42

Z. Zellforsch. 119, 309–320 (1971)

Cellular Organization in the Synganglion of the Mite *Macrocheles muscaedomesticae* (Acarina: Macrochelidae)

An Electron Microscopic Study*

Lewis B. Coons and Richard C. Axtell**
Department of Entomology, North Carolina State University, Raleigh, North Carolina, U.S.A.

Received April 23, 1971

Summary. The synganglion is bounded by an extracellular sheath and is divided into the cortex and the neuropile. The cortex contains two glial layers, each of which is composed of a distinctive type of glial cell, and three types of neurons. Type I is the least common and most electron dense, type II is most common, and type III represents neurosecretory cells with a larger volume of cytoplasm than in types I and II. Substantial areas of the neuron cell bodies are unsheathed. A third type of glial cell is found in the neuropile.

The first glial layer of the cortex, the perineurium, lies beneath the extracellular sheath and overlies the neuron cell bodies contributing to their ensheathment. In areas lacking neuron cells bodies, the perineurium overlies a second glial layer, the subperineurium, which is inflected inwards where a group of neuron cell bodies is encountered. The subperineurium contributes to the ensheathment of both the cell bodies of neurons and the nerve fibers. It is confluent with glial cells which arise within the neuropile. The neuropile contains nerve fibers and glial cells and is perforated by the esophageal canal, which is lined by the perineurium and subperineurium. Unsheathed nerve fibers contact each other in three ways: end-knob, longitudinal, and cross contacts.

Key-Words: Central nervous system — Mite — Ultrastructure.

Zusammenfassung. Das Synganglion wird von einer extrazellulären Scheide umkleidet und ist in Cortex und Neuropil gegliedert. Der Cortex enthält zwei Gliazellschichten (die jeweils durch einen bestimmten Zelltyp charakterisiert sind) und drei Typen von Neuronenzellkörpern. Neuronenzellkörper vom Typ I sind sehr elektronendicht und nur selten anzutreffen; Typ II ist am häufigsten vertreten; Typ III wird durch neurosekretorische Zellen repräsentiert, die zudem ein relativ größeres Plasmavolumen als Typ I und Typ II besitzen. Ausgedehnte Bereiche der Neuronenzellkörper sind nicht umhüllt. Außerdem wurde ein dritter Gliazelltyp im Neuropil gefunden.

Die äußere corticale Gliaschicht, Perineurium genannt, liegt unter der extrazellulären Scheide und überdeckt die Neuronen teilweise. In Gebieten, in denen Neuronenzellkörper fehlen, überlagert das Perineurium eine zweite Gliazellschicht, das Subperineurium. Diese Schicht kann sich ins Innere des Ganglions erstrecken, falls sie auf eine Neuronenzellkörpergruppe stößt. Das Subperineurium trägt sowohl zur Umhüllung der Neuronenzellkörper, als auch der Nervenfasern bei. Es steht in direktem Zusammenhang mit Gliazellen aus dem Inneren des Neuropils.

Das Neuropil umfaßt Nervenfasern und Gliazellen und umgibt den ösophagealen Kanal, welcher vom Perineurium und Subperineurium gebildet wird. Hüllenlose Nervenfasern treten in drei Arten miteinander in Verbindung, durch Endknöpfe, Längs- und Querkontakte.

* Supported in part by U. S. Public Health Service Research Grant EC-246 from the Environmental Control Administration, Training Grant ES-00069 from the National Institute of Environmental Health Sciences and by the Office of Naval Research. Paper No. 3320 of the Journal Series of the North Carolina State University Agricultural Experimental Station.

** We are indebted to Dr. R. F. Foelix for supplying the German translation of the summary and for reading the manuscript.

Introduction

One characteristic of the nervous systems of arthropods is a ventral chain of ganglia. Depending on the taxonomic group, these ganglia exhibit various degrees of fusion. Within the Acarina, this fusion results in a single mass or synganglion. The esophagus passes through the synganglion and divides it into a dorsal supraesophageal and a ventral subesophageal mass. Pharyngeal and cheliceral nerves originate from the supraesophageal mass, while pedipalpal nerves and nerves to the posterior organs and legs originate in the subesophageal mass.

The present study is a description of the different types of cells and their organization in the synganglion of the free-living mesostigmatid mite, *Macrocheles muscaedomesticae* (Scopoli). The discussion centers around a comparison to insect ganglia.

Materials and Methods

Only adult female mites were used in this study. They were obtained from colonies mass reared in the laboratory according to the methods of Rodriguez, Wade, and Wells (1962).

Tissue was fixed for 1 to 3 hours in 2.5% glutaraldehyde buffered in 0.05 M sodium cacodylate (pH 7.2) to which 0.15 M sucrose had been added. After a 12 hour wash in several changes of 0.05 M sodium cacodylate buffer (pH 7.2) containing 0.30 M sucrose, the tissue was postfixed in 1% osmium tetroxide in veronal acetate buffer (pH 7.2) containing 0.30 M sucrose. Following dehydration in a graded series of ethyl alcohols and propylene oxide, the tissue was infiltrated and embedded in Epon 812 according to the method of Luft (1961).

Tissue blocks were sectioned with a Reichert Om U2 ultramicrotome equipped with a diamond knife. Sections were stained in saturated uranyl acetate in 50% ethyl alcohol (10 minutes), and lead citrate (8 minutes) formulated according to Venable and Coggeshall (1965). Micrographs were obtained with either a Siemens Elmiskop II at 50 kV, or a Siemens Elmiskop I A at 80 kV.

Thick (0.5 μ) sections were used to maintain proper orientation. These were stained with a mixture of Azure II and Methylene blue (Richardson, Jarett, and Finke, 1960).

Results

1. Gross Morphology of Synganglion

The synganglion (Fig. 1), is located at the base of the gnathosoma and rests on the sternal shield. Like the ganglia of other arthropods, it consists of an outer cortical region and an inner neuropile. The cortical region is made up of layers of glial cells and cell bodies of neurons. The neuropile contains a mass of nerve fibers and glial cells and is traversed by the esophageal canal.

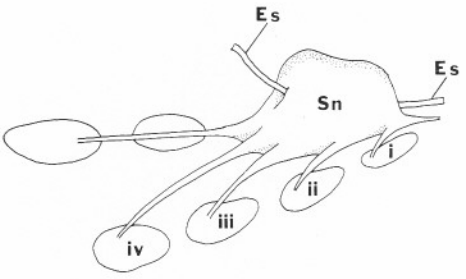
The complex of glial cells and their extensions forms two distinct layers in the cortex. An outer glial layer, the perineurium, underlies the extracellular sheath, and beneath the perineurium lies a second layer, the subperineurium (Fig. 2).

2. Cortex

a) Extracellular Sheath. The entire synganglion is bounded by a continuous extracellular sheath. It has a uniform thickness of about 800 Å and is composed of a structureless matrix (Figs. 3, 4).

b) Glial Cell Layers. The two glial cell layers are apposed to each other in areas of the cortex lacking groups of neuron cell bodies (Figs. 2, 4) but diverge whenever they are encountered (Figs. 2, 5). The perineurium overlies these groups

Fig. 1. Diagram of synganglion (*Sn*). Esophagus (*Es*) passes through synganglion. Numbered circles indicate relative positions of four pairs of legs. The only nerves shown are those to the legs and the pedipalpal nerve



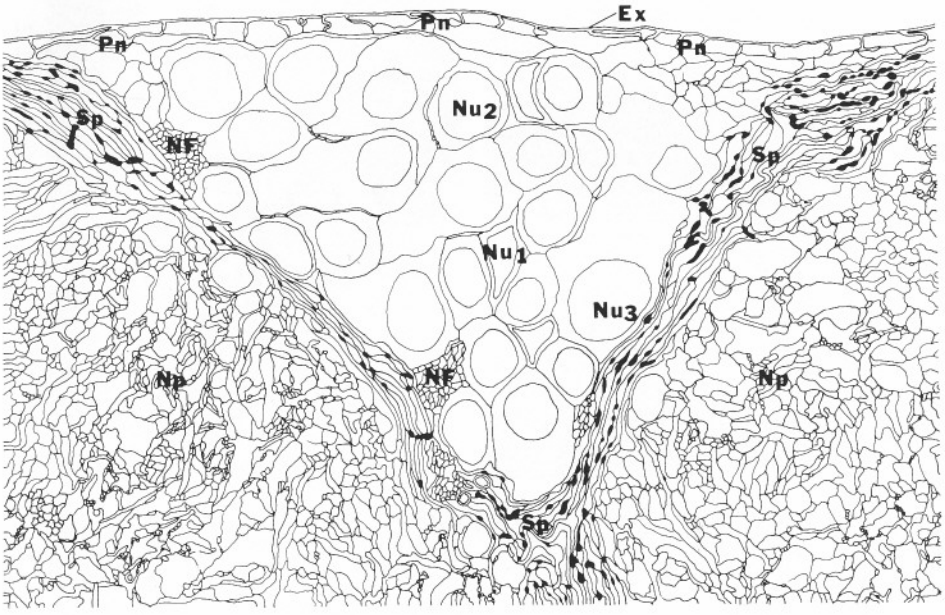
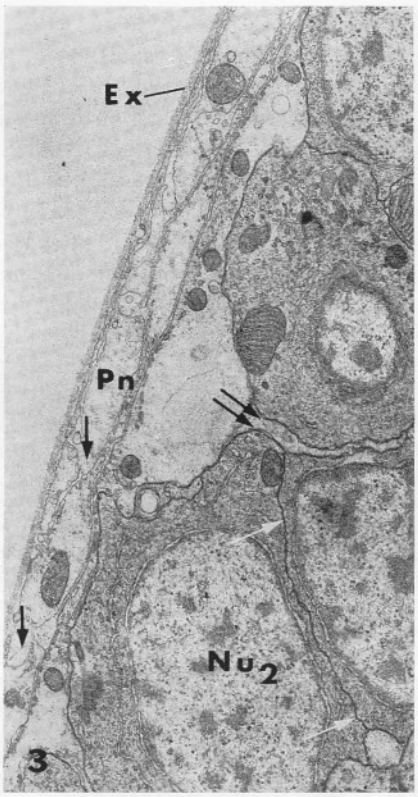
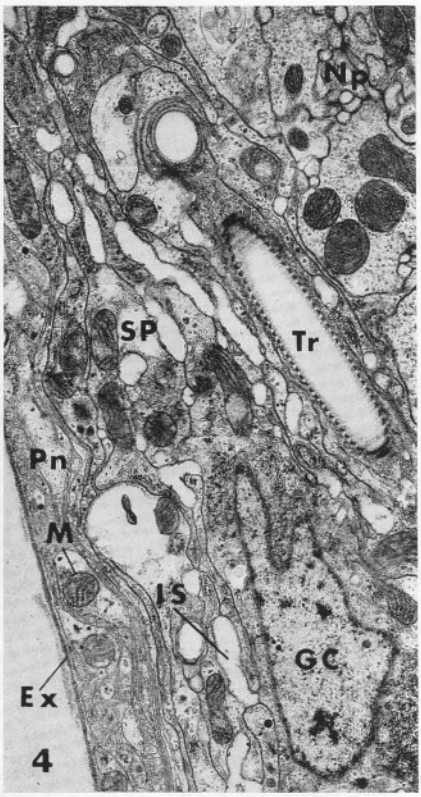


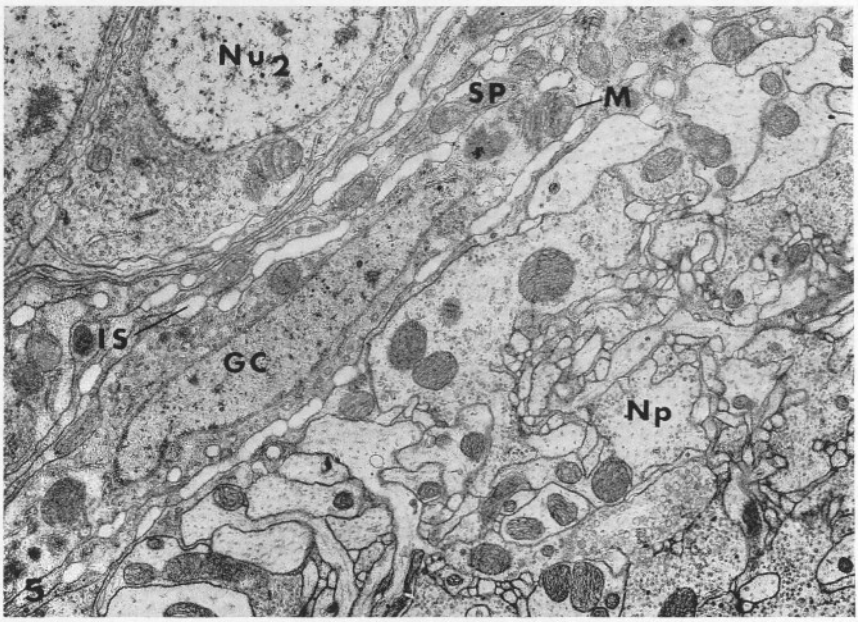
Fig. 2. Line drawing of part of the synganglion taken from a montage of electron micrographs. *Ex* Extracellular sheath; *Np* Neuropile; *NF* Nerve fibers; Nu_1 Type I neuron cell bodies; Nu_2 Type II neuron cell bodies; Nu_3 Type III neuron cell bodies; *Pn* Perineurium; *Sp* Subperineurium

and contributes to their ensheathment (Figs. 2, 3). The subperineurium is inflected inward from the perineurium and comes to occupy a position between the neuropile and the cell bodies of the neurons (Figs. 2, 5). Elements of the subperineurium extend into the neuropile (Fig. 2). The subperineurium contributes to the ensheathment of both the neuron cell bodies of the cortex and the nerve fibers of the neuropile (Figs. 6, 7).

The perineurium varies in width from about 0.25 μ to about 12 μ. The outermost cells differ in their topography from the innermost cells. Those just beneath the extracellular sheath are irregularly arranged with spaces between them (Fig. 3). The innermost cells have longer lateral walls and lack intercellular spaces (Figs. 3, 4). The cells of the perineurium often have a vacuolated appearance







Figs. 3–5

(Fig. 3), as do those of the grasshoppers *Locusta* (Ashhurst, 1959; Ashhurst and Chapman, 1961), and *Melanoplus* (Lane, 1968).

The subperineurium varies in width from about 0.5 μ to about 13 μ. The long lateral borders of these glial cells separate at intervals producing intercellular spaces (Figs. 2, 4). These spaces probably represent a form of the glial lacunar system first described by Wigglesworth (1960a) in the central nervous system of *Periplaneta americana* and subsequently found in the antennal lobe of the brain of *Locusta migratoria* (Schürmann and Wechsler, 1969), and the mushroom body neuropile of *P. americana* (Mancini and Frontali, 1967).

Elements of the tracheal system, which enter the synganglion through the perineurium, are found primarily in the subperineurium and in glial cells originating from within the neuropile (Figs. 4, 10).

The cell bodies of the cortical glial layers have an elongated shape (Figs. 4, 5). Mitochondria are common in cells of both layers (Figs. 4, 5). They also contain smooth and rough endoplasmic reticulum (Fig. 8), and glycogen inclusions (Figs. 6, 7).

c) Neurons. Cell bodies of neurons were arbitrarily designated as type I, type II, and type III. Type I cell bodies are much more electron dense and much less common than the other types (Fig. 6). The cell body is about 4 μ wide. A low volume of cytoplasm is present, most of the cell being taken up by the large nucleus. Although type I cell bodies were not commonly encountered, at least some were found in every synganglion studied.

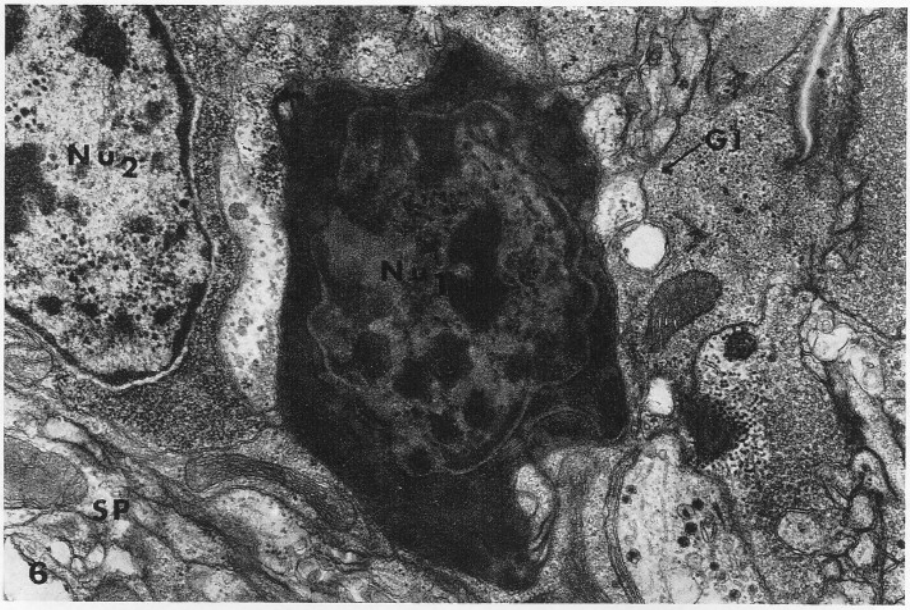
Type II cell bodies are the most common of the three types (Figs. 3, 7, 8). They measure about 5 μ in width. The volume of cytoplasm is low and the nucleus large as in type I cell bodies, but type II cell bodies have a more consistent oval shape and are less electron dense.

Type III cell bodies have the largest volume of cytoplasm of the three types (Figs. 7, 8) and vary in width from about 5 μ to 15 μ. These are neurosecretory cells. In connection with this function, profiles of rough endoplasmic reticulum and well developed Golgi bodies, as well as membrane bounded neurosecretory granules of different sizes and density are found within the cytoplasm. Electron dense material, similar to that of the neurosecretory granules, is found within the cisternae of the Golgi bodies (Fig. 9). Connections between what appears to be newly formed neurosecretory granules and the cisternae of the Golgi bodies are evident. This process of neurosecretory granule formation is similar to that described by Scharrer and Brown (1961) in the neurosecretory cells of the brain

Fig. 3. Cortical region. Extracellular sheath (*Ex*) borders outermost glial cells of the perineurium (*Pn*). Note extracellular spaces (black arrows). Type II neuron cell bodies (Nu_2) are ensheathed by perineurial cells (double black arrows). Ensheathment is incomplete (white arrows). ×10000

Fig. 4. Outermost area of cortical region. Perineurium (*Pn*), bordered by an extracellular sheath (*Ex*), lies next to subperineurium (*SP*). *GC* Glial cell body; *IS* Intercellular space; *M* Mitochondrion; *Np* Neuropile; *Tr* trachea. ×10000

Fig. 5. Area of cortex bordering neuropile (*Np*). Subperineurium (*SP*) is located between neuron cell bodies (Nu_2) and neuropile (*Np*). *GC* Glial cell body; *IS* Intercellular space; *M* Mitochondrion. ×10000



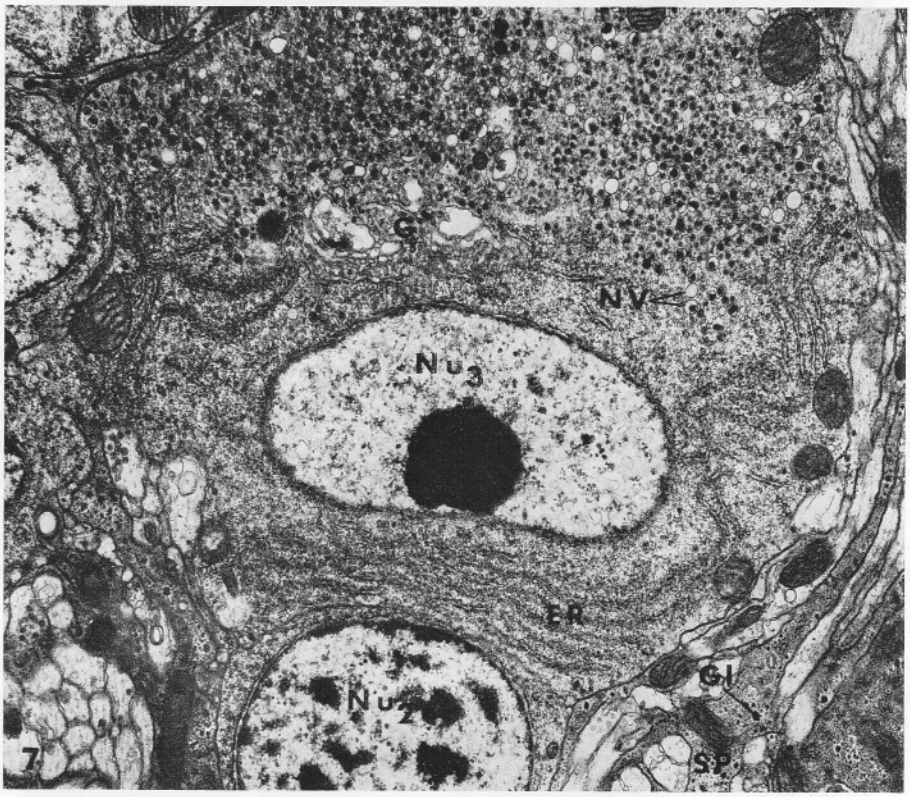


Fig. 6. Comparison of electron dense type I neuron (Nu_1) with type II neuron cell body (Nu_2). *Gl* Glycogen; *IS* Intercellular space; *NV* Neurosecretory vesicles; *SP* Subperineurium; *T* Trachea. ×14000

Fig. 7. Comparison of type II neuron cell body (Nu_2) with type III neuron cell body (Nu_3). *ER* Rough endoplasmic reticulum; *G* Golgi body; *Gl* Glycogen; *NV* Neurosecretory vesicles; *SP* Subperineurium. ×10000

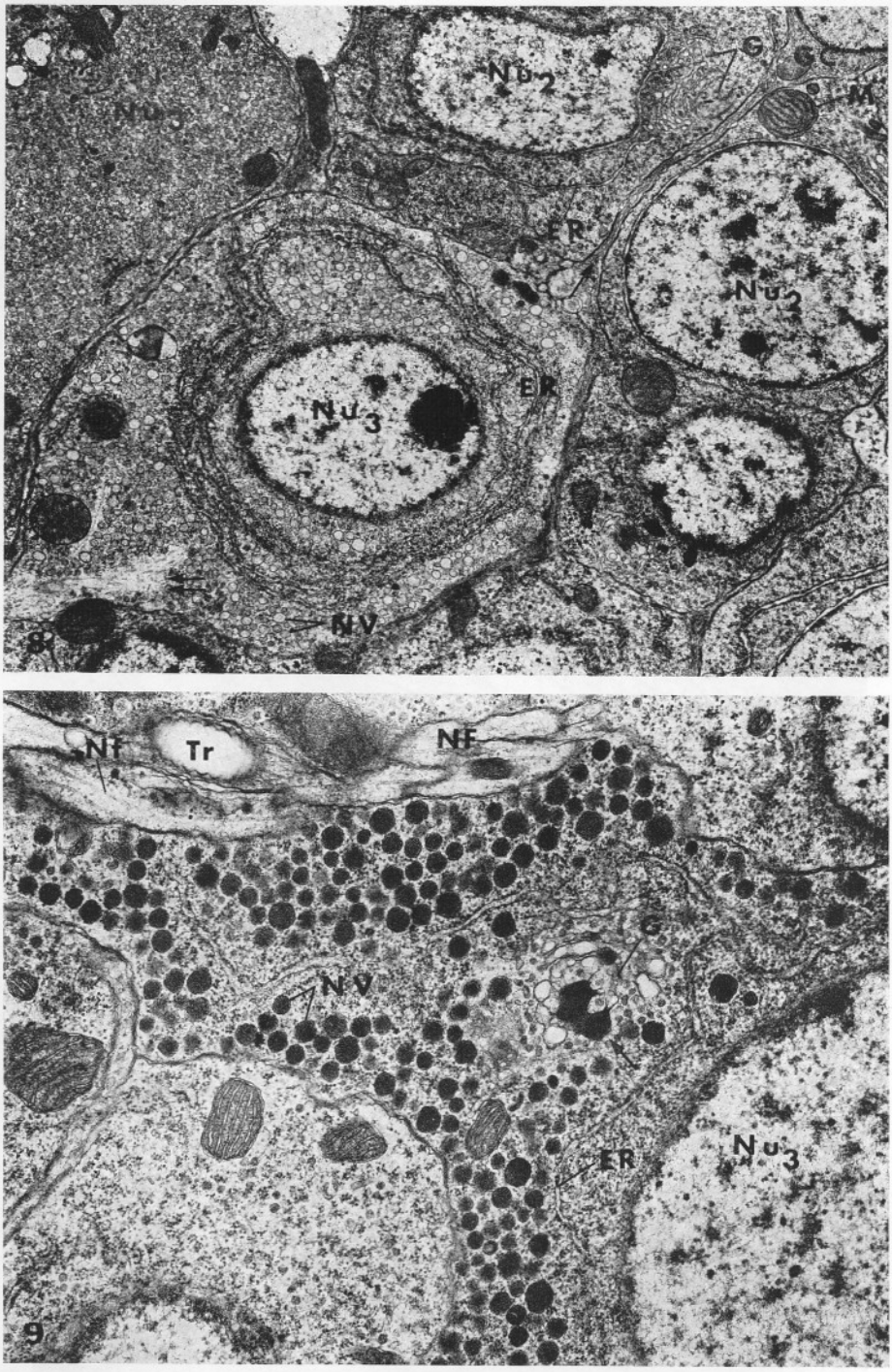
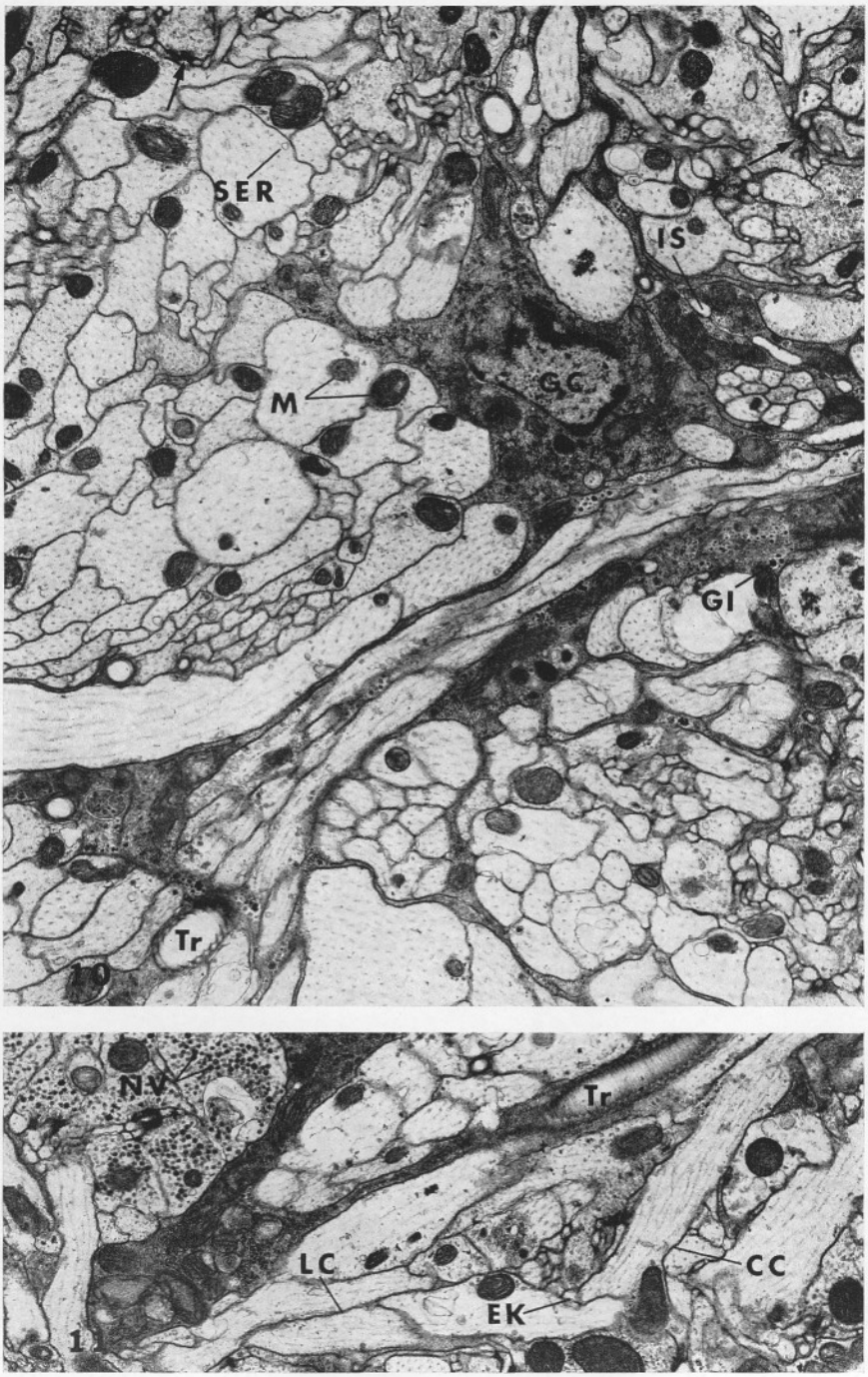


Fig. 8. Group of neuron cell bodies (Nu_2, Nu_3) with cellular detail labelled. Glial cell body (GC) sends extension with smooth endoplasmic reticulum (arrow) between several perikarya. Putative beginning of nerve fiber is identified by double arrows. ER Rough endoplasmic reticulum; G Golgi body; M Mitochondrion; NV Neurosecretory vesicles. $\times 10000$

Fig. 9. Cytoplasmic detail of type III neuron cell body (Nu_3) showing relationship between Golgi body (G), which contains dense material (arrow) similar to that of neurosecretory vesicles (NV). ER Rough endoplasmic reticulum; NF Nerve fiber; Nf Neurotubules; Tr Trachea. $\times 18000$



Figs. 10 and 11

of the earthworm, and since described in the parenchymal cells of the corpus cardiacum of the cockroach *Leucophaea maderae* (Scharrer, 1963), the medial neurosecretory cells of the brain of the blowfly *Calliphora erythrocephala* (Bloch, Thomsen, and Thomsen, 1966), and the peripheral neurosecretory cells of the stick insect *Carausius morosus* (Finlayson and Osborne, 1968). It has been suggested by Scharrer (1967), that the material incorporated into the neurosecretory granules is probably a product of the rough endoplasmic reticulum and is assembled in the Golgi bodies where a given amount is budded off into the cytoplasm.

Both electron dense (Fig. 9) and electron transparent granules (Fig. 8) are found in the neurosecretory cells. Although the majority of cells show only one type of granule, occasionally both electron dense and electron transparent neurosecretory granules are present (Fig. 7). The neurosecretory granules vary in size from about 500 Å to about 2000 Å.

Mitochondria, rough endoplasmic reticulum, free ribosomes, and Golgi bodies are found in all three types of neuronal perikarya (Figs. 7, 8, 9). As in the perikarya of insect neurons (Chiarodo, 1968; Beams, Sedar, and Evans, 1953; Hess, 1958; Trujillo-Cenóz, 1959; 1962), the free ribosomes and rough endoplasmic reticulum are not grouped into the parallel cisternae found in the vertebrate perikaryon. In the neurosecretory perikarya of this mite, the rough endoplasmic reticulum occurs in whorls (Fig. 8).

The glial ensheathment of the neuron cell bodies is incomplete, leaving one or more sides in contact with its neighbor (Fig. 3). Contact between cell neuron bodies and nerve fibers also occur. Morphological evidence of synapses were not observed in these areas.

3. Neuropile

a) Glial Cells. An extensive series of glial cells is found within the neuropile. They partially ensheath the nerve fibers and surround tracheal elements. These glial cells and their extensions come from two different sources, the subperineurium and from spider-like glial cells that arise within the neuropile (Fig. 10). These latter glial cells make up a third and distinct type of glial cell of the synganglion and are found exclusively within the neuropile. Extensions of these cells reach 8 μ. Within the cytoplasm are deposits of glycogen. The glial cells arising in the neuropile have intercellular spaces that are continuous with those of the subperineurium.

b) Nerve Fibers. Contacts between unsheathed nerve fibers (Fig. 11) may be divided into three categories (Chiarodo, 1968; Trujillo-Cenóz, 1959). Longitudinal contact is made by two parallel nerve fibers. End knob contacts are found at

Fig. 10. Area of neuropile containing glial cell body (*GC*) and its extensions. Arrows identify what are probably synaptic foci. *Gl* Glycogen; *M* Mitochondria; *SER* Smooth endoplasmic reticulum; *Tr* Trachea. ×10000

Fig. 11. Area of neuropile. *CC* Cross contact; *Ek* End knob contact; *LC* Longitudinal contact; *NV* Neurosecretory vesicles; *Tr* Trachea. ×10000

the end of one of the two nerve fibers making contact. Cross contacts are made when two fibers cross each other at right angles.

In cross section, the diameter of the nerve fiber varies from about 0.066 μ to 2.5 μ. Mitochondria, smooth endoplasmic reticulum (Fig. 10), and neurotubules (Fig. 9) are found throughout the nerve fibers. As Chiarodo (1968) noted, mitochondrial distribution is random, rather than being concentrated at the terminal end of the nerve fiber. Neurosecretory vesicles are found within some nerve fibers (Fig. 11). A thickened membrane, which is found between unsheathed nerve fibers (Fig. 10), probably represents the synaptic focus.

c) Esophageal Canal. The esophageal canal is bounded by an extracellular sheath which is continuous with that of the synganglion and surrounded by the same two glial cell layers found in the cortex. The glial cell layers of the esophageal canal bear the same relationships and have the same morphology as those in the cortex.

Discussion

The thin structureless extracellular sheath of *Macrocheles* is clearly different from the thick complex neural lamella of insects. Whether or not the absence of a complex neural lamella is a common characteristic of the Acarina is not known.

Although the mode of transfer and exact course of nutrients through insect ganglia is not known, it is commonly held that the glial cells are responsible for the nutrition of the neurons (Smith, 1967). Because of the similarity between the glial cells and neurons of both insects and this mite, it is reasonable to assume a trophic function for the glial cells of the synganglion. We could not show a transfer route of nutrient material through the cells of the synganglion.

The fine structure of the neuron cell bodies of the mite synganglion resembles those of insect ganglia thus far studied. The overall electron dense appearance of the type I cells is an exception, they have not yet been reported as occurring in insects. Hess (1958), using electron microscopy, and Wigglesworth (1960b), using light microscopy, found both "light" and "dark" neurons in the ganglia of *P. americana*. These "dark" cells, unlike those in the mite synganglion, had a higher electron density only in the cytoplasm.

In the central nervous systems of several vertebrates, workers have reported the presence of glial cells and neurons having a high electron density of both the cytoplasm and the nucleus (Mugnaini, 1965). In their general morphology, these cells more closely approximate the type I neuron cell bodies of the mite synganglion than the "dark" neurons described by Hess. In at least one study (Mugnaini, 1965), the occurrence of dark cells varied with the method of preparation and sometimes cells with an intermediate electron density were observed, raising the possibility of an artifact. The fact that these "dark" nerve cells occur in a variety of animals and that several standard methods were employed to prepare the tissue would seem to point away from the conclusion that all such cells are artifacts. In addition, cells with a high electron density of both the cytoplasm and nucleus have been observed in the mid-gut epithelium of the moth *Ephestia kühniella* (Smith *et al.*, 1969). It is suggested that they are replacement cells differentiating into an epithelial unit.

The glial ensheathment of the perikarya of this mite is incomplete resulting in large areas of contact between adjacent perikarya and between perikarya and nerve fibers. The few fine structural studies involving the perikarya of insect nerve cells have either not reported any interruptions of the glial sheath (Chiarodo, 1968; Hess, 1958), or have reported only small areas of interruption (Schürmann and Wechsler, 1969).

In the synganglion of this mite, the surrounding glial cell extensions do not invaginate into the neuronal perikarya. Such invaginations are commonly found in the ganglia of insects where they are believed to be involved in the transfer of trophic material (Smith, 1968).

Within the ganglia of insects a number of morphologically distinct glial cells have been described. Wigglesworth (1959) found at least four different types of glial cells in the central nervous system of the reduviid bug *Rhodnius prolixus*. Using Wigglesworth's classification system, Nordlander and Edwards (1958) have reported five different types of glial cells in the adult and larval brains of the monarch butterfly *Danaus plexippus plexippus*. Maddrell and Treherne (1967) reported two different glial cell types from the interganglionic connectives of the stick insect *Carausius morosus* and the cockroach *Periplaneta americana*. In the perineurium of the ganglia of these two insects, only one type of glial cell was found. The same general type of glial cell was described by Lane (1968) in the perineurium of the thoracic ganglia of the grasshopper *Melanoplus differentialis*. She also described neuroglial cells lying beneath the perineurial cells.

The synganglion of *Macrocheles* exhibits a paucity of cell to cell junctional specializations. In insect ganglia these junctions are common especially throughout the perineurium (Maddrell and Treherne, 1967), and within the neuropile (Smith, 1967).

The cellular organization in the synganglion of this mite has, in common with the ganglia of other arthropods, an extracellular sheath, a cortex containing glial cells and neuron cell bodies, and a complex neuropile. Differences lie primarily in the simplicity of the extracellular sheath, the amount of glial ensheathment of the perikarya, the arrangement of the glial cell layers and the relative lack of cell junctional specializations. Further ultrastructural studies, using other representatives of the Acarina, should yield valuable information on the similarities and differences between the synganglia of this large and economically important group of arthropods.

References

Ashhurst, D. E.: The connective tissue sheath of the locust nervous system: a histochemical study. Quart. J. micr. Sci. **100**, 501–412 (1959).

— Chapman, J. A.: The connective tissue sheath of the nervous system of *Locusta migratoria:* an electron microscopic study. Quart. J. micr. Sci. **102**, 463–467 (1961).

Beams, H. W., Sedar, A. W., Evans, T. C.: Studies on the neurons of the grasshopper with special reference to the Golgi bodies, mitochondria and neurofibrillae. Cellule **55**, 293–304 (1953).

Bloch, B., Thomsen, E., Thomsen, M.: The neurosecretory system of the adult *Calliphora erythrocephala*. III. Electron microscopy of the medial neurosecretory cells of the brain and some adjacent cells. Z. Zellforsch. **70**, 185–208 (1966).

Chiarodo, A. J.: The fine structure of neurons and nerve fibers in the thoracic ganglion of the blowfly, *Sarcophaga bullata*. J. Insect Physiol. **14**, 1169–1175 (1968).

Finlayson, L. H., Osborne, M. P.: Peripheral neurosecretory cells in the stick insect (*Carausius morosus*) and the blowfly larva (*Phormia terrae-novae*). J. Insect Physiol. **14**, 1793–1801 (1968).

Hess, A.: The fine structure of nerve cells and fibers, neuroglia, and sheaths of the ganglion chain in the cockroach *Periplaneta americana*. J. biophys. biochem. Cytol. **4**, 731–742 (1958).

Lane, N. J.: The thoracic ganglia of the grasshopper *Melanoplus differentialis:* fine structure of the perineurium and neuroglia with special reference to the intracellular distribution of phosphatases. Z. Zellforsch. **86**, 293–312 (1968).

Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961).

Maddrell, S. P. H., Treherne, T. E.: The ultrastructure of the perineurium in two insect species, *Carausius morosus* and *Periplaneta americana*. J. Cell Sci. **2**, 119–128 (1967).

Mancini, G., Frontali, N.: Fine structure of the mushroom body neuropile of the brain of the roach *Periplaneta americana*. Z. Zellforsch. **83**, 334–343 (1967).

Mugnaini, E.: "Dark cells" in electron micrographs from the central nervous system of vertebrates. J. Ultrastruct. Res. **12**, 235–236 (1965).

Nordlander, R. H., Edwards, J. S.: Morphology of the larval and adult brains of the monarch butterfly *Danaus plexippus* L. J. Morph. **126**, 67–94 (1968).

Richardson, K. C., Jarett, L., Finke, E. H.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. J. Cell Biol. **17**, 208–212 (1960).

Rodriguez, J. G., Wade, C. F., Wells, C. N.: Nematodes as a natural food for *Macrocheles muscaedomesticae* (Acarina: Macrochelidae), a predator of the house fly egg. Ann. Entomol. Soc. Amer. **55**, 507–511 (1962).

Scharrer, B.: Neurosecretion. XIII. The ultrastructure of the corpus cardiacum of the insect *Leucophaea maderae*. Z. Zellforsch. **60**, 761–796 (1963).

— The neurosecretory neuron in neuroendocrine regulatory mechanisms. Amer. Zoologist **7**, 161–169 (1967).

— Brown, E.: Neurosecretion. XII. The formation of neurosecretory granules in the earthworm *Lumbricus terrestris*. Z. Zellforsch. **54**, 530–540 (1961).

Schürmann, F. W., Wechsler: Elektronenmikroskopische Untersuchung am Antennallobus des Deutocerebrum der Wanderheuschrecke *Locusta migratoria*. Z. Zellforsch. **95**, 223–248 (1969).

Smith, D. S.: The trophic role of glial cells in insect ganglia. In: Insects and physiology (J. E. L. Beament, and J. E. Treherne, eds.), p. 189–198. London: Oliver & Boyd Ltd. 1967.

— Insect cells, their structure and function. London: Oliver & Boyd Ltd. 1968.

— Compher, K., Janners, M., Lipton, C., Wittle, L. W.: Cellular organization and ferritin uptake in the midgut epithelium of a moth, *Ephestia kühniella*. J. Morph. **127**, 41–72 (1969).

Trujillo-Cenóz, O.: Study on the fine structure of the central nervous system of *Pholus labruscoe* (Lepidoptera). Z. Zellforsch. **49**, 432–446 (1959).

— Some aspects of the structural organization of the arthropod ganglia. Z. Zellforsch. **56**, 649–682 (1962).

Venable, J. H., Coggeshall, R.: A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. **25**, 407–408 (1965).

Wigglesworth, V. B.: The histology of the nervous system of an insect, *Rhodnius prolixus* (Hemiptera). II. The central ganglia. Quart. J. micr. Sci. **100**, 299–313 (1959).

— The nutrition of the central nervous system of the cockroach *Periplaneta americana* L. The role of perineurium and glial cells in the mobilization of reserves. J. exp. Biol. **37**, 500–512 (1960a).

— Axon structure and the dictyosomes (Golgi bodies) in the neurons of the cockroach *Periplaneta americana*. Quart. J. micr. Sci. **101**, 381–388 (1960b).

Lewis B. Coons, Ph.D.
Department of Entomology
North Carolina State University
Raleigh, North Carolina, 27607
U.S.A.