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Studies with the Golgi method in central gangliogliomas and dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos disease)

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Summary. The rapid Golgi method, combined with current optical and electronmicroscopical techniques, was used in three central gangliogliomas and in one dysplastic gangliocytoma of the cerebellum to study the morphology of ganglionic cells. Gangliogliomas were composed of bipolar, fusiform and radiate cells with dense core and clear vesicles in the perikaryon and cellular processes, the number of each cellular type varying from one case to another. These features, together with the fact that isodendritic neurons are considered to be phylogenetically old neurons, suggest that these tumours are composed of «primitive» neurons that are not homogeneous with regard to their morphology. In contrast, ganglionic cells in dysplastic gangliocytoma are huge cells with long, stereotyped neurites that establish unique asymmetric contacts with neighbouring perikarya and neurites by means of claw-shaped processes covered with synaptic buttons. These morphological characteristics are different from those of any other neuron of the CNS.

Key words: Ganglioglioma, Ganglionic cell tumours, Dysplastic gangliocytoma, Cerebellum, Lhermitte-Duclos disease, Golgi method

Introduction

Central ganglionic cell tumours are composed of clusters of mature, large and often bizarre neurons separated by bundles of neuronal processes. Gangliocytoma refers to pure aggregates of ganglionic cells without significant glial content, whereas gangliogliomas are characterized by a mixed proliferation of abnormal neurons and glial cells (Russell and Rubinstein, 1989).

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Dysplastic gangliocytoma of the cerebellum is a particular type of gangliocytoma characterized by the proliferation of large ganglionic cells that grow throughout the cerebellar cortex replacing normal Purkinje and granule cells. The presence of a conspicuous myelinated plexus in the molecular layer, together with the apparent transformation of granule cells into large neurons in the transitional areas, have led to the consideration of granule cell hypertrophy as the most comprehensive explanation of the disease (Duncan and Snodgrass, 1943; Oppenheimer, 1955; Ambler et al., 1969; Reznik and Schoenen, 1983).

Studies with the Golgi method proposing to show the morphology of ganglionic cells in more detail are, however, scarce (Ambler et al ., 1969; Ferrer et al., 1979, 1983; Probst et al., 1979). For this reason, in the present work we describe the morphology of ganglionic cells, as revealed with the rapid Golgi method, in three central gangliogliomas and in one dysplastic gangliocytoma of the cerebellum in an attempt to study the neuronal types involved in central ganglionic cell tumours.

Materials and methods

The main clinical data of the people with ganglionic cell tumours in the present study are summarized in Table 1. Tumours were surgically removed and the postoperative course was good in all of these patients. Follow-up of the patients with gangliogliomas revealed no recurrence of the lesions after 7, 5 and 2 years in patients labelled 1, 2 and 3 respectively. The patient with dysplastic gangliocytoma of the cerebellum was healthy three years after the operation. Some characteristics of patients 1 and 4 have been previously reported (Ferrer et al., 1979, 1983).

The surgical specimens were rapidly fixed in 10% buffered formalin and representative samples were embedded in paraffin. Sections, 10 microns thick, were stained with H & E, cresyl violet (Nissl), Gross

Bielschowski and for the demonstration of GFAP according to the PAP method. After three days in formalin selected samples were impregnated according to the rapid Golgi method. The samples were immersed in 3% potassium bichromate - 1% osmium tetroxide (20/5; v/v) for 3-5 days. Immediately afterwards they were washed in 0.75% silver nitrate and dipped in fresh 0.75% silver nitrate solution. Finally, they were dehydrated in ethanol and shelled in paraffin. Sections, 70-100 microns thick, were obtained with a horizontal sliding microtome.

Small pieces of tissue from patients 2, 3 and 4 were also available for electronmicroscopical examination. The blocks were fixed with 2.5% glutaraldehyde for 24 h, postfixed with 2% osmium tetroxide for 2 h, dehydrated in ethanol and propyleneoxide and embedded in Epon. Semithin sections were stained with toluidine blue and ultrathin sections with uranyl acetate and lead citrate.

Results

Morphological findings were similar in the three gangliogliomas. Ganglionic cells were distinguished by their large cytoplasm with Nissl bodies and clear nuclei with prominent nucleoli. Glial cells were smaller in size and the chromatin was condensed; pleomorphis was mild or moderate and mitoses were absent. Ganglionic cells were distributed in clusters intermingled with bundles of neuronal processes and glial fibres (Fig. 1A). In some fields, however, neurons were randomly distributed, and in others they were arranged in a coarse fascicular pattern.

Binucleated neurons were common and multinucleated cells with nuclear bridges were seldom observed (Figs. 1B, C and D). These cells were not reactive for GFAP. These features, which have been repeatedly described in ganglionic cell tumours (Rubinstein and Herman, 1972; Mørk et al., 1979; Johannsson et al., 1981; Kayanaraman and Hederson, 1982; Demiere et al., 1986; Takahashi et al., 1989), indicate an abnormal nuclear division the significance of which remains unknown.

Haemorrhages, necrosis, vascular proliferation and endothelial hyperplasia were absent, but heavy lymphocytic infiltrates ocurred in case 3.

Electronmicroscopical examinations in cases 2 and 3 revealed a developed endoplasmic reticulum, clusters of ribosomes and numbers of mitochondria in the perikarya of neurons. Microtubules and intermediate filaments were common in neuronal processes. Asymmetric axondendritic synapses were common, but axosomatic synapses were very rare. Dense core vesicles, measuring about 60-200 nm, and clear vesicles 50-70 nm in diameter were present in the perikarya, neuronal processes and synapses. These characteristics are similar to those already reported in other central ganglionic cell tumours (Robertson, et al., 1964; Rubinstein and Herman, 1972; Probst et al., 1972; Kalyanaraman and Henderson, 1982; Takahashi et al., 1989).

Samples impregnated with the Golgi method exhibited a striking polymorphism of the neurons. In case 1 spindle-shaped, bipolar and fusiform neurons predominated in those fields with a fascicular pattern, but triangular, multipolar neurons of variable size also occurred in clumps (Fig. 2). In cases 2 and 3 fusiform and bipolar cells were the prevailing neuronal types (Figs. 3A, B). The axons emerged from the soma or from the proximal region of a dendrite and often their trajectory could only be followed a short distance. However, some axons had parallel courses to the dendrites of neighbouring bipolar and fusiform cells. As a rule, dendrites were thick and displayed few secondary proximal branches. Dendritic spines were very common and had long, thin pedicles and round spine heads. Thorn-like appendages were conspicuous in some cells (Fig. 2, cell D; Fig. 3C, D). The surgical sample of the patient with dysplastic gangliocytoma of the cerebellum was characterized by greatly enlarged convolutions occupied by huge ganglionar cells along the cerebellar cortex. The molecular layer exhibited a dense plexus of parallel myelinated fibres, and the white matter was reduced to a thin band of cystified tissue. Spongiosis was a conspicuous feature and calcospherites were encountered in the inner cortical level and underlying white matter (Fig. 4A). Normal cerebellar structures were absent. In paraffin sections, ganglionic cells had a large, pale nucleus and a prominent nucleolus and the cytoplasm was oval-shaped or polygonal, faintly eosinophilic and decorated with small numbers of peripheral Nissl granules. Binucleated neurons were not observed and mitoses were absent. Glial cells were scattered and looked like mere accompanying elements of the ganglionic cells. Intermediate cells were not observed.

Electronmicroscopical examination revealed large numbers of mitochondria in the soma and cellular processes, and neurites were filled with bundles of intermediate filaments measuring 7-10 nm in diameter. Synaptic profiles were often encountered. The presynaptic component was large, button-shaped, and established simple or double asymmetric synapses with the perikaryon and neurites of neighbouring cellular profiles. Synaptic vesicles were usually round and clear and measured about 60 nm in diameter (Fig. 4E), but dense-core vesicles measuring 70-150 nm in diameter were also observed. These ultrastructural features reflect those described in other cases (Gessaga, 1980; Beuche et al., 1983).

Ganglionic cells impregnated with the Golgi method had globular cell bodies and two or three stout dendrites with terminal claw-shaped appendages that surrounded the perikaryon and the thick cellular processes of neighbouring neurons as was revealed by contrast optics. The pattern of the dendritic arbor in some cells was reminiscent of normal granule cells despite striking differences in size. Most ganglionic cells, however, had dendrites directed towards the surface that had several medium-sized secondary branches ramifying profusely into small, varicose terminal branches, twigs and buds (Figs. 4B,C, 5).

The axon had a short descending trajectory in most

Table 1. Main clinical features in patients with ganglioglioma and dysplastic gangliocytoma of the cerebellum.

	Age (y)	Sex	Evolution (y)	Clinical symptoms and signs	CT scans	Pathology
patient 1	16	F	13	· hemihypotrophy (right side) · hemiparesis · mild mental retardation · generalized seizures	large, left fronto-parietal cyst with parasagittal nodule	ganglioglioma
patient 2	12	F	2	·cerebella ataxia · generalized hypotonia · hypereflexia · headache · papilledema	cystic expanssive lesion in the rostral vermis	ganglioglioma
patient 3	14	M	7	· seizures · "psychopatic" behaviour	left temporal space-occupying lesion	ganglioglioma
patient 4	63	M	2	· cerebella ataxia · slurred speech · headache	expansive lesion of the left cerebellar hemisphere	dysplastic gangliocytoma of the cerebellum

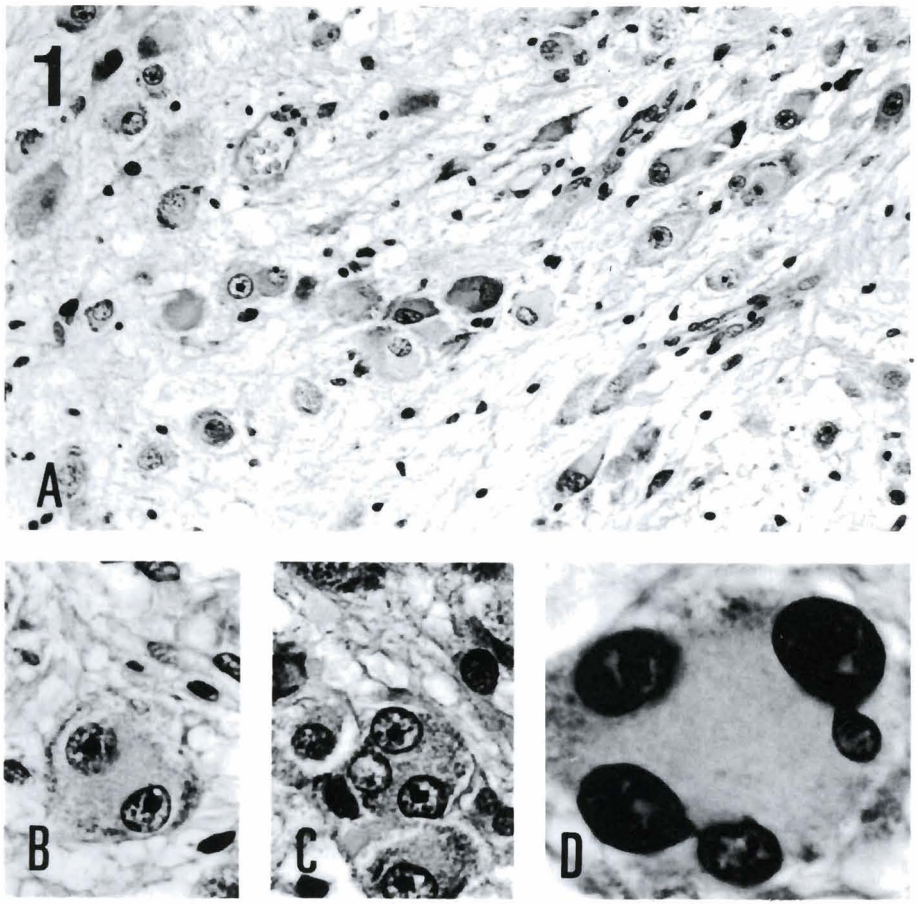

Fig. 1. General aspects in gangliogliomas. A. Aggregates of ganglionic cells with clear nuclei and prominent nucleoli. Nissl granules are found peripherically in the perikaryon of most cells (case 2). B and C: Binucleated and trinucleated neurons (case 3). D: Large multinuclear cell with nuclear bridges (case 3). A: Nissl stain × 160; B and C: H & E × 400; D: GFAP counterstained with H & E × 1,000

neurons, but later curved towards the surface, became thicker and reached, in most cases, the «molecular» layer where they divided into two tangential fibres. Along their ascending course thick neurites sent out at intervals bunches and clusters of bizarre collaterals with terminal buttons.

Claw-shaped appendages were also observed emerging from these neurites and, hence, the nature of isolated segments as dendrites or axons was difficult to recognize (Figs. 4D, 6).

Afferent and efferent fibres in the white matter were neither impregnated with current silver stains nor with the Golgi method. For this reason, ganglionic cells appeared isolated from the rest of the cerebellum.

Discussion

The present studies with the Golgi method demonstrate that impregnated neurons in three gangliogliomas are of three main types: bipolar cells, fusiform neurons and multipolar cells with long, scarcely branched dendrites (type I radiate neurons: Ramón-Moliner, 1968). The content of each type was variable from one case to another. It must be stressed that isodendritic neurons, both

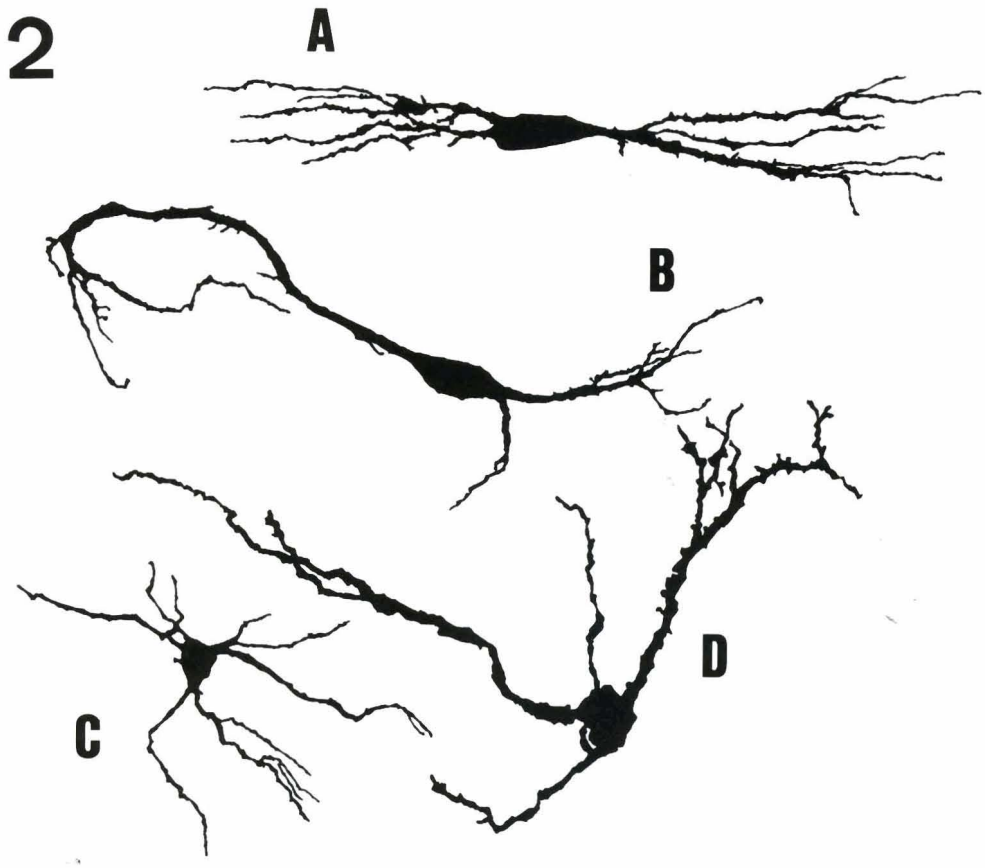

Fig. 2. Camera lucida drawing of ganglionic cells impregnated with the Golgi method (case 1). A. Fusiform neuron; B. Bipolar cell; C. Small radiate neuron; D. Multipolar neuron with long dendrites covered with dendritic spines. bar = 200 microns.

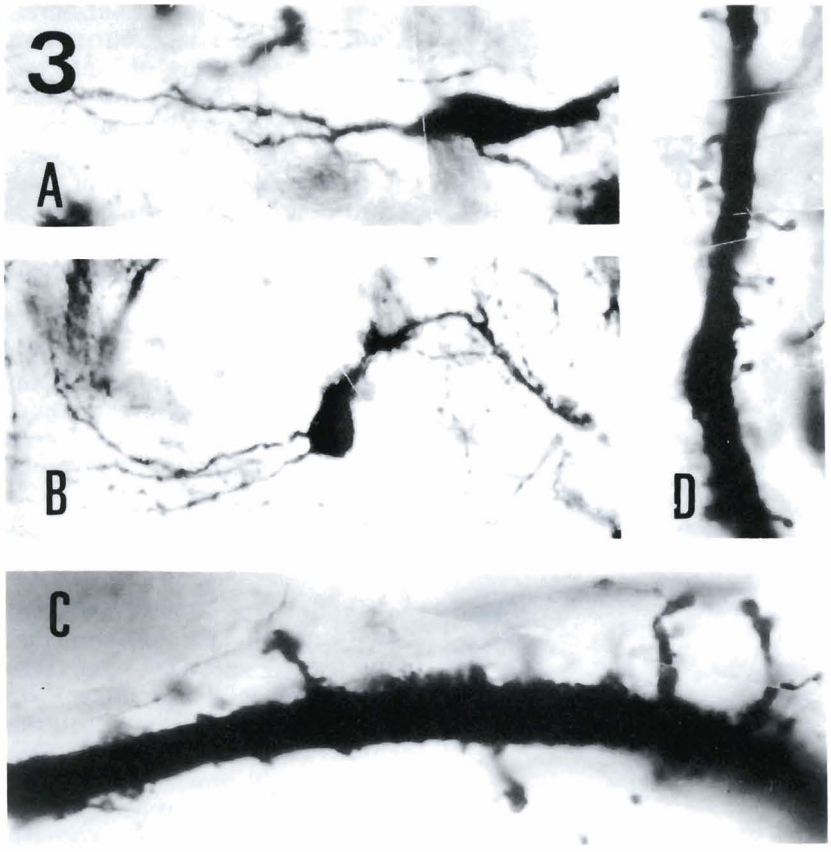

Fig. 3. Golgi-impregnated neurons in gangliogliomas. A. Fusiform neuron; B. Bipolar cell; C. Thick dendrite with thorn-like processes; D. Thick dendrite covered with spines. A, B and C: case 2, D: case 3. A and B × 200: C and D × 1,000

leptodendritic (bipolar and fusiform) and radiated, are mainly found in the phylogenetically oldest regions of the diecenphalon, mesencephalon and rhombencephalon (Ramón-Moliner, 1968; Ramón y Cajal, 1972, reprinted). For this reason, these types of neurons which are, for example, the core of the reticular formation and the predominating cells in the raphe nuclei, have been considered living fossils within the central nervous system of mammals (Ramón-Moliner, 1968). Based on these features, it may be suggested that ganglionic cell tumours: a.- contain neurons that are not homogeneous with regard to their morphology; and b.- are composed of «primitive» neurons.

These interpretations are supported by recent immunocytochemical studies that have demonstrated positive reactions to different neuropeptides and neurotransmitters in central gangliogliomas and gangliocytomas (Giangaspero et al., 1985; Izukawa et al., 1988; Takahashi et al., 1989). Neuropeptide expression was variable from one case to another and positive immunoreactivity for several markers was emphasized in one series (Takahashi et al., 1989). Moreover, tyrosine hydroxylase, an enzyme involved in catecholamine biosynthesis, has been demonstrated using the PAP method in 3 of 5 cerebral ganglionic cell tumours; one of which also exhibited serotonin (5-HT) immunoreactivity (Takahashi et al., 1989). It is known that neuropeptides are widely distributed in the nervous system, but catecholaminergic neurons, though present in the cerebral cortex (Gasper et al., 1987; Kosaka et al., 1987) do not prevail in the cerebral hemispheres; furthermore, 5-HT-containing neurons are almost limited to the raphe nuclei (see Emson, 1983 for references in the different chapters).

Particular conclusions are obtained in dysplastic gangliocytoma of the cerebellum. In case 4, although some neurons look like very large granule cells, as revealed with the Golgi method, the neuritic patterns of most of the ganglionic cells differ from those of

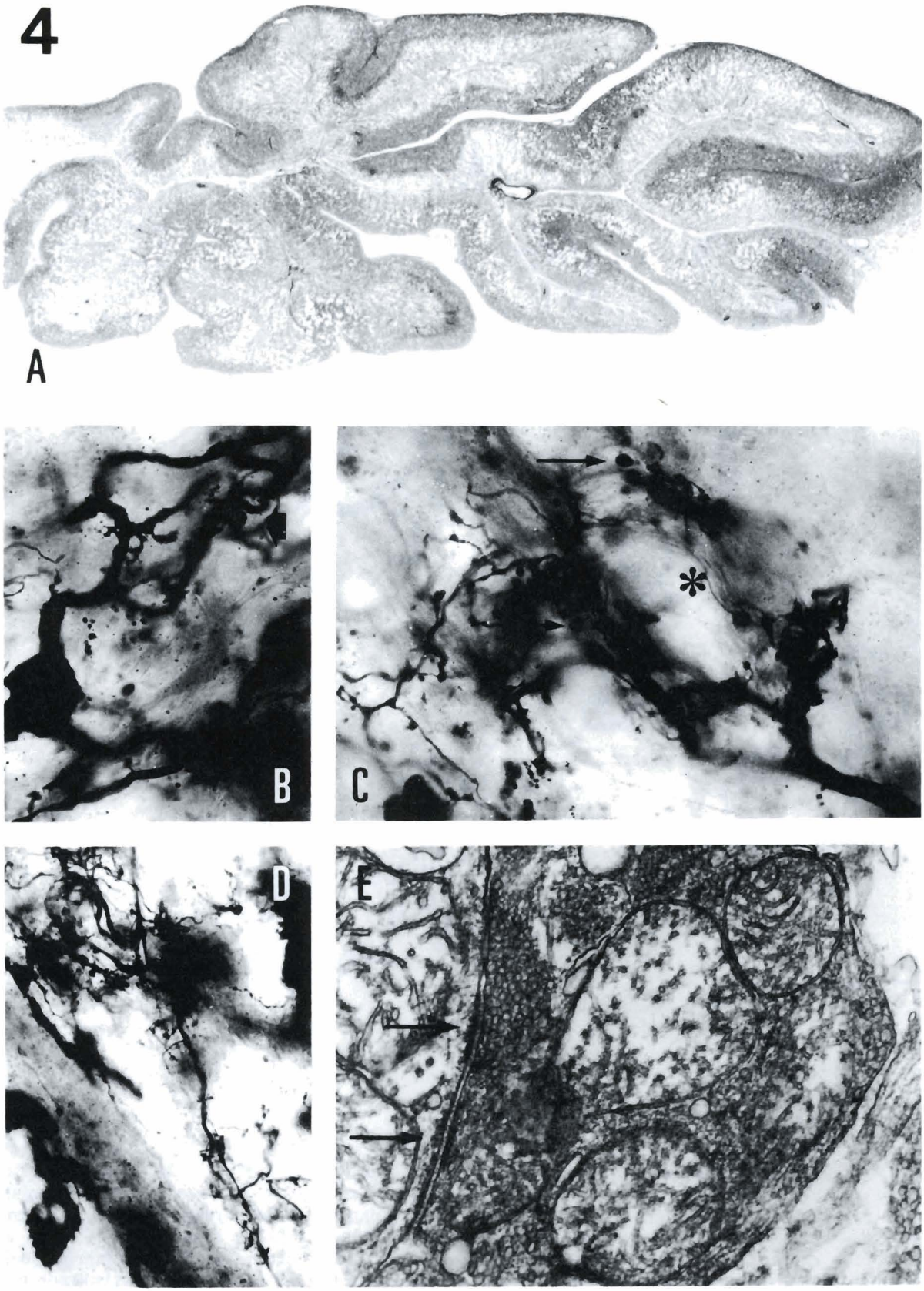

Fig. 4. Dysplastic gangliocytoma of the cerebellum. A. Sagittal section of the surgical sample showing enlarged convolutions, myelination of the upper cortical level, spongiosis of the cerebellar cortex and severe atrophy of the white matter. B. Ganglionic cell with thick dendrites and claw-shaped appendages (thick arrow). C. Thick neurite with multiple terminal branches with buttons (thin arrows) that form a basket (asterisk). D. Thin axon with many collaterals forming nest-like terminals. E. Double (arrows) asymmetric axosomatic synapsis; the button-like presynaptic component is filled with large mitochondria and numbers of clear vesicles. A: Luxol fast blue-Kluver Barrera × 10; B, C and D: Rapid Golgi method; B × 400; C and D × 1,000; E: × 34,000

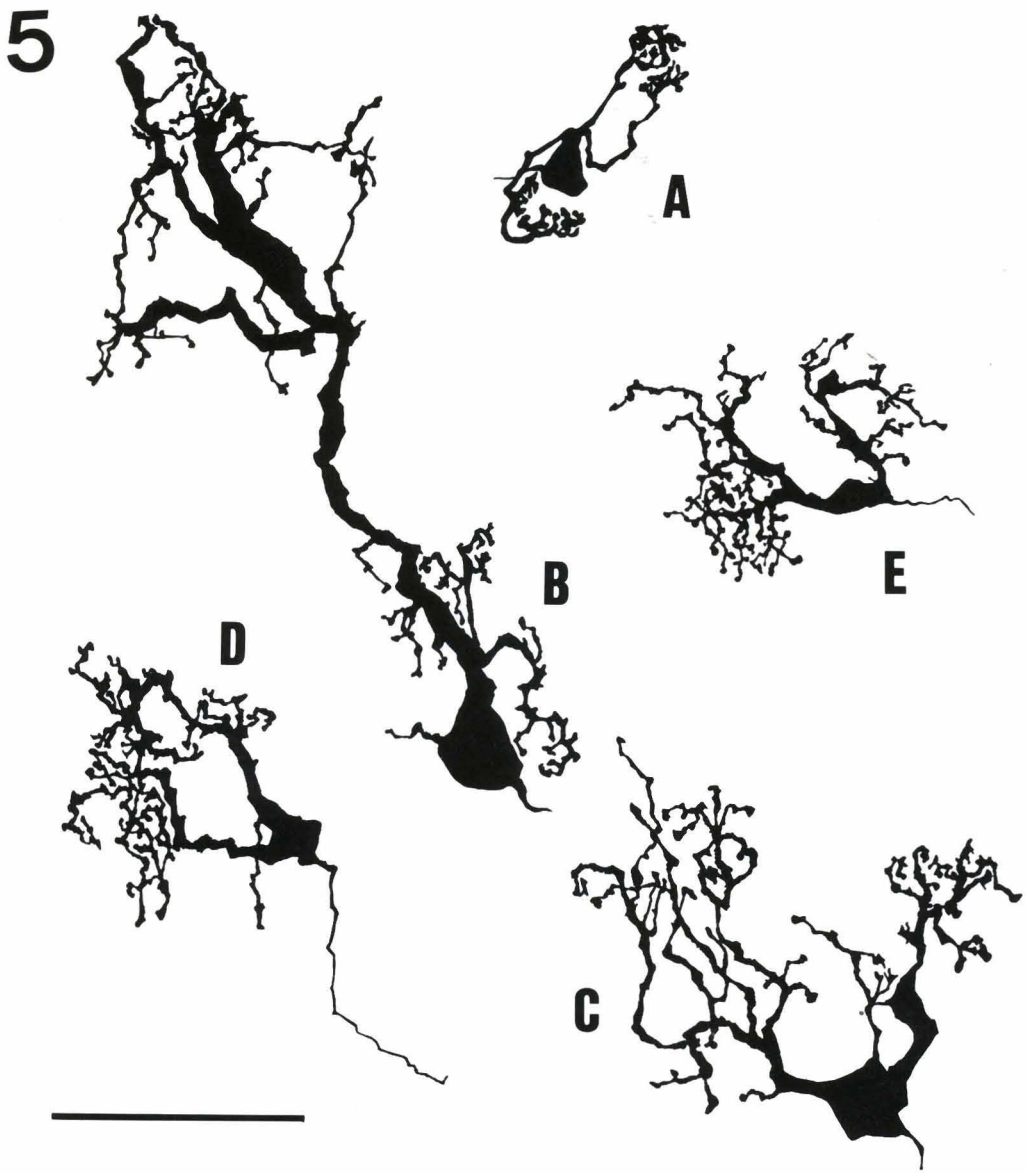

Fig. 5. Camera lucida drawing of ganglionic cells in dysplastic gangliocytoma of the cerebellum impregnated with the Golgi method. Cell A is the only one reminiscent of normal granule cells. Cells B to E exhibit thick dendrites with clusters of secondary branches and terminal tufts, twigs and buds, as well as claw-shaped processes. bar = 100 micron.

normal cerebellar neurons (Palay and Chan-Palay, 1974), as well as from granule and Purkinje cells in mutants or in experimentally-induced abnormalities (Caviness and Rakic, 1978; Sotelo, 1978). Recent immunocytochemical observations have revealed that ganglionic cells in these tumours express different neurofilament-related epitopes and cross-react with the pan-T-cell antibody anti-Leu-4 (Yachnis et al., 1988; Shiurba et al., 1988). These properties are similar to those in normal Purkinje cells because their perikarya and dendrites exhibit variable neurofilament immunoreactivities to distinct phosphorylated and non-phosphorylated epitopes, and are the only cerebellar neurons recognized by the pan-T-cell antibody (Garson et al., 1982; Yachnis et al., 1988; Shiurba et al., 1988). In contrast, although this occurs in cultured granule cells, neurofilament immunoreactivity is only transiently expressed by granule cells during a short time of development (Gilad et al., 1989; Cambray-Deakin and Burgoyne, 1986). Adult granule cells lack neurofilament expression (Burgoyne and Cambray-Deakin, 1988).

Furthermore, the present Golgi and ultrastructural observations indicate peculiar cellular appendages and specific cellular connections in dysplastic gangliocytoma. Claw-shaped cellular appendages in dendrites and neurites with terminal buttons establish asymmetric

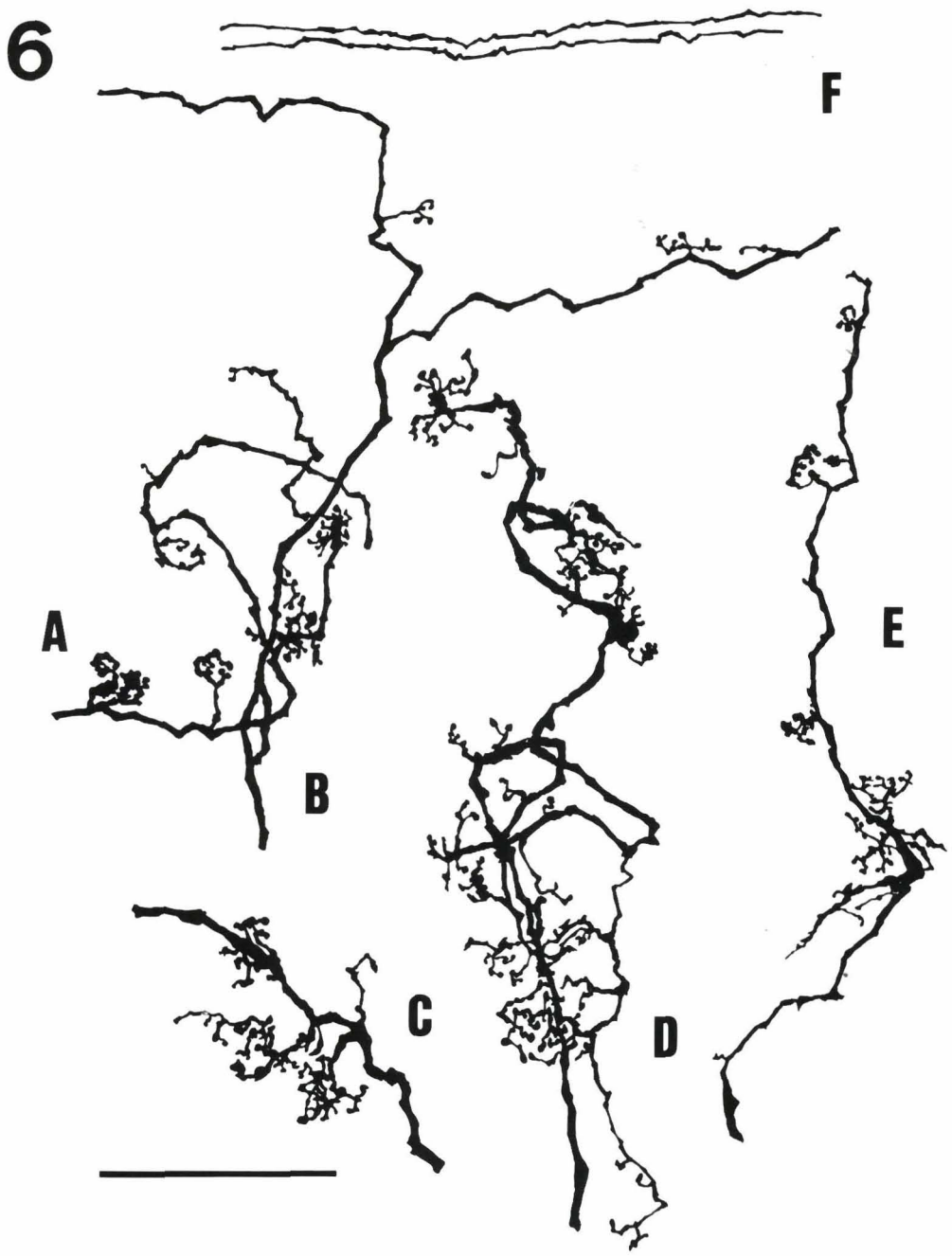

Fig. 6. Camera lucida drawing of neurites in dysplastic gangliocytoma of the cerebellum impregnated with the rapid Golgi method. Neurites have a tortuous course and give off variable numbers of collateral clusters and claw-shaped arborizations (A to E). F: parallel fibres in the upper border of the cerbellar cortex. bar = 100 microns.

synaptic contacts with the soma and neurites of neighbouring cells. This feature is consistent with the presence of anti-synaptic vesicle antibody (SV2 antibody) in the periphery of large cells and extracellular clusters in dysplastic gangliocytoma (Shiurba et al., 1988). This antibody usually decorates the periphery of Purkinje cells and the glomeruli in normal cerebellum. These features, as well as the lack of putative connections through the white matter, suggest that dysplastic gangliocytoma appears as an organized, self-regulated and complex proliferation of ganglionic cells displaying unique characteristics different from any other neuron of the central nervous system.

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