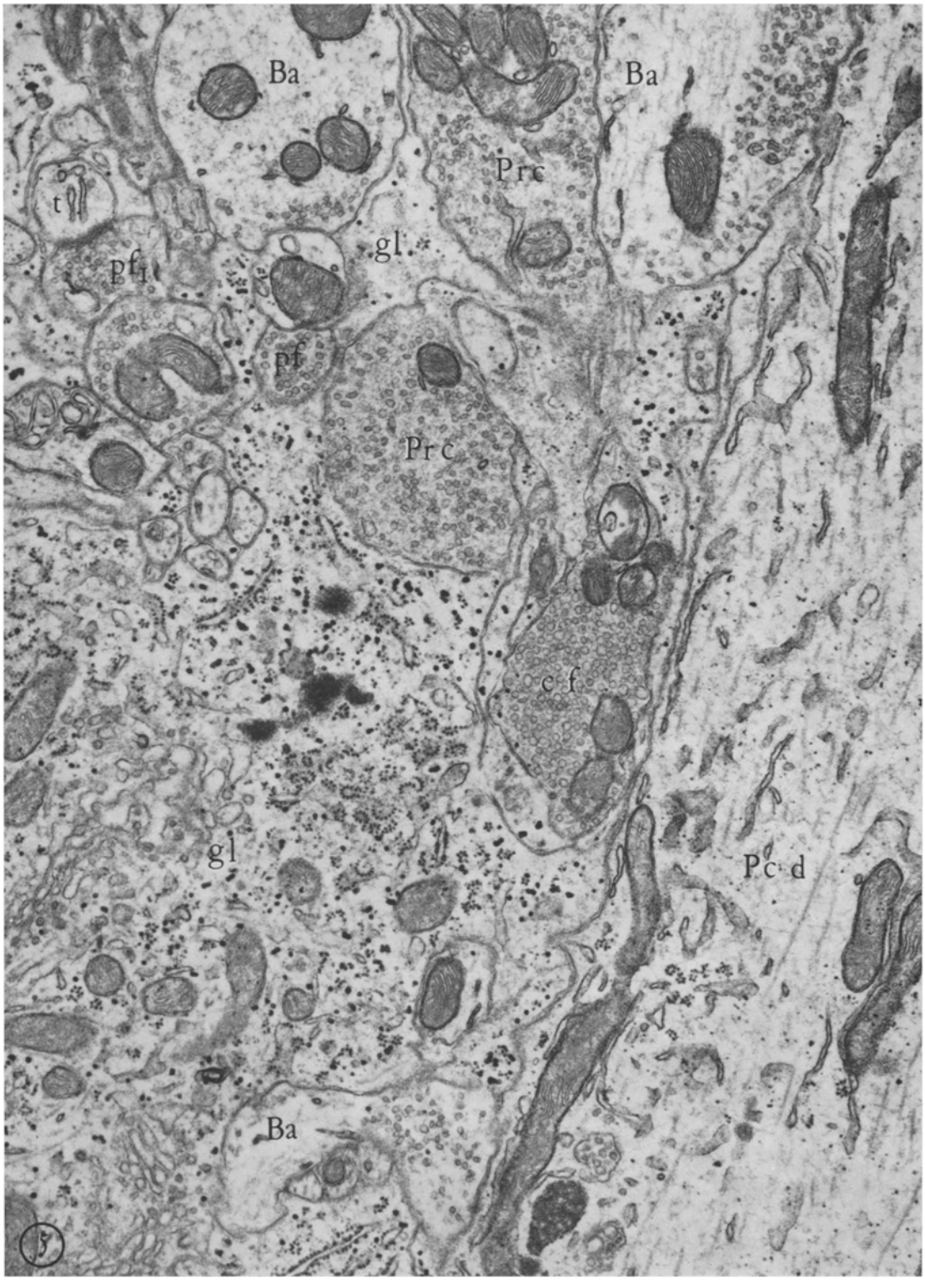


Fig. 5

Terminals belonging to recurrent collaterals of Purkinje cells are identified in the electron microscope by a constellation of characteristics that were first recognized by Lemkey-Johnston and Larramendi in 1968. Because the Purkinje cell axon and its collaterals are all myelinated, its terminal formations are most easily identified in electron micrographs when continuous with myelinated segments of the preterminal axons. These contain a mixture of neurofilaments, microtubules, and slender mitochondria oriented longitudinally. Sac-like distensions, about 0.8 μ wide, balloon out from these preterminal axons and contain a dark, filamentous matrix in which is suspended a loose array of synaptic vesicles, ranging in shape from flat and rectangular to elliptical or round. On the basis of these characteristics the terminals can be recognized in any plane of section, even when severed from their parent myelinated axons. Accordingly, the locations of these boutons were carefully mapped out in the montages of granular and molecular layers. Particular attention was paid to their synaptic relations with neurons in the vicinity, and to their frequencies of occurrence in the plexuses of the granular layer and the supra- and infraganglionic regions. From these electron micrographic maps, the three-dimensional aspects of these terminations were reconstructed, and such reconstructions were checked against the observations made on Golgi preparations in the light microscope. At this stage, camera lucida drawings of cerebellar neurons and their processes made from Golgi preparations were superimposed to produce composite diagrams (e.g., Figs. 3 and 4) that display the electron microscopic and light microscopic correlations.

Synaptic Relations of Recurrent Collaterals in the Molecular Layer. In the molecular layer, terminals belonging to recurrent collaterals of Purkinje cell axons have to be distinguished in the electron microscope from axons belonging to basket and stellate cells, parallel fibers, climbing fibers, and Lugaro cells. As is seen in Figs. 5–7, all of these axons with the exception of the last, which has yet to be identified in the electron micrographs, have cytological characteristics and synaptic patterns that set them apart from one another as well as from terminals of recurrent collaterals. We can summarize these distinguishing characteristics in the following way. The varicosities of parallel fibers are small and globular, contain a loose arrangement of round synaptic vesicles, and articulate with the thorns of Purkinje cell dendrites. Climbing fiber varicosities are large, distended sacs jammed with round synaptic vesicles and microtubules in a dense matrix. These, too, synapse with dendritic thorns of Purkinje cells. Basket and stellate fibers are recognized by their fleshy proportions, abundance of neurofilaments, and loose population of elliptical and round synaptic vesicles in a light matrix. The majority of these synapse on the shafts of Purkinje cell dendrites (see Chan-Palay and Palay, 1970).

Fig. 5. Boutons of recurrent collaterals in the molecular layer. Two profiles of recurrent collaterals (*Prc*) can be recognized by their dark axoplasmic matrix and their population of flat, elliptical, and round synaptic vesicles. In this same field, axonal profiles belonging to basket fibers (*Ba*), a climbing fiber (*cf*), and parallel fibers (*pf*) can be seen in the vicinity of a Purkinje cell dendrite (*Pcd*). A parallel fiber varicosity synapses with a Purkinje cell thorn (*t*). Extensive sheets of neuroglial cytoplasm (*gl*) containing glycogen are insinuated between the axons and dendrites in the field. (Lobule X, flocculus) × 25000

Examination by light and electron microscopy of many lobules confirms our observations in Golgi preparations that the supraganglionic plexus of recurrent collaterals is relatively sparse in the rat's cerebellar cortex. A few profiles of myelinated secondary, tertiary, and quartenary branches of collaterals can be found vertically oriented between the apical portions of the Purkinje cell somata. At a level of about 10–20 μ above the layer of Purkinje cells, these myelinated profiles bend away from the vertical and end. Fleshy, varicose terminals arising from these myelinated segments can occasionally be found in synapse with the dendritic shafts of medium-sized and smaller Purkinje dendrites and, more rarely, on dendritic thorns (Fig. 6). Synapses of recurrent collaterals with the somata of basket cells (Fig. 7) are equally rare. However, when terminals of recurrent collaterals are found, two or three club-shaped boutons are usually gathered upon the profile of a single basket cell perikaryon.

The endings of the recurrent collaterals are conspicuous by virtue of their dark, filamentous matrix and modest population of flat and round synaptic vesicles. The synapses of recurrent collateral terminals on Purkinje dendrites and on basket cells have synaptic complexes that do not conform to either of Gray's types (Gray, 1959). The synaptic interface is usually widened, with a dense, fibrous accumulation within the cleft. Both the pre- and the postsynaptic components have very shallow, filamentous densities adherent to cytoplasmic surfaces of their membranes (see Fig. 6).

The Infraganglionic Plexus. The infraganglionic area is the region of transition between the upper granular layer and the zone of Purkinje cells, through which all axons and dendrites entering or leaving the molecular layer must pass. The somata of the Purkinje cells are loosely arranged in a sheet, and even at their widest perimeter the cell bodies do not touch one another but leave enough space between them for the passage of neuronal and glial fibers between granular and molecular layers. The fusiform, short axon, intermediate cells of Lugaro can be found occasionally nestled in the spaces beneath Purkinje cells and more often, the cell bodies of Bergmann neuroglia occupy the corresponding spaces above Purkinje cell bodies. By far the most distinctive cytological arrangement in this area is the basket that cradles each Purkinje cell and ends in a pinceau around the initial segment of the axon; but these structures will not be described here. Instead, we shall focus on the network of recurrent collaterals and the synaptic patterns formed by their terminals with Purkinje cells, Lugaro cells, and other dendrites in the infraganglionic region.

In the electron micrographs, axonal profiles corresponding to the fleshy terminal branches of recurrent collaterals found in rapid Golgi preparations are more abundant in the infraganglionic region than anywhere else in the rat cerebellar cortex (Figs. 8–15). Unlike basket fibers, in which the axoplasm may be filled with neurofilaments and synaptic vesicles occur only near synaptic interfaces, the fleshy terminal branches of recurrent collaterals have synaptic vesicles dispersed over considerable lengths. Fig. 8 shows a long segment of a recurrent collateral containing slender mitochondria, fragments of the endoplasmic reticulum, neurofilaments, and microtubules, all oriented longitudinally in a dark matrix. Elliptical, flat, and round synaptic vesicles and an occasional 1000 Å dense core vesicle are strewn throughout the length of this profile. Cross-

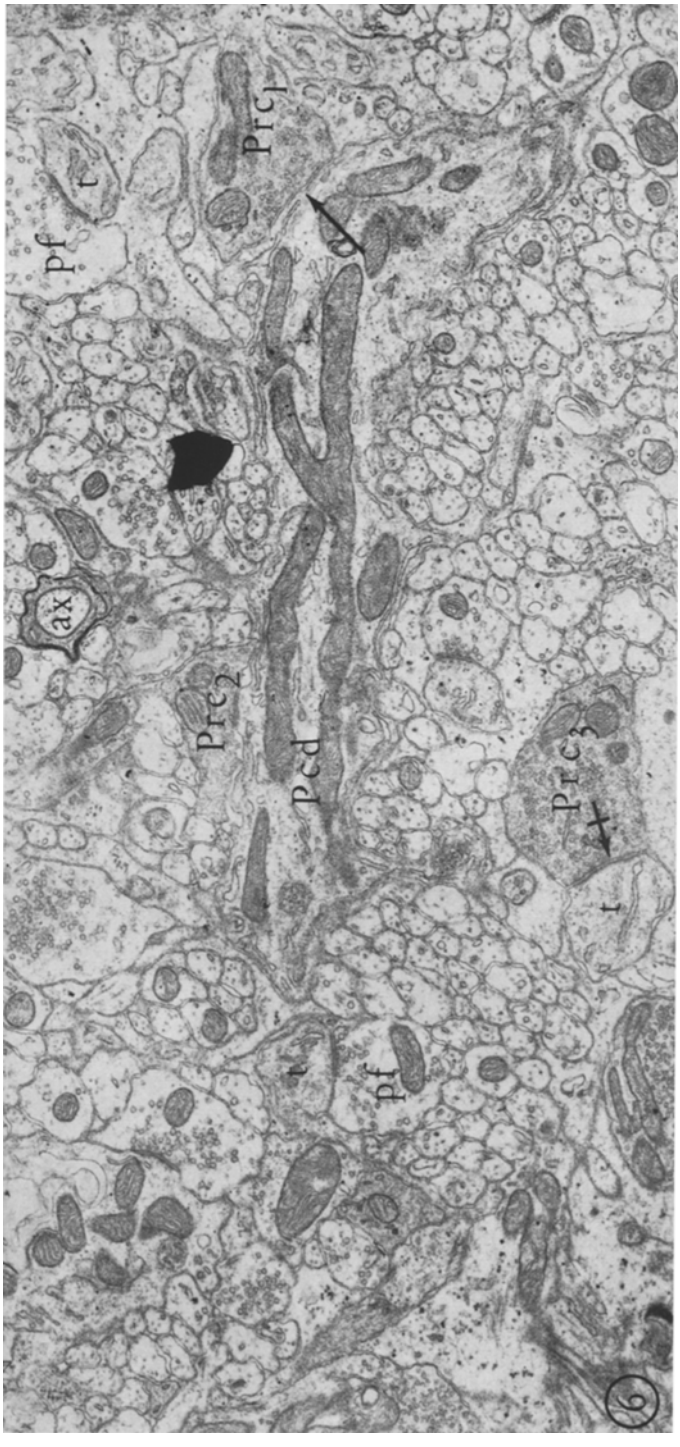


Fig. 6. Synapses of recurrent collaterals with the shaft and thorn of Purkinje cell dendrites. Boutons belonging to recurrent collaterals (*Prc*) in the supraganglionic plexus are seen in two types of synapses on Purkinje cell dendrites. Two endings (Prc_1, Prc_2) are seen to terminate directly on the shaft of the dendrite (→), whereas another bouton (Prc_3) synapses (⤙) on a Purkinje dendritic thorn (*t*). This last synapse differs from the synapses of parallel fibers (*pf*) on dendritic thorns (*t*). The synaptic cleft is widened and contains dense material, but the shallow densities adhering to both pre- and postsynaptic surfaces are nearly symmetrical. A cross section of a myelinated recurrent collateral is seen in the middle left of the figure (*ax*). (Lobule VIII, copula pyramidis.) × 15000

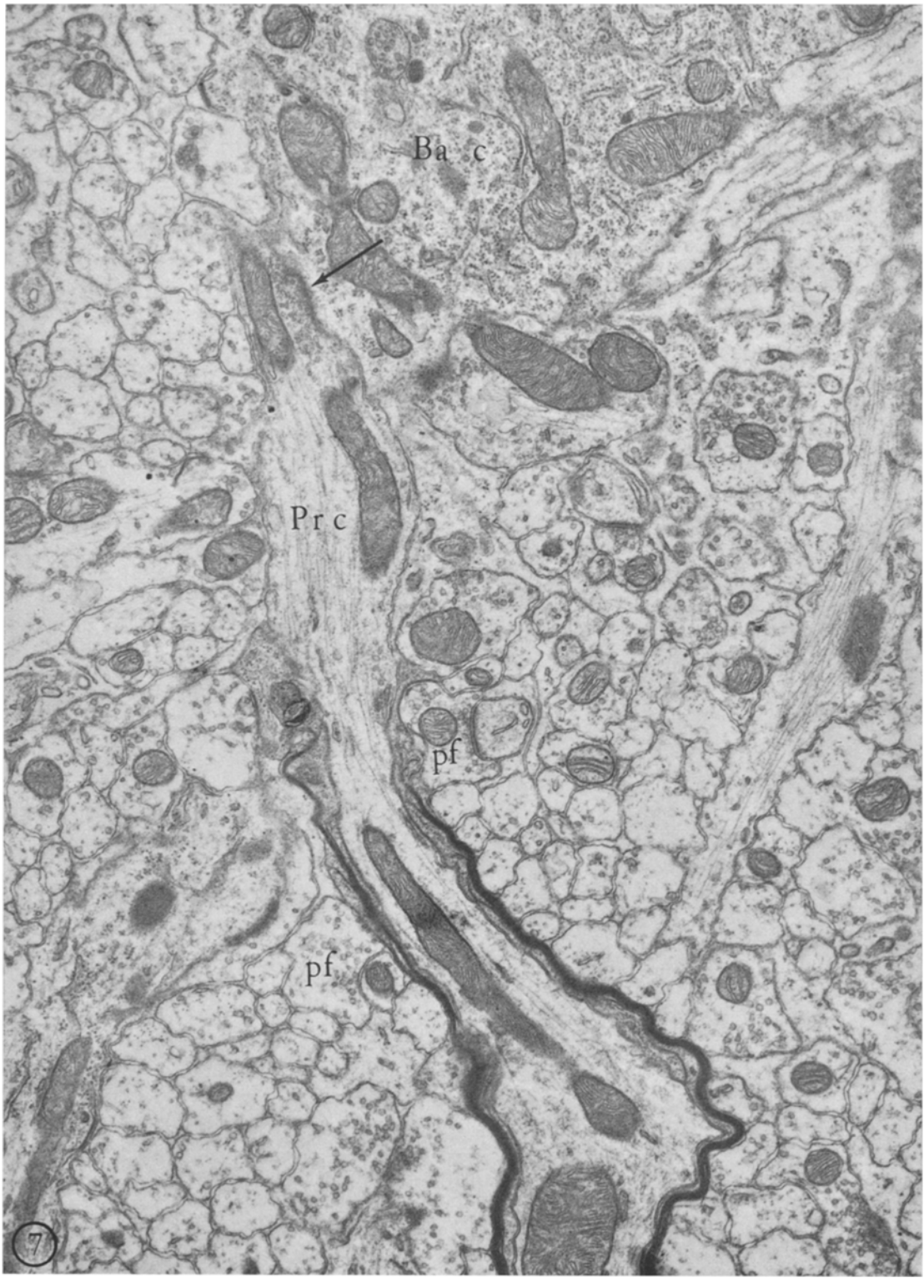


Fig. 7

sectional profiles of such terminations are also numerous and can be found in synapse with dendrites and somata of nearby neurons.

Synapses on Purkinje Cell Somata. Synaptic boutons from branches of recurrent collaterals penetrate the neuroglial sheath to form axo-somatic synapses on some Purkinje cells (Fig. 9). They are usually found in clusters on the surface of the cell. The boutons, covered by the neuroglial sheath of the Purkinje cell, are impressed lightly into the convexity of the cell surface. Each bouton is about 0.8–1 μ in diameter, containing at least several profiles of slender mitochondria and a moderately dense collection of polymorphic synaptic vesicles (Fig. 9). A profile of a bouton may have from one to three (arrows) small synaptic complexes on the part of its surface that is apposed to the perikaryon. The synaptic complexes differ only slightly in length (0.05–0.1 μ) whether the bouton is sectioned longitudinally or transversely; evidently the synaptic complex is circular in outline. In these spots the interstitial cleft is slightly widened from the usual 125 Å to about 230 Å and contains an accumulation of dense fibrillar material between synaptic membranes. On the pre- and postsynaptic surfaces very shallow symmetrical densities are observed. Just deep to this the hypolemmal cisterns of the Purkinje cell are evident (Kaiserman-Abramof and Palay, 1969). Thus these synaptic complexes between recurrent collateral and Purkinje cell are not consistent with either of Gray's types.

Synapses on Lugaro Cells. Considerable numbers of synaptic boutons belonging to recurrent collaterals have been found on the somata and initial portions of dendrites of Lugaro cells (Figs. 10 and 11). These cells can be recognized by their disposition in the cerebellar cortex, and by their fine structural characteristics. The fusiform or teardrop-shaped bodies of the Lugaro cells nestle beneath the Purkinje cells near the points of emergence of their initial axonal segments. The nucleus of Lugaro cell is roughly oval with a rounded base and lobulated superior aspect. Chromatin is dispersed throughout the nucleus with a tendency to clump near the nuclear membrane. A prominent nucleolus is characteristic, and there is usually a modest amount of cytoplasm. The perikaryon contains all the cellular organelles typical of neurons, in particular a well-developed Golgi apparatus. The Golgi apparatus not only surrounds the perinuclear zone, but also extends well into the dendrites, which are fleshy, straight, and of more or less uniform caliber, with almost no thorns. In favorable thin sections it is possible to find a dendrite issue from the apical end of the cell body, funneling microtubules, ribosomes, endoplasmic reticulun, and Golgi apparatus from the perikaryon (Fig. 10). The cell body and initial portions of dendrites are only partially or incompletely invested with the processes of neuroglial cells, through which axons have to penetrate in order to make synaptic contact. As is true of the axo-

Fig. 7. Recurrent collateral emerging from its myelin sheath to synapse on basket cell soma. The synaptic varicosity (*Prc*) belonging to a recurrent collateral in the supraganglionic plexus leaves the myelin sheath as a slightly distended sac filled with some neurofilaments, occasional tubules, and a population of polymorphic synaptic vesicles. It is attached to the soma of a basket cell (*Ba c*) with which it presumably synapses (→). The remainder of the field consists of parallel fibers (*pf*) in cross section and basket cell or other axons. (Lobule IV, vermis.) × 21000

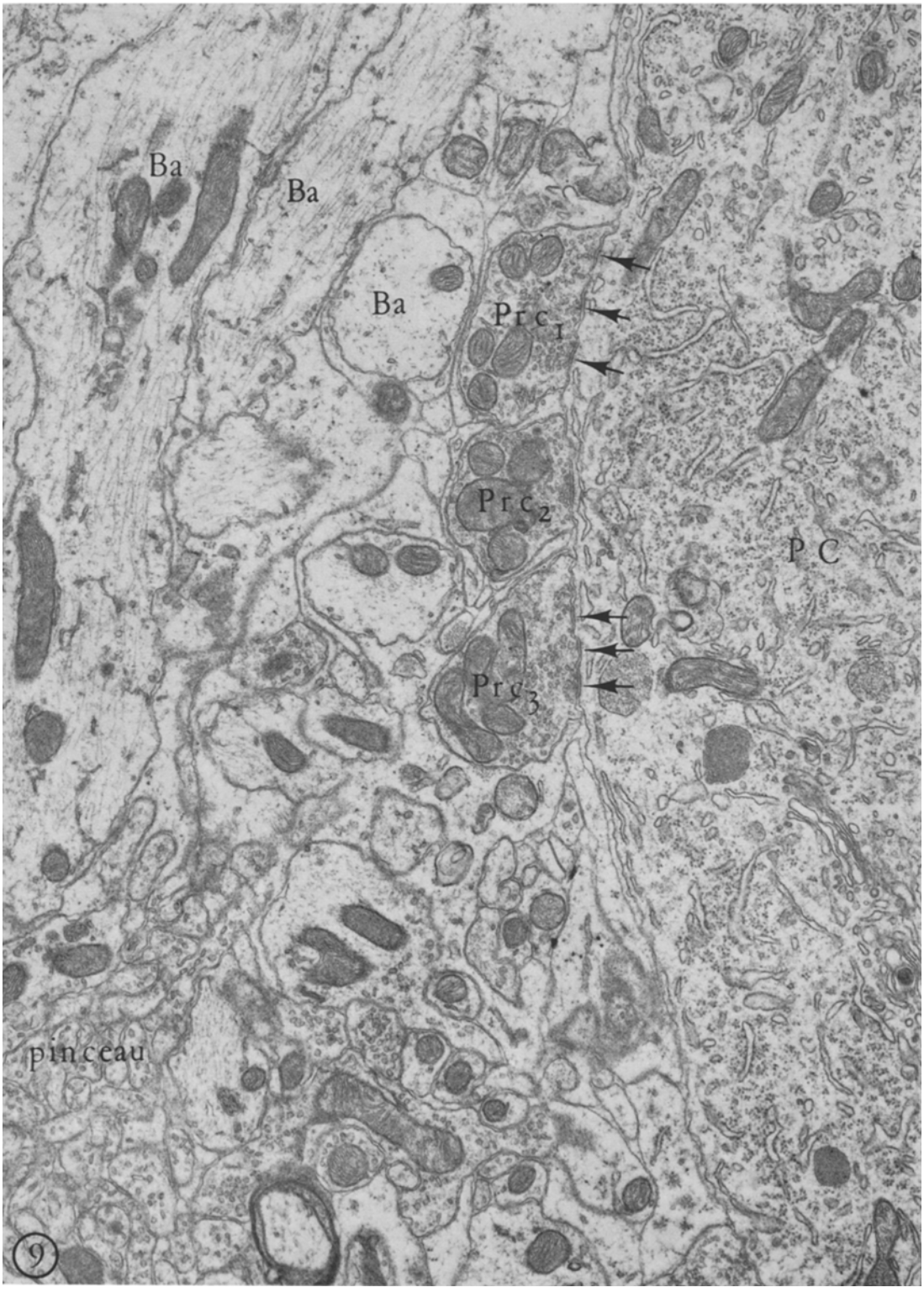


Fig. 7

somatic synapses on the Purkinje cell surface, there are usually clusters of several synaptic boutons from recurrent collaterals on each profile of the Lugaro cell soma. These terminations press against the cell surface, each forming a patch in which a series of small, circular synaptic complexes occur between the apposing cell membranes (Figs. 10 and 11). These macular synaptic complexes are separated by bare areas. These junctions are intermediate between Gray's type 1 and type 2. A widened interstitial cleft is usually present, with the apposing membranes parallel to each other. Shallow adherent densities are arranged symmetrically on the pre- and postsynaptic sides of the junction. Occasionally a round clump (about 0.1 μ in diameter) or aggregation of dense filamentous material can be found in terminal boutons that participate in these axo-somatic synapses (Fig. 11). The significance of these clumps is not known, but they may be akin to the proteinaceous aggregations present in the cytoplasm of many ostensibly normal neurons.

Other Complex Synaptic Arrangements. Besides the synapses with the somata and initial portions of dendrites belonging to Purkinje cells and Lugaro cells, terminals of recurrent collaterals participate in a number of complex synaptic arrangements with other dendrites in the infraganglionic area. These dendrites presumably belong to deep Golgi II cells or to granule cells in the vicinity. Two basic patterns of synaptic relationships prevail. Sometimes a recurrent collateral bouton engages in synapses with two or more dendrites (Figs. 12, 14, 15). Or, more commonly, a single dendrite receives several synapses and only one of these is a recurrent collateral bouton, the others being axons of granule cells or even mossy fibers (Figs. 13–15). Each of these two combinations of synapses may be found individually in the infraganglionic area (Figs. 12 and 13).

In Fig. 12 two dendritic profiles abut against and synapse with a large, rounded recurrent collateral bouton. The ending contains the dark, filamentous matrix, the numerous mitochondrial profiles, and the polymorphic synaptic vesicles so characteristic of these boutons. One of the synapses (arrow) has junctional complexes that conform to neither of Gray's types because of the widened interstitial cleft and symmetrical, shallow pre- and postsynaptic densities. The other synapse (asterisk) has complexes more typical of Gray's type 2 with only a very small widening of the interstitial cleft and symmetrical pre- and postsynaptic densities. These are to be compared to Fig. 13, in which a single dendritic profile (*d*) synapses with two axonal endings, only one of the boutons (*Prc*) belonging to the recurrent collateral network.

It is more usual, however, to find boutons of recurrent collaterals in the infraganglionic region that are involved in more complex arrangements of synapses than the examples cited above. In electron micrographs taken at the level of the pinceau, and particularly in sections in the frontal plane, one can find clusters of

Fig. 8. Terminal branches of recurrent collaterals in the infraganglionic region. A long, distended terminal (Prc_1) is identified by the dark appearance of its axoplasmic matrix and the polymorphic population of flat, elliptical, and round synaptic vesicles. Embedded in this matrix are neurofilaments, microtubules, occasional dense core vesicles, and slender mitochondria. Two other axons belonging to the recurrent collateral plexus (*Prc*) can also be seen in this field amongst the long profiles of basket axons (*Ba*). (Lobule IV, vermis.) $\times$ 17000

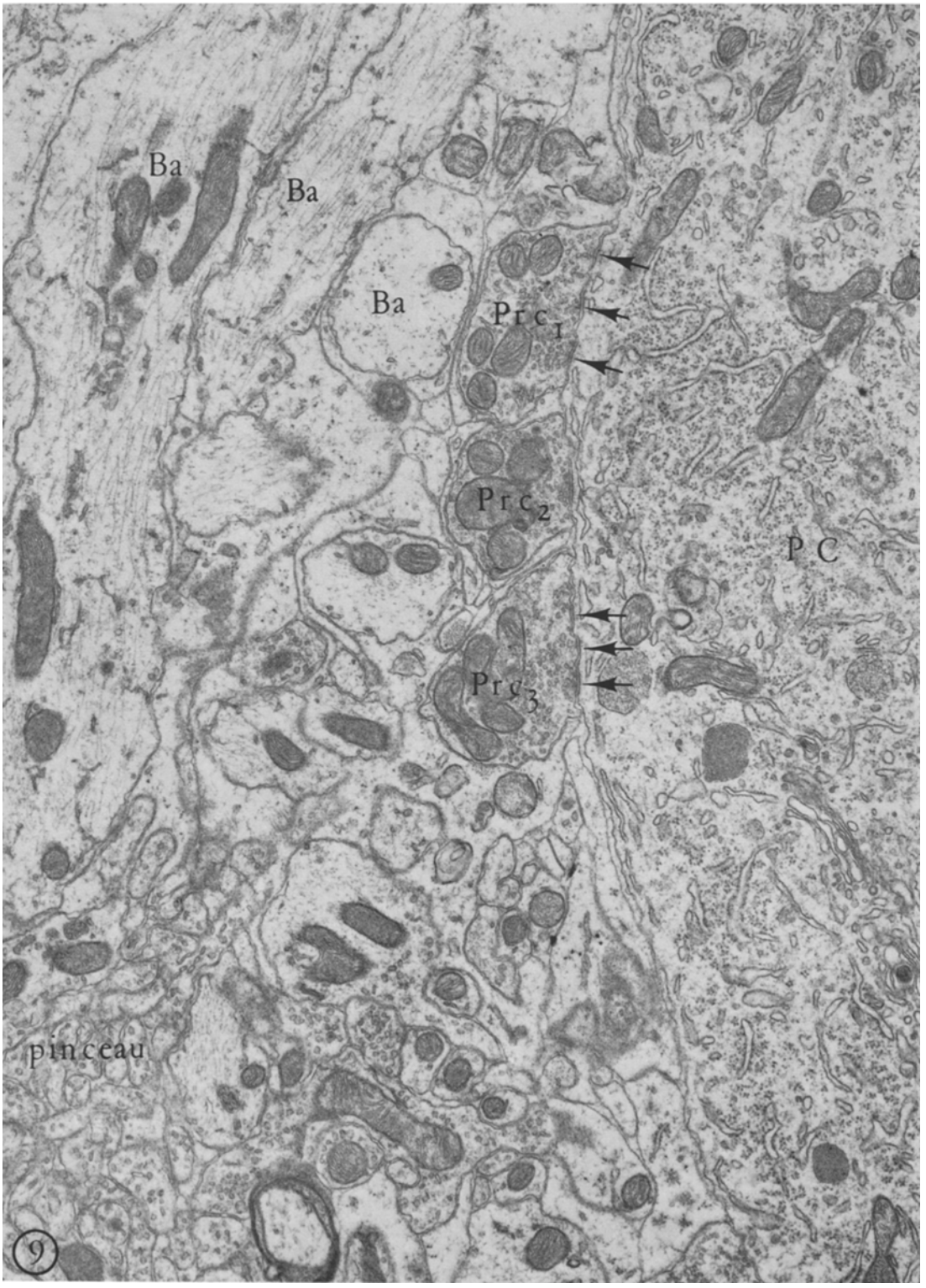


Fig. 9

several recurrent collateral boutons involved in several sets of combinations of the synapses seen in Figs. 12 and 13. Fig. 14 is a good example of this arrangement. The axonal profiles in the lower center belong to collaterals of the climbing fiber (*cf*) and have the characteristic dense population of round synaptic vesicles in a very dark matrix, with Gray's type 1 synapses (see Chan-Palay and Palay, 1971 a). These profiles are surrounded by dendrites and also by other axonal profiles that can be differentiated into two main types: Firstly, the familiar boutons of Purkinje recurrent collaterals marked by the presence of a dense matrix and polymorphic synaptic vesicles (asterisk). Secondly, boutons of Golgi II axons (*Ga*) distinguished by a population of flattened and elliptical synaptic vesicles loosely dispersed in a light, flocculent matrix. These axonal and dendritic combinations are often partially invested in processes of neuroglial cells, which dissect between sets of axons and dendrites, or incompletely encircle groups of them (see Fig. 14).

Complex synaptic arrangements similar to those just described have been observed to include profiles of mossy fibers (Fig. 15). In this case a large cluster of recurrent collateral boutons (*Prc*) is found amongst profiles of a mossy fiber (*MF*) and dendrites. Closer examination of this illustration shows that the recurrent collateral terminations that are present are involved in various combinations of synapses. For example, a recurrent collateral bouton (Prc_1) synapses with two dendritic profiles (d_1 and d_2), whereas two other recurrent collateral endings (Prc_2, Prc_3) are in synapse with other dendrites (d_3, d_4). Processes of neuroglial cells can be followed around the sets of axons and dendrites in this field.

These observations on electron micrographs of the infraganglionic region provide us with the synaptic relationships engaged in by the recurrent collaterals. Synapses are made with Purkinje cell somata and dendrites, Lugaro cell somata and dendrites, and with dendrites of deep Golgi II cells and perhaps of granule cells. These observations corroborate the complexity of the infraganglionic plexus which was only adumbrated by the tangle of varicose terminal branches seen with the light microscope in Golgi preparations (Figs. 1–4).

Terminations of Recurrent Collaterals in the Granular Layer. There are five principal categories of axons in the granular layer. Two of these connect the cerebellar cortex internally: firstly the short, unmyelinated, finely beaded axons of deep Golgi II neurons that ramify into almost all cerebellar glomeruli, and secondly, the thin axons of the granule cells (in the rat a very small number of these may be myelinated, Fig. 17) that ascend into the molecular layer. The next two of the five axonal systems represent the afferent inputs to the cerebellum, namely, the extensive mossy fibers and climbing fibers that enter from the white matter as myelinated axons. The remaining system, the myelinated axons of Purkinje cells and their recurrent collaterals, connects the cortex internally as well as providing the only efferent output of the cerebellar cortex.

Fig. 9. Synapses of recurrent collaterals on the soma of a Purkinje cell. Three profiles of boutons belonging to the infraganglionic plexus of recurrent collaterals (Prc_1, Prc_2, Prc_3) synapse on the soma of a Purkinje cell (*PC*). Two of the three terminals (Prc_1, Prc_3) each have three macular synaptic complexes (←) on the surface apposed to the Purkinje cell. In these spots the interstitial cleft is widened and contains dense fibrillar material. On the pre- and postsynaptic surfaces very shallow symmetrical densities are present. Just deep to this the hypolemmal cisterns of the Purkinje cell are evident. The free surface of each terminal is surrounded by a thin neuroglial sheath. Many thick basket cell axons (*Ba*) sweep around the terminals to end in the *pinceau* at the lower left. (Lobule IV, vermis.) × 17000

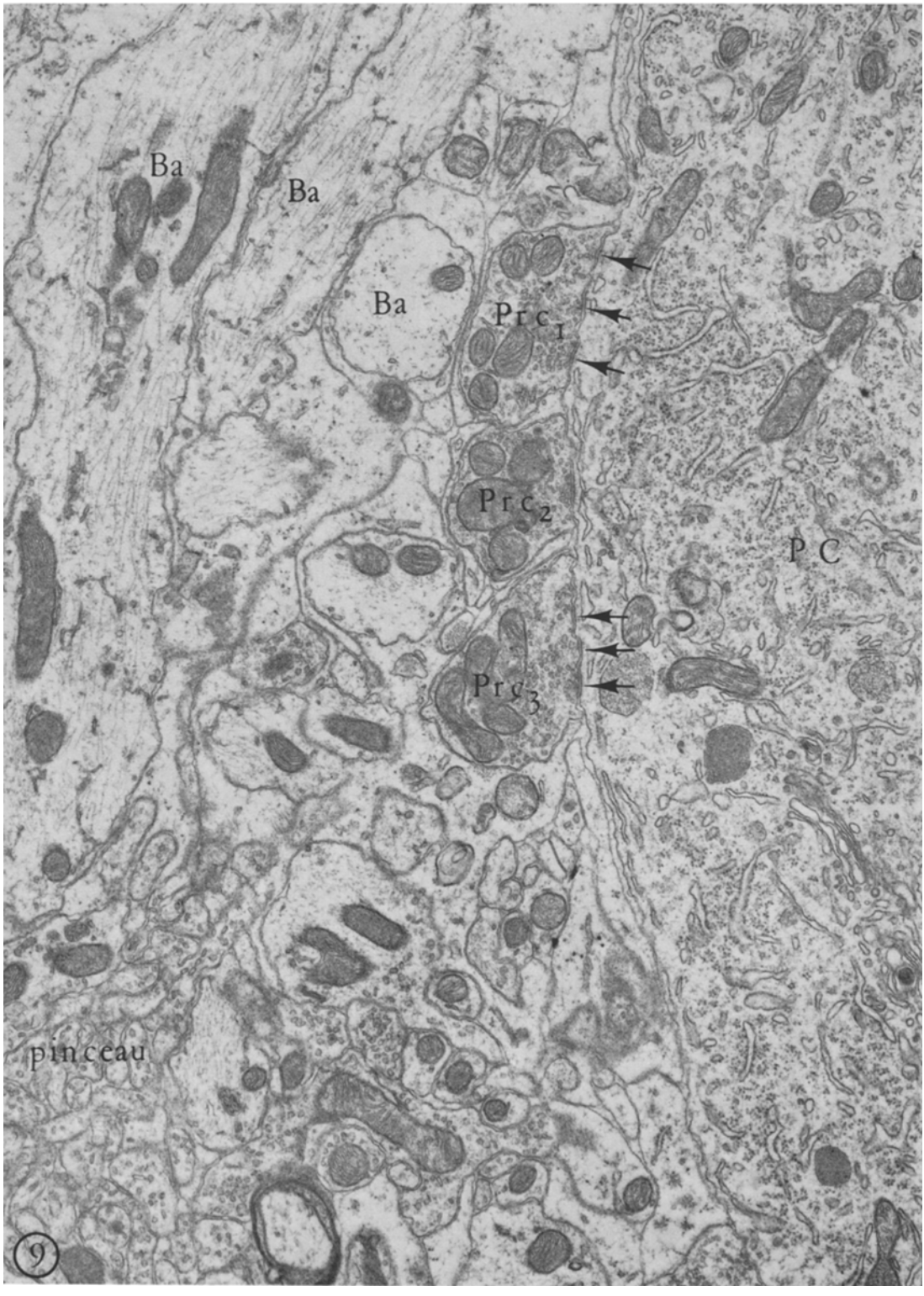


Fig. 10

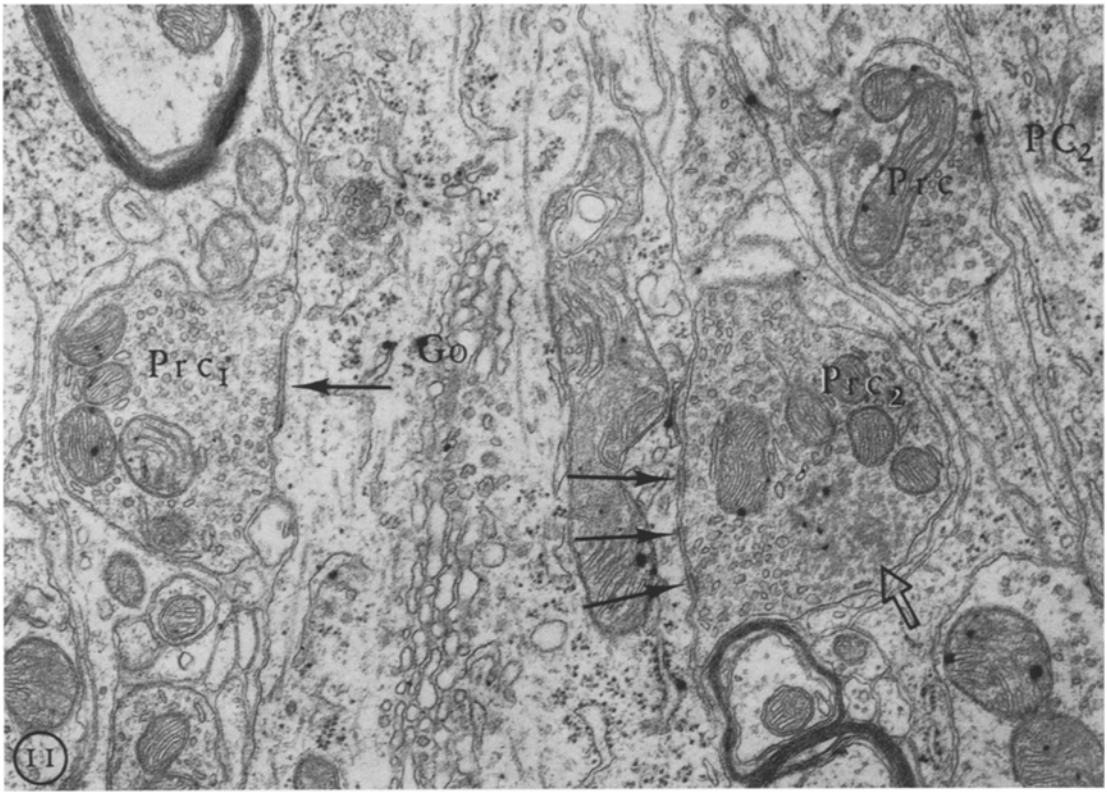
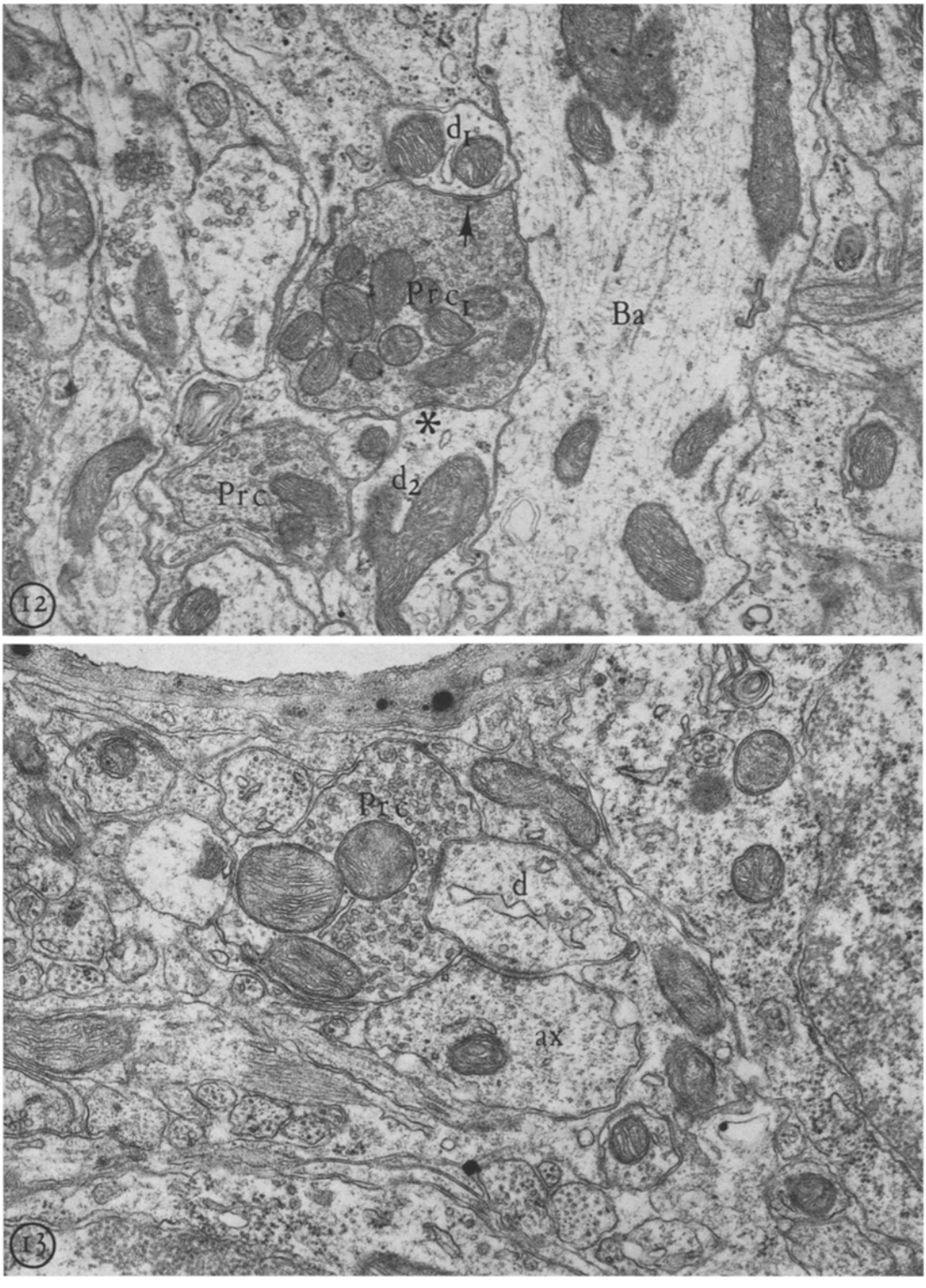


Fig. 10. Synaptic boutons of recurrent collaterals on the soma and dendrite of a Lugaro cell. The Lugaro cell (*LC*) is nestled beneath the somata of two Purkinje cells (PC_1, PC_2) and gives off a dendrite that extends to the top of the figure. The initial segment (*Ax*) of the Purkinje cell (PC_1) leaves the field at the bottom of the figure. This cell body is surrounded by many profiles of basket axons (*Ba*). The distinguishing features of the Lugaro cell are a nucleus with lobulated apical and smooth basal aspects, a prominent nucleolus (*nuc*), and a very well developed Golgi apparatus (*Go*). Four recurrent collateral varicosities (*Prc*) synapse on the surface of the Lugaro cell, and synaptic junctions are indicated by arrows. A detail of the field enclosed in the box is given in Fig. 11. (Lobule V, vermis.) × 17000

Fig. 11. The dendrite of a Lugaro cell synapsing with two boutons of the recurrent collateral plexus. The initial portion of the Lugaro cell dendrite (*LCd*) contains a prominent Golgi apparatus (*Go*) amongst the usual cellular organelles. Two boutons of recurrent collaterals (Prc_1, Prc_2) abut on the dendrite. The bouton on the left (Prc_1) shows a single long synaptic complex (←). The bouton on the right (Prc_2) shows a series of three short synaptic complexes (→). These junctions are typical of those made by recurrent collateral terminals, with the widened interstitial cleft, containing a dark material, and shallow, symmetrical pre- and postsynaptic densities. Bouton Prc_2 contains a dark proteinaceous clump (⇐). In the upper right hand corner a recurrent collateral bouton synapses on the soma of a Purkinje cell (PC_2). (Lobule V, vermis.) × 25000



Figs. 12 and 13

As described above, in rapid Golgi preparations a plexus of recurrent collaterals of Purkinje axons can be seen that ramifies in the middle and lower granular layer. Particularly in parasagittal sections, electron micrographs of the neuropil below the infraganglionic region often show long segments of large myelinated fibers belonging to recurrent collaterals. These fibers are oriented vertically in the granular layer and branch at irregular intervals. The twigs resulting from these branchings emerge from their myelin sheaths and expand into sacs containing polymorphic synaptic vesicles suspended in a dark, filamentous matrix (Fig. 16). Such fibers can be confused with certain slender axons that originate from deep granule cells but are also myelinated and form synapses *en passant* as they ascend through the granular layer. Fig. 17 shows such a granule cell axon emerging from its myelin sheath and synapsing with two dendrites. This synaptic varicosity with its round synaptic vesicles is similar to those found on parallel fibers in the molecular layer.

Synapses on Deep Golgi II Cells. A large number of the terminations of Purkinje recurrent collaterals are found on the somata and dendrites of deep Golgi type II neurons. It is not unusual to find the profile of such a cell (Fig. 18) dotted with terminal boutons of recurrent collaterals (arrows), clustered on the cell body and proximal portions of dendrites. As in synapses with Purkinje cells and Lugaro cells in the infraganglionic plexus described above, each bouton on the Golgi II cell displays a few small, separate, macular synaptic complexes. Each synaptic complex has a widened interstitial cleft, with a fibrous density between the apposed membranes, and symmetrical, thin pre- and postsynaptic densities. Thus, this is intermediate between Gray's types.

The Synapse en Marron. In two recent publications we described extensive synapses between the perikaryal surfaces of some deep Golgi II neurons and glomerular terminals belonging to climbing fibers (Chan-Palay and Palay, 1971a) and mossy fibers (Chan-Palay and Palay, 1971b) in the granular layer. Because the postsynaptic surface of the Golgi II neuron in the region of the synapse resembles the wrinkled surface of a chestnut, this form of axo-somatic articulation was referred to as the *synapse en marron*. The present investigation shows that boutons of recurrent collaterals of Purkinje axons also form extensive *en marron* synapses with some deep Golgi II cells (Fig. 19). The cytoplasm of the Golgi II cell at the synapse bears the distinctive postsynaptic fibrillar zone, and ribbons of synaptic complexes are typically arranged in the furrows between ridges that are bare of synapses. Because the synaptic complexes run longitudinally within the

Fig. 12. Recurrent collateral boutons of the infraganglionic region. The recurrent collateral bouton (Prc_1) in the center of the field synapses with two dendrites. The junction ($\rightarrow$) with the dendrite in the upper part of the figure (d_1) has a widened synaptic cleft, dense fibrillar interstitial material, and very shallow, symmetrical pre- and postsynaptic densities. The synapse ($*$) in the lower part of the figure (d_2) is more consistent with Gray's type 2, with very little widening of the synaptic cleft, dense interstitial material, and shallow pre- and postsynaptic densities. Another recurrent collateral profile (*Prc*) and a basket axon flank the synaptic arrangements described. (Lobule V, vermis) $\times$ 22000

Fig. 13. A dendrite (d) in the infraganglionic region in synapse with a Purkinje recurrent collateral (*Prc*) and another axon (ax). (Lobule X, vermis) $\times$ 30000

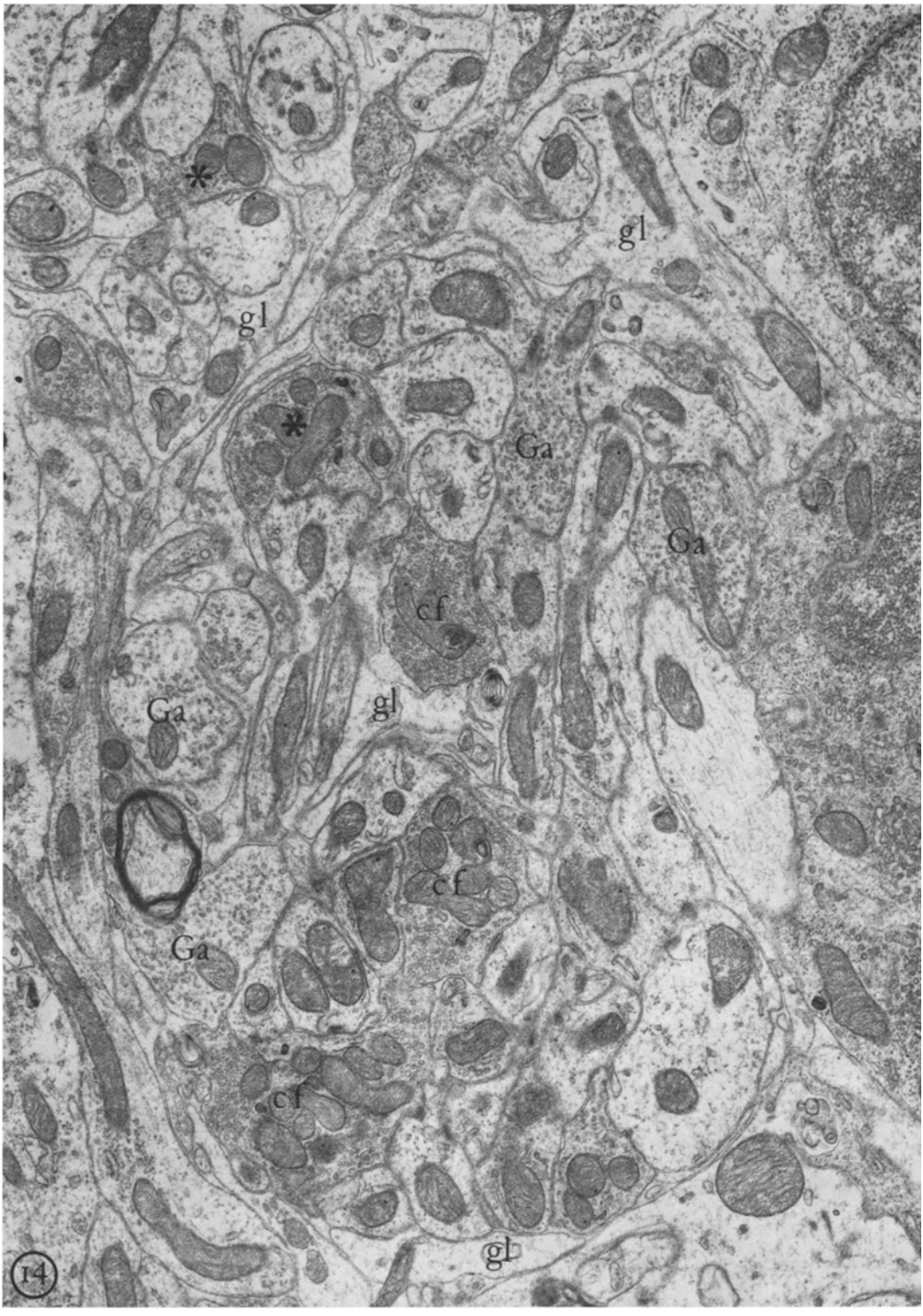


Fig. 14

furrows, where the synaptic complexes are cut parallel to the long axis of the furrows (Fig. 19, arrow) the electron micrograph shows a long, flat synaptic junction interrupted by a low ridge (Fig. 19, crossed arrow) bare of synapses. Where the section passes perpendicularly to the long axis of the furrow, a short, saucer-shaped synaptic complex is seen (Fig. 19, asterisk). The synaptic complex has a widened interstitial cleft filled with a filamentous density, slightly accentuated in the middle of the cleft. Both apposed membranes are parallel, and shallow pre- and postsynaptic densities are present. Thus, this synapse is also intermediate between Gray's type 1 and type 2. Unlike the situation described for mossy fiber and climbing fiber *en marron* synapses, the free sides of terminals of Purkinje recurrent collaterals are not engaged in the formation of those complex axo-dendritic arrangements that are found in glomeruli.

In 1965 Andres described stacks of tubular membrane systems in myelinated axons. These have been reported as being a criterion for the identification of profiles belonging to recurrent collaterals in electron micrographs of cerebellar cortex (Eccles *et al.*, 1967, p. 182; O'Leary *et al.*, 1968; Hámori and Szentágothai, 1968). We have not found these membrane systems in axons of the rat's cerebellar cortex.

Discussion

Historical Considerations. A contemporary investigation into the intrafolial course and terminations of recurrent collaterals belonging to Purkinje cell axons would not be complete without a thorough consideration of the writings of the earliest investigators on the subject. Camillo Golgi's original description in 1874 of the recurrent collateral system in the human cerebellum marked the important discovery that nerve fibers in the central nervous system possess many collaterals through which other neurons can be affected. The account, which was derived from his chrome-silver method, described the basic plan of the Purkinje axon recurrent collaterals in relation to the white matter and to the granular and molecular layers in the cortex. He says, "The fibers that bend upward in order to reach the molecular layer have a tortuous course, along which from place to place little fibers emerge that either lose themselves in the granular layer or go further upwards."

Several years later, Ramón y Cajal (1888, 1889, 1911) noted in a variety of animals and birds that after the third or fourth node the axons of Purkinje cells emit retrograde collaterals, which enter the granular layer. These collaterals were seen to ascend to the level of the Purkinje cells, where they traveled more or less horizontally across the folium under the cell bodies. Where bifurcations were abundant, these collaterals formed two concentric nerve fiber plexuses: the deep

Fig. 14. Other complex synaptic arrangements of Purkinje axon recurrent collaterals in the infraganglionic region. Profiles of recurrent collateral boutons (*) are found in various combinations of synapses with one or more dendrites in this field. These boutons can be differentiated from boutons belonging to axons of Golgi II cells (*Ga*), as the latter have a light axoplasmic matrix and a loose population of flat and elliptical synaptic vesicles. Three profiles of climbing fiber collaterals (*cf*) can also be seen in the center of the figure. Slips of neuroglial cytoplasm (*gl*) are insinuated between the clusters of axon-dendritic synapses and also partially invest the main central clump of processes. (Lobule V, vermis) × 17000

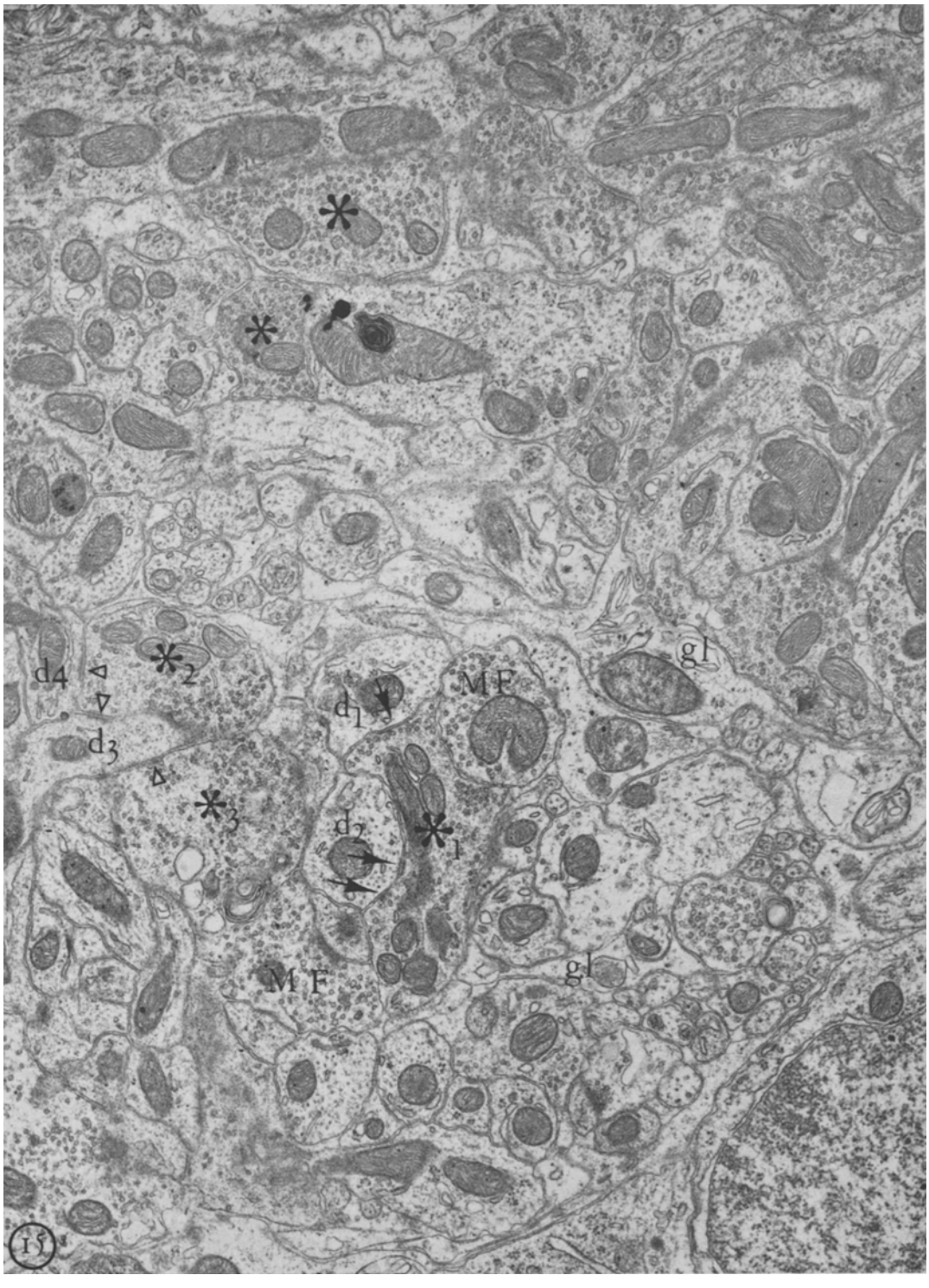


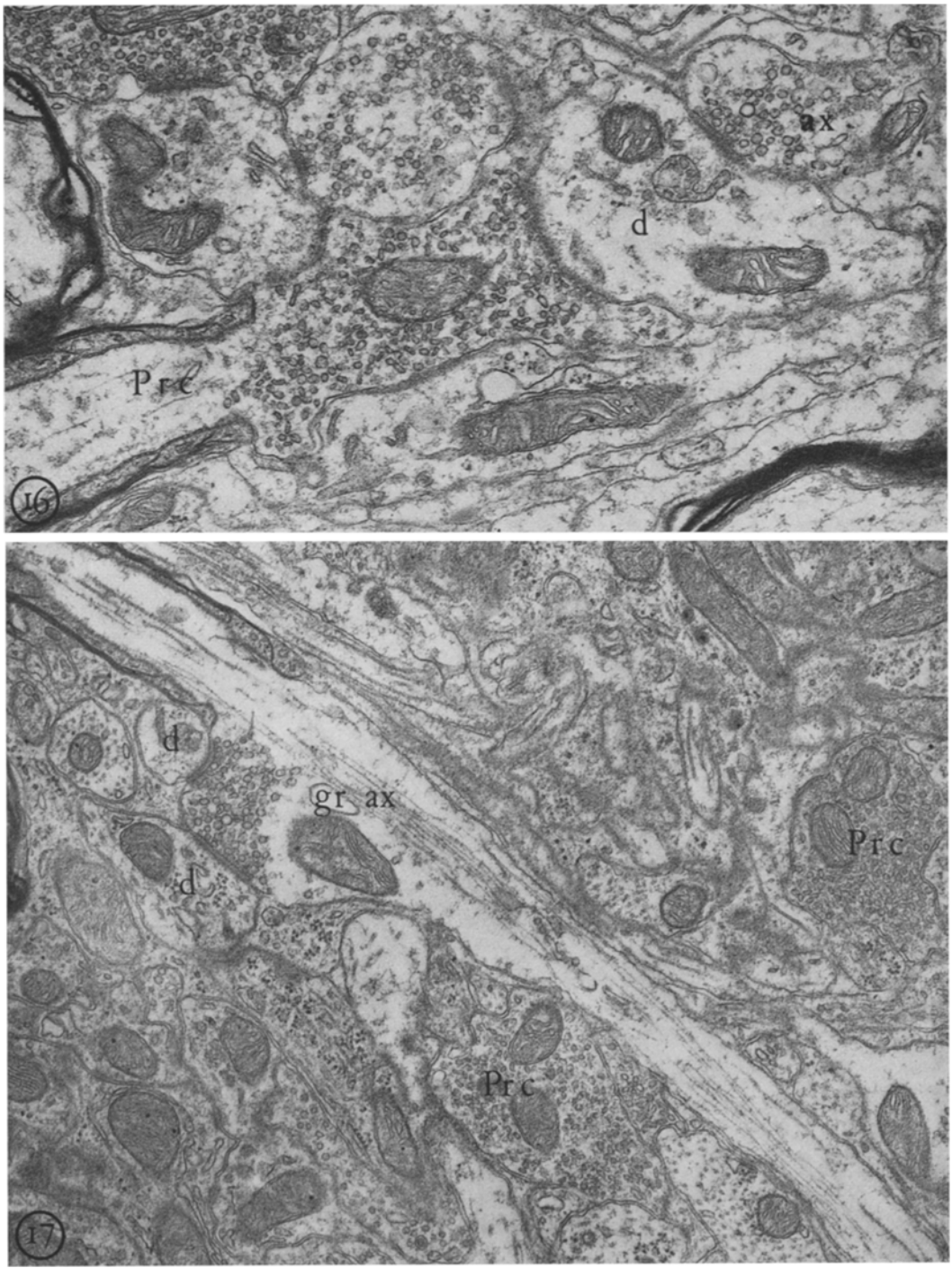
Fig. 15

plexus of secondary collaterals beneath the Purkinje cells, and a superficial plexus of tertiary collaterals above them. The collaterals of the deep plexus ended in relation to the bodies of Purkinje cells, whereas branches of the superficial plexus were seen to end in neurofibrillar rings or boutons on the primary dendritic trunks of Purkinje cells (Cajal and Illera, 1907). The superficial plexus consisted of myelinated branches that entered the molecular layer by passing between Purkinje cell bodies. These collaterals divided anew in the lower third of the molecular layer, and some of the branches continued for considerable distances in the long axis of the folium and sometimes could be traced for half the folial length.

It was also noted that the superficial plexus of recurrent collaterals varied in complexity with the species, being plentiful in man, sparse in the mouse, and rare in rabbits. Subsequently, Cajal (1912) presented additional observations from silver preparations of young dogs, cats, and man, and of animals with experimental lesions. He reported the presence of extensive pericellular plexuses made by recurrent collaterals of Purkinje cells around certain short-axon Golgi type II cells. There is no question as to Cajal's opinion on the identity of these cells; the description is very clear. Cajal states that neurons around which plexuses are found are *not* the larger, deep Golgi type II neurons of the granular layer but instead are the smaller, fusiform neurons found beneath Purkinje cells, whose axons traverse the granular layer to enter the white matter. We presume from the location of their cell bodies that Cajal meant the class of short-axon neurons called Lugaro cells. However, his description of the course of the axon of these cells differs from the observations of the present author and those of Fox *et al.* (1959) in that the axons of Lugaro cells ramify mainly in the molecular layer.

The description given by Golgi and Cajal on the network of recurrent collaterals was corroborated by their contemporaries, in particular Retzius (1892) and Kölliker (1896). Since then, neuroanatomists interested in the histology of the cerebellum, and in the Purkinje cell recurrent collateral system, have relied heavily upon Cajal's descriptions (Jakob, 1928; Jansen and Brodal, 1958). Jakob agreed with the account given by Cajal of the recurrent collateral plexuses in the supra- and infraganglionic regions. However, Jakob believed that the entire network was three-dimensional, with no obvious spatial orientation except perhaps in the frontal plane (1928, p. 151). This author was unwilling to speculate on the possibility that the boutons seen on the somata and dendrites of Purkinje cells in silver preparations might indeed be terminals of recurrent collaterals. In fact, this question of the terminations of branches in the recurrent collateral plexuses remained largely speculative (Fox *et al.*, 1954; Fox, 1959) until the advent of electron microscopy.

Fig. 15. Other complex synaptic arrangements of Purkinje axon recurrent collaterals in the infraganglionic region. The many recurrent collateral boutons (*) clustered in this region can be distinguished by their polymorphic synaptic vesicles and their dense axoplasmic matrix. The terminal marked $*_1$ is surrounded by two dendritic profiles (d_1, d_2), two profiles of mossy fibers, and other dendrites. The recurrent collateral bouton has three synaptic (→) junctions with d_1 and d_2. Two other recurrent collateral profiles, $*_2$ and $*_3$, are seen in several synapses (△) with two other dendrites, d_3 and d_4. Slips of neuroglial cytoplasm are insinuated between the axons and dendrites of the neuropil. (Lobule V, vermis) × 17000



Figs. 16 and 17

In the 50's and 60's the cerebellar cortex proved to be an ideal tissue for investigators of neuronal fine structure because of the almost crystalline rectilinear array of its cellular components. Several attempts were made with the electron microscope to identify the terminal arborization of the Purkinje axon recurrent collaterals (Hámori and Szentágothai, 1968; Eccles, Ito, and Szentágothai, 1967). But the identification of the terminations in these plexuses was not compelling until the work of Larramendi and Victor appeared in 1967, followed by that of Lemkey-Johnston and Larramendi, 1968, and Larramendi and Lemkey-Johnston in 1970. These last-mentioned authors reported on the basis of electron microscopic observations in the mouse cerebellum that the largest number of recurrent collateral profiles were observed in the molecular layer, presumably in the supraganglionic plexus, and that few synapses were found on Purkinje cells or their processes. Lugaro cells ("deep basket cells") showed synapses with recurrent collaterals (confirming earlier observations by Fox *et al.*, 1967; O'Leary *et al.*, 1968), and almost no synapses were formed with deep Golgi type II cells in the granular layer.

Species Differences in the Recurrent Collateral Network. As these accounts indicate, the networks of Purkinje axon recurrent collaterals in the cerebellum of various animals conform to a common architectural plan. The differences that have been found are usually minor, being displayed as quantitative variations in the extent of the plexuses previously described by Golgi and Cajal. Similarly, our findings in the rat cerebellar cortex are in accord with this basic plan of the recurrent collaterals. The intrafolial network of Purkinje axon collaterals were seen to form *three* fiber plexuses, one in the granular layer, a second profuse plexus in the infraganglionic region, and a sparse supraganglionic plexus in the molecular layer. The plexus of the granular layer is formed by terminal branches of collaterals that emerge from the lower reaches of the parent axon. The varicose fibers terminate as boutons on the somata and dendrites of deep Golgi II cells and on granule cell dendrites. The infraganglionic plexus is made up of collaterals that bend to run horizontally in the parasagittal plane for considerable distances just beneath the Purkinje cell bodies. Branches issue from these horizontal fibers, and their boutons terminate mainly on the body and dendrites of Purkinje cells and Lugaro cells. The sparse supraganglionic plexus is formed by branches that ascend between Purkinje cell bodies to enter the molecular layer. What few boutons are present in this plexus synapse on the shafts and, less commonly, on the thorns of Purkinje dendrites, and more rarely on the somata of basket cells.

Three issues remain to be discussed in comparing our account of the recurrent collateral plexus in the young adult rat with those of previous investigators on

Fig. 16. A recurrent collateral bouton (*Prc*) emerges from the myelin sheath to synapse with a dendrite (*d*) in the granular layer. The same dendrite also receives a synapse from another axon (*ax*). (Lobule V, vermis) × 30000

Fig. 17. The axon of a granule cell (*gr ax*) emerges from its myelin sheath and expands into an excrescence before continuing on its way through the granular layer. The excrescence bears round synaptic vesicles and is in synapse with two dendritic profiles (*d*). Two recurrent collateral boutons (*Prc*) are visible nearby. (Lobule IV, vermis) × 26000

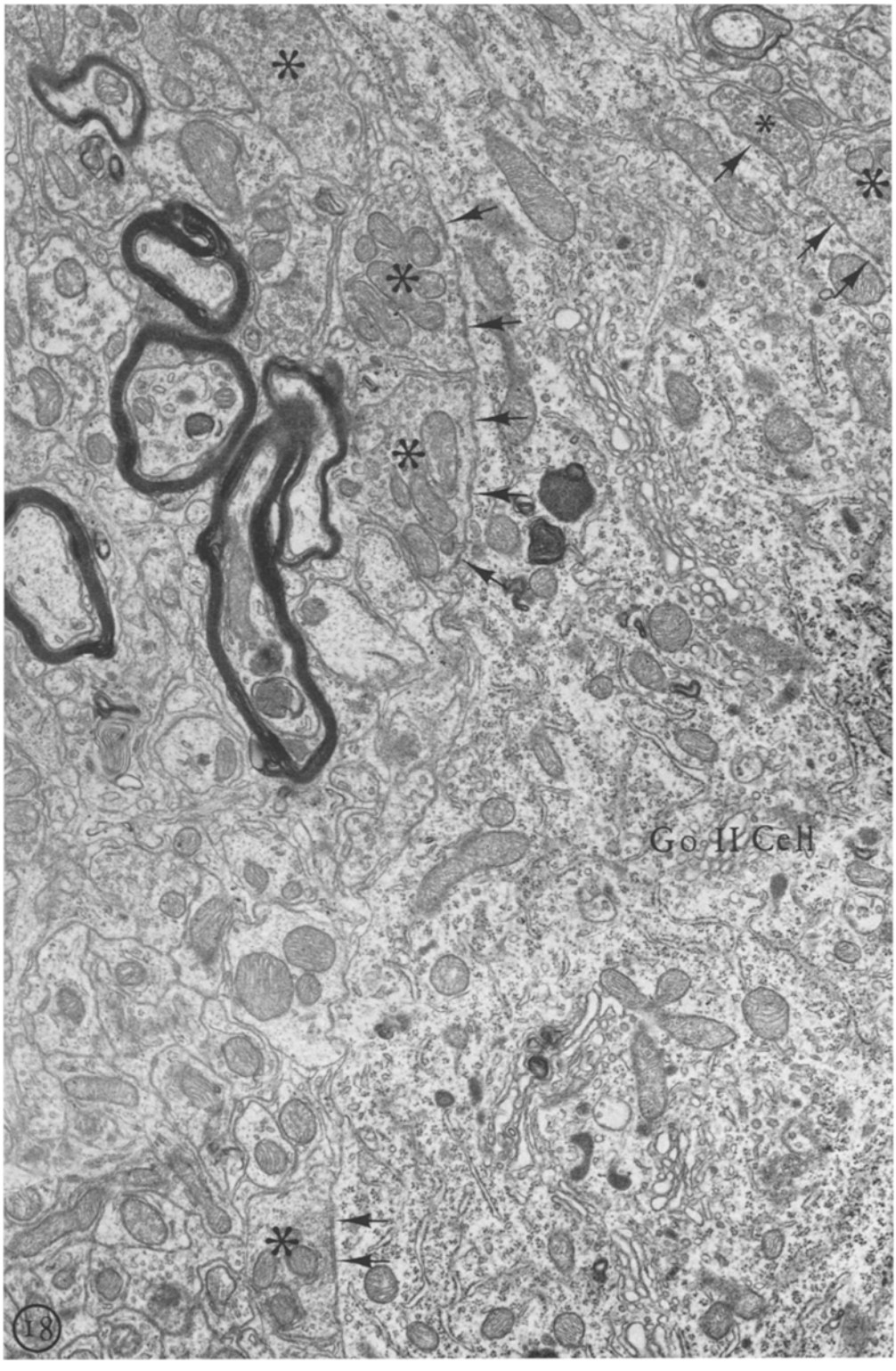


Fig. 18

infant animals of other species. These concern the supraganglionic plexus, the orientation of the entire plexus with respect to the axes of the cerebellar folia, and the existence of the plexus in the granular layer.

The supraganglionic plexus is remarkably sparse in the molecular layer of the rat, and, as was pointed out by Cajal, the extent of this plexus may be largely species-dependent. Furthermore, we were unable to trace in Golgi preparations any fibers in this plexus that extended parallel with the long axis of the folium, despite careful examination and reconstruction from serial sections of two entire folia (lobule IX, the ventral paraflocculus). This result contradicts Cajal's description of fibers in the supraganglionic plexus that travelled extensively in the long axis of the folium. These differences in observations might be another reflection of species variation consonant with the scantiness of the supraganglionic plexus in young adult rats compared with the infant animals examined by Cajal. Because of the lack of fiber components running parallel with the folium, the recurrent collateral network in the rat is not three-dimensional, but rather is confined to a thin parasagittal plane. Thus, the axonal plexus of Purkinje cells, like the dendritic arborization, is arranged as a fan spread out in a plane transverse to the folium.

The terminal plexus formed by recurrent collaterals surrounding deep Golgi type II neurons in the granular layer has been clearly identified both with light microscopy and electron microscopy in the rat cerebellar cortex. Moreover, we are reminded that Golgi in his earliest account of this axonal network in the human cerebellum described fine branches that lost themselves in the substance of the granular layer. It is difficult to reconcile our observations and those of Golgi, with Cajal's (1912) assertion that in the human, cat, and dog cerebellum the pericellular plexuses made by recurrent collaterals in the granular layer almost exclusively surround the short-axon fusiform neurons (Lugaro cells) beneath Purkinje cell bodies and definitely not the deep Golgi type II neurons.

Some Physiological Considerations. Data from physiological experiments in cats show that following juxtafastigial stimulation a weak inhibitory response is evoked in Purkinje cells, presumably through the antidromic activation of their recurrent collaterals (Eccles, Llinás, and Sasaki, 1966; Eccles *et al.*, 1967, p. 185). Similarly, a strong inhibition is produced in basket cells (Llinás and Ayala, 1967). The anatomy of the recurrent collateral networks in the young adult rat shows extensive contacts of varicosities in these collaterals with the dendrites and somata of Purkinje cells, deep Golgi II cells, a few basket cells, and the intermediate cells of Lugaro. Judging by the extent of the synapses of recurrent collaterals of Purkinje axons upon the dendrites and somata of Purkinje cells, one would suppose that these connections belong to a fairly strong negative feed-back system that may in some circumstances directly shut off Purkinje cell responses. This negative feed-back system, however, is at the same time connected with a positive feed-forward system involving synapses of recurrent collaterals

Fig. 18. A cluster of boutons of recurrent collaterals (*) in synapse with the soma and dendrite of a deep Golgi II cell (*Go II Cell*). These boutons form many small synaptic junctions with the surface of the Golgi II cell (→). (Lobule X, vermis) × 15000

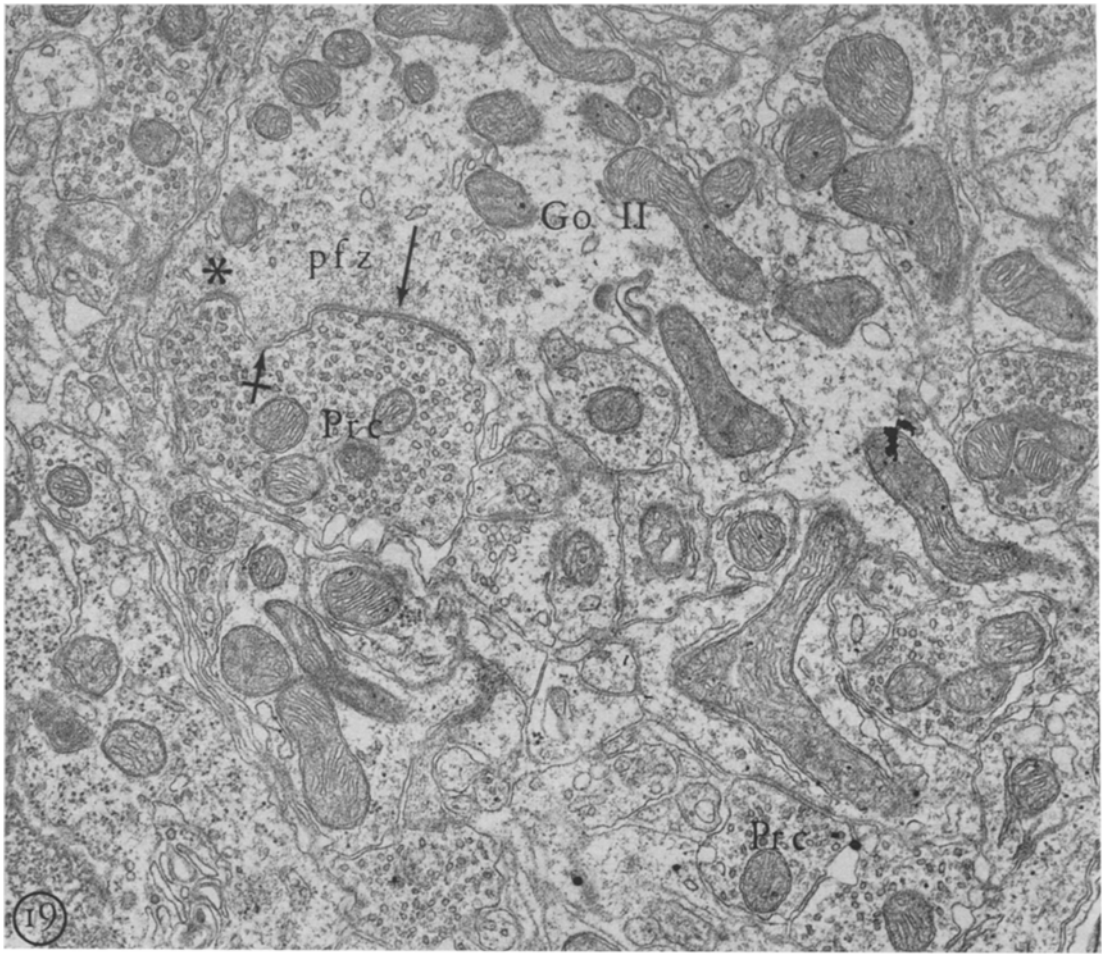


Fig. 19. The synapse *en marron* between a bouton of recurrent collateral and a deep Golgi II cell. The cytoplasm of the Golgi II cell (*Go II*) bears the distinctive postsynaptic fibrillar zone (*pfz*) and ribbons of synaptic complexes arranged in the furrows between ridges of the cell. A long, flat synaptic junction (→) is seen where the synaptic complex is sectioned in the long axis of the furrow. A short, saucer-shaped synaptic complex (*) is seen where the section passes across the axis of the furrow. The synaptic complex has a widened interstitial cleft filled with a filamentous density. Both apposed membranes are parallel, and shallow, symmetrical pre- and postsynaptic densities are present. The free sides of these recurrent collateral boutons are not engaged in the formation of glomeruli, unlike those of mossy fibers and climbing fibers engaged in synapses *en marron*. (Lobule X, vermis) × 22000

with deep Golgi II cells and some basket cells. Specifically, the inhibition of deep Golgi II cells would depress the inhibitory influences of these cells on glomeruli in the neuropil, resetting the circuits to allow incoming excitation from mossy and climbing fibers to affect granule cells. The inhibition of basket cells by recurrent collaterals would also depress basket cell control over the Purkinje cell itself. This relaxation of inhibitory influences would permit the restoration of

the Purkinje cell to its previously responsive state and allow afferent inputs to affect the Purkinje cell again.

The question of the role of Lugaro cells in the recurrent collateral circuits remains an enigma, partly because of the absence of physiological data on this cell type. We might speculate from their anatomy that these also function as coordinating neurons in the cerebellar circuits. Their location interposed between and beneath Purkinje cells, the orientation of their dendrites in the parasagittal plane, and the three-dimensional spread of their axons in the molecular layer are well suited to the task of gathering information from specific strips aligned at the junction of granular and molecular layers. We can only suppose that the messages so obtained are relayed to unidentified neurons in the molecular layer since the synaptic relations of Lugaro cells have yet to be described with the electron microscope.

In summary, recurrent collaterals from axons of Purkinje cells may provide a rapid monosynaptic feed-back mechanism for inhibitory control of the Purkinje cell responses. These collaterals may also contribute to a slower positive feed-forward circuit or resetting mechanism for the Purkinje cell that involves at least two synapses. Their inhibition may cause the release or depression of the inhibitory effect by deep Golgi II cells on surrounding granule cells, thus resetting them for subsequent responses to excitatory afferent input. Recurrent collateral inhibition of basket cells would also cause the disinhibition of Purkinje cells. The sequence that we have suggested in the operation of this scheme is only speculative, as the exact chronology for synaptic events in the recurrent collateral system is not available from physiological experiments already in the literature.

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