THE JOURNAL OF COMPARATIVE NEUROLOGY 339:159–173 (1994)

The Candelabrum Cell: A New Interneuron in the Cerebellar Cortex

JEANNE LAINÉ AND HERBERT AXELRAD
Laboratory of Neurophysiology, Faculty of Medicine Pitié-Salpêtrière, 75634, Paris 13, France

ABSTRACT

A new cell type is described in silver-impregnated sections of the rat cerebellar cortex, uniformly distributed through all the cerebellar folia. The soma is rather small, roughly pyriform, vertically oriented, and squeezed, in a sandwich-like manner, between the Purkinje cell somata. One or two thick dendrites arise from the upper pole of the cell body and course through the entire molecular layer, dividing into a few, slightly oblique, branches that can reach the pia mater. These dendrites are covered with irregularly distributed spines. Some more slender dendrites emerge from the lower part of the cell body, or from the proximal trunk of a molecular dendrite, and spread tortuously for a short distance in the upper granular layer. A thick initial segment emerges directly from the soma or from the proximal portion of a dendrite, the axon winding then horizontally through or just above the Purkinje cell layer. During this horizontal course it gives off vertically oriented beaded branches ascending through the major part of the molecular layer. These branches, rather closely spaced, occupy different parasagittal planes, separated by about 10 to 30 μm. This axonal arborisation can thus be compared with a candelabrum. The peculiar three-dimensional spread of the axonal collaterals suggests a functional relationship between these branches and the dendritic trunks of neighbouring Purkinje cells. A comparative analysis of the morphological differences between this candelabrum interneuron and the other corticocerebellar interneurons found in the vicinity of the ganglionic layer confirms the specificity of this new cell class.

Key words: rat, Golgi technique, molecular layer projection

Our understanding of the way information is processed in the vertebrate cerebellar cortex still rests on the classical morphological description of the cellular types of this structure by Ramón y Cajal ('11), the extent and precision of which allowed the physiological and electron microscopical breakthrough that opened, half a century later, the era of modern cerebellar studies (Eccles et al., '67; Palay and Chan-Palay, '74). Despite the wealth of data that have been published in the last 25 years on cerebellar morphology, our views about the elementary components that form this network and their interrelating circuitry have not changed. The cerebellar cortex is classically said to be made of five main neuronal types: the Purkinje cells (PCs, whose axons are the sole output of the cortex), the granule cells (which relay mossy fiber inputs), and three interneurons—the Golgi, stellate, and basket cells. Apart from the granule cells, all the neurons of the cerebellar cortex are inhibitory. A sixth neuronal type, the intermediate fusiform cell described by Lugaro (1894; Fox, '59; Palay and Chan-Palay, '74), although undeniably present, is generally less (or not) cited, in ignorance of the fate of its axonal terminations and in the absence of any physiological recording.

While studying, in Golgi-stained material, the projections of Lugaro cell axons in the molecular layer, we found some other axonal terminals that could be traced back to a previously undescribed neuron. This new interneuron has a pyriform soma squeezed, in a sandwich-like manner, between the PC somata. It is characterised by long ascending molecular dendrites, short basal granular ones, and an axon whose branches rise vertically throughout the molecular layer in different parasagittal planes, in a candelabrum-like fashion.

MATERIALS AND METHODS

Histological procedures

Albino male rats, aged 23 to 45 post-natal days, were deeply anaesthetised (60 mg/kg pentobarbital i.p.) and perfused through the ascending aorta with 4% paraformaldehyde in 0.1 M phosphate buffer. The cerebellum was processed according to two different silver-impregnation methods: 1) a variant of the rapid Golgi method in which the cerebellum is post-fixed for 48 hours in a solution of

Accepted September 8, 1993.

Address reprint requests to Dr. H. Axelrad, Laboratory of Neurophysiology, Fac. Medicine Pitié-Salpêtrière, 91 bd. de l'Hôpital, 75634, Paris 13, France.

0.19% osmium tetroxide-2.33% potassium dichromate, embedded in 4% agar, and immersed for 12 to 24 hours in 1% silver nitrate; 2) the single-section modification of the Golgi procedure elaborated by Gabbott and Somogyi ('84). In both cases the cerebellum was cut with a vibratome into 100 μm parasagittal serial sections. Once dehydrated the sections were mounted between two coverslips, to allow obverse-reverse analysis of the impregnated cells. Observations at high magnification with Nomarski optics, camera lucida drawings, and photomicrographs were performed with a Zeiss Axioplan microscope.

The rapid Golgi method has the advantage, especially in young animals, of often giving good impregnations of axonal structures, but these are frequently blurred by the superposition of too many impregnated elements. In contrast, the single section method impregnates fewer elements but sometimes produces beautiful selective impregnation of an isolated cell with a minimum of background staining.

Tridimensional computer reconstruction

To analyse the spatial spread of the dendritic and axonal branches, a schematic tridimensionnal (3D) reconstruction was performed. We used a fil-de-fer type rendering program, written in the laboratory by Dr. A. Crivat. This program allows colour labeling of different branches, rotations around all three cartesian axe, zoom procedures, and measurements. The x and y coordinate values of relevant points were calculated from parasagittal high-magnification camera lucida drawings, and the corresponding z coordinates were directly read on the fine adjustment of a Leitz Ortholux microscope. The origin was taken inside the volume envelope of the studied cell, usually a dendritic spine visible from both sides of the section.

RESULTS

The distinctive morphological features we shall describe are based on the study of a restricted sample of 15 cells from the vermis of seven different cerebella. These neurons had, as far as we could judge by thorough obverse-reverse inspection with Nomarski optics, a fairly complete impregnation. Some of the neurites were cut at the section's surfaces. As it is difficult to ascertain complete staining of silver-impregnated neurons, some thin dendritic branches and some axonal collaterals might have remained unstained. All of these cells reveal a great homogeneity in their different morphological characteristics and constitute, undoubtedly, a hitherto unrecognized neuronal type in the cerebellar cortex.

Cell body

The perikaryon, as can clearly be seen in the camera lucida drawings of Figures 1A–5A, is always located inside the PC layer. It is squeezed, either between the bulging parts of the PC somata or in the space left free between their upper poles, just at the level of the lower border of the molecular layer. This specific location is illustrated in Figure 2B with a photomicrograph focused on an impregnated soma sandwiched between two non-impregnated PC somata, clearly visible with Nomarski optics. The soma has usually a vertically elongated pear shape with smaller dimensions than the nearby PC somata. The cell body surface bears pedunculate or sessile spines.

Dendritic arborization

The dendritic pattern is characterised by the constant association of one or two long vertical molecular dendrites and of a few short oblique granular ones. The molecular dendrites emerge from the upper pole of the perikaryon as rather thick shafts displaying irregularly distributed pedunculate spines (Figs. 1C, 2C). They rise more or less vertically and divide at acute angles into oblique branches which may reach the pia matter. In the upper molecular layer, the distal branches can take a short horizontal course and are frequently richly covered with long, thin-necked spines or with spicular appendages (Figs. 1A, 5A). The dendritic tree spans roughly 150 μm in the parasagittal plane, whereas schematic 3D reconstruction shows that its mediolateral extent is restricted to less than 50 μm. This flattened aspect of the molecular dendritic tree is a feature it shares with most of the other dendritic trees spreading in this layer.

In our specimens, three to five dendrites emerge from the inferior part of the soma or from the proximal portion of a molecular dendrite and extend in the upper granular layer, where they eventually branch. These basal dendrites are rather thin and are covered with numerous spines. They meander horizontally or slightly obliquely at the interface between the upper granular and PC layers for a variable distance, but never extend far laterally or deeply in the granular layer (Figs. 1A, 5A). This downwardly oriented part of the dendritic arbor seems spatially spread as a more or less flared skirt centered by the neuron's soma. As calculated from our specimens the maximal span of the basal dendrites is in the order of 100 μm, but this *figure* must be taken with caution for in many cases these neurites appear to be cut at the section surfaces. A more precise analysis is underway to obtain an exact estimate.

Fig. 1. A candelabrum neuron impregnated by the rapid Golgi technique. Parasagittal sections in all figures. **A:** Camera lucida drawing. The pyriform cell body lies in the Purkinje cell (PC) layer. A single, stout dendritic shaft ascends from the upper pole and divides, giving oblique branches that rise throughout the whole molecular layer. These dendrites are richly provided with long thin-necked spines. At the lower pole of the soma three basal dendrites extend for a short distance in the ganglionic and granular layers. The thick conical initial segment of the axon emerges directly from the soma (arrowhead). This axon takes an oblique course through the molecular layer, while giving off ascending branches. At the mid-molecular layer level a horizontally directed collateral extends 95 μm further off (the two parallel bars indicate the interruption of the drawing). Note that the axonal arborisation spreads over an area totally overlapping that of the neuron's apical dendritic field. **B–E:** Photomicrographs illustrating details of this neuron (b–e in the camera lucida drawing). **B:** The perikaryon and the initial segment of the axon (arrowhead). Part of the ascending molecular dendrite is also visible, although out of focus. **C:** A molecular dendritic branch exhibiting long thin spines with bulbous endings. The spines of the molecular dendrites are, as a rule, more scattered than those on the dendrites of the basket and stellate molecular interneurons. **D,E:** Ascending and dividing branches of the axon with synaptic beadings and appendages. Calibration bar = 25 μm in A,B,D,E, 10 μm in C. In this and subsequent figures a black circle indicates sectioned neurites and an arrowhead indicates the initial segment of the axon.

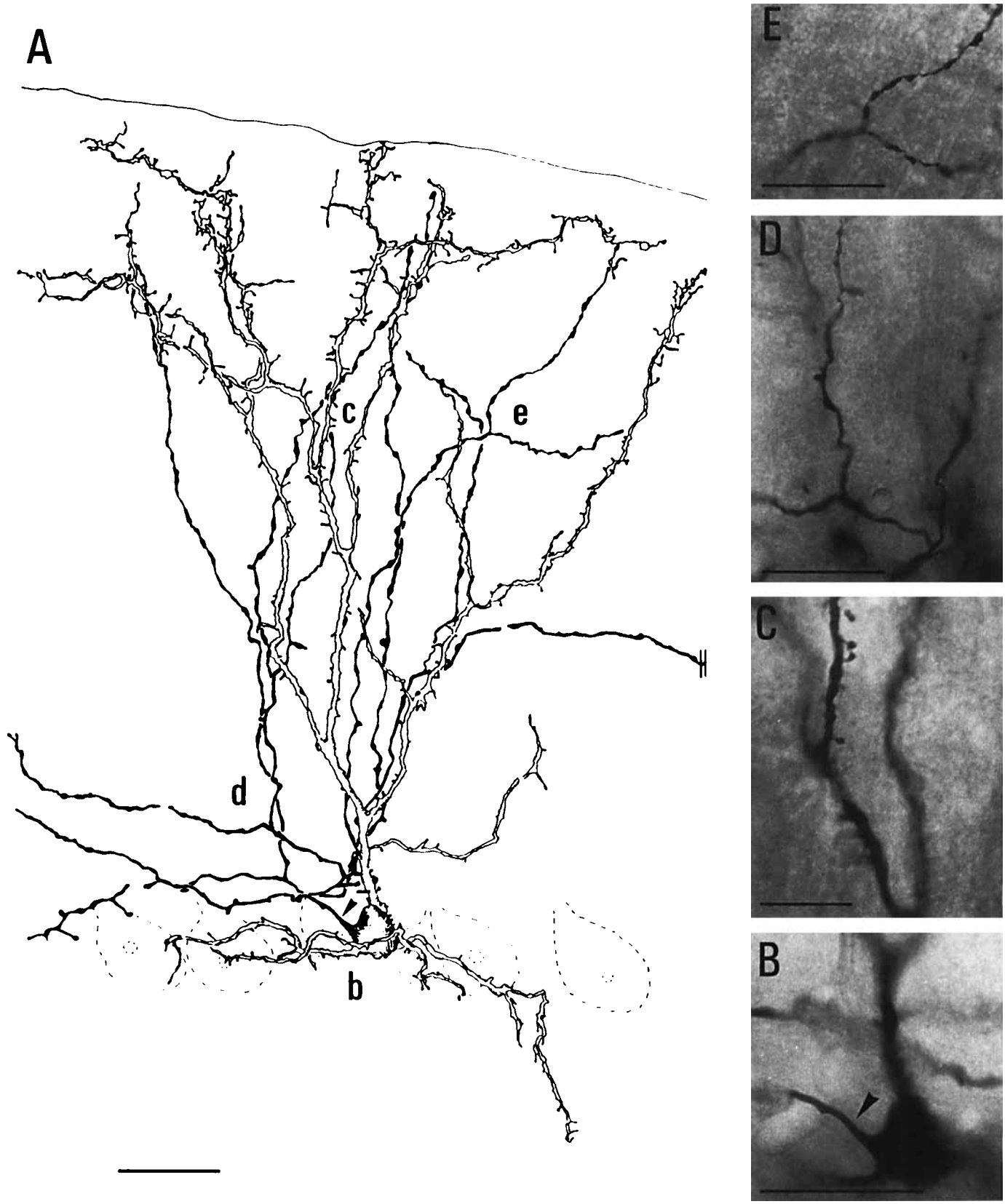

Figure 1

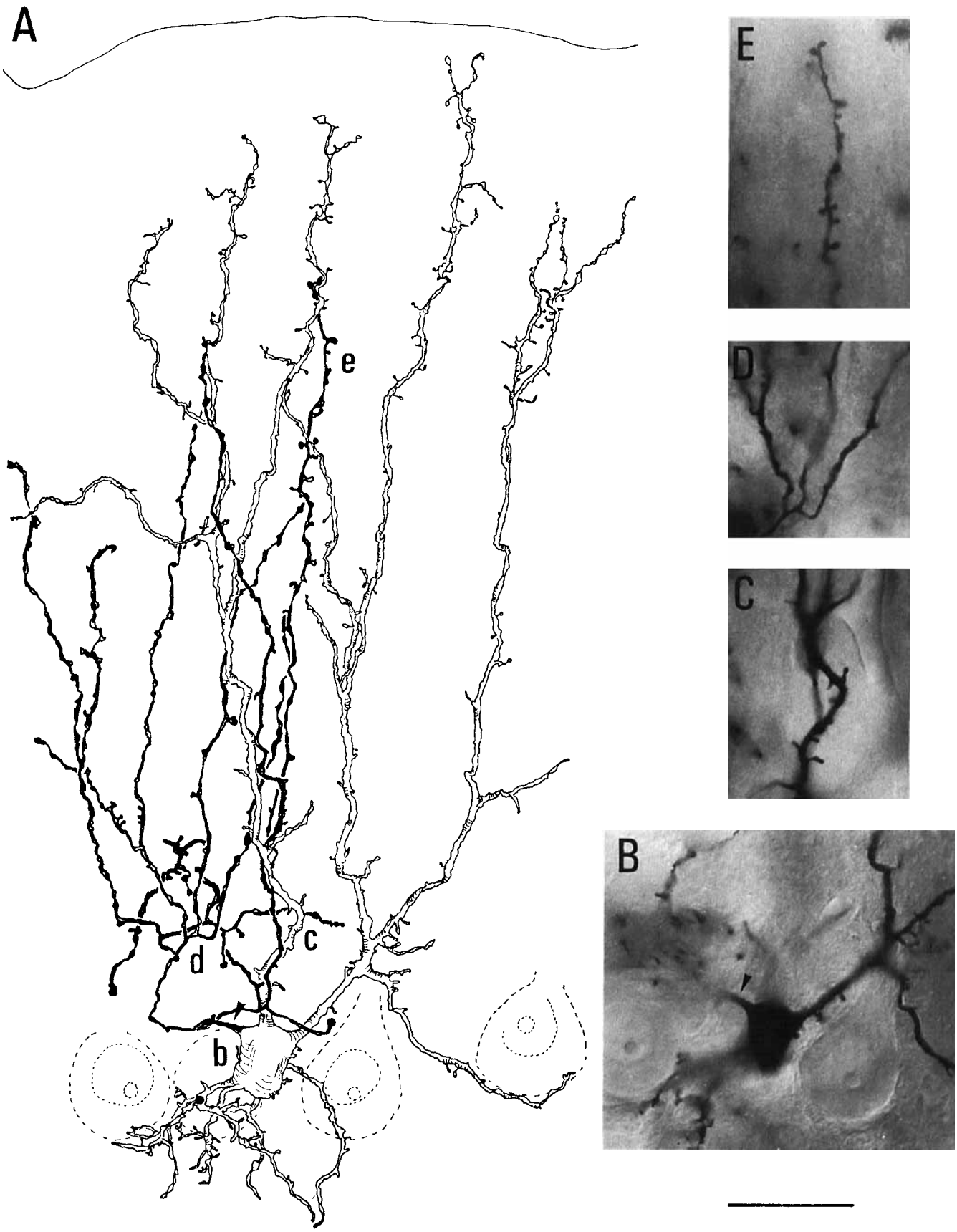

Figure 2

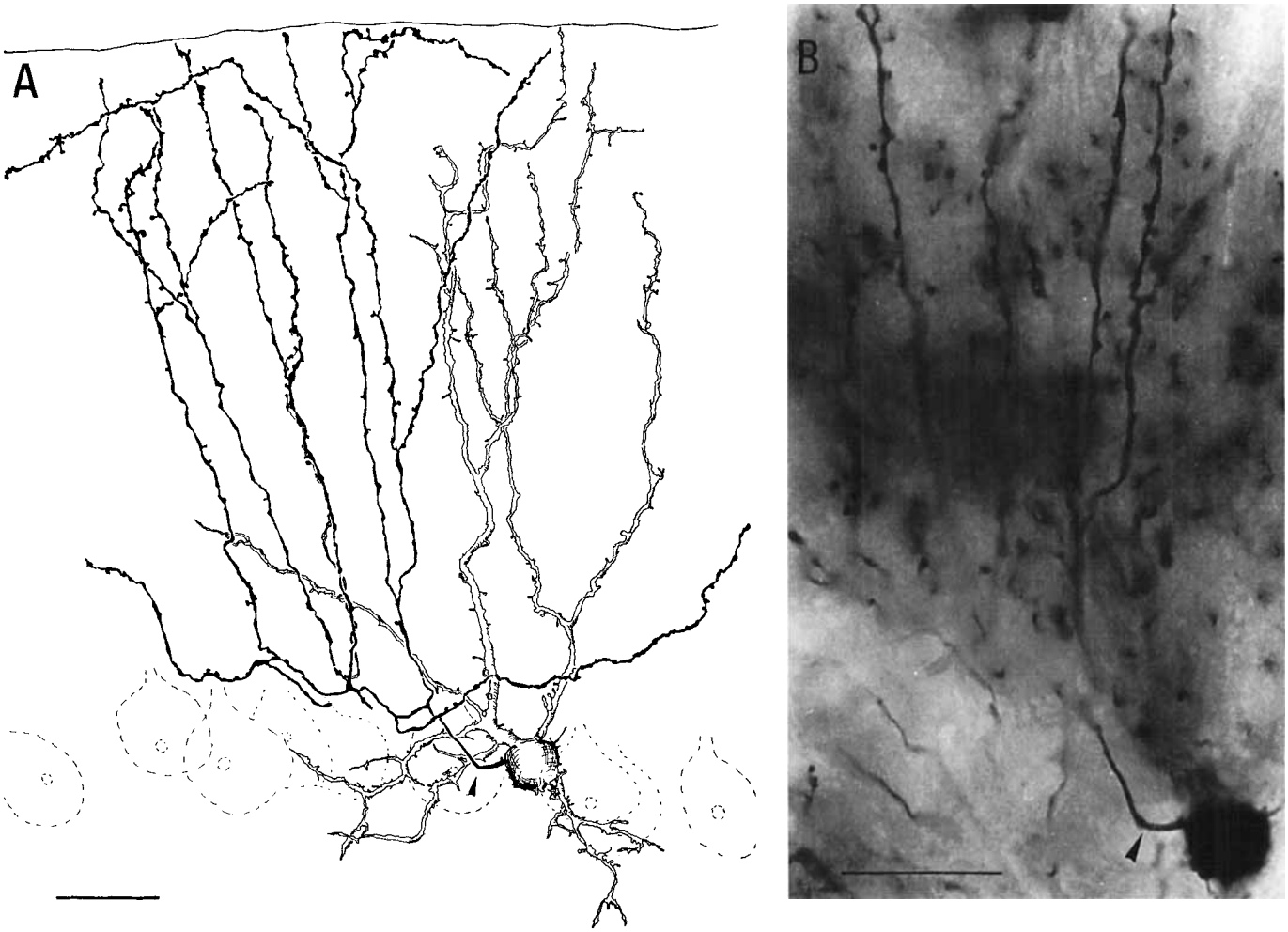

Fig. 3. **A:** This candelabrum cell (rapid Golgi technique) has a more spherical cell body, and two of its four basal dendrites originate from the proximal part of a thick molecular dendritic shaft. In this specimen the area of molecular layer covered by the axonal domain extends laterally from that of the dendritic arborisation, in contrast with the case illustrated in Figure 1. The vertical branches of the axon reach the pia mater, two of them running then horizontally, parallel to the folial surface. **B:** Photomicrograph illustrating the planar parasagittal alignment of the vertical ascending branches of this cell's axon, reminiscent of a candelabrum. Two of the collaterals and important portions of two others are exactly in the plane of focus. Their diameter is at least three times that of the vertically ascending portion of the granular cell axons. Calibration bars = 25 μm.

Axon

The initial segment of the axon originates directly from the perikaryon (Figs. 1B, 2B, arrowhead) or from the proximal part of a dendrite (Fig. 4A, arrowhead). It has a characteristic conical shape, rather thick in respect to the cell body. The axon then extends horizontally, usually coursing in the upper PC layer for a certain distance, winding around the different elements of the neuropil. During this somewhat contorted pathway, it gives off a series of vertical or slightly oblique branches (Figs. 1D, 2D, 3B). These upwardly coursing fibers sometimes divide (Figs. 1E, 2D), generally ending in the upper part of the molecular layer where they can, eventually, run parallel to the folial surface (Fig. 3A). Some other collaterals, in the inferior part of the layer, take a horizontal direction and may continue for a rather long distance (Figs. 1A, 3A, 4A). All these branches display a beaded aspect with, from place to place, more or less long appendages. Their diameter is much larger than the impregnated granule cell axons that can be seen gracefully ascending in the vicinity.

We have seen two distinct patterns for the spatial spread of this axonal domain. In certain cases the area delimited by the axonal branches will totally overlap the area spanned by the cell's dendritic tree (Fig. 1A), without, however, intermingling with the plane of distribution of these molecular dendrites. In some other cases, the axon spreads lateral to the dendritic arborisation, at a more or less great distance

Fig. 2. **A:** Camera lucida drawing of another specimen of candelabrum interneuron (rapid Golgi technique). There are two main ascending dendrites, and one of the five basilar dendrites originates from a molecular dendritic shaft. **B–E:** As for Figure 1. **B:** The peculiar location of the cell body inside the PC layer. The photomicrograph with Nomarski optics shows the perikaryon of the candelabrum cell sandwiched between two unstained PC somata. **C:** The proximal part of one of the ascending dendrites with long pediculate spines. **D:** Trifurcation of the axon at the lower part of the molecular layer. Each branch then ascends nearly vertically for the rest of its course. **E:** The beaded ending of a vertical axonal branch. Calibration bar = 25 μm.

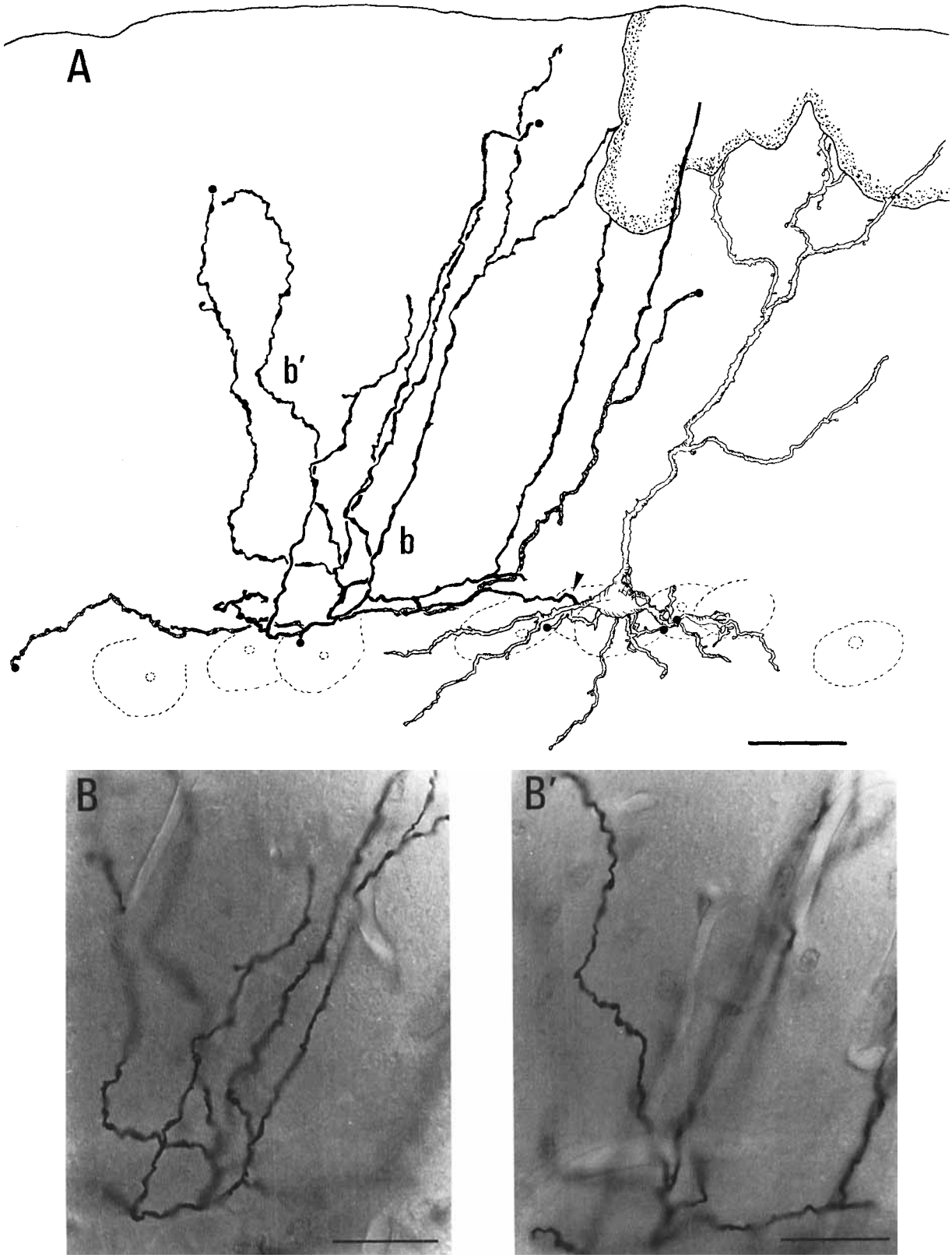

Fig. 4. **A:** Camera lucida drawing of a candelabrum interneuron impregnated with the single section Golgi procedure. The basal dendrites have a skirt-like disposition around the lower pole of the soma. As in Figure 3, the axonal domain spreads laterally from the somatodendritic arborization. It comprises eight vertical branches, organized in a candelabrum fashion, in different parasagittal planes (see text and Fig. 5C). **B,B':** Vertical branches of the axon (b–b' in A) ascending in two different planes. As in Figure 3B, the entire ascending branches, or important portions of them, are seen in the same plane of focus in B and B'. The distance between the plane of focus in B and the one in B', as read from the fine adjustment of the microscope, is in the order of 7 μm. Stippled zones in A, and in subsequent figures, indicate artifacts which obscure the termination of some neurites. Calibration bars = 25 μm.

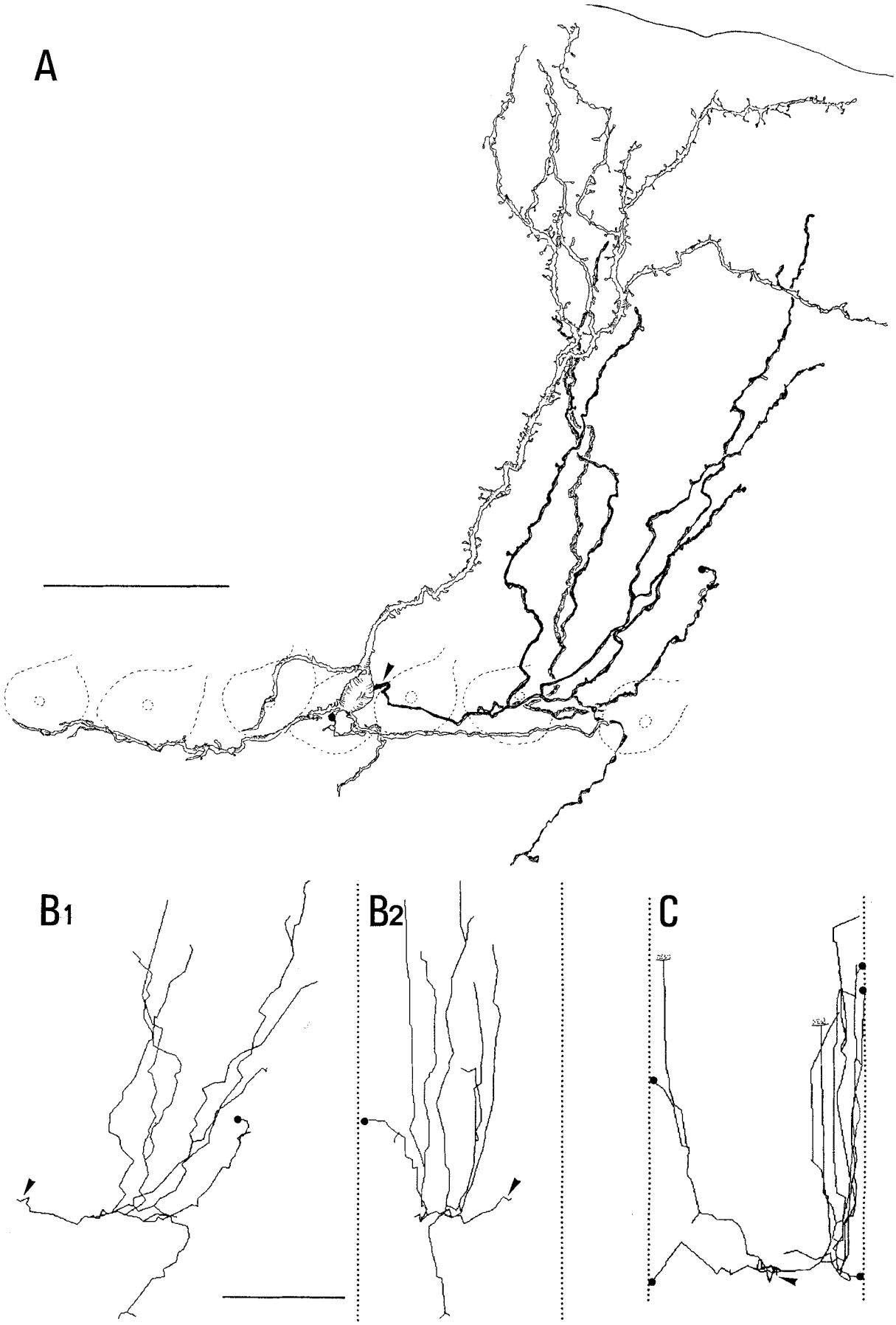

Figure 5 (See overleaf.)

(Figs. 3A–5A). Some intermediate cases can be seen (Fig. 2A). We did not find any correlation between the spatial pattern of the axonal domain and any other morphological feature of the neuron.

The 3D computer reconstruction of the axon reveals a specific feature of the axonal branches. Figure 5B1 is a parasagittal view of the computer reconstruction of the axon shown in Figure 5A. By a 90° rotation it is possible to view these collaterals in the transverse plane. This clearly shows that, as soon as they branch from the horizontal part of the axon, the fibers ascend vertically in different parasagittal planes. Each ascending branch, or group of nearby branches, runs parallel to another, separated by about 10–30 μm in the mediolateral direction (Fig. 5B2,C). This characteristic disposition reminiscent of a candelabrum may have functional implications. Indeed, it strongly suggests a direct relationship between these axonal ascending branches and adjacent PCs, whose dendritic trunks ascend vertically in the molecular layer and are separated from each other by distances of the same order of magnitude (Palay and Chan-Palay, '74; Berthie and Axelrad, unpublished observations).

Distribution

The hazardous impregnation of neuronal elements by the Golgi techniques does not allow any quantitative consideration on the relative frequency or topographic distribution of cell types. In our series of 24 cerebella, processed as indicated in the Materials and Methods section, we have, at present, indexed more than 120 candelabrum interneurons, selected on the morphological criteria described above. Many other partially stained elements (for instance axonal collaterals) were also encountered. These candelabrum cells seem, as far as can be told, present in the vermis as well as in the hemispheres, with a distribution among all lobules. We do not have the impression, in particular, of any coherent aggregation of these cells in particular zones.

Comparison with other interneuronal types

Although the features of the candelabrum interneuron are quite specific, it is necessary, when dealing with a new cell type in a structure as exhaustively analyzed as the mammalian cerebellar cortex, to compare carefully the morphology of these neurons with that of the other well-described cell types present in the vicinity. It could, indeed, be tentatively argued that what we term a candelabrum interneuron is but a subgroup of a known neuronal class or may even just be a particularly uncommon aspect of a familiar neuron, only partially stained.

If one excludes the Bergmann glial cells, which are easily recognised, there are three types of large interneurons that can be found at the approximate level of the ganglionic layer, where the candelabrum cell is located. These are, namely, 1) the intermediate fusiform cell of Lugaro, generally located just beneath the lower pole of PCs; 2) those Golgi cells having their cell body located at the uppermost part of the granular layer, or even inside the PC layer; and 3) the very low basket cells of the molecular layer. Despite the fact that the direction and termination of its axon are not known and therefore cannot be used to specify the cell type, the Lugaro cell cannot be confused with any other type of cerebellar neuron. It has, indeed, a very distinctive somatodendritic morphology: a horizontal elongated cell body with thick dendrites extending from each pole, thus bestowing on it the specific fusiform appearance (Fox, '59; Palay and Chan-Palay, '74).

Basket and Golgi cells are then the two only types of neurons with which the candelabrum cell could, eventually, be confused. In view of a comparative analysis of their somatodendritic and axonal morphology, we have selected nine specimens of each neuronal class. To ensure identical impregnation conditions, the selection was done in vermal sections of 45 day-old male Wistar rats, processed with the single section Gabbott and Somogyi method ('84). We selected only those basket cells located at the lowest level in the molecular layer and those Golgi cells present inside the PC layer, the latter cells representing only a small fraction of the Golgi cells found at the uppermost part of the granular layer. All neurons had an impregnated axon and could thus be faithfully identified. Figures 6–8 illustrate seven specimens of, respectively, the candelabrum, basket, and Golgi cell classes. We show here a restricted part of the camera lucida drawings, representing the soma and proximal portions of the dendrites and axon. For each case we have also outlined the non-impregnated PC somata adjacent to the studied interneuron and visible under Nomarski optics. Characteristic interneurons of different classes could be found in nearby folia in the same section: candelabrum cell f, g, e of Figure 6 and basket cells a, c, e of Figure 7.

At first glance differences between the somatodendritic appearance of candelabrum (Fig. 6) and Golgi cells (Fig. 8) are not obvious. A precise observation shows, however, that Golgi cells have a more globular soma and that their dendrites are more slender and more radially distributed. More fundamentally, these two cell types differ by the direction of their axon, the Golgi axon heading directly downwards towards the granular layer, whilst the axon of the candelabrum cell extends in a horizontal direction,

Fig. 5. **A:** Camera lucida drawing of another candelabrum interneuron stained with the single section Golgi method. There is one thick ascending molecular dendrite, branching in the upper part of the layer and covered with long thin spines. Two of the four granular dendrites extend for a certain distance at the inferior limit of the ganglionic layer. The area covered by the axonal branches is lateral to the apical dendrites. Note a rarely seen, thin, descending collateral of this axon. It penetrates deeper in the granular layer than usually do the basket cell "pinceaux." **B1:** A computer tridimensional (3D) reconstruction of the axon. Same parasagittal view as the camera lucida drawing in A. **B2:** A 90° rotation of the computer reconstructed axon allows a view from the transverse plane (right profile). This transverse view clearly illustrates the striking verticality of the ascending branches of the axon. They ascend in different planes, parallel to each other and to the dendritic trunks of PCs. At midmolecular layer level a branch is cut at the section's surface. The vertical dotted lines indicate the limits of the section. **C:** The 3D reconstructed axon of the candelabrum cell illustrated in Figure 4, also seen in the transverse plane after a 90° rotation. The ascending branches are, as in B2, vertical and occupy different parasagittal planes. Note that in the case of this axon most of the branches are segregated in a restricted territory whilst another branch ascends at a certain distance on the other side of the cell body (the arrowhead indicates the initial segment). Calibration bars = 50 μm.

Fig. 6. **a–g:** Renderings of the somatodendritic morphology of seven candelabrum cells. In view of a comparative analysis, the soma and proximal part of neurites are illustrated for low basket (Fig. 7) and high Golgi cells (Fig. 8). All the cells in these three figures are selected from cerebellar vermis processed with identical impregnation procedures. See text for commentaries. The pyriform cell body of the candelabrum cells lies exactly in the PC layer. The basilar dendrites extend systematically in the granular layer. Cells b and g are the same as in Figures 4A and 5A. Calibration bar = 50 μm.

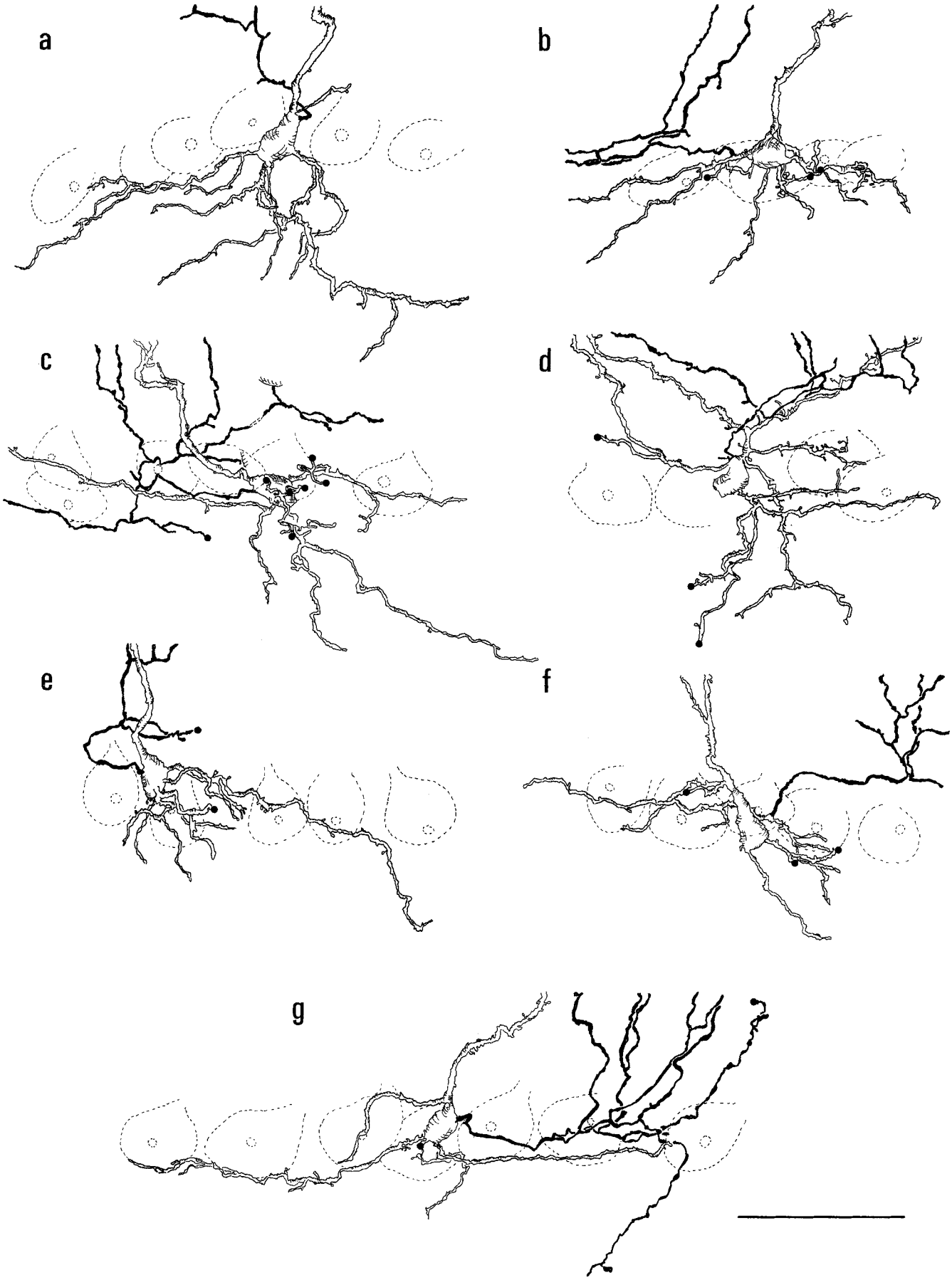

Figure 6

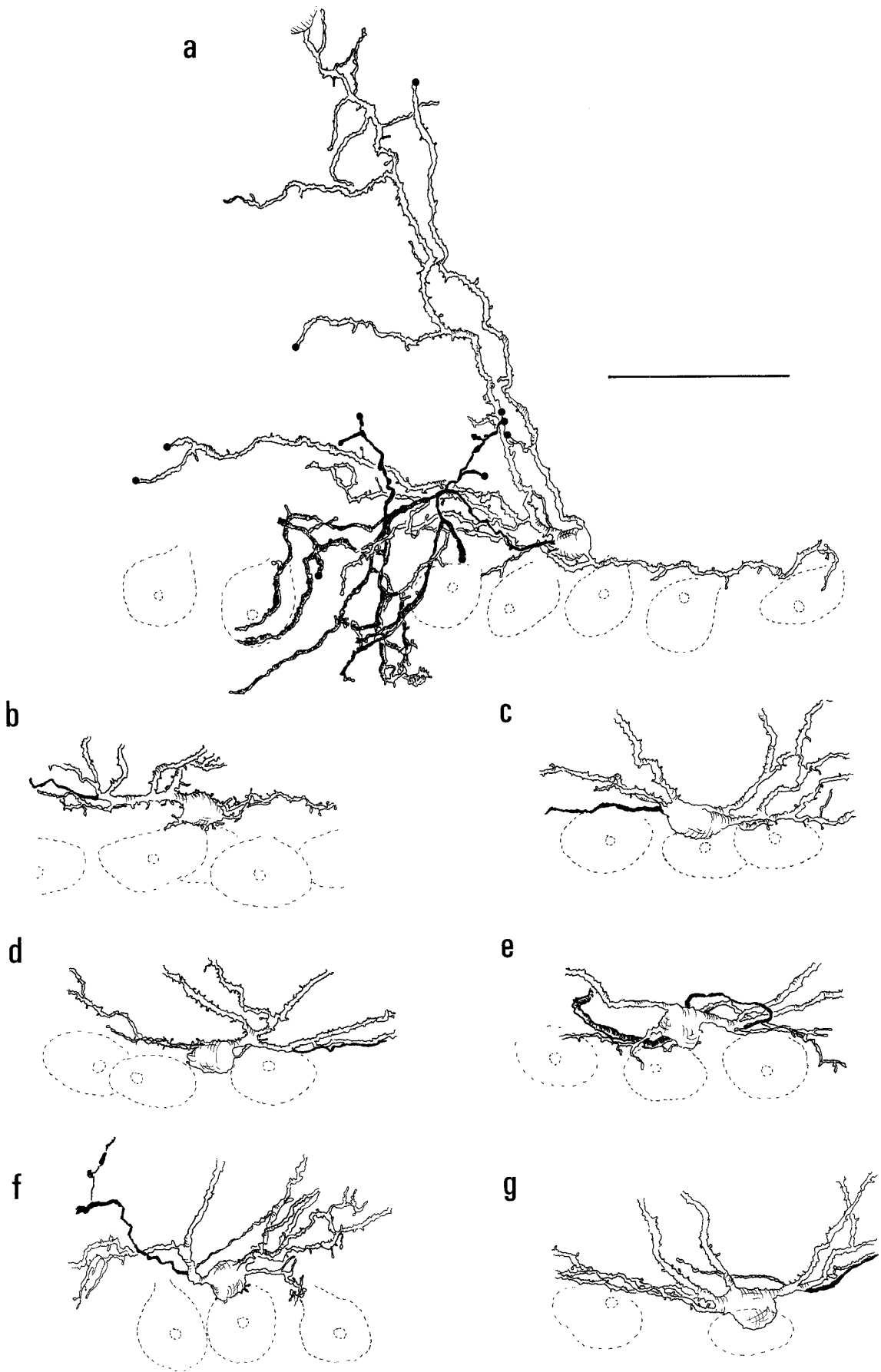

Figure 7

TABLE 1. Location of the Nine Somata of Candelabrum, Low Basket, and High Golgi Interneurons, With Respect to the Tangent to the Lower Pole of PC Somata (in μm)

Candelabrum cells	Basket cells	Golgi cells
4.10	11.70	4.10
6.20	14.40	5.50
7.50	15.10	6.90
8.90	18.50	7.20
9.60	19.20	8.90
9.90	22.60	9.60
11.00	23.60	11.60
12.30	24.00	12.30
16.40	25.40	12.30
Mean 9.54	19.39	8.71
S.D. 3.58	4.86	3.00

TABLE 2. Comparative Diameter in (μm) of the Horizontal Portion of the Impregnated Axons of Nine Candelabrum and Nine Low Basket Cells

	Distance from soma					
	0 μm	10 μm	20 μm	40 μm	60 μm	80 μm
Candelabrum cells	1.00	0.50	0.50	0.70	0.60	0.30
	1.00	0.50	0.30	1.00		
	1.40	1.00	0.70	0.70		
	1.40	0.70	0.50	0.70		
	1.40	0.60	0.70	0.50		
	1.60	0.70	0.70	0.50	0.70	0.70
	1.70	1.00	0.50	0.70	0.80	0.80
	1.70	0.70	0.40	0.50	0.50	0.70
	1.70	0.80	0.70	0.70	0.70	
Mean	1.43	0.72	0.56	0.67	0.66	0.63
S.D.	0.28	0.19	0.15	0.16	0.11	0.22
Basket cells	0.70	0.50	0.70	1.70	2.10	1.70
	0.90	0.50	0.70	0.70	2.10	1.70
	1.00	0.70	0.60	0.70	0.90	1.20
	1.00	0.80	0.70	1.00	1.70	1.40
	1.00	0.70	0.50	1.60	1.60	1.60
	1.20	0.90	0.50	1.00	1.70	1.40
	1.40	0.70	0.70	1.00	1.20	1.90
	1.40	0.90	1.00	1.70	1.40	1.90
	1.60	0.90	0.70	1.00	1.40	1.70
Mean	1.13	0.73	0.68	1.16	1.57	1.61
S.D.	0.29	0.16	0.15	0.40	0.39	0.24

either inside the ganglionic layer or in the supraganglionic plexus. As can be noted in Figure 7, our very low basket cells do not have any granular dendrites, in contrast with candelabrum cells. This is in keeping with the descriptions found in the literature about the dendritic tree of basket cells at different depths in the molecular layer (Mugnaini, '72; Rakic, '72; Palay and Chan-Palay, '74). These reports illustrate that very low basket cells have a dendritic arborisation extending high in the molecular layer, whereas the rare descending dendrites, when present, are very short and do not penetrate the granular layer (Mugnaini, '72: Fig. 25; Rakic, '72: Figs. 1A, 2A, 3A; Palay and Chan-Palay, '74: Fig. 158). Two other distinctive criteria of basket cells versus candelabrum cells are the location of the soma and the orientation of its long axis. The distances of each soma center from the tangent to the lower pole of the PCs for the three classes of neurons are given in Table 1. Despite the small sample, the locations of candelabrum and basket cells differ significantly ($P < .001$). This is not the case for candelabrum versus Golgi cells (but the sample may be too restricted). The orientations of the long axis of the cell bodies, in respect to the PC layer, are represented in Figure 9. Here, too, there is a clear-cut distinction between the basket and candelabrum cell classes, the first being exclusively horizontal, the second mainly vertical.

It then appears that the direction and distribution of their respective axons unequivocally differentiate candelabrum from high Golgi cells. On the other hand, three criteria unmistakably distinguish candelabrum and basket cells: 1) their location, inside versus just above the PC layer; 2) the orientation of the long axis of the soma, vertical versus horizontal; and 3) the constant presence versus total absence of basal granular dendrites.

To make sure that the candelabrum axon is not, in fact, a partially stained basket axon, we also compared the two types of axons, both coursing horizontally in the upper ganglionic layer or supraganglionic plexus. A low basket cell with a long axon is illustrated in Figure 10A (soma and proximal part of this cell are represented in Fig. 7c). Certain important differences pertaining to the axon exist between these cell types. First, whereas both give ascending branches into the molecular layer, only the basket axon has characteristic descending collaterals which encircle the lower pole of PC somata to form the baskets and finish around the PC axon initial segment in the pinceau formation. Second, the changes in the axon diameter, centrifugally from the soma, are different for the two cell types. We quantified these changes by measuring the axonal diameters at identical distances from the soma for both types of fiber (Table 2). When the means at each distance are compared, the difference between the two axonal types is clear: The candelabrum axon is larger at its origin than the basket axon (1.43 μm versus 1.13 μm) but, distally, it becomes thin and stays so for the entire measured length (0.56 μm at 20 μm, 0.63 μm at 80 μm). In contrast the basket axon is thin for only about 20 to 40 μm after its origin (about 0.70 μm) and becomes at least twice that thick from 40 to 50 μm onwards (about 1.50 μm). As it is known that with the Golgi techniques the precipitate completely fills the interior of the impregnated compartment, the differences in the measured diameters certainly indicate two different types of axon. As can also be seen in Table 2 for some candelabrum cells, it was not possible to measure the diameter of the horizontal portion of the axon further than 40 μm distally, because the axon gave off its collaterals quite proximally to the cell body.

In both cell types the axon emits ascending collaterals. These differ by their height in the molecular layer and by their distribution: The candelabrum collaterals are much more segregated spatially. We measured the vertical height of the ascending collaterals in both series of neurons (excluding the ones cut at a section limit or hidden by crystalline artifacts) and expressed it as a percentage of the height of the molecular layer in which they lie (Table 3). There is no statistical difference in the height of the molecular layer between the two samples. In contrast whereas the ascending collaterals of the basket cells ascend up to about 11% of the molecular layer those of the candelabrum interneurons ascend up to 66% ($P < .001$). We also analysed, with 3D computer reconstructions, the ascending trajectories of the basket collaterals. Figure 10B1 is a parasagittal view of a computer reconstruction of the seven ascending collaterals located between the two arrows in the basket cell of Figure 10A (for clarity, the horizontal

Fig. 7. **a–g:** Seven low basket cells. The cell body lies horizontally at the top of the PC somata. No dendrite extends into the granular layer. In a the axon has short ascending axon collaterals and the descending branches which characterise this cell type: They form baskets around PC somata and terminate as a pinceau around the initial segment of the PC axon. Cells a, c, and e are, respectively, from the same sections as candelabrum cells f, g, and e in Figure 6. Calibration bar = 50 μm.

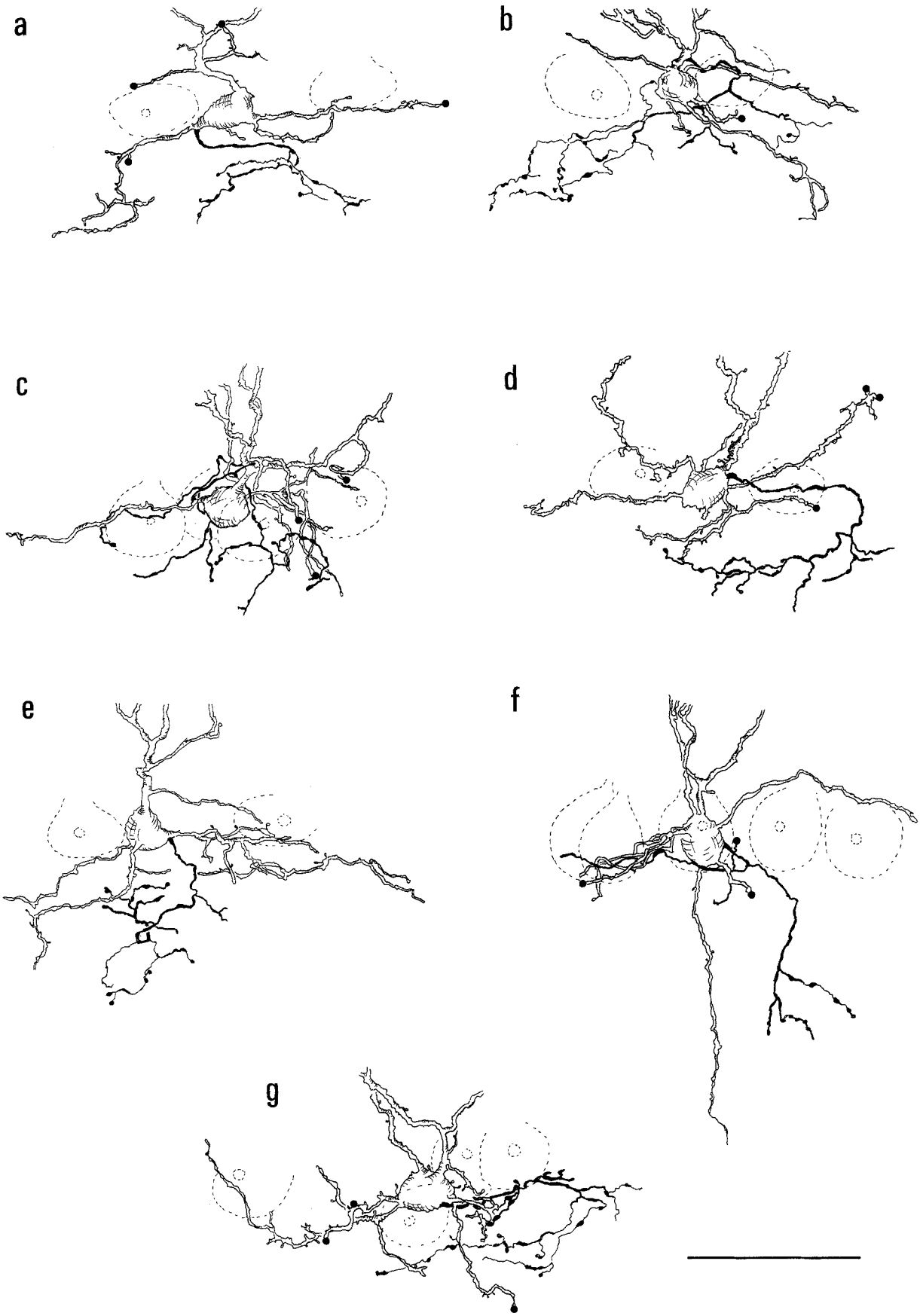

Figure 8

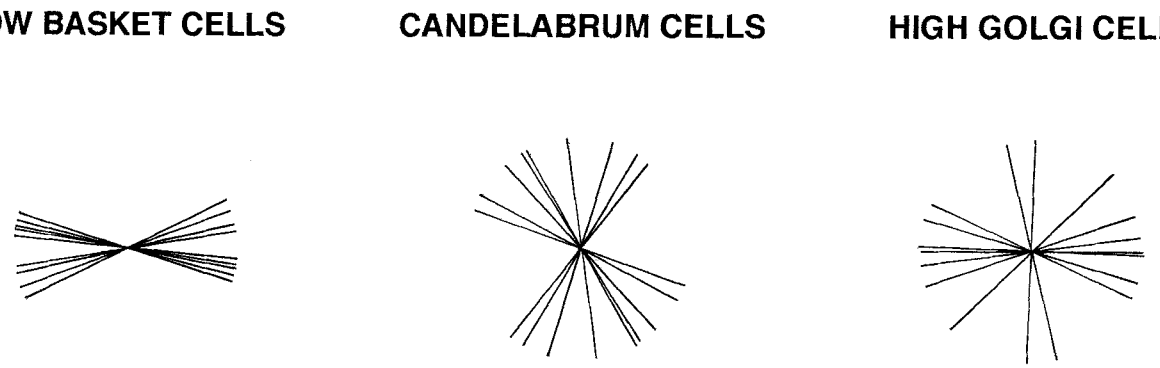

Fig. 9. Orientation of the great axis of the somata of the low basket, candelabrum, and high Golgi interneurons (n = 9). The monolayer of PC perikarya is taken, in each case, as the horizontal axis. See text for further explanation.

part of the axon is not shown). The transverse view, after a 90° rotation, is shown in Figure 10B2. As can be noted, these short collaterals have a very different ascending pathway from those of candelabrum cells (Fig. 5B2,C). Indeed, they appear in most cases contorted and oblique as soon as they branch from the main horizontal trunk. This type of trajectory has been found for the three other basket cell ascending collaterals we have reconstructed; another example is given in Figure 10C1–C2.

This analysis emphasizes two criteria differentiating the candelabrum cells axon ascending collaterals from those of the basket cells: 1) their relative height in the molecular layer and 2) their 3D spatial arrangement.

TABLE 3. Length of Ascending Collaterals Expressed as Percentage of Molecular Layer Height (n = 9 Neurons in Each Class)

Candelabrum cells	Low basket cells
0.48	0.05
0.53	0.05
0.59	0.08
0.60	0.09
0.67	0.10
0.73	0.11
0.74	0.12
0.79	0.14
0.84	0.24
Mean 0.66	0.11
S.D. 0.12	0.06

DISCUSSION

We present here the morphological description of a corticocerebellar neuron, the candelabrum cell, whose somatic location, dendritic arborisation, and peculiar pattern of axonal spread in the molecular layer support the classification as an entirely new cell type. The discovery of a previously unknown neuron in one of the most studied structures of the central nervous system requires some comment. We did not find, in a thorough survey of the main original studies describing the cellular types of the cerebellar cortex (Dogiel, 1896; Ramón y Cajal, '11; Estable, '23; Jakob, '28; Pensa, '31; Jansen and Brodal, '58; Eccles et al., '67; Fox et al., '67; O'Leary et al., '68; Mugnaini, '72; Palay and Chan-Palay, '74; Braak and Braak, '83), the slightest indication about a cell resembling the one we show here. Classification of neuronal types in silver-impregnated material relies upon three criteria: the location and shape of the soma, the typology of the dendritic arbor, and the pattern of axonal projections. To assign a given neuron reliably to a specific cell class it is necessary to dispose of all three criteria. As is well known to practitioners of silver-staining techniques, many cells are very often only partially stained, one of the most elusive parts being the myelinated axon. Our opinion is that the interneuron we describe here was missed in previous studies because of the superficial likeness between the somatodendritic features of the candelabrum interneuron and those of small, highly located, Golgi cells. Indeed, when the axon is not impregnated, both cell types can be differentiated only by a minute analysis (see Results). On the other hand we show, by a comparative analysis, that the candelabrum cell differs by too many morphological features from the basket cell to oppose the argument that the former neuron could only be an incompletely stained aspect of the latter cell. This is particularly true for their axon whose characteristic features are, for the basket cells, the descending collaterals and, for the candelabrum cells, the long vertically ascending collaterals.

As far as we can tell from our preparations, the candelabrum cell seems to be distributed in all parts of the cerebellum. This would imply that the classical basic circuitry of the cerebellar cortex, on which many models have been built (see Ito, '84), is oversimplified and should integrate the candelabrum cell, as well as the seldom mentioned intermediate fusiform cell of Lugaro.

Because extensive knowledge is available on the cerebellar cortex, it is possible to infer some of the principal plausible contacts of this candelabrum interneuron. The parasagittal orientation of the molecular dendrites transverse to the folium, as well as the pedunculate spines, is in favour of inputs from parallel fibers, by analogy with the other neurons having dendrites in the molecular layer. Some inputs may, evidently, also come from stellate cells and basket cells, as well as from climbing fibers. The restricted extent of the basal dendrites to the upper granular layer is in favor of synaptic contacts by the recurrent axon collaterals of PCs and by the ascending granule cell axons. As we mentioned in the Results section, the peculiar

Fig. 8. **a–g:** Seven high Golgi cells located inside the ganglionic layer. Their cell body is more globular and their dendritic distribution more radiated than is the case for the candelabrum cells. The direction and pattern of their axon differ drastically. Calibration bar = 50 μm.

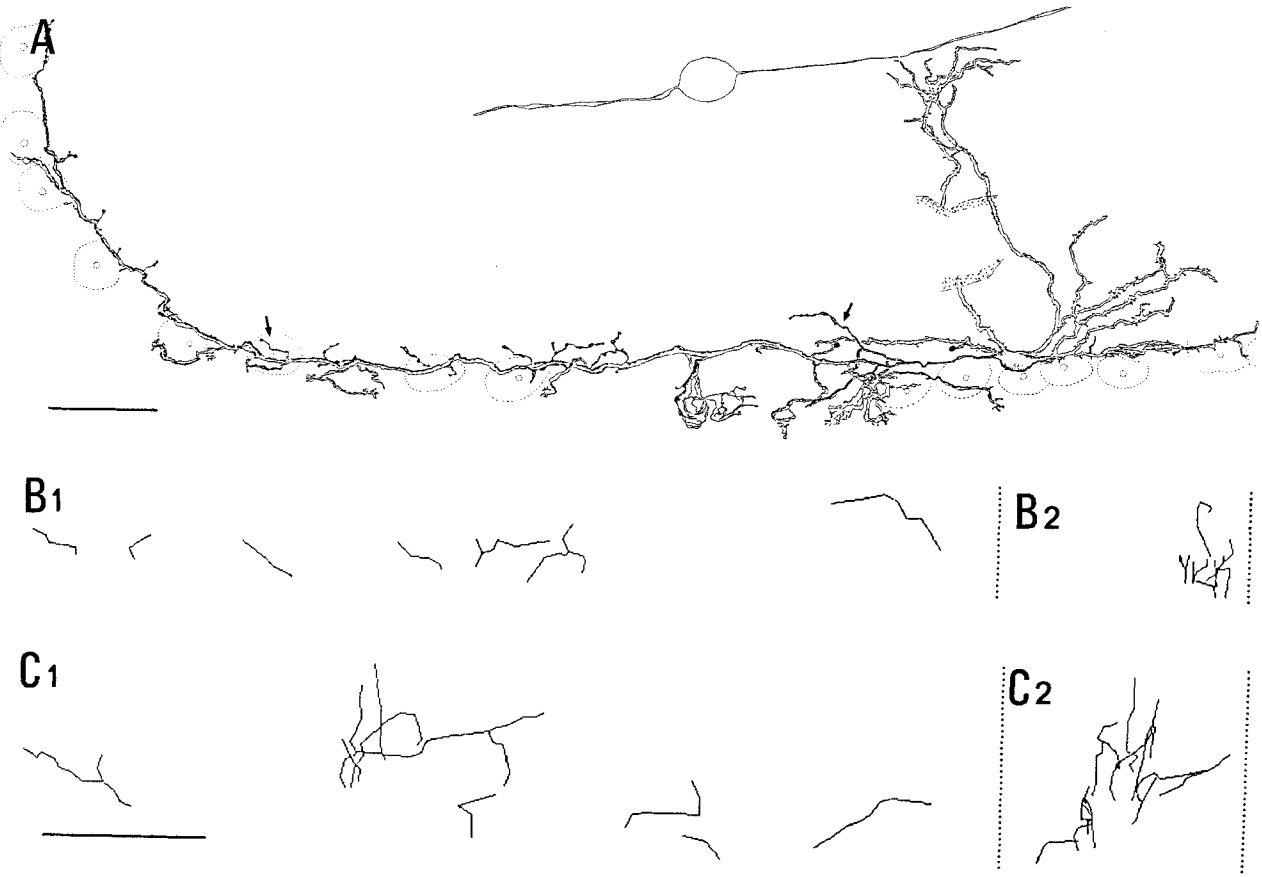

Fig. 10. **A:** Camera lucida drawing of an impregnated basket cell, located in the same section as the candelabrum cell of Figure 5A. The cell body lies at the lowest part of the molecular layer, with a typical fan-like organization of the dendrites and a long axon extending for a great distance in the supraganglionic plexus. Descending collaterals engage in the basket formation around the PC perikarya and terminate with the classical "pinceaux." Some short and thin ascending collaterals are also regularly emitted along the course of the axon. The two small arrows indicate the limits between which these ascending branches were computer reconstructed, in view of a comparison with the ascending branches of the candelabrum cell. **B1,B2:** Tridimensional reconstruction of the seven ascending collaterals specified above. B1: Parasagittal view. B2: Transverse view, after a 90° rotation. **C1,C2:** as B1, B2 for another basket cell axon. In contrast with the ascending axonal branches of candelabrum cells, these basket collaterals do not extend very high up in the molecular layer, and they have a contorted trajectory, from their origin onwards. Calibration bars = 50 μm.

3D spread of the axonal collaterals in the molecular layer strongly correlate with the relative positions of PC dendritic trunks. These could, therefore, very well be the post-synaptic target of the candelabrum axon. As shown, there are two different patterns of spatial organisation of the axonal domain: It can either totally overlap the area of the parent cell dendritic arborisation, or it can expand at a certain distance laterally. This would functionally mean either that the candelabrum interneuron can exert its effect on the neurons located in the immediate vicinity of its cell body and dendritic arborisation, thus receiving common parallel fiber input, or that it can modify the activity of neurons in a lateral region, themselves receiving a completely different set of parallel fibers.

However, a light microscopic observation of silver-impregnated material can give no precise information on the inputs and outputs of the neuron under study. Our next step will therefore be an electron microscopic analysis of selected specimens of silver-impregnated candelabrum cells, treated by the gold-toning technique (Fairén, '77).

ACKNOWLEDGMENTS

The authors express their sincere thanks to Mrs. M.E. Marc for expert technical help, to Dr. C. Sotelo for constant encouragement during the course of this study, and to Professor S.L. Palay for critical comments on a first draft of this manuscript. This research was supported by grant INSERM CRE 89 9001.

LITERATURE CITED

Braak, E., and H. Braak (1983) On three types of large nerve cells in the granular layer of the human cerebellar cortex. Anat. Embryol. (Berl.) *166*:67–86.

Dogiel, A.S. (1896) Die Nerven elemente im Kleinhirne der Vögel und Saügetiere. Arch. F. Mikrosk. Anat. *47*:707–719.

Eccles, J.C., M. Ito, and J. Szentágotai (1967) The Cerebellum as a Neuronal Machine. New York: Springer-Verlag.

Estable, C. (1923) Notes sur la structure comparative de l'écorce cérébelleuse et dérivées physiologiques possibles. Trab. Lab. Invest. Biol. (Madrid) *21*:169–256.

Fairén, A., A. Peters, and J. Saldanha (1977) A new procedure for examining Golgi impregnated neurons by light and electron microscopy. J. Neurocytol. *6:*311–337.

Fox, C.A. (1959) The intermediate cells of Lugaro in the cerebellar cortex of the monkey. J. Comp. Neurol. *112:*39–51.

Fox, C.A., D.E. Hillman, K.A. Siegesmund, and C.R. Dutta (1967) The primate cerebellum. Prog. Brain Res. *25:*174–225.

Gabbott, P.L., and J. Somogyi (1984) The "single" section Golgi impregnation procedure: methodological description. J. Neurosci. Methods *11:*221–230.

Ito, M. (1984) The Cerebellum and Neural Control. New York: Raven Press.

Jakob, A. (1928) Das Kleinhirn. In W.W. Mollendorf (ed): Handbuch der Microskopischen Anatomie des Menschen. Berlin: Springer-Verlag, Bd. IV/1, pp. 674–911.

Jansen, J., and A. Brodal (1958) Das Kleinhirn. In W. Bargman (ed): Handbuch der Mikroskopischen Anatomie des Menschen. Heildelberg: Springer, Bd IV/1.

Lugaro, E. (1894) Sulle connessioni tra gli elemente nervosi della corteccia cerebellare con considerazioni generali sul significato fisiologico dei rapporti tra gli elementi nervosi. Riv. Sper. Freniat. *20:*297–331.

Mugnaini, E. (1972) The histology and cytology of the cerebellar cortex. In O. Larsell and J. Jansen (eds): The Comparative Anatomy and Histology of the Cerebellum; The Human Cerebellum, Cerebellar Connections and Cerebellar Cortex. Minneapolis: Univ. of Minnesota Press, pp. 201–264.

O'Leary, J.L., J. Petty, J.M. Smith, M. O'Leary, and J. Inukai (1968) Cerebellar cortex of rat and other animals. J. Comp. Neurol. *134:*401–432.

Palay, S.L., and V. Chan-Palay (1974) Cerebellar Cortex. New York: Springer-Verlag.

Pensa, A. (1931) Osservazioni e considerazioni sulla struttura della corteccia cerebellare dei mammiferi. Reale Accad. Lincei Ser. VI, *5:*1–26.

Rakic, P. (1972) Extrinsic cytological determinants of basket and stellate cell dendritic pattern in the cerebellar molecular layer. J. Comp. Neurol. *146:*335–354.

Ramón y Cajal, S. (1911) Histologie du Système Nerveux de l'Homme et des Vertébrés. T.II. Paris: Maloine.