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A QUANTITATIVE ELECTRON MICROSCOPIC STUDY OF THE PURKINJE CELL AXON INITIAL SEGMENT

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Abstract—Quantitative electron microscopic examination of seventeen Purkinje cell axon initial segments has revealed the presence of a small but definite number of synapsing basket axon boutons on the initial segment, with an average of 3.5 axo-axonic synapses in the cat, and 2.9 in the rat. Although approximately 80% of the initial segment surface is covered by thin glial processes, 14.4% of the initial segment surface in the rat and 25.8% in the cat is contacted by synapsing and non-synaptic basket axons of varying size. It is suggested that the axo-axonic synapses represent a significant component of the powerful inhibition of the Purkinje cell by basket axons.

THE INHIBITORY nature of the basket cell to Purkinje cell synapse was demonstrated in 1963 (ANDERSEN, ECCLES & VOORHOVE, 1963). The main structural basis of the Purkinje cell inhibition by basket cells appears to be the Purkinje basket. The organization of this structure has been described in detail (PALAY, 1964*a*; HÁMORI & SZENTÁGOTHAI, 1965; FOX, HILLMAN, SIEGESMUND & DUTTA, 1967; MUGNAINI, 1972); the number of collaterals of basket cells contributing to one basket was estimated by SZENTÁGOTHAI (1965) to be around 50, giving chiefly axo-somatic synapses to the bottom part of the Purkinje cell somata. In addition HÁMORI & SZENTÁGOTHAI (1965) have observed that the initial segment of the Purkinje cell axon also receives numerous synapsing basket cell boutons, whereas the rarity of such axo-axonic synapses had been stressed for the rat (PALAY, 1964a, 1964*b*, 1967; PALAY & CHAN-PALAY, 1974). In view of the possible significance of direct contacts by inhibitory terminals in the initial segment region as the main site of the generation of the conducted spike (ARAKI & OTANI, 1955; COOMBS, CURTIS & ECCLES, 1957), it was deemed important to determine the number, and precise location of axo-axonic synapses as well as to study other ultrastructural characteristics of the initial segment region. Quantitative electron microscopy, based on 17 Purkinje cell initial segments, from both rat and cat, including reconstruction of the relevant details of some axons was used with this objective.

EXPERIMENTAL PROCEDURES

One cat and two rats were fixed by perfusion through the heart with 1.5% glutaraldehyde and 2% paraformaldehyde dissolved in 0.1 M sodium phosphate buffer (pH 7.2–7.4). After removing the brain from the skull, small tissue blocks were cut from the vermian lobule VII (Larsell) of the cerebellum and immersed in the same fixative for 5 h. Thereafter specimens were washed briefly in buffer, postfixed in phosphate buffered 2% OsO_4 solution, dehydrated, and embedded in Durcupan.

Electron microscopic serial section were cut in the transverse plane of the folium by an LKB ultramicrotome, using glass knives. Ribbons of 100–200 successive sections were mounted on formvar coated single-slotted grids with a 2 × 1 mm aperture. Sections were stained with aqueous uranyl acetate followed by lead citrate (REYNOLDS, 1963). Serial sections were studied by the JEM 100B electron microscope.

Purkinje cell axon initial segments, cut longitudinally were followed continuously from their origin to the beginning of the myelin sheath; approximately 60–100 sections were necessary to include their whole length. In seven cases every second section of the series were photographed. Photographic prints at a final magnification of 27,500 were used for both three-dimensional graphical reconstruction, and for membrane length measurements of these seven cases. Membrane length was measured with an electronic graphic calculator (Numonics corp., North Wales, Pa).

RESULTS

The initial segment (IS) of the Purkinje cell axon in characterized by a limiting membrane, undercoated with granular electron-dense material (Figs. 1, 3), (CHAN-PALAY, 1972; PALAY, SOTELO, PETERS, ORKAND, 1968). This undercoating starts immediately at the point of emergence of the axon from the cell body and can be traced to the beginning of the myelin sheath (Figs. 3a, b, d). The course of the IS varies from cell to cell. In the cat slight curves or bends may occur, while the course is more straight in the rat (Fig. 6). The lengths and diameters of the IS are given in Table 1. The length of eleven IS were measured under low magnification (8000×) whenever the plane of sectioning was favourable, i.e. when the emergence of the axon and the beginning of its myelin sheath could be seen only few sections apart. Although there seems to be no difference in the length of the IS between the two species, the IS of Purkinje cell in the cat appears to be considerably thicker than in the rat. It can be also seen that the IS gradually tapers

Abbreviation: IS, initial segment.

FIG. 1. Survey picture of the Purkinje cell axon initial segment as it leaves the cell body. A synapsing basket bouton (arrow) can be seen on the initial segment (the same with higher power in Fig. 1a). Axosomatic synapse is labelled (large arrow). The initial segment is surrounded by numerous middle size branches and small finger-like processes of basket cell axons, though the surface proper of the initial segment is covered predominantly by longitudinally arranged thin glial processes (open arrow). Occasionally the continuity between a finger-like process and a basket axon can be observed (arrowhead). Scale 1 μm in all pictures. (a) High power micrograph of axo-axonic synapse from Fig. 1. Coated pits (arrow) regularly occur on the initial segment, interrupting the electron dense undercoating of the axon membrane.

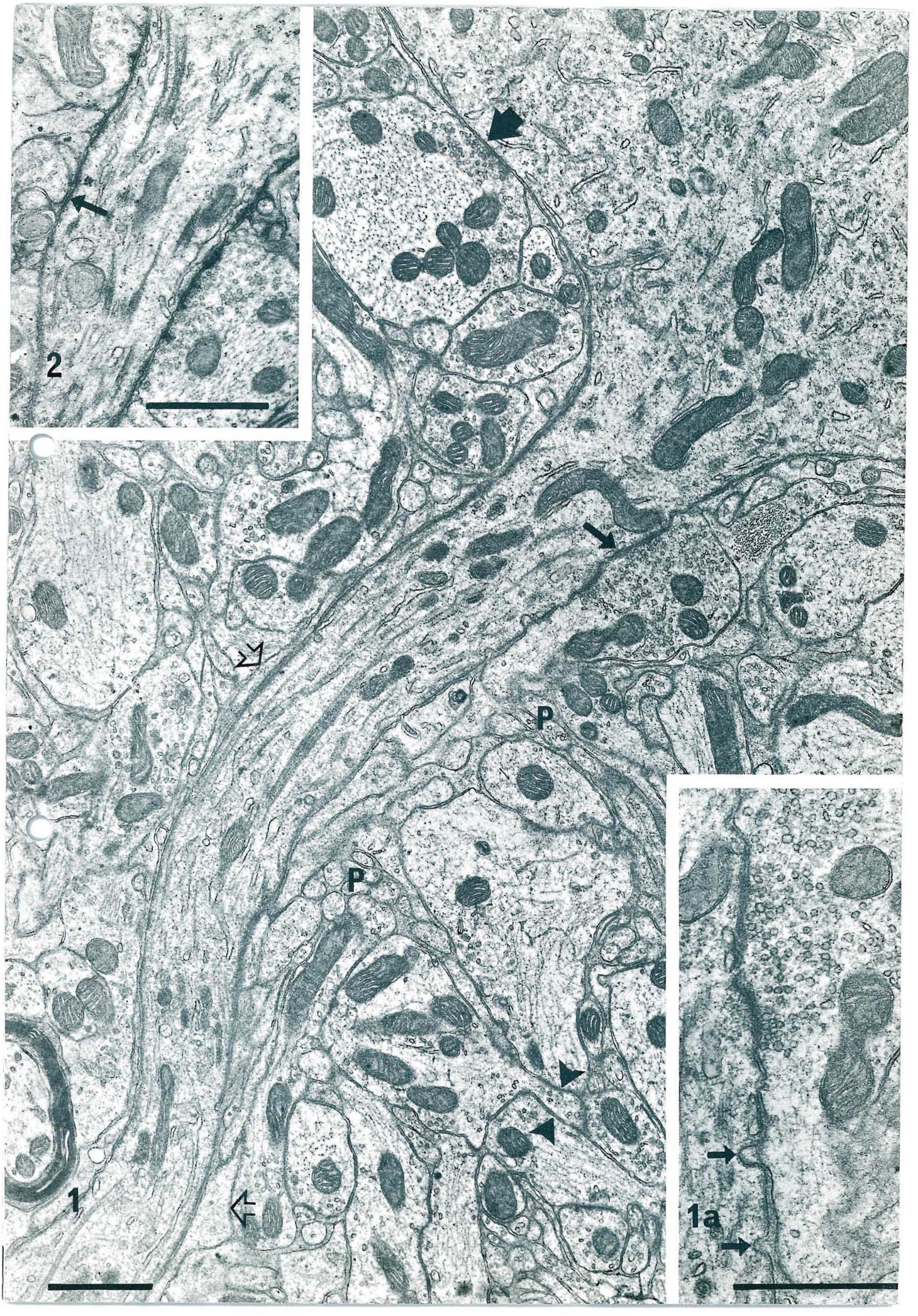
FIG. 2. Large basket cell bouton with an extended synaptic specialization on the cat Purkinje cell initial segment. A smaller bouton forms a synapse near to the emergence of the axon, and a vesicle-containing small process also contacts the axon surface (arrow).

FIG. 3a–d. Details of a cat Purkinje cell axon initial segment which was followed in serial sections from the origin until the beginning of the myelin sheath. (a) Emergence of the axon from the cell body. Arrow indicates a large basket bouton. (b) A small segment of the initial segment twisting among the basket cell axon collaterals in the 27th section from Fig. 3a. On the left side a large glial process (GL) separates the pinceau from the cerebellar glomeruli (G). (c) High power picture of the Purkinje cell axon shown in Fig. 3b. One small synapsing bouton (arrow) is visible even at this distance from the cell body. Another axon (A) contacting the initial segment was found in serial sections to establish no synaptic contact. (d) Beginning of the myelin sheath 34 sections away from Fig. 3b. Note that even this distal portion of the initial segment is surrounded by finger-like processes (P) of basket axons.

FIG. 4a–b. Serial sections of the cat Purkinje cell at the emergence of its axon. This initial segment was also reconstructed three dimensionally and is shown in Fig. 7a. (a) The boutons form synapses on the base of the soma (B1, B2) whereas a third is seen cut tangentially at the beginning of the initial segment (B3). (b) Eighteen sections away from Fig. 4b the axon providing bouton B1 can be seen descending and forming an additional bouton on the initial segment (B4). Another basket axon bouton on the initial segment is also seen in this section (B5).

FIG. 5. Tangential section of the initial segment of the rat Purkinje cell axon near to the cell body (PC). Two synapsing basket axon boutons on the initial segment are visible (arrows). Compare the size of these boutons to those of the cat. Note the longitudinal arrangement of thin glia processes to the initial segment (open arrow).

FIG. 6. General view of the pinceau of the rat Purkinje cell (PC) with the initial segment. Note that the whole pinceau is 'encapsulated' by large descending basket axons (– –) or glial preocesses (· · ·). The central area of the pinceau, especially in the vicinity of the initial segment is made up of small axon branches and terminal twigs of the basket axons, whereas the initial segment proper is covered predominantly by thin glial processes (open arrow).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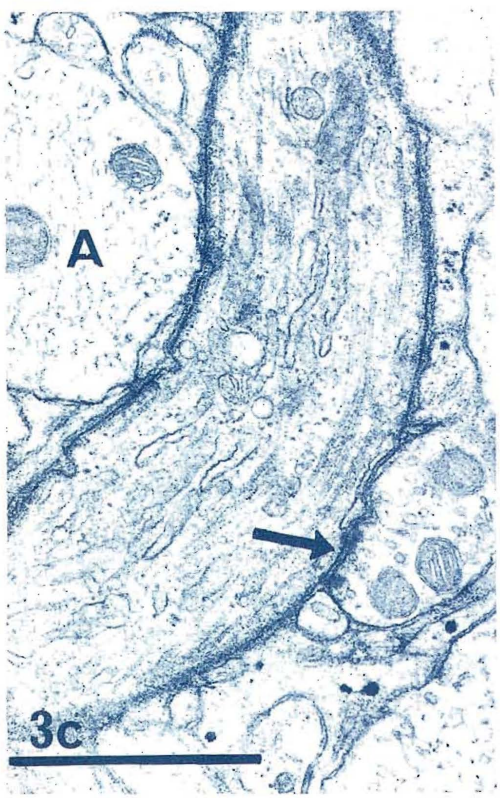
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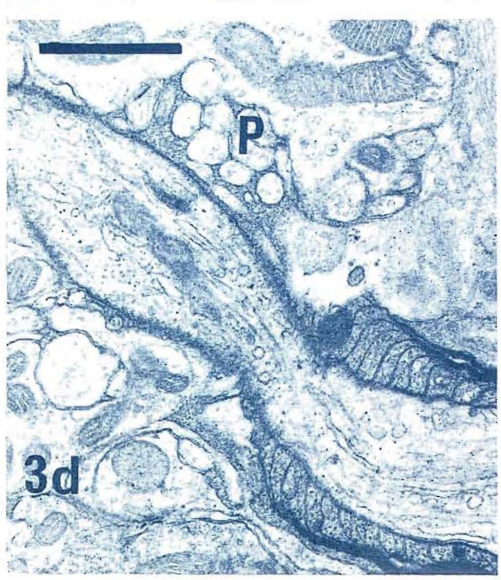
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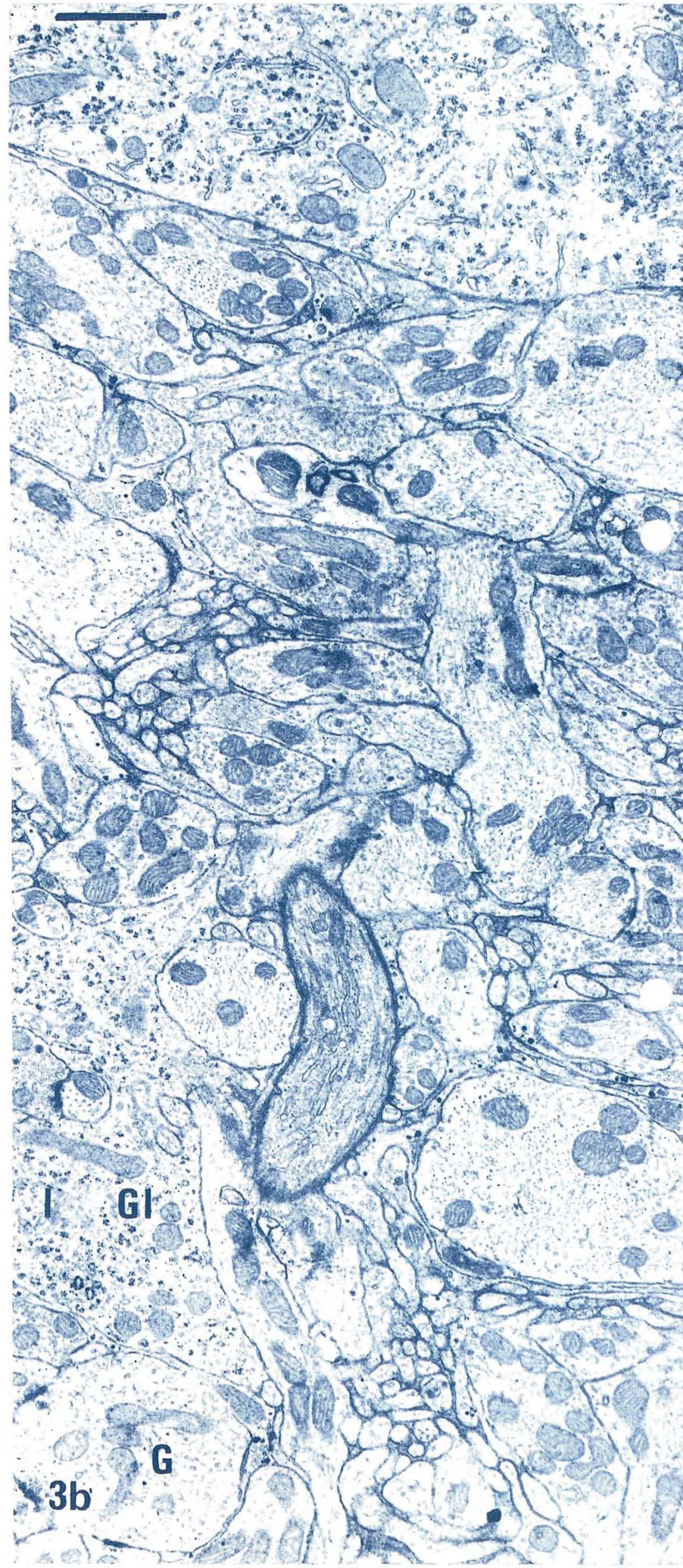
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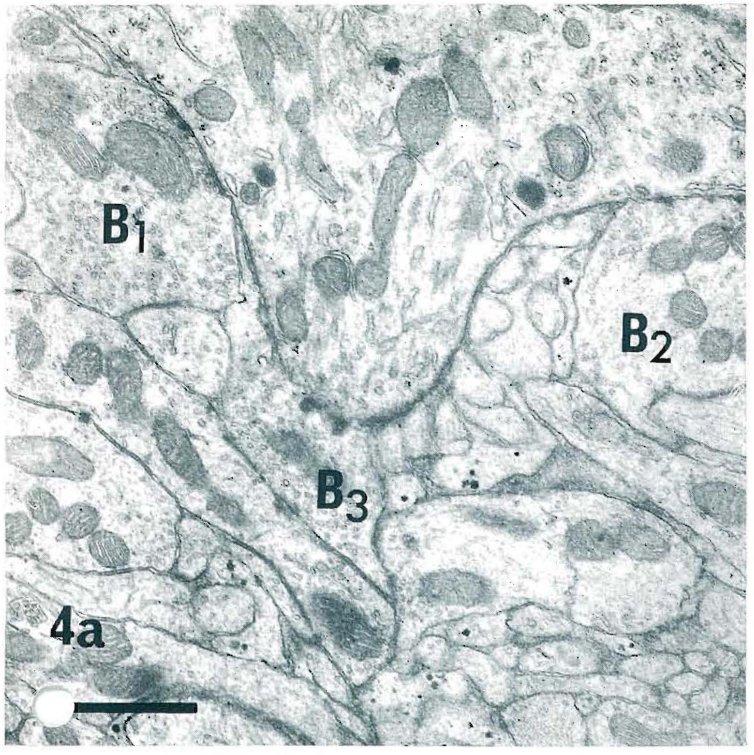
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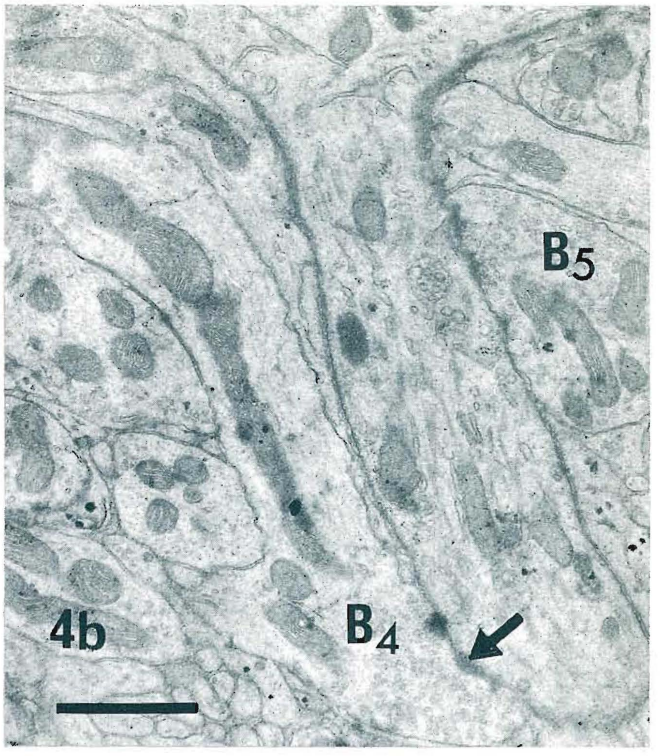
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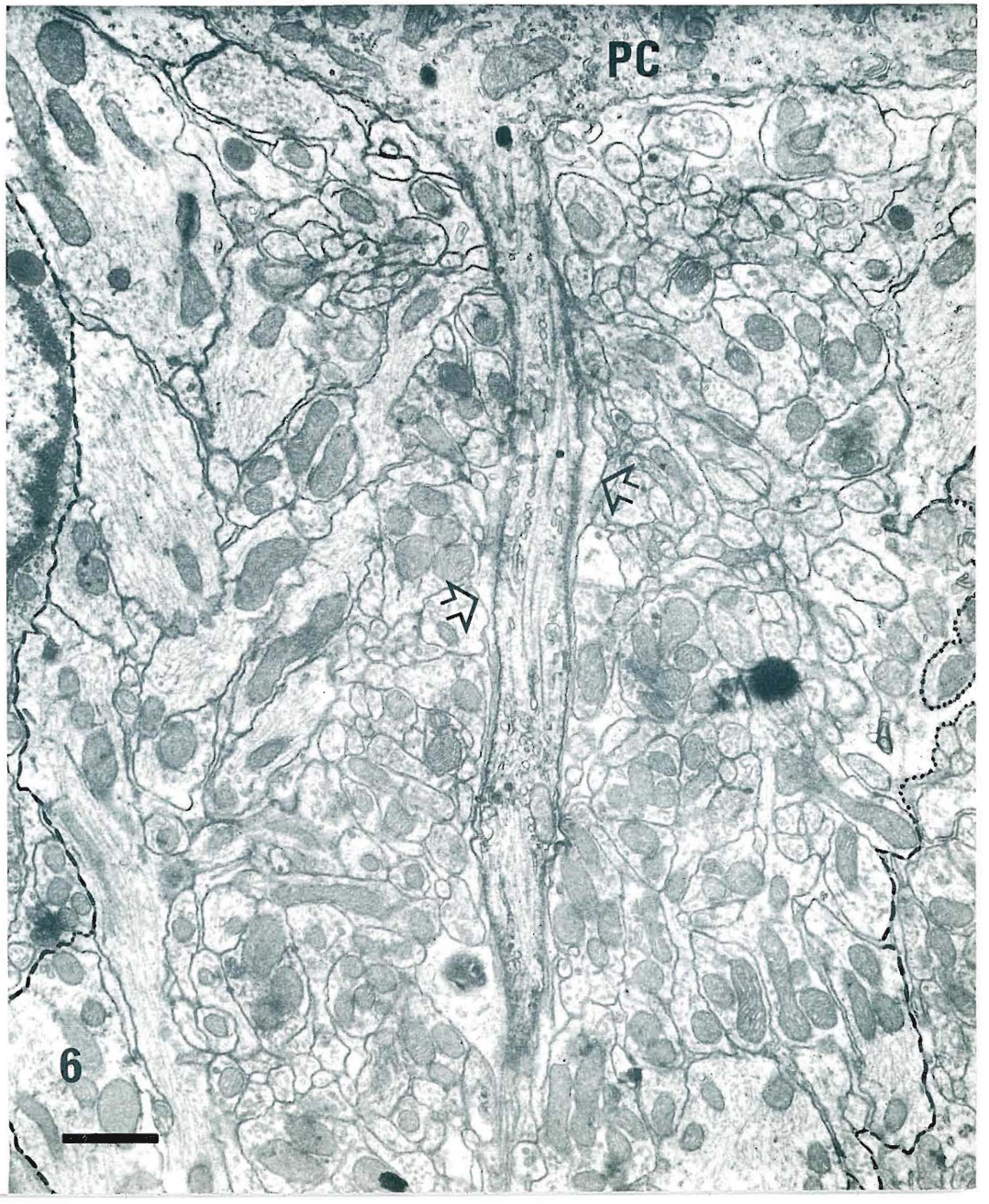
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TABLE 1. PARAMETERS OF THE INITIAL SEGMENT OF THE AXON OF CEREBELLAR PURKINJE CELLS

Species	Length of IS (μm)	Diameter of IS at origin (μm)	Diameter of IS at beginning of myelin (μm)	Total surface of IS (μm^2)	Surface of IS covered by processes	
					Glia	Basket axon
Cat	17.3 ± 1.3 (3)	1.31 ± 0.2 (5)	0.88 ± 0.28 (5)	59.8	74.2% (3)	25.8% (3)
Rat	17.1 ± 2.0 (8)	0.97 ± 0.15 (6)	0.73 ± 0.19 (6)	45.6	85.6% (4)	14.4% (4)

Values are mean ± S.E. The surface was calculated from the mean values as the superficies of a cone. The proportions of surface covered by glia and basket axons are expressed as mean values. Number of initial segments (IS) measured is indicated in brackets.

towards its entrance into the myelin sheath (Fig. 3d). Consequently the surface of the IS can be calculated roughly as the mantle of a truncated cone. In the rat, in four out of ten cases studied, small 0.3–0.4 μm spines were observed to protrude from the proximal (one third) portion of the IS. Such axonic 'spines' were never seen, however, in the cat.

The IS of the axon of the Purkinje cell is surrounded by branching axon collaterals of basket cells (Figs. 1, 3a, b), which build up the so-called pinceau beneath and around the base of the cell. Large, 1–2 μm dia. basket axons make up the boundary of this peculiar, composite structure (Fig. 6). As they descend they are tightly packed and organized in an approximately parallel array. In places where the large basket axons do not fill in the space completely, thin glial processes can be seen to intervene (Fig. 6). This outermost zone envelopes a convoluted, coiled mass of finer branches of basket axons, which become gradually thinner towards the IS. Between the few larger basket axons entering the inner space of the pinceau, small twigs and finger-like processes (0.1–0.3 μm in dia.) of other basket axons, some containing synaptic vesicles, fill in the space (Figs. 1, 3c, 6).

The IS is bordered essentially by two elements: glial and basket axon processes of varying size. An additional but not negligible part of the IS surface is in fact facing intercellular gaps between the contacting elements. Because of the limitations of the method used we did not measure the amount of this part of the membrane.

It can be seen from Table 1, that although the pinceau itself is poor in glial elements the IS is richly covered by glial processes. This glial sheath is not continuous but consists of thin bars and strips with their long axis parallel to that of the IS (Figs. 1, 5). There is some difference between the two species in the proportion of the IS membrane covered by glia, however, in both species a considerable proportion of the IS membrane is contacted by basket cell axons, their boutons and finger-like processes. Interestingly, although 3–12 basket axons in the cat and 2–6 basket axons on the rat, come into immediate contact with the IS, not all establish synaptic contact with it (Fig. 3c). The number of synapsing boutons and the longest extension of the specialized synaptic contact area detected on serial sections is given in Table 2. Not only are the basket boutons generally larger in the cat than in the rat but the synaptic spe-

TABLE 2. PARAMETERS OF SYNAPSING BASKET AXON BOUTONS ON THE INITIAL SEGMENT OF 17 PURKINJE AXONS

Species	Number of synaptic boutons on single IS	Number of IS observed	Mean	Longest extension of synaptic specialization area (μm)
Cat	1	1*	3.50 (6)	0.826 ± 0.326 (18)
	2	1		
	3	3		
	4	1		
	6	1		
Rat	0	1†	2.89 (9)	0.433 ± 0.118 (15)
	2	3		
	3	4		
	4	2		

* The pinceau of this initial segment was deformed by a capillary lying nearby.

† This initial segment had two lateral protusions several μm long not present under normal conditions. The protrusions were filled with myelin figures, dense bodies and vesicles, thus this could be a degenerating initial segment. Initial segments indicated by an asterisk or dagger were therefore, not included in the mean values.

Values in the last column are mean ± S.E. Synaptic specializations of 18 (cat) and 15 (rat) axo-axonic synapses were followed on consecutive serial sections and their longest extension was measured.

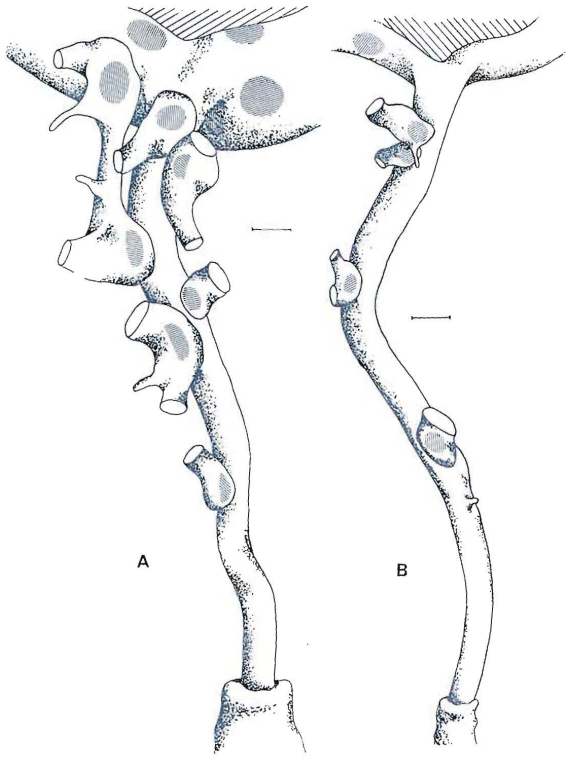

FIG. 7. Three dimensional reconstruction of the Purkinje cell axon initial segment of the cat (A) and that of the rat (B) to illustrate synapsing basket terminal distribution. Shaded areas demonstrate synaptic contacts by basket axons. Note that in the cat one basket axon establishes synaptic contact with both the soma and the initial segment of the Purkinje cell.

cialization area is also considerably larger (compare Figs. 1a and 2 with Fig. 5; see also Table 2). It is noteworthy that a relatively large part of the surface of the IS membrane is in actual synaptic apposition with basket cell terminals: 0.7–2% in the rat and 1–6% in the cat.

The distribution of boutons terminaux or boutons en passant along the IS is rather uneven. Most of the basket boutons can be observed on the proximal one third of the IS, although smaller boutons may also occur further down (Figs. 7(A), (B)). In some cases we were able to follow basket axon collaterals which in addition to synapsing on the soma descended and also established synapses with the initial segment (Figs. 4a, b).

DISCUSSION

It appears from this study that axo-axonic synapses between basket cell axon terminals and the IS of the Purkinje axon are not accidental but regular structures. The three-dimensional electron microscopical analyses of 17 IS has revealed the presence of a minor but nevertheless significant number of such syanapses (3.5 on the average in the cat, 2.9 in the rat). The surface of the IS occupied by specialized synaptic contact with presynaptic basket axon profiles is surprisingly large: about 2% of the total surface of the IS. This is not much less than the average on dendritic and soma surfaces of central neurons (for example 4–9% in the monkey lateral geniculate nucleus; PASIK, PASIK & HÁMORI, unpublished). The extension of the synaptic contact areas, as well as the size of the synapsing basket boutons are much smaller in the rat than in the cat. This may explain, at least partially, the contradiction between earlier statements on numerous such contacts in the cat (HÁMORI & SZENTÁGOTHAI, 1965) versus assurances that they occur only very occasionally in the rat (PALAY, 1964*a*,*b*, 1967; PALAY *et al.*, 1968). Obviously, the larger boutons and extended synaptic surfaces in the cat are more likely to appear in random sections, used exclusively earlier, than the smaller basket boutons and contact areas of the rat. The discrepancy between the two sets of observations has become less, more recently (PALAY & CHAN PALAY, 1974; GOBEL, 1971; MUGNAINI, 1972).

Measurements of the length of the Purkinje cell IS by our method resulted in approximately the same value, about 17 μm, in both the cat and the rat cerebellum. In light microscopic studies of Golgi material the length of the Purkinje cell IS was found to be about 30 μm by ECCLES, ITO & SZENTÁGOTHAI (1967), 50 μm by MUGNAINI (1972) and approx 40–50 μm by PALAY & CHAN-PALAY (1974). However, with the Golgi method the measurement of the length of the IS can not be expected to be quite accurate, due to difficulties in determining the point of the beginning of the myelin sheath.

Speculations about the functional significance of the localization of the inhibitory synapses in the axon hillock region go back to an early concept proposed by GESSEL (1940). Identification of the IS as the site of generation of the propagated spike potential (ARAKI & OTANI, 1955; COOMBS *et al.*, 1957) gave further emphasis to the possible localisation of inhibitory synapses on this strategic region of the neuron. Eventually, the inhibitory action exercised upon the Purkinje cells by the basket terminals (ANDERSEN *et al.*, 1963) became one of the best examples for the concept of a strategic localization of inhibitory synapses.

However, the highly elaborate structure, the so called 'pinceau' formed around the IS of Purkinje cells by basket axon terminals, is hard to explain in view of the many other cases of inhibitory pericellular basket terminals, where no such structures are present. Earlier it was postulated that this structure may be responsible for the slow build-up of basket cell inhibitory action (ECCLES *et al.*, 1967). It was speculated that transmitter released from basket axons in a relatively large space and partially remote from their target of the IS, could diffuse within the space surrounded by the glia and could therefore exert a long lasting tonic action. This idea was further supported by what appeared as an unusual amount of extracellular space between small processes of basket

axons (HÁMORI & SZENTÁGOTHAI, 1965). This hypothesis was recently criticised (PALAY & CHAN-PALAY, 1974) on the basis of the absence of both structural requirements; that is, the outer glial sheath and the larger extracellular space. However, observations on complete section series of several basket arrangements suggest that the outer mantle of the relatively bulky basket axon collaterals and the surrounding glial do, in fact, form a capsule, penetrated by relatively few extracellular channels toward the interior of the pinceau. Moreover, although the apparent extracellular space between cell processes described by HÁMORI & SZENTÁGOTHAI (1965) was a fixation artefact of techniques used at that time, there is obviously a relatively high proportion of extracellular space within the pinceau simply due to the small diameter of the numerous twisting twigs and finger-like processes of basket axons. This results, for obvious geometric reasons, in an extraordinary extension of membrane surfaces and correspondingly in an increased proportion of the extracellular space.

We can thus conclude, that the postulate of ECCLES *et al.* (1967) still has its structural basis although more physiological, quantitative morphological work is necessary to elucidate the function of the structures surrounding the IS.

Our quantitative measurements have also confirmed earlier observations (PALAY, 1964*a*; PALAY *et al.*, 1968) that the Purkinje axon IS is richly covered by glial processes. About 75% of the IS total surface in the cat, and 85% in the rat is contacted by an extremely thin sheath of glial strips and bars. The pinceau itself except for the very few thin glial processes passing through which originate from the glial cell bodies and are addressed exclusively to the IS, is otherwise void of glial elements. At present it is rather difficult to find a satisfactory explanation for the function of the peculiar glial sheath around the IS, although one possibility is that the large glial membrane surface may have a role in uptake and/or release of ions during the firing of the neuron.

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