Z. Anat. Entwickl.-Gesch. 136, 224—248 (1972)

The Stellate Cells of the Rat's Cerebellar Cortex*

Victoria Chan-Palay and Sanford L. Palay
Departments of Neurobiology and Anatomy, Harvard Medical School, Boston, Massachusetts

Received February 18, 1972

Summary. Stellate cells were studied in rapid Golgi preparations and in electron micrographs. These small neurons can be classified on the basis of their position in the molecular layer and the patterns of their dendritic and axonal arborizations as follows: (1) superficial cells with short, contorted dendrites and a circumscribed axonal arbor (upper third of the molecular layer); (2) deep stellate cells with radiating, twisted dendrites and with long axons giving rise to thin, varicose collaterals (middle third of the molecular layer); (3) deep stellate cells with similar dendrites and long axons giving collaterals to the basket around the Purkinje cell bodies (middle third of the molecular layer). An important characteristic of the stellate cell axon is that it generates most of its collaterals close to its origin. Even in long axon cells, only a few collaterals issue from the more distant parts of the axon. These forms contrast with the basket cell, which sends out long, straighter dendrites, and an extended axon that first emits branches at some distance from its origin. Furthermore, basket cell axon collaterals are usually stout in contrast to the frail, beaded collaterals of the stellate cell axon. The two cell types are considered to be distinct.

In electron micrographs stellate cells display folded nuclei and sparse cytoplasm with the characteristics usual for small neurons. Mitochondria are often the most conspicuous organelles because of their size and pleomorphism. The dendrites cannot be followed for long distances in thin sections because of their irregular caliber and course. Axons can be recognized on the basis of their appearance in Golgi preparations as short stretches of slender fibers distended at close intervals and running athwart the grid of the parallel fibers. These distensions, full of ovoid or flattened vesicles, synapse on the shafts of Purkinje cell dendrites and also on the dendrites of Golgi cells, basket cells, and other stellate cells. In all cases the synaptic complex occupies about a third of the junctional interface, the synaptic cleft is somewhat widened, and the pre- and postsynaptic dense plaques are thin and almost symmetrical.

Varicosities in the parallel fibers synapse with the soma and dendrites of stellate cells. These junctions display a widened synaptic cleft and asymmetrical pre- and postsynaptic densities. Junctions with climbing fibers (Scheibel collaterals) have also been seen.

The form of the stellate cell indicates that it plays a role in cerebellar circuitry different from that of the basket cell, although both cells are inhibitory. It is probably concerned with local effects on Purkinje cell dendrites within the field of its afferent parallel fibers.

Key words: Basket cells — Synapses — Electron microscopy — Golgi method — Inhibition — Axons — Dendrites.

Introduction

The stellate cells of the cerebellar cortex compose a class of small, polymorphous neurons lying in the outer two thirds of the molecular layer. They were described by several early students of cerebellar structure, Fusari (1883), Ramón y Cajal (1889), Ponti (1897), and Smirnov (1897). Smirnow was credited by Ramón y Cajal (1911) as having given the most complete description. He

* Supported by U. S. Public Health Service Research Grant NS03659 and Training Grant NS05591 from the National Institute of Neurological Diseases and Stroke.

was able to distinguish two kinds of stellate cells in the cerebellar cortex of dog, cat, and hare, according to the configuration of their axons. The first kind of stellate cell had a long, partly myelinated (*sic*) axon that arborized in a plane transverse to the long axis of the folium and gave off both ascending and descending collaterals. This cell was found almost exclusively in the outer two thirds of the molecular layer. The second kind of cell was smaller with a short, highly arborized axon, and it was found in the outermost third of the molecular layer. This classification was confirmed by Ramón y Cajal (1911), and has been followed by most subsequent authors (e.g., Eccles, Ito, and Szentágothai, 1967).

The stellate cells received very little further attention from microscopists until recent years. Most authors have considered them to be related to basket cells. Both basket cells and stellate cells are oriented in the same plane. They also occupy similar positions in the circuitry of the cerebellar cortex because they both receive synapses principally from the parallel fibers and they direct their output principally to the Purkinje cell. There is, however, a very important difference between the two cells in so far as the distribution of their axonal arborization is concerned. In large measure the axonal collaterals of the stellate cell impinge upon the shafts of the Purkinje cell dendrites, whereas the basket cell axon, in addition to having this disposition, participates in the construction of an elaborate plexus around the perikaryon of the Purkinje cell and in a remarkable periaxonal synapse, the pinceau, surrounding the initial segment of the Purkinje cell axon. Once this fundamental difference is noted, other differences between the two cell types become evident. In view of these morphological differences, it may be predicted that the functional role of these two cell types in the cerebellar cortex may be quite different.

The stellate cells, like the Golgi cells and the basket cells, have been implicated in the inhibitory control of the Purkinje cell. Inhibition by way of stellate cells has been documented in mammals (Anderson *et al.*, 1964; Eccles *et al.*, 1966a, b, c, 1967), reptiles (Llinás and Nicholson, 1969; Kitai *et al.*, 1969), amphibia (Rushmer and Woodward, 1971), cartilaginous fishes (Nicholson *et al.*, 1969; Eccles *et al.*, 1970). Especially interesting is the fact that stellate cells occur in the cerebella of all classes of animals that have been examined, whereas basket cells appear only in birds and mammals.

Stellate cells have not failed to attract the attention of electron microscopists. Several investigations in to cerebellar fine structure have shown various aspects of stellate cells in different animals, for example, mouse (Lemkey-Johnston and Larramendi, 1968a, b), frog (Sotelo, 1969, 1970), and monkey (Rakic, 1972). The present study provides an account of the morphology of the various types of stellate cells in the cerebellar cortex of the rat, and compares them with basket cells. The observations in the light microscope are based on Golgi preparations, and these are correlated with observations made on stellate cells in electron micrographs. The synaptic connections of both stellate cells and basket cells are described, and their functional significances are discussed in the light of recent physiological studies.

Materials and Methods

The following observations on the stellate cells of the rat cerebellar cortex were gathered from a large collection of rapid Golgi and Golgi-Kopsch preparations made from the brains

of rats ranging in weight between 180 and 325 g. Many of the animals had been perfused with aldehydes prior to immersion in Golgi solutions (Chan-Palay, 1971). Others were fixed directly by immersion or were perfused with Golgi solutions. The electron microscopic observations were made from an extensive collection of electron micrographs and montages of specific areas in the rat's cerebellar cortex. The regions examined include lobules IV, V, IX, and X in the vermis, lobule IX in the ventral paraflocculus, and lobule X in the flocculus, lobule VII in crus I and crus II, lobule VIII in the copula pyramidis and in the simple lobule (Larsell, 1952). The procedures involved in the preparation of these materials have been described in previous papers (Chan-Palay and Palay, 1970; Chan-Palay, 1971).

Results and Discussion

Light Microscopy

We shall begin this description of the stellate cells in Golgi preparations with the cells that reside in the outer third of the molecular layer near the pial surface of the folium. Small stellate cells are the only neuronal cell bodies present there; in contrast, larger stellate cells tend to lie in the middle and lower thirds of the molecular layer, where they are mingled with basket cells.

a) Superficial Short Axon Stellate Cells. The superficial stellate cells have small, fusiform somata about 5–10 μm in diameter. The appearance of their dendrites in Golgi preparations is typical of all stellate cells. They are irregular in caliber and very contorted, with many abrupt changes in direction, as if twisting and hooking around invisible obstacles in their course. The dendrites are relatively short and branch profusely, ending with twigs that run horizontally under the pial surface (Fig. 1). They are fitted with a few appendages in a variety of forms: lumpy, warty excrescences, short spicules, or tiny, round bulbs tethered to the dendrite by thin strands. The dendritic arrangements of stellate cells near the pial surface fall into two patterns. First, major dendrites can originate from opposite sides of the cell body, giving the cell a somewhat bipolar appearance. These dendrites extend either horizontally, that is, parallel with the pial surface (Fig. 2, *A*), or vertically (Fig. 3, *A*, *B*). Secondly, a cell can have dendrites that radiate from the cell body, ascend toward the pial surface, and then cascade downwards, often to reach below the level of the cell body (Fig. 3, *C*).

The axon of the stellate cell originates from the cell body or, less often, from a major dendrite, by way of a barely perceptible axon hillock. The initial segment is usually thin, straight, and smooth for about 10 μm, but it soon gives way to a meandering, branching axon. The axon bears thin, crooked collaterals, each

Fig. 1. A superficial stellate cell in a parasagittal section of rat cerebellar cortex. This small stellate cell lies in the upper third of the molecular layer near the pial surface. The many contorted branches of its dendritic tree arise from three main dendrites which radiate from the cell body. The dendritic surfaces display a few short spicules or tiny round bulbs on thin threads. The axon (*ax*) begins as a thin, smooth initial segment which soon meanders from its original course, breaking up into many fine, beaded collaterals. The entire plexus arborizes within the field of the dendrites. Rapid Golgi preparation. Camera lucida drawing. Section 90 μm thick. 100× oil immersion objective

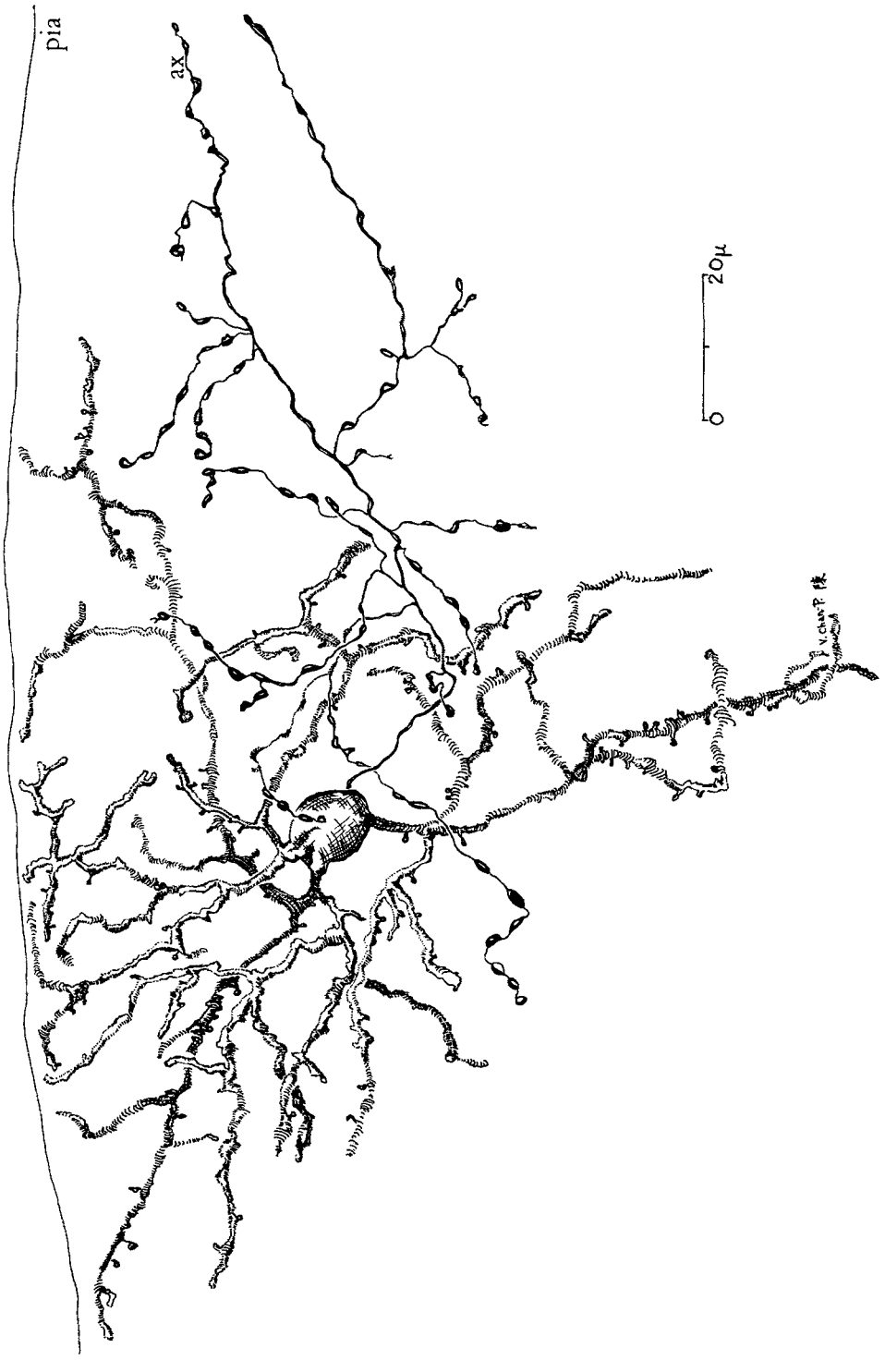


Fig. 1

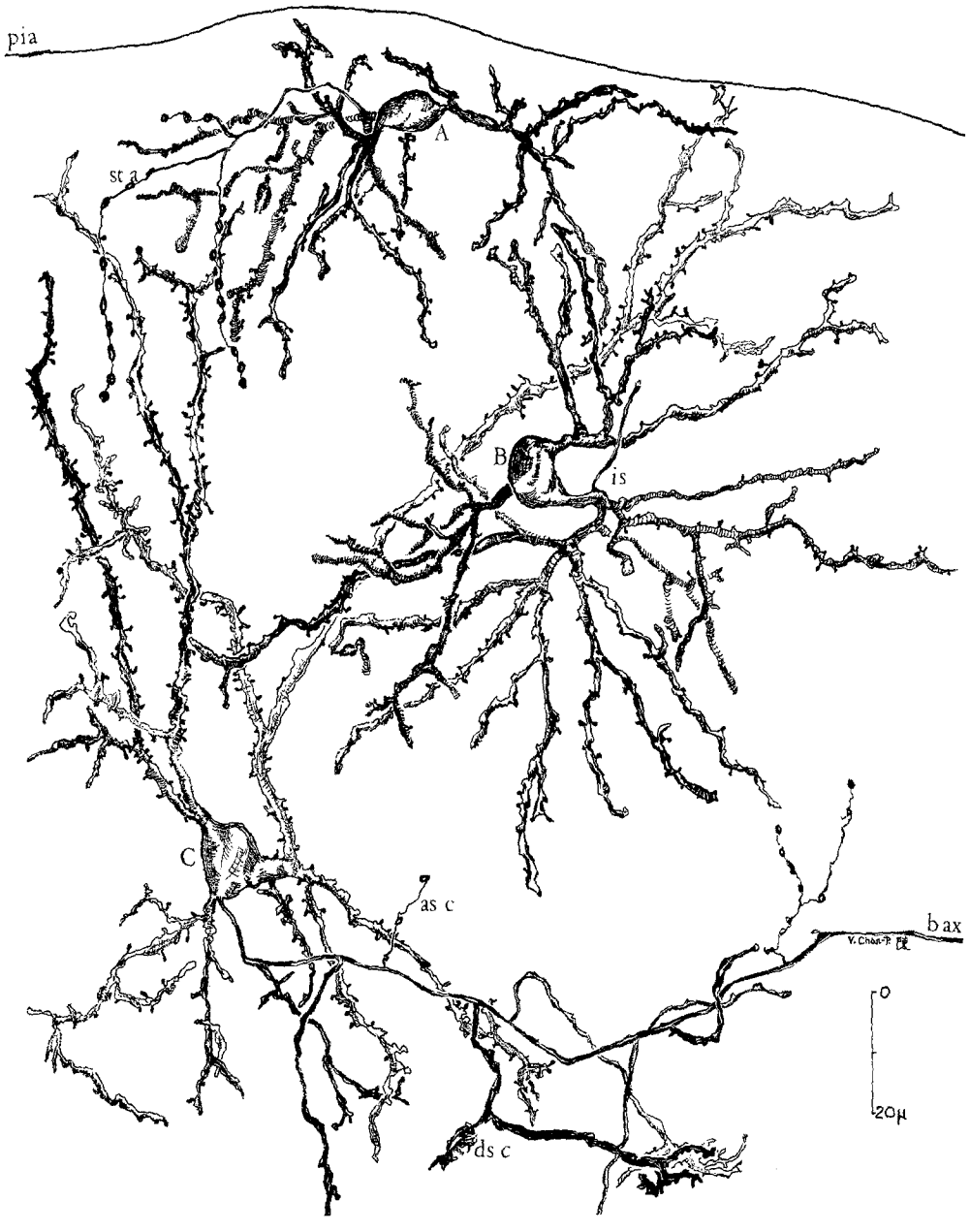


Fig. 2. A comparison of the dendritic arrangements of a superficial and a deep stellate cell with that of a basket cell in a parasagittal section of the molecular layer. The superficial stellate cell (*A*), lying just underneath the pial surface, gives rise to short, crooked dendrites that issue from either pole of the cell body and run horizontally for a limited distance. The axon ramifies simply into three beaded collaterals (*st a*). The deep stellate cell (*B*) lies in the middle third of the molecular layer, and its relatively long, contorted dendrites radiate from the cell body, bearing a few spines. Except for the initial segment (*is*), the axon has not been illustrated here. The basket cell (*C*) lies in the lower third of the molecular layer, and its long,

arising from it at approximately right angles or somewhat less. The collaterals are short, tenuous, varicose threads resembling strands of loosely strung, imperfect pearls (Figs. 1–5). The axon of the superficial stellate cell is usually fairly short, and is confined to the same restricted fields as its dendrites (Figs. 1–2). The axon can loop back and forth through a depth of about 30–40 μm in this field (Fig. 3, *A*), giving off varicose collaterals at irregular intervals. The axons and dendrites of most superficial stellate cells both generate fairly simple arborizations, as is illustrated in Figs. 2*A* and 3*A*, *B*, *C*. However, some stellate cells in the outer third of the molecular layer have been encountered with complicated, highly branched dendrites and more extensive axonal arborizations (Fig. 1). These cells are not considered to be another class of stellate cells, but simply a more complicated version in the spectrum of stellate cell morphology.

All stellate cells, like the other interneurons of the molecular layer, the basket cells, have their dendrites and axons spread out only in the plane that lies transverse to the axis of the folium (Fig. 4). When viewed in Golgi preparations sectioned parallel with the axis of the folium, the profiles of these cells are about 40 μm wide.

b) Long Axon Stellate Cells. As one descends deeper into the middle third of the molecular layer, stellate cells with very long axons are encountered. These cells (Figs. 2, *C* and 3,*D*, 4) are distinct from the superficial short axon stellate cells, and constitute the second type, or the deep long axon stellate cells. The dendrites in Golgi preparations are, as in the superficial cells, contorted, with a few spiny appendages. The major dendrites emerge from the cell body and give rise to a large number of branches, which radiate outwards into the molecular layer, dividing frequently. The axon can extend for lengths of up to 450 μm (Fig. 3, *D*), always running in the parasagittal plane, transverse to the axis of the folium. A typical example of such a deep stellate cell is seen in Fig. 5. In the first third of its traverse after the initial segment the axon gives rise to a number of ascending and descending, tenuous, and varicose collaterals. The ascending collaterals sometimes branch again to produce a simple plexus. The descending collaterals always branch repeatedly, giving the resulting arborization the appearance of a thin, rather short beard. The main stem of the axon emerges from the cell body and continues along at approximately the same level in the molecular layer as it occupied at its beginning. The number of collaterals emitted from the remaining two thirds of the axonal stem decreases as the last varicosity that signals the tip of the axon is approached.

relatively straight dendrites extend across the entire layer to reach up towards the pial surface. The dendrites of this cell are particularly thorny, and they branch at their tips to run for short distances sometimes just under the pia. The smooth, thick axon (*b ax*) gives rise to many thick descending collaterals (*ds c*) that form the pericellular basket and pinceau. A few thin, beaded ascending collaterals (*as c*) are also illustrated. Rapid Golgi preparation. Camera lucida drawing. Section 120 μm thick. 100× oil immersion objective

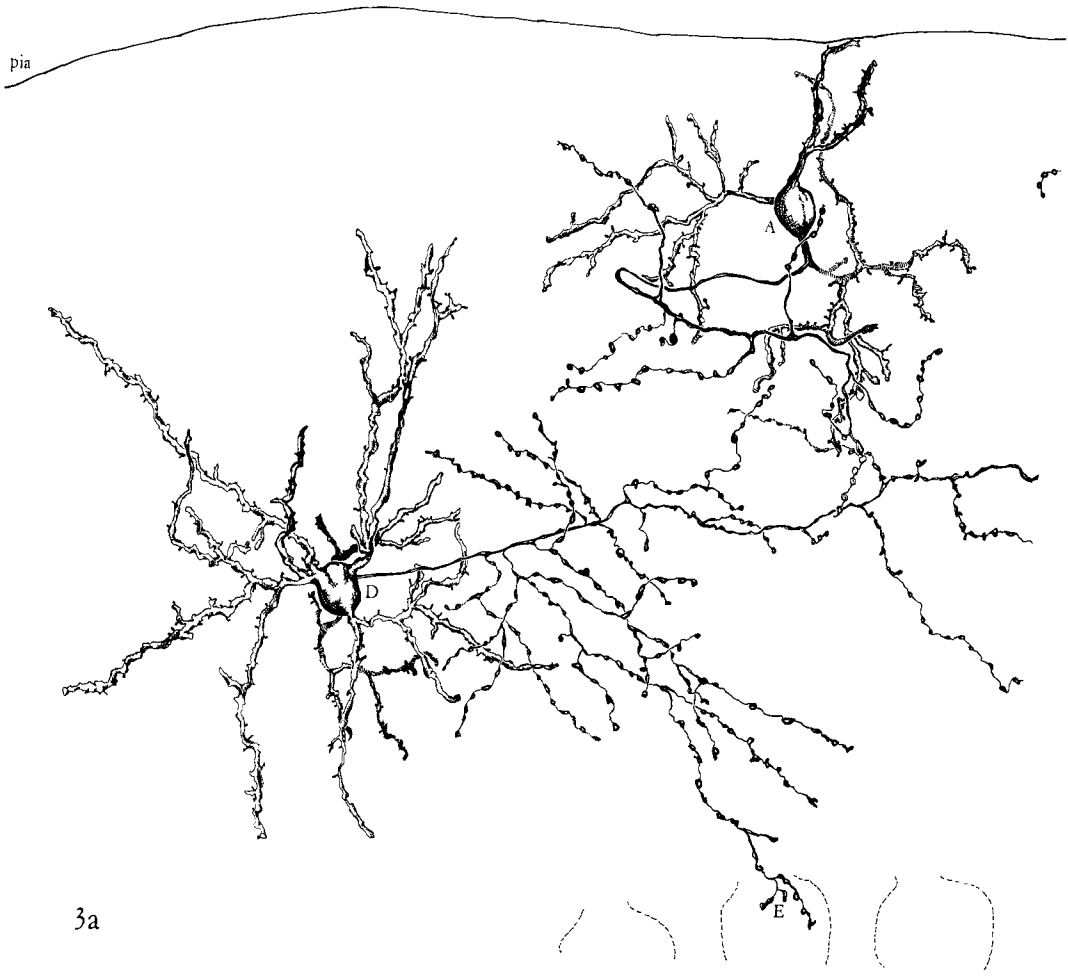
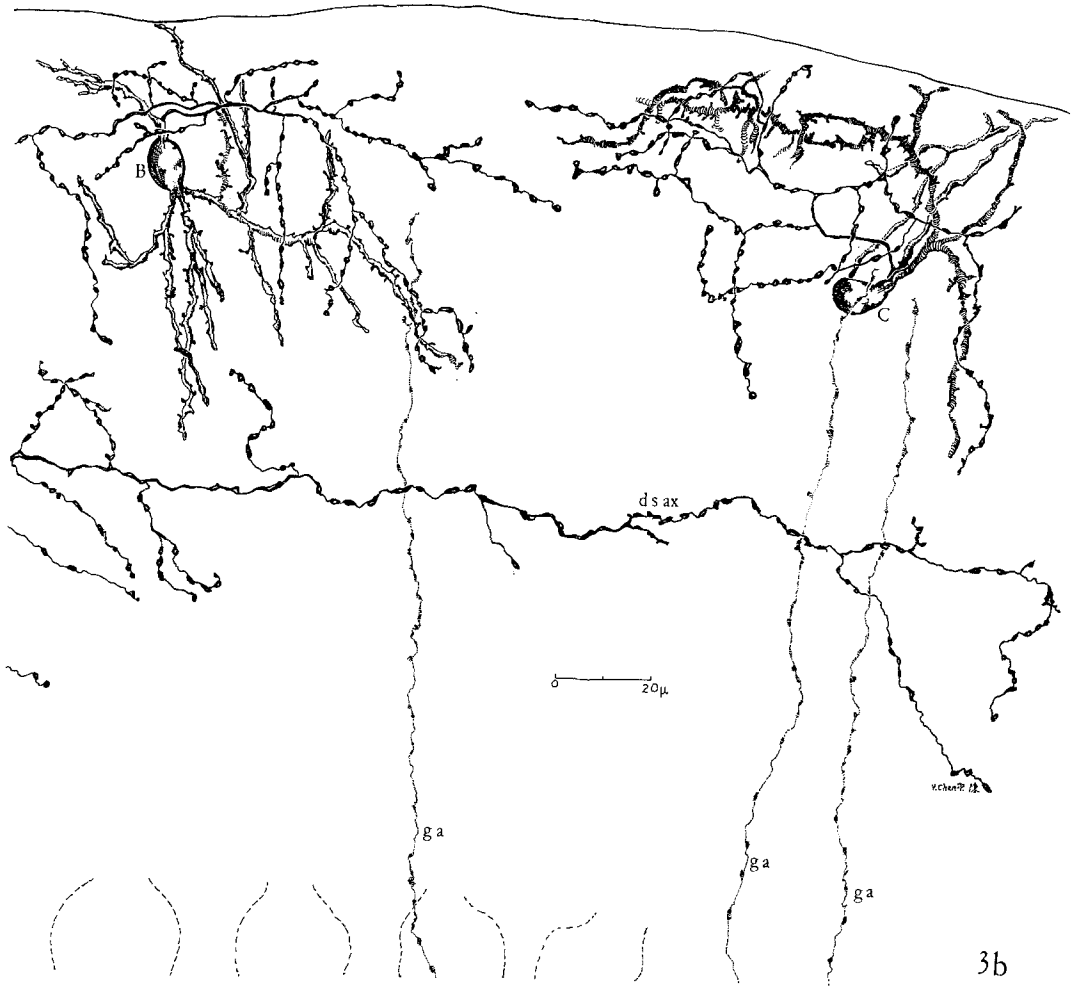


Fig. 3a and b. Three superficial stellate cells and a deep long axon stellate cell in the molecular layer. These three examples of short axon superficial stellate cells (*A*, *B*, *C*) are simpler than that illustrated in Fig. 1. Cell *C* has dendrites that issue from the cell body and ascend nearly to the pial surface, then cascade downwards sometimes to reach below the level of the cell body. The axonal arborization is distributed in a tangle of beaded threads around these dendrites. Ascending axons of granule cells (*ga*) come into relation with the dendrites and cell body of stellate cells *B* and *C*, respectively. The long axon stellate cell (*D*) has a typical axonal plexus. The initial part of the axon gives rise to a thin bush of beaded collaterals, some ascending, but most descending. One of the descending collaterals (*E*) reaches down to branch in the region of the pericellular basket of a Purkinje cell soma. The remainder of this axon (*d s ax*) continues across the folium for about 450 μm, giving off occasional collaterals in its traverse. Camera lucida drawing, sagittal section. Section 120 μm thick. 100× oil immersion objective

In rare instances a branch from one of the descending collaterals issuing from the axon of the deep stellate cell continues as a long, thin, varicose thread down to the level of the cell body of a Purkinje cell (Fig. 3, *E*), where it divides into



three or four shorter branches. It is not clear from Golgi preparations whether these stellate axon collaterals actually come into synaptic contact with the Purkinje cell body or only contribute to the pericellular basket around it and its initial segment.

c) Basket Cell. It is pertinent at this stage to describe the typical basket cells in order to point out the similarities and differences between them and the long axon deep stellate cells. Fig. 2 illustrates the axonal and dendritic patterns of a superficial stellate cell, the dendrites of a deep stellate cell, and the dendritic and axonal arrangements of a basket cell.

Basket cells lie almost exclusively in the lower third of the molecular layer at the level of major Purkinje cell dendrites and their branches. The squat cell body (about 10 μm across) throws out long dendrites and an axon of extraordinary complexity. Unlike the dendrites of stellate cells, those of basket cells spread out in a vertical direction from the points and upper sides of the cell body

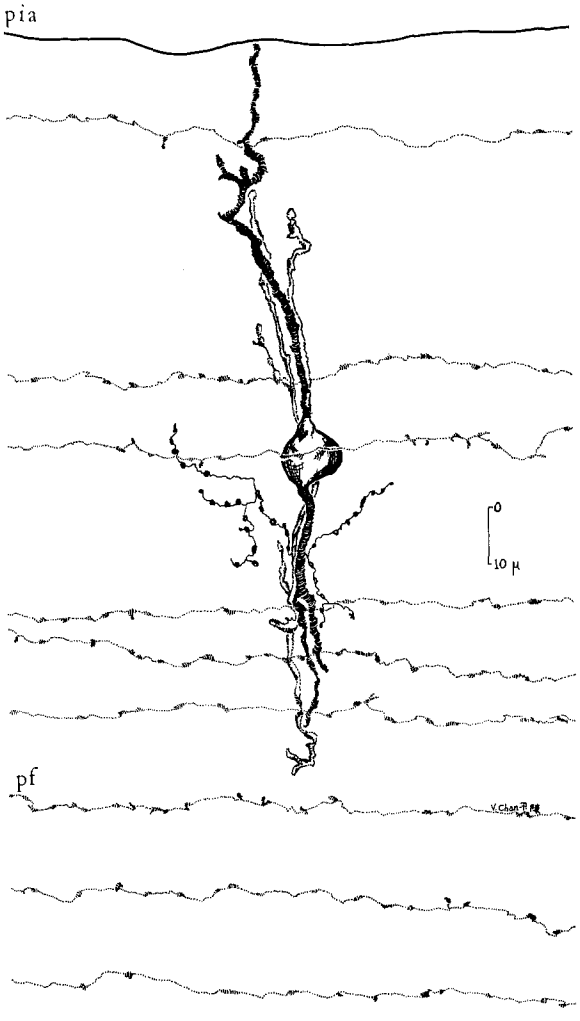


Fig. 4. Stellate cell in a section parallel to the longitudinal axis of the folium. The axons and dendrites of this stellate cell project a narrow profile when viewed in this plane of section. Parallel fibers (*pf*) running along the axis of the folium come into relation with the dendrites and soma of this cell. Camera lucida drawing. Section 90 μm thick. 100× oil immersion objective

Fig. 5. Deep long axon stellate cell. This cell lies in the molecular layer midway between the pial surface and the Purkinje cell layer. It is a typical deep stellate cell, as its axon (*ax*), which traverses about 320 μm across the folium, gives off numerous descending and a few ascending beaded collaterals in its initial portion. Farther out along the axon, the number of collaterals is small. Parasagittal section. 90 μm thick. Camera lucida drawing. 100× oil immersion objective

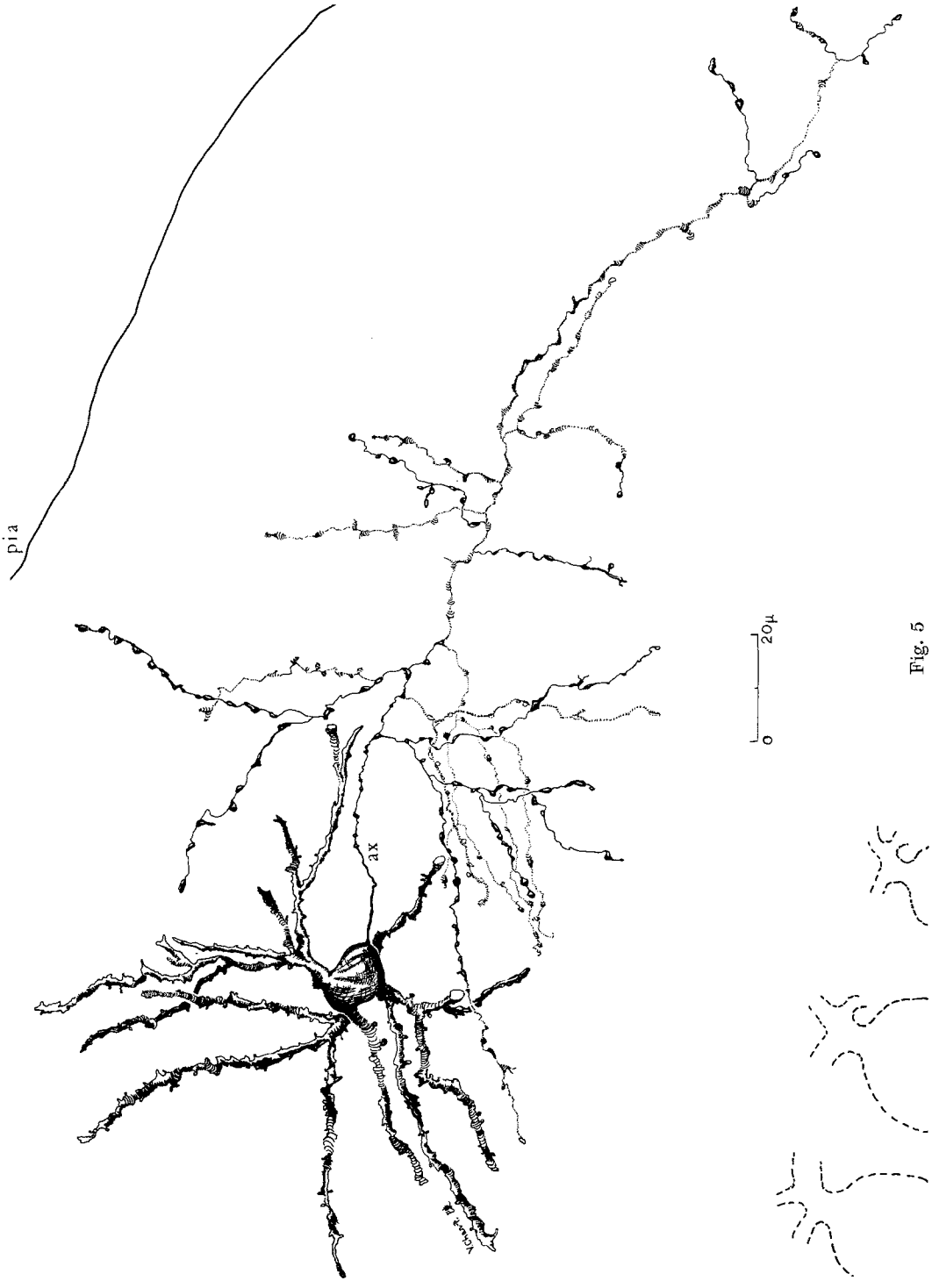


Fig. 5

and thus produce a fan-shaped field in the parasagittal plane. Many of them extend horizontally for some distance above the Purkinje cells before turning upwards. Compared to the very contorted dendrites of stellate cells, the basket cell dendrites are relatively straight, with some irregularities in diameter and less frequent abrupt changes in direction along their lengths. The dendrites are long, often reach the pial surface, and thereupon branch at their tips to run horizontally before recurving downwards for a short distance. The dendrites that project towards the granular layer are usually shorter and more curved. Dendrites of basket cells can have filopodia or stiff, short spines, but the number of these appendages varies greatly from cell to cell and in different Golgi preparations. Usually in the same preparation, however, the dendrites of stellate cells have fewer spines than those of basket cells.

The axon of a basket cell, like that of the deep stellate cell, quickly assumes a horizontal course in a plane transverse to the longitudinal axis of the folium. The initial segment of the axon is extremely slender and smooth in contrast to the rest of it. Generally it is fairly straight, but it sometimes arcs and loops apparently around other cells or large dendrites before it arrives at its definitive level. At the site of its first bifurcation, the axon abruptly doubles or even triples in caliber. From this point on, as the main horizontal stem of the axon crooks its way among the lower dendrites of Purkinje cell trees, it emits a succession of descending and ascending collaterals. Each descending collateral is a thick, sinuous process that branches and rebranches as it cascades over the primary dendrites of the Purkinje cell to reach the soma, where it contributes to the pericellular basket. The stout, stubby branches continue beyond the cell body as a thick, branching tangle to form the pinceau around the initial segment of the Purkinje axon. These basket axon branches differ markedly in form from the tenuous, varicose threads of the deep stellate cell axon that contribute to the pericellular basket.

Just as there is no difficulty in identifying the small neurons in the upper third of the molecular layer as stellate cells, usually there should be no difficulty in identifying the neurons in the deeper molecular layer as basket cells. However, as Ramón y Cajal (1911, p. 25) remarks, two kinds of basket cells can be distinguished: (a) one with an axon which sends all of its descending collaterals into baskets, even its terminal arborization, and (b) the other with an axon which gives off descending basket collaterals in the beginning of its course and which during the rest of the course gives off only thin ascending collaterals to the molecular layer. Our own preparations confirm these observations.

Thus, in Golgi preparations the neurons of the molecular layer can be classified as either stellate cells or basket cells on the basis of their position in the molecular layer, their dendritic arrangements, and, most conclusively, their axonal arborizations. Stellate cells comprise:

(1) Superficial cells with short, circumscribed, contorted dendrites and limited axonal fields (upper third of the molecular layer);

(2) Deep stellate cells with radiating, contorted dendrites and long axons with thin, varicose collaterals (middle third of the molecular layer);

(3) Deep stellate cells with radiating, contorted dendrites and long axons with varicose collaterals that contribute to the pericellular basket (middle third of the molecular layer).

Basket cells, in contrast, have long, straighter, more extensive dendrites and comprise (a) cells with many thick descending collaterals (lower third of the molecular layer) and (b) cells with few descending collaterals and many ascending collaterals (lower two thirds of the molecular layer). These categories, however, are not intended to be absolute, as morphological variants within any spectrum can be found.

In a recent paper on the development of neurons in the molecular layer of the monkey's cerebellar cortex, Rakic (1972) observes that the small neurons of this layer dispay different dendritic patterns according to their position in the grid of parallel fibers. Our observations in the rat's cerebellar cortex generally agree with those reported by Rakic, so far as the dendritic patterns are concerned. But since he did not consider the axonal arborizations of these cells, he could not differentiate between stellate and basket cells. Our own studies indicate that since the axonal trees generated by these two cells are markedly different, they cannot be assimilated into a single cell type.

In the middle third of the molecular layer there are long axon stellate cells with collaterals that enter the pericellular basket (type 3) and basket cells with few descending and many ascending collaterals (type b), and there may be difficulty in distinguishing between them on the basis of theirs overall axonal pattern alone. However, the differences between the dendritic patterns of stellate and basket cells, described above, and the fact that the descending collaterals of stellate cells are usually tenuous, varicose threads rather than thick branches may aid in making this distinction. We believe, therefore, that basket and stellate cells do not merge into one cell type, but are distinct. This belief is strengthened by the fact that in electron micrographs two different kinds of axonal profile are found. One of these can be ascribed to basket cells, the other to stellate cells (see below).

Supportive evidence for the distinction between these two classes of cells is found in a recent paper on the quantitative analysis of the cerebellar molecular layer in cats (Palkovits *et al.*, 1971). By comparing the ratio of the lengths and breadths of cell somata with their volume, these authors sow that neurons of the molecular layer separate into two classes. The stellate cells are distinctly smaller with a greater length to breadth ratio, and basket cells tend to be larger and rounder. By these criteria, numerous stellate cells and few basket cells were recorded in the upper half of the molecular layer, whereas many more basket cells and fewer stellate cells were found in the lower half of the layer.

Electron Microscopy

Because of the large variety of basket and stellate cells in the deeper part of the molecular layer, it is best to begin a description of the fine structure of the

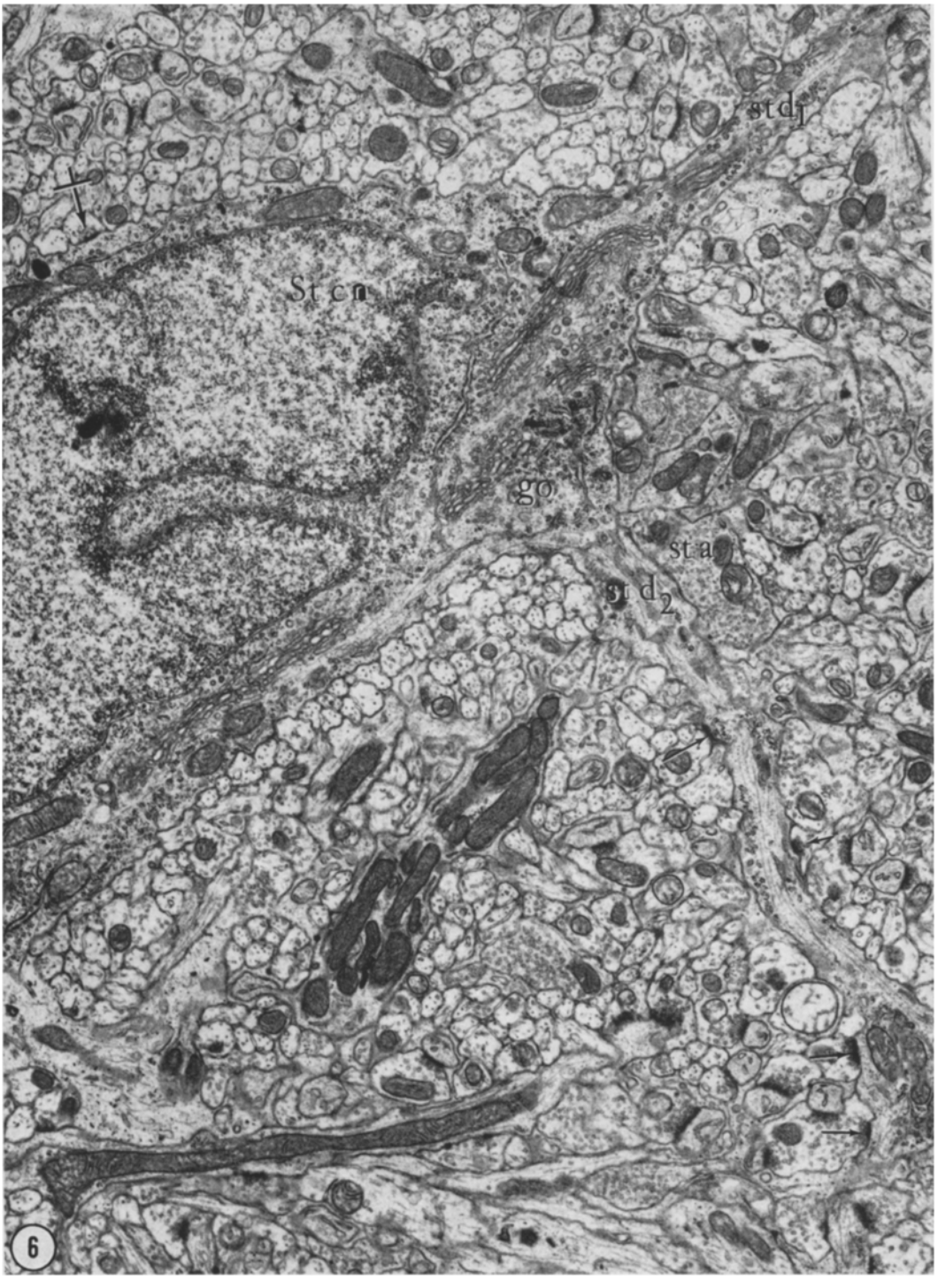


Fig. 6

stellate cells with the superficial group. These cells can be securely identified as stellate cells since they are the only neurons in the outer third of the molecular layer of the adult cerebellar cortex. The following account has been made from electron micrographs of such cells, and in specific cases from those of deeper stellate cells when they could be distinguished from basket cells. Superficial stellate cells generally have small, elliptical perikaryal profiles that are occupied almost entirely by their nuclei. The nuclei are characteristically rounded, with one or several shallow indentations (Fig. 6). The deeper cell bodies can be larger, up to 12 μm in diameter, and they have more voluminous cytoplasm. They tend to be more ellipsoidal than the superficial stellate cells and can even be multipolar. The nuclei of these cells are more likely to have deep and complicated indentations than those of the superficial cells. In all, the chromatin is usually homogeneously dispersed throughout the karyoplasm except for a thin, irregular marginal condensation. In a few cells one or two large masses of heterochromatin occur in this marginal zone or nearer the center. The nucleolus is also frequently marginal in position.

The Cell Body

As in most small neurons, the cytoplasm of the stellate cell is rather scanty and simple. The nuclear envelope often throws out irregular streamers that join with the granular endoplasmic reticulum. This organelle is represented by a few ramifying tubules or cisternae, sometimes very long and stringy, and usually randomly dispersed. Although its outer surface is studded with ribosomes arranged here and there in single and double rows, surprisingly long stretches of the surface are free of any granules. In the cytoplasmic matrix, ribosomes are arranged in rosettes of four to six members, loosely scattered about not only in the vicinity of the granular endoplasmic reticulum but also in the membrane-free zones and in the nuclear indentations. It should be noted that ribosomes are frequently attached to the outer wall of the nuclear envelope. The agranular reticulum is confined to a few vagrant tubules and occasional subsurface cisternae.

The Golgi apparatus, consisting of stacked agranular, fenestrated cisternae and associated vesicles, presents a highly variable configuration from one cell to another. In some stellate cells it is fragmented into small aggregates of cisternae together with clustered vesicles. These are dispersed over the perikaryon and

Fig. 6. The dendrites and soma of a superficial stellate cell in the neuropil of the outer molecular layer. This cell has an elliptical perikaryon almost entirely occupied by an indented nucleus. The nucleus (*St c n*) contains a nucleolus and homogeneously dispersed chromatin with a thin marginal condensation. Two dendrites (*st* d_1 and *st* d_2) emerge from a common cone of perikaryon in which a prominent Golgi apparatus (*Go*) is present, and pursue crooked paths out of the plane of this section. Profiles of numerous parallel fibers abut against the perikaryal surface, but only one makes a synapse with it ($-/\rightarrow$). The lower dendrite (*st* d_2) receives four synapses with parallel fibers ($\rightarrow$) and abuts against the axon of a stellate cell (*st a*). Spiny branchlets belonging to Purkinje cell dendrites and many thorns in synapse with parallel fibers fill the rest of the field in this illustration. Parasagittal section. $\times 8000$

appear in the roots of the major dendrites, apparently as disconnected bodies. In other stellate cells, the Golgi apparatus is organized into a coherent shell of overlapping cisternae entraining swarms of vesicles of different sizes. This shell is applied like a cap to the nucleus opposite the origin of one of the dendrites, and it can extend out into the dendrite for five or ten microns. Sometimes, in fortunate sections, a single centriole or a pair is encontered in the vicinity of the Golgi apparatus. A few lysosomes and multivesicular bodies are strewn about the cytoplasm, but are generally inconspicuous.

In low magnification micrographs (Fig. 6) the most prominent organelles in the cytoplasm of stellate cells are the mitochondria. Although these structures are sparse, their size and construction makes them stand out in the meager perikaryon. They tend to be small rods or globules, ranging from 0.2 to 0.4 μm in diameter and 0.8 to 1.6 μm in length. Occasionally they can be up to 9 μm long, forming a branching, curved rodlet lying parallel to the nuclear envelope, looking as if a number of small mitochondria had coalesced. The mitochondrial matrix is dense and the cristae are highly pleomorphic, varying from the usual transverse plates in small mitochondria to undulating oblique, longitudinal, or even tubular cristae in the larger examples.

Microtubules and neurofilaments are also scarce, often hidden from view in low power micrographs by the crowding of polysomes and other organelles into the thin rim of perinuclear cytoplasm. Microtubules appear singly, running around the nucleus and assembling into groups at the bases of the dendrites or the axon.

The Dendrites

The larger dendrites begin as conical extensions of the perikaryon with all of the cytoplasmic organelles funneling through the narrow orifice and aligning themselves parallel to the longitudinal axis of the dendrite. The Golgi apparatus (Fig. 6) is frequently a major component of the proximal segment of the dendrite, nearly filling it with a longitudinal stream of fenestrated cisternae, tubules, and vesicles. Mitochondria are aligned along the edges of this stream, and it is surrounded by a sleeve of matrix in which microtubules run longitudinally and tags of the rough endoplasmic reticulum or groups of polysomes appear. Farther out, the cytoplasm of the dendrite clears as the Golgi apparatus drops out. Now the most conspicuous components are the mitochondria. sinuous, long, and surrounded by a leash of parallel microtubules and occasional neurofilaments. The agranular endoplasmic reticulum appears as sparse tubules in the thicker parts of the dendrite, and polysomes are infrequent. The cytoplasm of the smaller dendrites has this same composition from their origins onwards.

Because of the irregular caliber and crooked, ramifying course of the dendrites, they cannot be followed continuously for long distances in the thin sections used for electron microscopy. Stretches 6 to 10 μm long are frequently encountered, running athwart the grid formed by the parallel fibers (Figs. 6 and 9). These correspond to the profiles expected from the Golgi preparations. They have irregular contours (Fig. 9), frequent bifurcations, closely spaced varicosities, and sometimes thin, tapering appendages filled with a fibrous matrix that match the

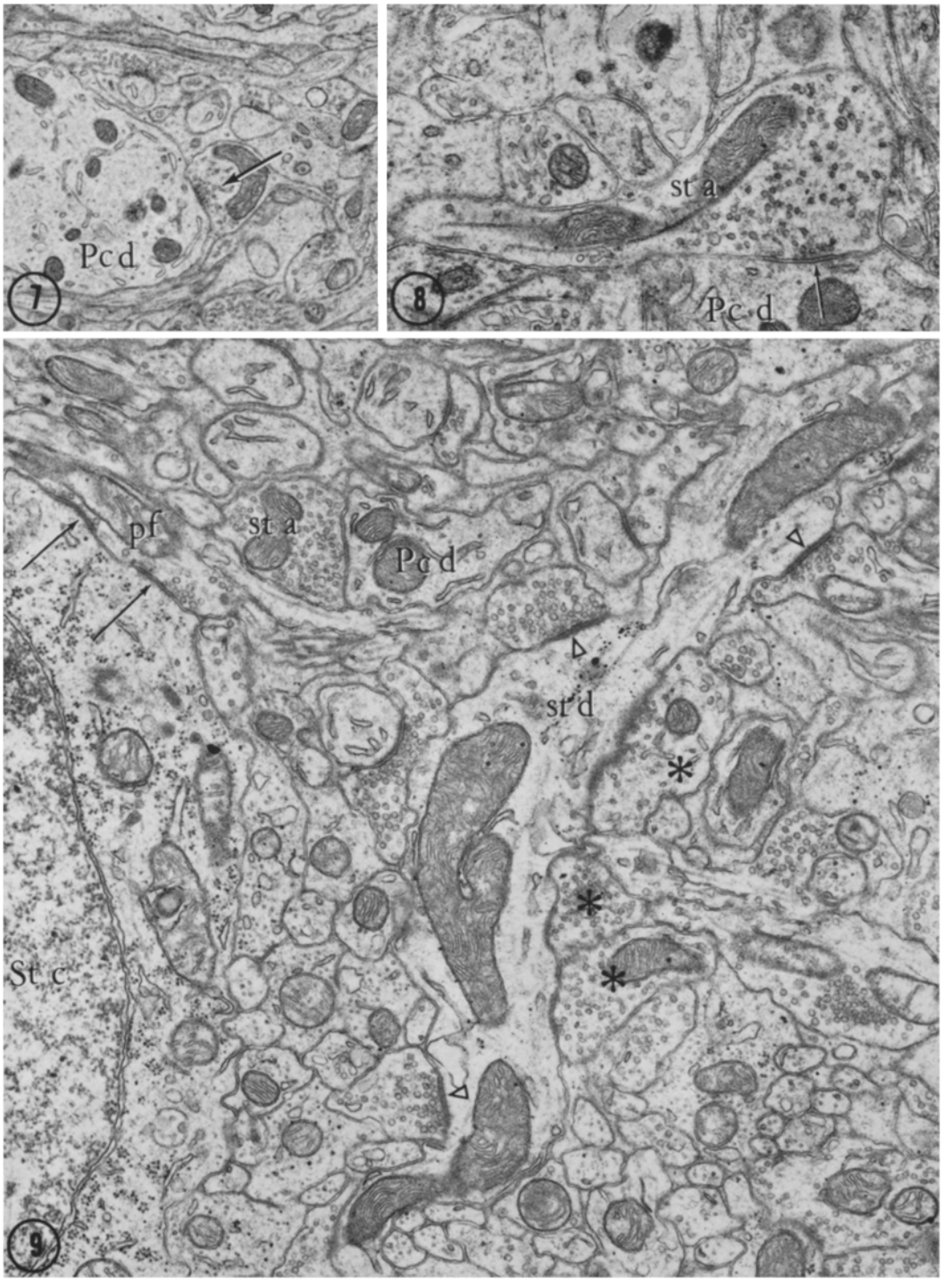
spicules of the Golgi preparations. The stellate cell receives most of its synapses on these more peripheral dendritic branches.

Synaptic Connections of Perikaryon and Dendrites

As neither the cell body nor its processes are ensheathed by neuroglial cells, the dendrites traverse a sea of parallel fibers, which, lying in direct apposition to them, are the principal afferents to the stellate cells. Many of these fibers simply pass by without developing any synaptic specializations; others expand into synaptic varicosities that impinge upon the shaft or the appendages of the dendrite (Fig. 9). Synapses occur singly or in clusters, with lengthy bare intervals in between. The parallel fiber varicosity encloses a loose aggregate of round synaptic vesicles. Only one third to one half of the junctional interface is involved in the synaptic complex. In this zone, the interspace or synaptic cleft is enlarged by a slight doming of the presynaptic membrane, while the postsynaptic membrane remains flat. Dense, filamentous material crossing the synaptic cleft, together with asymmetrical pre- and postsynaptic cytoplasmic densities, make the whole complex dark and conspicuous. The synapse resembles those made by the parallel fiber on the thorns of Purkinje cell dendrites, except that the postsynaptic dense plaque is usually not so thick in the stellate cell dendrite as in the Purkinje cell thorn. Nevertheless, the appearance of the synaptic junctions made by the parallel fiber on these two very different sites is very nearly the same.

Synapses of the parallel fibers on the somata of stellate cells, however, tend to be somewhat different from those on the dendrites. There is a greater variation in the thickness of the postsynaptic density, and the synaptic cleft is not usually widened. Such differences are not consistent from cell to cell in the same section, and are probably not significant. Nearly all of the parallel fiber endings are synapses *en passant*; the fibers synapsing on the cell body can contact neighboring dendrites in the same way. Although the cell body and the proximal segments of the dendrites are surrounded by parallel fibers, very few of them synapse with these portions of the cell. This observation is in agreement with the findings of Lemkey-Johnston and Larramendi (1968b). Profiles of the entire cell body display only from one to five synaptic endings (Figs. 6, 9), although the whole of the cell outline can be in contact with parallel fibers.

There is another type of axon terminal synapsing on the dendrites of the stellate cells that is encountered much less frequently than the parallel fiber ending. This is the ending of axons from other stellate cells and occasionally the endings of ascending collaterals from basket cells. Such contacts were first brought to notice in a paper of the Scheibels (1954) dealing with the climbing fiber, and were further described in a summary of their observations in Jansen and Brodal's review of cerebellar structure (1958). Stellate cell terminals effect *bouton* or *en passant* synapses (Figs. 6, 9) with the shafts of the dendrites and with the cell bodies. In neither site are they numerous. The Scheibels (1954) also described junctions between climbing fibers and stellate cells or stellate cell dendrites. We have seen these junctions a few times, and usually on the cell bodies of deeper stellate cells.



Figs. 7—9

The Axon

The initial segment of the axon originates from a funnel-shaped protuberance on the cell body or one of the dendrites. A few terminals of other stellate cell axons are located upon this small axon hillock. The initial segment, from 0.45 to 0.6 μm across, contains the usual fasciculi of microtubules that stream into it from the cell body, as well as fragments of the rough endoplasmic reticulum, isolated ribosomes, and small lysosomes. The characteristic undercoating of the plasmalemma begins beyond the point where the axon has assumed its definitive caliber. Although our electron micrographs show stellate cell initial segments of considerable length, we have not had the good fortune to follow any of them into the more distal parts of the axon. In all of our examples of initial segments that are continuous with the stellate cell body or one of its dendrites, the initial segments are enclosed in a thin neuroglial sleeve. It is not clear whether this sheath is a general characteristic of all stellate cell initial segments or is merely a fortuitous finding, because in each case a spiny branchlet of the Purkinje cell crosses the trajectory of the initial segment. The sheath about the axon may be only an offset from that around the Purkinje cell dendrite. The observation is mentioned for two reasons. First, the remainder of the axonal arborization is not ensheathed in neuroglia. Second, the initial segments of basket cell axons and Purkinje cell axons are similarly ensheathed by neuroglia.

The collaterals of the stellate cell axon can be identified in electron micrographs on the basis of their similarity to the axons seen in the Golgi preparations. The impregnated axon is a thin, beaded thread with few branches but pursuing a tortuous, highly irregular course across the path of the parallel fibers. In the upper molecular layer parallel fibers and stellate cell axons are the only axonal units present, and in electron micrographs of the outer third of the molecular layer numerous axonal profiles are encountered that answer to the description of the stellate cell axon (Figs. 7, 8, 10). These profiles are of narrow fibers, about

Fig. 7. A varicosity (→) of a stellate cell axon synapses on the shaft of a Purkinje cell dendrite (*P cd*). The profile of the axon of a stellate cell shows a bulbous termination and a thin connecting thread. The varicosity contains elliptical synaptic vesicles aggregated against the presynaptic surface. Frontal section. ×15000

Fig. 8. Stellate cell axon terminal synapsing with a Purkinje cell dendrite. The thin connecting portion contains a couple of microtubules and a long, slender mitochondrion which continues into the varicosity (*st a*). The varicosity contains flattened and elliptical synaptic vesicles and a dense cored vesicle, suspended in a light matrix. The synaptic complex (→) occupies only about a third of the junctional interface. ×22000

Fig. 9. Synapses of parallel fibers and stellate axons on a stellate cell soma and a stellate cell dendrite. The stellate cell (*st c*) body at the left receives two synapses (→) from a parallel fiber (*pf*) that sweeps by. A stellate cell dendrite (*st d*) wends its crooked way diagonally across the field, and upon its surface there are four synapses with parallel fibers (▷) and three with stellate axons (*). At the top of the figure midway between the stellate dendrite and the cell body profile, another stellate axon terminal (*st a*) synapses upon the shaft of a Purkinje cell dendrite. ×22000

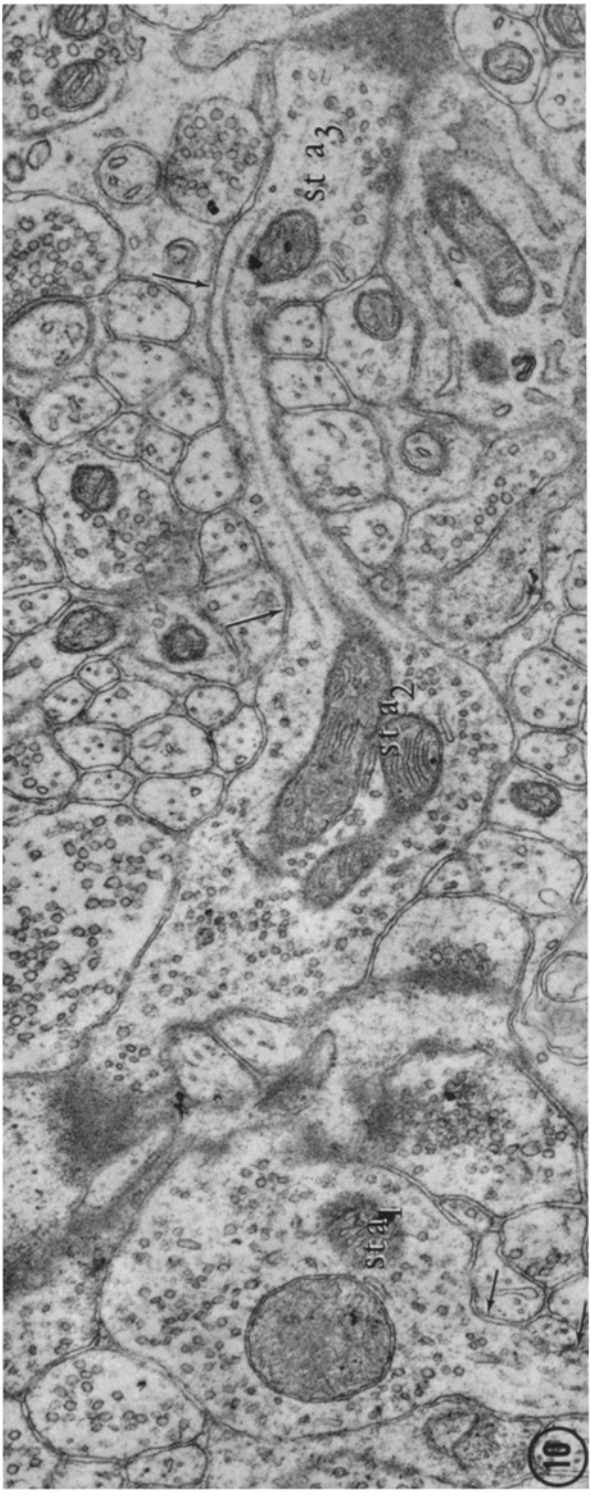


Fig. 10. The varicose stellate cell axon. The three varicosities of this stellate cell axon (*st* a_1, *st* a_2, *st* a_3) contain mitochondria and flattened and elliptical synaptic vesicles, suspended in a light matrix. The tenuous connecting portions between varicosities (*arrows*) contain a microtubule or two and an occasional synaptic vesicle in the same light, flocculent background. ×29000

0.1 μm in diameter, that distend at closely spaced intervals into wide bays, 1.5 to 2.0 μm across. The fibers twist and turn, bending into a different direction with each expansion. Consequently, they cannot be followed in thin sections for more than a few microns; however, as they twist in and out of the plane of the section their trail can often be picked out in a micrograph as a disconnected chain of tailed circular or elliptical profiles. The fine, thread-like connecting links are, of course, often missed by the thin sections. They contain one or two microtubules, and occasionally a long mitochondrion, which run from one varicosity to the next. The varicosities usually include loose aggregates of small, slightly flattened or ovoid synaptic vesicles along with one or two elongated or branched mitochondria.

Synaptic Connections

The varicosities make synaptic contact with the shafts of dendrities belonging to Purkinje cells (Figs. 7, 8), and end, very rarely, upon a thorn of a Purkinje cell dendrite. They also contact the somata and dendrites of stellate cells (Figs. 6, 9) and the dendrites of Golgi cells and basket cells. In all cases the structure of the contact is the same, with the synaptic complex occupying about a third of the junctional interface. The presynaptic ending contains a light, flocculent matrix in which mostly flattened and some elliptical synaptic vesicles are suspended. A few of the vesicles cluster near the synaptic interface. The synaptic cleft is wider than the usual interstitial space and is traversed by fine filaments. On the cytoplasmic sides of the apposed surface membranes a thin, delicate fringe is attached more or less symmetrically. The junction is intermediate between Gray's two types (Gray, 1959). The endings of basket cell axons on the same kinds of postsynaptic structures can be differentiated from those of stellate cell axons, because the former contain a set of neurofilaments and a scattering of elliptical vesicles, which aggregate at the junctional interface.

The Purkinje Cell Pericellcluar Basket

In two instances we have seen electron micrographs of axonal profiles with the characteristics of the stellate axon in the region of the basket surrounding the Purkinje cell. In one, the profile comprises two elongated varicosities connected by a thin thread containing one or two microtubules, a couple of neurofilaments, and a large mitochondrion that continues into both varicosities. There are flattened synaptic vesicles in the enlargements, and the junctional interface of one of them contains three short synaptic contacts with the soma of the Purkinje cell against which it abuts. The synaptic junctions themselves are similar to those described above. These axons may represent stellate cell contributions to the pericellular basket like that illustrated in Fig. 3, *D*, *E*. We have not yet observed any axonal profile that fits the description of stellate axons in the pinceau or in the surrounding infraganglionic neuropil.

The connections of stellate cells that we have observed in electron micrographs are summarized in Fig. 11. The illustration was constructed by superimposing camera lucida drawings of Purkinje cells, stellate cells with either short

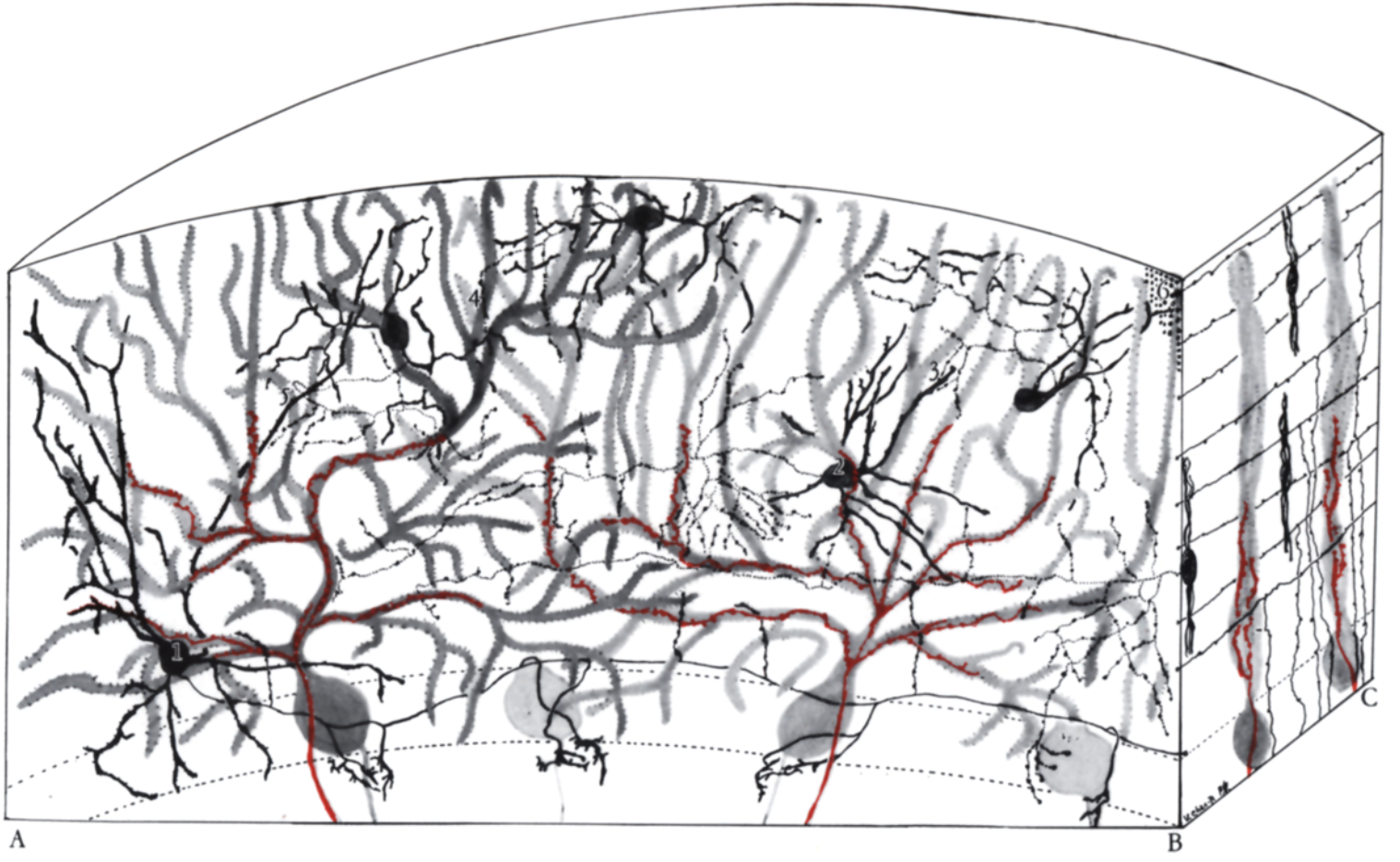


Fig. 11

or long axons, basket cells, climbing fibers, and parallel fibers within the molecular layer in planes parallel to the long axis of the folium and transverse to it. The synaptic relationships between these various neuronal elements are indicated (numbers 1–6), but the dendrites of Golgi cells in the molecular layer have been omitted in order to simplify the illustration.

Physiological Considerations

Upon activation, following parallel fiber stimulation, the axonal plexuses of the basket cell inhibit Purkinje cell activity. This inhibition is effected by the descending collaterals that form the pericellular baskets and pinceaux around several successive Purkinje cells (Anderson *et al.*, 1964; Eccles *et al.*, 1966a, b, c, 1967). In addition, ascending collaterals run on the Purkinje cell dendrites, to drape and entwine over major branch points in company with the climbing fiber, thus providing the inhibition necessary for regulating and modulating local portions of the dendritic tree (Chan-Palay and Palay, 1970). This inhibition serves to counteract climbing fiber and parallel fiber excitation of the Purkinje cell. Owing to the disposition and length of the basket axon in the parasagittal plane, these effects of both descending and ascending collaterals are carried over long distances across the folium and to a certain extent laterally, affecting several successive Purkinje cell trees. The parallel fiber input to the basket and Purkinje cell dendrites, however, travels along the length of the folium at right angles to the path of the basket axon.

Like basket cells, stellate cells have also been implicated in the inhibitory circuits of the cerebellar cortex. On superficial consideration, basket cells and deep stellate cells, with their long axons running in the parasagittal plane, appear to be quite similar and might be thought of as simple variations of a single cell type. More serious study, however, indicates that these two cells incorporate different principles of form and that their roles in the circuitry and physiology

Fig. 11. Diagram showing the interrelations between Purkinje cell dendritic tree, climbing fiber, basket cell, and superficial and deep stellate cells. Two Purkinje cells (gray), two climbing fibers (red), a basket cell black with black axon), three superficial stellate cells (black somata with dotted axons), and one deep stellate cell (black, with dotted axon) are shown in the parasagittal plane (*A–B*). Two Purkinje cells (gray), two climbing fibers (red), a series of parallel fibers (black), and three stellate cells (black), one with a long axon (dotted line), are seen on edge in a view parallel with the axis of the folium (*B–C*). The interrelations of the processes of these cell types have been observed in electron micrographs and are reconstructed in this figure to provide the equivalent arrangement at the light microscope level. The terminal arborizations of the climbing fibers contact Purkinje cell dendrites and also the primary dendrites and somata of basket cells (*1*) and deep stellate cells (*2*). The axon of the basket cell traverses the folium, giving rise to ascending collaterals and to the pericellular baskets and pinceaux around the Purkinje cell bodies. The axons of both superficial and deep stellate cells synapse with the dendrites of other stellate cells (*3*), Purkinje cells (*4*), and basket cells (*5*). Stellate celis, basket cells, and Purkinje cells also come into synaptic relation with the numerous parallel fibers that course through this field (*6* and in plane *B*, *C*)

of the cerebellar cortex should be quite different. Both cells are spread out in the parasagittal plane, and they present an extremely narrow profile to the stream of parallel fibers impinging on them in the longitudinal axis of the folium. Both cells have axons that course in the parasagittal plane and synapse on Purkinje cells. These similarities should not obscure the great differences between them. Basket cells have long, radiating dendrites that spread out in the molecular layer, receiving inputs from all levels and over a wide, fan-like expanse. Stellate cell dendrites radiate within a more circumscribed field, close to the cell body. Their contorted dendrites receive inputs from a comparatively restricted stream of parallel fibers. Basket cell axons project over a long distance, usually skipping the one or two Purkinje cells in the vicinity of their perikarya. In contrast, the axon of the stellate cell ramifies profusely close to the cell body, synapsing mainly on the Purkinje cells in the immediate vicinity, and then continues onward with only a few additional twigs. This mode of arborization is epitomized still further by the more superficial stellate cells, the axonal ramifications of which are confined entirely to the vicinity of the cellbody. There is one other point of difference. Even though some collaterals of stellate cell axons contribute the pericellular plexus around the Purkinje cell, they have a completely different form from the terminal arborization of the descending basket cell collaterals. Their sparseness, their simple varicose form, and their absence from the pinceaux indicate that they cannot have the efficacy of the basket cell fibers.

The fine anatomy, therefore, suggests that the inhibition resulting from stimulation of a stellate cell axon is confined to the Purkinje cells and dendrites in the region immediately around the cell body of the stellate cell. In other words, a parallel fiber volley would elicit principally local inhibitory activity from both types of stellate cells and a much weaker inhibition of successive Purkinje cell dendritic fields in the parasagittal plane. In terms of the analysis proposed by Eccles *et al.* (1967, p. 208, Fig. 114), this would mean that the inhibition evoked by stellate cell activity would be largely "on beam", rather than "off beam". Because of its limited axonal arborization, the stellate cell, unlike the basket cell, could not be effective in inhibiting the discharges of the Purkinje cell. It might, however, be important in resetting the membrane potential of the Purkinje cell dendrites after the passage of parallel fiber excitation. On the basis of their local effects, therefore, we would like to suggest that stellate neurons deserve to be considered in cerebellar circuitry as distinct from basket cells. The fact that stellate cells are phylogenetically older than basket cells supports this view.

References

Anderson, P., Eccles, J. C., Voorhoeve, P. E.: Postsynaptic inhibition of cerebellar Purkinje cells. J. Neurophysiol. **27**, 1138–1153 (1964).

Chan-Palay, V.: The recurrent collaterals of Purkinje cell axons: A correlated study of the rat's cerebellar cortex with electron microscopy and the Golgi method. Z. Anat. Entwickl.-Gesch. **134**, 200–234 (1971).

Chan-Palay, V., Palay, S. L.: Interrelations of basket cell axons and climbing fibers in the cerebellar cortex of the rat. Z. Anat. Entwickl.-Gesch. **132**, 191–227 (1970).

Eccles, J., Ito, M., Szentágothai, J.: The cerebellum as a neuronal machine. Berlin-Heidelberg-New York: Springer 1967.

Eccles, J. C., Llinás, R., Sasaki, K.: The inhibitory interneurones within the cerebellar cortex. Exp. Brain Res. **1**, 1–16 (1966a).

Eccles, J. C., Llinás, R., Sasaki, K.: Parallel fibre stimulation and the responses induced thereby in the Purkinje cells of the cerebellum. Exp. Brain Res. **1**, 17–39 (1966b).

Eccles, J., Llinás, R., Sasaki, K.: The action of antidromic impulses on the cerebellar Purkinje cells. J. Physiol. (Lond.) **182**, 316–345 (1966c).

Eccles, J. C., Tábotiková, H., Tsukahara, N.: Responses of the Purkinje cells of a selachian cerebellum (Mustelus canis). Brain Res. **17**, 57–86 (1970).

Fusari, R.: Sull'origine delle fibre nervose nello strato molecolare delle circonvoluzione cerebellari dell'uomo. Atti Reale Accad.-Sci. (Torino) **19**, 47–51 (1883).

Gray, E. G.: Axosomatic and axo-dendritic synapses of the cerebral cortex: An electron microscopic study. J. Anat. (Lond.) **93**, 420–433 (1959).

Jansen, J., Brodal, A.: Das Kleinhirn. Handbuch der Mikroskopischen Anatomie des Menschen, Band IV/1 (Hrsg. W. Bargmann). Berlin-Göttingen-Heidelberg: Springer 1958.

Kitai, S. T., Shimono, T., Kennedy, D. T.: Inhibition in the cerebellar cortex of the lizard, Lacerta viridis. In: Neurobiology of cerebellar evolution and development (R. Llinás, ed.), p. 481–492. Chicago: AMA-ERF Institute for Biomedical Research 1969.

Larsell, O.: The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat. J. comp. Neurol. **97**, 281–356 (1952).

Lemkey-Johnston, N., Larramendi, L. M. H.: Morphological characteristics of mouse stellate basket cells and their neurological envelope: An electron microscopic study. J. comp. Neurol. **134**, 39–72 (1968a).

Lemkey-Johnston, N., Larramendi, L. M. H.: Types and distribution of synapses upon basket and stellate cells of the mouse cerebellum. J. comp. Neurol. **134**, 73–112 (1968b).

Llinás, R., Nicholson, C.: Electrophysiological analysis of alligator cerebellar cortex. A study on dendritic spikes. In: Neurobiology of cerebellar evolution and development (R. Llinás, ed.), p. 431–466. Chicago: AMA-ERF Institute for Biomedical Research 1969.

Nicholson, C., Llinás, R., Precht, W.: Neural elements of the cerebellum in elasmobranch fishes: Structural and functional characteristics. In: Neurobiology of cerebellar evolution and development (R. Llinás, ed.), p. 215–243. Chicago: AMA-ERF Institute for Biomedical Research 1969.

Palkovits, M., Magyar, P., Szentágothai, J.: Quantitative histological analysis of the cerebellar cortex in the cat. III. Structural organization of the molecular layer. Brain Res. **34**, 1–18 (1971).

Ponti, U.: Sulla corteccia cerebellare della Cavia. Monit. zool. ital. 8, 36–40 (1897).

Rakic, P.: Extrinsic cytological determinants of basket and stellate cell dendritic pattern in the cerebellar molecular layer. J. comp. Neurol. In press.

Ramón y Cajal, S.: Sur l'origine et la direction des prolongations nerveuses de la couche moléculaire du cervelet. Int. Mschr. Anat. Physiol. **6**, 158–174 (1889).

Ramón y, Cajal, S.: Histologie du système nerveux de l'homme et des vertébrés (trans. by L. Azoulay), vol. 2. Paris: Maloine 1911.

Rushmer, D. S., Woodward, D. J.: Inhibition of Purkinje cells in the frog cerebellum. I. Evidence for a stellate cell inhibitory pathway. Brain Res. **33**, 83–90 (1971).

Scheibel, M. E., Scheibel, A. B.: Observations on the intracortical relations of the climbing fibers of the cerebellum. J. comp. Neurol. **101**, 733–763 (1954).

Smirnow, A. E.: Über eine besondere Art von Nervenzellen der Molecularschicht des Kleinhirns bei erwachsenen Säugetieren und beim Menschen. Anat. Anz. **13**, 636–642 (1897).

Sotelo, C.: Ultrastructural aspects of the cerebellar cortex of the frog. In: Neurobiology of cerebellar evolution and development (R. Llinás, ed.), p. 327–371. Chicago: AMA-ERF Institute for Biomedical Research 1969.

Sotelo, C.: Stellate cells and their synapses on Purkinje cells in the cerebellum of the frog. Brain Res. **17**, 510–514 (1970).

Dr. V. Chan-Palay
Department of Anatomy
Harvad Medical School
25 Shattuck Street
Boston, Massachusetts 02115 (U.S.A.)