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THE FINE STRUCTURE OF CORPORA PEDUNCULATA IN *GRYLLOTALPA GRYLLOTALPA* L. (INSECTA, ORTHOPTERA)

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Among the most frequently investigated parts of Insect brain are the corpora pedunculata. There are various conceptions regarding their functional interpretation. DUJARDIN's (1850) assumption that the corpora pedunculata represent higher association centers ("intelligence centers") is generally accepted. Inspite of numerous examinations (LANDOLT, 1965; LANDOLT and RIS, 1966; STEIGER, 1967; MANCINI and FRONTALI, 1967; 1970; FRONTALI and MANCINI, 1970; SCHÜRRMANN, 1970; 1971), the connections of neurons have not been revealed submicroscopically, furthermore, the opinions concerning the mechanism of impulse transmission are again divergent.

The present paper is intended to publish the observations gained on the corpora pedunculata of an Orthoptera species not yet investigated electron microscopically, since they differ in some aspects from those of other authors made on other species.

Material and methods

Adult specimens of *Gryllotalpa gryllotalpa* L. collected from their natural environment during December and January were used. Usually they were investigated within several days of collection.

The animals were killed by decapitation without anaesthetization. The chitin was removed from the ventral side of the head, in order to reach the brain.

The brain was fixed in Susa fixative and embedded in paraffin for histology. Serial sections were cut in various planes and stained with routine histological methods.

For electron microscopy the brain was fixed in toto by two ways:

1. Two per cent OsO_4 buffered with 0,2 M s-collidine at pH 7,2 (BENNETT and LUFT, 1959) for 30 min at 0°C and 10 min at room temperature.

2. Four per cent glutaraldehyde dissolved in 0,2 M s-collidine buffer of pH 7,2 for 2 hours at 0°C then postfixation with OsO_4 as described under 1.

The fixation was followed by dehydration in ethanol and propylene oxide and embedding in araldite (Durcupan ACM, Fluka). Ultrathin sections were cut on an LKB Ultrotome III and stained with uranyl acetate and lead citrate (Reynolds, 1963). Micrographs were taken with a TESLA BS 413A electron microscope.

In order to achieve an exact orientation, half-thin sections were prepared from the araldite-blocks and investigated in phase contrast or after toluidine blue staining in light microscope. Ultrathin sections were cut always from the areas having been identified at light microscopic level. For selection of the suitable areas the "mesa" technique was applied.

Results

The general construction of corpora pedunculata in *Gryllotalpa gryllotalpa* L. is similar to that of Hymenoptera and Orthoptera investigated so far. They consist of a perikaryon layer, a calyx, a pedunculus and the alpha and beta lobes *(Fig. 1)*. The perikaryon layer and the calyx form a close unity with each other, localized in the postero-dorsal area of the brain, somewhat laterally from the median plane. The pedunculus runs from it in a dorso-ventral direction and continues in the two lobes. One of the latter, the alpha lobe turns again in dorsal direction forming an acute angle with the pedunculus and terminating just under the dorsal surface of the brain. The beta lobe first maintains the direction of the pedunculus then turns into a medial one and almost contacts with the opposite beta lobe near the ventral side of the brain at the median line.

Similarly as in other Orthoptera species (Panov, 1966) the corpora pedunculata display only one calyx on each side. Ultrathin sections were taken from the areas indicated in *Fig. 1*.

There were no essential differences in the ultrastructure of brains fixed by different methods apart from a somewhat better preservation achieved by double fixation. Therefore our description concerns the results obtained by both methods.

1. *Globuli cells*

The globuli cells *(Fig. 2)* are densely packed to each other just under the brain surface. Their diameter measures generally 10—15 μ, however, in some cases it reaches even 20 μ. Their nuclei are relatively large and of oval or round shape, the nuclear substance is uniformly granulated, there are no accumulations of chromatin in them. The cytoplasm of the globuli cells forms a narrow strip around the nucleus, it is very poor in organelles. Only a few mitochondria and Golgi complexes of poor composition can be seen. The endoplasmic reticulum is extremely poor in membranes, and many free ribosomes may be seen. Centrioles and lysosome-like granules also occur now and then.

The cells either directly contact each other or are separated by a narrow glial layer *(Fig. 2)*. It is conspicuous that in both forms of contacting the cell membranes are broken at some places, small vesicles are present in the surroundings of such regions and the cytoplasm of the adjacent cells seems to be mixed freely *(Fig. 2.)*. It also appears that the disappearance of the cell membranes has not been completed, however, they show an intense loosening up.

The cells are of unipolar appearance in sections. Their process running toward the glomerular layer can only be followed during a short distance even in the case of a suitable section plane. These processes form bundles in the vicinity of the calyx neuropile. We failed to observe axons of outer origin between the globuli cells, neither were axo-somatic synapses found.

2. *Calyx neuropile*

The characteristic components of calyx neuropile are the glomeruli *(Fig. 3)*. Their central element is an end knob of about 3—6 μ diameter, filled with numerous clear vesicles of 300—400 Å in diameter. Dense-core vesicles (DCV) of 800—1500 Å also occur randomly among the vesicles. Numerous mitochondria with abundant cristae are also present in the end knob. The preterminal part of the axon belonging to the end knob contains a lot of microtubuli.

Numerous thin nerve fibres form a plexus around the end knob. Ribosomes can often be observed in these processes, therefore, they can be considered dendritic. Synaptic vesicles are not characteristic elements of these fibres. The end knob shows synaptic contacts with the thin fibres at several places, characterized by thickening of the membranes and certain accumulation of a dense substance on both sides, furthermore, by widening out of the intercellular space and clustering of the synaptic vesicles in the presynaptic part near the contact. However, most part of the contacting membranes display no synaptic specialization. We failed to observe typical "tight junctions" between the contacting membranes. Thin fibres infolding into the end knob can often be seen.

The glomeruli become increasingly scarce in the deeper layers of the calyx neuropile. Here the fibre bundles gather forming an anatomical transition between the calyx neuropile and the stem of corpora pedunculata, i.e. the pedunculus.

3. *Pedunculus*

The pedunculus consists mainly of thin axons *(Fig. 4)* being densely packed. The majority of them reach about 1 μ or less in diamter. The structure and content of the axons are the same as in other Orthoptera (Schürmann, 1970), however, the separation of the zones is less definite. The occurrence of interaxonal contacts considered as synapses is also similar. In the region of the origin of the alpha lobe the pedunculus contains axon bundles of perpendicular direction as compared to the main fibres running obviously towards the alpha lobe.

4. *Alpha lobe*

In the area of the alpha lobe near the pedunculus *(Fig. 5)* three type of nerve processes can be distinguished. The structure of the first (1) is identical with that ot fhe thin axons of the pedunculus, it forms bundles at some places and contains small synaptic vesicles and microtubuli. The second type is larger in size, it displays one or more mitochondria besides the synaptic vesicles (2). The third type (3) is of much greater diameter than the first two. It contains irregular membrane profiles showing various degrees of fragmentation and destruction as well as vesicles. Mitochondria and multivesicular bodies also occur in it. The surface of such processes is uneven and the limiting membrane is also fragmented and destructed at several places.

In cases of proper section plane the transition of the thin axons into the large varicosities could be observed, and what is more, we have pictures where the large axonal varicosity goes over again into a thin axon on the opposite side.

Synaptic contacts of peculiar structure can also be seen between these fibres. Two or three axons contact with each other forming a relatively small area where clusters of vesicles and accumulation of a substance of high electron density can be found. These contacts are usually of symmetric structure, they show no polarization. Apart from these, usual synapses being pre- and postsynaptically differentiated also occur.

Receding from the pedunculus less and less thin fibres can be found *(Fig. 6)* and the large varicosities become predominant. In the part of the alpha lobe of the most dorsal localization the structure is unusually irregular. The varicosities here are extremely large, they cannot be distinguished individually because of the confluations. Disintegrating membranes can often be seen in this region.

Although not very frequently, synaptic contacts can also be observed here. Glial processes containing glycogen granules also occur.

5. *Beta lobe*

The beta lobe *(Fig. 7)* is a direct anatomical continuation of the pedunculus. Accordingly, bundles of thin peduncular axons can be followed in it for a long distance. As against to the alpha lobe, however, the structure of the beta lobe is much more homogeneous. Although the thin axons show varicosities even here, those are uniformly smaller in diameter than in the alpha lobe and their structure is also more regular. They contain many synaptic vesicles, mitochondria, microtubuli and sometimes dense-core vesicles. Varicosities of large diameter were observed very rarely. The structure of synaptic contacts is similar to that described in the alpha lobe.

Discussion

Since the first description of Trujillo-Cenoz and Melamed (1962) numerous papers have been published on the fine structure of corpora pedunculata of Insects. Most of the investigations were carried out in Hymenoptera, however, several papers concern Orthoptera species, too. The *Gryllotalpa gryllotalpa* L. has not been investigated so far.

Our investigations have revealed that the corpora pedunculata are of similar general construction in this species as in the other Insects. Apart from the fact that only one calyx is present on each side, the anatomical distribution is identical. The globuli cell layer and the calyx neuropile have been investigated most frequently.

The most conspicuous feature of the globuli cells of *Gryllotalpa gryllotalpa* is the compact organization where the cells contact closely with each other either directly or through a narrow glial sheath. Independently from the method of fixation, the cell membranes are completely intact at some places, however, elsewhere they show some kind of loosening up allowing the cytoplasm of the adjacent cells to be mixed. Such types of intercellular contacts have also been observed in ants (LANDOLT, 1965; LANDOLT and RIS, 1966). The latter authors demonstrated by means of serial sections that these are really existing membrane incontinuities and not artifacts of sectioning. In our case such loosenings up were observed between both directly contacting nerve cells and those separated by glial layer. It should be noted that membrane discontinuities of similar character have also been described in the procerebrum of Gastropoda (ZS.-NAGY and SAKHAROV, 1970), although there they occurred only between nerve and glial cells.

Some of the authors attribute a significance to the soma-somatic contacts from the point of view of the intercellular exchange of macromolecular substances and are on the opinion that the vicinity of the cell nucleus plays an important role in that (LANDOLT and RIS, 1966). In our material the close vicinity of the nucleus, the place of intercellular contact could not always be observed. This contradicts to some extent to the assumption related above. Nevertheless, the possibility of intercellular exchange of substances cannot be denied.

A morphological similarity exists between the globuli cell layer of corpora pedunculata and the procerebrum of Gastropoda. In spite of this fact, the difference seems to be significant, for the former does not contain axo-somatic synapses, whereas the latter does, most probably with a catecholaminergic mediation (ZS.-NAGY and SAKHAROV, 1969; 1970).

The calyx neuropile has also been investigated by numerous authors (TRUJILLO-CENOZ and MELAMED, 1962; GOLL, 1967; STEIGER, 1967; MANCINI and FRONTALI, 1967; LAMPARTER et al., 1969; FRONTALI and MANCINI, 1970; SCHÜRMANN, 1971). Its construction is similar in the species investigated by us, however, we consider the failure of typical "tight junctions" after both OsO_4 and double fixation as a significant difference. A further difference is that the "dark" and "bright" presynaptic endings observed by STEIGER (1967) in the calyx neuropile of *Formica rufa* and *Camponotus ligniperdus* cannot be distinguished. The glomeruli of *Gryllotalpa gryllotalpa* are rather similar to those of *Camponotus* in as much as the "intrinsic" fibres are present in the end knob. The significance of the ,,intrinsic" fibres is unclear, they may be absent in species closely related to those where they are abundant (STEIGER, 1967).

The structure of the pedunculus has been investigated in detail in *Achaeta domesticus* (Gryllidae) by SCHÜRMANN (1970). Our findings are similar to his with the only difference that the zonal distribution of the fibres failed to appear, or was very weak.

The alpha lobe has been investigated by means of electron microscopy only in *Periplaneta americana* (MANCINI and FRONTALI, 1967; FRONTALI and MANCINI, 1970). As compared to this species, the structure of the alpha lobe of *Gryllotalpa gryllotalpa* is significantly different. Types 1 and 2 of axons described by the above authors cannot be distinguished. The transitions between thin and thick axons indicate that the different axonal forms may represent various parts of the same axon. As far as the axonal content is concerned, the varicosities strongly differ from the other axons, i.e. fragmental membrane details occur in them together with vesicular components. The irregular shape of the varicosities is conspicuous and difficult to explain. On the basis of usual electron microscopic interpretations one can regard this phenomenon as an artifact. Since, however, they occur even in places where well-preserved axons, mitochondria, etc. also appear, it is difficult to imagine them simply as technical artifacts. It can be assumed that the axonal membranes are more labile for some reasons than those of other nerve processes, and this is why they are destructed during the fixation. This, however, may reflect real differences as compared with those axons being better preserved by fixation.

The vesicular components also differ from those described in *Periplaneta*. The small, clear vesicles are abundant even in *G. gryllotalpa*, whereas the small "granular vesicles" and the large "semi-dense granules" appear only randomly.

The structure of the alpha lobe depends on the distance from the pedunculus, where the section was cut.

On the basis of these results one can conclude only very cautiously as regards the synaptic organization of the alpha lobe.

The structure of the beta lobe is considered to be similar as that of the alpha lobe in the literature (Mancini and Frontali, 1967; 1970; Frontali and Mancini, 1970). In our case, there is an other situation, since the beta obe is of significantly different construction than the alpha.

Summary

1. The structure of globuli cells is similar to the forms described in other Orthoptera and Hymenoptera. There are no axo-somatic synapses in the globuli cell layer. Intersomatic membrane discontinuities can be observed.

2. The calyx neuropile displays an expressed glomerular structure. The thin axons invaginating into the end knob are present even in this species. Typical "tight junctions" could not be observed. Synaptic vesicles of 300–400 Å are predominant in the end knobs, while dense-core vesicles of 800–1500 Å occur only rarely.

3. The structure of the pedunculus is also similar to that of other Orthoptera with the difference that the separation of zones is less expressed.

4. Alpha lobe: its structure differs from that of species investigated so far. One part of the peduncular thin axons enters the alpha lobe, where large varicosities and a medium sized axon form can also be found. Receding from the pedunculus, the latter two forms become more and more predominant.

5. Beta lobe: its structure differs from that of the alpha lobe, i.e. it contains large varicosities only randomly and the structure is more homogeneous and uniform.

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GRYLLOTALPA GRILLOTALPA L. (INSECTA, ORTHOPTERA) CORPORA PEDUNCULATAJÁNAK FINOMSZERKEZETE

Zs.-Nagy Imre és *Muskó Ilona*

Összefoglalás

1. A globulussejtek struktúrája hasonló más Orthoptera és Hymenoptera fajokban leírt formákhoz. Axo-szomatikus szinapszisokat a globulussejtek rétege nem tartalmaz. Interszomatikus membrán-fellazulások megfigyelhetők.

2. A kehelyneuropil glomeruláris szerkezete kifejezett. A végbunkó belsejébe betüremkedő vékony rostok ennél a fajnál is megtalálhatók. Tipikus ,,tight-junction''-t nem sikerült megfigyelni. A végbunkóban 300—400 Å nagyságú szinaptikus vezikulák dominálnak, a 800—1500 Å átmérőjű dense-core vezikulák ritkán fordulnak elő.

3. A pedunculus szerkezete is hasonló más Orthoptera fajokéhoz, azzal a különbséggel, hogy a zónák elkülönülése kevésbé kifejezett.

4. Alfa lebeny: szerkezete eltér az eddig vizsgált fajokétól. A pedunculus vékony axonjainak egy része belép az alfa lebenybe. Itt varikózus tágulatok és egy közepes méretű axonforma is található. Távolodva a pedunculustól egyre inkább az utóbbi két forma dominál.

5. Béta lebeny: szerkezete eltér az alfáétól, amennyiben nagyobb axontágulatok csak elvétve találhatók s az egész sokkal homogénebb és egyöntetűbb képet mutat.

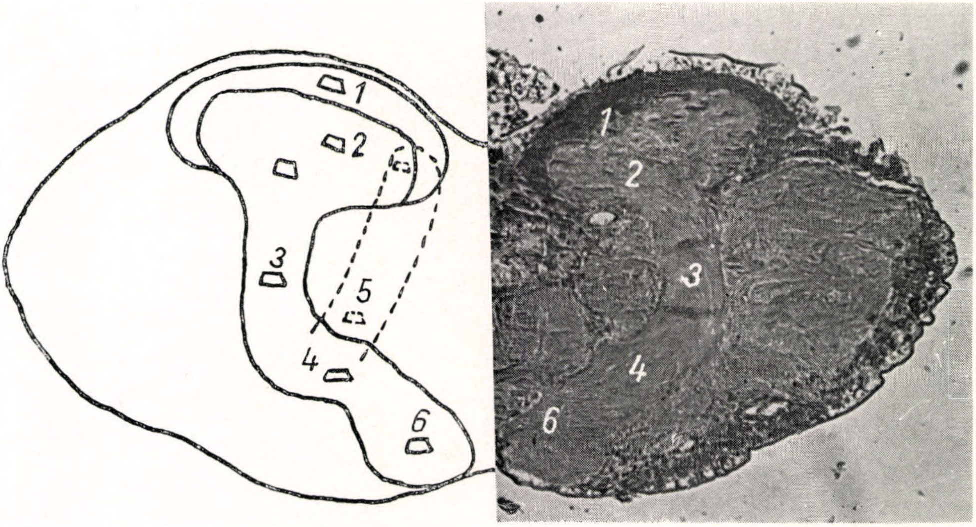

Fig. 1. The schematic drawing of the brain (a) and its light microscopic picture (b) in a horizontal section. 1 — perikaryon layer; 2 — calyx neuropile; 3 — pedunculus; 4 — junction of the alpha and beta lobes; 5 — alpha lobe; 6 — beta lobe. The quadrangles indicate the places where ultrathin sections were prepared from. × 76

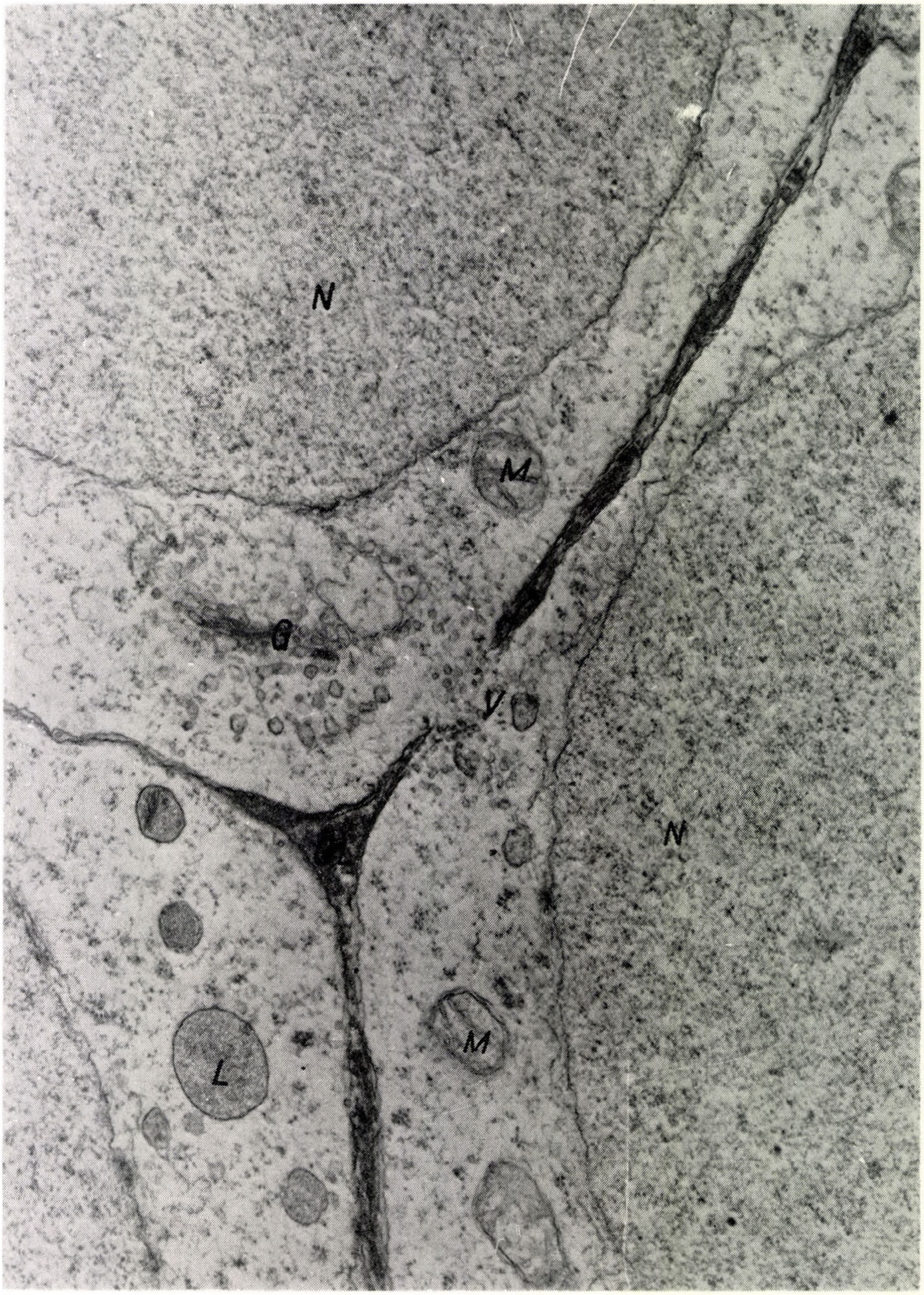

Fig. 2. The place of contact of three cells from the perikaryon layer. N — nucleus; G — Golgi complex; M — mitochondrium; GL — glial process; L — lysosome. Arrow indicates the discontinuity of the cell membranes. V — vesicles. Fixation: OsO_4. $\times$ 25 000

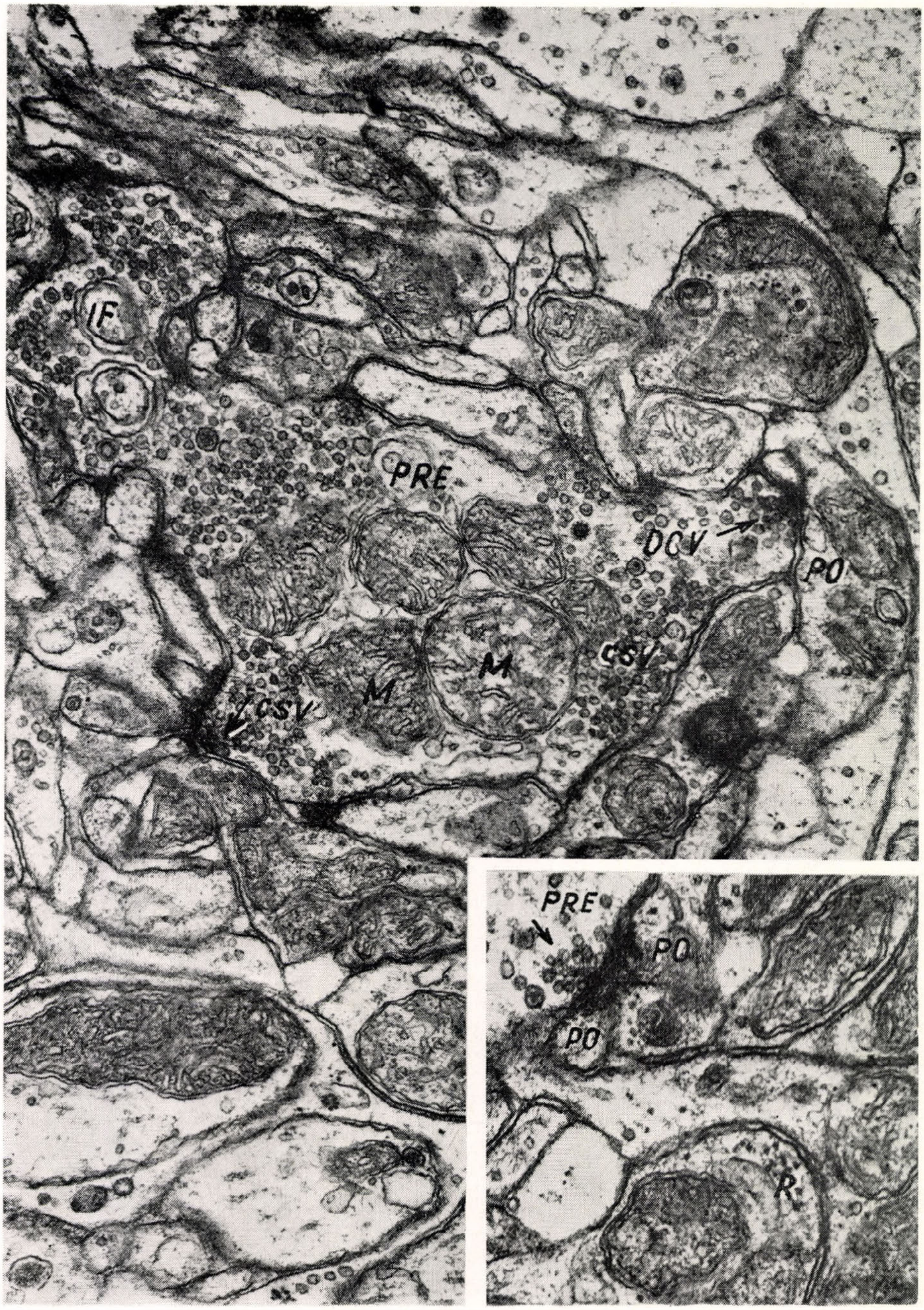

Fig. 3. The picture of a glomerulus from the calyx neuropile. Presynaptic end knob (PRE) can be seen centrally, surrounded by postsynaptic fibres (PO). M — mitochondrium; CSV — clear synaptic vesicles; DCV — dense-core vesicles; IF — "intrinsic" fibre; Arrows indicate the synapses. Fixation: OsO_4, $\times$ 30 000. Inset: enlarged view of a synapse where the postsynaptic fibres contain ribosomes (R). Fixation: OsO_4, $\times$ 48 500

Fig. 4. Detail of the pedunculus. M — mitochondrium; CSV — clear synaptic vesicles; N — neurotubuli; GL — glial process; AX — transections of axons; Arrows indicate places considered to be synapses. Fixation: OsO_4, $\times$ 16 500

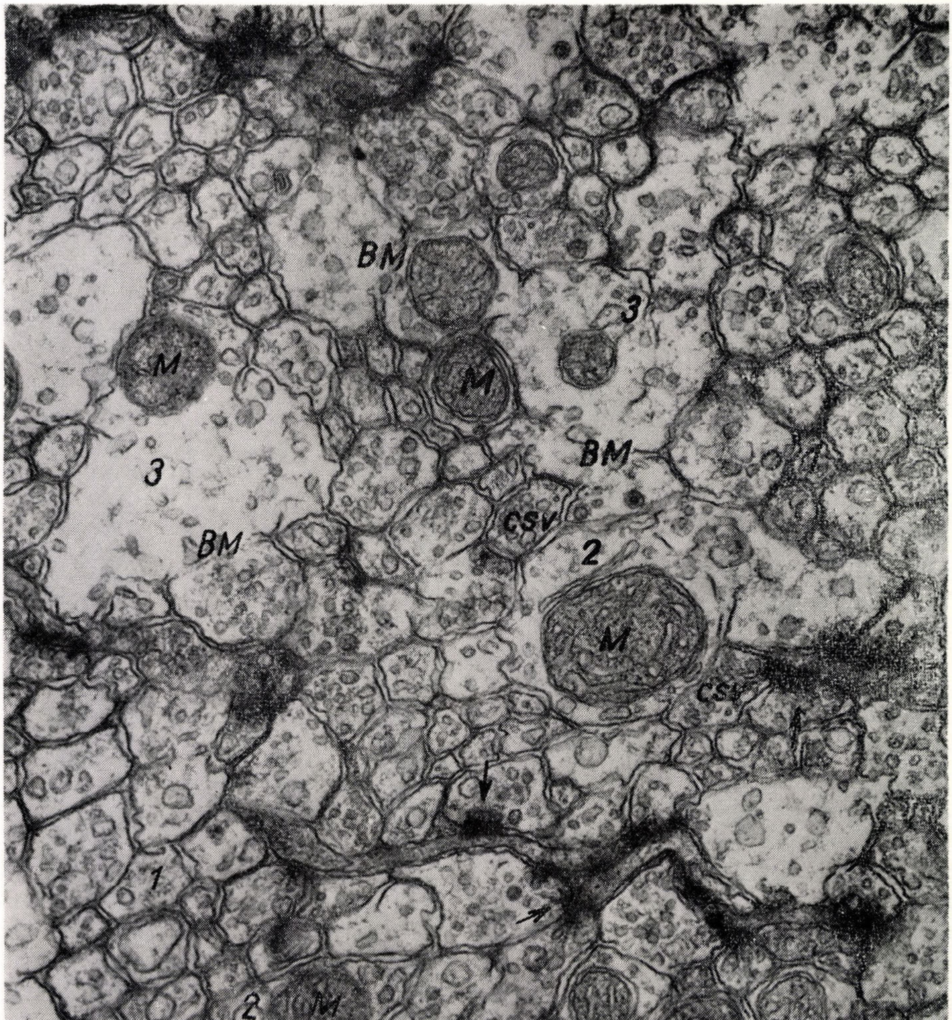

Fig. 5. Alpha lobe near to the branching out from the pedunculus. M — mitochondrium; CSV — clear synaptic vesicles; BM — broken membranes. Arrows indicate synapses. 1, 2 and 3 — the types of axons (see the text). Fixation: OsO_4, $\times$ 30 000

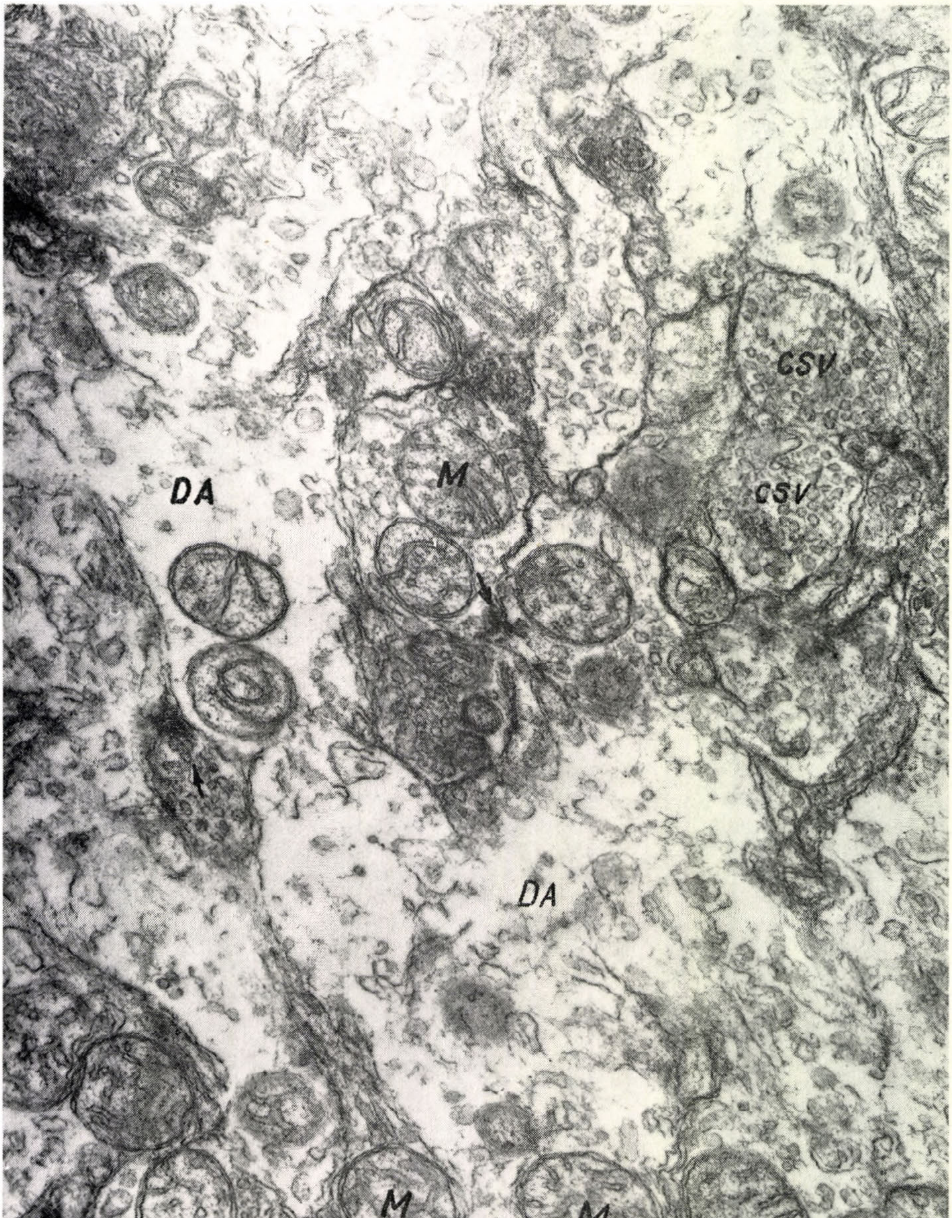

Fig. 6. Part of the alpha lobe, farthest from the pedunculus. M — mitochondrium; CSV — clear synaptic vesicles. DA — destructed areas. Arrow indicates a synapse. Fixation: OsO_4, $\times$ 25 000

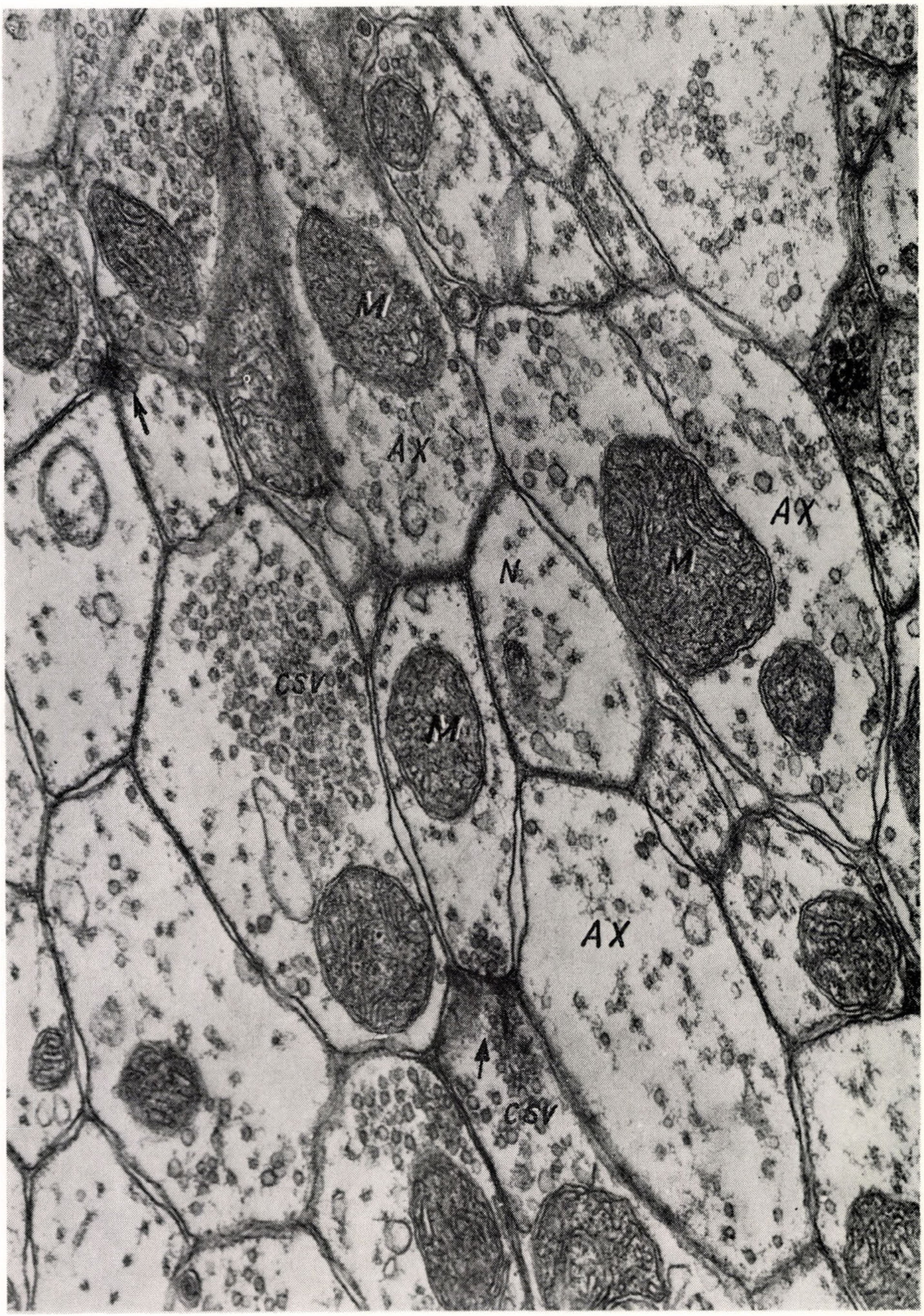

Fig. 7. Detail of the beta lobe. The axonal profiles (AX) are of uniform size. M — mitochondrium; N — neurotubuli; CSV — clear synaptic vesicles; GL — glial process. Arrows indicate the synapses. Fixation: OsO_4, $\times$ 25 000