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The Neuronal Organization of Cerebellar Lobe C1 in the Mormyrid Fish Gnathonemus Petersii (Teleostei)

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Summary. The corpus cerebelli of mormyrid fishes is strongly developed and differentiated into four lobes: C1–C4. Although all of these lobes contain the characteristic cerebellar layers: granular, ganglionic and molecular, they show distinct architectonic differences. A previous study revealed that the ganglionic layer of C1, in addition to Purkinje elements contains conspicuous giant cells. In the present paper the results of a further analysis of C1 are reported. This analysis is based on serially sectioned brains of *Gnathonemus petersii*, stained according to Nissl, Bodian and Häggquist. Semi-thin sections were stained with p-phenylenediamine. Routine EM techniques were used to visualize synaptic relations. Mossy fibres and granule, Golgi, Purkinje and stellate cells are located characteristically throughout C1. It appeared that the giant cells of a previous study represent the largest elements of a population which has been termed now the eurydendroid cells. The average size of the latter is somewhat larger than that of the Purkinje cells, but both groups of cells show a considerable overlap in the size of their somata. Purkinje cells and eurydendroid cells are present throughout the ganglionic layer and both have a flattened, sagittally oriented, dendritic tree that extends into the molecular layer. Yet, the eurydendroid cells (EC) display the following characteristics which distinguish them from Purkinje cells (PC): (1) In EC the Nissl substance is dispersed diffusely throughout the soma, whereas in PC it tends to be concentrated around the nucleus, (2) The soma/nucleus ratio for EC is distinctly larger than for PC, (3) The dendritic trees of EC extend over a larger stretch of the molecular layer than those of PC, hence the term EC, (4) The dendrites of EC are more widely spaced and oriented less strictly parallel to each other than those of PC, (5) The dendrites of EC are somewhat irregular in outline and not covered with spines, in contrast to those of PC, (6) The axons of EC are oriented radially and join bundles of coarse fibres which leave the cerebellum whereas the axons of PC extend and ramify within the ganglionic layer, (7) The somata of EC, contrary to those of PC, are enveloped by a dense axonal plexus which forms numerous synaptic terminals on them. The numerical ratio of EC: PC was 1:5.5. The circuitry in C1 and the possible functional roles of its constituent neurons are discussed. It is pointed out that in this lobe the axons of PC impinge on EC and that the latter constitute its output system.

Key words: Cerebellum — Mormyrid fishes.

Introduction

In teleost fishes the cerebellum is well developed and consists of two principal parts: the corpus cerebelli and the valvula cerebelli. The former is massive and situated dorsal to the rhombencephalon; the latter is a pouch-like structure which projects forward under the tectum in the mesencephalic ventricular cavity. It has been known since the time of Sanders (1882) that in one group of teleosts, the mormyrids, the cerebellum attains amazing dimensions. In these forms the valvula has grown out of the ventricle of the midbrain to become a superficial structure which covers virtually all other parts of the brain. The corpus cerebelli of mormyrid fishes is also strongly developed and differentiated into four lobes,

which have been termed C1 through C4 (Nieuwenhuys and Nicholson, 1969a; Fig. 1). The valvula as well as the various lobes of the corpus of the mormyrid cerebellum contain the three characteristic layers: granular, ganglionic and molecular, yet these various parts show distinct architectonic differences. Previous studies (Nieuwenhuys and Nicholson, 1969a, b; Nieuwenhuys *et al.*, 1973) have shown that the ganglionic layer of lobe C1, in addition to Purkinje elements, contains conspicuous giant cells. Closer scrutiny revealed that these giant cells represent merely the largest elements of a group of cells which, on the basis of the relatively wide extent of their dendritic trees, may be termed the eurydendroid cells. The present study analyses the neuronal organization of cerebellar lobe C1 in the mormyrid fish *Gnathonemus petersii*. Particular attention will be paid to the Purkinje and the eurydendroid cells, and to the place of these elements in the circuitry of this lobe.

Material and Techniques

Young specimens of *Gnathonemus petersii* were used, their total length ranging from 80–150 mm. The animals were anaesthetized in a 0.025% solution of M.S. 222 (Sandoz). The brains were exposed and after fixation removed, embedded in paraffin and cut in serial sections. One transverse and three sagittal series were stained with cresylechtviolet. One transverse, three sagittal and one horizontal series were impregnated according to Ziesmer's (1951) modification of the Bodian technique. Two sagittal series were stained according to Häggquist (1936). Twenty-nine brains were prepared by the rapid Golgi technique. These brains were embedded in celloidin and sectioned at a thickness of 80 μm, twenty-two in the sagittal, three in the transverse and four in the horizontal plane.

Material for 1 μm and ultrathin sections was prepared as follows. After anaesthesia in a 0.025% solution of M.S. 222 (Sandoz), the animals were perfused through the heart with a 3% glutaraldehyde solution in a 0.1 M phosphate buffer (pH 7.2). Following perfusion pieces of several parts of the cerebellum were dissected out. These pieces were washed in a mixture of a 0.1 M phosphate buffer and a 25% sucrose solution (proportion 2:1), before postfixation in a 2% solution of OsO_4 in the same buffer. Specimens were then dehydrated and embedded in epon.

Lobe C1 of one specimen was sectioned horizontally at a thickness of 1 μm. Selected sections of this series were stained with a 1% solution of p-phenylenediamine in ethanol (Holländer and Vaaland, 1968).

From each block to be examined by electron microscopy 1 μm sections were stained with a 0.1% solution of toluidine blue and examined in the light microscope. This was done to ensure the presence of Purkinje or eurydendroid perikarya in the thin sections and to obtain correct orientation. Thin sections were then taken from appropriate blocks, stained with uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope.

A continuous sagittal Nissl series, cut at a thickness of 20 μm, was employed for measuring the size of the perikarya of Purkinje and eurydendroid cells, for counting the total numbers of these cells and for the preparation of a three dimensional reconstruction of lobe C1. For the measurements 100 Purkinje and 100 eurydendroid cells were randomly selected from this series, projected with a drawing prism and drawn at a linear magnification of 1000 times. In these drawings the size of the somata was determined by averaging their largest diameter in two directions perpendicular to each other. The data obtained for both cell types were plotted in a histogram (Fig. 7). The diameter of the nuclei of the same neurons were also measured and for each cell the ratio soma/nucleus was determined. These data were also plotted in a frequency histogram (Fig. 8).

In the sagittal Nissl series lobe C1 encompassed 66 sections. Using a drawing prism these sections were projected and the outlines of lobe C1 and of the external surface of its granular layer were drawn on transparant paper at a magnification of 280 times. The positions of Purkinje and eurydendroid cells were plotted in these drawings. In order to avoid inclusion of one soma in more than one drawing, only those somata were plotted in which a nucleolus was

clearly visible. For Purkinje cells, many of which contain two nucleoli (Fig. 4), double representation was avoided by looking to see if the entire nucleus was present in the section and, if not, by careful superposition of the drawing of the pertinent section with those of the preceding and the following section. By counting the Purkinje and eurydendroid cells plotted in the drawings of C1 the total number of the cells of these two types was determined.

The drawings just mentioned were also used to prepare a three dimensional reconstruction, showing the position of the eurydendroid cells in lobe C1, as follows: the outlines of the external surface of the granular layer were transferred from the drawings to sheets of styrofoam and then these sheets were cut with a hot wire along these outlines. Each sheet of styrofoam was laid on its corresponding drawing and the position of the somata of the eurydendroid cells was marked by sticking pins with large, black heads into the edge of the sheet. The sheets were then stacked so as to form a model showing the external surface of the granular layer of C1 and the distribution of the eurydendroid cells within the ganglionic layer (Fig. 9). Finally, the model of the external surface of the granular layer was flattened out by applying the following procedure. The model was photographed from all sides at a standard distance and in such a fashion that the surface area to be photographed was oriented perpendicular to the optical axis of the camera. The resulting 22 photographs were then put together in a montage. Fig. 10 is based on this montage.

Abbreviations

ax pl	axonal plexus	lob caud cb	lobus caudalis cerebelli
C1–4	central lobes 1–4 of the cerebellum	lob lin lat	lobus linea lateralis
cfm	central fibre mass	lob trans cb	lobus transitorius cerebelli
cr cb	crista cerebellaris	mf	mossy fibre
d tr	dendritric trunk	ml	molecular layer
eu	eurydendroid cell	n	nucleus
Go	Golgi cell	P	Purkinje cell
ggl 1	ganglionic layer	st	stellate cell
gr	granule cell	telenc	telencephalon
gr 1	granular layer	valv cb	valvula cerebelli

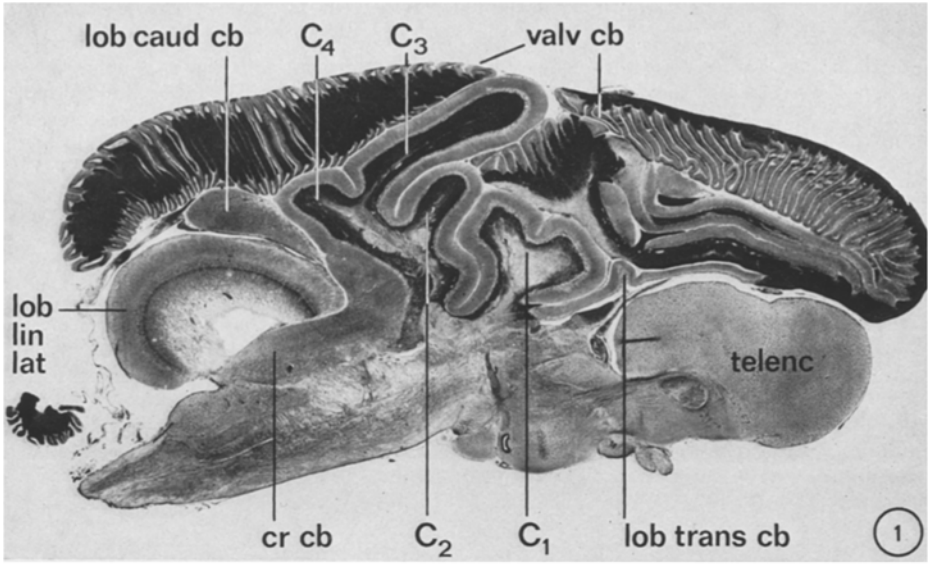
Results

1. Overall Structure

Figs. 1 and 2 show that lobe C1 in sagittal sections is somewhat mushroom-shaped. Rostrally it passes, *via* a thin lamella—the lobus transitorius cerebelli—over into the valvula and caudally it is continuous with lobe C2. Since C1 is laterally bounded by the pedunculi valvulae, huge fibre masses which connect the valvula with the remainder of the brain, this lobe essentially consists of a folded strip of cerebellar tissue. Thanks to this structural peculiarity the external surface of the granular layer (Fig. 9) could be unfolded into a plane (Fig. 10).

2. The Layers and Their Constituent Elements

a) The Granular Layer. As its name implies this layer consists mainly of densely packed small, granular cells. Centrally it borders upon a compact fibre mass; peripherally its boundary with the ganglionic layer is fairly sharp (Fig. 2). Numerous Golgi cells are present in the granular layer. Mossy fibres terminate within it and coarse fibres, originating from the ganglionic layer, traverse it in a predominantly radial direction. The latter fibres assemble in the central fibre mass of C1 where they constitute bilateral, rostrocaudally arranged, groups of bundles (Fig. 11).



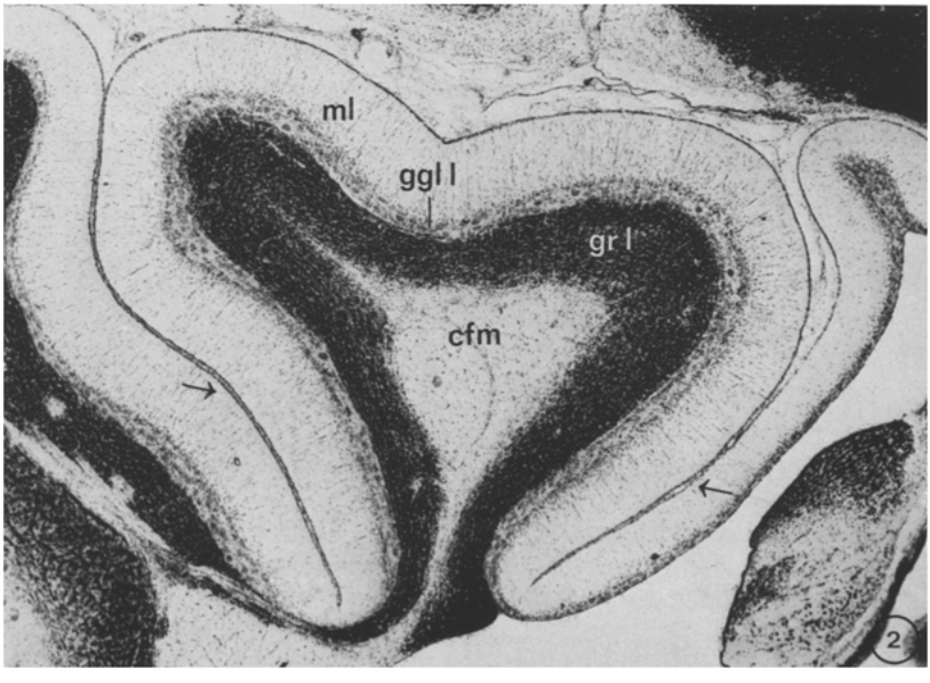


Fig. 1. Sagittal section through the brain of Gnathonemus petersii. Bodian Ziesmer. ×16
Fig. 2. Sagittal section through lobe C1. The arrows indicate the position of Fig. 11. Nissl preparation. ×50

The somata of the granular cells have a diameter of 3–4 μm. These elements have 3–6 short, often tortuous dendrites which terminate in a knob or as a small "claw". Their axons, which originate either from the soma or from one of the dendrites, ascend toward the molecular layer where they bifurcate in the charac-

teristic T-shaped junction to form a parallel fibre. It should be mentioned that the axons of the most laterally situated granular cells of C1 do not bifurcate but simply arch medially after reaching the molecular layer. It is noteworthy, too, that in C1 many parallel fibres and their parent axons are myelinated.

The Golgi cells are found throughout the granular layer, but the majority of these elements are in the immediate vicinity of the interface with the ganglionic layer. In the continuous Nissl series employed for the determination of the total number of Purkinje and eurydendroid cells the Golgi cells were also counted. Their total number appeared to be 721 and 400 of these occupied a superficial position. The somata of the Golgi cells are round or fusiform and measure on the average 8 by 10 μm, whereas the diameter of their nuclei is 6–7 μm. The dendrites of the Golgi cells are directed peripherally and ramify in the ganglionic layer and in the deeper zone of the molecular layer. In the deeper Golgi cells the dendrites originate from a single apical dendritic trunk, but in the superficial elements two or more main dendrites may arise from the soma. The delicate axons of the Golgi cells ramify and terminate in the granular layer (Fig. 6).

The mossy fibres fan out from the central fibre zone and form a plexus which spreads throughout the granular layer. At irregular intervals they form small, fusiform dilatations, which make contact with the dendrites of granule cells.

There is evidence (see below) that the coarse fibres which traverse the granular layer of C1 constitute the output system of this lobe. The bundles in which these fibres assemble constitute together the tractus cerebellotegmentalis mesencephalicus which, after having left the cerebellum, decussates in the caudal part of the mesencephalic tegmentum. This system was first described by Stendell (1914) who observed that its fibres decussate and then arch rostrally and terminate in a cell mass which he considered as a primordial red nucleus.

We studied the fibre pattern of the tractus cerebellotegmentalis mesencephalicus in horizontally cut semi-thin sections through the basal part of C1. These sections were stained with p-phenylenediamine according to Holländer and Vaaland (1968), a technique which shows the myelin sheaths of the fibres very clearly (Fig. 11). Using a drawing apparatus a section—its approximate level is indicated by the arrows in Fig. 2—was projected and the outlines of the myelin sheaths of all fibres within the bundles of the fibre system in question were drawn at 1000 × magnification. The calibre of the fibres was taken as the smallest diameter of their outlines (Table 1). The data recorded will be discussed below in relation to other findings.

b) The Ganglionic Layer. This layer has a width of 100–130 μm. Its borders with both the granular and the molecular layers are distinct; the latter is characterized by the inception of a pattern of vertical stripes, the so called palisade pattern (Figs. 3, 12). The term ganglionic layer has been introduced since this layer contains, apart from Purkinje cells, another type of large element: the eurydendroid cells. In addition to the perikarya and the initial dendritic ramifications of these large neurons the ganglionic layer harbours the somata of numerous smaller cells. The latter are concentrated in the outer one-third of the layer (Fig. 12). A dense plexus of sagittally oriented, beaded axons is present in the ganglionic layer and

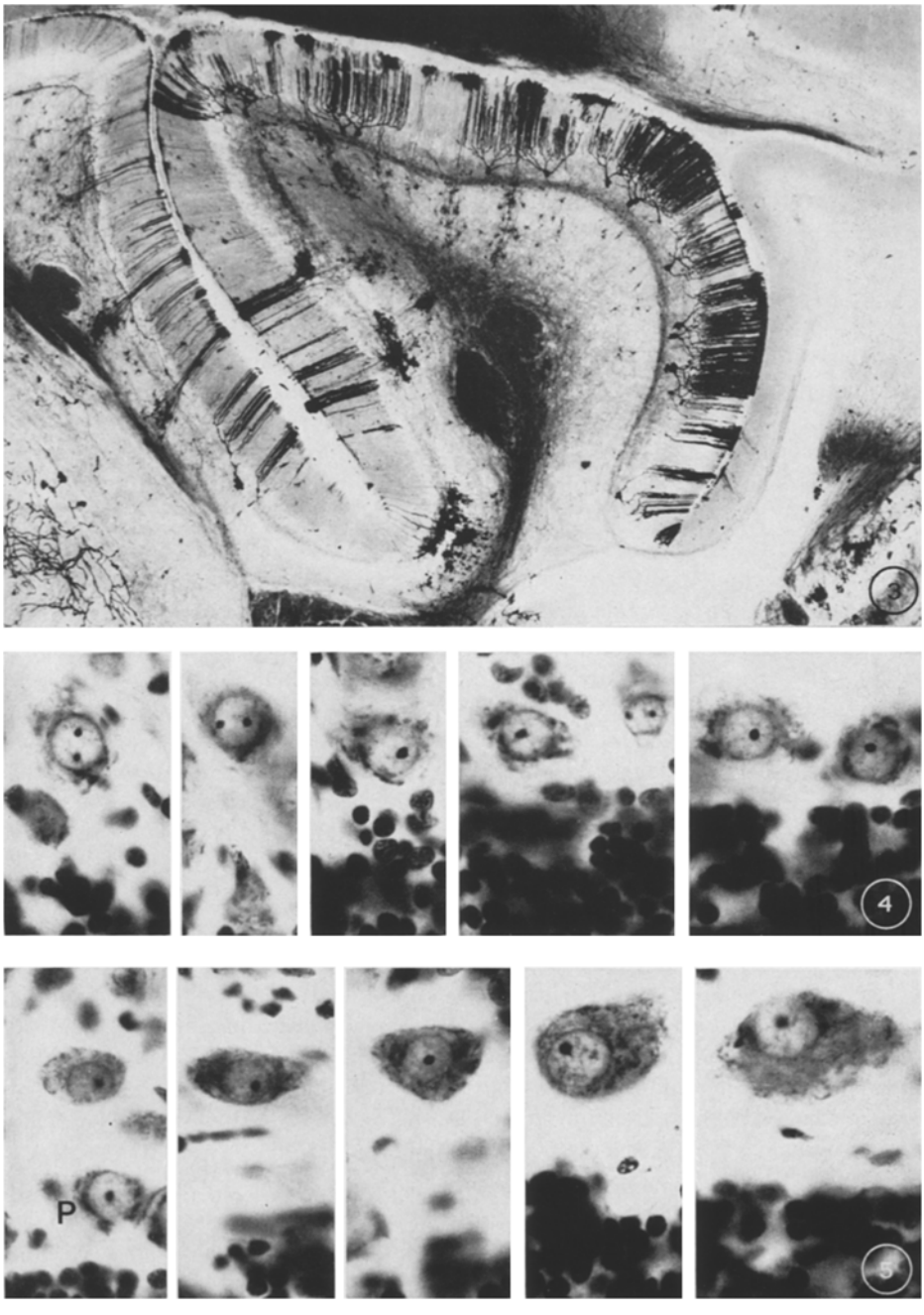


Fig. 3. Sagittal section through lobe C1, showing a number of impregnated Purkinje and granular cells. Rapid Golgi technique. ×48

Fig. 4. Cell bodies of Purkinje cells, sampled from sagittal Nissl preparations. ×850

Fig. 5. Cell bodies of eurydendroid cells, sampled from sagittal Nissl preparations. ×850

Table 1. Number and calibre of fibres present in the bundles of the tractus cerebellotegmentalis mesencephalicus. Cumulated number in brackets

Diameter in μm	10	9	8	7	6	5	4	3	2	1
Left side	1	—	6	18	52	131	154	251	488	324
	(1)	(1)	(7)	(25)	(77)	(208)	(362)	(613)	(1101)	(1425)
Right side	—	—	7	22	61	121	166	208	537	198
			(7)	(29)	(90)	(211)	(377)	(585)	(1122)	(1320)
Total number	1	—	13	40	113	252	320	459	1025	522
	(1)	(1)	(14)	(54)	(167)	(419)	(739)	(1198)	(2223)	(2745)

the delicate axons of the granular cells traverse this layer radially on their way to the molecular layer. The various cellular elements mentioned will now be described. However, a number of structural and ultrastructural characteristics of the Purkinje and eurydendroid cells will be discussed in a later section.

The Purkinje cells are irregularly scattered throughout the entire ganglionic layer. One frequently finds two and occasionally three Purkinje somata on top of each other. The total number of Purkinje cells in lobe C1 of one specimen was 6866. The somata are rounded, fusiform or pear-shaped. Their average size is 16 by 12 μm; the diameter of their nuclei is about 8 μm. The Purkinje somata contain distinct clumps of Nissl substance which tend to concentrate around the nucleus (Fig. 4). From their upper half three to five main dendrites arise. These ascend vertically or obliquely through the ganglionic layer. From their main dendrites short or long secondary dendrites originate and these in turn give rise to tufts of tertiary branches which ascend rigorously parallel to each other through the molecular layer toward the external surface (Figs. 3, 6). It is this peculiar arrangement of the tertiary dendrites of the Purkinje cells which produces the palisade pattern of the molecular layer (Nieuwenhuys and Nicholson, 1967). As in all gnathostomes the dendritic trees of the mormyrid Purkinje cells are flattened and sagittally oriented.

The eurydendroid cells, like the Purkinje elements, are found at all levels of the ganglionic layer. They are, however, much less numerous than the latter: in the same specimen in which the Purkinje cells were counted, their total number was 1249. In order to study the distribution of the eurydendroid cells a three dimensional reconstruction of lobe C1 has been prepared (Fig. 9). A chart based on this model shows the position of all of the eurydendroid cells in plan view (Fig. 10). The preparation of the model and the chart has been described in the section *materials and techniques*. The chart reveals that eurydendroid cells are present throughout the ganglionic layer of C1 and that distinct concentrations of these cells do not occur. It appears, however, that the elements under discussion in the rostral wall of C1 are more numerous than in the dorsal and caudal walls.

The cell bodies of the eurydendroid cells are fusiform, triangular or rhomboid-shaped with their long axis oriented sagittally (Fig. 5). They measure from 12.5 by 10 to 27 by 17.5 μm and are slightly larger than the Purkinje cells (Fig. 7).

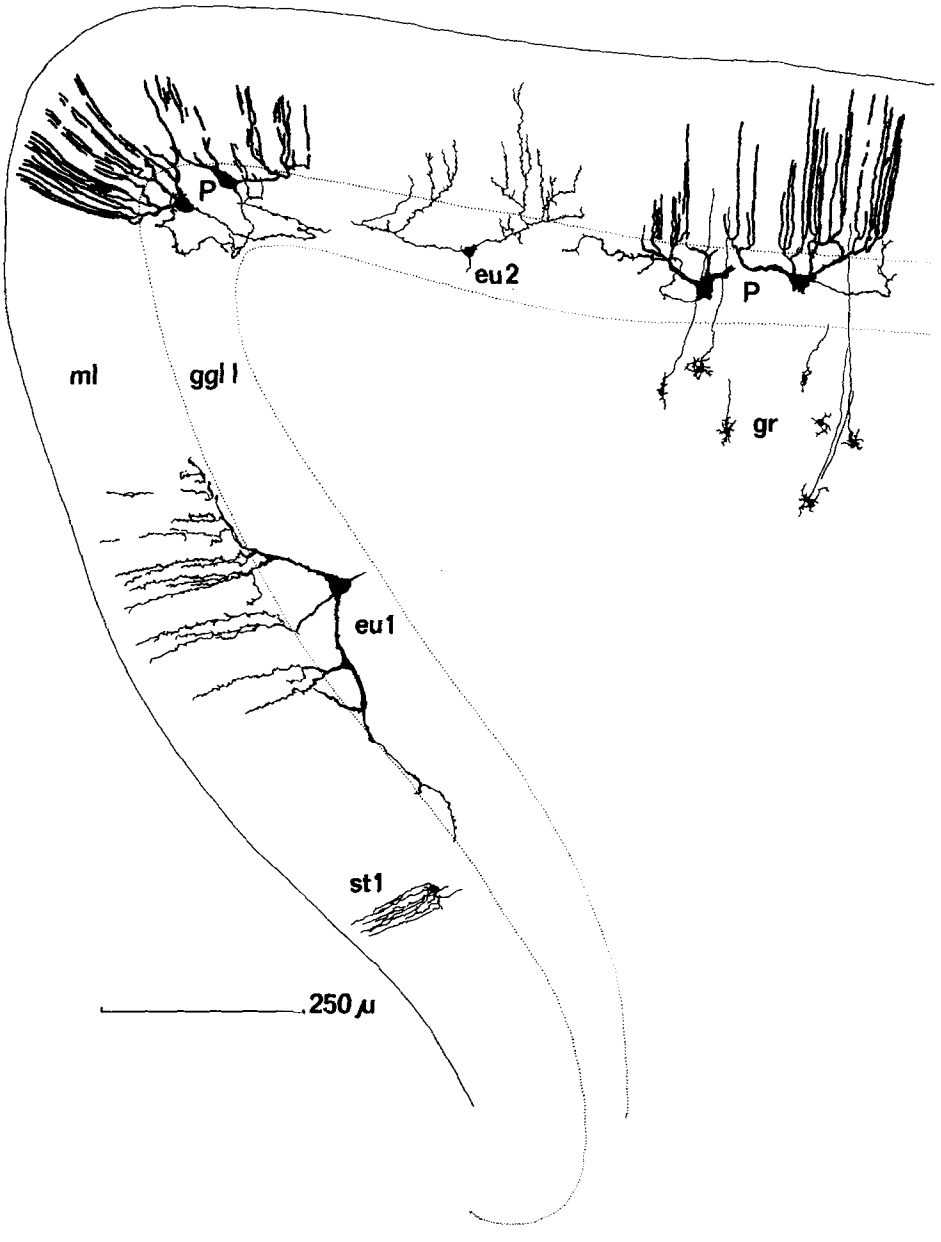
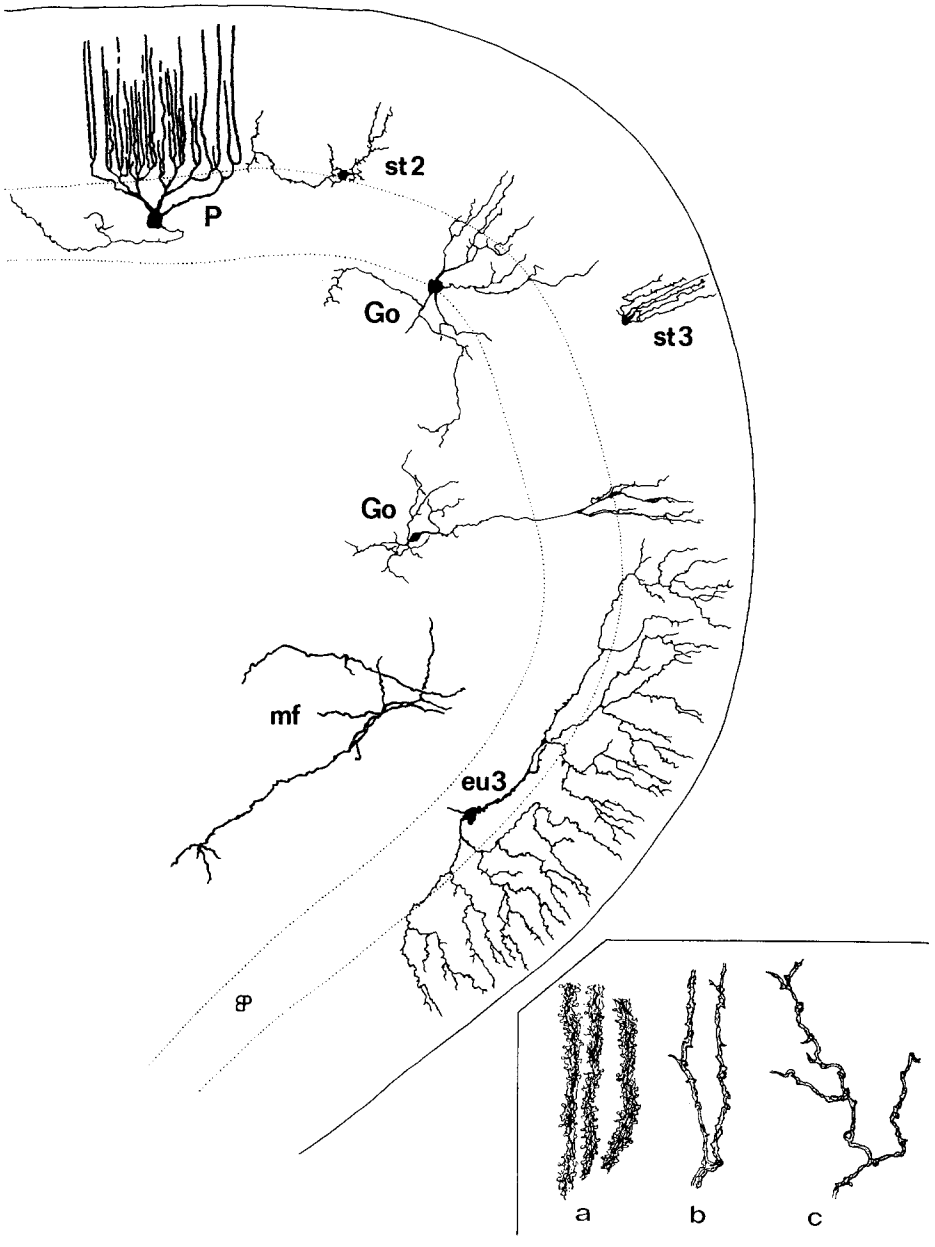


Fig. 6 a—c. Composite drawing of neurons observed in sagittally sectioned Golgi preparations.

Since their nuclei are consistently smaller than that of the Purkinje cells their average soma/nucleus ratio is considerably larger than that of the Purkinje cells (Fig. 8).

The eurydendroid cells can be clearly distinguished from the Purkinje cells because of the diffuse spread of the Nissl substance in their somata. Within this



Insert shows (a) dendrites of a Purkinje cell, (b) dendrite of eu 1 and (c) dendrite of eu 3. × 1270

Nissl substance local condensations occur, but these are less distinct than in the Purkinje cells and, moreover, they are not preferentially located around the nucleus (Figs. 4, 5). The dendritic trees of eurydendroid cells also have a much larger sagittal extension than those of Purkinje cells (Fig. 6). Their name is based upon this distinctive feature (eurys, Gr, is: wide). In general the eurydendroid

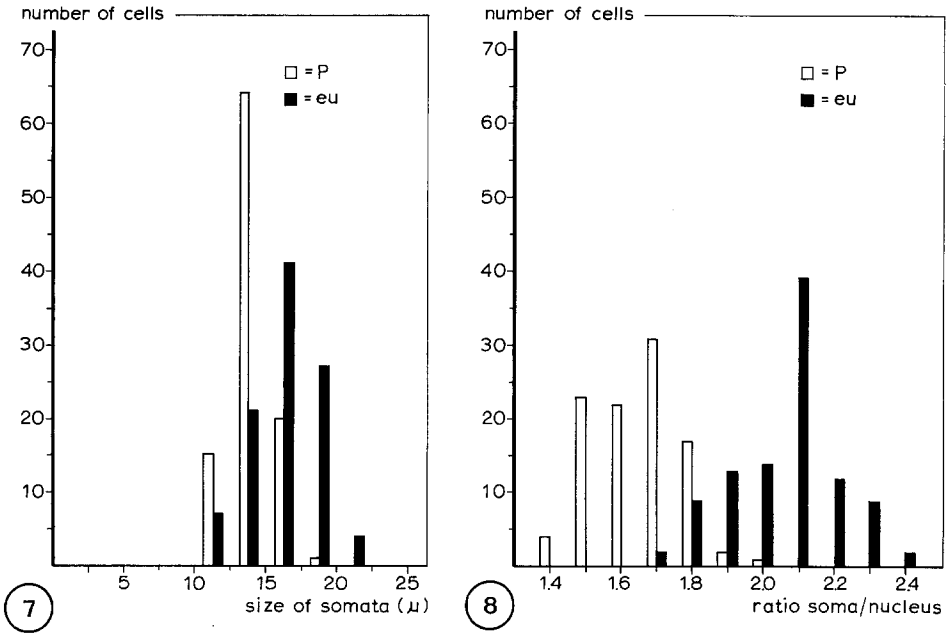


Fig. 7. Histogram showing the size distribution of the somata of 100 Purkinje and 100 eurydendroid cells sampled from sagittal Nissl sections throughout the lobe. The cell size abscissa intervals are in 2.5 μm units. These values were tested with Wilcoxon's two sample test. With a level of significance of 5%, P appeared to be smaller than 10^{-6}

Fig. 8. Histogram to compare the ratio soma/nucleus of the same Purkinje and eurydendroid cells as employed for the preparation of Fig. 7. The abscissa class intervals are in units of 0.1

cells have two main dendrites which arise from opposite poles of the soma. In the largest elements one or two additional dendrites may arise from their upper aspect. The main dendrites—their diameter is clearly correlated with the size of the soma—issue side branches which, either directly or after some further ramification, enter the molecular layer. Within the latter these branches show more bifurcation and adhere less strictly to the palisade pattern than those of the Purkinje cells (Fig. 6).

The small neurons which constitute a separate sub-layer in the outer one-third of the ganglionic layer require further analysis; they will be tentatively considered as deep stellate cells. Their cell bodies are rounded and have a diameter of 8–9 μm; the diameter of their nuclei is about 6 μm. In their cytoplasm small fragments of Nissl substance are clearly visible. Our interpretation of these elements as stellate cells is tentative since our Golgi material has yielded only a single—incompletely impregnated—stellate cell which, with regard to size and position, corresponds to the neurons observed in Nissl and Bodian material (Fig. 6; st 2). This element has thin, somewhat irregular dendrites that extend into the molecular layer. The horizontal process, passing to the left and dichotomizing into an ascending and a descending branch, probably represents a part of its axonal system. In Bodian

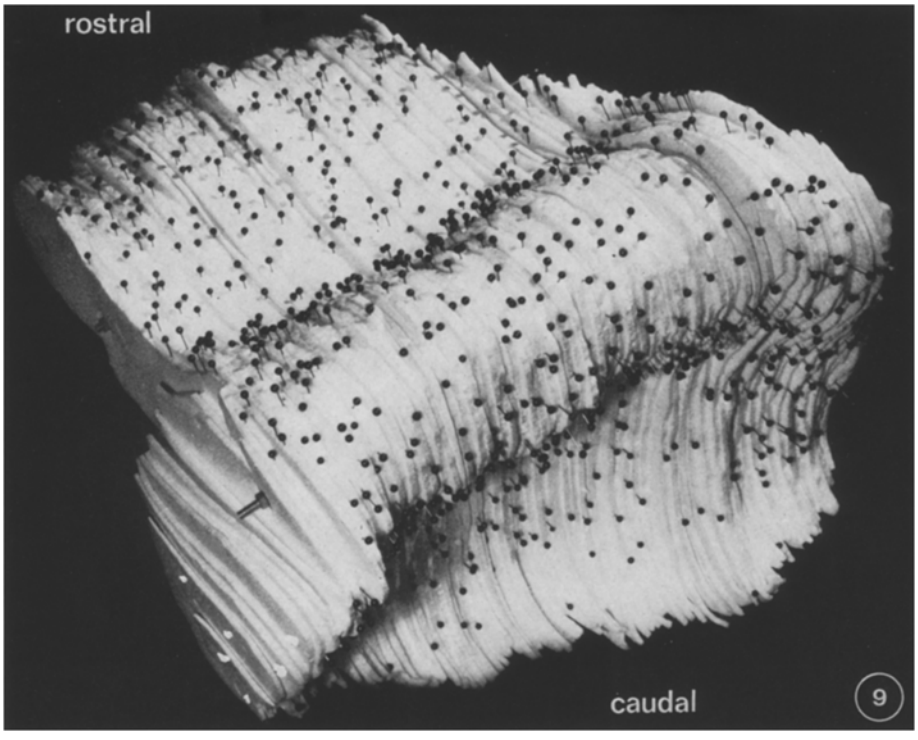
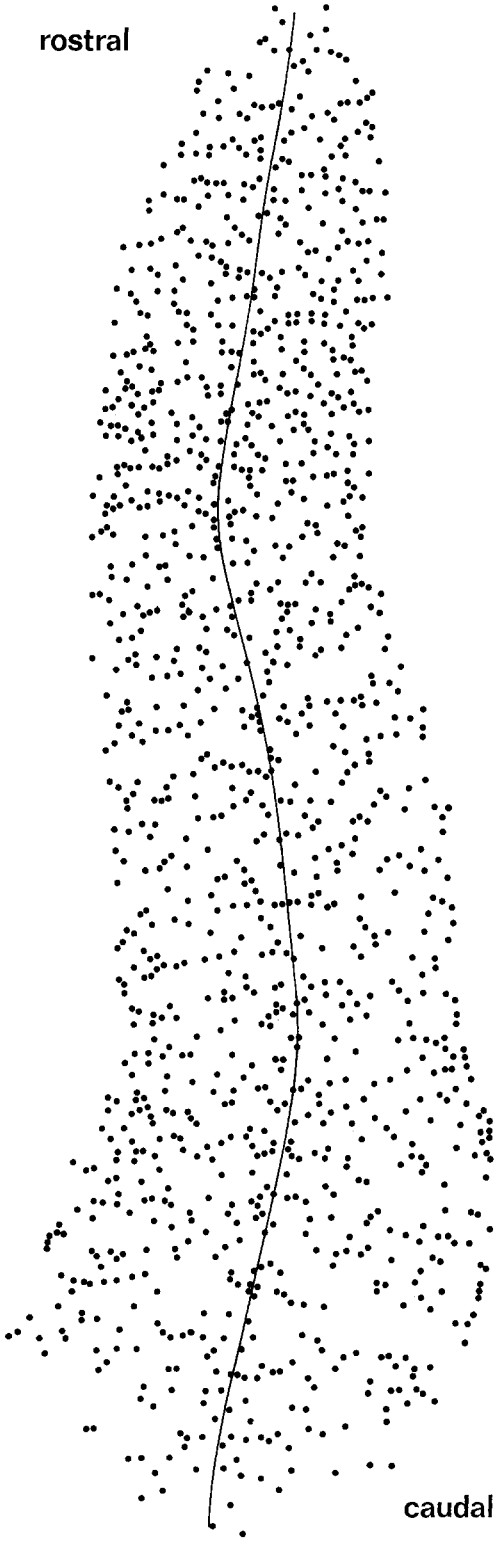


Fig. 9. Three dimensional reconstruction of the external surface of the granular layer of lobe C1. The position of the somata of the eurydendroid cells is indicated by pin's heads. The reconstruction has been prepared from a continuous, sagittal Nissl series

preparations the somata of the cells under discussion are covered by numerous small aggregations of argentophilic material. Preliminary ultrastructural observations show that the latter correspond to synaptic terminals.

In addition to the elements just discussed the ganglionic layer contains the somata of another type of small cell. In semi-thin sections stained with toluidin blue only the rather dark nuclei of these elements can be observed. These nuclei are often bean-shaped or lobulated; their diameter is about 5 μm. Except for some chromatin aggregates beneath the nuclear envelope their karyoplasm is homogeneous. These cells are found throughout the ganglionic layer, often as the satellites of Purkinje cells. They are probably a type of glia: the Golgi epithelial cells. The processes of these cells extend as Bergmann's fibres into the molecular layer. Glial elements of this type have been clearly observed in all parts of the corpus cerebelli and in the valvula as well (Nieuwenhuys and Nicholson, 1969b).

c) The Molecular Layer. The molecular layer forms a continuous sheet of neuropil in which the peripherally oriented dendrites of Purkinje, eurydendroid, Golgi and deep stellate cells articulate synaptically with the distal parts of the granule cell axons. The latter constitute the parallel fibre system. Apart from a wealth of dendritic and axonal processes the molecular layer contains scattered,



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small neurons, the superficial stellate cells (Fig. 6: st 1, 3). The somata of these elements are of a round or vertically oriented ellipsoid shape; they measure about 6 by 8 μm. Their dendrites show little sagittal extension and consist of ascending branches which adhere to the palisade pattern. We failed to trace the axons of these elements.

The dendritic trees of the Purkinje, eurydendroid and stellate cells are flattened and spread in the sagittal plane. The parallel-fibres, on the other hand, are oriented transversely and thus pass at right angles through the dendritic fields of the Purkinje cells. This orthogonal pattern is found in all gnathostomes; however, in mormyrids the "geometrization" of the molecular layer is even further advanced. Previous studies (Nieuwenhuys and Nicholson, 1967, 1969b) have shown that in most parts of the mormyrid cerebellum, including lobe C1, the straight terminal dendrites of the Purkinje cells are aligned in strict rows which alternate with thin sheets of parallel fibres. For an ultrastructural analysis of this remarkable "lattice" pattern we refer to Kaiserman-Abramof and Palay (1969).

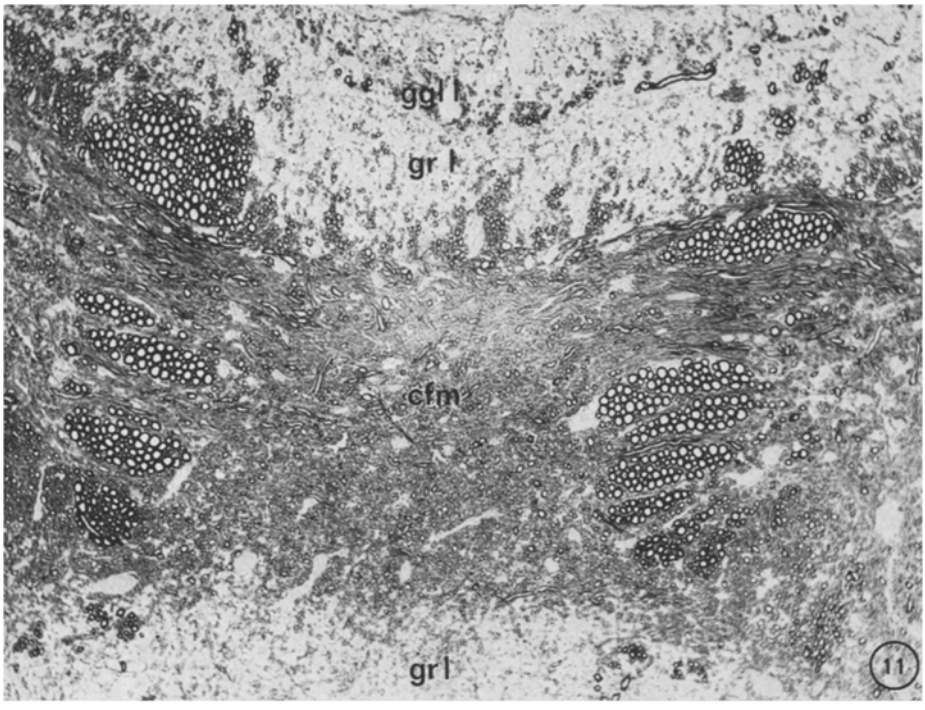
3. Purkinje and Eurydendroid Cells

In the present study special attention has been paid to the two types of large cells present in lobe C1, *i.e.*, the Purkinje and the eurydendroid cells. The somata of both types are situated in the ganglionic layer and both have a flattened dendritic tree that extends into the molecular layer. However, it has already been mentioned that between these two cell types distinct differences exist with regard to, among other things, the distribution of Nissl substance in their somata and the spread of their dendritic arborescences. Some further structural differences between the Purkinje and eurydendroid cells will now be discussed and the place which both cell types occupy in the circuitry of C1 will be considered.

Golgi and preliminary ultrastructural studies showed that the vertically oriented terminal dendrites of the Purkinje cells are densely covered with spines (inset Fig. 6: a). The enlarged terminal parts of the spines are in synaptic relation with bulbous swellings of the parallel fibres. The latter may be inserted in the main axis of the parallel fibres themselves, but more frequently they are situated at the end of very short collateral branches which extend from the parallel fibre sheets toward the Purkinje dendrites. The dendrites of the eurydendroid cells are somewhat irregular, but bear no spines (Fig. 6, inset b, c). Electron micrographs from sections cut tangentially through the molecular layer show that the diameters of the largest branches of the eurydendroid cells surpass that of the shafts of the Purkinje dendrites. Contrary to the latter they contain no subsurface cisterns. Around their perimeter the dendrites of the eurydendroid cells are in synaptic contact with terminal knobs of parallel fibres.

The axons of the Purkinje cells emerge from the inferior pole or from the side of the somata. Quite often they describe a small loop and then travel a horizontal course for a variable distance. It is noteworthy that our Golgi material revealed

Fig. 10. The external surface of the granular layer of lobe C1, with the position of the perikarya of eurydendroid cells marked upon it, flattened out into a plane



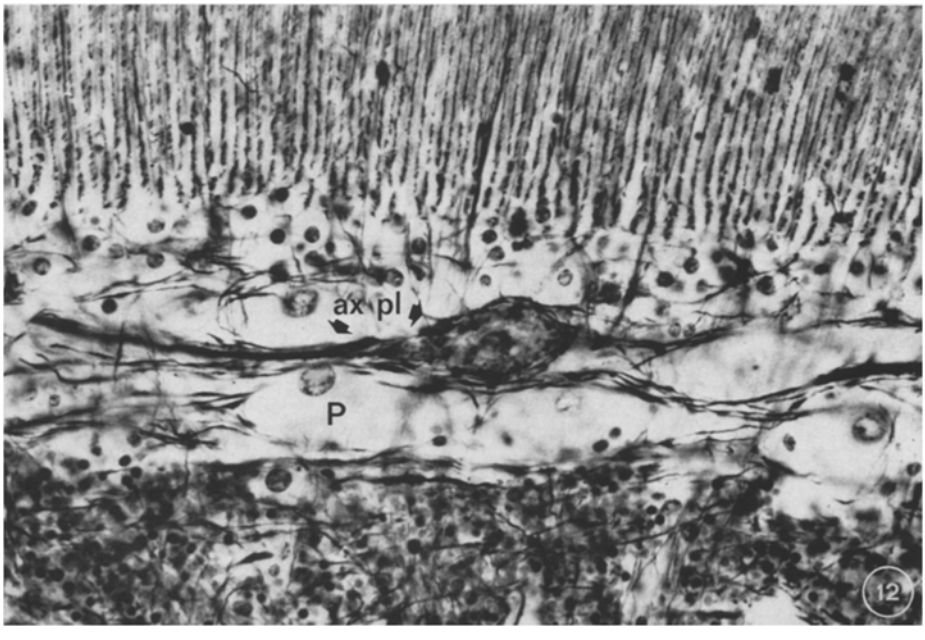


Fig. 11. Horizontal section through lobe C1, showing bilateral bundles of coarse fibres. The approximate position of this section is indicated in Fig. 2. Semi-thin (1μm) epon section stained with paraphenylenediamine. ×170

Fig. 12. Sagittal section through the ganglionic layer of lobe C1, showing an eurydendroid cell. Its soma and dendritic trunks are invested in an axonal plexus. Bodian-Ziesmer preparation. ×480

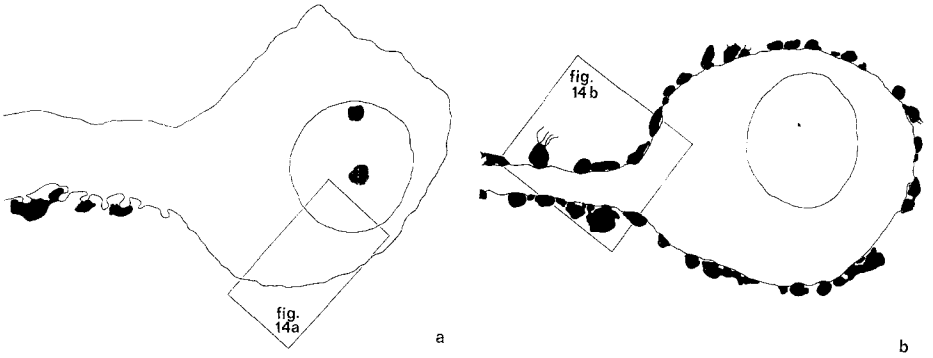
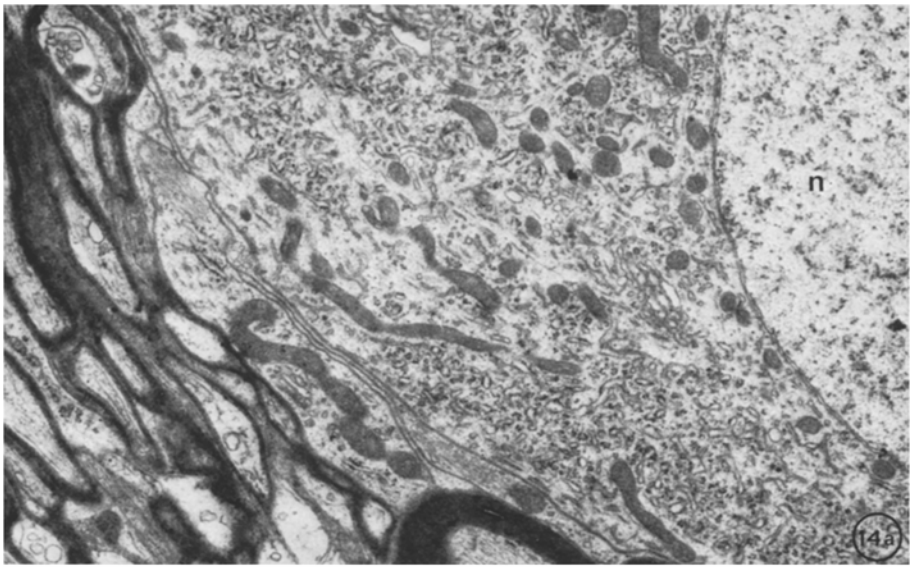


Fig. 13 a and b. Tracings of the somata of (a) Purkinje cell and (b) eurydendroid cell, prepared from montages of electronmicrographs. Axon terminals are shown in black

more than 25 Purkinje axons in C1 and not a single one appeared to enter the granular layer. The axons under discussion are often smooth initially, but soon develop distensions along their length. At irregular intervals they issue collaterals of varying lengths which, like their parent axons, are often beaded. Often collateral or terminal branches of the Purkinje axons ascend toward the most superficial part of the ganglionic layer and sometimes even enter the deepest zone of the molecular layer (Fig. 6). Semi-thin sections stained with p-phenylenediamine revealed the presence in the ganglionic layer of numerous sagittally running thin myelin sheaths with a diameter of about 2 μm. Most probably these myelin sheaths envelop the smooth initial parts of the Purkinje axons. Bodian-Ziesmer preparations reveal that the ganglionic layer contains an extensive plexus of axons which also are preferentially sagittally oriented. These axons are richly provided with fusiform swellings. Although there is no direct proof that they originate from Purkinje cells, comparison of the silver and Golgi pictures leave no doubt that this is the case. A previous study (Nieuwenhuys and Nicholson, 1969b) reported that the somata and the very argentophilic dendritic trunks of the eurydendroid cells (then termed giant and fusiform elements) are densely surrounded by this axonal plexus, whereas the Purkinje somata lack such an axonal investment (Fig. 12).

The relations just sketched strongly suggest that the Purkinje axons synapse with the eurydendroid cells, but of course the silver material does not permit conclusive identification of synapses. Therefore the somata of the large cells of the ganglionic layer were subjected to an electron microscopical analysis. Purkinje somata were easily recognized through the presence of numerous hypolemmal cisterns (Kaiserman-Abramof and Palay, 1969). They are ensheathed in glia lamellae and rarely have synaptic endings on their surface (Figs. 13a, 14a). The somata and dendritic trunks of the eurydendroid cells, on the other hand, are surrounded by a great number of axon terminals many of which synapse on their surface (Figs. 13b, 14b). Some of these synaptic enlargements arise directly from myelinated axons. They frequently show several separate junctional complexes. At the active zones both pre- and postsynaptic components have shallow densities of about equal size and staining intensity. The synaptic cleft is widened and filled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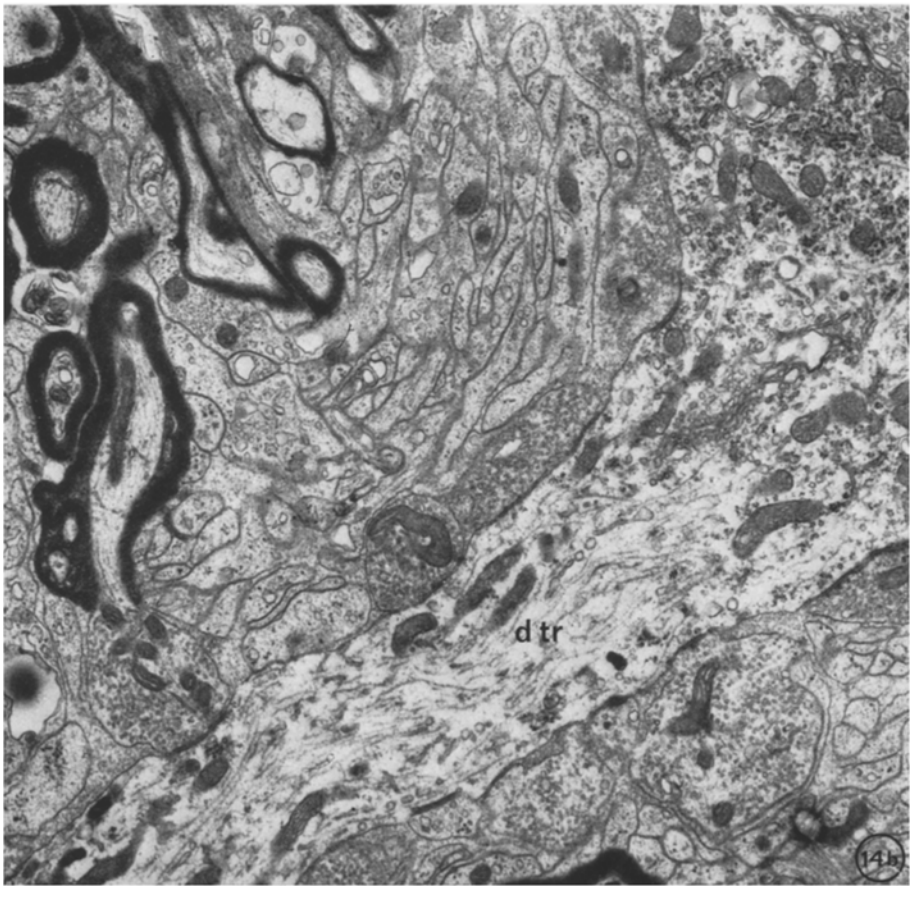
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with dense material. Round or elliptical synaptic vesicles are dispersed throughout the terminals in a rather dense matrix. The morphological appearance of these boutons terminaux is very similar to that of the endings of the main axons and recurrent collaterals of mammalian Purkinje cells (Larramendi and Lemkey-Johnston, 1970; Chan-Palay, 1971, 1973). We conclude that in lobe C1 of the mormyrid cerebellum the Purkinje axons impinge upon the somata and the main dendrites of the eurydendroid cells. It should be added that the rare synapses observed on the somata of Purkinje cells closely resemble those on the eurydendroid elements. Preliminary observations have shown that the same holds true for the synaptic endings upon the surface of the deep stellate cell somata. This indicates that at least a part of the ascending collateral and terminal branches of the Purkinje axons terminate upon the deep stellate cells.

The axons of the eurydendroid cells emerge from the basal side of these elements and are directed toward the granular layer. In Golgi preparations they invariably appear to end abruptly at a short distance from the soma (Fig. 6). It is known that such sudden failure in the impregnation of axons often coincides with myelinisation. Indeed, in sagittally cut Häggquist sections it has been repeatedly observed that the axons of eurydendroid cells after having entered the granular layer, acquire a coarse myelin sheath (Nieuwenhuys and Nicholson, 1969b). These myelinated fibres pass radially in the lobe and finally join the tractus cerebellotegmentalis mesencephalicus of Stendell (1914), which has already been discussed. These observations suggest that the axons of the eurydendroid cells constitute the output system of C1. Since we cannot prove this directly, we tried to derive some further evidence on the destination of the axons of these elements from a comparison of their total number present in C1 with the numbers of coarse fibres present in the tractus cerebellotegmentalis mesencephalicus.

It has already been mentioned that the size of all of the fibres present in the tractus cerebellotegmentalis mesencephalicus has been determined in a horizontal section, at the level indicated in Fig. 2. The results of this analysis are represented in Table 1. It will be seen that the tract in question does not contain a sharply delimitable contingent of coarse fibres. It will be clear that in a numerical comparison of the fibres in the bundle and the eurydendroid cells only the cells situated dorsal to the level of the section in which the fibre pattern was analysed should be taken into account. In order to approach this number as close as possible we introduced into our model of C1 (Fig. 9) a plane corresponding to the level of this section. It appeared that of the total number of 1249 eurydendroid cells present in the model 983 elements were situated dorsal to this plane. This number falls between the total numbers of fibres larger than 4 μm and 3 μm and thus supports the thesis that the coarser fibres in the tractus cerebellotegmentalis mesencephalicus of Stendell derive from eurydendroid cells. Fibres with diameters of 3 μm or less are not only present within, but also beyond the territories of the tractus cerebellotegmentalis mesencephalicus. They probably represent mossy fibres.

Fig. 14 a and b. Electronmicrographs showing (a) part of the soma of a Purkinje cell and (b) part of the soma and the proximal portion of a dendritic trunk of an eurydendroid cell. ×11000. The position of these figures is indicated in Fig. 13

Finally a brief comment should be made on the presence of climbing fibres. In a previous paper (Nieuwenhuys and Nicholson, 1969b) it was reported that in Golgi material small groups of short, stubby projections are present on the somata and the proximal dendrites of the Purkinje cells. Kaiserman-Abramof and Palay (1969) identified these gemmules at the ultrastructural level and noted that they are in synaptic contact with varicose axons that follow the main dendrites of the Purkinje cells. The above mentioned authors tentatively identified these axons as climbing fibres. The groups of dendritic projections just referred to have also occasionally been observed by us on Purkinje elements present in C1 (Fig. 13A), but up to now our material has not yielded conclusive evidence for the presence of climbing fibres in this lobe.

Discussion

In the preceding section evidence has been presented for the occurrence of the following neuronal elements in lobe C1 of the mormyrid cerebellum: mossy fibres, granule, Golgi, Purkinje, eurydendroid and stellate cells. We will now design a tentative circuit for this lobe and speculate on the functional roles of the various neuronal elements. The pattern of connectivity has been summarized in Fig. 15 at three different levels of schematization. Fig. 15c shows that the known and probable connexions may be represented as an open loop superimposed on which are four different closed circuits containing the interneurons.

The open loop which, in terms of number of synaptic interruptions, forms the shortest path through C1 consists of the mossy fibres, the granule cells and the eurydendroid cells. The mossy fibres, which constitute the only positively identified input system to C1, ramify in the granular layer. Their branches have varicosities that enter into synaptic contact with the distal ends of a number of granule cell dendrites. The ultrastructural studies of Kaiserman-Abramof and Palay (1969: C3, valvula) have shown that the varicosities of the mossy fibres constitute the core of synaptic glomeruli the structure of which is much simpler than in higher vertebrates. The axons of the granule cells ascend toward the molecular layer where they either divide in T-fashion or simply bend horizontally to become parallel fibres. The latter are arranged in highly regular, transversely oriented, sheets. They synapse with dendrites of all of the neuronal elements present in C1, except for their own parent neurons, the granule cells. The open loop is completed by the eurydendroid cells the axons of which leave C1, constituting the tractus cerebellotegmentalis mesencephalicus. Physiological experiments in cats and in several nonmammalian vertebrates (Eccles *et al.*, 1967; Llinás and Hillman, 1969; Nicholson *et al.*, 1969; Llinás and Nicholson, 1969) have shown that the mossy fibre-granule cell—parallel fibre system forms excitatory synapses on all elements which extend their dendrites in the molecular layer. It is reasonable to assume that this also holds true for the mormyrids. The physiological properties of the eurydendroid cells are entirely unknown; yet the fact that these elements constitute the output system of C1 renders it likely that they, notwithstanding their superficial position, are comparable to cells of the deep cerebellar nuclei of other vertebrates[1]. Since the latter are known to be excitatory in nature

1 In a previous paper (Nieuwenhuys and Nicholson, 1969b) this comparison has been further substantiated.

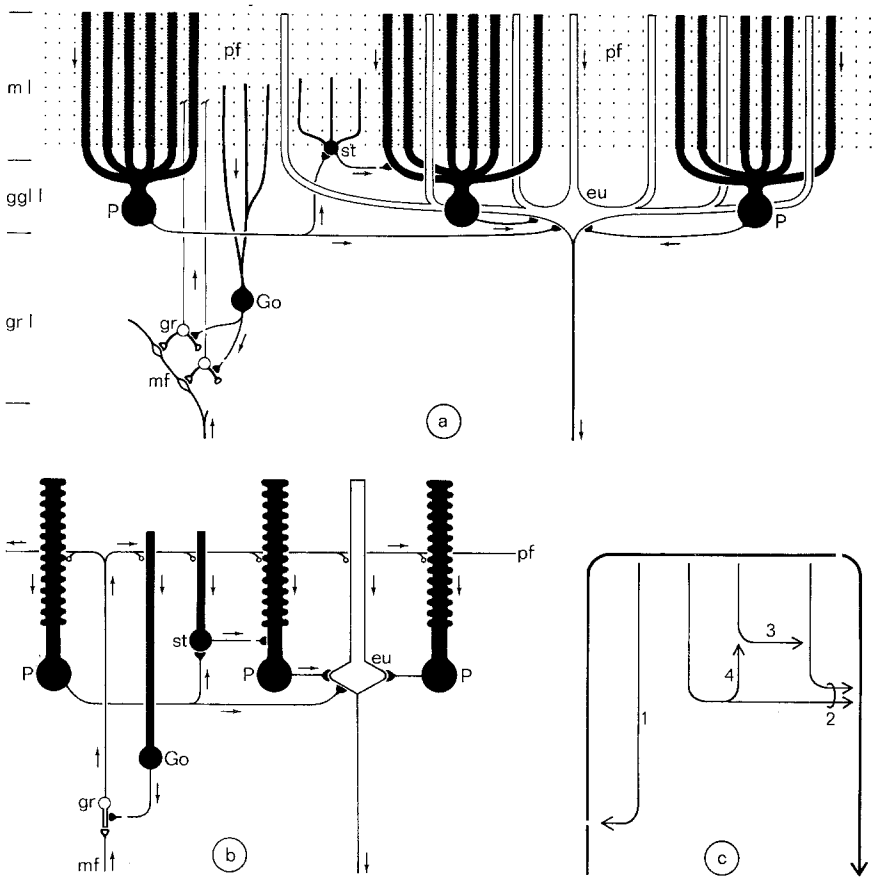

Fig. 15 a—c. The neuronal elements in lobe C1 and their relationships. (a) semi-diagrammatic drawing illustrating the mossy fibre and the five neuron types identified; (b) diagram of the pattern of connectivity; (c) highly diagrammatic representation of the main loop through C1 (heavy arrow) and the various circuits superimposed on this loop. Further explanation in text. In all figures the arrows indicate the directions of impulse propagation. In a and b all cells supposed to be inhibitory are shown in black. Interrupted axons indicate connexions not yet established with certainty

(Eccles *et al.*, 1967) it seems plausible that the eurydendroid cells are also excitatory.

Before discussing the various circuits in C1—numbered 1 through 4 in Fig. 15c—it should be emphasized that the initial part of all of these paths is formed by the excitatory granule cell—parallel fibre system (Fig. 15).

1. The dendrites of the Golgi cells extend into the molecular layer whereas their ramifying axons terminate in the granular layer. Our Golgi material did not reveal which structures are contacted by their terminals, but it is probable that they synapse with the dendrites of granular cells, as has been described for various other vertebrates (Eccles *et al.*, 1967; Hillman, 1969; Sotelo, 1969). Physiological experiments (Eccles *et al.*, 1967; Llinás and Nicholson, 1969; Shi-

mona, 1973) have shown that the Golgi cells inhibit the granule cells. Thus the circuit closed by these elements represents a negative feed back system.

2. The Purkinje cells derive their main input from the parallel fibres and their axonal system converges upon the somata and proximal dendrites of the eurydendroid cells. According to the current concepts of the physiology of the cerebellum (Eccles *et al.*, 1967; Llinás and Hillman, 1969; Llinás and Precht, 1969b; Kidokoro, 1969) the Purkinje cells have a direct inhibitory action upon all their target cells. If this also applies to the mormyrid cerebellum the eurydendroid cells receive their excitatory input from the parallel fibres, whereas the Purkinje cells act upon them as a feed forward inhibitory system.

3. Nissl and Bodian preparations have revealed numerous small neurons in the most superficial part of C1. So far our Golgi material has yielded only a single impregnated cell of this type (Fig. 6: st 2). The dendrites of this element extend into the molecular layer; its axon passes for some distance horizontally and then ramifies likewise in the molecular layer. Provided that these observations may be generalized for all of the small neurons of the ganglionic layer, C1 contains a well developed system of deep stellate cells. In other lower vertebrates these elements make synapses with the dendrites of the Purkinje cells (Hillman, 1969; Sotelo, 1970) and mediate a strong inhibition on the latter (Nicholson and Llinás, 1969; Nicholson *et al.*, 1969; Shimona, 1973). Following excitation of a narrow beam of parallel fibres the deep stellate cells exert an inhibitory action upon the Purkinje cells which flank the beam of excitation. The net effect of this "off beam" inhibition of the intrinsically inhibitory Purkinje cells, will be a disinhibition of the target neurons of the latter.

4. Axons or axon collaterals of the Purkinje cells ascend to the superficial part of the ganglionic layer, where they enter into synaptic contact with the deep stellate cells. Such relations have not been described as yet for other lower vertebrates; however, it is well known that in mammals recurrent collaterals of Purkinje cells synapse with the functional equivalents of the deep stellate, *i.e.* the basket cells (Larramendi and Lemkey-Johnston, 1970; Chan-Palay, 1971). The experiments of Llinás and Precht (1969a, cat) revealed that the recurrent Purkinje collaterals produce a definite inhibitory action on the basket cells. The authors conclude that these collaterals act via the basket cells by means of disinhibition as a recurrent facilitatory system upon the Purkinje cells. It is conceivable that in lobe C1 the system constituted by Purkinje axons/collaterals—stellate cells—Purkinje cells plays a similar functional role.

Placing the eurydendroid cells centrally in the circuitry of C1 our speculations on the functional significance of the various neuronal elements in this lobe may be summarized as follows (cf. Fig. 15):

1. The eurydendroid cells are driven by the mossy fibre—granule cell—parallel fibre system. This excitatory input is modulated by the Golgi cells which form a negative feed back from the parallel fibres to the granule cell dendrites.

2. The Purkinje cells inhibit the eurydendroid cells.

3. The deep stellate cells inhibit the Purkinje cells, thus exerting a facilitatory action on the eurydendroid cells.

4. Ascending axons and collaterals of Purkinje cells act, via deep stellate and Purkinje cells, as a disfacilitatory system upon the eurydendroid cells.

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