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Morphology of the Golgi-Impregnated Lugaro Cell in the Rat Cerebellar Cortex: A Reappraisal With a Description of Its Axon

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ABSTRACT

We present a detailed description of the somatodendritic and axonal features of the Golgi-impregnated Lugaro cell in the rat cerebellar cortex. This neuron, present throughout the cerebellum, is characterized by a fusiform cell body located at the border between the granular and Purkinje cell layers, horizontal bipolar dendrites extending in the parasagittal plane and an axon projecting exclusively to the molecular and granular layers. A quantitative analysis of 77 Lugaro cells confirms the somatodendritic homogeneity of this cell type. Computer reconstructions showed that the bipolar dendrites spread like an horizontal X centered by the cell body, implying that Lugaro cells are arranged in a flat lattice just beneath the Purkinje cell layer. Fifty-seven Lugaro cells with sufficiently impregnated axons displayed a common pattern of organization with, nevertheless, a certain variability of the axonal trajectory. Some axons projected directly into the molecular layer with a profuse plexus spreading just above the parent cell. Other axons first traveled downwards into the granular layer, sometimes even reaching and passing into the white matter. They always hooked back to reascend through the granular layer and also finally terminate in the molecular layer, near and sometimes at a distance from the parent cell. In a few samples, one or two remote axon collaterals were seen to extend longitudinally in the lower molecular layer for a few hundred microns, in the same direction as the parallel fibers. In all cases a few collaterals projected into the granular layer.

In view of its dense afferentation by Purkinje cell recurrent collaterals and its profuse inhibitory projection in the molecular layer, the Lugaro cell could act as a feedback interneuron on the corticocerebellar output.

Indexing terms: cerebellum, interneuron, Golgi staining, molecular layer projection

The histology of the cerebellar cortex has been extensively studied with the Golgi techniques, and its neuronal composition is commonly assumed to be made of six different neuronal types, with an apparently repetitive connectivity: the Purkinje, granule, basket, stellate, Golgi, and Lugaro cells (Ramón y Cajal, 1911; Eccles et al., 1967; Fox et al., 1967; Mugnaini, 1972; Palay and Chan-Palay, 1974). Two new neuronal types have recently been described in this structure: the monodendritic or unipolar brush cell (Altman and Bayer, 1977; Hockfield, 1987; Rogers, 1989; Munoz, 1990; Mugnaini and Floris, 1994; Berthié and Axelrad, 1994) and the candelabrum cell (Lainé and Axelrad, 1994). Among the classical cells, the "intermediate cell" of Lugaro (Golgi, 1874; Lugaro, 1894; Fox, 1959; Palay and Chan-Palay, 1974; Sahin and Hockfield, 1990) has not yet gained full status as a member of the corticocerebellar network. It is, even nowadays, often omitted in the listing of the neuronal types of the cerebellar cortex and is never taken into account in the many models of this part of the central nervous system (see Ito, 1984). This can be ascribed essentially to two complementary reasons: (1) its axonal projection is not exactly known and has been subject to controversy since the very first morphological analysis of this structure with the Golgi techniques, and (2) no functional recording of this cell type has ever been obtained with electrophysiological techniques.

Lugaro, one century ago, specified this particular neuronal type as "intermediate" because he had impregnated some cells with a soma located at the upper border of the granular layer and an axon ascending into the molecular layer where it bifurcates and takes a horizontal direction parallel to the long axis of the folium and to the parallel fibers. On the other hand, Ramón y Cajal (1911) described a fusiform neuron, in the same location as that indicated by Lugaro, but with an axon descending obliquely through the

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granular layer, right into the white matter where it seems to become a centrifugal fiber ("il semble se transformer en fibre centrifuge"). In 1959, Fox confirmed, on a unique fusiform neuron in the monkey cerebellar cortex, the axonal projection described by Lugaro, but in 1974, Palay and Chan-Palay described and illustrated Lugaro cells with a descending axonal pattern identical to that of Ramón y Cajal (they nevertheless state in their monograph that some of these neurons with purely molecular projections can be seen). It therefore appears that the uniqueness of the somatodendritic morphology and localization of the Lugaro cell has been sufficiently characterized, but that it has been assigned two different axonal patterns, each implying a very different functional role in the corticocerebellar network. If the Lugaro cell has axonal projections that remain inside the granular and molecular layers of the cortex, it should be classified as an interneuron. Conversely, if the Lugaro cell projects outside the cortex by means of the white matter, it should be considered as an output neuron, in parallel with the Purkinje cell axonal pathway.

In the present Golgi study, we bring complementary data on the location and morphology of the Lugaro cell's soma and dendrites, including the tridimensional spread of the latter. Furthermore, we describe the axonal projections and show that they remain, in all cases, inside the cerebellar cortex. The apparent discrepancy between the observations of Lugaro and Ramón y Cajal is indeed due to the variability of the axonal trajectory. The axon can either directly project to the molecular layer, as stated by Lugaro, or first take a descending course through the granular layer, even reaching the white matter as stated by Ramón y Cajal, before systematically making a hairpin turn to reascend in the cortex and give a profuse molecular layer arborization in the vicinity, and sometimes at a distance, from its parent cell body. The Lugaro cell must therefore be considered as a corticocerebellar interneuron.

MATERIALS AND METHODS

Histological procedures

Young albino male rats (13 to 45 postnatal days) were deeply anaesthetized (60 mg/kg pentobarbital i.p.) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The cerebellum was processed following two different silver impregnation methods: the classical rapid Golgi method, and the single-section Golgi procedure elaborated by Gabbott and Somogyi (1984; for details, see Lainé and Axelrad, 1994). In both cases, the cerebellum was cut into 100-μm parasagittal serial sections, and the sections were mounted between two coverslips to allow obverse-reverse analysis of the impregnated cells.

Data analysis

Observations, camera lucida drawings, and photomicrographs were performed with a Zeiss Axioplan microscope. Selected neurons were drawn at high magnification and reconstructed from serial sections when needed. In view of minimizing variability factors, all quantitative data given in the results section were calculated from a homogeneous group of Lugaro neurons selected in respect to: (1) the rat strain, sex and age (45 postnatal days Wistar male rats); (2) the Golgi technique used to impregnate the cells (single section procedure); and (3) their vermian location in the cerebellum. No correction for shrinkage was made.

Tridimensional computer reconstructions of some of the neurons were performed with a fil-de-fer type program written in the laboratory for a 486 PC by Dr. A. Crivat. The x and y coordinate values of relevant points were digitized from the parasagittal high magnification camera lucida drawings, and the corresponding z coordinates were directly read on the fine knob of a Leitz Ortholux microscope. The origin was systematically taken inside the cell envelope, for instance, a spine visible from both sides of the section.

RESULTS

General considerations

The unique morphology of the typical Lugaro cell, fusiform soma prolonged at each pole by thick dendritic shafts extending transversally to the folium (Figs. 1C,D; 4A; 6A), and its significant location, directly beneath the Purkinje cell layer (Figs. 1A,B; 4A; 6A), allow an easy recognition of this cell type when parasagittal Golgi sections are scanned. This class of neurons can be clearly differentiated from the other large neuronal types that are found in the neighbourhood. The Purkinje and candelabrum cell somata that lie in the ganglionic layer are more rounded, as are the Golgi cells of the upper granular layer, and all these neurons give off dendrites radiating in more vertical directions than the fusiform neurons. Although fewer are seen than other cerebellar neuronal types, Lugaro cells are not a rare encounter. The elusiveness and randomness of the Golgi techniques make these inappropriate for a precise evaluation of relative population numbers and it is not possible, therefore, to approximate numerically their density directly from our data. However, for 30 cerebellar vermises, stained with the single-section procedure (see Materials and Methods), and for a significant number of neurons, a rough ratio of 1:25 was found between impregnated Lugaro and Purkinje neurons. This estimate is not very far from the one found in the cat with a selective immunocytochemical approach (Sahin and Hockfield, 1990). The Lugaro cell type does not appear to be segregated in particular regions of the cerebellar cortex. It is commonly found in all folia of the vermis and hemispheres, and must therefore be considered as a regular constituent of the corticocerebellar network. It should be mentioned, however, that in some instances there may be two or three Lugaro cells impregnated very near to each other, hypothetically indicating spatial clusterings of these neurons (see Discussion).

Somatodendritic location and morphology

If one relies on the classical morphological criteria to identify Lugaro cells, i.e., mainly their characteristic fusiform appearance (Ramón y Cajal, 1911; Fox, 1959; Palay and Chan-Palay, 1974; Sahin and Hockfield, 1990; Lainé et al., 1992), these neurons are in most cases located in the lower part of the ganglionic layer or at the level of the infraganglionic plexus. This location can be quantitatively assessed by measuring the distance from the center of each Lugaro cell soma to the line connecting the inferior pole of the nearby Purkinje cell somata, as seen with Nomarski optics. Data for 77 neurons are illustrated in the histogram of Figure 2A. The centers of the cells are mainly located either directly between the lower rounded part of the Purkinje cell bodies (as in Figs. 1B; 8A; 9B; 11) or at the level of the Purkinje cells initial axon segment (Figs. 6; 9A; 10; 12). In addition, some typical fusiform neurons can also

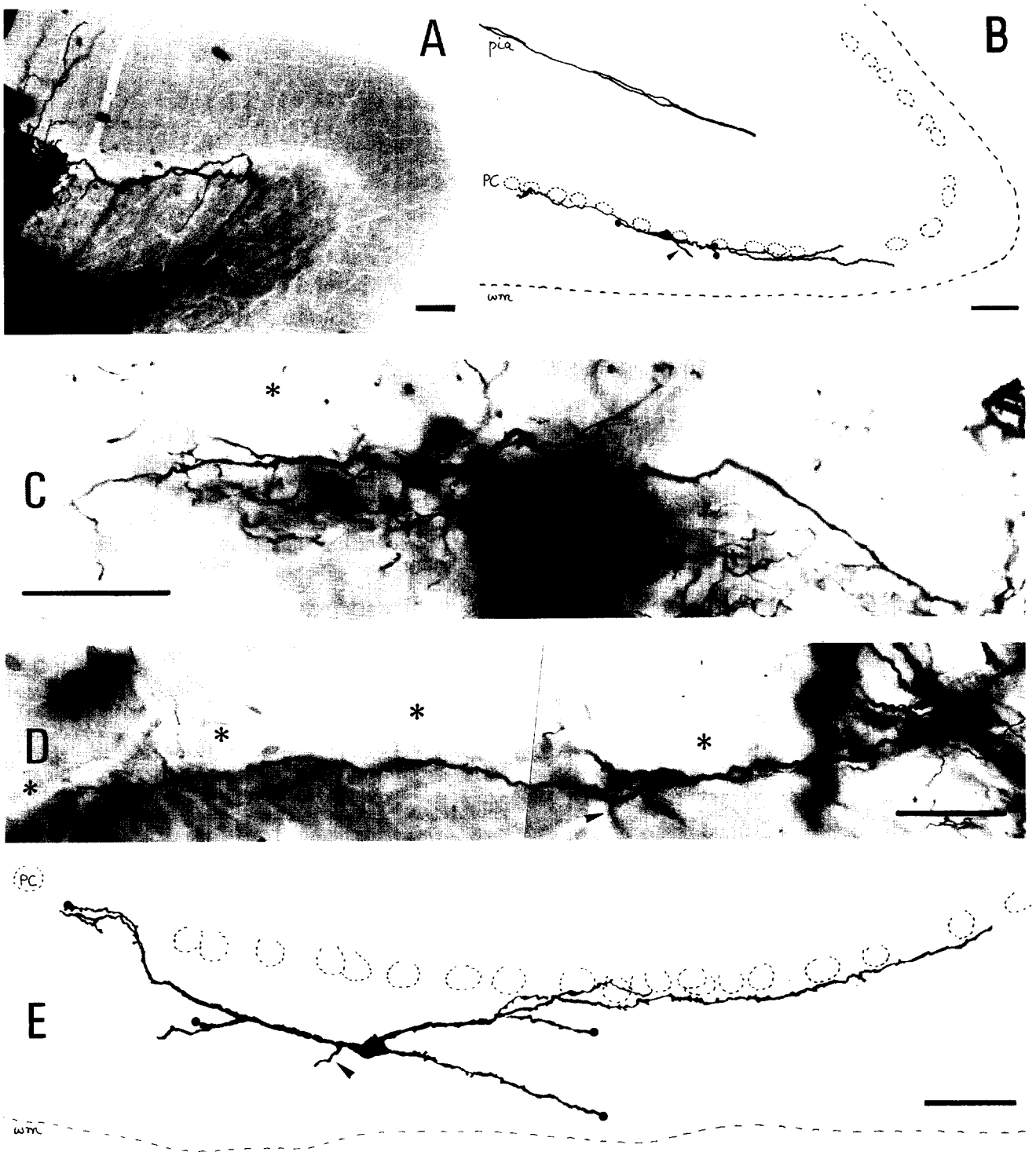

Fig. 1. Typical Golgi-impregnated Lugaro cells in parasagittal sections of the cerebellar vermis. **A,B:** Small magnification photomicrograph (A) and camera lucida drawing (B), that show the characteristic location and the bipolar elongated aspect of the Lugaro cells. The soma of these neurons is usually located just at the border between the granular and Purkinje cell layers and is prolonged at each pole by thick dendrites, coursing straighforwardly under the Purkinje cells somata. **C,D:** Photomicrographs that illustrate the long fleshy dendrites emerging from the opposite poles of the horizontal fusiform soma and coursing in the infraganglionic plexus. These dendrites are rather poorly ramified and covered by sparse protruding spines. Asterisks indicate overlying Purkinje cells somata. **E:** Camera lucida drawing of a rarely seen deep Lugaro cell. The soma is located in the middle of the granular layer and the two main polar dendrites ascend obliquely towards the upper granular layer where they course horizontally for some distance. A third slender dendrite is cut en route towards the white matter. A, B, D, E: Single section Golgi method, young adult rat, folium VId, IXc, I,VII respectively. C: Rapid Golgi method, 24-day-old rat, folium IXc. In these and subsequent figures, an arrowhead indicates the initial segment of the axon and a black circle signals a sectionned neurite; abbreviations here and in the following figures: pia, pia mater; wm, white matter; PC, Purkinje cell. Scale bars = 50 μm.

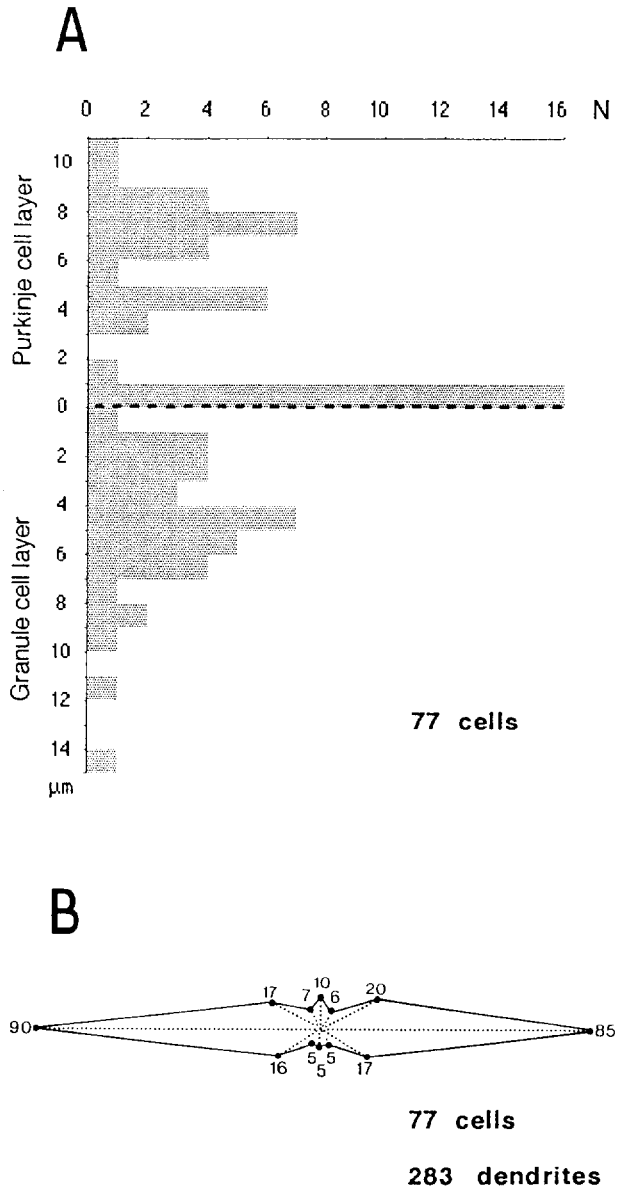

Fig. 2. Quantified representation of the location and dendritic bipolarity of Lugaro cells. **A:** Histogram of the distance between the center of each Lugaro cell soma and the tangent line to the inferior poles of neighbouring Purkinje cells somata, as seen with Nomarski optics. This line is represented by the dotted line crossing the x (distance) axis at origin. The Lugaro cell somata located above this line are found inside the Purkinje cell layer between these cells' rounded inferior poles, those located beneath this line are in the infraganglionic plexus in between the basket cells pinceaux. **B:** Radial histogram of the dendritic emergence. The great axis of the somata is horizontal. The dendrites are counted according to the angle made by their proximal portion with the major axis of the soma. More than 80% of the dendrites emerge at, or close to, the opposite extremities of the soma.

be spotted more deeply, scattered at different levels in the granular layer (Figs. 1E, 13, 14). Their polar dendrites bend slightly and ascend progressively towards the infraganglionic plexus where they continue to spread laterally. These neurons represent only a small fraction of the typical Lugaro cell population and are not included in the histogram of Figure 2A.

The Lugaro cell perikaryon is mostly elliptical or roughly triangular in shape (Figs. 1E, 10C), with average great and small diameters of, respectively, 16.1 µm (n = 77; σ = 2.7) and 9.5 µm (n = 77; σ = 1.5). Its major axis is usually oriented parasagitally and horizontally, parallel with the Purkinje cell layer. This elongated shape is enhanced by the thick dendrites that prolong each pole of the elliptical soma, thus blurring the exact limits between cell body and dendritic origin. The distinctive somatic bipolarity is featured in Figure 2B, in which the origin of each dendrite, independently of its length or thickness, is represented in a radial histogram: for 77 neurons, more than 80% of the 243 dendrites emerge from the opposite extremities of the soma. These polar dendrites are rather fleshy, their diameter being in the range of 1.5 µm to 4.5 µm at a 5 µm distance from the estimated origin. They stretch for a long distance, while narrowing regularly, just below the Purkinje cell somata, and their terminal course often slants either downwards into the granular layer or upwards into the low molecular layer (Figs. 1A, 6, 10A). Some other features can complement this basic morphological pattern. Not uncommonly, one or two slender dendrites are emitted by the soma or a major dendritic shaft and descend more or less vertically through the granular layer (Figs. 4, 8, 9B), sometimes even reaching the white matter (Fig. 10B). More rarely, one of the major dendrites may ascend vertically through the major part of the molecular layer either directly, as soon as it leaves the soma (Figs. 10A, 14), or after coursing for a certain distance horizontally (Figs. 4, 12). It remains that the strong tendency of the Lugaro cell's dendrites to extend towards, reach and stay in the immediate vicinity of the ganglionic layer is a most significant morphological feature (see for instance Figs. 1E, 10C, 13). In all cases, the dendritic processes are rarely ramified, their shafts remaining undivided for a long distance. When they do divide, the bifurcations take place rather near the beginning of the dendrite at acute angles. The terminal portion most often breaks up into a few thin and undulating ramifying branchlets, especially near the inferior pole of the Purkinje cell somata (Figs. 1E, 4, 8, 9). The spine coverage of Lugaro cell dendrites is usually rather low, the difference in the shapes of the spines, pedunculate (Fig. 5D) or sessile (Fig. 7C), suggesting the existence, at the dendritic level, of at least two different synaptic inputs.

Spatial spread of the dendrites

The important lateral extension of the dendrites from each side of the Lugaro cell soma has been reported in prior studies (Palay and Chan-Palay, 1974). However, in our sample of cells there is a continuum between short and long dendritic spans. The shortest of them (Figs. 8, 9) display rather slender and tortuous dendrites with a total length of 100 to 200 µm, passing beneath 6 to 10 Purkinje cell bodies. The dendrites of the longer cells are thicker, with a more direct course, and spread for distances exceeding 300–400 µm (Figs. 1B–D), that is, at least 15 to 20 Purkinje cell bodies. Some cells with extremely long dendrites (above 600–700 µm) are also encountered. No relationship could be found between the length of the dendrites and the topography of the part of the folium where the cell is located: flat, concave or convex. This seems to discard any significant local mechanical influence on the length of these neurites during development.

The elongated appearance of the polar dendrites is most evident in the parasagittal plane and is enhanced by the

two-dimensional projection mode used for illustrations. This representation is, however, misleading, since the different dendritic branches located on the same side of the cell do not, as might be concluded from the sole examination of the parasagittal camera lucida drawings, follow neighbouring courses in a strict proximity with one another. In fact, their respective paths diverge more or less as soon as they originate from the soma. This point is clearly illustrated in Figure 3A,B with three-dimensional computer reconstructions that have undergone a 90° rotation of the dendritic tree from their original parasagittal representation (respectively, neurons of Figures 1B and 11). They are thus viewed in the horizontal plane, i.e., in a plane parallel to the pial surface and to the Purkinje cell layer. The divergent paths of the main dendrites appear then quite strickingly: they draw an X shape centered by the cell body (soma not shown). These cells having been impregnated by the single section procedure, their neurites can be followed only within the section limits indicated by the two horizontal lines, a full black circle indicating the cut neurites. The same type of spatial spread of the dendrites in the horizontal plane was found for the other 6 computer-reconstructed neurons. In some of our sections, cut horizontally to the pial surface, impregnated Lugaro cells exhibited the same X-shape configuration as seen in the horizontal three-dimensional reconstructions. This is shown in the photomontage of Figure 3C. The elliptical soma of this Lugaro cell is prolonged, at each pole, by dendrites which diverge with an acute angle of about 45°, thus displaying a nearly perfect X. In some other cells, this feature is less recognizable, either because the angle between dendrites is much wider, as in Figure 3D, or because sections are cut more or less obliquely in respect to the Purkinje cell layer (Fig. 3E,F). This planar distribution of the Lugaro cell's dendrites in a horizontal plane, just beneath and parallel to the layer of Purkinje somata, is unique in the cerebellar cortex.

The axon

General features. The axonal arborization of Lugaro cells is seldom well impregnated beyond the initial segment in adult rats (over 30 postnatal days), in contrast with the other corticocerebellar interneurons. This is in favor of the Lugaro cell axon being myelinated, as it is well acknowledged that the presence of a myelin sheath generally impedes silver impregnation, and as it has been demonstrated that oligodendrocytes are present at the molecular layer level of the cerebellar cortex (Sotelo, 1967; Palay and Chan-Palay, 1974). We obtained 57 Lugaro cells impregnated with a significant portion of their axon in rats aged 13 to 45 postnatal days, 17 of which were from 14 to 20-day-old cerebella. We have shown in a prior study that Lugaro cells differentiate early when compared to basket and stellate cells (Lainé et al., 1992), their somatodendritic morphology being already recognizable as soon as 5 postnatal days. More important, their morphological characteristics appear mature as soon as 15 postnatal days. The same holds true for the axon. Indeed, the different types of projection patterns that were seen in young animals were confirmed by the few adult impregnated arborizations we found, entitling us to pool all the data in a single group.

Figures 4B and 6B illustrate camera lucida drawings of typical Lugaro cell axonal arborizations as they appear in parasagittal sections. The axon descends obliquely in the granular layer while giving off ascending collaterals which, by successive bifurcations, invade the molecular layer above the soma and dendrites of the parent cell with a complex branched and beaded plexus. In addition to the molecular plexus, some collaterals project exclusively in the granular layer, as is the case in Figure 6B. Both these features, profuse intricate plexus expanding through the molecular layer and more or less sparse projections to the granular layer, are common to all Lugaro cells axons. Each axon originates from the perikaryon or from a proximal dendritic trunk, with a robust conical initial segment prolonged by a stout and smooth stem 0.8 to 1.3 μm in diameter. The axon then usually emits 3 to 4 collaterals in succession, often at irregular intervals. It may happen, especially when the first branching takes place inside the ganglionic or low molecular layers, that a number of collaterals arise from a single plurifurcation, as in Figure 9A, in which the axon gives off five branches at a single site, 45 μm distant from its origin. All the collaterals are rather regular in caliber, in the order of 1 μm, that is, about the same diameter as the parent stem. They are then far larger than the thin granule cell axons crossing in the vicinity.

Variability of the axonal trajectory. A striking characteristic of this neuron is the variability of the axonal trajectory. A comparative analysis shows that Lugaro cell axons can be divided into two groups, according to their direct or indirect trajectory to the molecular layer. In the first case (34 out of 57), the axon takes an ascending course, not far from its origin, to immediately enter the molecular layer where it breaks into a profuse local plexus. In the indirect configuration (23 out of 57), the axon heads downwards in the granular layer while giving off upwardly directed branches which invade the overlaying molecular layer. After travelling to a more or less great depth in the granular layer, the distal part of the axon also turns upwards to reascend and terminate in the molecular layer. Some axons of the indirect subtype can approach and even enter the white matter in the center of the folium, but in all instances, they shortly leave it to reascend towards the molecular layer quite distally from their origin. No axonal collateral was seen coursing inside the white matter corticofugally, in direction of the deep nuclei.

The axons of the directly ascending subgroup (Figs. 8, 9) always exhibit an horizontal or upwardly directed initial

Fig. 3. The spatial spread of the Lugaro cells dendrites in the infraganglionic plexus, as seen in an horizontal view, i.e., parallel to the pial surface and to the Purkinje cell layer. **A,B:** Fil-de-fer computer reconstructions of the dendrites rotated by 90° from the parasagittal plane (soma not shown). Same cells as in Figures 1B and 11, respectively. The different polar dendrites clearly take divergent paths soon after their origin. The horizontal lines indicate the section limits. **C,D:** Photomontages of two Lugaro cells impregnated in horizontal cerebellar sections. They confirm the dendritic pattern found by rotating the computer-reconstructed neurons in A,B. At each pole the dendrites deviate from each other with acute angles in a V manner, thus drawing an X centered by the cell body. Note that in D, parallel fibers (arrows) can be seen running in the molecular layer immediately above the impregnated neuron. **E,F:** Camera lucida drawings of two Lugaro cells impregnated in sections cut obliquely in respect to the Purkinje cell layer. The Purkinje cell somata appear as a sheet instead of a single row. The angle between two polar dendrites is more acute than in the preceeding strictly horizontal views, especially in E. Young adult rats, single section Golgi technique. Scale bars = 50 μm.

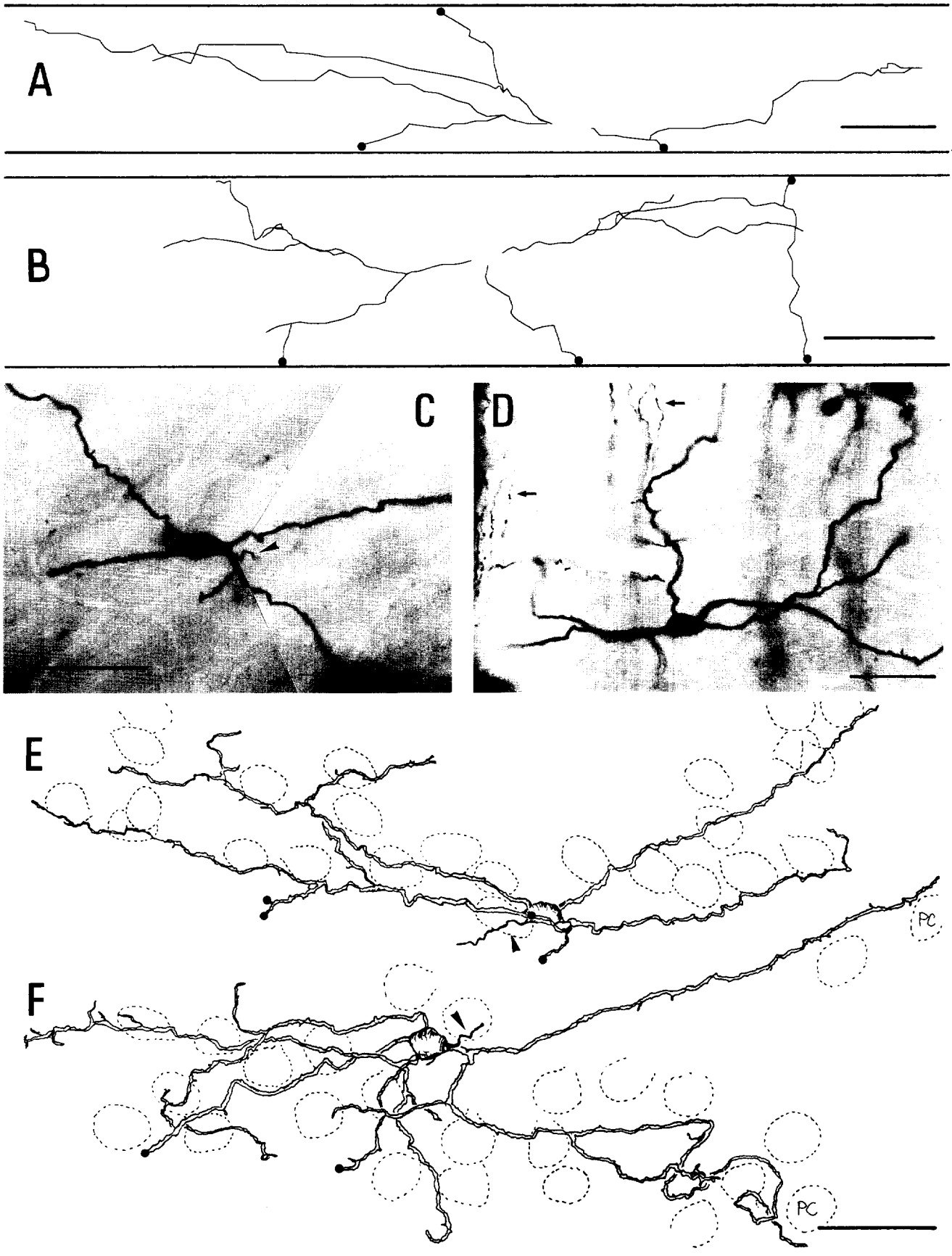

Figure 3

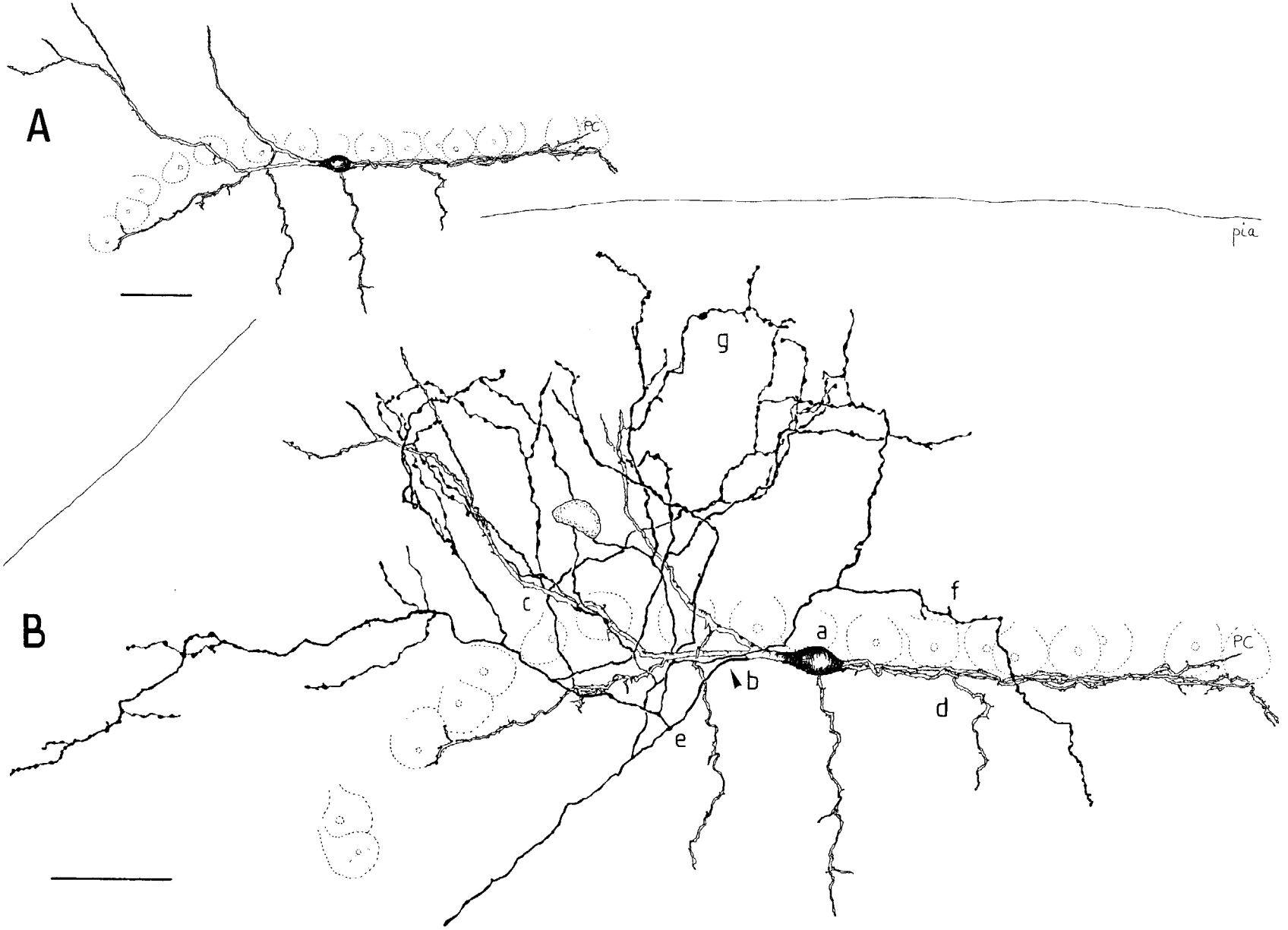

Fig. 4. Camera lucida drawings of a Lugaro cell impregnated with its axon. **A:** Separate drawing of soma and dendrites to show the location of the neuron and its dendritic spread in both the granular and molecular layers. **B:** The axon originates from one of the polar dendrites and heads downwards in the granular layer where the distal part failed to impregnate. It gives off ascending collaterals that ramify profusely in the molecular layer. One of the molecular branches gives a descending collateral terminating in the upper granular layer. Parasagittal section, rapid Golgi technique, 18-day-old rat, folium IXa. a–g indicate details illustrated in Figure 5. In this and subsequent figures, stippled zones indicate crystalline artifacts that obscure a portion of a neurite. Scale bars = 50 μm.

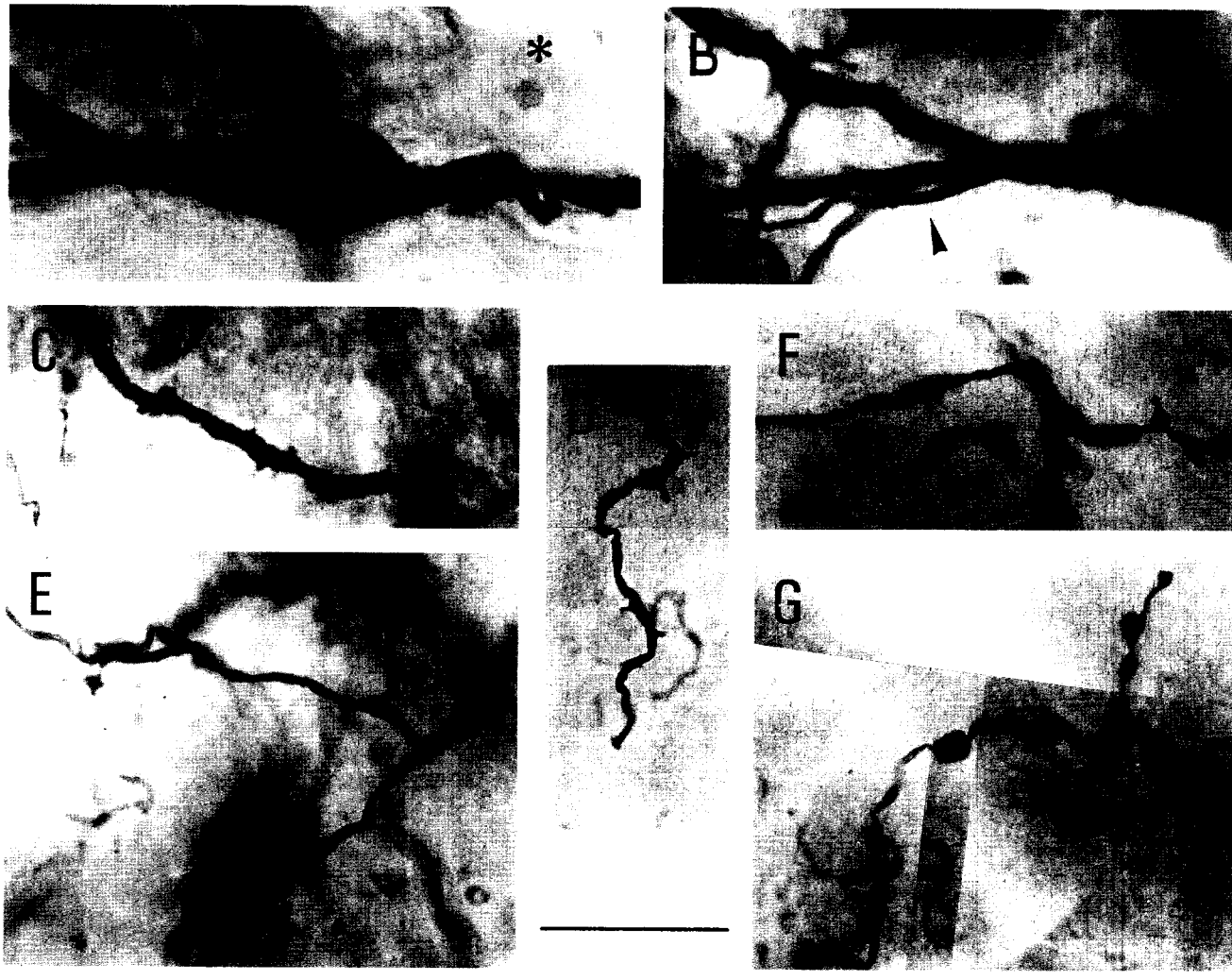

Fig. 5. High magnification micrographs illustrating details of the Lugaro cell drawn in Figure 4. **A:** The fusiform soma is prolonged by thick dendritic shafts at each pole and is located at the interface between the granular and Purkinje cell layers. The nucleoli of two Purkinje cell somata located just above are distinctly visible (asterisks in A and B). **B:** The initial segment of the axon (arrowhead), with a classical conical shape, originates from the unique left dendrite, just before its bifurcation. **C:** Sessile spines on the molecular portion of a dendrite. **D:** Necked spines on a granular dendrite. **E:** Mode of bifurcation of the axon in the granular layer that gives off two ascending branches. **F:** An axonal branch coursing above the upper pole of Purkinje cell somata. **G:** The beaded ending of an axonal collateral in the high molecular layer with large rounded swellings. Scale bar = 20 μm for A–G.

segment. They wind more or less horizontally for a short distance between the Purkinje cell somata and emit successive ascending fibers. These collaterals are rather thick and rise obliquely into the molecular layer where they branch in a dichotomous manner, forming a complex plexus which occupies the inferior two-thirds (Figs. 8, 9A) or even the entire thickness of the molecular layer (Fig. 9B). The axonal ramifications display numerous closely spaced rounded varicosities. Especially before their termination, the collaterals can arise from a trifurcation, the terminal branches usually appearing as a succession of globular enlargements connected by slender threads (Figs. 5G, 7A, 8). This direct axonal pattern also includes granular layer projections, as some descending collaterals are seen to terminate below the infraganglionic plexus (Figs. 8A, 9, small arrows), and as varicosities are always present along the axonal shaft while it courses at the border between granular and Purkinje cell layers (Fig. 8B). An interesting observation is that these direct molecular layer projections are most often associated with Lugaro cells displaying an overall small dendritic field, this being the only correlation found between any two morphological traits of the Lugaro cells in our series.

The axons of the indirect subgroup have initial segments that, in contrast with those of the direct subgroup, engage immediately downwards into the granular layer. During their descending and somewhat oblique course, the fibers emit successive collaterals at irregular intervals. The trajectories of the successive branches always obey a same principle: they hook back in a more or less open arciform curve, sometimes dividing, reascend and systematically terminate inside the molecular layer where they ramify repeatedly (Figs. 4, 6, 10A,B). The individual trajectory of these axons through the granular layer may vary significantly. The axon can either remain in the upper half of this layer as in Figures 4, 6 and 10A, either approach and travel

Fig. 6. Camera lucida drawings of another typical Lugaro cell impregnated with its axonal arborization in the cerebellum of a young adult rat. **A:** Soma and dendrites drawn separately, evidencing the elongated bipolar pattern characteristic of this cell type. In this case, the dendrites remain inside the granular layer. **B:** The axon originates from the soma and breaks up in a rich plexus projecting both to the granular and molecular layers. All the branches display richly beaded profiles. Parasagittal section, single section Golgi procedure, young adult rat, folium V. a–d: Details illustrated in Figure 7. Conventions as in Figure 1. Scale bars = 50 μm.

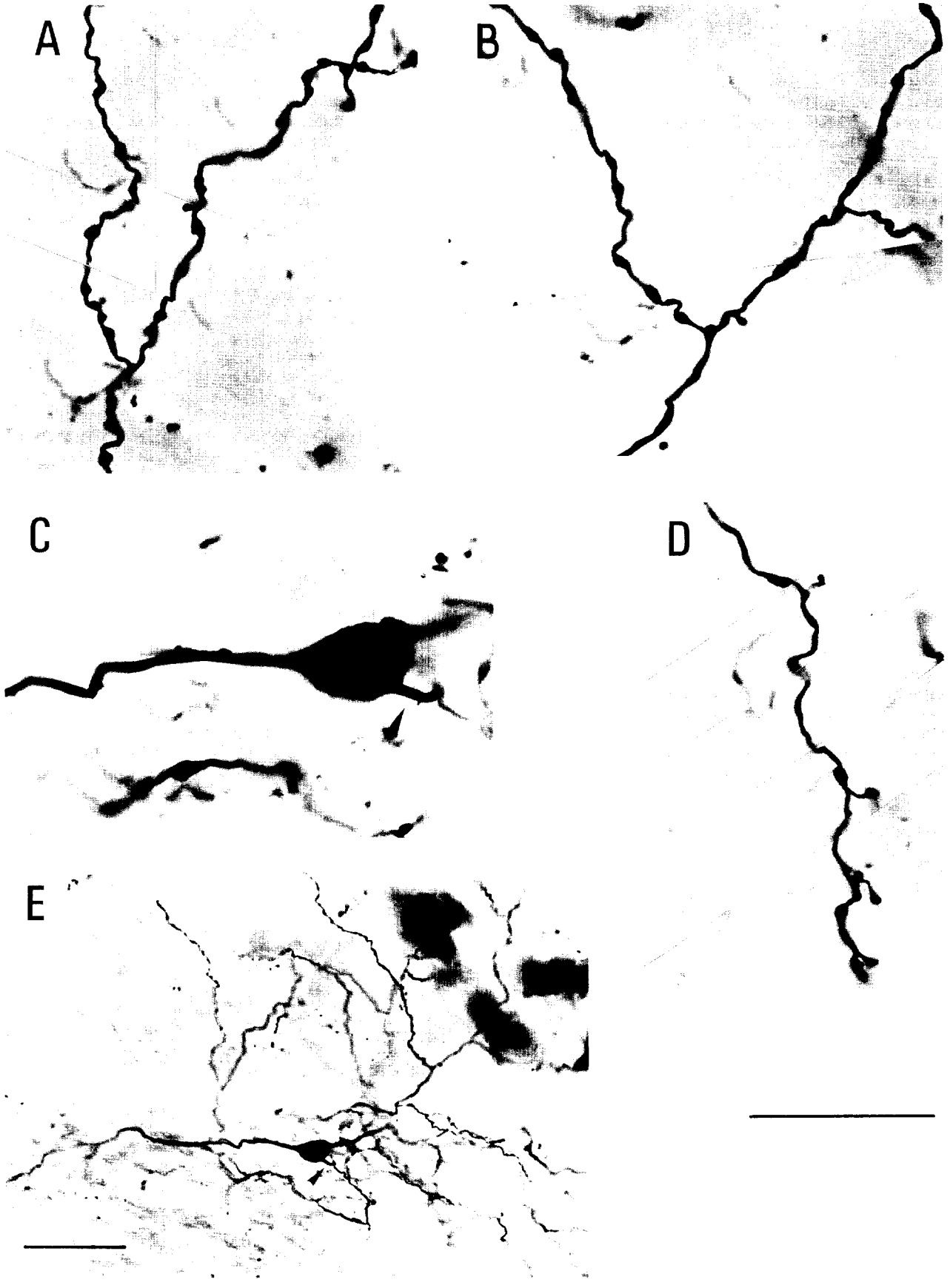

Fig. 7. Same cell as in Figure 6. **A,B:** Photomontages of the thick varicose ramifications of two axonal collaterals in the inferior third of the molecular layer. **C:** The ovoid pericaryon from which emerge the axonal initial segment and two dendritic trunks. Part of a granular branch of the axon is visible beneath the left dendritic shaft, bearing large varicosities. **D:** Beaded terminals of an axonal collateral in the middle granular layer. **E:** Low magnification photomicrograph of the whole cell. Conventions as in Figure 1. Scale bar for A–D = 25 μm, bar in E = 50 μm.

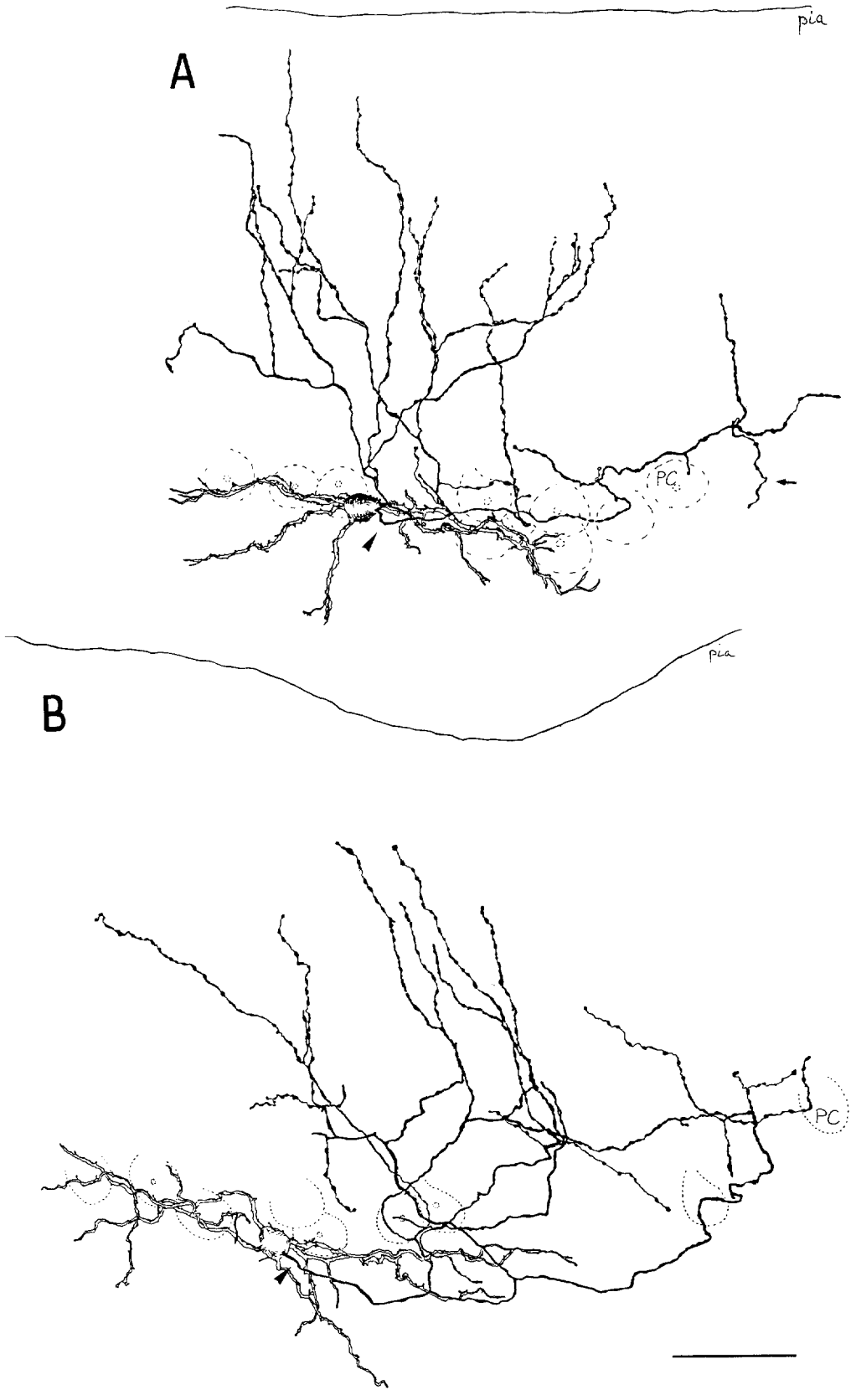

Fig. 8. Camera lucida drawings of two Lugaro cells with a directly ascending axon. **A:** The axon originates from the soma and runs horizontally in between the Purkinje cells somata. It emits successive collaterals that ascend and ramify in the molecular layer. These give off beaded oblique branches, two of which reach the superior one-third of the molecular layer, whilst the descending portion of another ends in the granular layer (small arrow). Note that most of the molecular spread of the axon is located just above the somatodendritic field of its parent neuron. **B:** The pattern of the axonal arborization in this neuron is quite similar to the one in A. A complex arbor branches in the molecular layer exactly above its somatodendritic spread, and an horizontal collateral spreads laterally in the Purkinje cell layer and branches in short collaterals projecting in, or near, the ganglionic layer. Parasagittal sections, rapid Golgi technique, 3-week-old rats, folium IV and V respectively. Conventions as in Figure 1. Scale bar = 50 μm.

alongside the white matter as in Figure 10B,C, or even enter the latter for a certain distance as in Figures 11–14. In the case of particularly complete impregnations, the molecular layer terminal projections exhibit the same varicose intricate ramifications as those of the direct group of axons. Indirect axons also have granular layer projections: some short collaterals are indeed confined to this layer (Figs. 6, 7D, 10A,B, 11–13), and there are varicose, presumably synaptic, enlargements scattered en passant along the axonal shafts (Figs. 11, 14).

Distally projecting fibers. As noted in the Introduction, Lugaro (1894: Plate IX, Fig. 12) and Fox (1959: Plate 2) on the one hand, Ramón y Cajal (1911: Fig. 35) and Palay and Chan-Palay (1974: Fig. 118) on the other, illustrate quite different axonal patterns for this cell type: lengthy fibers coursing in a mediolateral direction in the lower molecular layer in the first case, granular layer fibers touching the white matter in the second. In fact, both configurations are seen among Lugaro cell axons and seem to constitute two distinct types of distally projecting collaterals.

The long descending axonal stem is but a particular case of the indirect subgroup described above. In our specimens, this type of axon originates from a soma located near the apex of the folium and courses obliquely through the entire depth of the granular layer down to the central white matter. Usually the Golgi impregnation stops there (Fig. 11), as was the case for Ramón y Cajal's (1911) and Palay and Chan Palay's (1974) specimens. We were fortunate enough to obtain a few such axons more completely impregnated (Figs. 12–14). In all cases, these axons can be seen to change their course inside the white matter and to reascend laterally through the granular layer, heading towards the molecular layer located on one of the sidewalls of the same folium. In one case, the axon is even seen to enter the low molecular layer (Fig. 14). All these long fibers appear to have a terminal field located in about the same parasagittal plane as the parent cell, but are distal by more than 200–400 μm in a rostrocaudal direction. No evidence whatsoever of a putative corticofugal branch was found.

The other distally projecting fibers, those coursing laterally in the molecular layer parallel to the parallel fibers, were originally seen by Lugaro and Fox in horizontal sections and are difficult to distinguish in parasagittal ones. We used rotated three-dimensional reconstructions to identify this type of collateral. Figure 15 A1, A2 shows computer reconstructions of the Lugaro cell axon of Figure 4. This has been rotated by 90° from the parasagittal plane so it can be viewed, respectively, in a transverse plane (i.e., parallel to the longitudinal axis of the folium and perpendicular to the pial surface), or in a horizontal plane (i.e., parallel to the pial surface). The arrows indicate the direction of the parallel fibers. In a transverse view (Fig. 15, A1), the axonal plexus in the molecular layer appears as intricate as in the parasagittal view, without any obvious geometrical regularity. One molecular collateral (Fig. 15, A1, asterisk) is seen to have its origin at a low level and to gradually ascend while moving away in the same direction as the parallel fibers. When seen horizontally (Fig. 15, A2), the same collateral appears to extend in the mediolateral direction for more than 100 μm. The local axonal arborization has a grossly elliptical envelope with a parasagitally oriented great axis, parallel to the spread of the Purkinje cell dendritic trees. In Figure 15, B1, B2, the transverse and horizontal views of the axon of another Lugaro cell (not illustrated) also displays such a lengthy mediolateral branch. As is the case for all the other Lugaro neurons, this cell has an axonal plexus projecting in the molecular layer above the parent soma, the location of which is indicated by the axon's initial segment (Fig. 15, B1, B2, arrowhead). From this local projection a longitudinal collateral (Fig. 15, B1, B2, asterisk) courses just above the Purkinje cell layer, gives off ascending molecular branches and ends about 200 μm laterally from its origin.

In summary, Lugaro cell axons display a local (direct or indirect), profuse, molecular layer projection that is located just above the parent cell soma and dendrites. They also display some granular layer collaterals. Both these projections are present in all the impregnated axons in our series. Moreover, two types of distal molecular projections are also found. One of them is a long fiber coursing through the depth of the granular layer, and even in the white matter, before it reascends into the molecular layer, in about the same parasagittal plane but at some distance from the soma in the rostrocaudal direction. The other is an entirely molecular longitudinal fiber coursing for a certain distance, in the mediolateral direction, that is parallel to the parallel fibers. It is not clear for the moment how these different molecular projections, the constant local one and the two types of distal ones, combine at the single cell level. In all cases, the axon remains within the cerebellar cortex and the Lugaro cell must therefore be considered as a purely corticocerebellar interneuron.

DISCUSSION

In the present study we have examined, in a significant number of neurons, the somatodendritic and axonal features of Golgi-impregnated Lugaro cells of the rat cerebellar cortex. We describe here for the first time the particular X-shaped spatial spread of the dendrites, and the different trajectories and terminal projections of the axon. The elusiveness to impregnation of the axon and the striking variability of its trajectory had lead to a long-standing uncertainty about the exact status of this neuron. The Lugaro cell appears in fact to be a morphologically homogeneous and ubiquitous neuronal type which is located and projects exclusively inside the cerebellar cortex. It must therefore be considered as a regular class of corticocerebellar interneurone.

The Lugaro cell type

The morphological identification of a neuronal class requires that the cells share common characteristics in respect to three classical criteria: soma location, somatodendritic typology and axonal pattern. We show that the Lugaro cells, which are found in all parts of the cerebellum, fulfill all these criteria and must therefore be considered as a distinct cell class: (1) they are nearly always located at the upper border of the granular layer, just beneath the monolayer of Purkinje cell somata, only very few neurons being scattered in the depth of the granular layer; (2) they have a bipolar fusiform shape, the soma being elongated in a parasagittal direction from which the long dendrites radiate in a diverging manner, extending in a flattened horizontal X underneath the ganglionic layer; and (3) their axon projects into the molecular layer with a constant profuse local plexus and some apparently inconstant distal fibers, while a few sparse projections to the granular layer are also systematically found. This pattern, which we illustrate and describe for a great number of axons, stresses

the intermediate role played by this neuron between the granular layer, where it receives its inputs, and the molecular layer, to which the major axonal branches project (Lugaro, 1894).

Our description of the soma confirms and extends prior reports on Golgi-impregnated material (Lugaro, 1894; Fox, 1959; Palay and Chan-Palay, 1974), or on material stained with a specific antibody (Sahin and Hockfield, 1990). The three-dimensional analysis of the spatial extent of the polar dendrites has shown that it forms a flat X centered by the cell soma. This underlines an important specificity of this cell type. It is indeed the only interneuron in the corticocerebellar network which has an horizontally flattened dendritic arbor, in strong contrast with all the dendritic trees of the molecular layer, which expand in a vertical parasagittal plane, or with those of the granular layer, which are inscribed in conical (Golgi cells) or spherical (granule cells) volumes. Lugaro cells are then organized in a sort of lattice at the level of the infraganglionic plexus, thus sampling the activity of the fibers crossing their given territory. A study using restricted dextran-biotin injections in the rat cerebellar cortex confirms, in the labelled Lugaro cells, all the above morphological features, including the axonal pattern (work in progress). Its characteristics clearly differentiate this neuron from other corticocerebellar interneuronal classes. It is, in particular, clear that one cannot consider Lugaro cells as displaced basket cells. These latter are, indeed, characterized by fan-shaped dendrites raising in a vertical parasagittal plane through the molecular layer, and an axon extending also in the parasagittal plane, with a number of descending collaterals which contact the lower half of the Purkinje cell soma and form a specific basket-like net (for a quantitative analysis of Golgi-impregnated basket cells morphology, see Lainé and Axelrad, 1994).

Considering the homogeneity of their morphological characteristics, as well as their omnipresence in the different folia of the rat cerebellum, Lugaro cells must be considered as a constant element of the corticocerebellar network. These neurons have been found in birds (O'Leary et al., 1968) as well as in numerous mammals (rodents: O'Leary et al., 1968; Palay and Chan-Palay, 1974; felines: Lugaro, 1894; Ramón y Cajal, 1911; Mugnaini, 1972; primates: Fox, 1959; Christ, 1985; human: Golgi, 1874; Jakob, 1928; Braak and Braak, 1983). In all these species, they likely maintain the same essential features considering that, in our Golgi preparations of chicken, cat, rabbit, monkey, and human cerebelli, they do not seem to differ, at least qualitatively, from those established in the rat. This cell type must then be added to the "basic cerebellar circuit" that has been shown to exist from reptiles to birds and mammals (Llinás and Hillman, 1969).

During rat cerebellar postnatal development, Lugaro cells differentiate as early as Golgi cells (Lainé et al., 1992), thus suggesting that both cell types originate from the ventricular neuroepithelium. Lugaro cells may in fact be among those lately formed "large neurons" located superficially in the granular layer, near the Purkinje cell layer, and presumed to be Golgi cells by Altman and Bayer (1978). In an elegant recent study using retroviruses carrying the β-galactosidase gene to analyse the fate of the dividing progenitors in the white matter of the immature postnatal rat cerebellum, no mention is made of Lugaro neurons. This appears astonishing because Golgi interneurons were labelled in the granular layer, alongside the different interneurons of the molecular layer (Zhang and Goldman, 1996). Further studies are then necessary to ascertain the embryological origin and exact migration route of the Lugaro cell class.

Somatodendritic variability

Some of the above-mentioned morphological criteria can undergo variations and deserve a special mention. The vast majority of the cells are located in a narrow zone, immediately between or under the inferior poles of the Purkinje cell somata, whereas a very small minority of them are scattered throughout the entire depth of the granular layer. This had been noted in earlier Golgi or immunocytochemical studies (Golgi, 1874; Jakob, 1928; Fox, 1959; Eccles et al., 1967; O'Leary et al., 1968; Palay and Chan-Palay, 1974; Braak and Braak, 1983; Rogers, 1989; Sahin and Hockfield, 1990). The functional consequences of these rather displaced somata might not be that important, for we show that the dendrites have a strong tendency to extend towards and reach the infraganglionic plexus, thus allowing these neurons to receive the same mix of inputs as the regularly located Lugaro cells. On the other hand, even in the very rare cases where the dendrites of some of the displaced cells do not reach their usual location, it is most likely that the Purkinje cell axon recurrent collaterals, and the ascending portion of the granule cells axon, which are known to constitute the main inputs to this cell type (Palay and Chan-Palay, 1974), can easily find their assigned postsynaptic target.

Lugaro cell dendrites have systematically been described as very long. From the large number of impregnated neurons in our study, it appears that the extent of the dendritic field varies in a wider range than previously reported. Lugaro cells with short dendrites can often be found, which share all the typical features of the Lugaro cell type, especially somatodendritic bipolarity and molecular axonal projection. They are characterized by a somewhat restricted dendritic expansion, with a global parasagittal span in the order of 100–150 μm, in contrast with the 600 to 700 μm of the longest ones. These great differences in dendritic length may be the explanatory ground for the observation that Lugaro cells are not always regularly interspersed in the cerebellar cortex. As we pointed out in the Results, the Golgi method is not well suited to give quantitative data about the relative proportions of different

Fig. 9. **A:** A Lugaro cell with a particularly dense and intricate direct axon. In contrast to the axons illustrated in the other figures, which emit a succession of collaterals, this axon gives off its collaterals by means of a unique plurifurcation of 5 branches at 45 μm from the axon hillock, at the level of the apex of the Purkinje cells somata. This mode of collateralization by plurifurcation is not exceptional in the Lugaro cells' axon. The emerging branches then loop horizontally between the Purkinje cell somata before ascending obliquely into the molecular layer where they ramify and become varicose. Note that the section is not strictly parasagittal. **B:** Same kind of axonal arborization as in Figure 8, in this case projecting through the entire thickness of the molecular layer. The black circles indicate two collaterals that take a direction parallel to the parallel fibers, but are cut at the section surface. Unfortunately, these were impossible to follow with certainty in the neighbouring section, due to an excessive number of stained elements. Small arrows in A and B indicate in each case, a collateral ending in the granular layer. Parasagittal sections, rapid Golgi technique, 3-week-old rats, folium VI and IV, respectively. Conventions as in Figure 1. Scale bar = 50 μm.

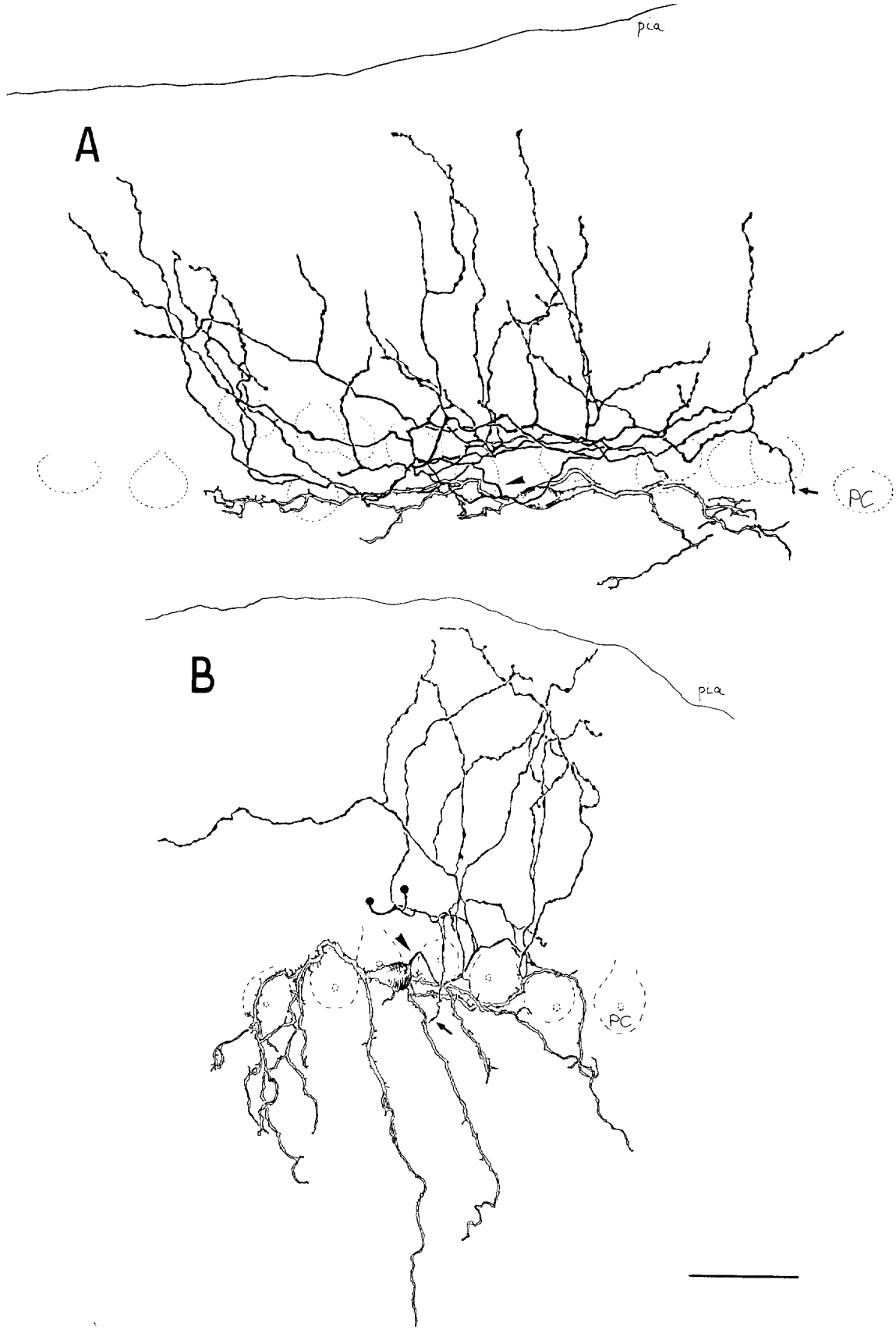

Figure 9

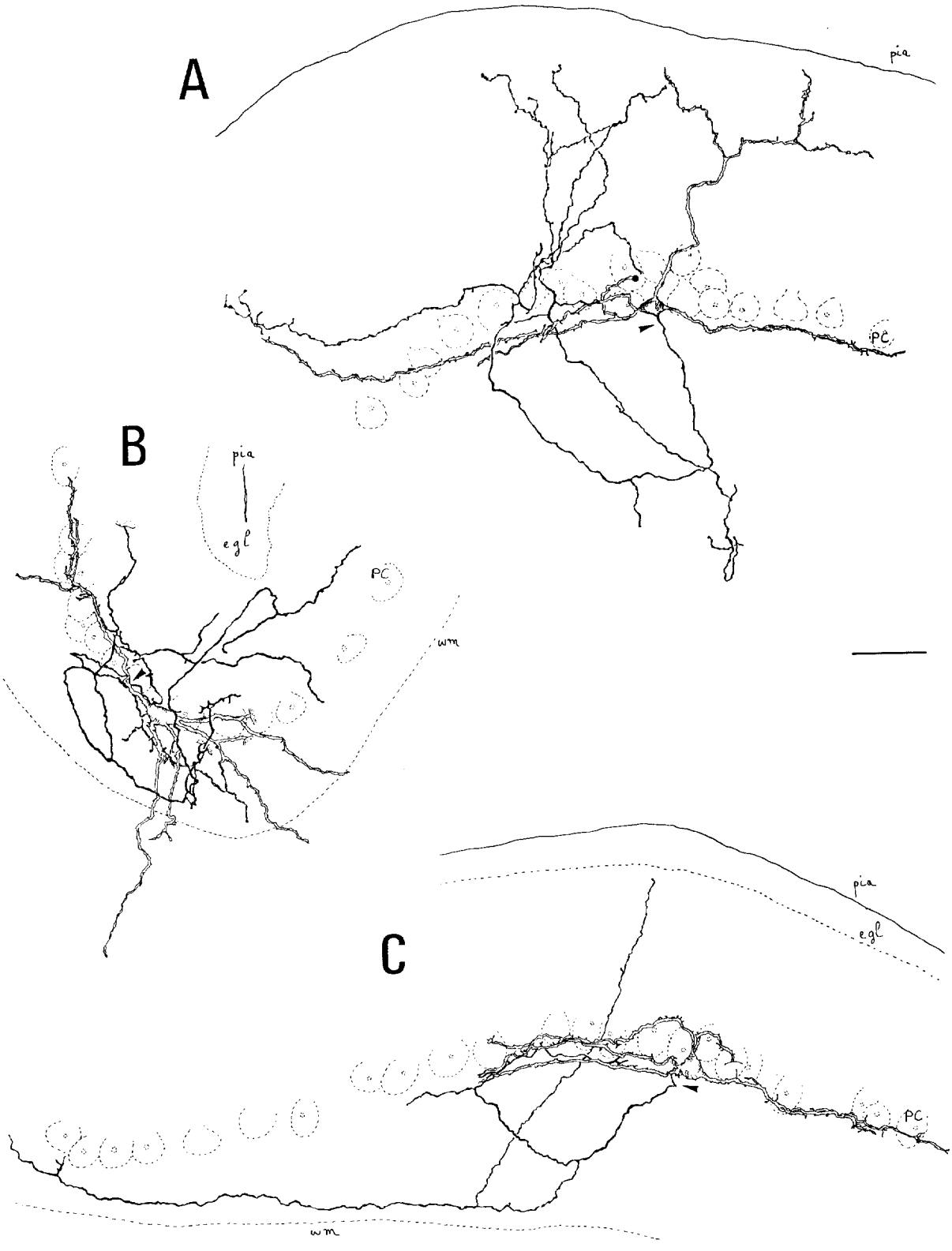

Fig. 10. Lugaro cells with an axon projecting to the molecular layer after several arciform or U shape turns in the granular layer. **A:** The somatodendritic pattern of this Lugaro cell is unusual, for a stout dendrite emerges from the upper convexity of the ovoid cell body and ascends vertically through the molecular layer. Its axon, only partially stained, originates from the soma and has a descending vertical course in the granular layer. It gives off several collaterals and, at midgranular layer level, makes a hairpin turn to reascend, but is then no longer impregnated. Only two arciform collaterals are well stained. These ascend obliquely, divide in the ganglionic layer and project throughout the inferior two-thirds of the molecular layer. **B:** Another indirect axonal pattern with collaterals making arciform turns before dividing and ascending in the molecular layer. They also give a few short beaded collaterals in the granular layer. **C:** The axon of this incompletely stained neuron descends obliquely and emits two ascending collaterals. It then runs horizontally into the deep granular layer close to the white matter and gradually ascends into the granular layer. The impregnation ends near the Purkinje cell layer, at a few hundred microns from its origin. Parasagittal sections, rapid Golgi method. A: 29-day-old rat, folium IXa. B and C: 13-day-old rat, folia II and IXc. egl, external granular layer in this and subsequent figures. Conventions as in Figure 1. Scale bars = 50 μm in A, C, 45 μm in B.

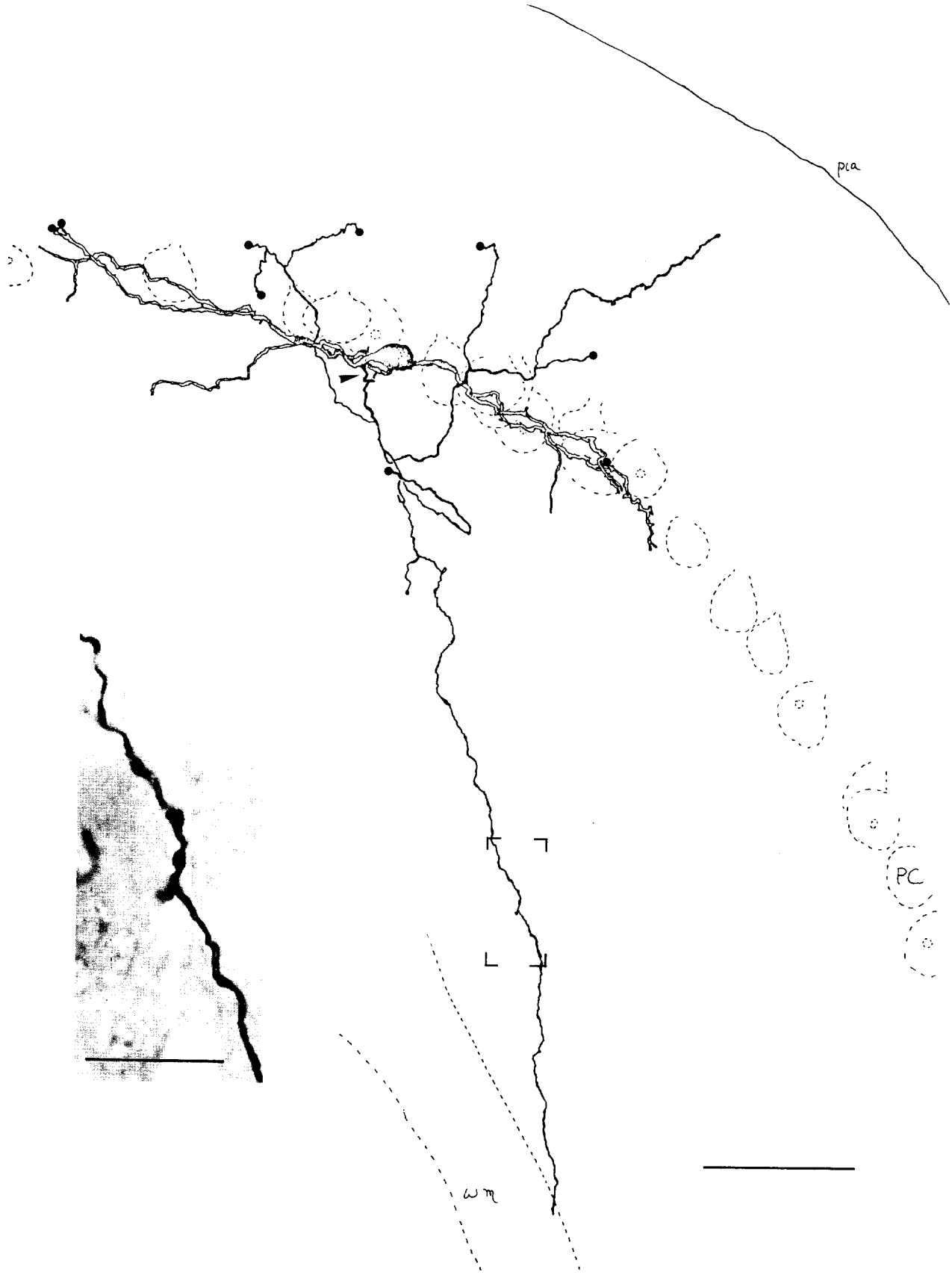

Fig. 11. Camera lucida drawing of a Lugaro cell whose axon reaches the white matter. The axon descends obliquely through the entire granular layer. During its course it branches in local or ascending collaterals. It then enters the white matter where its impregnation stops short. The unimpregnated distal portion can be faintly seen for a few more microns with Nomarski optics. **Inset:** High magnification micrograph of a granular portion (frame corners on the drawing) of the descending axonal shaft that shows several, presumably synaptic, varicosities. Young adult rat, folium I, single section Golgi procedure. Conventions as in Figure 1. Scale bar = 50 μm, 10 μm in inset.

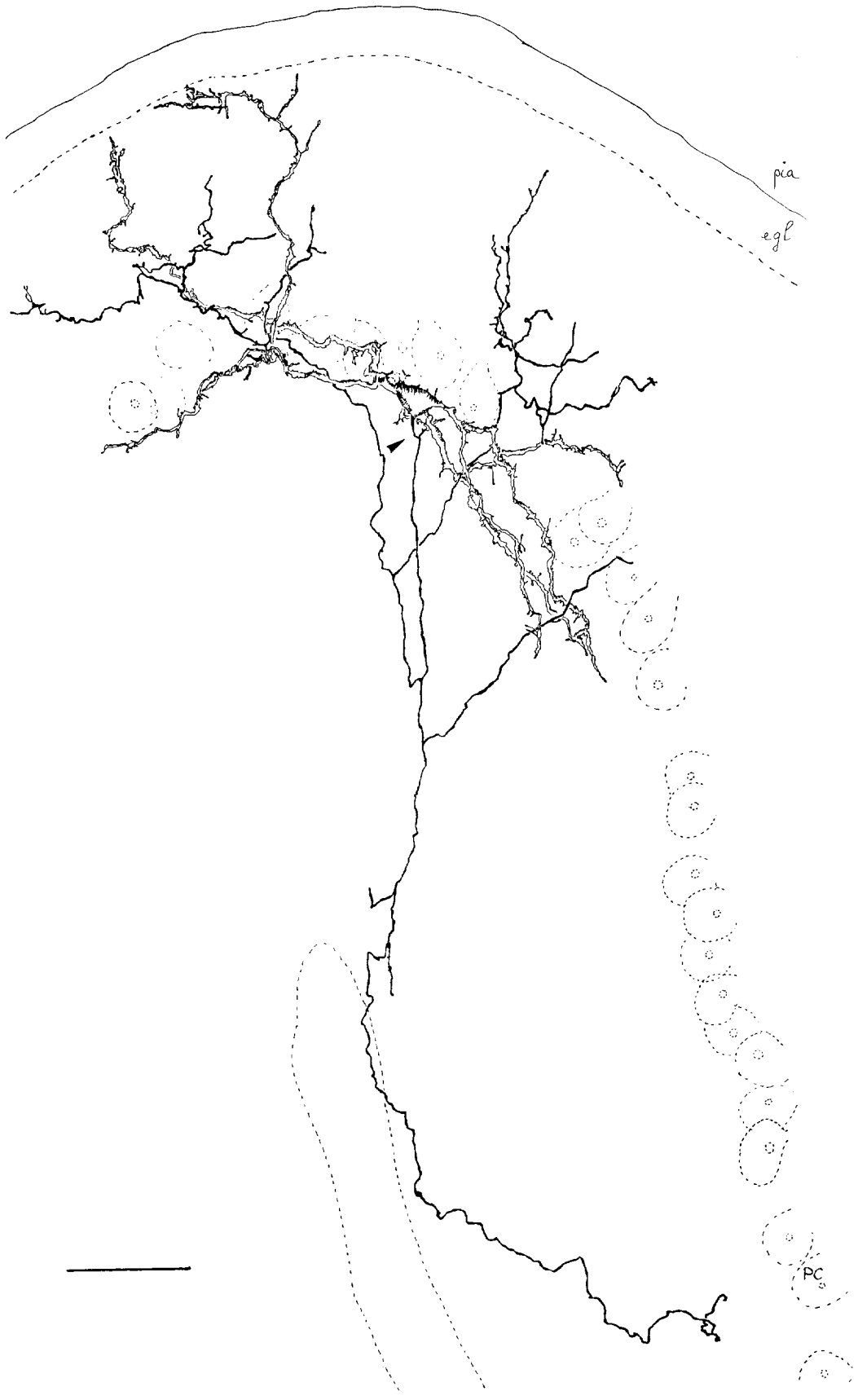

Fig. 12. A Lugaro cell whose axon courses for a short distance in the white matter before reascending in the upper granular layer at more than 400 μm from its origin. The axon emerges from the cell body located at a lobule summit. It descends vertically and gives off, at acute angles, several collaterals that ascend and project into the molecular layer just above the parent cell dendrites. The descending branch enters the white matter for some tens of microns and then curves to ascend obliquely through the granular layer, in the direction of the molecular layer located on the side of the same lobule. Its impregnation unfortunately stops near the Purkinje cell layer. Rapid Golgi technique, 13-day-old rat, folium IX a. Conventions as in Figure 1. Scale bar = 50 μm.

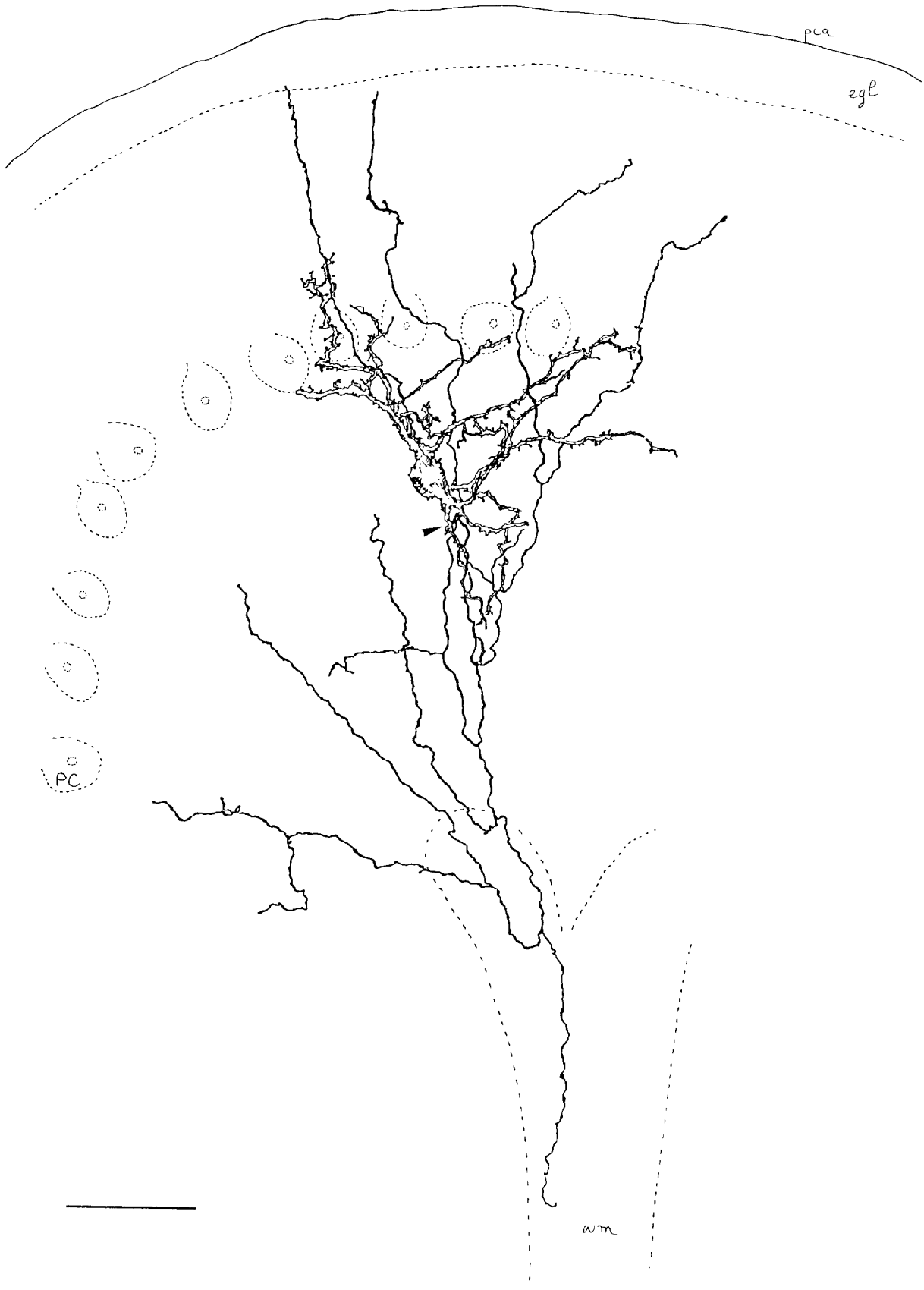

Fig. 13. A Lugaro cell whose axon makes a U-shape turn in the white matter. The fusiform soma is unusually located at a certain distance from the Purkinje cell layer and is obliquely oriented. Note that the majority of its bipolar dendrites head towards and reach the infraganglionic layer. The axon descends into the white matter while emitting successive collaterals which make several hairpin turns to reascend towards the molecular layer. A beaded collateral emerges from the U in the white matter, tapers and ends a little lower by a terminal bouton. Rapid Golgi technique, 13-day-old rat, folium IV. Conventions as in Figure 1. Scale bar = 50 μm.

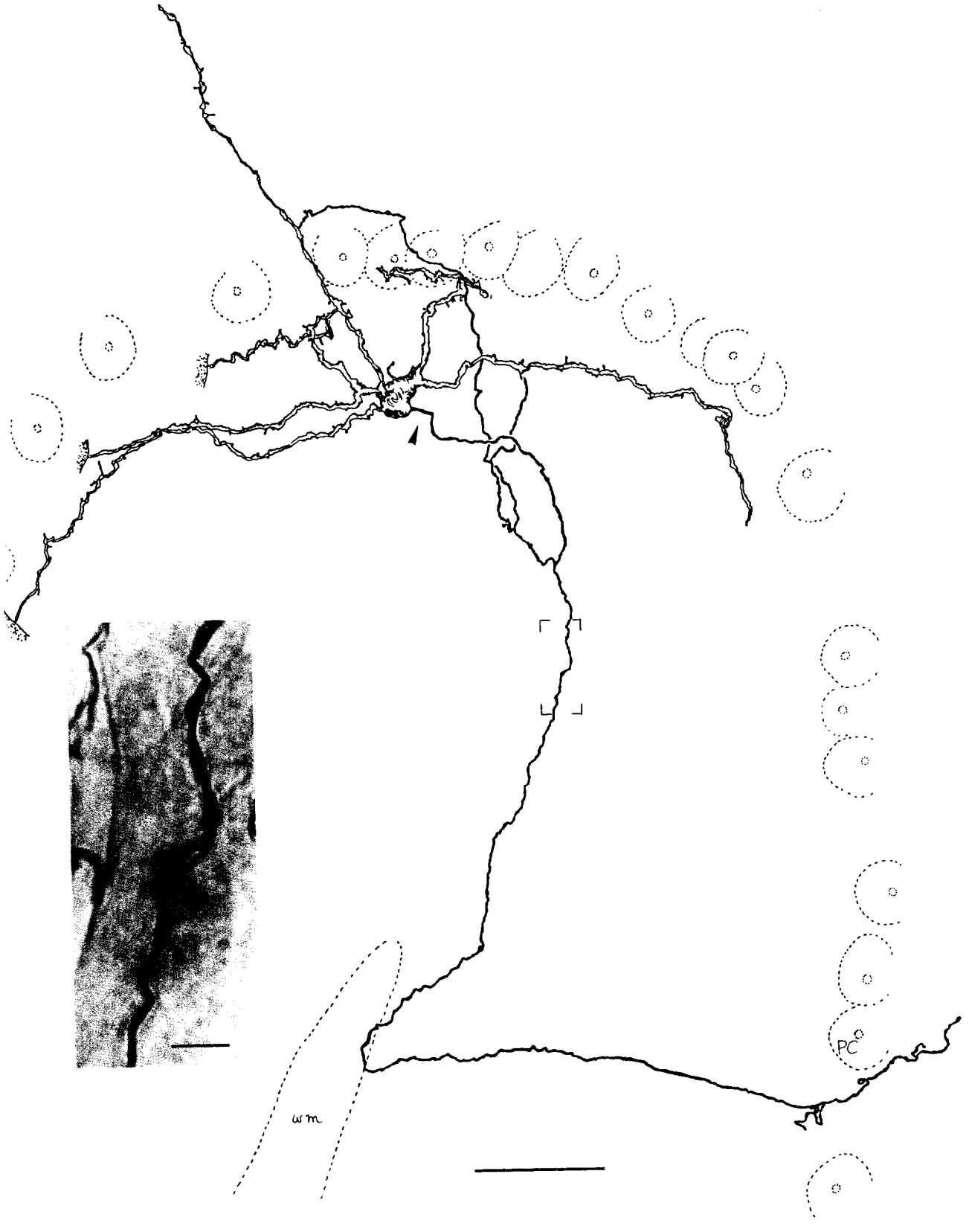

Fig. 14. Camera lucida drawing of another example of Lugaro cell whose axon projects into the molecular layer after a U-turn in the white matter. Five dendrites originate from the soma, all of which, but one, reach the infraganglionic plexus, the last one ascending vertically in the molecular layer. The partially stained axon displays the same pattern as in the neurons illustrated in the three preceding figures: a descending trajectory giving off ascending collaterals, a short portion inside the white matter and a terminal portion reascending the granular layer finally to enter the molecular layer at more than 300 μm from the parent soma. **Inset:** High magnification micrograph (frame corners on the drawing) of several enlargements of the axon in the granular layer. Rapid Golgi technique, 36-day-old rat, folium VIa. Conventions as in Figure 1. Scale bar = 50 μm, 10 μm in inset.

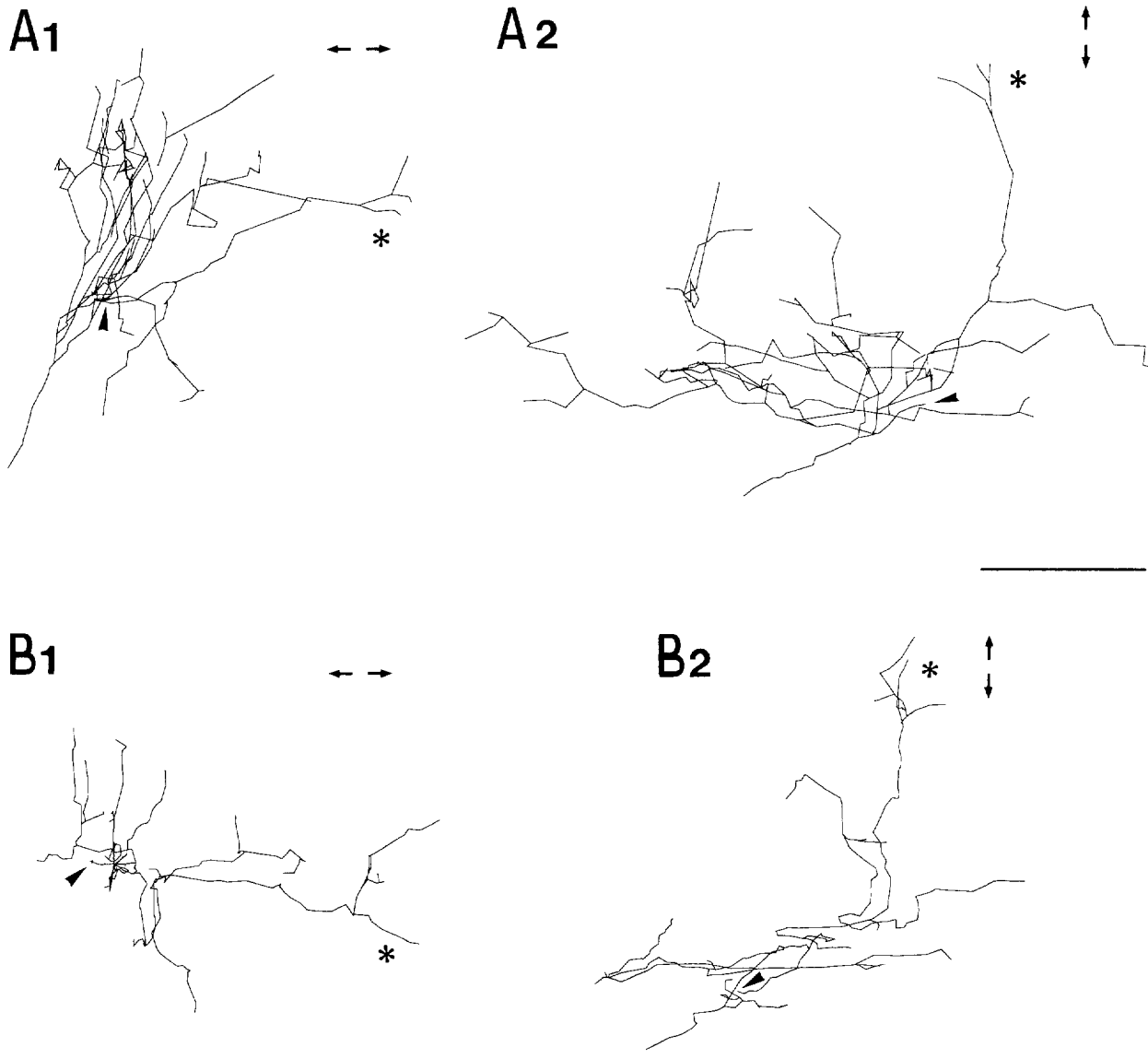

Fig. 15. Computer reconstructions of two Lugaro cell axonal arborizations demonstrating distal projecting collaterals, parallel to the longitudinal axis of the folium. A1, A2: Same neuron as in Figure 4. **A1:** View of the axon in the transverse plane, after a 90° rotation from its original orientation in the parasagittal plane. The axonal projection invades the major part of the molecular layer, just above the parent cell body, with a dense and complex plexus. One collateral, marked by an asterisk in A1 and A2, is seen to ascend obliquely in the molecular layer while moving away in a direction parallel to the longitudinal axis of the folium. **A2:** The same axonal plexus viewed in an horizontal plane. The axonal plexus covers a roughly elliptical area with a parasagittal great axis. The longitudinal collateral projects to a site more than 150 μm away from the main plexus. **B1, B2:** The axonal tree of another Lugaro cell (not illustrated) viewed, respectively, in the same transverse and horizontal planes as in A1, A2. This axon also displays both a cluster of molecular collaterals overlaying the parent cell body, and a lengthy longitudinal collateral (asterisk in B1 and B2). This latter, after a hairpin turn in the granular layer, runs in the low molecular layer in the same direction as the parallel fibers. After emitting en route some collaterals, it ends more than 200 μm away from its origin. Arrows indicate the direction of the parallel fibers, i.e., the long axis of the folium. Conventions as in Figure 1. Scale bar = 100 μm.

cell types in a given structure. In a rough estimate, we found that in our series there were about 25 times more impregnated Purkinje cells than Lugaro cells. Immunocytochemical studies, using antibodies against specific proteinic components of Lugaro cells, can give more reliable indications upon the relative number of this neuronal type in the cerebellar cortex. Lugaro cells are the only corticocerebellar cells specifically labelled by Cat 301 and Cat 304, two monoclonal antibodies raised against different epitopes of the same surface chondroitin sulfate proteoglycan. These mainly label the soma and proximal dendrites of the neurons, whose frequency was evaluated in the cat cerebellum to be about one Lugaro cell (Cat 301-positive) to 20 or 30 Purkinje cells (calbindin-positive; Sahin and Hockfield, 1990). In spite of a species difference, both density evaluations are quite close, indicating that there are far less Lugaro interneurons than Purkinje cells, i.e., the output neurons of the structure. This is in strong contrast with the molecular layer interneurons which are much more numerous than Purkinje cells (Palkovits et al., 1971; Korbo et al.,

1993). One consequence of such an overall low ratio is that the distribution of Lugaro cells in the cortex, if regular, may delineate hypothetical corticocerebellar modules with some common operational properties. Here again, the Golgi techniques, on account of their versatility, are not suited to answer the important question of the regularity of distribution. We sometimes found sections in which, as reported above, occasional clusters of two nearby Lugaro cells could be seen. This implies that in such occasions, the dendrites of nearby located Lugaro cells partially cover the same granular layer territory. This was confirmed in rat preparations labelled with an antibody against calretinin, a calcium-binding protein particularly expressed in two corticocerebellar neuronal types, unipolar brush cells and Lugaro cells (Rogers, 1989; Arai et al., 1991; Floris et al., 1994). Such preparations showed a seemingly unequal repartition of the anti-calretinin-labelled fusiform cells. These can be found in clusters of 2 to 3 Lugaro somata very close to each other in some parts of the cortex, the distribution being more regular in other parts or, still in other locations, stained Lugaro perikarya may even be absent (unpublished observations). Thus, the horizontal lattice formed by the extended dendrites of Lugaro cells beneath the Purkinje cell layer is likely not regular, a fact that can only in part be attributed to the difference in dendritic lengths pointed out above.

About the axon

The main contribution of this study is the description of the different trajectories and termination sites of the Lugaro cell axon. As is mentioned in the Introduction, the descriptions by Lugaro and Ramón y Cajal differed as to the fate of the axon, an uncertainty that lasted with later descriptions, and likely a consequence of the variability of its trajectory. We show here the great homogeneity of the axonal distribution pattern. It always ascends to the molecular layer, either directly or after an indirect route through the depth of the granular layer, which also systematically receives some projections. The axon never leaves the cerebellar cortex. This last point was confirmed by a thorough examination of vermal sections following horseradish peroxidase (HRP) injections in the deep cerebellar or vestibular nuclei. No retrogradely labelled fusiform neuron was found in the uppermost granular layer.

All Lugaro cells have a local projection in the molecular layer just above the parent cell. Some of the neurons also display distal projections. One such distal projection is but a particular case of the indirect axon which courses down to the low granular layer, and even the white matter, before reascending to an area in the molecular layer located laterally but in the same parasagittal plane as that of the local projection. This implies that the postsynaptic cells of both distal projection sites receive distinct inputs, specially from different parallel fibers, thus indicating that the Lugaro cell exerts a cooperative action between functionally different areas. The presence, in some cells, of longitudinal collaterals of the axon coursing in the lower molecular layer, parallel to the parallel fibers, appears as quite unique. These collaterals emit terminal ramifications in the molecular layer at different parasagittal locations in the folium, distant mediolaterally from the cell of origin by one to several hundreds of microns. These fibers were first described by Lugaro (1894) in the cat and by Fox (1959) in the monkey, and are confirmed in the present study in the rat. However, the lengths indicated by these authors (2,000 μm for Lugaro and 940 μm for Fox) are much longer than those we found. Two factors may explain this discrepancy. First, there may be a species difference, the rat collaterals being shorter than those of cats and monkeys. Second, such long fibers may just have impregnated incompletely, or not at all, in our preparations. Indeed, in some cases, we were able to follow for several hundred microns longitudinal axonal fibers endowed with the morphological characteristics described in the Results. Unfortunately, it was not possible to trace them back to their parent cell. In any case, these fibers, extending straightforwardly in the direction of the long axis of the folium, are unusual in the cerebellar cortex, only the parallel fibers having the same type of orientation. Molecular layer interneurons have parasagittally oriented axons, parallel to the dendritic trees of the Purkinje cells and transverse to the folium. Climbing fibers also have such a parasagittal organization (see review in Ito, 1984). It could well be that this type of Lugaro cell axon collateral allows transfer of information between modules located in different parasagittal bands evidenced by immunolabelling (reviewed in Hawkes et al., 1992), an hypothesis that will be tested by simultaneously labelling the Lugaro cells and the boundaries of different bands.

We cannot, at present, decide whether the Lugaro cell has a unique type of axonal pattern, more or less completely impregnated in each of our specimens, and thus revealing only one or the other of the projection sites, or if there are indeed different subclasses of molecular axonal projections. An argument in favor of the latter hypothesis stems from the initial direction of the primary axonal shaft. Indeed, in the case of directly ascending axons, the beginning of the axon is horizontal or points unambiguously upwards (Figs. 8; 9), with no hints at descending branches, whereas the initial segment of axons with descending distal branches heads downwards as soon as it leaves the soma (Figs. 10A,E; 11–13).

The Lugaro cell in the corticocerebellar network

In spite of what has been mentioned in the Introduction about it not yet being fully recognized as a neuronal type per se, some indications do exist in the literature about different characteristics of Lugaro cells, other than their Golgi morphology. Lugaro cells are putative inhibitory interneurons because they have been shown to be immunopositive to γ-aminobutyric acid (GABA) in the rat (Gabbott et al., 1986; Aoki et al., 1987). Some of these neurons may also be glycinergic, as Ottersen et al. (1987) described and illustrated in the baboon, some glycine-like immunoreactive fusiform cells with features typical of Lugaro cells. These authors also signaled glycine-like immunoreactive fibers in the deep molecular layer, the characteristics of which hint at Lugaro cell axons. Moreover, the colocalization of GABA and glycine-like immunoreactivities in fusiform cell bodies, located in the infraganglionic plexus, was illustrated in the mouse by Takayama (1994), who also noted numerous glycine positive fibers in the molecular layer.

Lugaro cells' synaptic afferences have been investigated by several electron microscopic studies. Lemkey-Johnson and Larramendi (1968a,b) were the first to consider a category of "very low basket cells" whose characteristics are, as also pointed by Palay and Chan-Palay (1974), clearly those of Lugaro cells. Indeed, these neurons have elongated cell bodies in the Purkinje cell layer or just below it and they show, at the electronmicroscopic level, a completely differ-

ent synaptic recovering than other stellate and basket neurons (see in particular Figure 132 in Larramendi, 1969). They quantitatively ascertained the predominance of inhibitory inputs from Purkinje cell recurrent collaterals (95%), relatively to the excitatory inputs from the ascending part of the granule cell axons (5%), on the somata of Lugaro cells (Lemkey-Johnson and Larramendi, 1968b). Later on, these authors (Larramendi and Lemkey-Johnson, 1970), as well as Chan-Palay (1971) and Palay and Chan-Palay (1974), confirmed and strongly underlined this outstanding high density of Purkinje cell recurrent collateral synapses on Lugaro cell somata and proximal dendrites, the only portions identifiable on pure morphological grounds at the electron microscopical level. They also confirmed the much rarer excitatory synapses made by the ascending portion of granule cell axons, a synaptic input that may explain the slight immunopositivity of Lugaro cells to the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptor subunit GLUR2/3 (Petralia and Wenthold, 1992). All these observations are in keeping with several light microscopic Golgi and immunocytochemical studies which point to the strong density of nestlike structures formed by the recurrent collaterals of several neighbouring Purkinje cells around fusiform somata in the upper granular layer (De Camilli et al., 1984; Marin-Padilla, 1985; Hawkes and Leclerc, 1989; Rogers, 1989), and may be the reason for the systematic attraction of the Lugaro cell dendrites towards the infraganglionic plexus that we show in the Results. The Lugaro cells thus appear to have an elective role in the sampling and integration of the outputs converging from a great number of neighbouring Purkinje cells.

The postsynaptic targets of Lugaro cells' projections remain at present hypothetical. The organization of their molecular axonal arborization is multiramified with a plexus of intricated oblique branches, thus showing no stringent spatial relationship with the vertically oriented molecular layer dendrites. This axonal arborization is then noticeably different from the candelabrum cell axon, the other large nonmolecular interneuron which projects into the molecular layer, whose branches ascend vertically in the parasagittal and transversal planes in a manner reminiscent of the Purkinje cell dendritic tree (Lainé and Axelrad, 1994). Lugaro cells may then contact molecular interneurons, and more plausibly basket cells, since the Lugaro cell axonal plexus mainly spreads into the inferior two-thirds of the molecular layer. Golgi cell dendrites are also present in the molecular layer and could therefore be a postsynaptic site to Lugaro cells axons.

Receiving their main inputs from Purkinje cells and exerting their effect on basket cells, which themselves inhibit Purkinje cells, the Lugaro cells would thus mediate a disinhibitory feedback loop modulating the activity of the cerebellar cortex output neuron. This mode of operation is reminiscent of the mode of operation of Golgi cells which mediate an inhibitory feedback loop centered on the structure's input relay neuron, the granule cell. Moreover, cross-talk between Lugaro and Golgi cells could be present as Golgi cells may be Lugaro cells' putative targets in the granular layer. Indeed, at least some Lugaro cells seem glycine-like-immunoreactive (see above), and glycinergic synaptic currents have been evidenced in a recent patch-clamp study of Golgi cells, with arguments against the involvement of other Golgi cells inputs for these currents (Dieudonné, 1995).

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