HISTOLOGICAL STUDIES ON THE SUBOESOPHAGEAL GANGLION OF

Acheta domesticus (Linn.) (Orthoptera: Gryllidae)

by

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ABSTRACT

The arrangement and interconnections of neurons in the insect nervous system is extremely complex and has only in a few cases been worked out in detail. In particular, very little is known of the organisation of the suboesophageal ganglion, even though it is involved in the control and regulation of sensory and motor activities during important feeding processes. Acheta domesticus (Linn.), a relatively generalised insect, has been selected as the subject of a detailed histological analysis of the suboesophageal ganglion. The analysis of cellular composition deals with the ganglion sheath and other types of glial cells, and then distinguishes large and medium sized motor neurons and small association neurons. The distribution of these is mapped and their histological characteristics are described.

The neuropile, fibre tracts and glomerular bodies are described and illustrated in the adult suboesophageal ganglion, together with an analysis of the major axonal pathways that it contains and the connections between the ganglion, other regions of the central nervous system and the peripheral sense-organs. This description of the topographical histology of the ganglion has been followed by selective silver impregnation of individual neurons and axonal connections using those modifications of the Golgi technique found most suitable after a detailed comparison of alternative procedures. About ten main types of axon terminations were recognised and their fields and internal relationships recorded.

The resulting neurological maps of the ganglion will, it is hoped, provide a structural basis on which the electrophysiological investigation of selected functional units may proceed.

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I GENERAL INTRODUCTION

Although there are many studies of the structure of the central nervous system of insects, relatively few have been made of the histological structure of the suboesophageal ganglion. This is particularly striking if one compares the available information on the suboesophageal ganglion with that on the histology of the brain, optic lobes and retina of insects, both in respect of their cellular composition and fine structure, as well as in the attempts which have been made to identify structural and functional patterns of neuronal interconnection.

Much general information on the cellular composition of the insect central nervous system is provided by Zawarzin (1924a & b), Bierbrodt (1942), Pipa et al (1959), Wigglesworth (1959), Smith & Treherne (1963), Trujillo-Cenóz (1960), Fielden (1963), Seabrook (1968), Huddart (1971) and Ali (1973 & 1974), who have described in varying detail using different techniques the nerve sheath and perilemma, the glial cells, neuropile, fibre tracts and the glomerular bodies. In terms of the detailed structure and individual relationships of neurons and their terminations, Sanchez (1915 & 1919), Cajal & Sanchez (1915), Pedler & Goodland (1965), Trujillo-Cenóz (1960 & 1965), Trujillo-Cenóz & Melamed (1966) and Melamed & Trujillo-Cenóz (1967) may be cited.

The observations reported in this study have been made on sections of the suboesophageal ganglion of Acheta domesticus (Linn.). The aim of the work has been to provide, as far as possible, a detailed histological analysis of the suboesophageal ganglion of a relatively generalised Pterygote insect and thus ultimately to contribute towards a fuller functional understanding of that region of the nervous system which is directly involved in the

important sensory and motor control of feeding behaviour, as well, perhaps, as contributing indirectly to other aspects of nervous integration.

The studies fall into two parts, with seven main sections.

1. General Histology

   (A) The analysis of major patterns of cellular composition.

   (B) Neuropile and fibre tracts.

   (C) Glomerular bodies.

   (D) Axonal pathways.

2. Detailed analysis of individual neurons (Golgi impregnation procedures)

   (A) The different types of dendritic endings.

   (B) The various kinds of branched endings.

   (C) Axons and their branches and terminations in the suboesophageal ganglion.

For studying the general histology and cellular composition of the ganglion, the normal techniques of staining serial sections with Mallory's phosphotungistic haematoxylin and Mallory's connective tissue stain were very useful. To investigate the neuropile, fibre tracts and major axonal pathways, the osmium tetroxide technique of Wigglesworth (1959), followed by ethyl gallate treatment gave quite good results.

For the description of the more detailed structure of individual neurons, Golgi impregnation techniques were used with minor changes in some aspects of the procedures and these gave good results. Two further impregnation techniques were also tried; a double-impregnation procedure and a mixed impregnation method of silver nitrate and osmium tetroxide:

II HISTORICAL REVIEW

Investigations of the general histology of the insect nervous system has been very largely directed towards the brain, optic lobes and the thoracic and abdominal ganglia. Surprisingly little attention has so far been paid to the suboesophageal ganglion in insects. Such information as exists is summarised on a systematic basis in the following account:

1. General description of the ganglion.

Among many brief general descriptions of the suboesophageal ganglion, Myers (1928), for example, described the suboesophageal ganglion and ventral nerve cord in Melampsalta sericea (Cicadidae). He mentioned that the ovoidal suboesophageal ganglion is connected to the first posterior ganglionic mass by a pair of long stout connectives, and added that the first ganglionic mass lies largely in the prothorax. Hilton (1937), claimed that in Gryllus assimilis the suboesophageal ganglion has only one pair of nerves to the neck muscles. Nesbitt (1941), has described the suboesophageal ganglia of several Orthopteroid insects, i.e. Conocephalus, Dissosteira, Ceuthophilus, Romalea, Termopsis, Anisolabis, Mantis and Gryllus; he also described the brains and the ventral ganglionic chain, and gave a brief description of the thoracic ganglia and the abdominal ganglia. Sanchez (1941), gave a very brief description of the suboesophageal ganglion in Apis. Ehnbom (1948) and Power (1950), have described the suboesophageal ganglion of Trichopteran insects and some other families, and Drosophila respectively. In Locusta migratoria, Albrecht (1953), has described the suboesophageal ganglion and the ventral nerve cord. Henry (1958), studied some cervical nerves from the suboesophageal ganglion and also described the prothoracic ganglion in some Orthoptera, Neuroptera, Coleoptera and Hymenoptera. Schmitt

(1959), described the position and relations of the suboesophageal ganglion in the grasshopper Dissosteira carolina; he added that there are two fine cervical nerves leaving the suboesophageal ganglion. Mill (1964), studied the structure of the central nervous system of the abdomen in Aeshna juncea and Aeshna cyanea. Vasvary (1966), gave a general description of the ventral nerve cord and the suboesophageal ganglion in Tibicen chloromera (Cicadidae).

2. General histology:

(A) Standard techniques:

Wigglesworth (1957 & 1959), introduced the use of osmium tetroxide as a fixative and stain for the tissues of the central nervous system. His technique has since been used by others with good results in studying the type of glial cells, ganglion cells and neuropile, including fibre tracts, glomerular bodies and axonal pathways. Two other standard staining procedures used for studying the cellular components and various types of neurons are Mallory's phosphotungstic haematoxylin and Mallory's connective tissue stain (Hughesdon's modification) as recommended by Drury & Wallington (1967).

(B) Perilemma cells and other glial cells:

Wigglesworth (1959), studied the neurilemma, perineurium and glial cells in Rhodnius. Smith & Treherne (1963), and Seabrook (1968), also described the ganglion sheath, perineurium (type I glial cells) and neurilemma (neural-lamella) in Periplaneta americana and Schistocerca gregaria. Ali (1973), described the neurilemma, perineurium, glial cells and various types of neurons in Pieris brassicae.

(C) The various types of neurons (ganglion cells):

The origins of the motor and sensory fibres in Aeshna nymphs were described by Zawarzin (1924). Pyle (1941), Johansson (1957), Wigglesworth (1959), Fielden (1963) and Heywood (1965), divided the

N.B. Here and elsewhere in this thesis, the term 'fibre tract' refers to a large number of axons, not necessarily all of the same functional nature, which form large, micro-anatomically distinct bundles. The term 'axonal pathway' (p.7, etc.) refers to the path followed by an individual axon. It will be clear by a comparison of Table 1 and 2 that some, but not all pathways, can be referred to particular tracts.

ganglion cells into association and motor neurons, depending on their size. Trujillo-Cenóz (1960), studied some of the neurons and glial cells in Armadillidium vulgare (Crustacea-Isopoda), and recognised that the neurons and glial cells are arranged at the periphery of the ganglion. Chiarodo (1963 & 1968), described three cell types in the thoracic ganglion of Sarcophaga bullata. Cohen & Jacklet (1967) and Young (1960), studied the distribution of motor neurons in the meso-thoracic ganglion of Periplaneta americana, while Huddart (1971), investigated the neurons of the prothoracic ganglion of Carausius morosus. Ali (1973), like Heywood (op. cit.), divided the ganglion cells into association and motor neurons, depending on their size. Gregory (1974), studied the neuron cell body groups in the meso-thoracic ganglion of adult male Periplaneta americana.

(D) Neuropile; fibre tracts and glomerular bodies:

Descriptions of the neuropile, to include neuronal pathways and glomerular bodies started early, e.g. Hilton (1911a & b), who traced some neuron pathways in the ventral ganglia of Corydalis. Zawarzin (1924a & b), described some sensory fibres passing to the higher centre in Aeshna larvae, and also recognised glomerular bodies during his study of the histology of the brain. Hanström (1928 & 1940), gives extensive accounts of the cephalic nervous system in the lower Exopterygote insects, including many references to earlier work, but confined largely to the centres and major pathways and glomerular bodies within the brain. Bierbrodt (1942) recognised some more heavily staining glomerular bodies when studying the histology of the brain in Panorpa larvae. Pipa et al (1959) found in the thoracic and first two abdominal ganglia of the adult cockroach Periplaneta americana, seven pairs of longitudinal tracts and ten commissural tracts. Trujillo-Cenóz (1960), found in Armadillidium vulgare (Crustacea-Isopoda), using 1% OsO_4 fixation, that neurons and glial cells are arranged at the periphery of the ganglion while the central region

is a dense assembly of inter-woven neuropile fibres. Fielden (1963), has studied the neuropile, the axons and the peripheral nerves in Anax. Mill (1964), mentioned some sensory fibres, motor and inter-neuron cells, and some transverse and longitudinal tracts of fibres during his study of the neuropile in Aeshna. Ali (1967), recorded some fibre tracts in the suboesophageal ganglion of adults Pieris brassicae, and described also the ventral, lateral and dorsal glomerular bodies. Seabrook (1968), found in the seventh abdominal ganglion of the locust Schistocerca gregaria, seven transverse commissures and six longitudinal connective tracts, and also described and illustrated seventy-six other neuron tracts. Huddart (1971), stated that in Carausius morosus, some axons from cortical neurons pass into the central neuropile of the ganglion and many eytoplasmic strands from neuroglial cells also penetrate into the neuropile. Ali (1974), has described the glomerular bodies in the brain and the suboesophageal ganglion of the adult and larval stages of Pieris brassicae.

(E) Axonal pathways:

Hilton began in 1902 to study the sense cells and their sensory fibres leading to connections with neurons in the ventral ganglia. In 1911 a & b, he also studied the sensory tracts and some of the axonal pathways of motor and association neurons in the ventral ganglia of Corydalis. Zawarzin (1924 a & b), also described the types of the neurons and their fibres, he made a survey of some of the long fibres and cells in the central nervous system of Aeshna nymphs. Pumphrey & Rawdon-Smith (1937), mentioned some giant fibres in the abdominal cord of Periplaneta americana. Pyle (1941), traced various axonal pathways in the thoracic ganglia of Ephestia kuehniella and Power (1943, 1946 a & b), has described the distribution of tracts and certain specific fibres in the brain of Drosophila melanogaster. Power (1948), has also studied the fibre tracts and the axonal pathways and their distri-

bution during his investigation of the thoracico-abdominal nervous system of adult Drosophila melanogaster. Ehnbom (1948), traced some axonal pathways in the suboesophageal ganglion of adult Trichoptera. Cook (1951), indicated some giant fibres during his study of the nervous system of Locusta migratoria, while Hughes (1953), traced some of these fibres in dragon fly nymphs. Satija (1958 a), studied the brain and thoracic nerve cord, with special reference to the nervous pathways, including those descending from the brain in Locusta migratoria. Similar studies were also continued by Satija (1958 b, c & d) on the brain and thoracic nerve cord of Aeshna nymphs, Apis and Calliphora. Guthrie (1961) has made a detailed study of the topography of the nervous system in Gerris, with information relating to the individual neurons, by using Palmgren's - silver - on - the - slide technique (Palmgren, 1948 & 1955), and the rapid silver nitrate method of Golgi. He also gave a detailed description of the structure of the central nervous system including the suboesophageal ganglion. Mill (1964), mentioned some of the axonal paths of motor neurons and inter-neurons during his work on the structure of the central nervous system of the abdomen in Aeshna; he gave also a description of some cell groups and associated fibre tracts. Seabrook (1968), described and illustrated seventy-six axonal tracts in the seventh abdominal ganglion of the desert locust Schistocerca gregaria, and also (Seabrook, 1970), traced about a hundred neurons within the terminal ganglionic mass of the same species.

3. Cytology and ultrastructure:

Most studies of the detailed histology of the central nervous system of insects have used Golgi impregnation techniques or other methods derived from them. The majority of these studies deal with the descriptive histology of the brain, optic lobes and retina of insects and the techniques are often dependent on work in other

animal groups, especially vertebrates.

(A) Light-microscopy:

Towards the end of the last century and beginning of this century, detailed neurohistological studies were started by Cajal (1892, 1909 & 1910), followed by Zawarzin (1913), Sanchez (1915, 1918 & 1919) and others, all using similar procedures of Golgi impregnation. Cajal & Sanchez (1915), distinguished in their light-microscopic observation of the insect intermediate retina between giant monopolar neurons and small or medium sized monopolars.

Work of this kind has been revived and greatly extended in the past decade, especially by Strausfeld & Blest (1970). Parallel to this there has been considerable work on the vertebrate nervous system, for example, Boycott,Gray & Guillery (1961), reported a study by light- and electron-microscopy of the central nervous system of Lacertidae while Boycott & Dowling (1969), reported more information on the distribution of synapses in three dimensions, and on the spatial relationships of the different types of nerve cells; they emphasised that light-microscopy is necessary to provide this type of information.

(B) Electron-microscopy:

Most recent studies of the fine structure of the central nervous system carried out by electron-microscopy have employed modifications involving osmium tetroxide fixation allied with Golgi techniques. See, for example, Pedler & Goodland (1965); Trujillo-Cenóz (1960 & 1965); Trujillo-Cenóz & Melamed (1963 & 1966); Melamed & Trujillo-Cenóz (1967). Trujillo-Cenóz (1959), studied the fine structure of the ganglionic chain in Pholus labruscoe (Lepidoptera) and gave some early electron-micro-

graphs of the insect nervous system. The same author (1960) reported further electron-microscope studies of distinctive nerve fibres in the abdominal ganglia of Armadillidium vulgare (Crustacea-Isopoda). Trujillo-Cenóz (1965), also made electron-microscope observations on the intermediate retina of the adult flies Sarcophaga bullata and Lucilia sp. Boycott et al (1961) used both electron- and light-microscopy in studying the synaptic structure of the central nervous system of some Lacertidae. Trujillo-Cenóz & Melamed (1965), provided an exclusively ultrastructural account of the organation and nervous connection of the Ommatidia of Diptera. Dowling & Boycott (1965 & 1966), investigated synaptic contacts by electron-microscopy during their studies of the inner plexiform layer of the primate retina. Guillery (1970), concluded in his paper on the light- and electron-microscopy of normal and degenerating axons, that in general the light-microscopical methods cannot be used to show the synaptic relations of degenerating axons. This is possible in only a limited number of regions, where the axon terminals can be clearly recognised by their characteristic morphology and where their special relationship to well defined postsynaptic structures can be demonstrated. Electron-micrographs are needed to allow a more reliable identification of synaptic relationships. He pointed out, however, that both light- and electron-microscopical methods allow ready identification of degenerating axons. These studies demonstrate incidentally that the degenerative reaction of axons is not the same in all fibre systems, at least in the vertebrates species studied by Guillery.

One may note again the close relationship in techniques and general concepts between those working on vertebrate nervous systems and those on the insects.

(C) Methods and techniques:

a. Silver impregnation methods.

Several methods of studying different histological aspects of the nervous system of insects and other animals have been developed but some of the most important are derived from modifications of the Golgi techniques and of osmium tetroxide, or of both techniques together. Available methods include Kenyon's method (1896), Vigier's technique (1908), Cajal's method (1910), the double-impregnation method of Kallius (1910), Holmes's methods (1943, 1947), Palmgren (1948), the Golgi-Kopsch method (Lee, 1950), Romanes (1950), Golgi cox techniques (Scholl, 1953), and a modification of the double-impregnation procedure of Kallius (Strausfeld & Blest, 1970). Trujillo-Cenóz (1959), studying the fine structure of the ganglionic chain in Pholus labruscoe (Lepidoptera), after fixation in 1% osmic acid in a veronal buffer, pH 7.9, found that the neuropile appears as punctate or structureless substance when stained with anilin dyes, whereas the Golgi method and methylene-blue method reveal the existence in this region of two kinds of neuron prolongations equivalent to the axons and dendrites of vertebrate neurons. He stressed that it was impossible in his investigation to find any structural characteristic enabling him to define a nerve fibre either as a dendrite or as an axon. Blest (1961), mentioned some modification of Holmes's silver nitrate method (Holmes, 1943), for insect central nervous system histology. Guthrie (1961), referred to several metallic impregnation techniques during his studies of the detailed anatomy of the nervous system in Gerris. The silver-on-the-slide method of Holmes (1947), and Romanes (1946 & 1950), were found less satisfactory than that of Palmgren (1948), which gave some excellent results. He also found that the rapid Golgi silver-bichromate process occasionally gave good results (5% success) but the Golgi-cox mercury-bichromate method gave a much

higher percentage of successful preparations (50 to 70% success). When Guthrie (1962), was studying the regenerative growth of nerve axons in Periplaneta americana, a simple, rapid and invariably successful staining method was required to allow the state of the regenerating nerve to be judged. Well-oxidised Hansen's Trioxyhaematin was used for this purpose, rather than a silver technique; it gave a dark brown colour to the fibres and so defined them clearly. Trujillo-Cenóz & Melamed (1963), used a fixative solution of 1% Os O_4 in veronal buffer at pH 7.4 to study the fine structure of the optic lobe of Musca domestica and Calliphora vomitoria. They employed electron-microscope observations preceded by a short description of the most significant histological features of the intermediate retina as revealed by the Golgi method. This showed short processes arising from the monopolar prolongations from radial penetrations between the photoreceptor fibres. The same authors (1964), also fixed the nerve centre of the spider Lycosa erythrognatha in formaldehyde and osmium tetroxide. Trujillo-Cenóz and Melamed (1965), used a fixative solution of 10% paraformaldehyde or 5% glutaraldehyde in phosphate buffer, and 1% Os O_4 in phosphate buffer when studying the compound eyes of Sarcophaga and Lucilia. Mill (1964), referred to several techniques during his studying of the abdominal nervous system of Aeshnid nymphs. These included Palmgren's silver technique (Palmgren, 1948 & 1955), and the rapid Golgi technique as described by Carleton & Leach (1938). Colonnier (1964), outlined various methods derived from Golgi techniques in his studies of the tangential organisation of the visual cortex of some vertebrates, i.e. Golgi rapid techniques, Golgi-cox methods (Sholl, 1953), Golgi-Kopsch methods, (Lee, 1950), a glutaraldehyde modification of the Golgi-Kopsch method, the Cajal method for neurofibrils (Cajal & Roman, 1910), and Holmes's axon technique (Holmes, 1943). He gave a full description of the glutaraldehyde

modification of the Golgi-Kopsch impregnation method which gave good results. Colonnier also described the dendritic fields of some cells after using some of the other Cajal techniques.
Trujillo-Cenóz (1965), working on the structural organisation of the intermediate retina of Sarcophaga bullata and Lucilia sp., used a fixative solution of 1% Os O_4 in veronal buffer or 2% Os O_4 in insect Ringer. Blackstad (1965), used Golgi preparations for mapping experimental axon degeneration by electron-microscopy. Ramon-Moliner (1967), gave a brief discussion of sources of error in the study of Golgi-impregnated material. Boycott & Dowling (1969), found that after initially inadequate results with the Golgi-rapid and Golgi-cox methods of impregnation, they obtained good results with Colonnier's modification of the Golgi-Kopsch method. They pointed out that Colonnier's method employs glutaraldehyde as the primary fixative instead of formaldehyde, and that it regularly gave good impregnation of most types of primate retinal neurons, particularly in the central area of the retina. The same authors also reported that a rapid Golgi method gave good impregnation of a few cells, whereas Colonnier's modification stained more cells and was more generally consistent; they found the Golgi-cox method was unsuccessful. Strausfeld & Blest (1970), stated that the Golgi-Colonnier selective silve procedure was used successfully to visualise the neurons in their study of the optic lobes of Lepidoptera. They also described in detail some of the non-selective and selective silver impregnation techniques and indicated some other stains which could be used in the general reconstruction of brain regions. Strausfeld (1970), reviewed some Golgi studies on insects, and examined the optic lobes of Diptera both by variants of the Golgi-Colonnier selective staining techniques and by reduced silver procedures. Strausfeld found that Colonnier's (1964) method works especially well on the Syrphidae, giving beautifully clear impregnation of the neural elements of both

the optic lobes and mid-brain regions. Young (1973), tried to follow pathways through from receptors and receptor analysers to the various types of higher and lower motor centres and then all the way to effectors by using Cajal's staining method (Cajal, 1917), and by Golgi preparations following the Kopsch technique (Kopsch, 1899), after fixation in formal-bichromate as described by Stephens (1971). His studies were conducted on the optic lobe of the Cephalopods Loligo vulgaris and Loligo pealeii and he found that Golgi methods were as fickle with this as with other material. Fortunately, however, Cajal's silver methods succeed admirably with these organisms and with the aid of some successful Golgi-Kopsch preparations it was possible to discover much about the organisation through which visual signals are analysed in these molluscs.

b. Recent developments in technique.

The general impression from this review of metallic impregnation techniques is that the modified Golgi-Colonnier method used by Strausfeld and his co-worker offered one of the most hopeful methods and it was the basis on which much of the present work was carried out.

The older histological techniques for studying the structure of the nervous system try to resolve single neurons or patterns of neurofibrillae by the Golgi method and its modifications. In more recent years some very specific techniques have been developed for the structural investigation of the central nervous system. Some of these diverse modern procedures include backfill techniques or the injection of cabalt salts or special dyestuffs into the neuropile or selected cell bodies. Other techniques include, for example, Heimer's (1970) method of silver impregnation of degenerating axoplasm in plastic-embedded material, which bridges the gap between light- and electron-microscopy, and the experimental

tracing of fibre connections. Advantageous as these recent methods have proved to be for the study of central nervous connections, Heimer added that light-microscopic techniques alone are inherently unable to provide pictures of synaptic relationships detailed enough to satisfy. Although the modern silver methods may enable the investigator to identify accurately the regions of synaptic distribution of most fibre systems, they fail to disclose the nature of the postsynaptic elements involved in the connections. That degenerating 'boutons terminaux' can be identified by electron-microscopy, together with the associated postsynaptic structures, is, therefore, a finding of prime importance that has already led to productive combinations of experimental work with light- and electron-microscopic studies. Heimer emphasised, however, his experience that a proper selection of tissue fragments for electron-microscopy of degenerating 'boutons terminaux' is impossible without the assistance of adequate light-microscopic techniques. He used fixation by buffered formalin or buffered glutaraldehyde-formalin and the tissue was prepared for ultrastructural examination, following the customary procedure of rinsing in phosphate buffer, with post fixation in 1 - 2% buffered osmium tetroxide solution.

In tracing pathways with radioactive horseradish peroxidase (see La Vail & La Vail, 1972), it was found that differentiating neurons may respond to changes in their peripheral fields by atrophy or hypertrophy, though the mechanisms by which the neuron cell body recognises its target field are poorly understood. One possible mechanism involves the uptake of material by the axon terminal and its transport in a centripetal or retrograde direction to the cell body. La Vail & La Vail also reported the uptake and retrograde transport of horseradish peroxidase by axons of two different neuronal populations in the central nervous system of the chick: from terminals in the retina to their cell bodies and from

terminals in the optic tectum to the retinal ganglion cell bodies. They injected horseradish peroxidase in Ringer's solution into the eye and fixed in glutaraldehyde and formaldehyde in phosphate buffer at pH 7.4. Cowan et al (1972), used amino acids (tritiated Leucine), readily incorporated into proteins which are then transported along the axon; they added that this method is more useful for tracing fibre pathways, especially where the number of fibres is low. They also demonstrated that the autoradiographic tracing of axonally transported radioactive materials can be used as a neuroatomical method for determining the origin, course and termination of fibre pathways within the central nervous system of some vertebrate species. The basis of the method is that radioactively labelled amino acids and certain other precursor substances injected into specific regions of the brain are taken up and incorporated into protein and other macromolecules by neuronal somata in these regions, then transported along the axons where the label can be demonstrated by autoradiography. Methods of this kind have not yet been used in the insects.

In resolving single neurons, electrophoretic uptake of (i) Procion dyes and (ii) cobalt salts into axons or cell bodies has been employed. Stretton & Kravitz (1968), in a study of the specificity of neuronal connections in the lobster abdominal ganglia, electrophoretically injected the dye Procion-Yellow M_4 Rs into identified cell bodies. This dye spreads into the fine branches of the cells, survives fixation and routine histological procedures, and so permits the construction of cell shapes through examination of serial sections of ganglia. This method of dye injection should have general applicability in studies where a knowledge of the geometry of specific cells is important. They also stated that no fully satisfactory method exists for tracing the course of individual neurons within the neuropile. Certain conventional neuroanatomical

methods, such as staining with methylene blue or silver impregnation, will apparently stain cells in their entirety but the success of these methods depends on their staining a random minority of nerve cells, so that the probability of visualising any particular cell is quite low. Stretton & Kravitz also found that the four most satisfactory dyes were Procion Brown H_3 Rs, Brilliant Red H_3 BNs, Navy Blue H_3 Rs, and Yellow M_4 Rs. Procion Yellow M_4 Rs is more fluorescent than the other three and they used it exclusively. Pitman et al (1972), used intracellular cobalt injection in their studies of branching in central neurons, in the metathoracic ganglion of the cockroach Periplaneta americana by light- and electron-microscopy. Cobalt chiloride can be injected into an identified nerve cell body in an insect ganglion and allowed to react there with ammonium sulphide, so staining the soma and its branches with a black precipitate. The stained cell body and its branches throughout the neuropile are visible both by light- and electron-microscopy. They found that it was essential in analysing the cellular basis of behaviour to be able to identify the individual neurons involved and to visualise the geometry of their interacting, branched processes. One of the most useful of the classical techniques for observing individual neurons has, of course, been the Golgi method and its variations. These have been powerful tools because only a small proportion of a neuronal population is impregnated and those neurons that have been so stained are visualised entirely. Although this method gives much cellular detail and has been extended from the light-microscopic level to the ultrastructural level, it has the major disadvantage that neurons are stained more or less randomly and that the results can be notoriously capricious. Injection methods are, therefore, potentially more valuable and are increasingly being employed. Llinás (1973), used Procion Yellow and Cobalt salts for studying structural and functional relationships in the vertebrate central

nervous systems. Llinás also considered that the Procion Yellow staining techniques pioneered by Stretton & Kravitz (1968), and the more recent cobalt method (Pitman et al, 1972), should be invaluable in the further analysis of structure-function relationships at the single cell level. He found that injection of Procion Yellow often caused the nucleus to be stained more conspicuously than the rest of the neuronal soma. This may be because the nucleus has a more basic pH than the cytoplasm. Llinás (1973), also pointed out that Procion Yellow can be used for several types of investigations in the central nervous systems: e.g., identification of neurons and axons, detailed morphology and its relation to neuronal integration, connectivity of neuronal nets, the three-dimensional study of axonal convergence and in neuropharmacological studies. Llinás also pointed out that this use of the dye has been extremely important in examining the relationship between the distribution of a telodendrion and the functional property of the synapse. It generates on a given target cell. Llinás added that cobalt back-filling in the vertebrate nervous system had been very capricious but led, when successful, to intriguing results. Gregory (1974), used Bodian's silver stained sections (Bodian, 1936), and Procion Yellow staining to examine the general neuroanatomy and details of the nerve roots in the mesothoracic ganglion of adult males of Periplaneta americana. He summarised the general structure of the ganglion and also gave details of seven pairs of longitudinal fibre tracts, ten transverse commissures and three new oblique tracts of characteristic shape. Gregory's work applies an improved (Bodian) silver technique and a new use of Procion Yellow to display whole tracts of nerve fibres instead of single ones (cf. p. 14), and he examines four aspects of the neuroanatomy: identification of the roots of the peripheral nerves, definition of neuron cell body groups, and the recognition of longitudinal and transverse nerve fibre tracts. Altman & Tyrer (1974), used the

cobalt filling method in studies of the development of neuronal connections implicated in the flight of crickets and locusts, examining changes in the flight neurons as they acquire their adult function. Székely & Callyas (1975), described different ways of filling and staining nerve cells in the vertebrate nervous system with cobalt salts. They also found by the axonal filling of nerves in the dorsal and ventral roots in the lumber region of frogs (Rana esculenta), that cobalt salts may move as far as 15 to 20 mm away from the site of filling, if sufficient time is allowed for the procedure. Goodman (1974), described an axonal filling method with cobalt chloride in his work on the anatomy of the ocellar interneurons in the brain of the locust, Schistocerca vaga. Strausfeld & Obermayer (1976), reported the results of experiments with cobalt chloride introduced into cut axons of the thoracic ganglia of three species of Diptera (Calliphora erythrocephala, C. vomitoria and Musca domestica). They also described the conditions in which such "transynaptic" diffusions rarely occur, including conditions where cobalt, chloride diffusion can be accelerated and enhanced so that the resulting profiles appear identical to Golgi stained element. Strausfeld & Hausen (1977), pointed out that most of our knowledge of the structure of the central nervous system is derived from classical histological techniques, which resolve single neurons that are unpredictably impregnated by the Golgi method, or its modifications. Upon these methods many of the modern procedures for structural investigation have been established. They added, however, that with rare exceptions their modern methods consistently fail to demonstrate complete populations of interneurons, constituting a morphological class, or to reveal each cell in its entirety and allow comparisons with neurons that have been resolved by the Golgi method. Strausfeld & Hausen also described a simple histological application of the cobalt technique that meets requirements for the structural analysis of neuronal

ensembles within the intact neuropile. Their method can also be used to demonstrate nerve cell constituents of tracts, in addition to the general architectural features of the neuropile. The application of cobalt chloride or cobalt acetate by passive injection, as applied to insect nervous systems, is potentially a powerful tool for resolving large populations of neurons in the central nervous system in other phyla.

Golgi studies have shown that insect neuropile contain diverse and sometimes exceedingly complex cell shapes (Strausfeld, 1976). Application of cobalt chloride or cobalt acetate by passive injection into the neuropile demonstrates that even though several thousand nerve cells may be included in a single synaptic region, their arrangement can be described as simple, and obeying distinct rules of organisation.

(D) Studies related to fine structure and histology:

Several papers deal with different aspects of the fine structure of the insect nervous system and that of related invertebrate groups but no papers were found reporting specifically on the insect suboesophageal ganglia.

a. Nerve fibres and nerve cells.

Meyer (1957), studied the structure of nerve fibres in Hirudo medicinalis. Edwards et al (1958), studied the fine structure of the nerve and neuromuscular junction in the wasp leg. Hess (1958a & b), has described the fine structure of nerve cells and fibres, and the neuroglia of the ganglion chain in Periplaneta americana. Trujillo-Cenóz (1960), reported brief observation on the fine structure of a distinctive type of nerve fibre found in the abdominal ganglia of Armadillidium vulgare (Crustacea-Isopoda). Strausfeld & Blest (1970), used Golgi-Colonnier selective silver

procedure successfully to show up neurons in the optic lobe of some Lepidoptera. They found three main types of nerve cells (classes I, II and III), the so-called perpendicular, tangential and amacrine cells respectively, with many types of neurons in each class.

b. The fine structure of synapses, and synaptic and dendritic fields.

There are several investigations dealing with the fine structure of synapses and the synaptic & dendritic fields in insects and other animal groups. Robertson (1953 & 1955), studied the ultrastructure of the synapses in crayfish nerve fibres. Robertis & Bennett (1955), described synapses in the earthworm ganglia. Hubel & Wiesel (1959 & 1962), studied the dendritic field of some neurons and the receptive fields of single neurons in the cat. Young (1960), dealt with the dendritic field of some neurons in the optic lobe of Cepholopods and the same author (1966), paid considerable attention to the correlation of the shapes of dendritic fields with the neural basis of visual discrimination in Octopus and other animals. Boycott, Gray & Guillery (1961), studied the synaptic structure and its alteration with environmental temperature in the nervous system of Lizards. Trujillo-Cenóz & Melamed (1963), studied the fine structure of the synapses occurring between the photoreceptors and the second optical neuron synapse (the monopolar cells) in the retina of Musca domestica and Calliphora vomitoria. The same authors (1964), also reported preliminary studies on the fine structure of the synaptic contacts in the visual system of the spider Lycosa erythrognatha. Colonnier (1964), described the dendritic fields of some neurones during his studies on the tangential organisation of the vertebrate visual cortex. Brown (1965), and Brown & Major (1966), reported some dendritic fields of the retinal ganglion cells of the rat and cat. Dowling & Boycott (1965 & 1966), described the

synaptic contacts of five main cell types in the primate retina, while the same authors (Boycott & Dowling, 1969), added information on the three-dimensional distribution of synapses and the spatial relationships of the different types of nerve cells. Strausfeld (1976), gave many details of the structure of the neuropile and the shapes of neurons, callaterals and synaptic and dendritic fields in his book Atlas of an Insect Brain. He added that his atlas attempts to acquaint the reader with the shapes and dispositions of the neuropile regions and single nerve cells.

The variety of species studied and the fact that they include only a few insects indicate the extent to which work on the detailed neurohistology of insects can benefit from that done on other groups.

4. Investigations on the suboesophageal ganglion:

Although much important work has been carried out describing and studying the central nervous systems in insects and other animals, it is the brain, thoracic and abdominal ganglia, optic lobes and retina which have been most intensively studied; relatively little attention has so far been paid to the suboesophageal ganglion, especially to its histological features. Hilton (1911 b), made some remarks on the motor and sensory tracts of Corydalis and has described some fibres running from the suboesophageal ganglion into the supraesophageal ganglion. He mentioned that some of these cross in the commissure and run up the opposite side while others run up the same side. He pointed out that the suboesophageal ganglion has considerable connections with the brain, but the connectives coming to it from below are larger than those connecting it above. Myers (1928), gave very brief description of the position of the suboesophageal ganglion in Melampsalta sericea; the rounded suboesophageal ganglion is connected to the first ganglionic mass by a pair of long stout connectives. Snodgrass (1935), mentioned in his

Principles of Insect Morphology (Chapter XVI), that the ventral nerve mass of the head is composed of the united ganglia of the primitive ganathal segments. He further pointed out that the histology of this composite ganglion has been very little studied by precise neurological methods, which is perhaps surprising because of the long-standing dispute as to the number of segments comprising the insect head. He added that the suboesophageal ganglion innervates the mandibles, the hypopharynx, the maxillae, the labium, the salivary ducts and at least some of the muscles of the neck. The longitudinal nerve tracts that enter or traverse the suboesophageal ganglion are of great importance, since they contain the connective fibres between the sensory centres of the head and the motor centres of the body, but Snodgrass remarks how little detailed information we have concerning them. The suboesophageal ganglion is also an inhibitory centre of the nervous system, since its removal results in the same somatic reflexes being more readily excited by external stimuli. Hilton (1937), mentioned that the suboesophageal ganglion of Gryllus assimilis has only one pair of nerves to the neck muscles. Nesbitt (1941), has also described the suboesophageal ganglion of some orthopteroid insects, i.e. Conocephalus, Ceuthophilus, Romalea, Dissosteira, Diapheromera, Mantis, Anisolabis and Gryllus. Since the suboesophageal ganglion is composed of the united ganglia of the three primitive gnathal segments, it follows that, though fused, it must continue to supply the three segments bearing the mouth parts with the sensory and motor nerves. This being the case, there are three large pairs of nerves, the anterior pair to the mandibles and their musculature, the middle to the maxillae, and the posterior to the labium. Other smaller nerves are present, sometimes arising separately, sometimes in conjunction with one of the larger. These supply the hypopharynx, the salivary ducts and the muscles of the neck. Sanchez (1941), gave another brief description

of the suboesophageal ganglion in Apis. Ehnbom (1948), studied the central nervous systems of various orders of insects and described the suboesophageal ganglia in each case. He mentioned that externally the suboesophageal ganglion is completely homogenous and its morphology varies from a long and narrow shape to spheroidal and egg-shaped. Histologically he found that ganglion cells form a broad zone ventrally and laterally which decreases dorsally, and on the dorsal side they are represented only by some scattered cells which are situated along the median furrow that divides the neuropile into a right and a left lobe. Ehnbom considered that the best histological survey of the suboesophageal ganglion is obtained from sagittal sections, where the original division into three pairs of ganglia: (mandibular, maxillary and labial) is very clear. He also found that the whole length of the suboesophageal ganglion is intersected by three pairs of horizontal tracts which pass from the cerebrum via the circum-oesophageal connectives to enter the ventral nerve cord. These tracts come from the protocerebrum, deutocerebrum and tritocerebrum. The suboesophageal ganglion is also traversed by a number of nerve-bundles that pass in all directions and from its ventral surface there issue three pairs of nerves which are at first undivided: the nervus mandibularis, nervus maxillaris and the nervus labialis. Power (1950), has very briefly described the suboesophageal ganglion in Drosophila and Albrecht (1953), that of Locusta migratoria. He pointed out that the suboesophageal ganglion is generally held to be derived by the fusion of separate maxillary, mandibular and labial ganglia; it is situated above the mouth parts and beneath the tentorium in the midline of the head. It is elongate oval in shape, flattened dorsally and convex ventrally, and connected to the brain by two long stout, circum-oesophageal connectives. He found that this ganglion gives off eight pairs of nerves, leading to: the mandibular muscles, hypopharynx, maxillae, labium and salivary gland with

another three pairs extending to the muscles of the neck. Nerves leaving the suboesophageal ganglion supply structures such as the labium and maxillae which contain numerous muscles and also numerous sensory receptors (cf. Kubra, 1978), and must presumably contain both sensory and motor tracts. Henry (1958), described the nerves given off from the suboesophageal ganglion in Melanoplus sp. (Orthoptera), and in Coleoptera. Henry mentioned that the cervical muscles are innervated from a nerve arising from the suboesophageal ganglion. Schmitt (1959), reported that in Dissosteira carolina the posterior portion of the suboesophageal ganglion may usually be seen projecting slightly from beneath the tentorial bridge; two fine nerves (the cervical nerves), leave it here and pass dorsally. He added that the paired interganglionic connectives from the suboesophageal ganglion pass beneath the crossed prosternal muscles of the cervical sclerites to the prothoracic ganglion. Imms (1957), and Richards & Davies (1977), gave a very brief description of the suboesophageal ganglion in their General Textbook of Entomology, and Imm's General Textbook of Entomology, agreeing that it is formed by the fusion of the ganglia of the mandibular, maxillary and labial segments, and gives off paired nerves supplying their respective appendages. Guthrie (1961), in his investigation of the anatomy of the nervous system in Gerris stated that the suboesophageal centre is formed by the intimate fusion of three ganathal neuromeres, mandibular, maxillary and labial, early in development with the result that the components and difficult to identify in the adult. He also found that the suboesophageal neuromeres are so closely integrated in Gerris that only in sections passing through certain parts of the central nervous system can they be distingiushed histologically. He described the size of each centre, and some fibre tracts which enter the ventral neuropile. He also mentioned some long-fibred elements in the dorsal longitudinal tract which

have terminals in the maxillary, mandibular and labial centres and other elements which connect the dorsal region with the protocerebral lobes. A few complete intersegmental elements, with fibres in the prothoracic and the gnathal centres, could also be traced. The suboesophageal ganglion may also connect with the corpora allata and supply the labral nerve (Willey, 1961 in Periplaneta). Wigglesworth (1972), reviews studies on the functions of the suboesophageal ganglion in his Principles of Insect Physiology. Vasvary (1966) mentioned that an early publication by Dufour (1833) describing the ventral nerve cord in Cicada orni, did not recognise the suboesophageal ganglion which, according to Myers (1928), may have been mistaken for the brain. Vasvary (op. cit.) described the suboesophageal ganglion of another Cicada, Tibicen chloromera; he recorded the position of five pairs of nerves from its lateroventral surface, and three pairs from the ventral area of the ganglion. Although dealing with a Hemipteran, Vasvary's description is similar to that of Albrecht (1953) on Locusta migratoria. Ali (1974), has described the suboesophageal ganglion in larvae and adults of Pieris brassicae; she also illustrated the neuron clusters, fibre tracts and glomerular bodies of the ganglion in the developmental stages of the same insect.

III MATERIAL AND METHODS

1. Insects used:

Live specimen of Acheta domesticus (Linn.) were obtained from a biological supplier (Xenopus Ltd., Holmesdale Nursery, Midstreet, South Nutfield, Redhill, England). From these insects a culture was established.

2. Rearing techniques:

Ghouri & McFarlane (1958), described a rearing technique using glass jars, each containing a group of ten to twelve adults or twenty to twenty five nymphs. This technique was also followed by Kubra (1978). The first two authors kept the insects at 28°C and 40% relative humidity, and each culture jar was supplied with a dish of moistened sand.

In the present work a mass culture rearing technique was used, slightly different from that described by Ghouri & McFarlane (1958). Mass rearing colonies (150 to 200 in early nymphal stages, or 100 of the late instars and adults) were kept in plastic containers, each measuring 35 X 25 centimetres and 20 centimetres in height. The cages were maintained in the laboratory at a normal temperature of 22 to 28°C and humidity was not specially controlled. Each culture cage was supplied with a petri dish 7 centimetres in diameter and $1\frac{1}{2}$ centimetres high. In each dish was a layer of moistened cotton wool and an inverted glass tube filled with water, stoppered with cotton wool and surrounded with cotton wool to keep it in a vertical position. This provided a sufficient supply of water and was replaced every two days. The diet used was that described by Brown (1973), and Kubra (1978); it consisted of 75% wheat germ (Bemax), 2% dried skimmed milk, 5% yeast. In the

mass rearing technique this diet was satisfactory, with some slices of fresh apple to increase the humidity; some fermentation of the fruit may occur, but this is not a disadvantage. The insects were provided with sufficient food every two days. Pieces of folded newspaper were provided to increase the available space for movement by the nymphs and for movement and mating of the adults. The adult cages containing 50 females and 50 males were provided with another circular glass dish 14 centimetres in diameter and 2 centimetres high. A thick layer of moistened sand was placed in the dish, and sprayed with water every day to keep the sand moist. This provided a suitable site for the female to lay eggs. Sometimes if the temperature reached 28°C nymphal and adult cages were provided with a third small dish containing some stones and filled with water. The top of each cage was covered with muslin. The insects were transferred to fresh containers every two weeks. After three weeks oviposition the sand dishes were removed to another container and kept moistened until hatching.

3. Dissection of the ganglion:

Adults were anaesthetically killed for dissection and histological treatment by immersing them in the fixative solution or 70% alcohol or by abdominal injection with the fixative solution. The suboesophageal ganglion was then dissected out by immersing them in 70% alcohol; the head, thorax and abdomen were opened and the ganglion removed under a stereoscopic dissecting microscope.

4. Techniques used:

To analyse the cellular composition of the ganglion, excised material was fixed in Bouin's solution for subsequent staining in Mallory's phosphotungistic haematoxylin (Drury &

Wallington, 1967), or in Mallory's connective tissue stain (Hughesdon's modification; R.G. Davies, pers. comm.). The material was embedded in paraffin wax and serial transverse longitudinal (parasaggital and sagittal) and frontal (horizontal) sections were cut at 5 - 7 µm, a thickness which was most satisfactory for the present purposes. Sections were mounted on slides and stained after dewaxing them and bringing them to distilled water. Material fixed and stained by these two methods was mounted in Canada Balsam or D. P. X.

To study the neuropile, fibre tracts and axonal pathways, Wigglesworth's (1957 & 1959) method of osmium tetroxide fixation, followed by ethyl gallate treatment, was used. The material was fixed at 0 - 4C° for 2 hours in 1% osmium tetroxide buffered at pH 7.2 with cacodylate buffer. This was followed by saturated picric acid in water for four hours or overnight to complete the hardening of the tissues. The material was then transferred to a saturated solution of ethyl gallate in 0.25% aqueous cresol and stored in this for 3 - 24 hours. The sections were then embedded in paraffin wax and cut at 5 - 7 µm (normally 6 µm was most satisfactory). Material fixed in osmium tetroxide and stained by ethyl gallate was mounted in D. P. X. only. This procedure is a simplified version of Wigglesworth's original method, which had been found satisfactory by Ali (1973 & 1974).

5. Results:

(A) With Mallory's phosphotungistic haematoxylin:

All the structures appear in various shades of dark grey and greyish-lilac; nuclei are dark blue; the depth of the colour could usually be controlled easily by varying the period of time that the sections were left in 95% ethanol. The nerve fibres and axonal

tracts show less distinctly than with Mallory's connective tissue stain.

(B) Mallory's connective tissue stain (Hughesdon's modification):

The neurilemma stains bright blue; the nerve fibres and tracts show more distinctly blue and dark blue; neurons and nuclei are red and blue; neuropile light blue and red; the fibre tracts are sometimes easy to identify by their blue or dark blue colour.

(C) Osmium tetroxide technique:

All structures appear in various shades of grey, dark grey and black; the nuclei are black; neurons are greyish, grey and black; cell-boundaries are clear and nerve fibres show more distinctly than in other preparations, with the tracts usually very clear.

All photograph negatives in this part have been taken with the aid of the light photmicroscope (Carl Zeiss) at magnifications between X 50 and X 200. The drawings were made on co-ordinate paper with the aid of a square eye piece, using standard light-microscopy.

6. Other techniques:

To study the finer details of the histological structure of the suboesophageal ganglion other techniques were employed; these will be discussed in detail in the appropriate section elsewhere (p. 155).

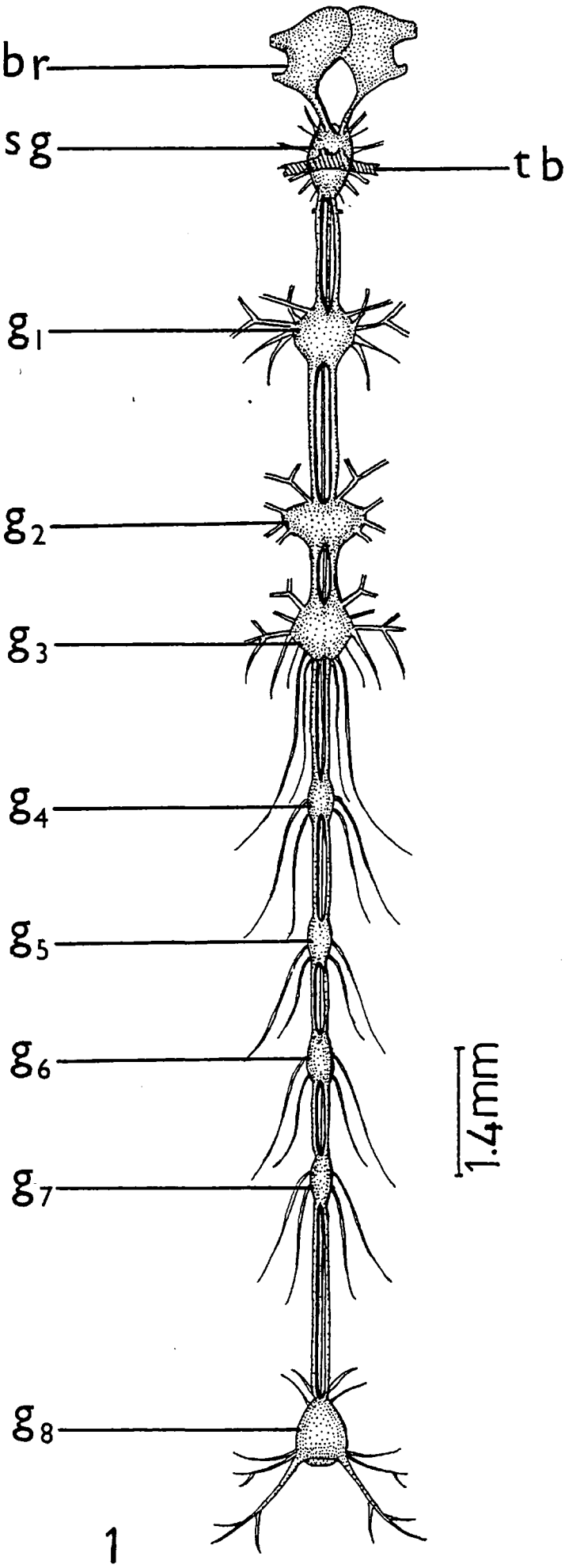
IV GENERAL DESCRIPTION OF THE VENTRAL NERVE CORD IN *Acheta domesticus* (Linn.)

1. Description of the suboesophageal ganglion:

The middle portion of the suboesophageal ganglion is covered by the tentorial bridge (tb, Fig. 1). It is situated above the mouth parts, below the beginning of the midgut and beneath the tentorium. It is oval in shape, slightly flattened dorsally, and convex ventrally. A pair of stout circum-oesophageal connectives (cc, Figs. 2 & 3), connect the suboesophageal ganglion to the brain, passing around and over the oesophagus. Another pair, the first thoracic connectives (thc, Figs. 2 & 3), lie closer together, one on each side of the median longitudinal nerve (mln), and connect the suboesophageal ganglion to the first thoracic ganglion.

The suboesophageal ganglion gives off seven pairs of nerves, as shown in ventral view (Fig. 2). Four pairs of nerves arise from the lateroventral surface and one pair from the anteroventral area. The first pair of nerves, of moderate size (mdn), arises from the anterior lateroventral surface of the ganglion and innervates the mandibular muscles. A second pair of nerves (hpn), arises from the anteroventral area and innervates the hypopharynx; these are very small and more slender than the first pair. The third pair of large nerves (mxn), supplying the maxillae, arises from the lateroventral surface of the ganglion. The fourth pair, also large (lbn), supplies the labium, and arises laterally and a little behind the middle of the ganglion. The fifth small pair of nerves (sgln) arises from the posterior lateroventral surface of the ganglion and innervates the salivary glands (labial glands).

Fig. 1. Dorsal view of the ventral nerve-cord and the Brain in the adult of Acheta domesticus.



1

A dorsal view of the suboesophageal ganglion is shown in Fig. 3. Two pairs of nerves (n_1, n_2), very small and slender, arise from that side and supply the cervical muscles (dorsal cervical muscle). The first pair (n_1) comes off from the anterodorsal surface, while the second pair (n_2) arises from the posterior laterodorsal surface.

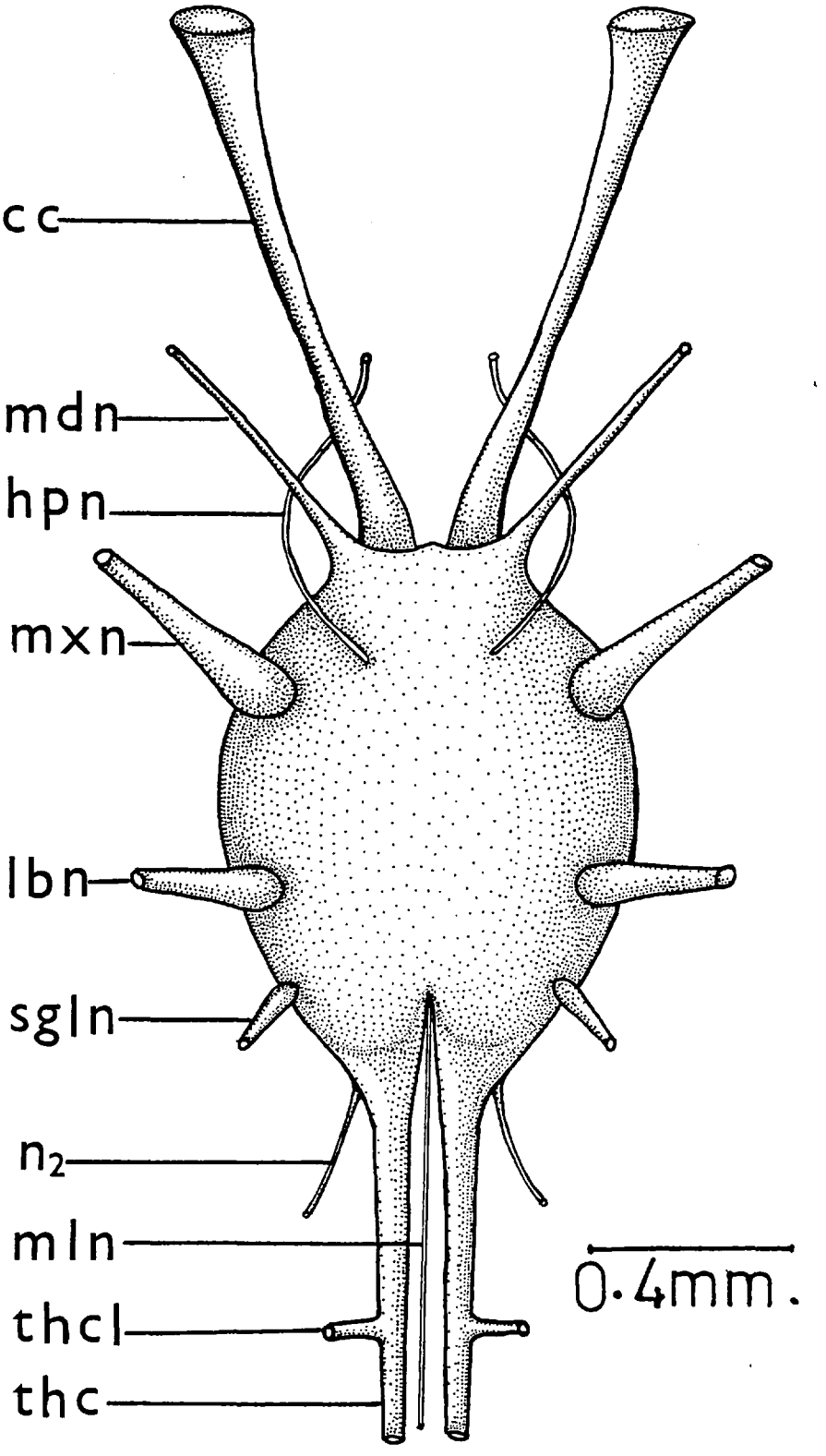
A pair of large nerves (th cl), originates laterally from each interganglionic connective and each joins another nerve which arises from the prothoracic ganglion.

2. The thoracic and abdominal ganglia:

The three thoracic ganglia (g_1 - g_3, Fig. 1), are each situated in the body segment which they innervate, as shown in the dorsal view of the ventral nerve-cord and the brain of Fig. 1. The metathoracic ganglion and the first two abdominal ganglia of Acheta domesticus are fused together.

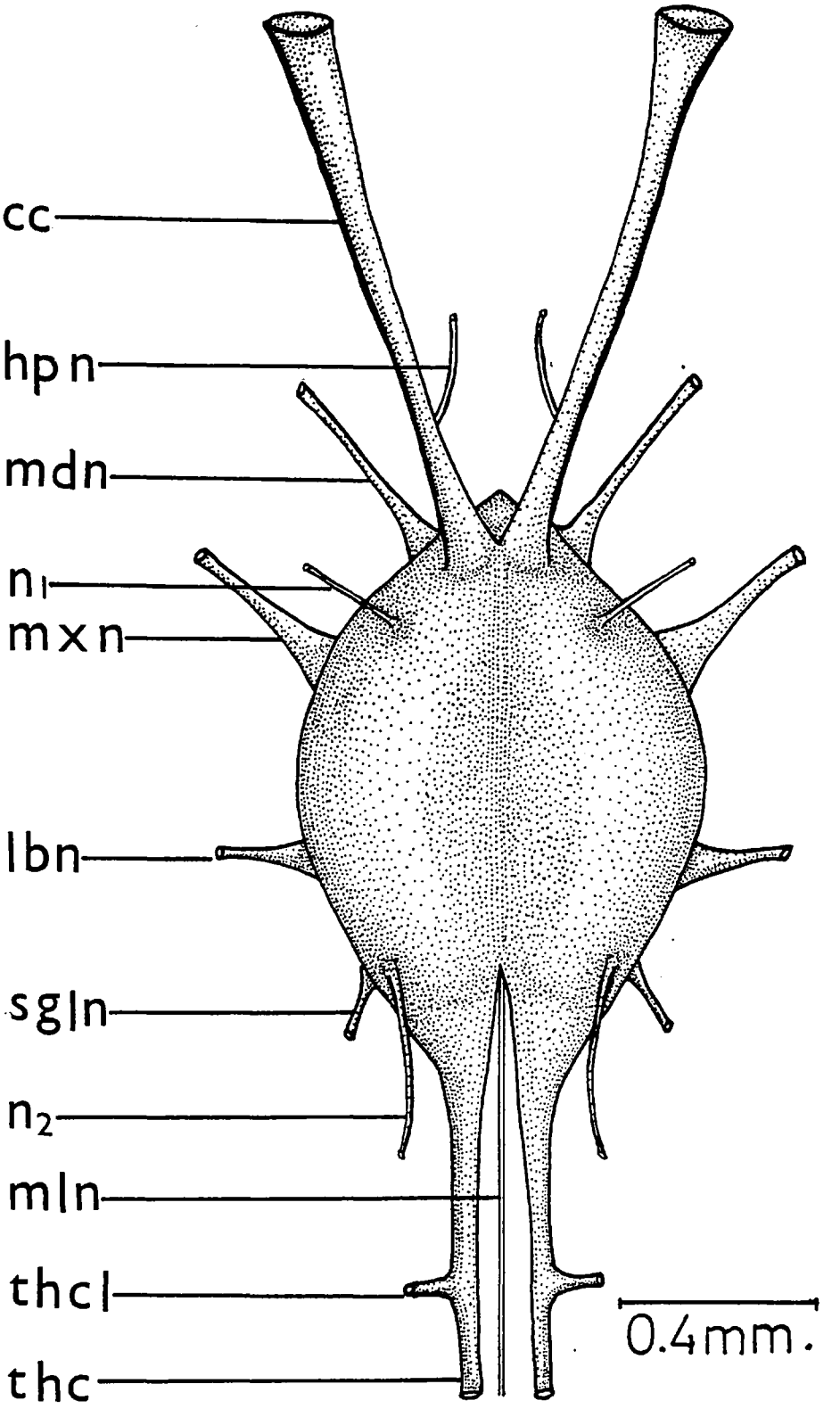
Acheta domesticus has five abdominal ganglia (g_4 - g_8) situated respectively in abdominal segments two, three, four, five and eight (Fig. 1). The first two abdominal ganglia are fused with the metathoracic ganglion, while g_4 to g_7 supply abdominal segments three, four, five and six; the last ganglion g_8 supplies the remaining abdominal segments (i.e. seven, eight, nine, ten and eleven).

Fig. 2. Suboesophageal ganglion of the adult of Acheta domesticus (ventral view).



2

Fig. 3. Suboesophageal ganglion of the adult Acheta domesticus (dorsal view).



3

The suboesophageal ganglion and the ventral nerve cords are described very briefly in some insects, e.g. in Melampsalta sericea by Myers (1928), and in Apis by Sánchez (1941). The suboesophageal ganglion of some Trichoptera has been investigated by Ehnbom (1948), and is also metnioned by Power (1950), in Drosophila, but unfortunately no details are given.

Myers (1928), described the brain and the ventral nerve cord of Melampsalta sericea, where the round suboesophageal ganglion is connected to the first ganglionic mass by a pair of long stout, well-separated interganglionic connectives; the first ganglionic mass lies largely in the prothorax. Hilton (1937), figures the ventral nerve cords of some Orthoptera and mentions that Mantis religiosa L. has five ventral ganglia, though Nesbitt (1941), stated that all the specimens of Mantis religiosa L. which Hilton dissected had only four abdominal ganglia. Nesbitt (1941), also stated in his descriptions of the ventral ganglionic chain in Gryllus assimilis Fabr., that the suboesophageal ganglion has only one pair of nerves to the neck muscles, which leaves from the extreme posterior part of the ganglion. He added that the first two abdominal ganglia are fused with the metathoracic ganglion and five free abdominal ganglia are located respectively in segments two, three, four, five and eight. Albrecht (1953), in the central and peripheral nervous system of Locusta migratoria, described the suboesophageal ganglion and the ventral nerve cord. Henry (1958), described the cervical muscles and their nerves in Melanoplus sp. She stated that the dorsal cervical muscle bundles are supplied by a nerve arising near the base of the nerve to the salivary gland. In Harpalus sp., the muscle bundles of the neck are innervated by a nerve arising from the suboesophageal ganglion in very close proximity to the salivary gland nerve. Henry also

found that in Agulla adnixa (Raphidiidae), there arises from the ventral nerve cord which connects the suboesophageal and the prothoracic ganglia a pair of nerves which pass to the salivary glands in the prothorax. Schmitt (1959), studied the cervicothoracic nervous system of a grasshopper, Dissosteira carolina, and found that the suboesophageal ganglion may usually be seen from beneath the tentorial bridge; two fine nerves, the cervical nerves, leave the suboesophageal ganglion posterolaterally and pass dorsally. Vasvary (1966), studied the suboesophageal ganglion in the male annual cicada Tibicen chloromera (Walker), where the ventral nerve cord consists of a suboesophageal ganglion, prothoracic ganglion, and a thoracic or abdominal ganglionic mass. He found no ganglia in any of the abdominal segments, while the prothoracic ganglion supplies nerves to some of the muscles of the cervical area and prothorax. Guthrie & Tindall (1968), gave a general account of the nervous system in Periplaneta americana. They described the brain, the suboesophageal ganglion with all the nerves given off from this ganglion; they also described the thoracic and abdominal nervous system in detail.

V HISTOLOGICAL COMPOSITION (GENERAL HISTOLOGY)

1. Introduction

The object of the present work is to give a description and analysis of the cellular composition of the suboesophageal ganglion, including the perilemma cells and other glial cells, the various types of neurons (ganglion cells), and the fibre tracts, glomerular bodies and axonal pathways of the neuropile.

There are many papers dealing with the structure of the central nervous system of insects, though relatively few have previously dealt with the histological structure and the cellular composition of the suboesophageal ganglion. Smith & Treherne (1963), and Seabrook (1968), described the cell bodies of the ganglionic cortex and the nerve sheath (neurilemma and perineurium). Wigglesworth (1959), and Ali (1973) have described in detail the perilemma and other glial cells. For this study and analysis of the cellular composition, preparations stained in Mallory's phosphotungstic haematoxylin and Mallory's connective tissue stain were employed. For other descriptions of the neuropile and fibre tracts, in various species, see Trujillo-Cenóz (1960), Fielden (1963) and Huddart (1971). Ali (1974), recorded 30 fibre tracts in the suboesophageal ganglion of the adult of Pieris brassicae. Fibre tracts are also described by Hilton (1911 b), Pipa et al (1959), Zawarzin (1924 a & b), Mill (1964) and Gregory (1974). Zawarzin (1924 a) and Bierbrodt (1942) described some of the glomerular areas in Aeshna and Panorpa larvae respectively. Ali (1967 & 1974), described a ventral and dorsal glomerular body in the thoracic ganglia of the adult of Pieris brassicae and the glomerular body in the suboesophageal ganglion of the fifth instar larva in the same insects. Descriptions of

axonal pathways are given by, among others, Hilton (1911 a), Zawarzin (1924 a & b), Ehnbom (1948), Guthrie (1961) and Ali (1974).

In the present work, for studying the neuropile fibre tracts and axonal pathways, Wigglesworth's methods of osmium tetroxide fixation, followed by ethyl gallate treatment were helpful.

2. Analysis of cellular composition:

The cells comprising the suboesophageal ganglion are of four main types. Three of these are glial cells corresponding to the types I, II and IV of Wigglesworth (1959) and Ali (1973), while the fourth type of cells are neurons. These cells may be distinguished by their position, shape and size of nuclei and cell bodies. Heywood (1965) mentioned briefly some characteristics of these cells in Pieris brassicae during metamorphosis and Ali (1973), has given accounts of characteristics of similar cells in the third abdominal ganglion in larval stages and the adult of Pieris brassicae.

Description of the cells. The cells are as follows:-

(A) Perilemma cells (Ganglion sheath):

Two layers constitute the perilemma, the layer of perineural cells (type I glial cells), surrounded by a non-cellular layer: the neural lamella or neurilemma. Among others, Smith & Treherne (1963) and Seabrook (1968), have described the neuropile, covered by a cortex of neurocytes, and enclosed in the nerve sheath, comprising an outer non-cellular neural lamella secreted by the underlying perineurium.

a. The neural lamella (neurilemma).

The neural lamella (nl) (Figs. 4, 5 and 16), is thick and more distinct in Mallory's stain preparations, where it appears blue in colour and measures between 1.4 μm and 4.2 μm in thickness (see Figs. 9, 10, 11, 17 and 18). Wigglesworth (1959), has pointed out that in Rhodnius larvae (fifth or last larval stage), the fibrous sheath contains collagen fibrils and often measures as much as 0.75 μm in thickness. Ali (1973), says that in the central

nervous system of Pieris brassicae, the neural lamella is not very distinct in the early larval stages but becomes so in the later instars, though it is not more than 2 μm thick.

b. The perineurium (Type I glial cells).

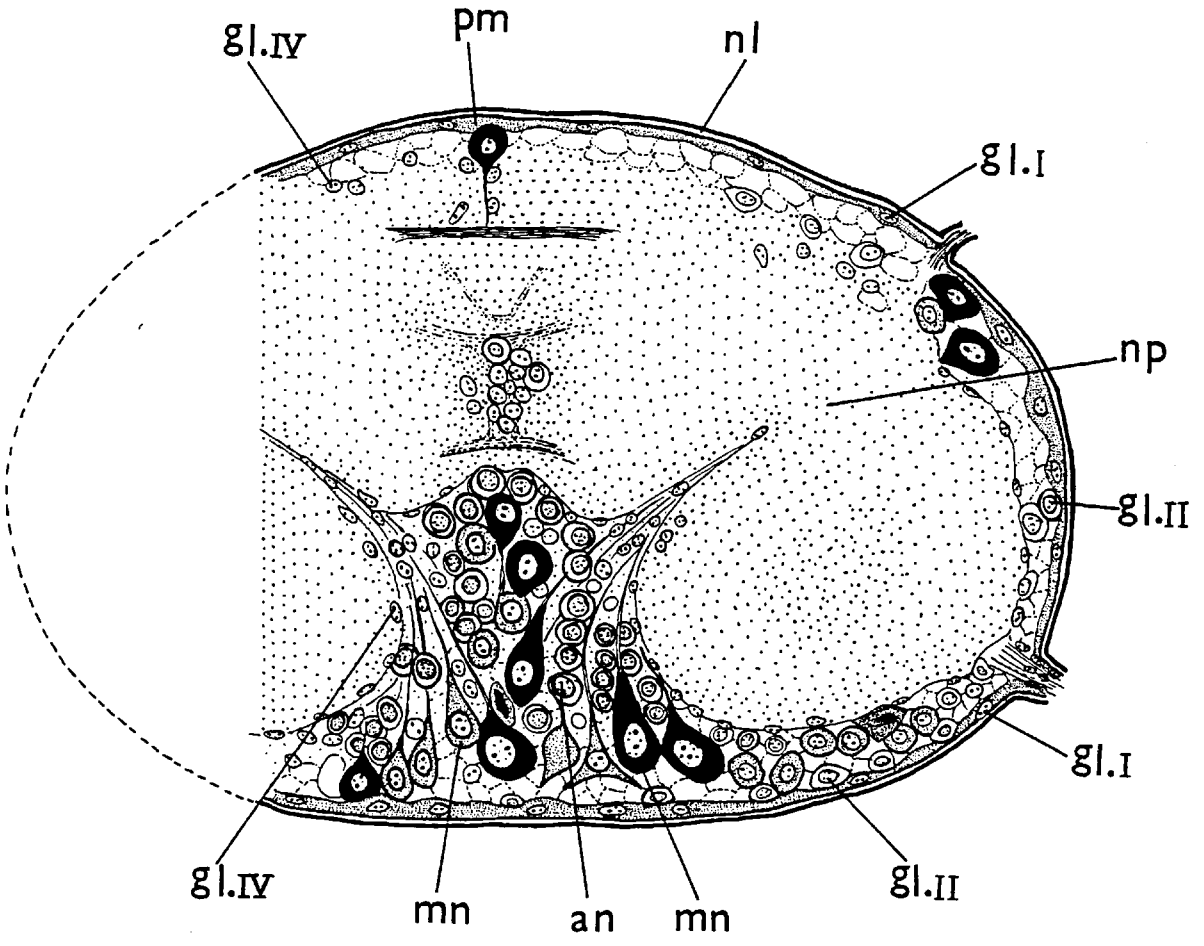
Type I glial cells (glI) form the perineurium (pm). These are found around the main body of the ganglion (Figs. 4, 5 and 16) and are small, with small nuclei. Ali (1973), reports that the nuclei are small, with irregularly scattered chromatin granules and no distinctly recognisable nucleoli. In Acheta these cells are more abundant near the interganglionic connectives and also near the junction of the connectives with the ganglia; sometimes they do not appear as a regular layer around the ganglion;and at other points the perineurium is several cells thick (see Figs. 10, 11, 12 and 14). This layer is most readily seen in Mallory's stain preparations and phosphotungstic haematoxylin preparations, and not very well defined in slides stained with osmium-ethyl gallate (Figs. 11 & 18).

(B) Type II glial cells:

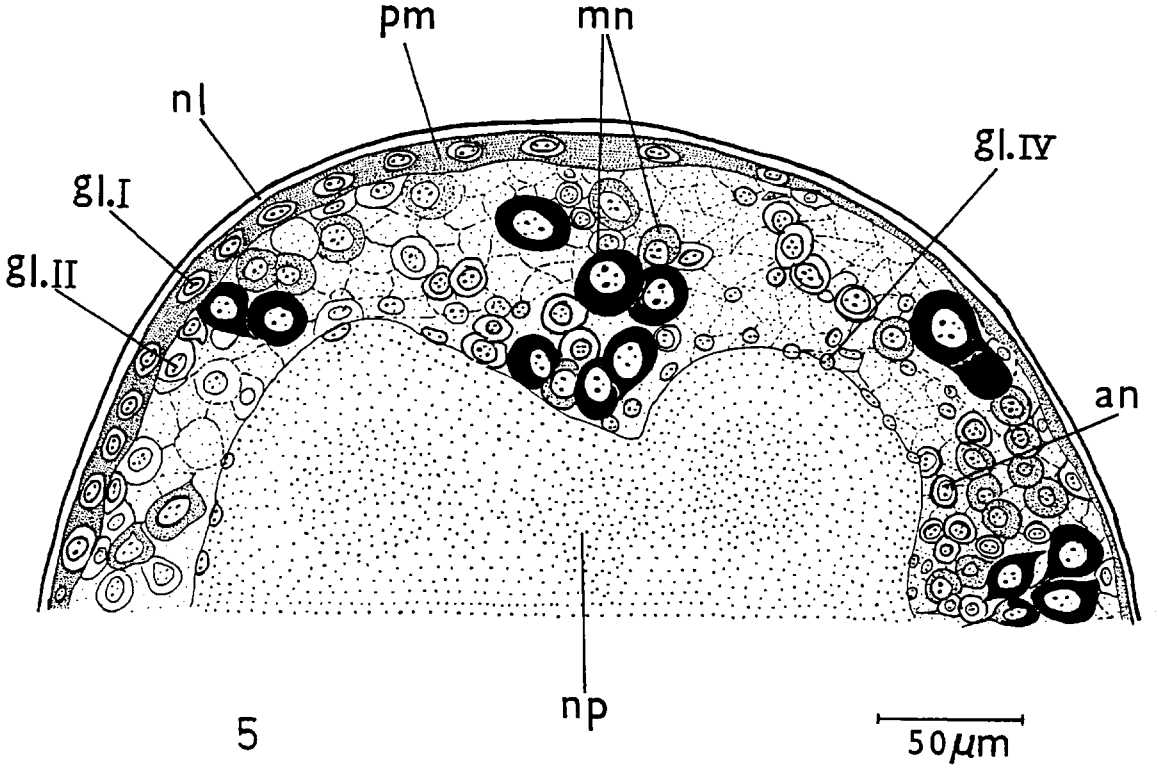
Type II glial cells (glII) are similar to those reported by Wigglesworth (1959) and Ali (1973). They are simply scattered among the neurons that lie between the perineurium and the neuropile (Figs. 4, 5 and 16). The nuclear chromatin is scattered beneath the nuclear membrane. The cells are medium in size and their nuclei are spheroidal or ovoid in shape (see Figs. 10, 12 and 13).

Fig. 4. Transverse section through suboesophageal ganglion, showing neurilemma, perineurium (glial cells) and neurons.

Fig. 5. Part of parasagittal section through suboesophageal ganglion, showing three types of glial cells, some motor and association neurons.


gl.IV
pm
nl
gl.I
np
gl.II
gl.I
gl.IV
mn
an
mn
gl.II
100 µm

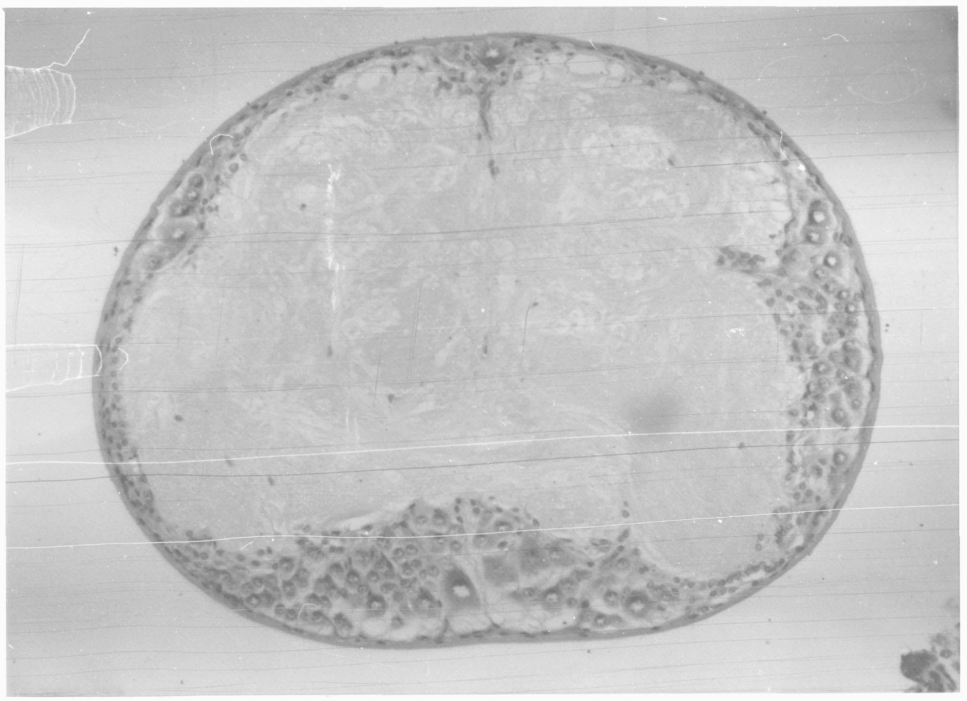
4


pm
mn
nl
gl.IV
gl.I
gl.II
an
np
50 µm

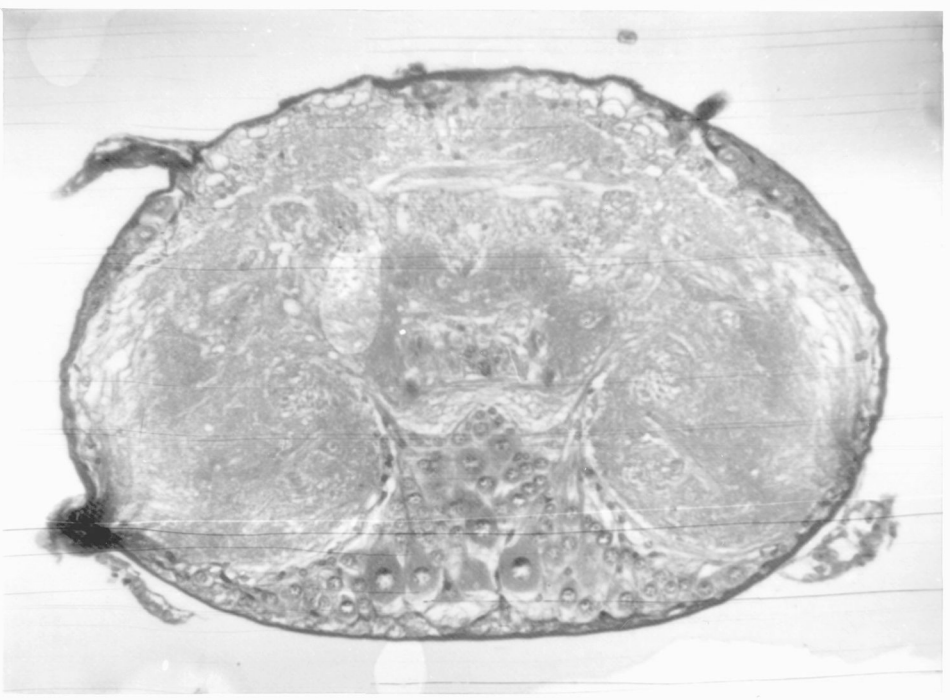
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Fig. 6. Light-micrograph of transverse sections through suboesophageal ganglion showing neurilemma, perineurium and neurons, stained by Mallory's phosphotungstic haematoxylin (X 200).

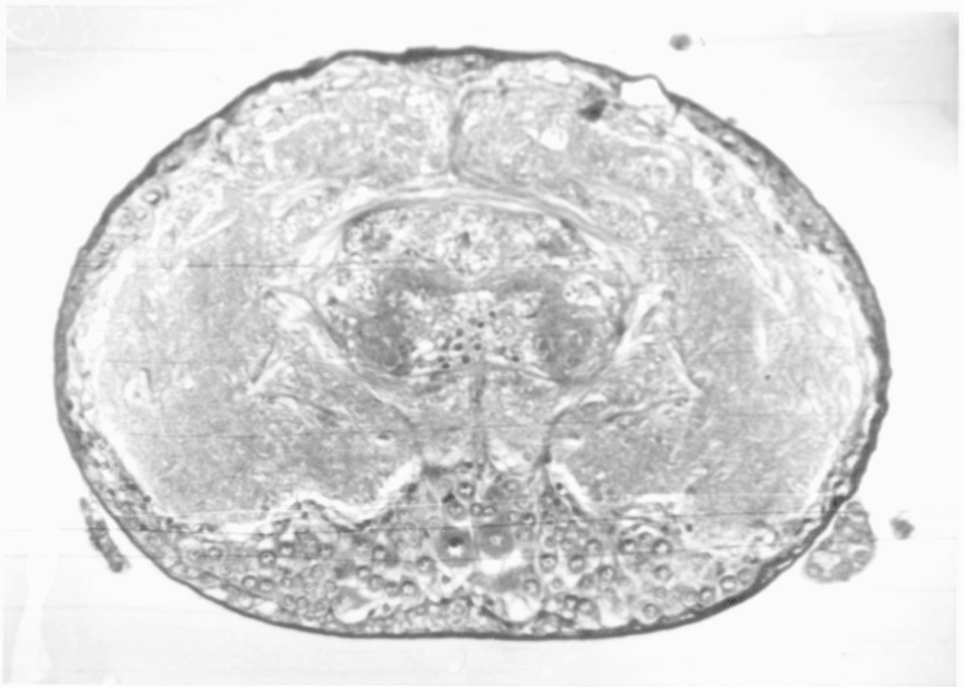
Figs. 7, 8 & 9. Light-micrographs of transverse sections through the suboesophageal ganglion showing some structures of the neurilemma, perineurium and some neurons and fibre tracts, stained by Mallory's connective tissue stain (Hughesdon's modification) (X 200).



6



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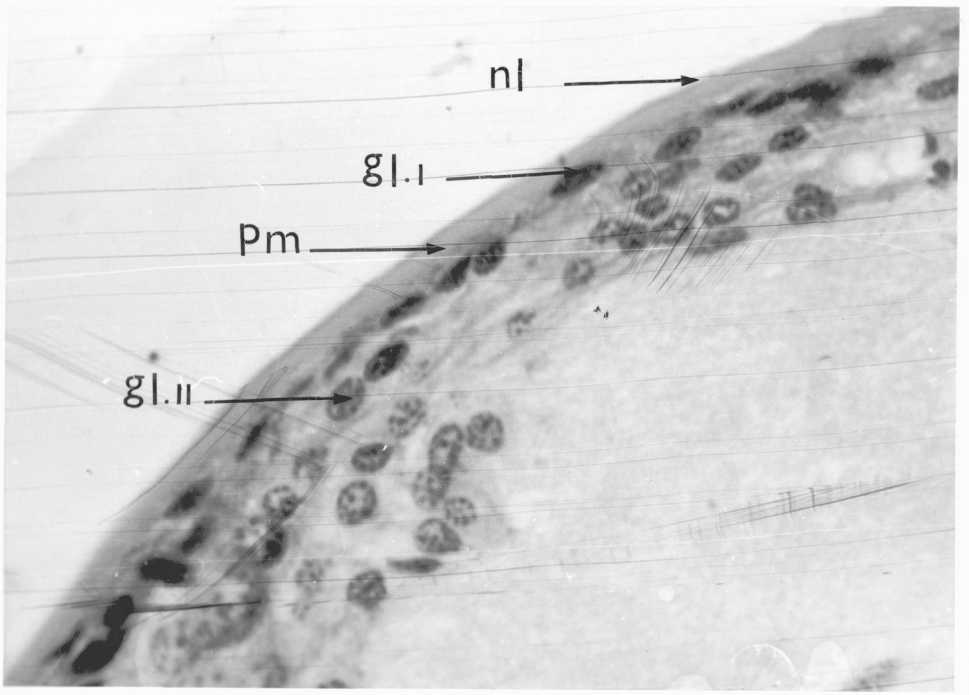


9

Fig. 10. Light-micrograph showing types of glial cells and neurilemma, type I glial cells (perineurium), and type II glial cells (X 800).

Here and elsewhere , the abbreviations used on the figures are listed on p. 298 .

Fig. 11. Light-micrograph of parasagittal section showing the neurilemma, perineurium (type I glial cells) and some neurons, stained by osmium tetroxide stain (X 320).

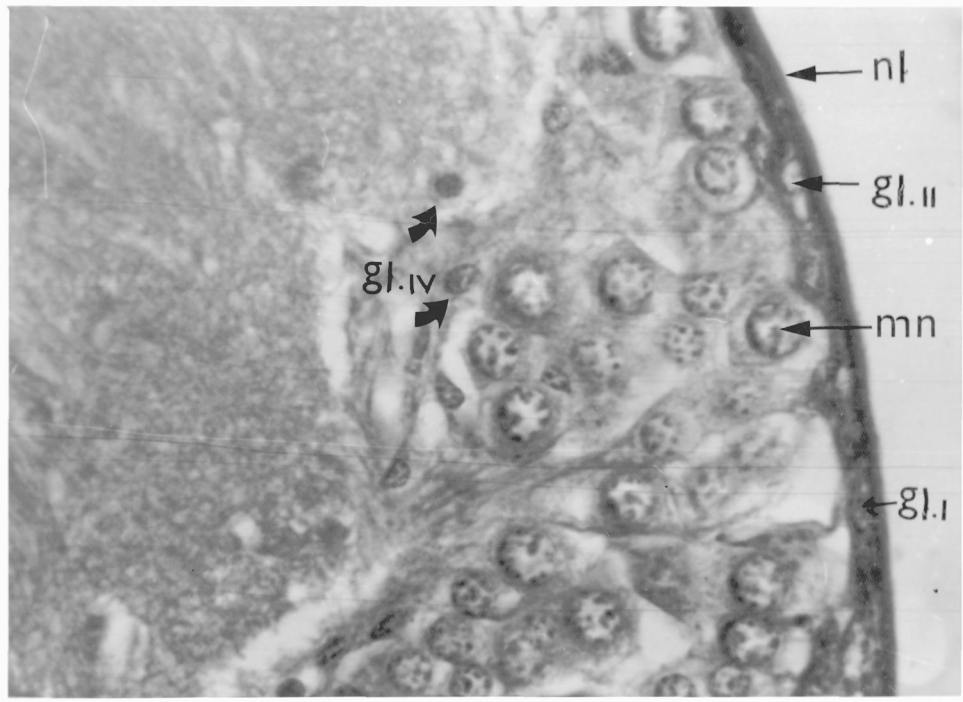


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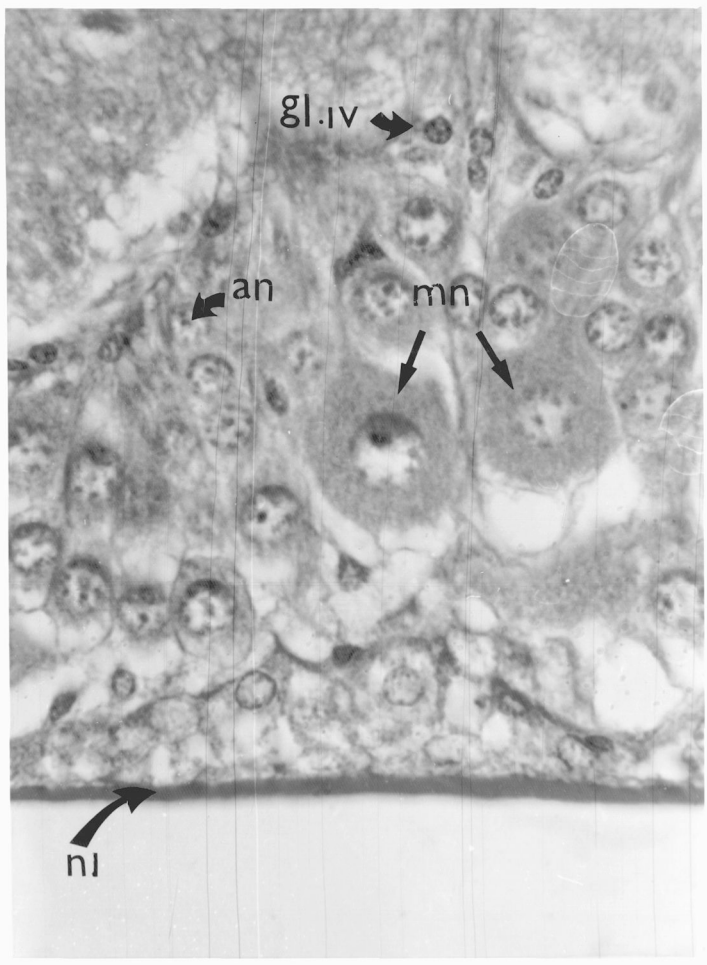


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Figs. 12 & 13. Light-micrograph showing three types of glial cells, neurilemma and some neurons in sections stained by Mallory's connective tissue stain (X 800).

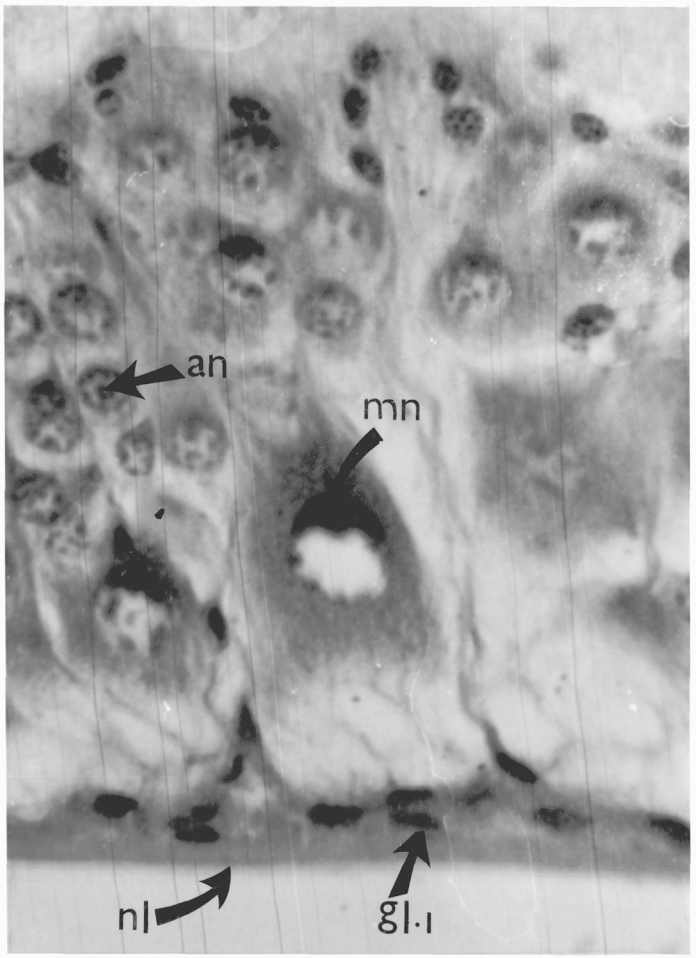


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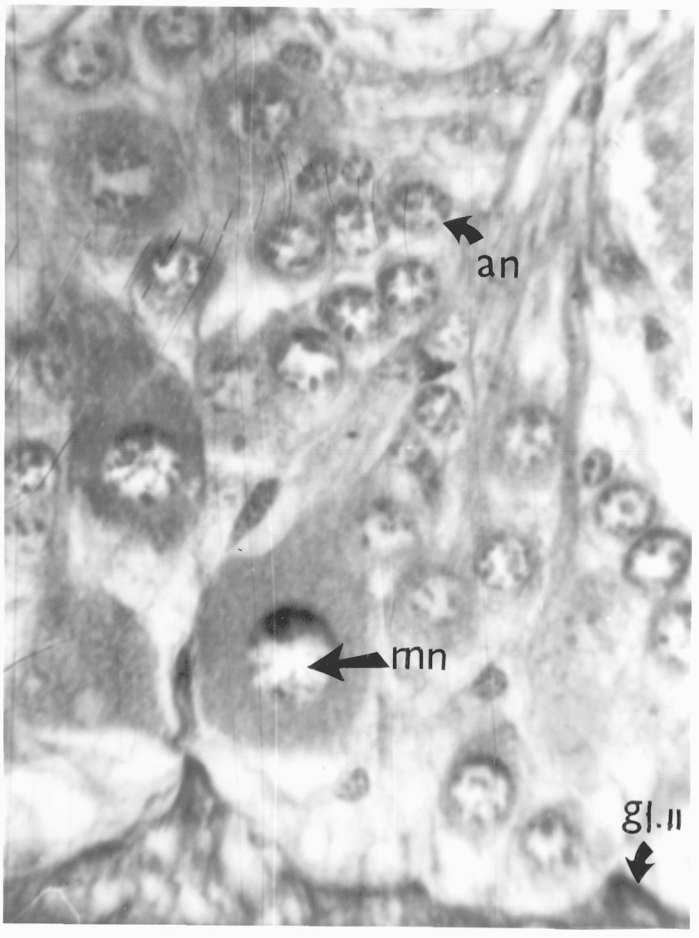


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Figs. 14 & 15. Light-micrograph showing types of glial cells, some neurons (motor, association neurons) and neurilemma in sections stained by Mallory's connective tissue stain (X 800).



14



15

(C) Type IV glial cells:

These correspond to the type IV glial cells of Wigglesworth (1959) and Ali (1973). They surround the neuropile, separating it from the neurons (glIV; Figs. 4, 5, 12 and 13), and they may also surround some of the glomerular bodies (Figs. 16, 17, 18, 19 and 20). Individual type IV cells can sometimes be seen scattered in the neuropile (np) where they may occur in great numbers, especially when associated with the axons running through the inter-ganglionic connectives, and with those peripheral axons which run through or around the neuropile. These cells are small and their nuclei are elongate or ovoidal, with scattered chromatin.

(D) The various types of neurons (ganglion cells):

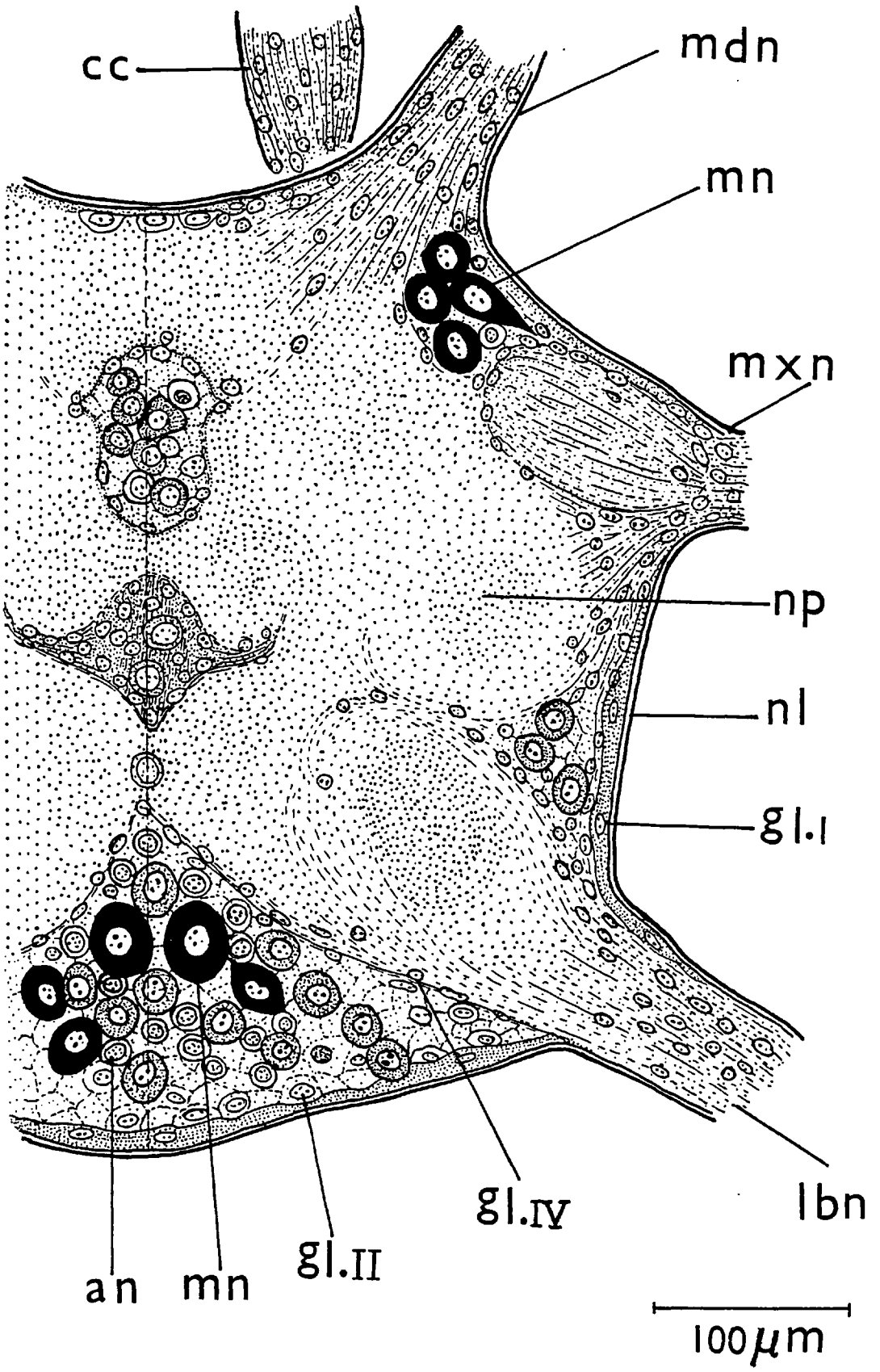
Neurons are the fourth type of cells described here and usually lie in the ganglionic cortex, i.e. between the neuropile and the perineurium: they can be divided readily into three different categories on the basis of their size and correspond partly to those described by Ali (1973), Pyle (1941), Johansson (1957), Wigglesworth (1959), Fielden (1963) and Heywood (1965), who all divided the ganglion cells into association and motor neurons, depending on their size. Zawarzin (1924), has described the origins of the motor and sensory fibres in Aeshna nymphs. Chiarodo (1963, 1968), has described three basic cell types in the cortex of the thoracic ganglion of the blowfly Sarcophaga bullata, distinguishing them by their size and cytological detail. Cohen & Jacklet (1967) and Young (1969), have studied the distribution of large and small motor neurons and gave a cell map of the large motor neuron of the mesothoracic ganglion of Periplaneta americana. Huddart (1971), has divided the neurons of the pro-thoracic ganglion of Carausius morosus into three groups, the

giant neurons (34 to 38 μm in diameter) and two smaller size groups (4 to 8 μm and 14 to 18 μm). Gregory (1974), has summarised the general structure of the mesothoracic ganglion of adult males of Periplaneta americana (L.), and also studied the neuron cell body groups. In Acheta the largest motor neuron (mn) cell bodies are around 30 - 40 μm in diameter; their length sometimes reaches 60 μm while their width may attain 40 μm. The medium sized cells are between 20 - 30 μm, and the smallest ones (an; the association neurons), are around 10 - 20 μm. The nuclei of the large and medium-sized motor neurons are similar except in size; they usually have two distinct nucleoli (Ali, 1973), with darkly staining chromatin, but sometimes they lack recognisable nucleoli and show only scattered chromatin. The boundaries of the motor neuron cells are very distinct (Figs. 7, 12, 13, 14, 16, 19 and 20), and they have dark-staining dense cytoplasm, especially in Mallory's stain and osmium tetroxide/ethyl-gallate preparations, where their axons can be seen distinctly (see Figs. 11, 15, 17, 18, 19 and 20).

The internuncial neurons (association neurons, inter-neurons) and their nuclei are very different and smaller than those of the motor neurons. Normally the nuclei of the association neurons are 7 - 13 μm in diameter and are spheroidal in shape, with scattered chromatin and no recognisable nucleolus; they are usually surrounded by a thin layer of cytoplasm, and the cell bodies are usually scattered among the motor neurons, or sometimes arranged in groups towards the neuropile (as will be described in Section V on fibre tracts). A latero-ventral group of association neurons can be seen in Figs. 21, 22, 33 and 34, with their fibre tracts running through the neuropile, and another ventral group is shown in Figs. 4 and 23.

In general the structure of the ganglion is such that there are few neurons to be seen on the dorsal side while ventrally and laterally there is a thick layer of cells, thickest in the mid-ventral region of the ganglion.

Fig. 16. Frontal section through the suboesophageal ganglion, showing glial cells, neurons and mandibular, maxillary and labial nerves; showing also large numbers of type IV glial cells associated with the axons running through the interganglionic connectives and with the peripheral axons which run through or around the neuropile.

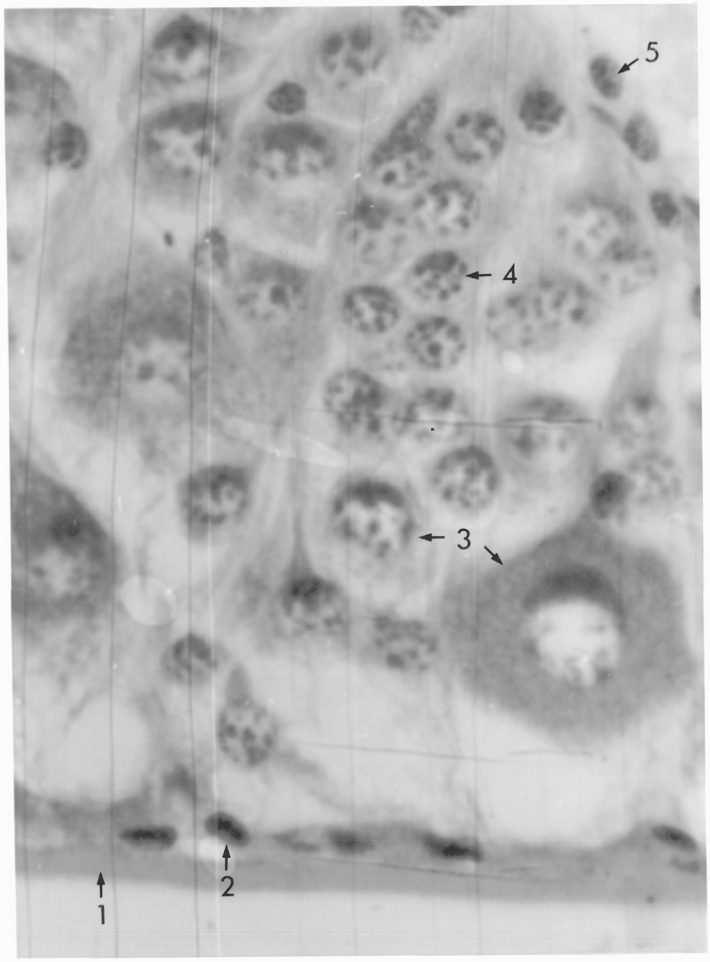


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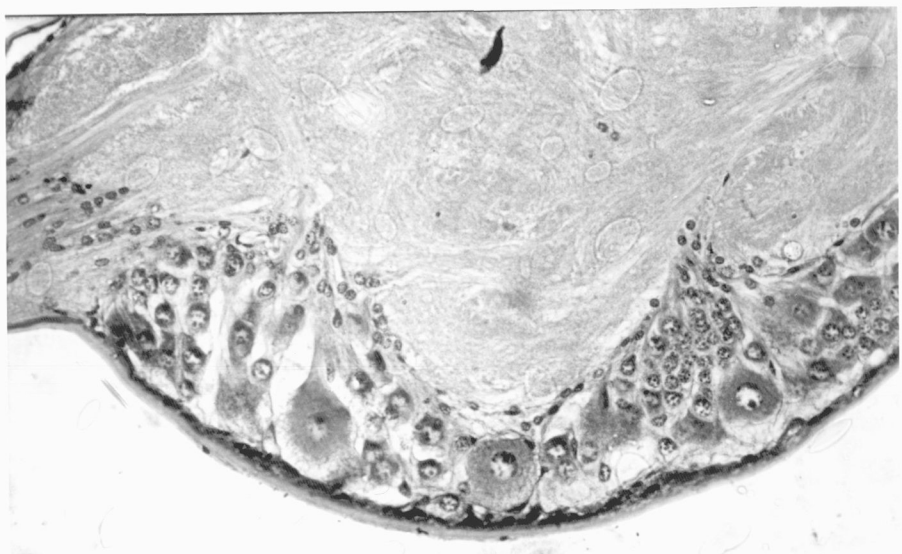
Fig. 17. Light-micrograph showing types of glial cells, the neurilemma and some neurons as stained by phosphotungstic haematoxylin (X 800).

1. neurilemma
2. perineurium (type I glial cells)
3. large and medium sized motor neurons
4. association neurons
5. some type IV glial cells.

Fig. 18. Light-micrograph of parasagittal section stained by osmium tetroxide showing the neurilemma (black), some motor and association neurons and type IV glial cells (X 320).



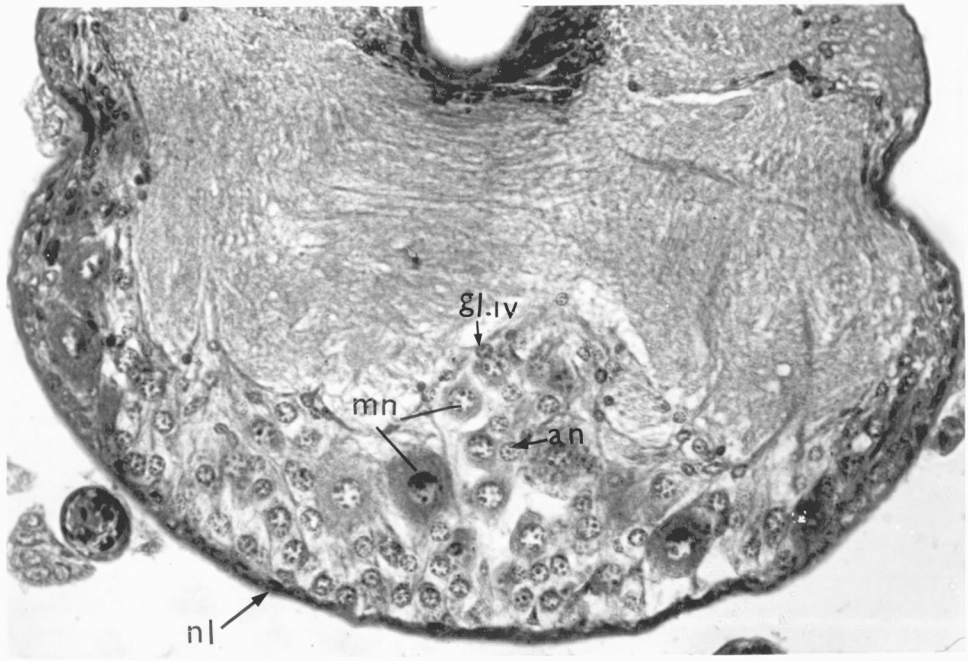
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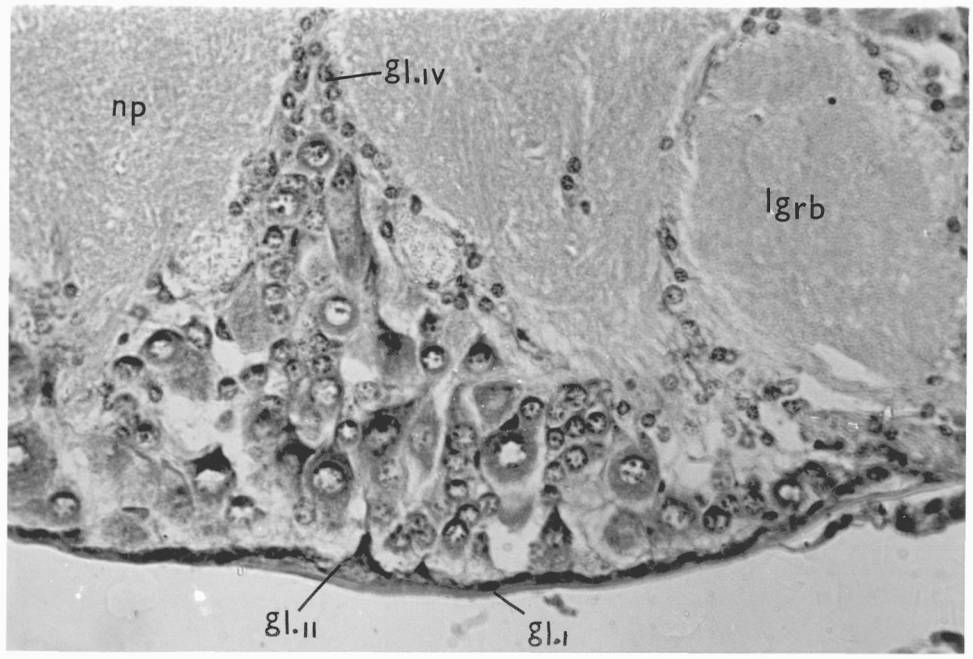
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Fig. 19. Light-micrograph of transverse section stained by osmium tetroxide technique showing large and medium sized motor neurons, association neurons, type IV glial cells *and* neurilemma , stained black, other structures black and grey, and neuropile stained grey (X 320).

Fig. 20. Osmium tetroxide preparations to show the three kinds of neurons, type IV glial cells, neuropile and lateral glomerular body (X 320).



19



20

3. Neuropile and fibre tracts:

The neuropile (np) occupies the central part of the ganglion, covered by a cortex of cell bodies or neurocytes (Figs. 19, 20, 57 and 58). In some regions it includes some individual type IV glial cells (see Figs. 51, 53 and 54). The neuropile contains axons of different diameters, running in different directions, sometimes transversely or longitudinally. Some of these axons form distinct fibre tracts which are very easy to recognise. It also contains some glomerular regions. Trujillo-Cenóz (1960), recognised that in Armadillidium vulgare (Crustacea-Isopoda), the neurons and glial cells are arranged at the periphery of the ganglion while the central region is a dense assembly of interwoven fibres. The neuropile is usually stained blue in Mallory's preparations, dark grey in osmium-ethyl-gallate preparations and is usually rather light in haematoxylin preparations.

In general the neuropile is a very complex area; axons and various fibre tracts from the neurons pass into the central neuropile area. Some of these fibre tracts can be followed clearly but others are not easy to recognise. Huddart (1971), stated that in the stick insect Carausius morosus, axons from cortical neurons pass into the central neuropile area of the ganglion and many neuro-glial cytoplasmic strands also penetrate into the neuropile.

The distribution of readily identifiable tracts and major pathways is described in Table 1. In some cases the neurons giving rise to these fibre tracts can also be identified, but the complexity of the neuropile is so great that although the origins of these tracts may usually be seen clearly, their destination is often less clear once they have entered the neuropile.

Fielden (1963), also found that in nymphs of Anax the axons occupying the ventral part of the peripheral nerves are difficult to trace after they enter the ventral neuropile.

The fibre tracts described in Table 1 are generally numbered from the anterior to the posterior region of the ganglion, as seen in transverse sections and it should also be noted that in general there are three major groups of fibre tracts: transverse, longitudinal and oblique. Relatively few previous works have analysed the system of fibre tracts in the ventral ganglion. Ali (1967), recorded 30 fibre tracts in the suboesophageal ganglion of the adult of Pieris brassicae; Hilton (1911a) traced neuron pathways in the ventral ganglia of Corydalis; Pipa et al (1959), found that altogether seven pairs of longitudinal tracts are distinguishable within the thoracic and first two abdominal ganglia of adult Periplaneta americana, and ten commisural tracts were also present. Zawarzin (1924a & b) and Mill (1964), reported in Aeshna a few sensory fibres passing to the higher centres. Seabrook (1968), described the structure of the seventh abdominal ganglion of the desert locust Schistocerca gregaria. He reported seven transverse commissures and six longitudinal connective tracts, and also described seventy-six other intraneuronal tracts. Gregory (1974), gives some details of seven paired longitudinal fibre tracts, ten transverse commissures and three new oblique tracts during his studies of the general structure of the mesothoracic ganglion of adult males of Periplaneta americana. In Acheta domesticus the 42 suboesophageal tracts described in Table 1 can be classified into the following major groups:-

(A) Transverse tracts crossing the mid-line (transverse commissures):

The most numerous of these make up st_3, st_{2a} and st_{2b}. From their position in the body of the ganglion they can be called the first and second anterodorsal commissures and the anteroventral commissure respectively. Tracts st_{15}, st_{16} and st_{17}, st_{21} are also well defined; according to their position they can be called respectively the first and second central commisures, and the dorsocentral and ventrocentral commissures. Similarly, tracts st_{23}, st_{33} and st_{25}, st_{38} might be called the first and second posterodorsal commissures and the first and second posteroventral commissures respectively.

(B) Longitudinal connective tracts:

Tracts st_4, st_{27}, st_{28}, st_{29}, st_{30}, st_{31}, st_{32} (a, b and c), st_{34} and st_{39} are longitudinally directed. Most of these tracts run through the whole length of the ganglion and represent the intraganglionic portions, the anterior and posterior connectives of the ventral nerve cord.

(C) The oblique group of tracts:

Some of these are oblique and some partly transverse and partly oblique. Most of the remaining tacts of the ganglion fall into this group. They appear mostly to be given off by motor or association neurons, so that some of them are mixed tracts, i.e. the component axons are connected either with motor or with association neurons. Tract st_{35} is very clearly an oblique tract and is given off by a ventral group of association neurons.

Table 1.

Fibre tracts in the suboesophageal ganglion of the adult Acheta domesticus

No.	Origin	Course of tract	Other remarks
st_1	From ventrolateral group of association neurons.	Runs dorsalwards; cannot be followed beyond the dorso-lateral part of the neuropile below st_3.	Figs. 21, 22, 33, 34.
st_{2a}	From lateral region of the neuropile.	The fibres run dorsally, forming a transverse commissure between the two halves of the neuropile.	Figs. 21, 23 The second anterodorsal commissure.
st_{2b}	From ventrolateral region of the neuropile.	Runs ventrally, crossing the mid-line and forming a transverse commissure between the two sides of the neuropile.	Figs. 22, 23, 34. The antero-ventral commisure.
st_3	From dorsolateral region of the neuropile.	The fibres form a commissure between the two circumoesopha-geal connectives.	Figs. 21 to 23, 34. A trans-verse antero-dorsal commissure.
st_4	From ventral part of neuropile; a longitudinal tract.	Runs ventrally on either side of the midline near the boundary of the neuropile.	Figs. 21 to 23, 34, 35.

No.	Origin	Course of tract	Other remarks
st_5	From a lateral group of motor neurons.	Runs ventralwards around the boundary of the neuropile, passing the mid-ipsilateral neuropile; some other fibres passing towards a ventrolateral area containing association and motor neurons.	Fig. 22.
st_6	From ventral association neurons.	Runs dorsalwards along the ventro-lateral periphery of the neuropile.	Fig. 23.
st_7	From mid-ventral motor and some mid-ventral association neurons.	Runs vertically dorsalwards in the midline, dividing the ganglion into right and left halves.	Fig. 23.
st_8	From hypopharyn-geal nerve.	Runs dorsalwards to centre of neuropile and disappears near st_{2a}.	Figs. 22, 23, 36. An intraganglionic connection of the hypo-pharyngeal nerve.
st_9	From a dorsolateral group of motor neurons.	The fibres run ventrally from the dorsolateral motor neurons into the maxillary nerve.	Figs. 24, 37.
st_{10}	From a ventral group of motor neurons.	Runs dorsolater-ally then curves ventrally again and runs into the maxillary nerve.	Fig. 24. Very close to st_9.

No.	Origin	Course of tract	Other remarks
st_{11}	From the ventral group of motor neurons.	Runs dorsalwards and cannot be followed beyond the middle of the neuropile.	Figs. 24, 37.
st_{12}	From a ventrolateral group of motor neurons.	Runs dorsomedially and associated with fibres of st_{13}, forming with it a connection between the two groups of neurons.	Figs. 24, 25, 37.
st_{13}	From a ventral group of association neurons.	Runs dorsalwards, and turns laterally then curves down, apparently forming connection with tract st_{12}.	Figs. 24, 37.
st_{14}	From ventrolateral association and some motor neurons.	Runs dorsolaterally along with fibres st_9.	Fig. 24.
st_{15}	From maxillary nerve.	Runs dorsalwards to middle of ganglion and passes across the midline.	Figs. 24 to 27, 38, 39. It forms middle fibre of maxillary nerve (the first central commissures).
st_{16}	From ventrolateral region of neuropile and maxillary nerve.	Runs dorsalwards and curves in the middle, passing across the midline.	Figs. 25 to 27, 38, 39. Forms the second central commissures.

No.	Origin	Course of tract	Other remarks
st_{17}	From dorsal side of the neuropile.	The fibres run towards either side of the mid-line on the dorsal aspect of the ganglion, forming a commissure between the two dorsolateral regions of the neuropile.	Figs. 25 to 29, 39 to 41. Forms the dorsocentral commissure.
st_{18}	From ventral part of neuropile.	The fibres run dorsalwards and curve laterally to associate with the fibre tract of st_{16}.	Fig. 25.
st_{19}	From the anterior cervical nerve.	Runs ventralwards and curves laterally, perhaps reaching the laterocentral motor neurons.	Figs. 24, 25. Possibly in whole or in part a motor nerve tract.
st_{20}	From the lateral neuropile and maxillary nerve.	The fibres run dorsalwards to the dorsolateral region of the neuropile.	Figs. 26, 27.
st_{21}	From the neuropile on the ventral border of the ganglion.	Runs close to the ventral boundary of the neuropile on either side of midline.	Fig. 26. Forms the ventrocentral commissure.
st_{22}	From a group of mid-ventral and mid-dorsal motor and association neurons.	Runs vertically, dorsal and ventralwards, dividing the ganglion into a right and left half.	Figs. 27, 28, 39, 40, 42 to 44, 46.

No.	Origin	Course of tract	Other remarks
st_{23}	From dorsal part of neuropile.	The fibres run below st_{17} and st_{33}, crossing the midline on either side of the neuropile and curving down to reach fibres coming from st_{24}.	Figs. 28 to 30, 40, 42 to 44, 46. It forms the first posterodorsal commissures.
st_{24}	From a mid-ventral group of motor neurons and some ventrolateral association neurons.	Runs dorsolaterally to reach st_{23} in middle of the ganglion.	Figs. 28, 40. Axons and synaptic regions very distinct.
st_{25}	From the neuropile on ventral side of the ganglion.	Runs ventrally, crossing the midline then curves very close to boundary of st_{24}.	Figs. 28, 29, 40, 41. Forms the first posteroventral commissure.
st_{26}	From the mid-central part of the ganglion	Runs ventrolaterally on either side of the midline, crossing st_{24} and st_{35} and runs down in the direction of maxillary and labial nerves.	Figs. 28 to 30.
st_{27}	From the centre of the ganglion it runs longitudinally on either side of the midline.	The fibres run longitudinally on either side of midline but curve near centre of ganglion to run more or less transversely then return as a longitudinal tract and run into the posterior connectives.	Figs. 29, 30, 42 to 46, 53, 54, 59 to 65. Some fibres of the two corresponding tracts cross each other near the posterior end then run into the contralateral posterior connectives - see Figs. 60, 61, 65.

No.	Origin	Course of tract	Other remarks
st_{28}	From circumoesophageal connectives, as a longitudinal tract.	The fibres pass laterally on either side of the ganglion through its whole length and run into the posterior connectives.	Figs. 29, 30, 42 to 46, 52 to 56, 60 to 65.
st_{29}	From the circumoesophageal connectives lateral to st_{27}, as a longitudinal tract.	The fibres pass on either side of the midline below st_{28} and run through the whole length of the ganglion into the posterior connectives.	Figs. 29, 30, 43 to 46, 52 to 65.
st_{30}	From the circumoesophageal connectives, between st_{28} and st_{32c} and lateral to st_{32a}, as a longitudinal tract.	The fibres run laterally through the whole length of the ganglion and pass into the posterior connectives.	Figs. 29 to 32, 43 to 46, 54.
st_{31}	From dorsal side of ganglion, as a longitudinal tract.	Runs on either side of midline below st_{32b}; possibly enters the posterior connectives.	Figs. 29 to 32.
st_{32}	Three groups of fibres, forming st_{32a}, $_b$ and $_c$, all from the circumoesophageal connective and forming a longitudinal tract.	(a) The fibres run lateral to st_{30}, peripheral to the neuropile and laterally	Figs. 29, 30, 42 to 46. Contains giant fibres.
		(b) The fibres run dorsally above st_{31}.	Figs. 29, 30, 42, 45. Contains giant fibres.

No.	Origin	Course of tract	Other remarks
st_{32} (cont.)		(c) The fibres run dorsally above st_{30}.	Figs. 29, 30, 43 to 46.
		Generally all these three fibre tracts run dorsolaterally, along the periphery of the neuropile, through the whole length on both sides of the ganglion. They join the anterior and posterior connectives; usually the three fibre tracts join together before leaving or entering the connectives at the anterior and posterior end of the ganglion.	
st_{33}	From the dorsal part of the neuropile.	Runs dorsally below st_{17}, crossing the midline and running laterally around st_{30} on either side of the ganglion.	Figs. 28 to 30, 46. The second posterodorsal commissure.
st_{34}	From the dorsal part of the ganglion, as a longitudinal tract.	Runs close to and on either side of the midline, below st_{31} and st_{32} and lateral to st_{30} and st_{28}.	Figs. 29 to 32.
st_{35}	From a ventral group of association neurons.	Runs towards the dorsolateral part of the neuropile.	Fig. 29.

No.	Origin	Course of tract	Other remarks
st_{36}	From labial nerve.	Runs dorsalwards and turns with two branches around st_{29} and st_{28}.	Figs. 30, 43, 44.
st_{37}	From lateral association and motor neurons.	Runs to the midline with two branches around st_{30} and passes towards st_{23}, with which it becomes associated.	Figs. 31, 30.
st_{38}	From the ventral side of the neuropile.	Runs ventrally, crossing the midline then runs dorsally and curves around st_{29} and st_{27}.	Fig. 31. Forms the second posteroventral commissure.
st_{39}	From the ventro-lateral side of the neuropile as a longitudinal tract.	The fibres run on either side of the midline lateral to st_{28} and st_{29} and join st_{29} and st_{27} near the posterior end.	Figs. 31, 32, 46, 48.

4. Glomerular bodies:

In the adult of Acheta three conspicuous pairs of glomerular bodies are found in the neuropile of the suboesophageal ganglion, while near the anterior central region of the ganglion there is an additional unpaired glomerular structure. This latter centre has been called the anterior central glomerular body (cgrb, Figs. 24, 36, 37, 57 & 58). Zawarzin (1924 a & b) and Bierbrodt (1942), are among many who illustrate some glomerular bodies in the brains of Aeshna and Panorpa larvae; their figures resemble the heavily staining areas found in the suboesophageal ganglion of Acheta.

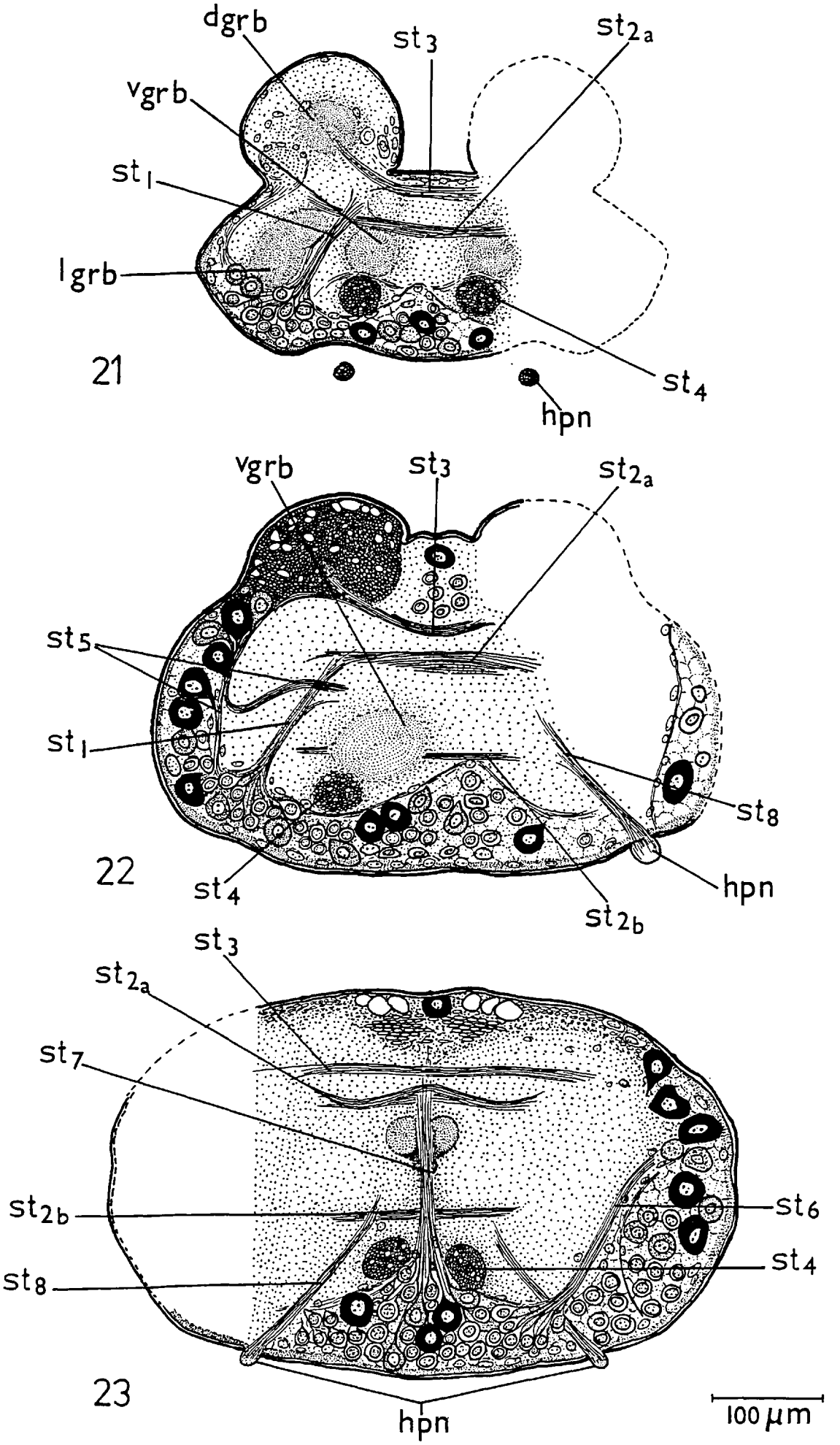
The most anterior pair of glomeruli appears towards the ventral side of the ganglion (vgrb, Figs. 21, 22, 35 to 37). The second pair is more dorsal, and can be called the dorsal glomerular body (dgrb). Starting from the point at which the circumoesophageal connectives join the suboesophageal ganglion dorsally, (Fig. 21), this pair extends near the midline in the centre of the ganglion. Fibre tract st_3 breaks up in the dorsal glomerular body (Figs. 21 to 23). The third pair of glomeruli is lateral, and referred to here as the lateral glomerular bodies (lgrb, Figs. 20, 21, 35 & 36). They increase in size towards the centre of the ganglion, where they make up a large mass occupying the lateral region, and are always prominent where the maxillary nerve enters the ganglion. Ali (1967 & 1974), found in the thoracic ganglia of the adult Pieris brassicae (Lepidoptera), only traces of a ventral glomerular body, but a lateral pair is prominent, as is the dorsal glomerular body near the posterior end of the ganglia, while in the larval stages two pairs of glomerular bodies were observed in the mid-ventral and lateral regions. Ali also found in the suboesophageal ganglion of the fifth instar larva that there is a pair of large dorsal glomerular bodies, as well as small lateral and

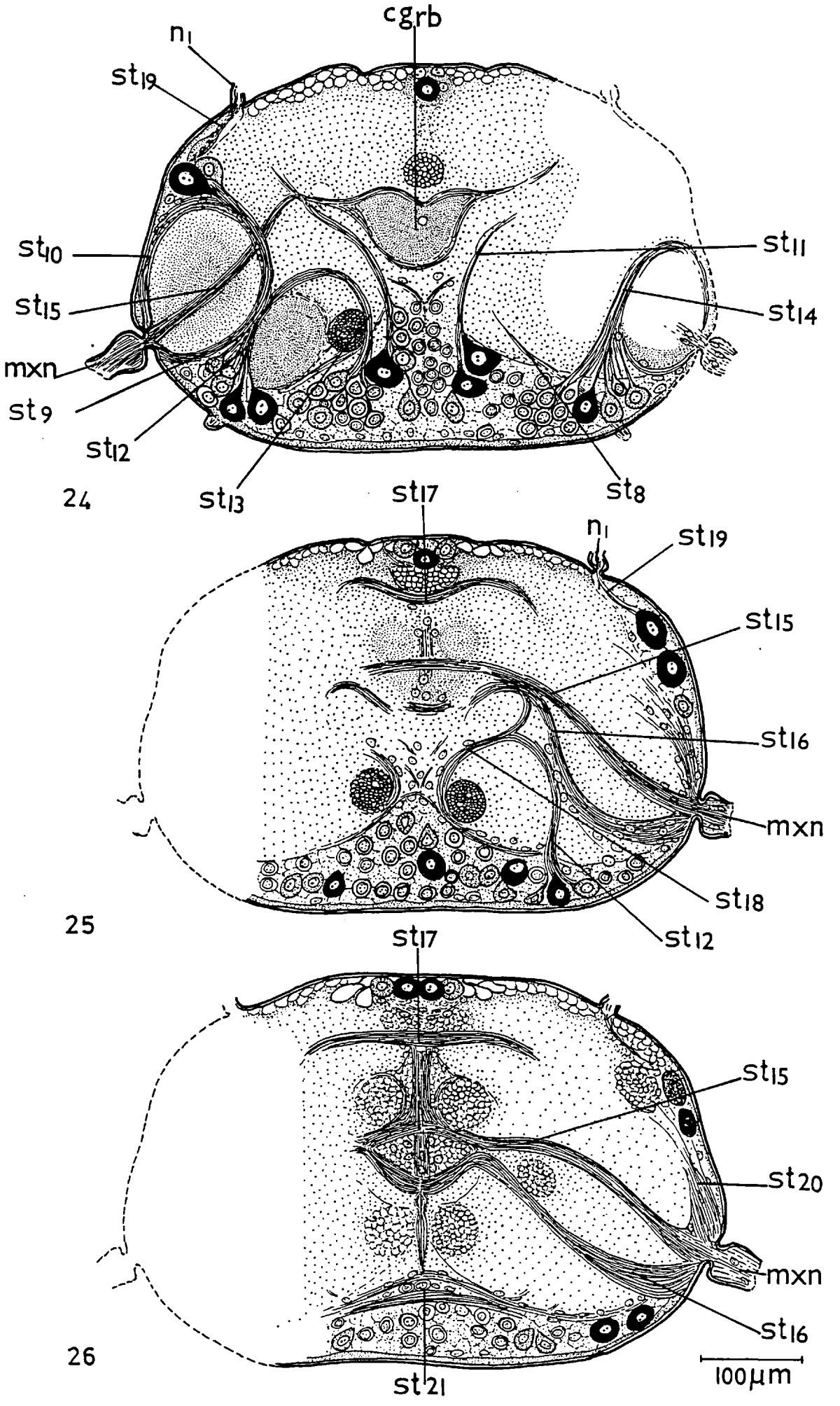
ventral pairs. The lateral pair lies towards the centre of the ganglion, and the ventral pair is not very well developed.

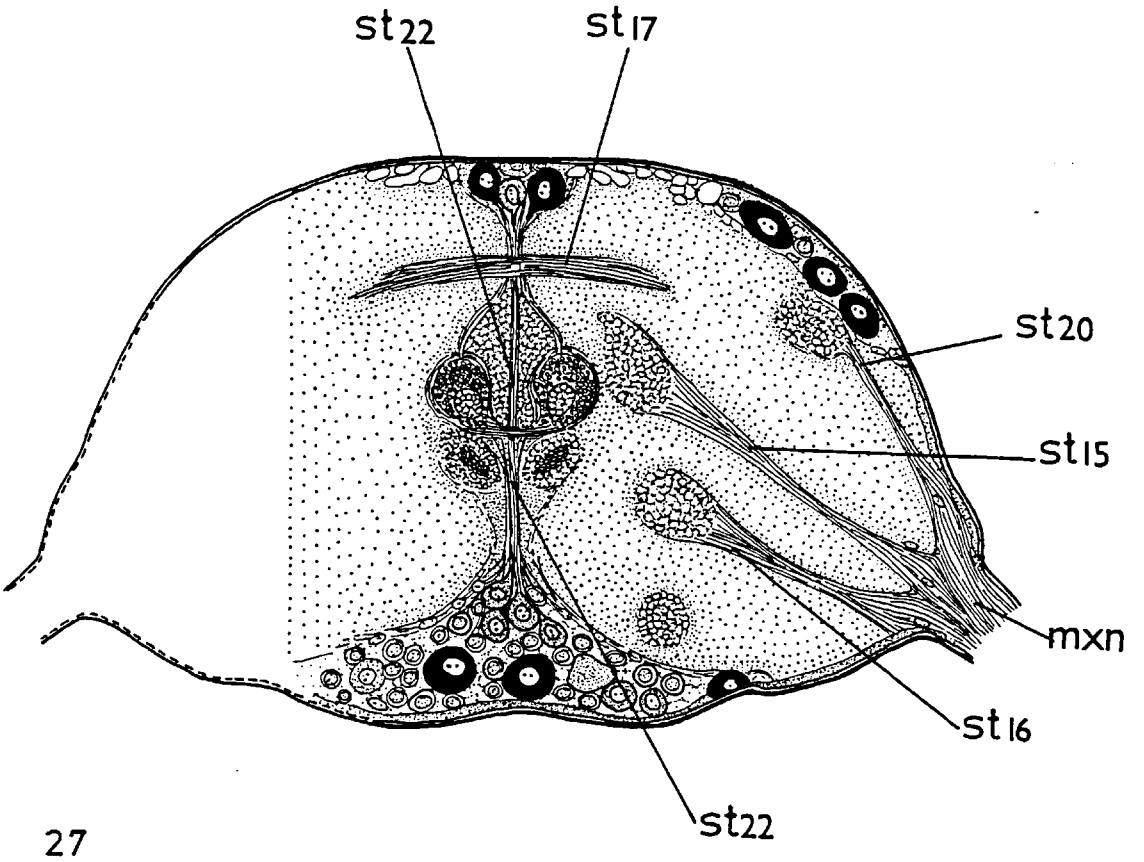
The central glomerular body of Acheta is well developed and appears very distinct before the middle of the ganglia, (Figs. 24, 36, 37, 57, 58 & 65). This glomerular centre does not extend behind the middle of the ganglia.

The glomerular regions of the neuropile may appear dark or pale staining, depending upon their development; within them the axons can sometimes be seen breaking into smaller branches.

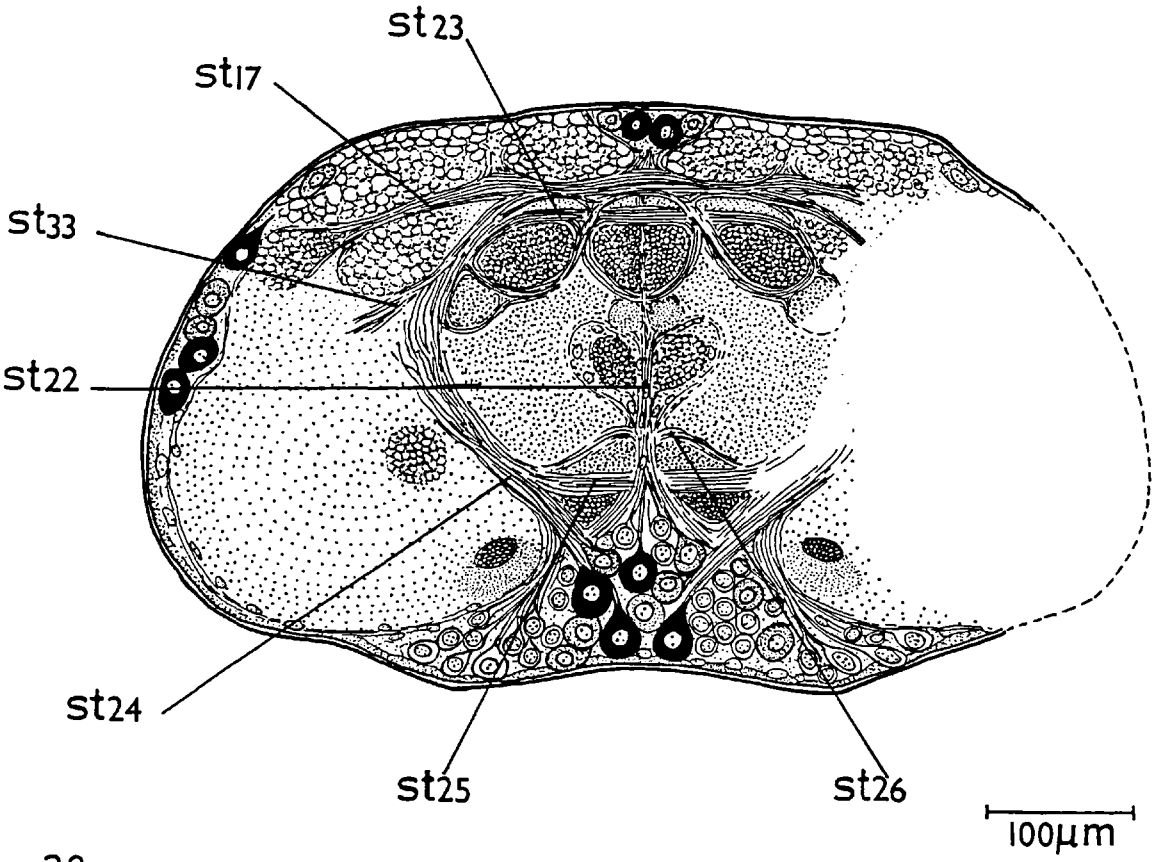
Figs. 21 to 32. Transverse serial sections of suboesophageal ganglion of adult Acheta domesticus showing the neuron groups and fibre tracts listed in table 1. Cells with dark cytoplasm represent large motor neurons; cells with stippled cytoplasm represent medium-sized motor neurons; cells with white cytoplasm and stippled nuclei are association neurons.


dgrb
st3
st2a
vgrb
st1
lgrb
21
st4
hpn
vgrb
st3
st2a
st5
st1
st8
22
st4
st2b
hpn
st3
st2a
st7
st2b
st6
st4
st8
23
hpn
100 µm

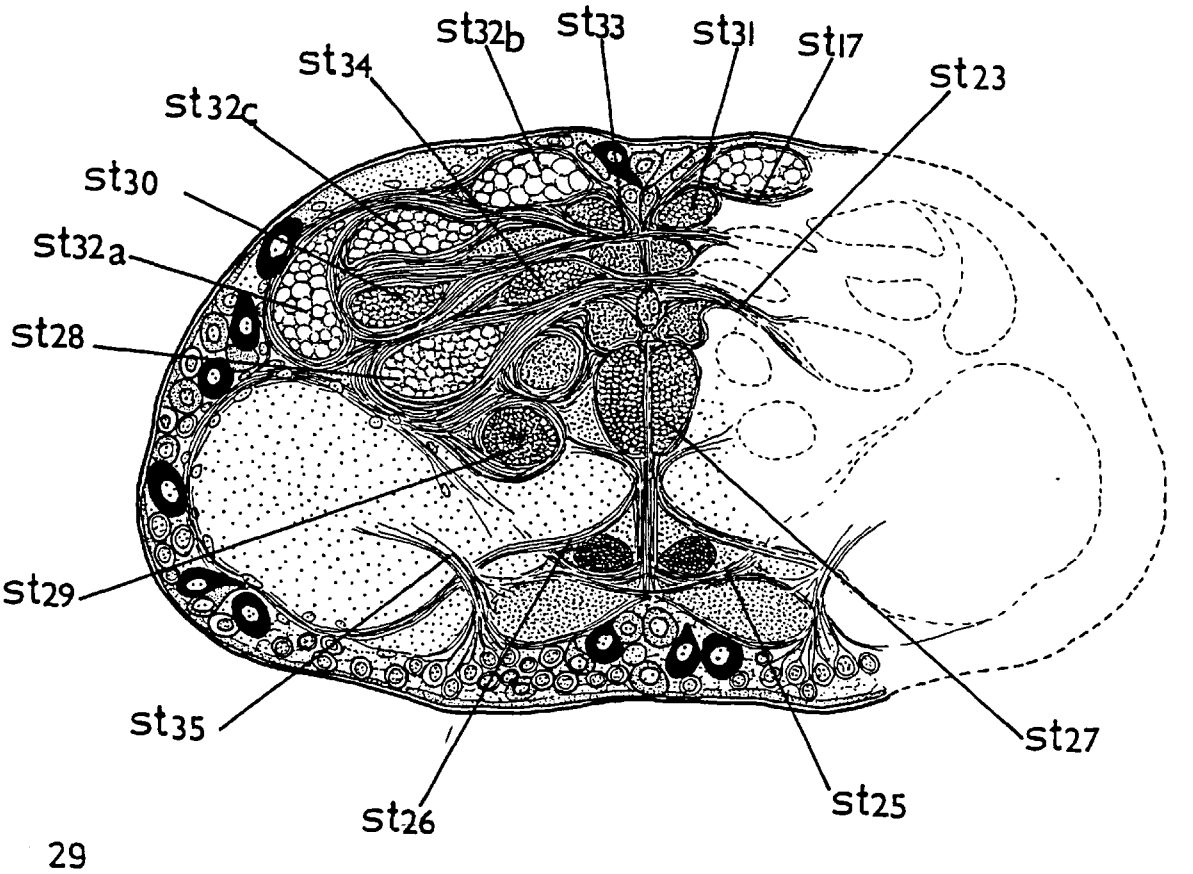

cgrb
n1
st19
st10
st11
st15
st14
mxn
st9
st12
st13
st8
24
st17
n1
st19
st15
st16
mxn
st18
st12
25
st17
st15
st20
mxn
st16
st21
26
100µm


st22
st17
st20
st15
mxn
st16
st22

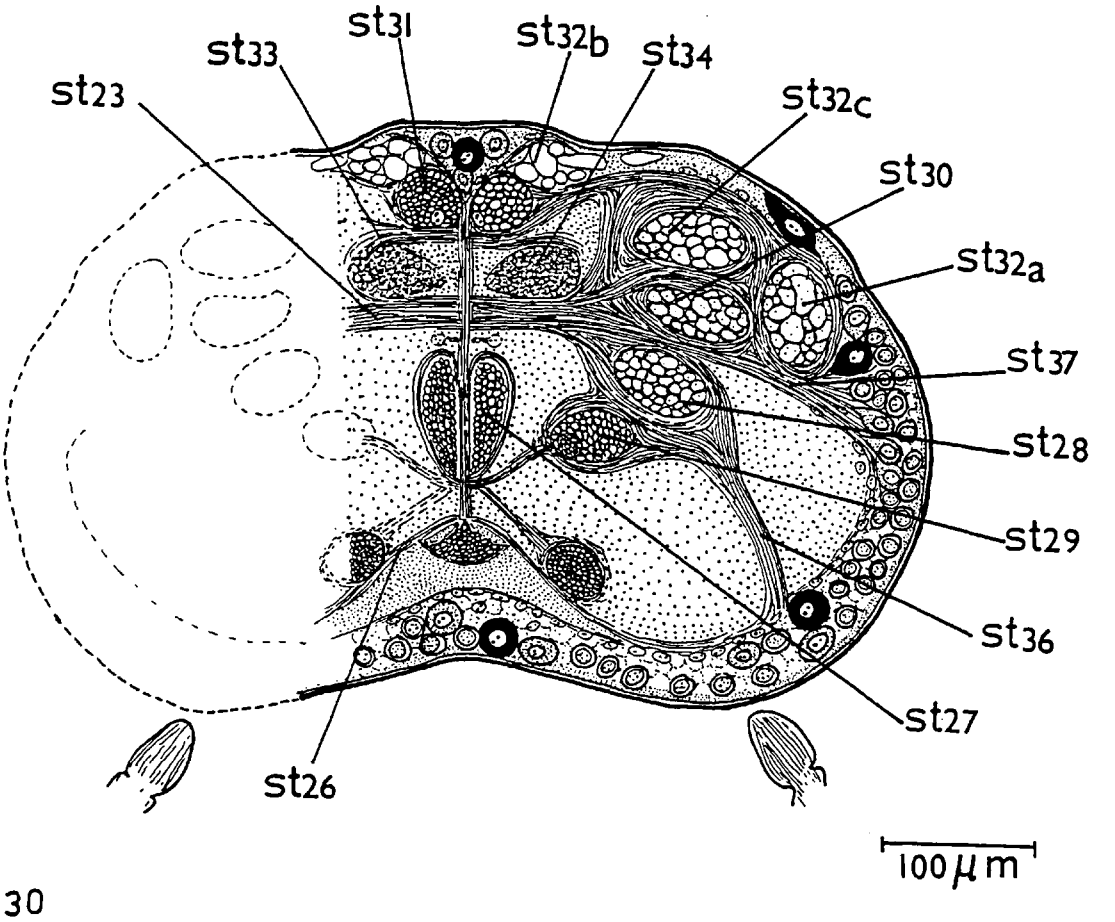
27


st23
st17
st33
st22
st24
st25
st26
100µm

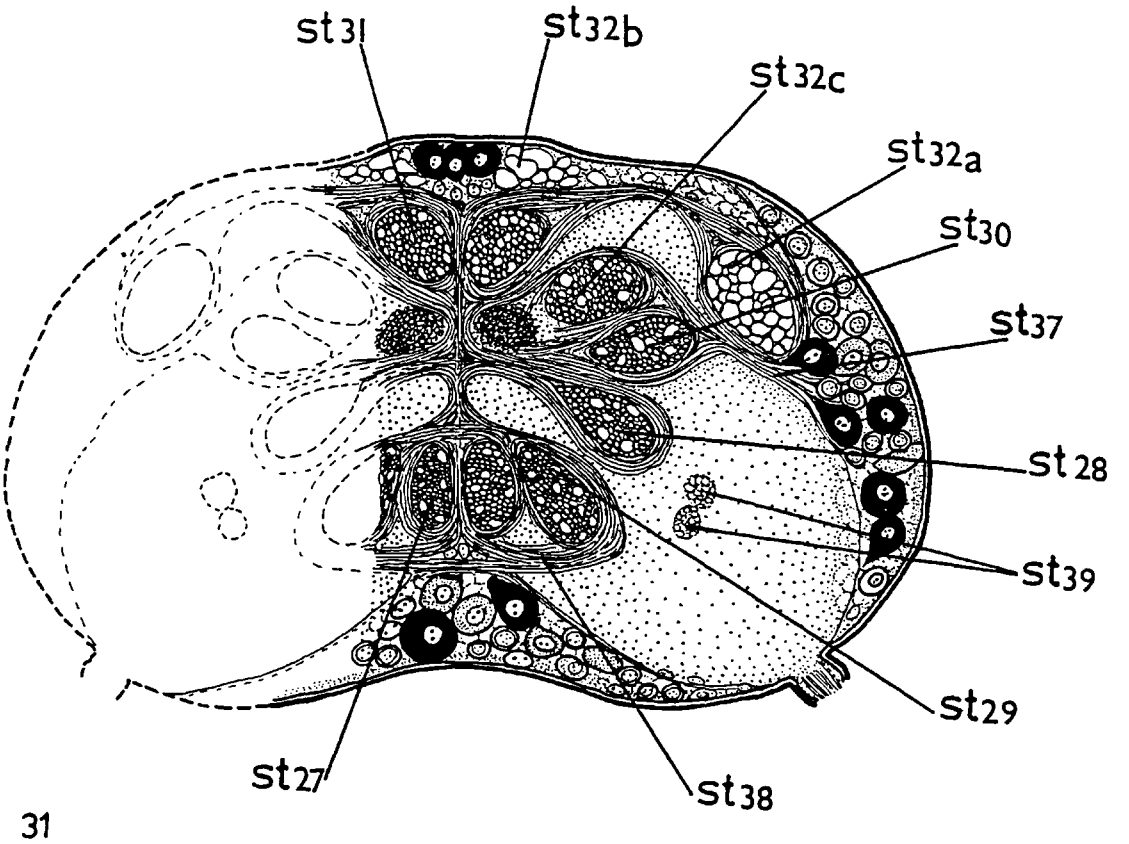
28


st32b
st33
st31
st17
st34
st23
st32c
st30
st32a
st28
st29
st35
st26
st25
st27

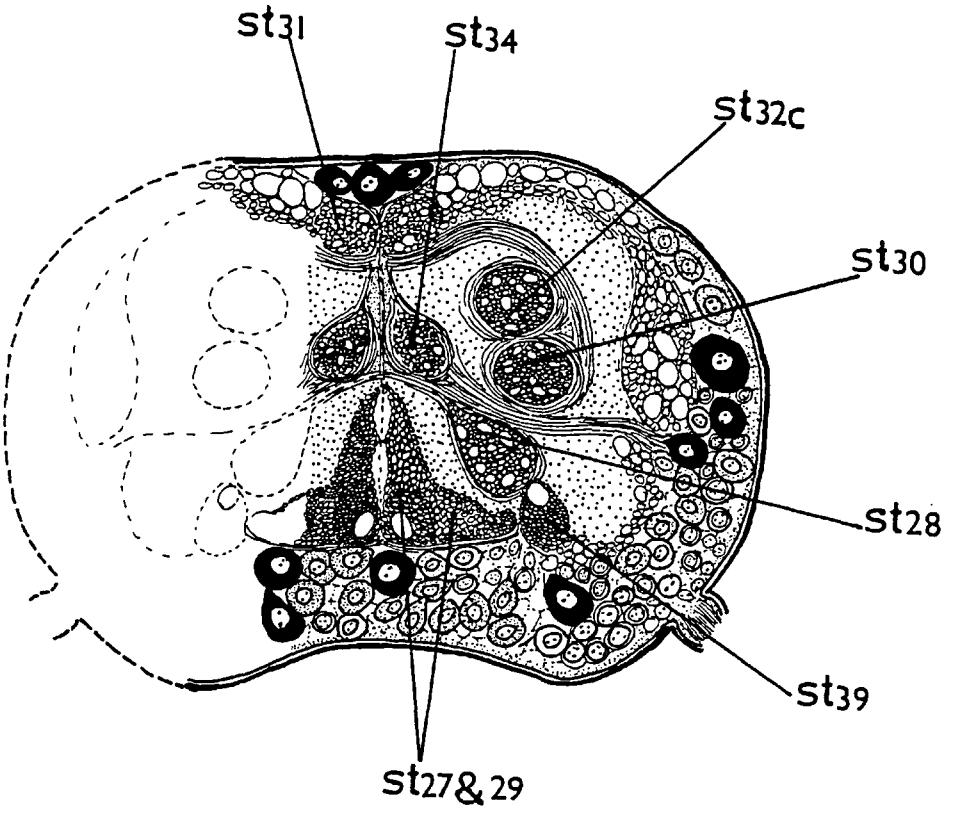
29


st31
st33
st32b
st34
st23
st32c
st30
st32a
st37
st28
st29
st36
st27
st26
100μm

30


st31
st32b
st32c
st32a
st30
st37
st28
st39
st29
st27
st38

31

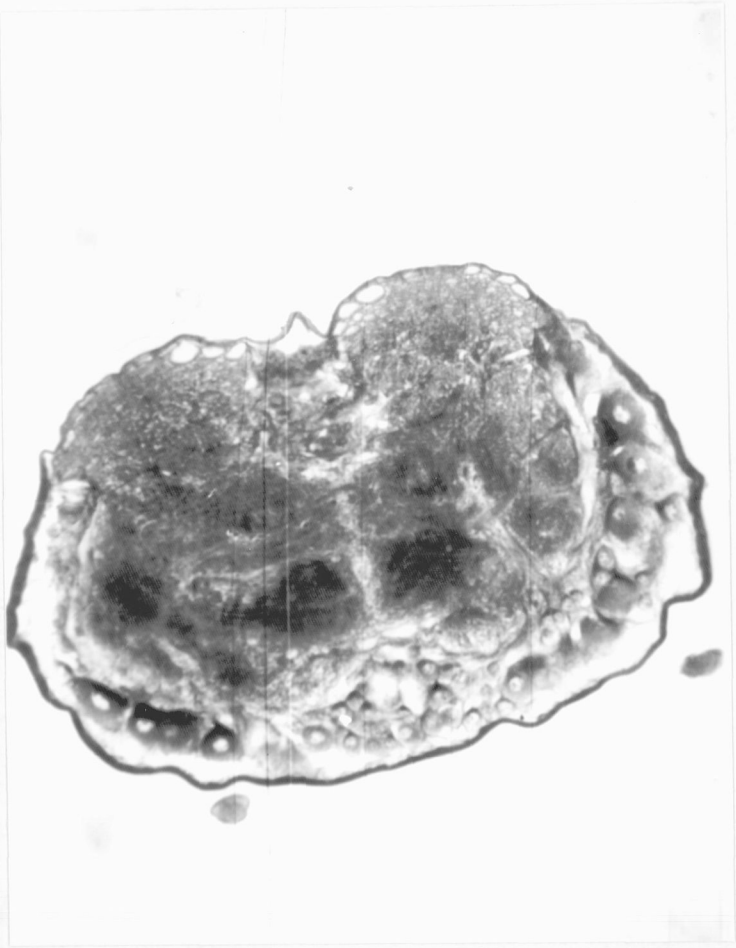

st31
st34
st32c
st30
st28
st39
st27&29

32

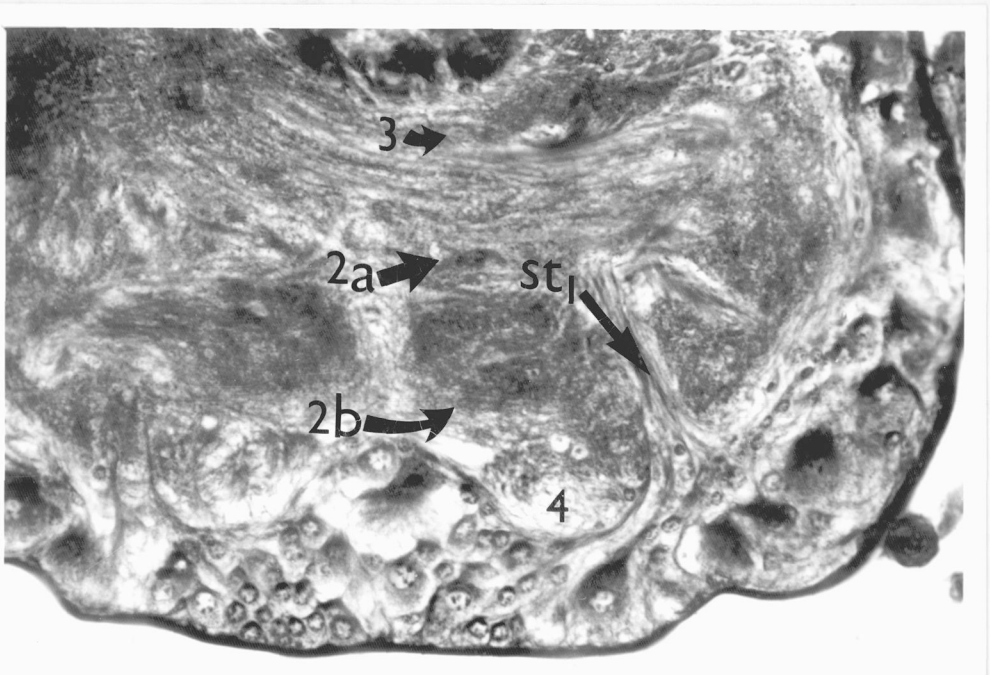
100μm

Fig. 33. Light-micrograph of transverse section stained by osmium tetroxide technique showing motor and association neurons and fibre tract st_1 (X 200).

Fig. 34. Light-micrograph of transverse section stained by osmium tetroxide to show some neurons and fibre tracts st_1, st_2 a & b, st_3 and st_4 (X 320).



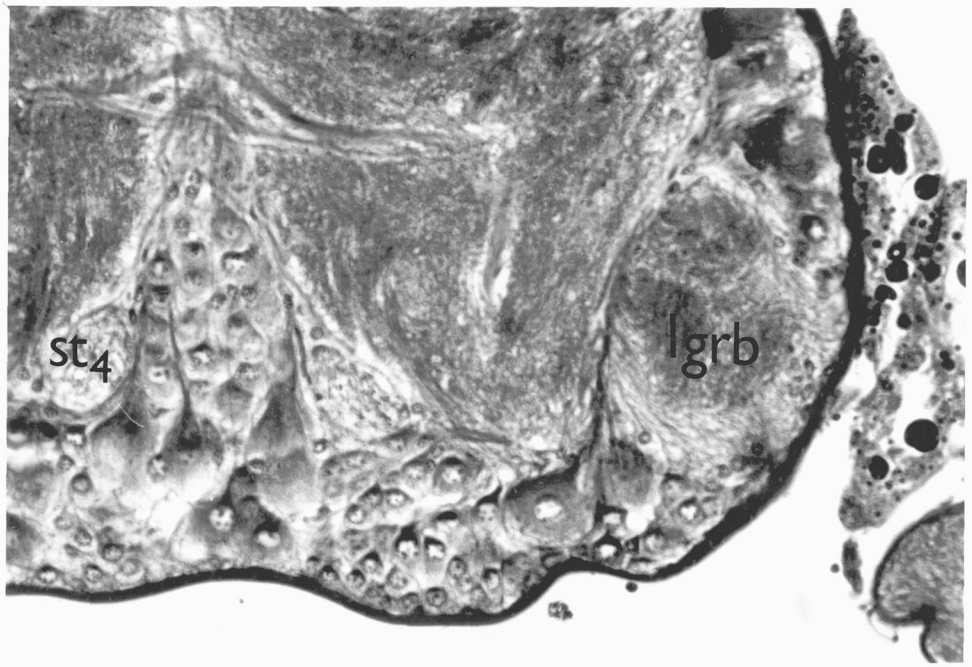
33



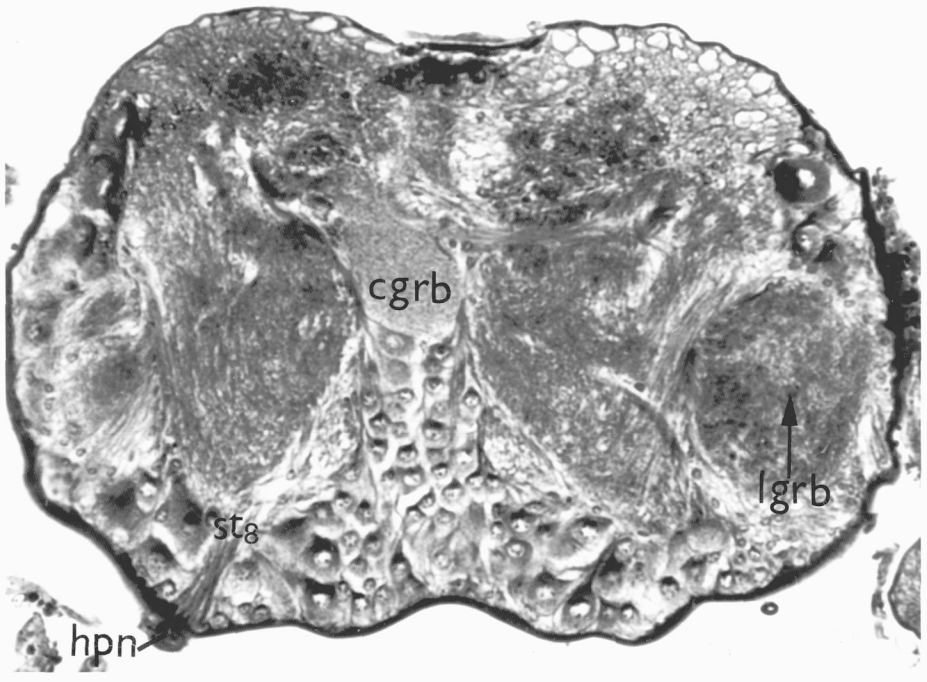
34

Fig. 35. Light-micrograph of transverse section stained by osmium tetroxide to show two axons from a large motor neuron in the lateroventral region (axons 18 & 19); also shows fibre tracts st_4 and the lateral and ventral glomerular body. (X 320).

Fig. 36. Light-micrograph of transverse section stained by osmium tetroxide showing beginning of fibre tract st_8 (from the hypopharyngeal nerve), the lateral glomerular body and the central glomerular body. (X 200).



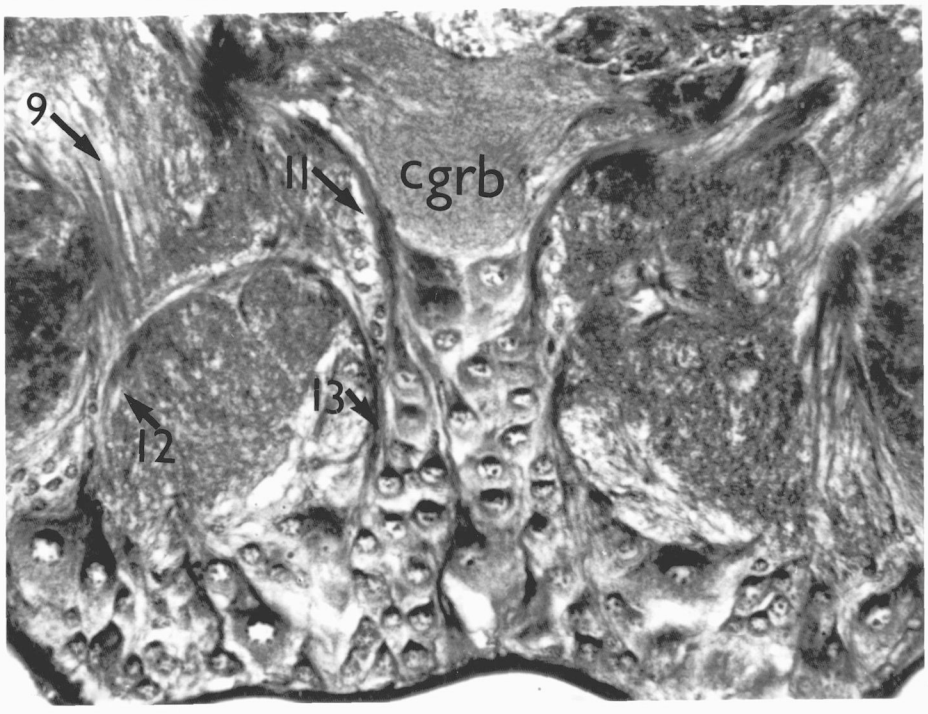
35



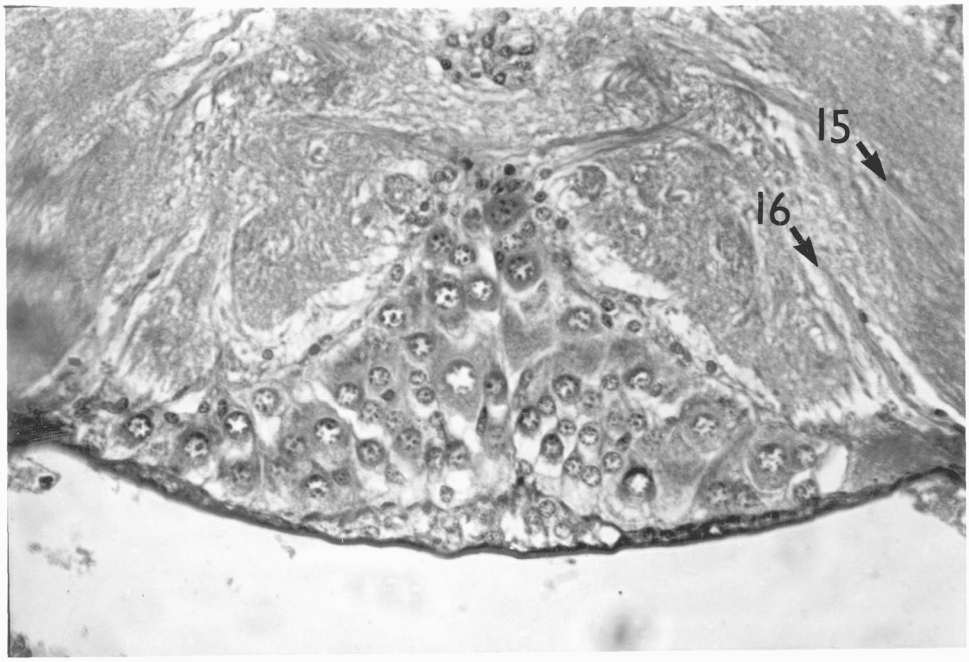
36

Fig. 37. Light-micrograph of transverse section stained by osmium tetroxide showing fibre tracts, a part of st_9, st_{11}, st_{12}, st_{13}, the ventral glomerular body and the central glomerular body. (X 320).

Fig. 38. Light-micrograph of transverse section stained by osmium tetroxide method showing fibre tracts st_{15} and st_{16} and some neurons. (X 320).



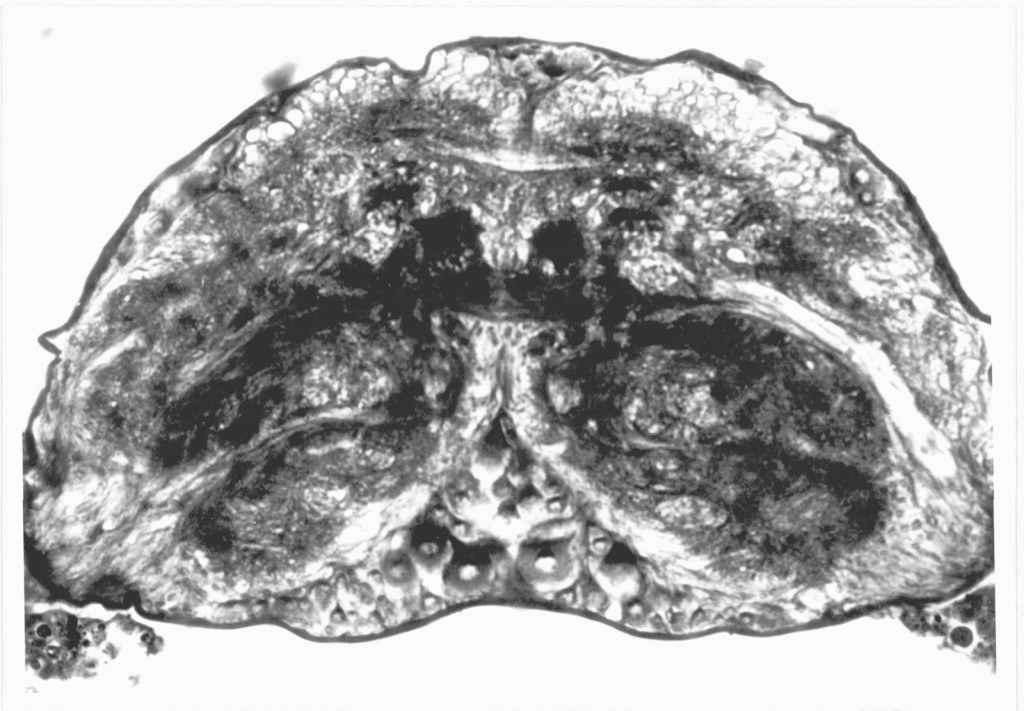
37



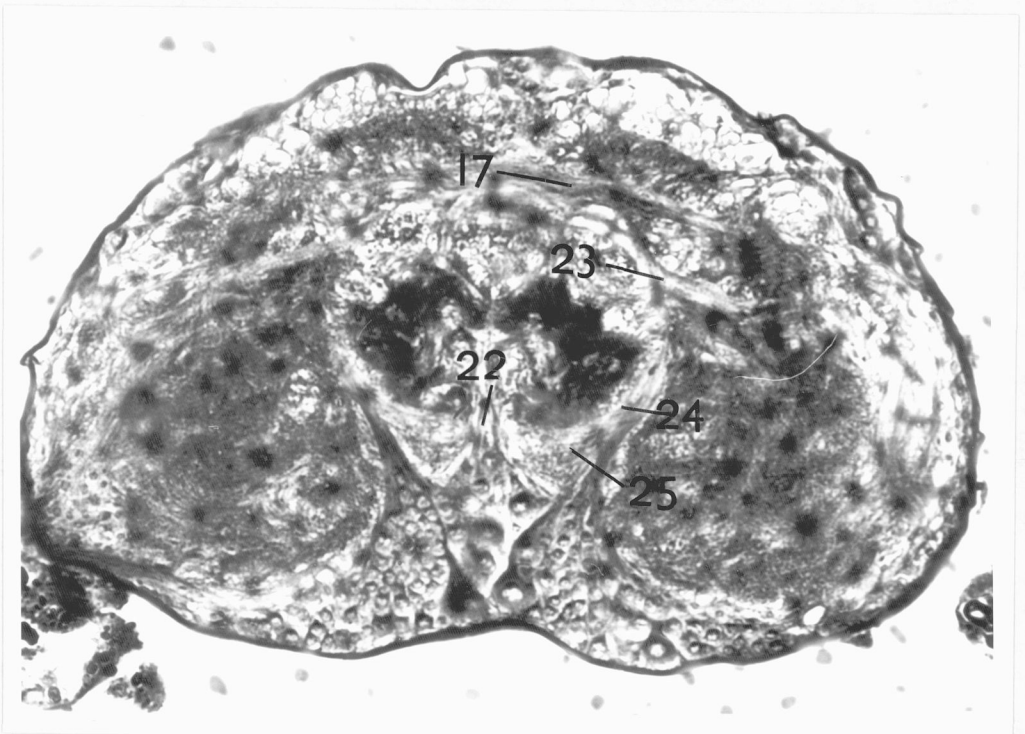
38

Fig. 39. Light-micrograph of transverse section stained by osmium tetroxide method showing fibre tracts st_{15}, st_{16}, st_{17} and st_{22}. (X 200).

Fig. 40. Light-micrograph of transverse section stained by osmium tetroxide showing fibre tracts st_{17}, st_{22}, st_{23}, st_{24} and st_{25}. (X 200).



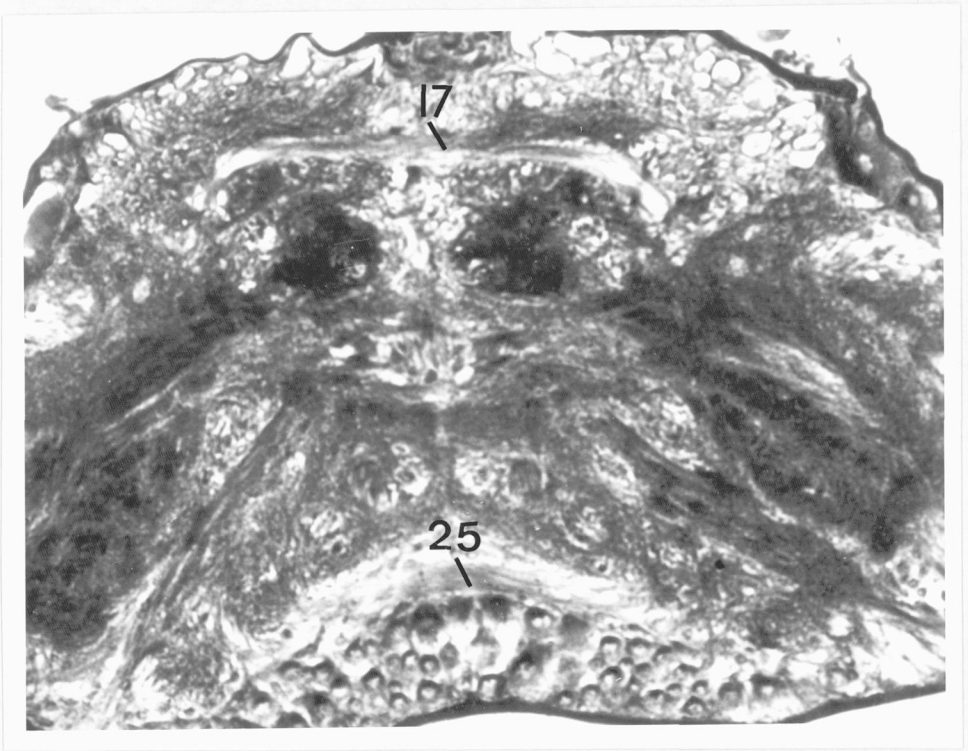
39



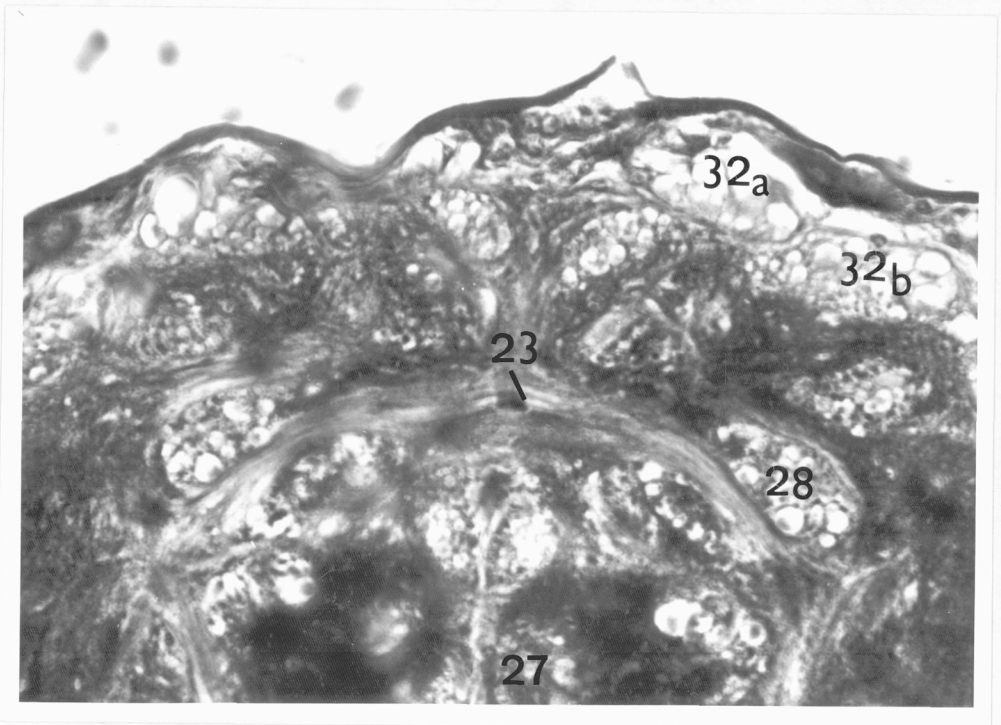
40

Fig. 41. Light-micrograph of transverse section stained by osmium tetroxide showing fibre tracts st_{17} and st_{25}. (X 200).

Fig. 42. Light-micrograph of transverse section stained by osmium tetroxide showing fibre tracts st_{23}, st_{22} and longitudinal tracts st_{27}, st_{28}, st_{32a} and st_{32b}. (X 320).

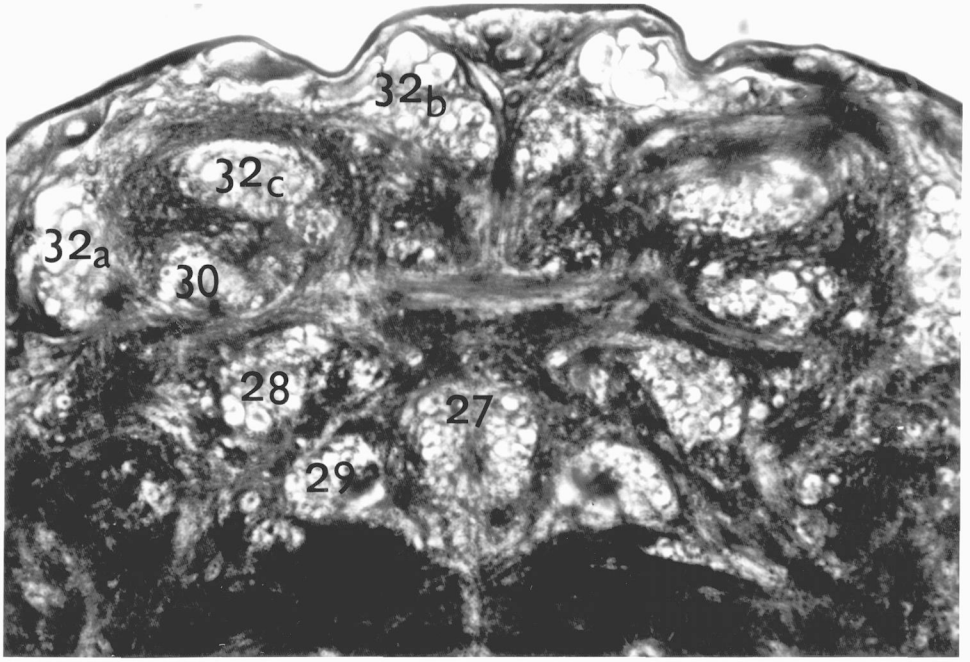


41

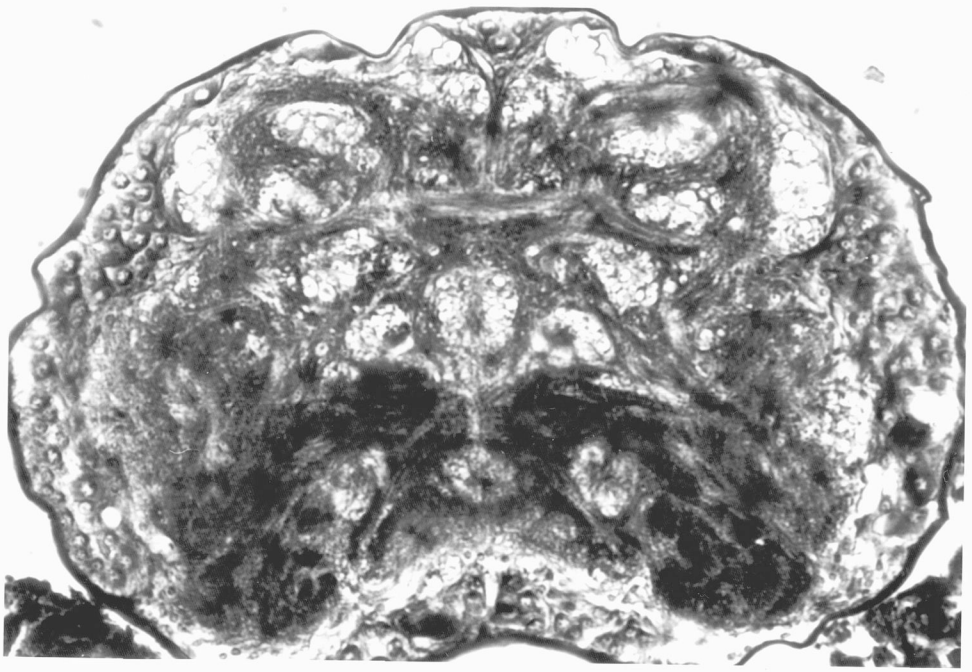


42

Figs. 43 & 44. Light-micrograph of transverse section stained by osmium tetroxide showing part of fibre tract st_{22}, st_{23}, st_{36} and the longitudinal tracts st_{27}, st_{28}, st_{29}, st_{30}, st_{32a} and st_{32c}. (X 320 & 200 respectively).



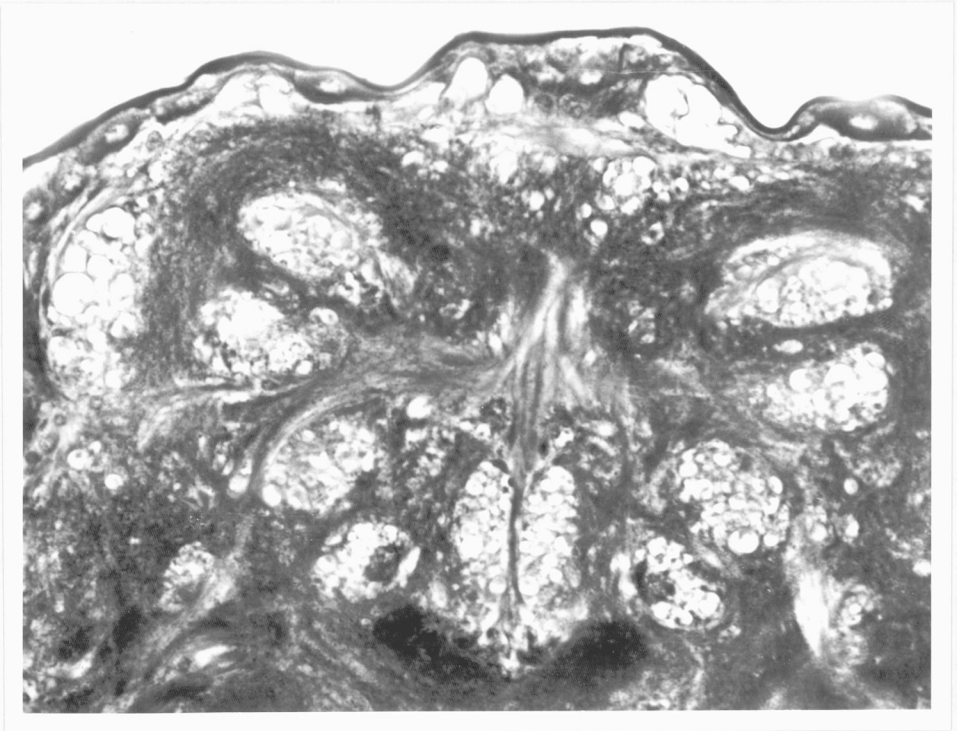
43



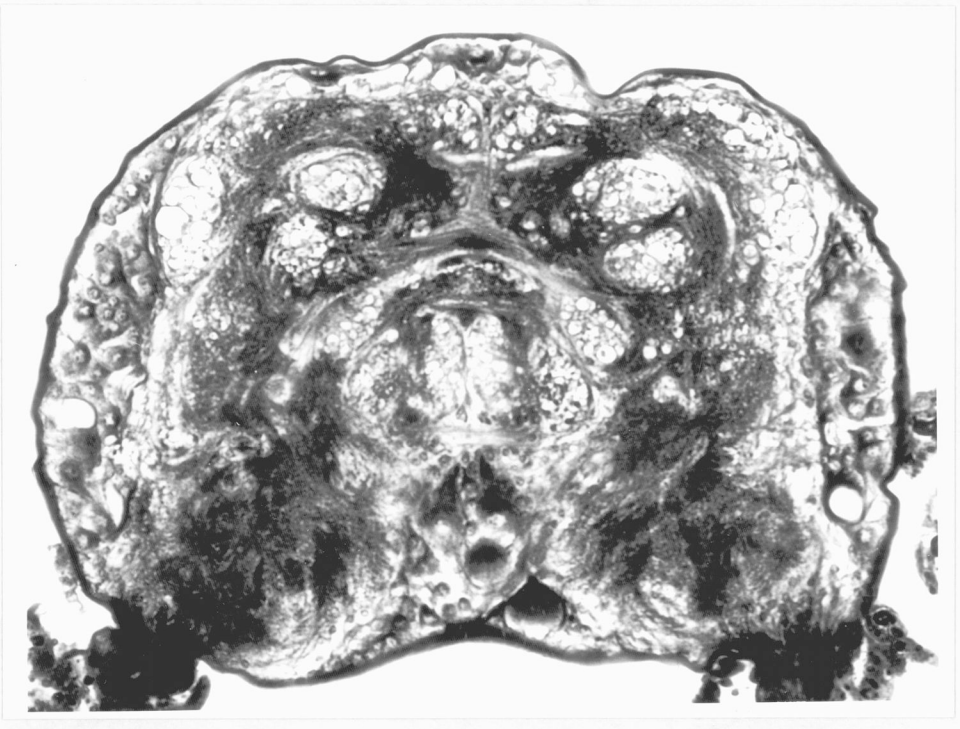
44

Fig. 45. Light-micrograph of transverse section stained by osmium tetroxide showing longitudinal fibre tracts st_{27}, st_{28}, st_{29}, st_{30}, st_{32a}, st_{32b} and st_{32c}. (X 320).

Fig. 46. Light-micrograph of transverse section stained by osmium tetroxide showing some of the transverse and longitudinal fibre tracts (st_{22}, st_{23}, st_{33} and st_{27}; st_{28}, st_{29}, st_{30}, st_{31}, st_{32a}, st_{32c} and st_{39}). (X 200).



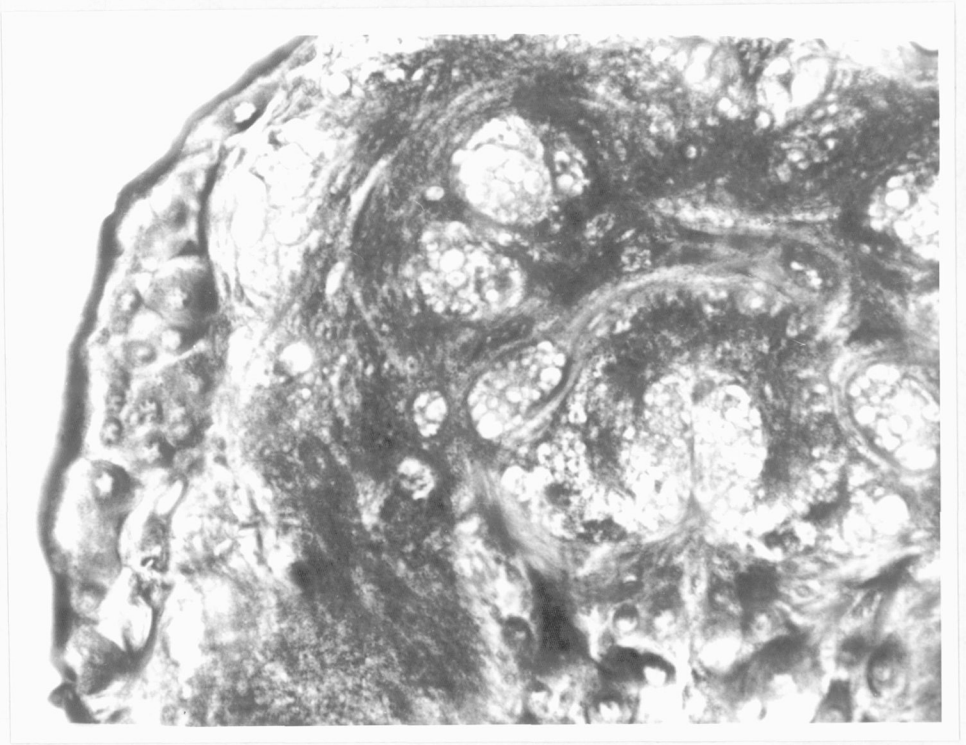
45



46

Fig. 47. Light-micrograph of transverse section stained by osmium tetroxide showing some of the longitudinal fibre tracts (st_{27}, st_{28}, st_{29}, st_{30}, st_{32a} and st_{32c}). (X 320).

Fig. 48. Shows the posterior end of the ganglion (transverse section), stained by osmium tetroxide, showing the beginning of the approximation of longitudinal tracts; it also shows fibre tract 39. (X 200).



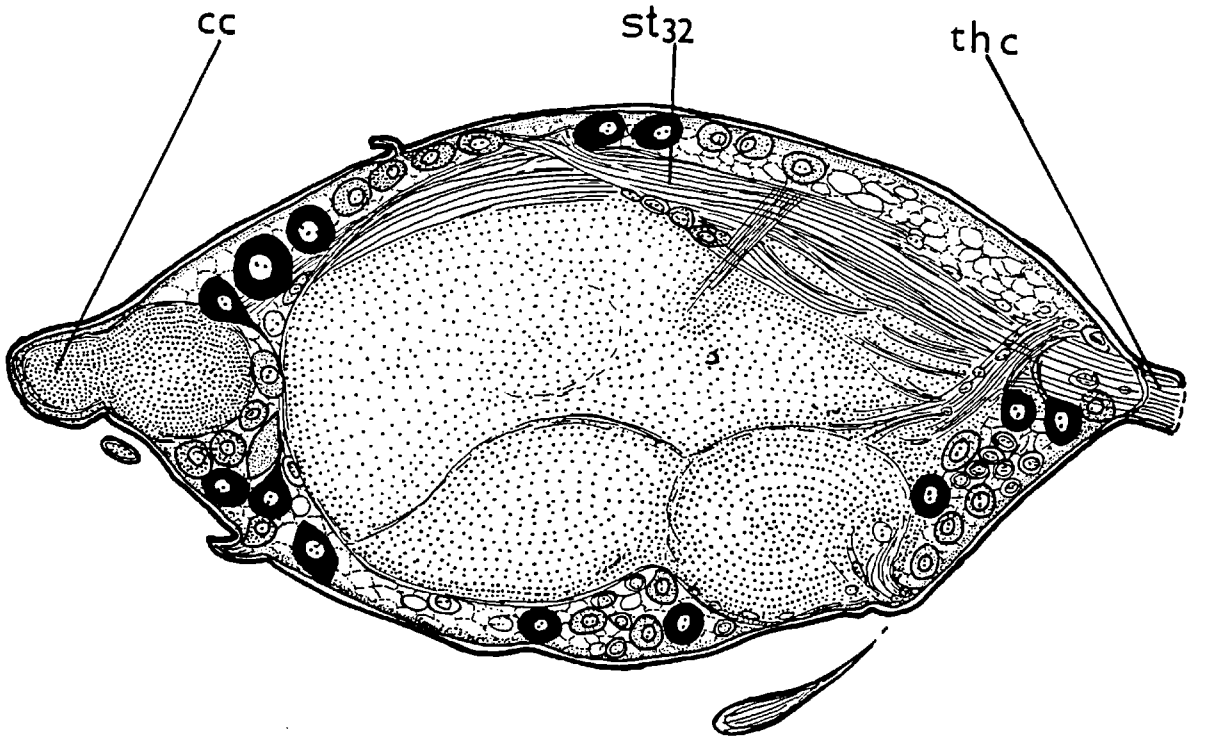
47



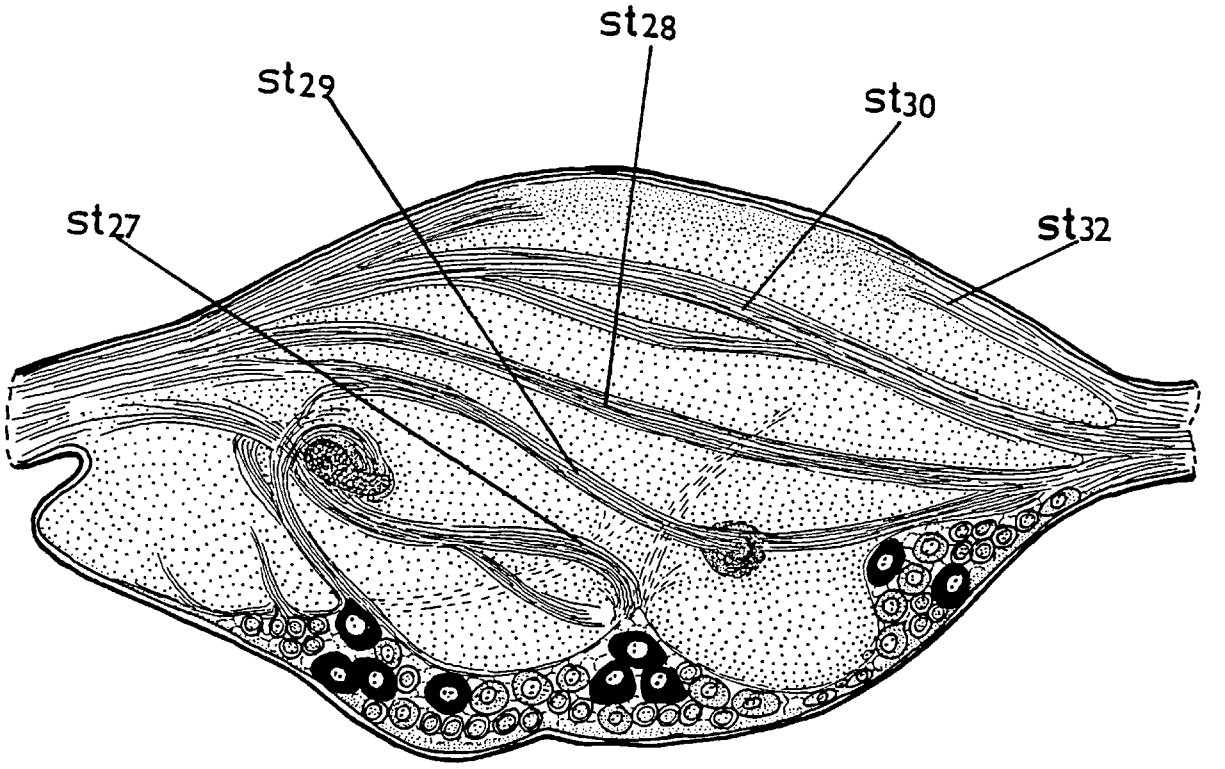
48

Fig. 49. Parasagittal section, showing the groups of fibre tracts st_{32}.

Fig. 50. Sagittal section through suboesophageal ganglion, showing some longitudinal tracts.



49

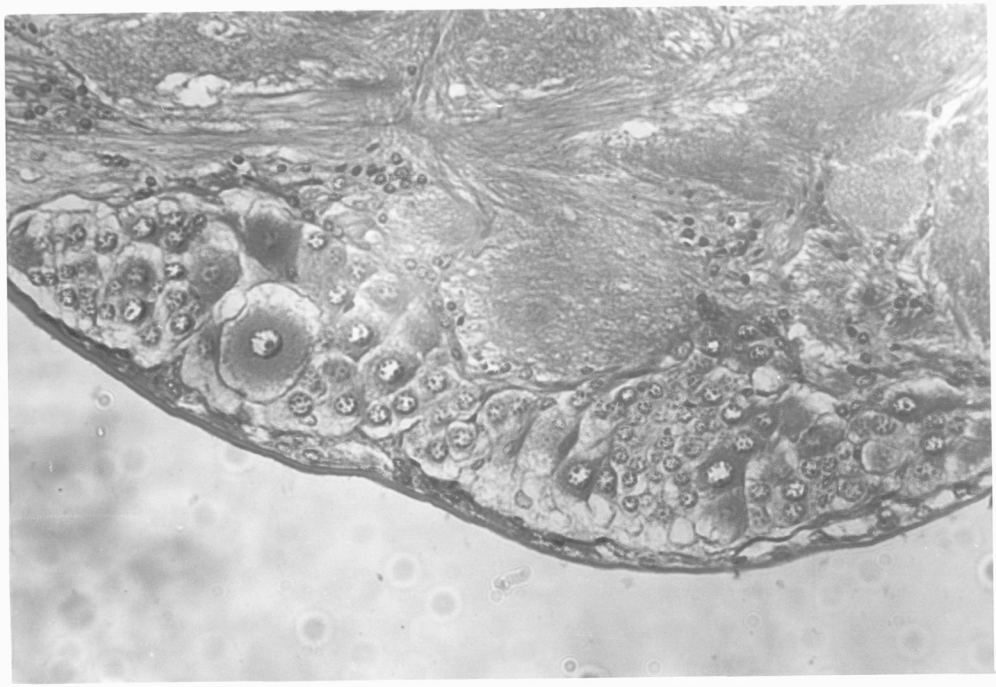


50

100 µm

Fig. 51. Light-micrograph of part of parasagittal section stained by osmium tetroxide, showing neurilemma, types I & IV glial cells, some motor and association neurons and some longitudinal fibre tracts. (X 320).

Fig. 52. Light-micrograph of parasagittal section showing neurilemma, some types of neurons and start of longitudinal fibre tracts st_{28}, st_{29} and st_{32}, as stained by osmium tetroxide. (X 200).

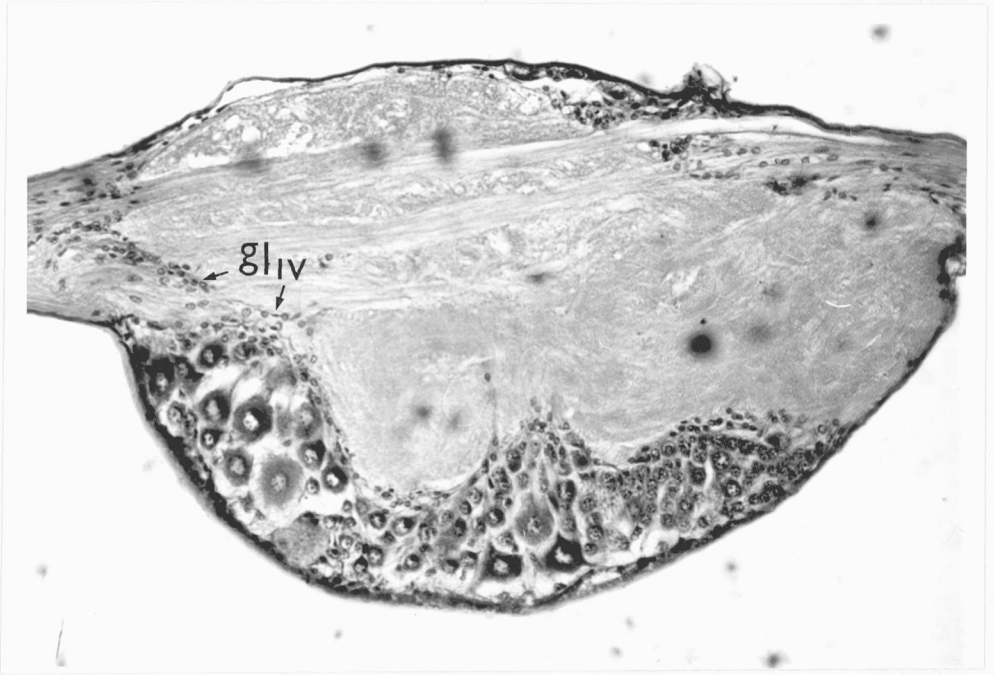


51

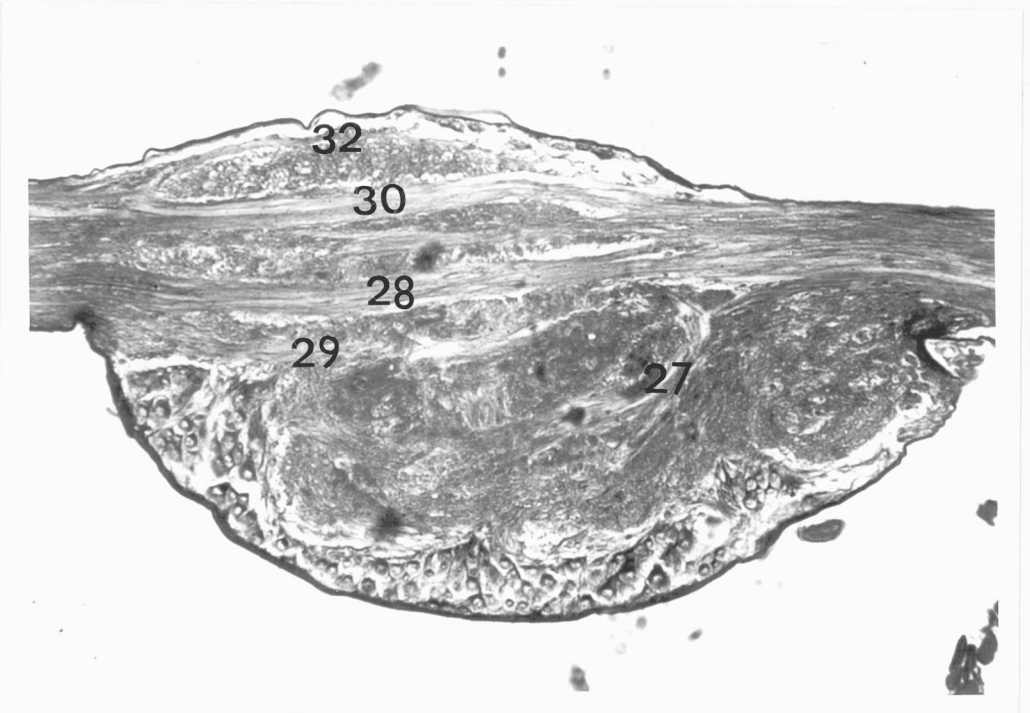


52

Figs. 53 & 54. Light-micrographs of parasagittal sections as stained by osmium tetroxide showing types of neurons, type IV glial cells and longitudinal fibre tracts st_{27}, st_{28}, st_{29}, st_{30} and part of st_{32}. (X 200).



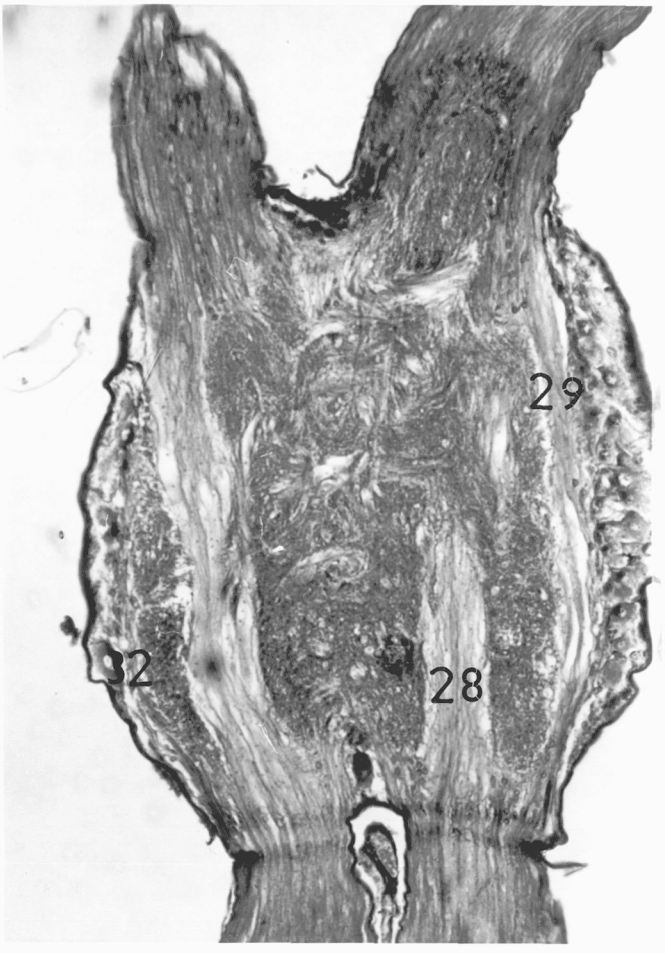
53



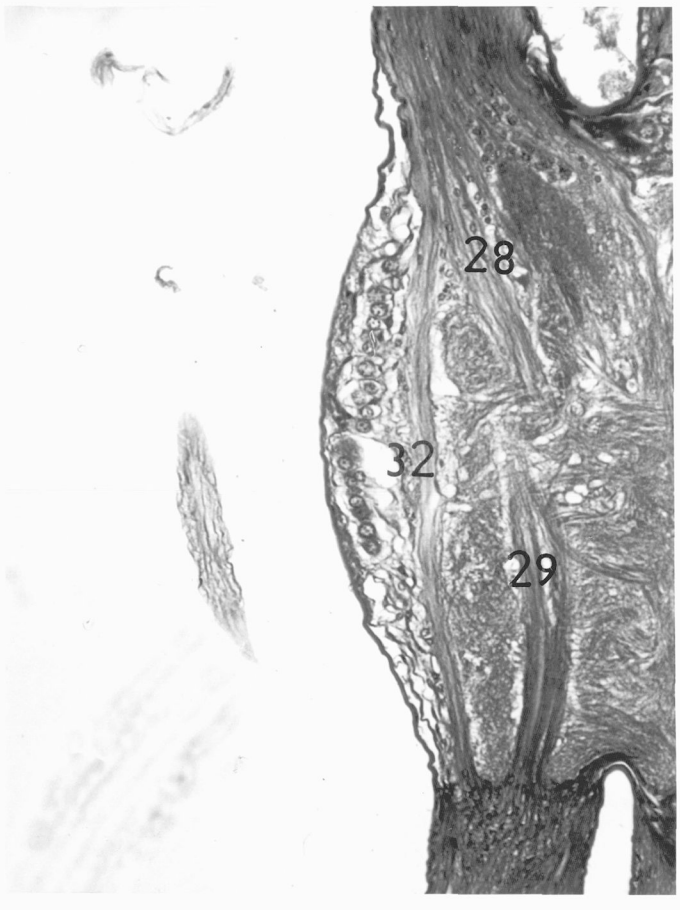
54

Fig. 55. Light-micrograph of frontal section showing some neurons and longitudinal fibre tracts st_{28}, st_{29} and st_{32}, as stained by osmium tetroxide. (X 200).

Fig. 56. Light-micrograph of frontal section showing longitudinal fibre tracts st_{28}, st_{29} and st_{32}. (X 200).

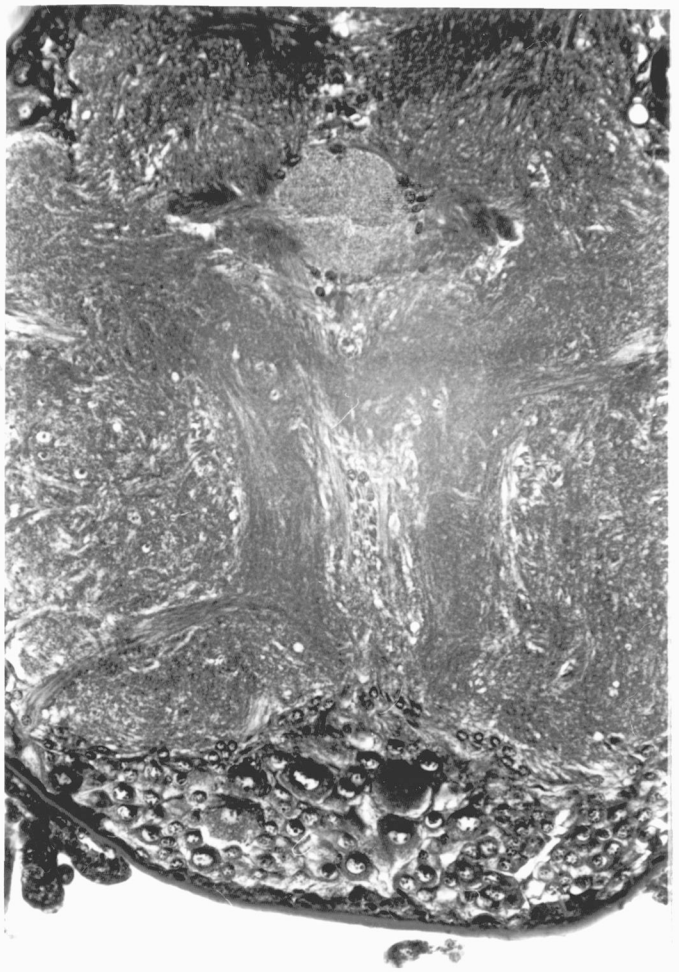


55

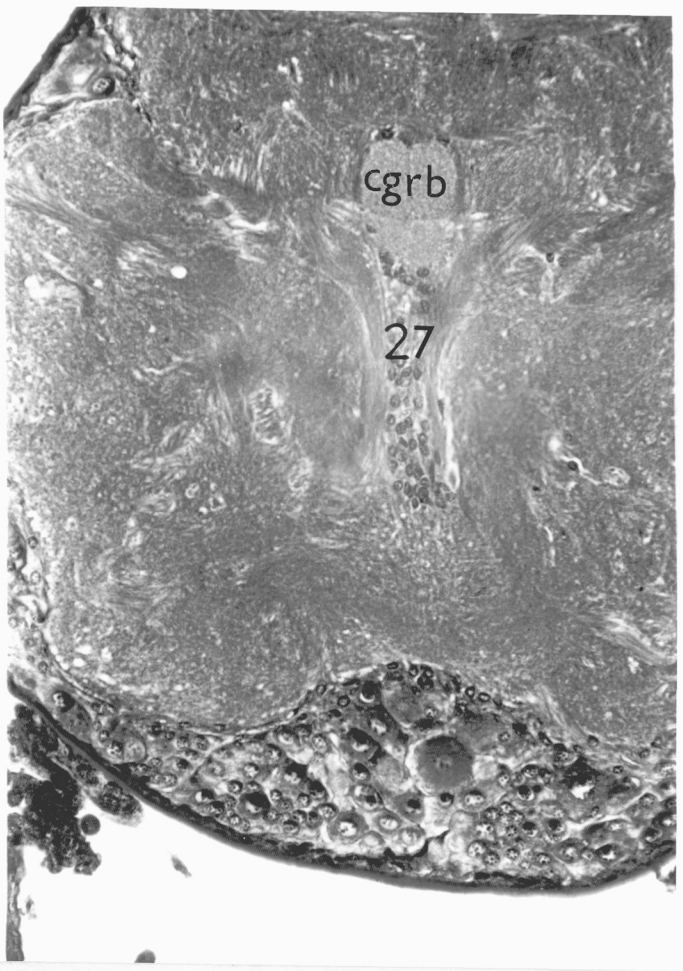


56

Figs. 57 & 58. Light-micrograph of frontal section showing neurons and neurilemma, neuropile and central glomerular body and start of longitudinal fibre tracts st_{27}. (X 320).



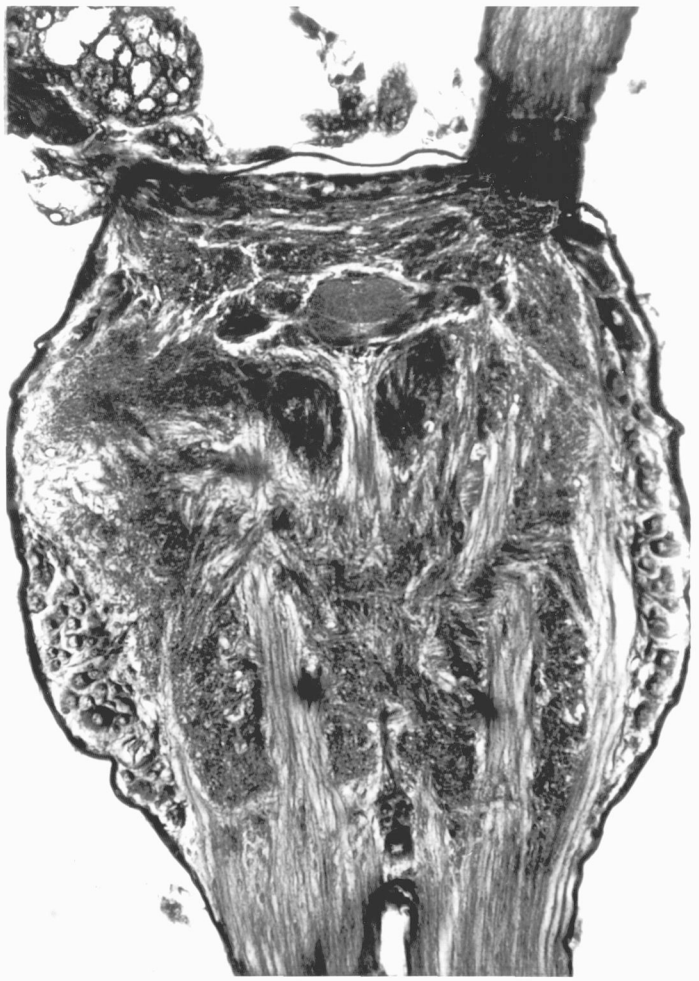
57



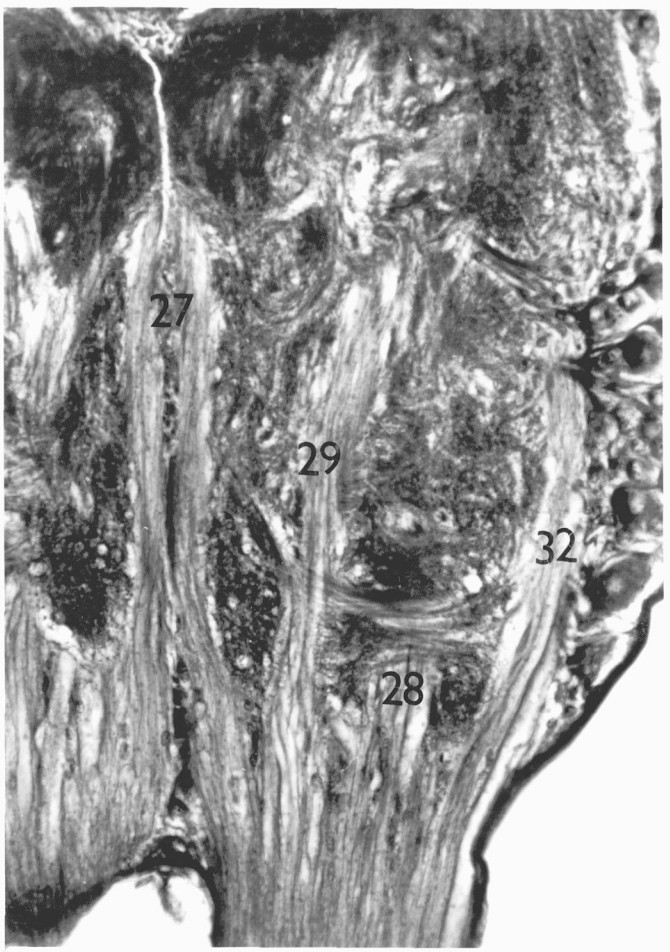
58

Fig. 59. Light-micrograph of frontal section showing the central glomerular body and longitudinal fibre tracts st_{27}, st_{29} and st_{32} as stained by osmium tetroxide. (X 200).

Fig. 60 Light-micrograph of frontal section showing the beginning of longitudinal fibre tracts st_{27}, st_{28}, st_{29} and st_{32} as stained by osmium tetroxide. (X 320).



59



60

Figs. 61 & 62. Light-micrographs of frontal section showing the longitudinal fibre tracts st_{27}, st_{28}, st_{29}, st_{32} and the central glomerular body, as stained by osmium tetroxide. (X 200 & 320 respectively).

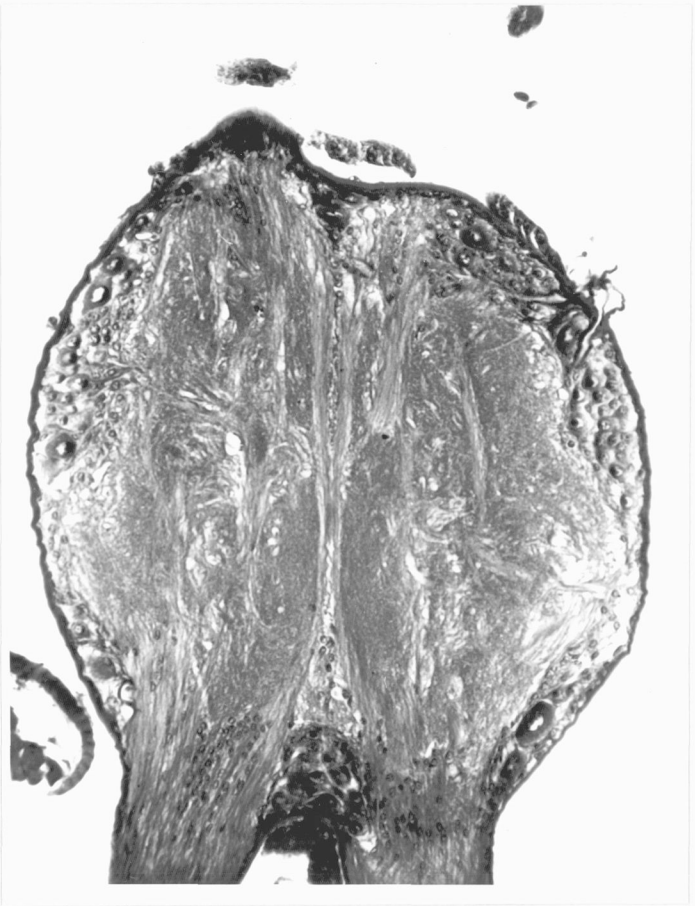


61

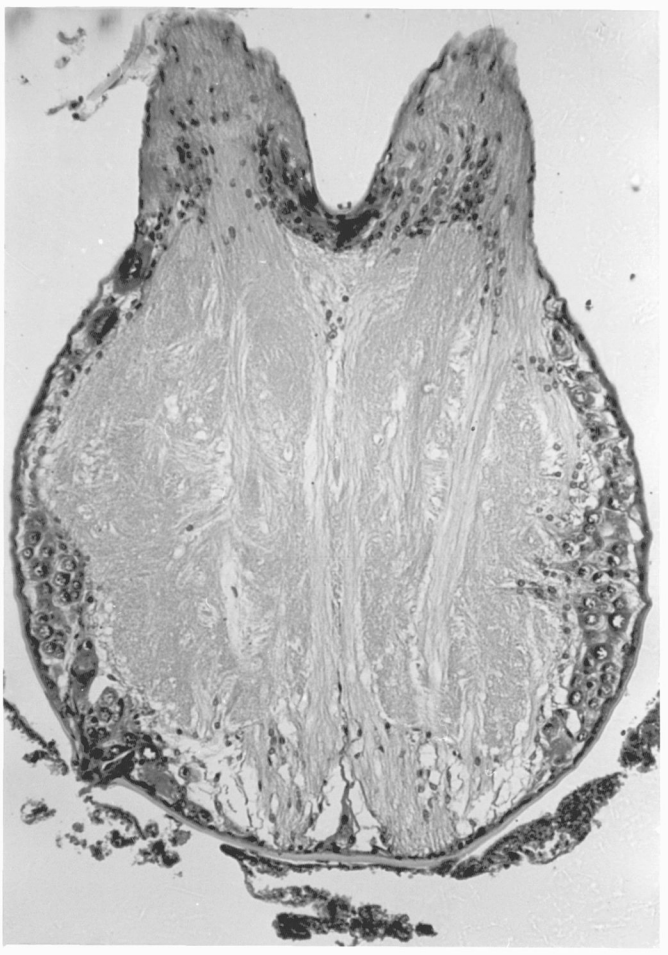


62

Figs. 63 & 64. Light-micrographs of frontal section showing longitudinal fibre stracts st_{27}, st_{28} and st_{29}. (X 200).

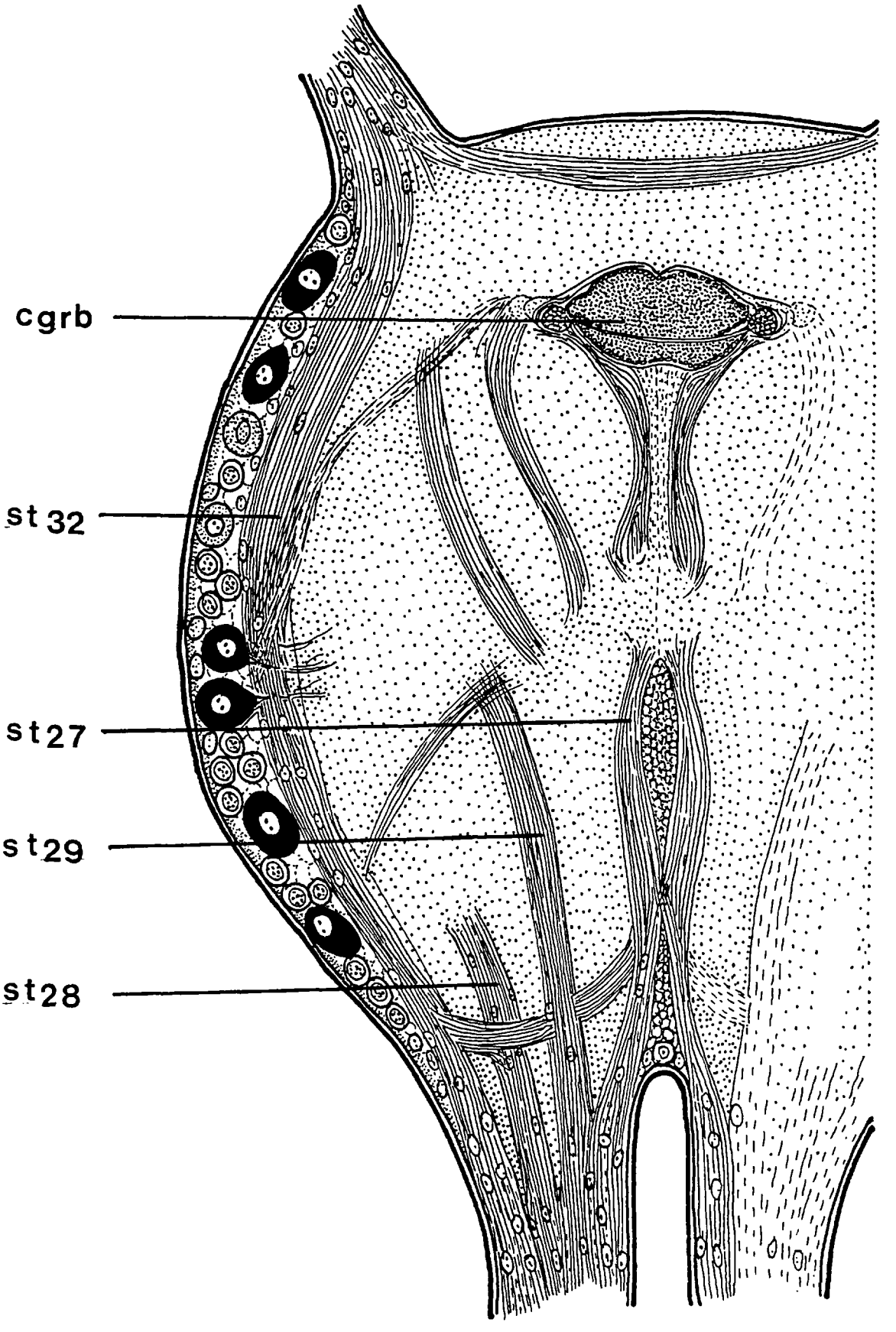


63



64

Fig. 65. Frontal section through suboesophageal ganglion, showing some neurons and longitudinal tracts.



65

5. Axonal pathways:

The following part is a description of some axons traced within the suboesophageal ganglion of Acheta domesticus (L.) after staining by the osmium ethyl gallate technique of Wigglesworth (1957). The neurons and axons have been illustrated mainly from transverse and frontal sections, with some others from a series of vertical sections. Although several papers have been concerned with the structure of the insect central nervous system studies concerning the structure and axonal pathways in the suboesophageal ganglion are very few. The suboesophageal ganglion of an adult Trichopteran has been investigated by Ehnbom (1948). Axonal pathways have been traced by Hilton (1911a) in the ventral ganglia of Corydalis, and by Pyle (1941), in the thoracic ganglia of Ephestia, while Zawarzin (1924a & b) described the types of the neurons in the nymphs of a dragonfly. Guthrie (1961), has studied the topography of the nervous system in Gerris, with some information relating to individual neurons. Mill (1964), described the structure of the eighth abdominal ganglion of Aeshna, with mention of some neuron tracts.

One hundred and twelve neurons and axons were observed in the present investigation, but there are many further axons visible which could not be traced either to a cell body or, on the other hand, to the point of arborization within the neuropile. It is very difficult to follow individual fibres throughout their length and dendrite branches could not easily be located. Guthrie (1961), has already pointed out that the fibres of interneurons were difficult to distinguish throughout their length, and he added that the most successful methods used in his study of Gerris were Palmgren's "silver-on-the-slide" technique and the rapid silver nitrate method of Golgi. Cohen & Jacklet (1967), studied the distribution

of the motor nerve cell bodies in the metathoracic ganglion of the cockroach Periplaneta americana, recognising 3000 neurons in the ganglion. Seabrook (1968), described and illustrated 76 axonal tracts in the seventh abdominal ganglion of the desert locust Schistocerca gregaria, while in 1970 he referred to a hundred neurons within the terminal ganglionic mass of the same species.

Table 2 and Figs. 66 to 71 represent those neurons which have been located in various series of transverse sections. Neurons have been listed in figures as they appear from the anterior to the posterior ends of the ganglion. Fig. 66 represents neurons 1 - 8, i.e. those which have been located in transverse section of the ganglion extending from the anterior part to the level of the hypopharyngeal nerve. Fig. 67 represents neurons 9 - 24 compiled from series of transverse sections extending from the hypopharyngeal nerve to the level of the maxillary nerve, while Fig. 68 shows neurons 25 to 43, compiled from a series of sections extending from the region of the maxillary nerve to the level of the labial nerve. The last figure in the transverse section series is Fig. 69, which represents neurons 44 to 53, located between the level of the salivary nerve and the posterior end of the ganglion.

Table 2

Neurons 1 - 53 of the suboesophageal ganglion as seen in the adult of Acheta, compiled from a series of transverse sections.

No.	Origin	Course of axon	Other remarks
1	From an interneuron of the anterior ventro-lateral group.	The axon passes to the middle region of ipsi-lateral neuropile where it disap-pears.	Fig. 66; Axon passing through fibre tracts st_1.
2	An interneuron of the anterior ventro-lateral group.	The axon runs dorsally crossing the midline to the middle of the contralateral neuropile where it disappears.	Fig. 66; The axon runs via tract st_1 then curves via st_{2a} to cross the midline.
3	A motor neuron, its cell body lying in the anterolateral region of the ganglion	Axon runs ventrally to the ventro-lateral region of the ganglion.	Fig. 66.
4	An interneuron of the anteroventral group.	The axon passes laterally and curves dorsal-ward via tract st_1 crossing the mid-line through tract st_2 to the central region of the contralateral neuropile.	Fig. 66; Its dendrites are distributed in the mid-contralateral neuropile.
5	A motor neuron of the anterolateral group	The axon runs ventral to the ventral glomeru-lar body and collaterals run to the ventro-lateral region of neuropile.	Fig. 66; Dendrites arborize in the ventral glomerular body.

No.	Origin	Course of axon	Other remarks
6	A motor neuron of the same group as 5.	Runs ventral to and distributed throughout the ipsilateral lateral neuropile.	Fig. 66.
7 ?	A sensory neuron from the hypopharyngeal nerve.	Runs dorsally, and turns slightly to enter longitudinal tract st_4.	Fig. 66.
8 ?	A sensory neuron from the hypopharyngeal nerve.	The axon passes dorsally to the ventral glomerular body.	Fig. 66. Dendrites arborize in the ventral glomerular body.
9 ?	A sensory neuron from the hypopharyngeal nerve.	Runs dorsally to enter the central glomerular body, collateral leaves to join the ventral glomerular body.	Fig. 67; Dendrites arborize in the central glomerular body and ventral glomerular body.
10	A motor neuron of the ventrolateral group.	The axon runs dorsally and arborizes in the middle region of the ipsilateral lateral neuropile.	Fig. 67.
11	A motor neuron of the ventrolateral group.	Axon runs dorsally and to the middle region of the neuropile, then curves down to the ventral ipsilateral region of the ganglion.	Fig. 67.
12	A motor neuron of the mid-ventral group.	The axon passes dorsally to the dorsal ipsilateral neuropile.	Fig. 67; Collateral runs through the second anterior dorsal commissure (tract st_{2a}).

No.	Origin	Course of axon	Other remarks
13	A motor neuron of the mid-ventral group.	The axon passes dorsally to the central glomerular body.	Fig. 67.
14	An interneuron of the of the mid-ventral group.	The axon runs dorsally to enter the central glomerular body.	Fig. 67.
15	An interneuron of the median ventral group.	The axon runs dorsally to the dorsal ipsilateral neuropile.	Fig. 67.
16	An interneuron of the ventral group.	The axon passes to the laterodorsal region of the ipsilateral neuropile.	Fig. 67.
17	A motor neuron of the dorsolateral region of the ganglion.	Its axon passes ventrally and curves to enter the maxillary nerve.	Fig. 67; The neuron curves around the lateral glomerular body.
18	A motor neuron located in the ventrolateral part of the ganglion.	The axon runs dorsally and curves down around the lateral glomerular body to enter the maxillary nerve.	Fig. 67.
19	A motor neuron of the ventrolateral group.	The axon runs dorsally and curves to enter the central glomerular body in which it arborizes.	Fig. 67.
20	An interneuron of the median ventral group.	Runs dorsally then curves down to enter the maxillary nerve.	Fig. 67.

No.	Origin	Course of axon	Other remarks
21	A motor neuron of the mid-ventral group.	The axon passes dorsally, then curves down to the maxillary nerve	Fig. 67; The axon runs down via tract st_9.
22	A sensory neuron contributing to the maxillary nerve.	The axon passes into the central glomerular body in the middle region of the ganglion.	Fig. 67; Axon runs via tract st_9.
23	A sensory neuron contributing to the maxillary nerve.	Runs dorsally to enter the second anterior dorsal commissure with collateral branches to the central glomerular body.	Fig. 67; The axon runs through tract st_{15}.
24	A motor neuron lying between the lateral motor neuron group.	The axon runs dorsally passing into the anterior cervical nerve.	Fig. 67; The axon runs via tract st_{19}.
25	A motor neuron situated in the ventral group.	The axon passes dorsal to reach tract st_{16}.	Fig. 68; This axon probably enters and continues with st_{16}.
26	A motor neuron of the mid-dorsal group.	The axon runs ventrally and curves to enter the anterior cervical nerve.	Fig. 68.
27	An interneuron situated in the mid-dorsal group.	The axon passes down to the ventral part of the ganglion.	Fig. 68; The axon runs through tract st_{22}.

No.	Origin	Course of axon	Other remarks
28	A motor neuron of the mid-dorsal group.	The axon runs downwards to the ventral part of the ganglion	Fig. 68; The axon runs through tract st_{22}.
29	A motor neuron of the mid-ventral group.	The axon runs upwards to the mid-dorsal part of the ganglion.	Fig. 68; Axon runs via tract st_{22}.
30	An interneuron lying in the mid-ventral group.	Its axon passes dorsally to the mid-dorsal region of the ganglion.	Fig. 68; Axon runs via tract st_{22}.
31	A sensory neuron contributing to the maxillary nerve.	The axon extends into the median ipislateral neuropile and enters the longitudinal tract st_{29}.	Fig. 68; Axon runs through st_{16}.
32	A sensory neuron contributing to the maxillary nerve.	The axon passes to the ipsilateral dorsal neuropile then enters longitudinal tract st_{28}.	Fig. 68; It runs via tract st_{15}.
33	A sensory neuron contributing to the maxillary nerve.	Its axon runs dorsally, to enter the longitudinal tract st_{32a}, in the dorsolateral side of the ganglion.	Fig. 68; The axon runs via tract st_{20}.
34	A motor neuron of the mid-ventral group.	Its axon passes dorsally to the median part of the ipsilateral neuropile.	Fig. 68.
35	An interneuron lying in the ventral group.	The axon runs dorsally to the centre of the ipsilateral neuropile.	Fig. 68.

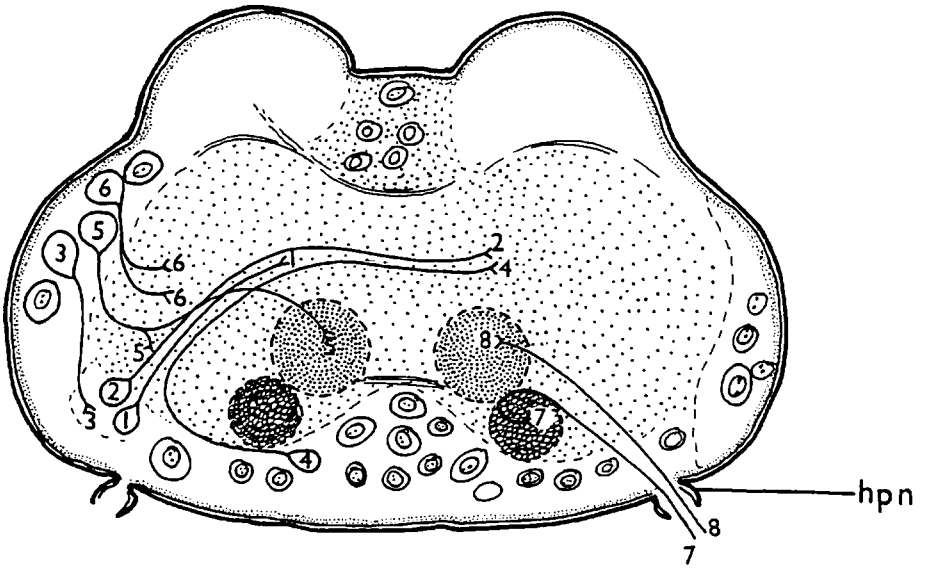
No.	Origin	Course of axon	Other remarks
36	An interneuron of the mid-ventral group.	The axon passes laterally to the lateral region of the ipsilateral neuropile.	Fig. 68.
37	An interneuron situated within the ventral group.	The axon passes to the lateral region of the ipsilateral neuropile.	Fig. 68.
38	A motor neuron of the lateral group.	The axon runs laterally, crossing the midline to the contralateral neuropile then curving ventralwards to run into the labial nerve.	Fig. 68.
39	An interneuron of the lateral group.	The axon runs the same way as that of neuron 38.	Fig. 68
40	A sensory neuron contributing to the labial nerve.	Axon passes dorsally to the ventral region of the neuropile.	Fig. 68
41	A neuron from the labial nerve.	The axon passes to enter longitudinal tract st_4.	Fig. 68; The axon runs via tract st_{26}.
42	A motor neuron lying in the lateral group.	The axon passes dorsally along the margin of the neuropile to enter longitudinal tract st_{32b}.	Fig. 68
43	A motor neuron lying in the lateral group.	The axon runs dorsally to enter longitudinal tract st_{32c}.	Fig. 68.

No.	Origin	Course of axon	Other remarks
44	A sensory neuron contributing to the salivary nerve.	The axon runs to the mid-ventral part of the ganglion and enters longitudinal tract st_{27}.	Fig. 69.
45	A sensory neuron contributing to the salivary nerve.	The axon enters longitudinal tracts st_{39}.	Fig. 69.
46	A motor neuron situated in the lateral group.	The axon runs ventrally, passing into the posterior cervical nerve.	Fig. 69.
47	A neuron contributing to the posterior cervical nerve.	Runs dorsal to the dorsal region of the ganglion.	Fig. 69; Probably enters longitudinal tracts st_{32b}.
48	A motor neuron lying in the lateral group.	The axon passes through tract st_{37} to the mid-dorsal region of the neuropile; a collateral is given off to enter longitudinal tract st_{34}.	Fig. 69.
49	A motor neuron lying in the lateral group.	The same course as axon 48, to the mid-dorsal region of the ganglion	Fig. 69; The axon also runs via tracts st_{37}.
50	A motor neuron of the mid-ventral group.	The axon runs dorsally to enter longitudinal tract st_{27}.	Figs. 69, 70; The axon runs along the midline.
51	A motor neuron situated in the mid-dorsal group.	Its axon descends to the mid-centre of the neuropile.	Figs. 69, 70; The axon runs along the midline.

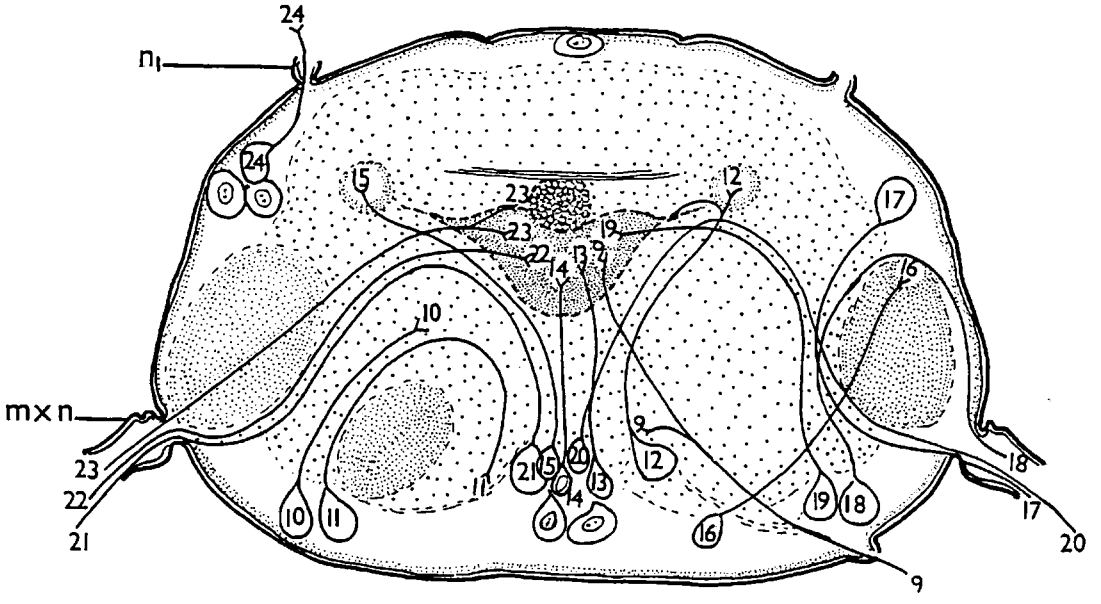
No.	Origin	Course of axon	Other remarks
52	A motor neuron of the same motor cells appearing in the middle of the ganglion near the posterior end.	The axon runs ventrally to the mid-ventral part of the ganglion.	Figs. 69, 71; The axon runs along the mid-line.
53	A motor neuron of the mid-central group.	It is an ascending axon running to the mid-dorsal part of the ganglion.	Fig. 69; The axon runs along the mid-line.

Fig. 66 Neurons 1 - 8 of the suboesophageal ganglion, compiled from a series of transverse sections extending from the anterior part to the level of the hypopharyngeal nerve.

Fig. 67. Neurons 9 - 24 of the suboesophageal ganglion compiled from a series of transverse sections extending posteriorly from hypopharyngeal nerve to the level of the maxillary nerve.



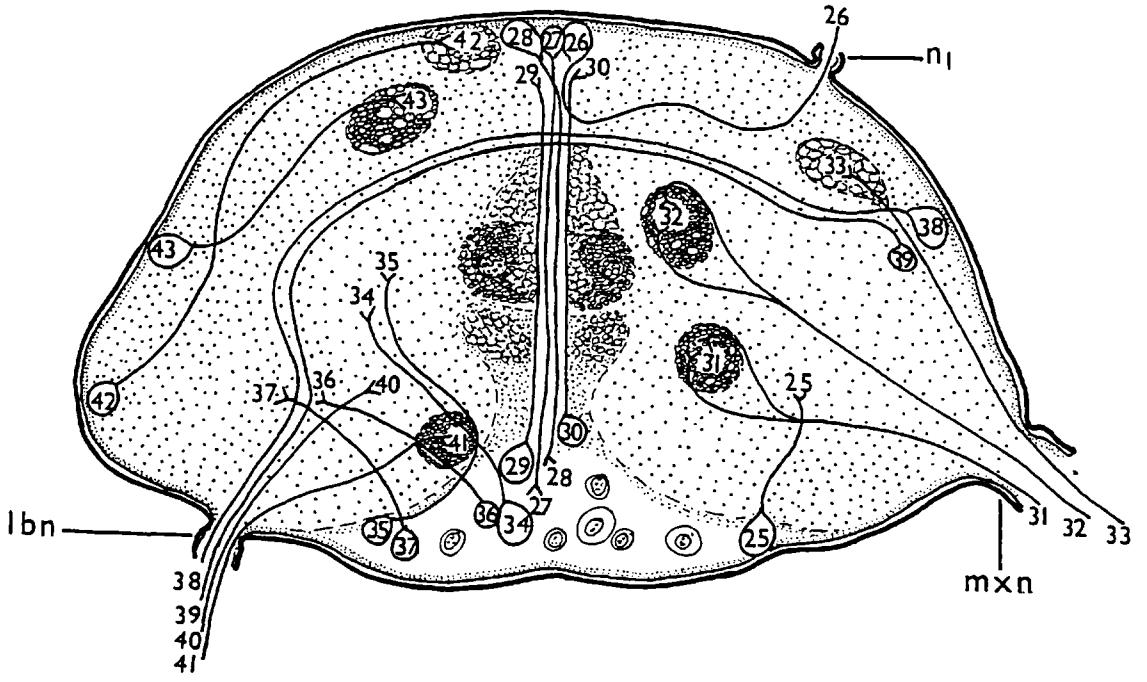
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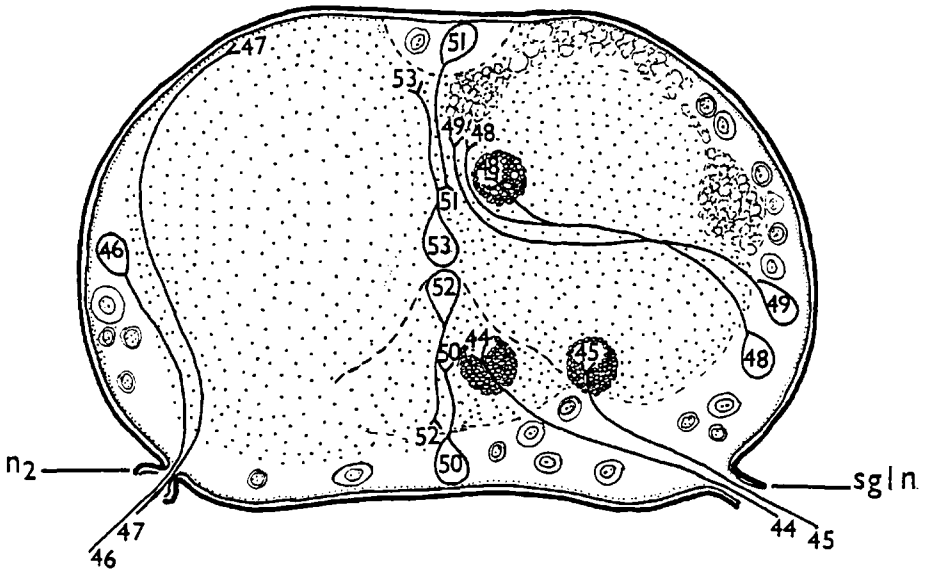
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Fig. 68. Neurons 25 - 43 of the suboesophageal ganglion compiled from a series of transverse sections extending posteriorly from the maxillary nerve to the level of the labial nerve.

Fig. 69. Neurons 44 - 53 of the suboesophageal ganglion, compiled from a series of transverse sections extending from labial and salivary nerve to the posterior end of the ganglion.


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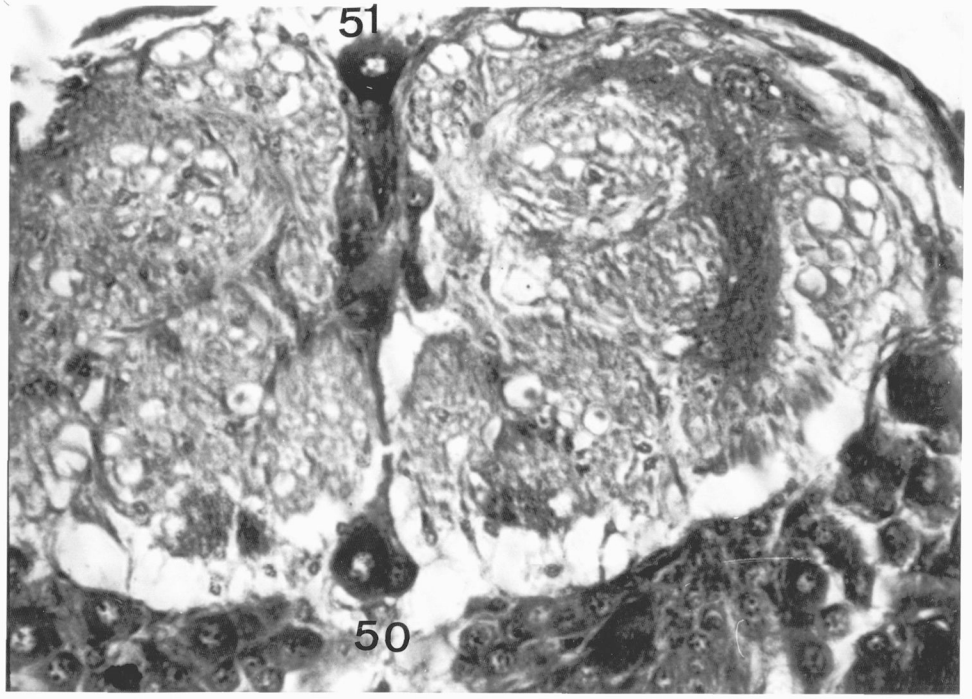

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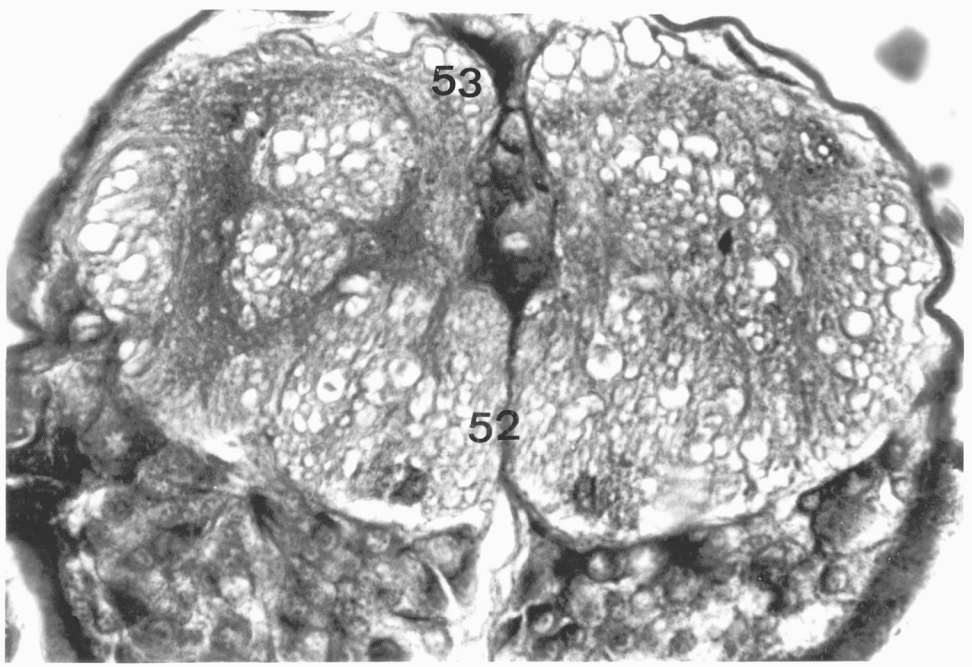
100 μm

Fig. 70. Light-micrograph of transverse section stained by osmium tetroxide showing posterior end of the ganglion and the motor neurons Nos. 50 & 51 with their axons. (X 320).

Fig. 71. Light-micrograph of transverse section stained by osmium tetroxide showing posterior end of ganglion and the central motor neurons and their axons Nos. 52 & 53. (X 320).



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Table 3, and Figs. 72 to 75, illustrate those neurons which have been located in a series of frontal sections. The axons listed in the figures are as they appear gradually from dorsal to ventral through the ganglion. Figs. 72 and 75 represent neurons 54 to 67, located in the dorsal part of the ganglion, while those which appear in the central part (neurons 68 to 80) are illustrated in Figs. 73 and 75. Neurons 81 to 93 have been compiled from the series of sections in the ventral part of the ganglion and are illustrated in Figs. 74 and 75.

Table 3

Neurons 54 to 93 of the suboesophageal ganglion
as seen in the adult of Acheta
compiled from a series of frontal sections

No.	Origin	Course of Axon	Other remarks
54	A neuron from higher centres of the nervous system.	The axon passes to the middle of the ipsilateral dorsal neuropile; a collateral is given off to the anterior and middle regions of the contralateral neuropile.	Figs. 72, 75; The axon passes via longitudinal tract st_{29}.
55	A neuron from higher centres of the nervous system.	The axon runs ventrally to the posteromedian end of the ganglion.	Figs. 72, 75; It is a neuron of longitudinal tract st_{27}.
56	A neuron of unknown position associated with the longitudinal tract st_{27}.	The axon passes through the whole ganglion.	Figs. 72, 75.

No.	Origin	Course of Axon	Other remarks
57	A neuron of unknown position associated with longitudinal tract st_{32}.	The axon passes through the whole length of the ganglion.	Figs. 72, 75.
58	An ascending neuron from the first thoracic ganglion.	The axon passes to the lateral posterior region of the dorsal neuropile.	Figs. 72, 75; The axon passes via longitudinal tract st_{32}.
59	A neuron from higher centres.	The axon runs posteriorly and curves to arborize in the anterior region of the ipsi-lateral dorsal neuropile.	Figs. 72, 75.
60	A neuron of unknown position associated with longitudinal tract st_{27}.	The axon passes through the whole ganglion; an anterior collateral is given off which passes to the anterior region of the dorsal contra-lateral neuropile.	Figs. 72, 75.
61	A neuron from the higher centres	The axon passes to the mid-lateral region of the ipsi-lateral dorsal neuropile.	Figs. 72, 75.
62	A neuron ascending from the first thoracic ganglion.	The axon passes to the posterior region of the ipsi-lateral dorsal neuropile.	Figs. 72, 75.
63	A neuron ascending from the first thoracic ganglion.	The axon runs up and curves to the posterior region of the mid-dorso-ventral contra-lateral neuropile.	Figs. 72, 75.

No.	Origin	Course of Axon	Other remarks
64	An interneuron located in the lateral group.	The axon runs to the mid contralateral neuropile then curves, descending through the first thoracic connectives.	Figs. 72, 75; This axon enters the first thoracic connective via longitudinal tract st_{27}.
65	A motor neuron located in the posterior lateral group.	It is an axon descending to the contralateral side of the neuropile, and then curving down to enter the first thoracic connectives; a collateral is given off to the middle posterior region of the ipsilateral neuropile.	Figs. 72, 75.
66	A motor neuron of the posterior lateral group.	The axon passes to the middle posterior region of the contralateral neuropile.	Figs. 72, 75.
67	An ascending neuron from the first thoracic ganglion or a lower centre.	Passes up to the ipsilateral antero-dorsal neuropile.	Figs. 72, 75; The axon passes via tract st_{29}.
68	A neuron of unknown position associated with longitudinal tract st_{29}.	The axon passes through the ganglion.	Figs. 73, 75.
69	A neuron ascending from the first thoracic ganglion	The axon passes to the anterior region and enters the anterocentral glomerular body.	Figs. 73, 75; The axon runs through tract st_{29}.

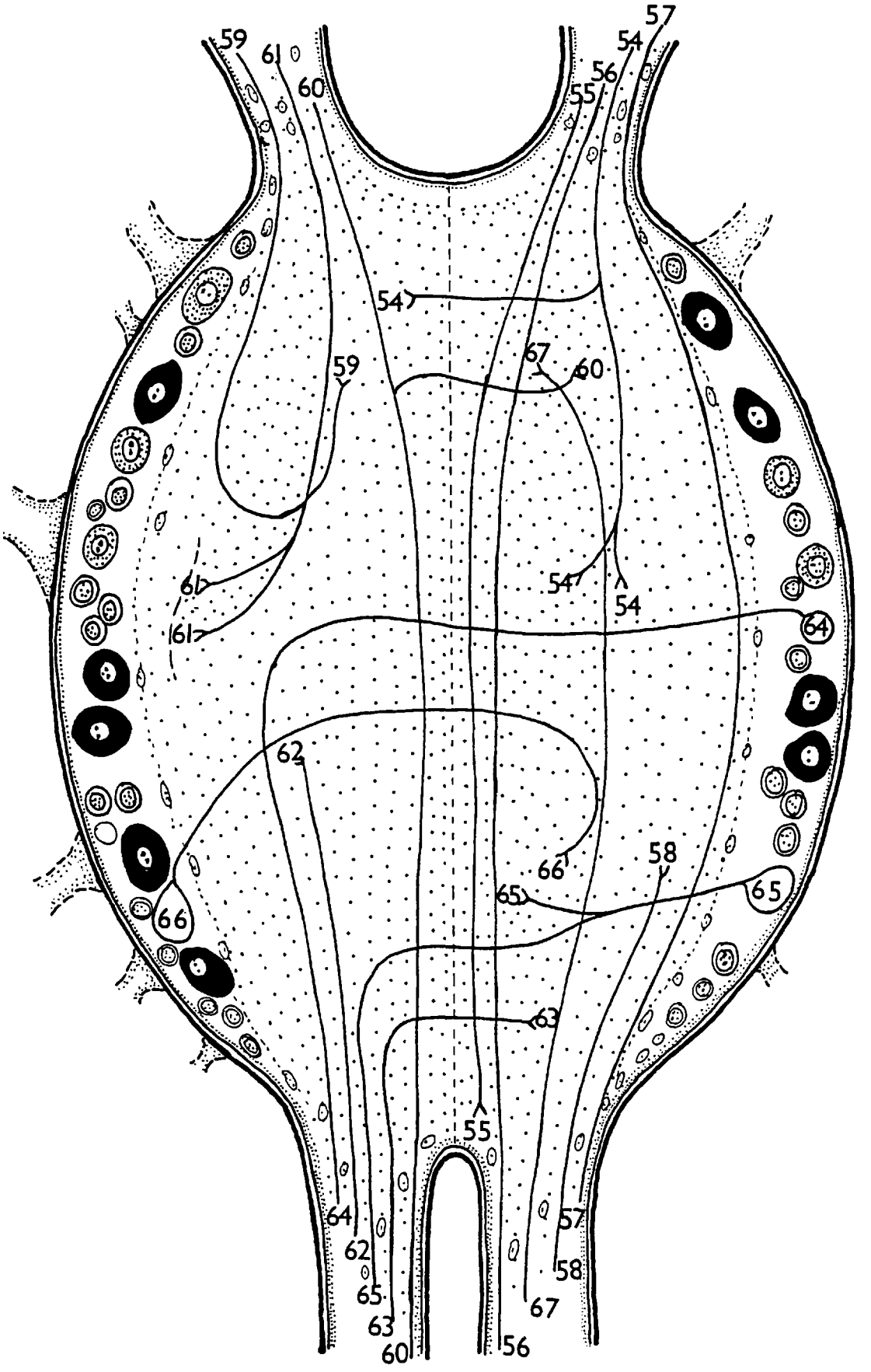
No.	Origin	Course of Axon	Other remarks
70	An ascending neuron from the first thoracic ganglion or a lower centre.	Passes to the centre of the ipsilateral neuropile.	Figs. 73, 75.
71	A motor neuron of the lateral group.	The axon ascends to the anterocentral glomerular body.	Figs. 73, 75.
72	A motor neuron situated in the lateral group	The axon passes to the centre of the ipsilateral neuropile.	Figs. 73, 75.
73	An ascending neuron from the first thoracic ganglion	The axon passes upwards crossing to the opposite side, and runs anteriorally to enter the anterior central glomerular body.	Figs. 73, 75; The axon runs with longitudinal tract $s\iota_{27}$.
74	A motor neuron located in the anterolateral part of the ganglion	The axon passes lateralwards to the anterolateral region of the contralateral neuropile.	Figs. 73, 75.
75 ?	A sensory neuron of the mandibular nerve.	Axon passes to the anterolateral region of the contralateral neuropile.	Figs. 73, 75.
76 ?	A sensory neuron from the salivary nerve.	The axon runs to the middle region of the ipsilateral neuropile.	Figs. 73, 75.
77 ?	A sensory neuron from the salivary nerve.	The axon passes to the middle region of the ipsilateral neuropile.	Figs 73, 75.

No.	Origin	Course of Axon	Other remarks
78	A motor neuron situated in the antero-lateral part of the ganglion.	The axon runs to the middle of the ganglion and enters the anterior central glomerular body.	Figs. 73, 75.
79	A sensory neuron contributing to the salivary nerve.	Passes to the middle region of the ipsilateral neuropile then curves down (before the midline) and disappears shortly before the ventral connectives.	Figs. 73, 75.
80	A sensory neuron contributing to the salivary nerve.	Runs to the mid-line and passes to the contralateral neuropile then disappears in the middle of the posterior region.	Figs. 73, 75.
81	A neuron from the group of motor and association neurons which is situated in the median antero-ventral region of the ganglion.	The axon passes anteriorly and bifurcates, sending a branch into each of the mandibular nerves.	Figs. 74, 75.
82 ?	A sensory neuron from the mandibular nerve.	The axon passes to the middle region of the ipsi-lateral ventral neuropile; a col-lateral is given off to the anterolateral region of the ipsi-lateral neuropile.	Figs. 74, 75.

No.	Origin	Course of Axon	Other remarks
83 ?	A sensory neuron from the mandibular nerve.	The axon runs to the anterior region of the ipsilateral ventral neuropile.	Figs. 74, 75.
84	A motor neuron located in the latero-ventral group.	The axon passes to the middle postero-ventral region of the ipsilateral neuropile.	Figs. 74, 75.
85	A motor neuron located in the antero-ventral part of the ganglion.	Axon enters the hypopharyngeal nerve.	Figs. 74, 75.
86 ?	A sensory neuron from the hypopharyngeal nerve.	The axon passes to the anteroventral region of the ipsilateral neuropile.	Figs. 74, 75.
87 ?	A sensory neuron from the labial nerve.	Passes to the posteroventral part of the neuropile; collateral is given off to the ipsilateral lateral neuropile.	Figs. 74, 75. The first branch, which is given off in the postero-ventral region, perhaps enters the transverse tract.
88	A neuron from the labial nerve.	The axon passes to the middle posteroventral region of the ipsilateral neuropile.	Figs. 74, 75.
89	A neuron from the labial nerve.	Axon passes to the anteroventral centre of a motor and association neuron group.	Figs. 74, 75; In fact it is not clear whether the axon is coming from or going to the labial nerve.

No.	Origin	Course of Axon	Other remarks
90 ?	A sensory neuron from the maxillary nerve.	The axon passes to the lateral region of the neuropile.	Figs. 74, 75.
91 ?	A sensory neuron from the maxillary nerve.	The axon passes to the lateral region of the neuropile.	Figs. 74, 75; This axon comes from a small branch of the maxillary nerve.
92	A motor neuron of the anteroventral centre group.	The axon passes into the maxillary nerve.	Figs. 74, 75.
93	Probably a sensory neuron contributing to the maxillary nerve.	The axon passes laterally and bifurcates to enter the mid central area of of the motor and association neuron group.	Figs. 74, 75; It is not clear whether the axon is coming from or going to the maxillary nerve.

Fig. 72. Neurons 54 - 67 of the suboesophageal ganglion, compiled from a series of doros-frontal sections.

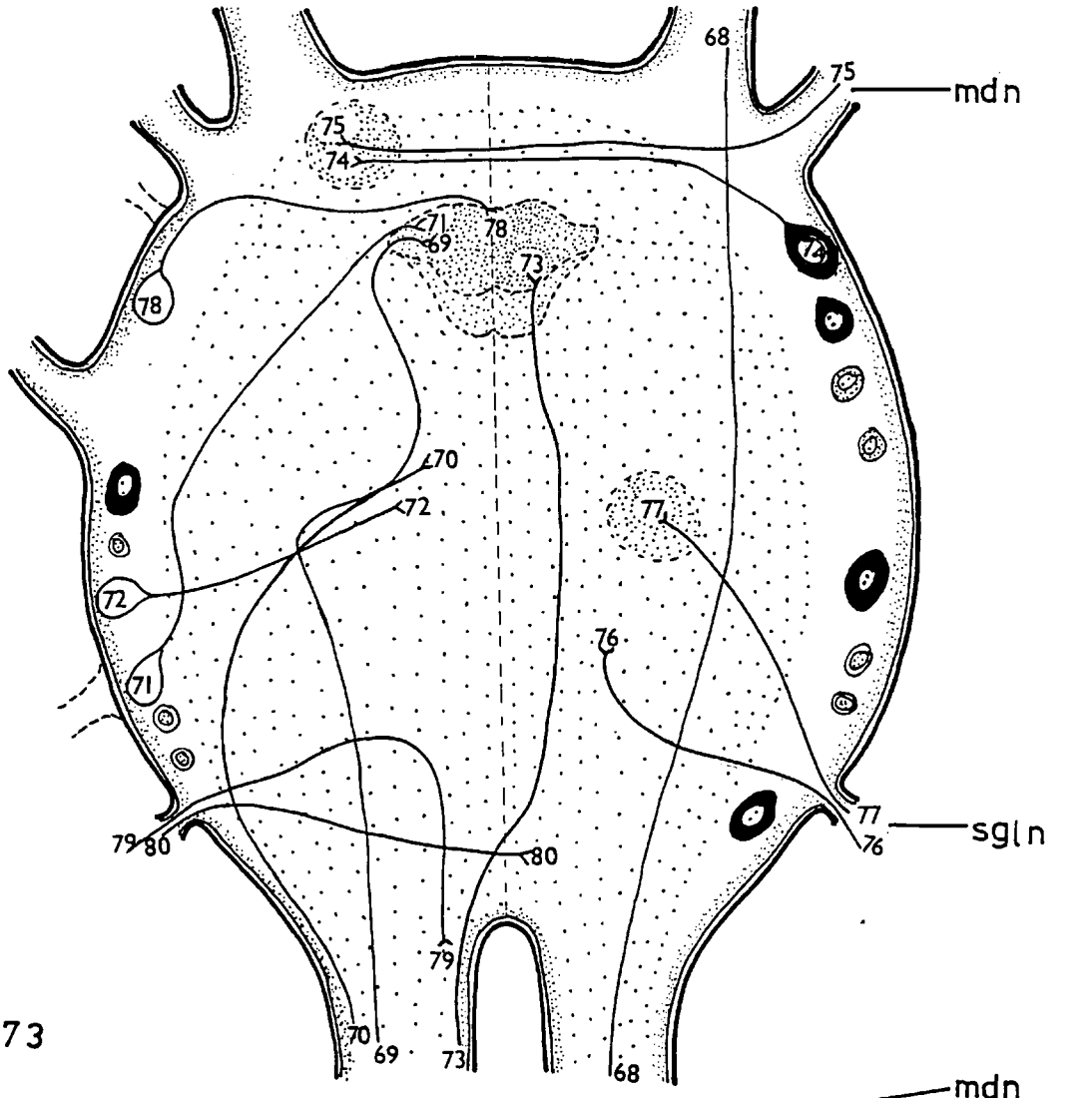

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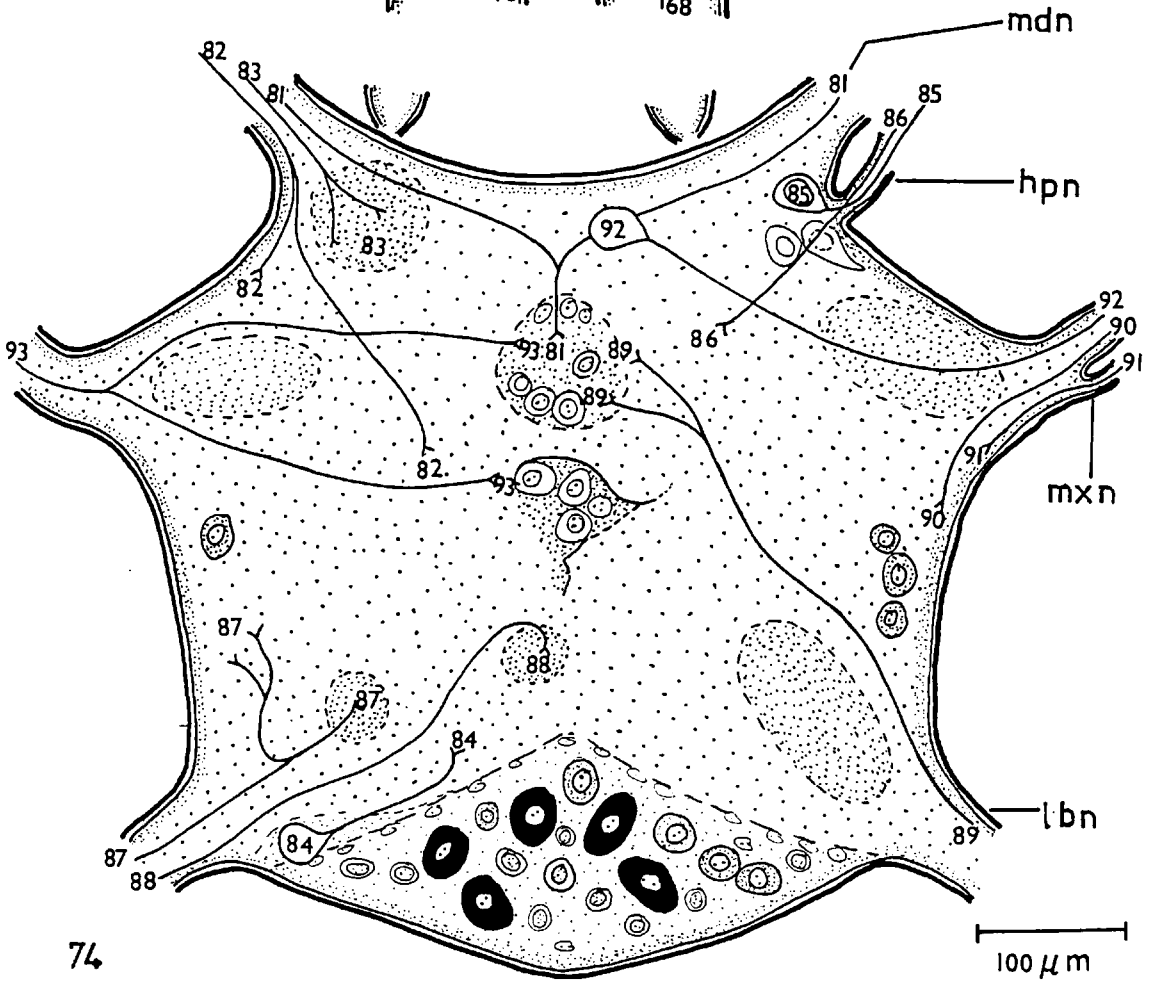
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Fig. 73. Neurons 68 - 80 of the suboesophageal ganglion, compiled from a series of mid-frontal sections (the median part).

Fig. 74. Neurons 81 - 93 of the suboesophageal ganglion, compiled from a series of ventro-frontal sections (the ventro-frontal part).

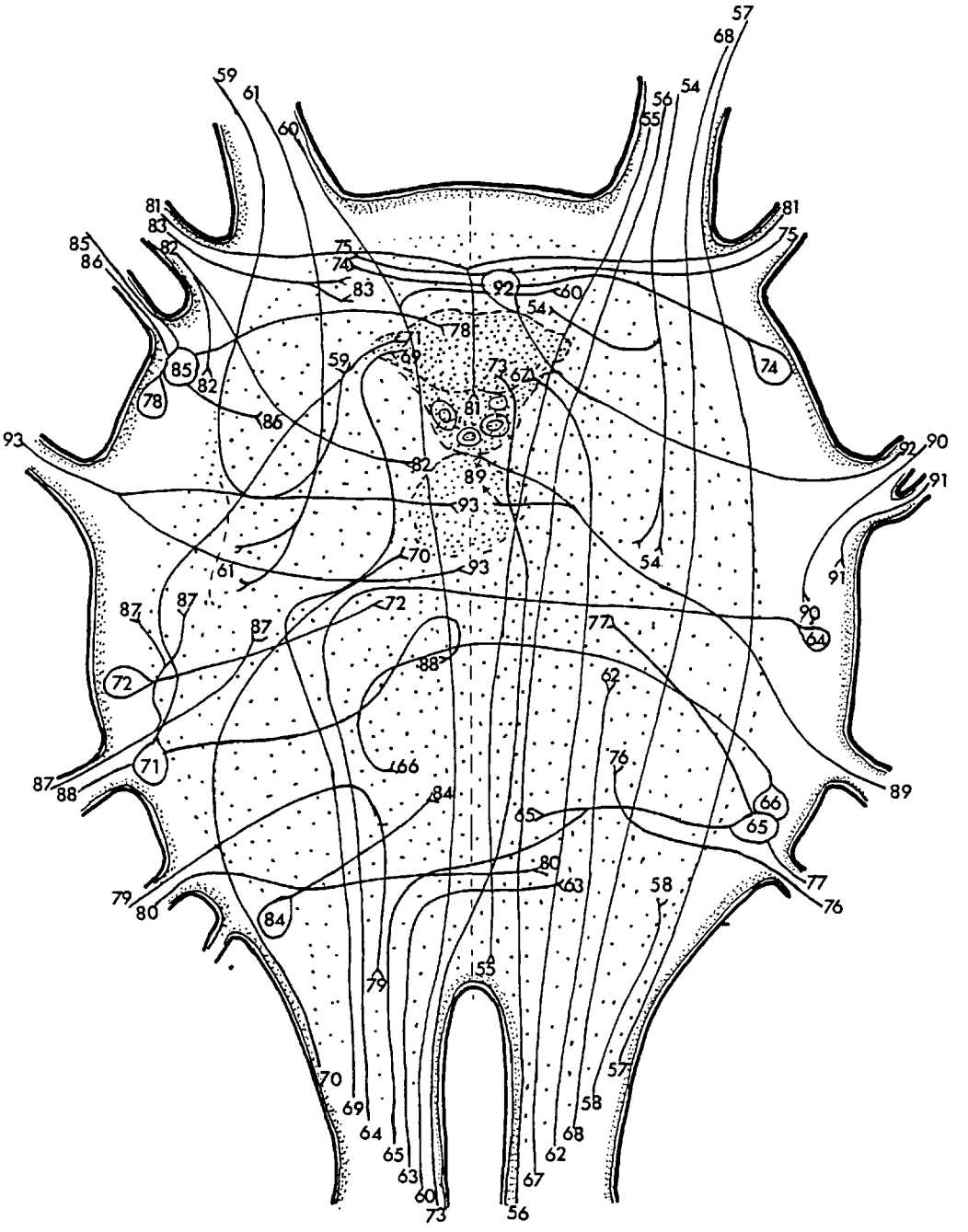


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Fig. 75. Neurons 54 - 93 of the suboesophageal ganglion, compiled from a series of frontal sections (dorsal to ventral, through the ganglion).



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100 μm

Table 4 lists some neurons which were observed in a series of vertical sections. Fig. 76 illustrates neurons 94 to 112, i.e. those which have been identified in vertical sections through the whole ganglion.

Table 4

Neurons 94 - 112 of the suboesophageal ganglion, as seen in the adult of Acheta, compiled from a series of vertical sections.

No.	Origin	Course of Axon	Other remarks
94	A motor neuron lying in the anteroventral group.	The axon passes dorsally to the anterior middle region of the ipsilateral neuropile.	Fig. 76.
95	An interneuron situated in the posteroventral group.	The axon passes dorsalwards to the postero-median region of the ipsilateral neuropile.	Fig. 76.
96	A neuron from the first thoracic ganglion.	The axon passes to the posterior ipsilateral ventral neuropile.	Fig. 76.
97	A motor neuron located in the mid-ventral group.	Its axon turns dorsally and disappears in the anterior region of the ipsilateral ventral neuropile.	Fig. 76.
98	An interneuron lying in the ventral group.	The axon passes anterodorsally to the mid-anterior region of the ipsi-lateral neuropile.	Fig. 76.

No.	Origin	Course of Axon	Other remarks
99	A neuron in the posterior ventral nerve cord, from the first thoracic connective.	The axon passes to the back of the anterior region of the ipsilateral ventral neuropile.	Fig. 76.
100	A neuron of unknown position, associated with longitudinal tract st_{29}.	Its axon passes through the ganglion via longitudinal tract st_{29}.	Fig. 76.
101	A neuron, also of unknown position, associated with longitudinal tract st_{28}.	The axon passes through the ganglion, joining the anterior and posterior connectives.	Fig. 76.
102	A neuron in the brain, associated with the anterior connective.	The axon runs posteriorly and disappears in the middle dorsal region of the ipsilateral neuropile.	Fig. 76; This axon runs through longitudinal tract st_{30}.
103	An interneuron of the anterior latero-ventral group.	The axon runs dorsal to the anterior region of the ipsilateral neuropile.	Fig. 76.
104	A neuron posterior to the suboesophageal ganglion, associated with the first thoracic connective.	The axon passes to the mid dorsal region of the ipsilateral neuropile.	Fig. 76; Axon passes via longitudinal tract st_{32}.
105	A motor neuron lying in the posteroventral group.	The axon passes to the mid-posterior region of the ipsilateral neuropile.	Fig. 76.

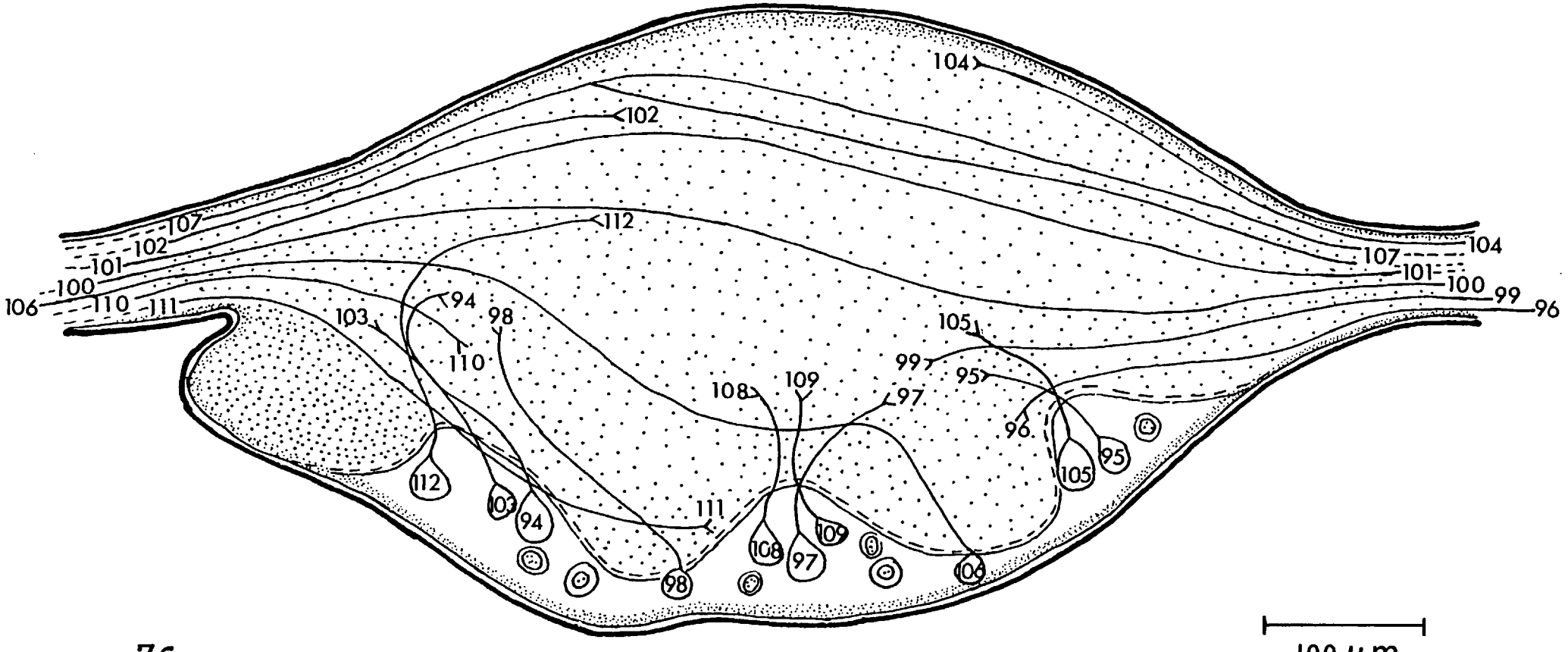
No.	Origin	Course of Axon	Other remarks
106	An interneuron situated in the ventral group.	The axon runs anteriorly, passing to the contralteral neuropile and entering the circum-oesophageal connective.	Fig. 76.
107	A neuron, of unknown position, associated with longitudinal tract st_{30}.	The axon passes through the ganglion, dividing into two branches before entering the posterior connective.	Fig. 76.
108	A motor neuron of the mid-ventral group.	The axon passes dorsally to the middle anterior region of the ventral neuropile.	Fig. 76.
109	An interneuron located in the mid-ventral group.	The axon passes dorsally to the ventral neuropile in which it disappears.	Fig. 76.
110	A neuron in the brain, associated with the anterior connective.	The axon passes to the ventral region of the ipsilateral neuropile.	Fig. 76.
111	A neuron in the brain, associated with the anterior connective.	The axon passes to the ventral region of the ipsilateral neuropile.	Fig. 76.
112	A motor neuron located in the anterior ventral group.	Its axon passes dorsally to reach longitudinal tract St 29 and then disappears among its fibres.	Fig. 76.

Three general points should be noted in connection with this list of 112 axonal tracts. Firstly, despite the difficulty of tracing such tracts, I am reasonably confident that all the above have been clearly recognised and are based on detailed examinations of several series of sections. There are no doubt many other axons and parts of axons present which cannot be traced in full and are not, therefore, recorded above; those listed represent a minimum number of well defined pathways. Secondly, the possibility exists when making these observations that some of the pathways recognised in, say, a transverse series will be recorded again in analyses based on frontal or sagittal sections. Whether this is so will depend partly on the direction of the pathway in relation to the plane of the section. Where possible I have tried to avoid referring to a given pathway under two numbers in the Tables above, but it is obviously difficult to be quite certain on this point. Thirdly, there is the difficulty of identifying the origins and terminations of axons where these lie outside the suboesophageal ganglion. Further histological analysis might confirm or dispose some of the suggested sites of origin or termination which have been indicated tentatively above. There are, however, considerable difficulties in establishing such connections, especially as the major regions of the nervous system would have to be processed separately and the linkages between them established afterwards. Alternatively, physiological techniques would be required, though again there is the difficulty of associating physiologically recognisable pathways with those demonstrated histologically.

ADDENDUM

Since the preceding work was completed, Altman & Kien (1979) have published the first account of a few axonal pathways revealed by cobalt chloride filling in the suboesophageal ganglion of Locusta migratoria. In particular they refer to (a) two fibres innervating the salivary gland; (b) sensory axons from the labium; (c) two motor neurons supplying the cervical musculature; (d) sensory axons from setae on the dorso-occipital regon of the head.

Fig. 76. Neurons 94 - 112 of the suboesophageal ganglion, compiled from a series of vertical sections.


104
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112
103
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109
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98
106
105
95
100 μm

76

VI DETAILED ANALYSIS OF INDIVIDUAL NEURONS (SILVER IMPREGNATION TECHNIQUES)

1. Introduction:

There are relatively few studies of the histology or fine structure of the suboesophageal ganglion in insects. There are, however, many descriptions of the histology of the Brain, Optic Lobes and Retina of insects and other animals, some of which describe appropriate microscopical and ultrastructural techniques. Near the end of the last century some studies of the correlation of central neural structures and functions were started by Cajal (1892, 1909, 1910), Sánchez (1915, 1919) and Cajal & Sánchez (1915), who used Golgi preparation methods or other techniques derived from Golgi preparations to study the histology of the optic lobes. More recently many methods derived from Golgi or osmium tetroxide techniques have been used to study the detailed light-microscope histology of the central nervous system and these have subsequently been extended into electron microscopy (Pedler & Goodland, 1965; Trujillo-Cenóz, 1960 & 1965; Trujillo-Cenóz & Melamed, 1963, 1966; Melamed & Trujillo-Cenóz, 1967).

Trujillo-Cenóz used a Golgi method of selective impregnation for most of his preparation, while Zwarzin (1913), had used both the Golgi and methylene-blue procedures in his studies on Aeshna larvae. Guthrie (1961), mentioned some silver and Golgi techniques which gave good results during his studying of the detailed anatomy of the nervous system of Gerris. Trujillo-Cenóz & Melamed (1963), worked on the fine structure of the second optic neurone synapse in the Retina of Musca domestica and Calliphora vomitoria, using a rapid Golgi process. Dowling & Boycott (1965, 1966), described the synaptic contacts of the five main cell types

of the primate retina as observed with the electron microscope, using some Golgi methods, but they did not give much information as to the distribution of synapses or the relationships of the different types of nerve cells, while the same authors (Boycott & Dowling, 1969), stated that for information on the distribution of the synapses in three dimensions, light microscopy remains necessary.

During the last ten years, modifications of the Golgi-kopsch technique (Lee, 1950), have been used to study the distribution of synapses or the different types of neurones and their spatial relationships. Boycott & Dowling (1969), reported that after their initially inadequate results using the Golgi-Cox method of 1891 (as described by Sholl, 1953), and the rapid Golgi methods (Gatenby & Painter, 1937) they obtained good results with Colonnier's modification of the Golgi-kopsch method (Colonnier, 1964). Strausfeld & Blest (1970), also stated that Golgi-Cox and rapid Golgi methods failed to give satisfactory results during their studies. They also reported some of the non-selective silver impregnation techniques, for example the modification of Holmes' silver method (Holmes, 1943), as used by Blest (1961), as well as Fraser-Rowell's modification (Rowell, 1963), which was used for revealing fibre tracts. They found that for Lepidoptera and Diptera this technique did not reveal sufficient fine detail and that several other variants of Golgi techniques were unsuccessful. The technique they finally adopted was that devised by Colonnier (1964), which gave good results. Strausfeld & Blest (1970), also reported that the Golgi-Cox and rapid Golgi procedures failed to give satisfactory results while another modification of the double-impregnation procedure (Kallius, 1910), revealed only a few components clearly against a deep brown background of unimpregnated tissue.

2. Methods and technique:

The observations reported in this section have been made on sections of the suboesophageal ganglion of Acheta domesticus (Linn.) stained by silver impregnation techniques.

(A) Golgi impregnation procedure:

A variation of the Golgi silver impregnation procedure (Colonnier, 1964), was used. Colonnier's technique has also been employed by Boycott & Dowling (1969), and Strausfeld (1970), yielding good results. A number of different procedures were tried by varying certain factors. As shown in Table 5, such variations were attempted repeatedly until successful results were obtained.

Table 5

Variations in the techniques tested showing the factors altered and the criteria used to assess the quality of the preparations. Indications of the results obtained for some specific variations are listed in the remarks column.

Factors Involved	Variation in preparations	Optimum procedure	Remarks
Age of insects	less than 24 hrs, 24 hrs; older than 24 hrs.	24 hrs. or less if possible	Generally young insects gave good results but less than 24 hrs old poor results due to the damage of ganglion. More than 24 hrs old = poor sections and only few details
Period of pre-fixation	2 hrs; 4 hrs; 6 hrs; 12 hrs; 15 hrs; 24 hrs; and 48 hrs.	12 - 15 hrs	12 - 15 hrs gave best results; other durations gave poor results with dark sections.
pH of pre-fixation	pH 6.9 7.2 7.3	between pH 7.2 & pH 7.3	At pH 6.9 dissections were not clear; some sections had shrunk, others were dark.
Fixation period	24 hrs; 36 hrs; 48 hrs and 72 hrs.	36 hrs.	36 hrs. gave very clear sections; other times gave dark preparations with brown bundles of tracts and not many dendrites shown.
Temperature of fixation of silver impregnation	20°C	20°C	Always carried out at 20°C in the dark.

Factors involved	Variation in preparations	Optimum procedure	Remarks
Duration of silver impregnation	24 hrs; 36 hrs; 48 hrs and 72 hrs.	48 - 72 hrs.	48 - 72 hrs. showed no differences; less than 48 hrs. gave hazy, grey sections.
Embedding a) 2% Celloidin b) 4% Celloidin c) 8% Celloidin	4, 6 and 7 days	4 days	Not much difference but minimum of one week gave a chance for 8% Celloidin to penetrate the ganglion tissues better.
Hardening a) The hardening of the half Celloidin blocks by chloroform vapour	2 hrs; 3 hrs; 4 hrs; overnight	3 - 4 hrs.	More than 4 hrs. makes the blocks very hard and causes them to shrink. Such blocks crack or divide when ready for cutting or during cutting.
b) The hardening of the whole Celloidin block after inserting the object.	4 hrs; 6 hrs; overnight	6 hrs.	6 hrs. hardening with chloroform vapour is sufficient to keep the blocks in good condition, without over-hardening, for at least three weeks under cedar wood oil until they are cut.
Clearing in cedar wood oil + a few drops of absolute alcohol and chloroform	4 hrs; 6 hrs; 12 hrs. or more	12 hrs. or more	12 hrs. gave very clear blocks ready for cutting; it was possible to store the blocks under the mixture for three weeks before cutting.

Factors involved	Variation in preparations	Optimum procedure	Remarks
Cutting Thickness of sections	25, 40, 60, 80 and 100 µm	Between 60 and 80 µm	60 µm gave good sections which were easy to handle.
Mounting	D. P. X., Canada balsam, Styrolite and Permount.	Styrolite and Permount	Styrolite and Permount are clear media and the Celloidin was not milky, as with D. P. X. Canada balsam gave dark brown sections after a week. Sections mounted in Styrolite and Permount did not darken for a long period - over 6 months.

Explanation of Table 5 and the criteria used to select the optimum procedures for the preparation of material used in detailed study of the fibre tracts and dendrites.

a. Age of insect:

Three different ages were tried, viz. less than 24 hours, 24 hours and older than 24 hours (3, 4 & 5 days). 24 hours old or less if possible gave better sectioning with clear details. When using insects less than 24 hours old it was very difficult to dissect out the ganglion and all the nerves without damaging the tissues, or the branches given off from the ganglion. This is because at this age the ganglia are still hardening as their tissues continue postembryonic differentiation. Insects older than 48 hours were poorly impregnated and showed very little detail; only a few dendrites were impregnated. Strausfeld & Blest (1970), stated that in Sphinx ligustri and Pieris brassica the fixation of young animals in buffered glutaroldehyde resulted in excellent impregnation of a number of neurons localised within a small portion of the suboesophageal ganglia, whereas animals more than 5 days old often show fewer impregnated neurons.

b. Prefixation:

Prefixation was carried out in glutaraldehyde, buffered to pH 6.9 - 7.3. This solution was made by mixing 17.5 mls glutaraldehyde [approximately 25% $CH_2(CH_2.CHO)_2$, as supplied by B.D.H. Chemicals Ltd., England] with 25 mls of 0.8% sodium hydroxide, 25 mls of 2.7% potassium dihydrogen orthophosphate, and 32.5 mls distilled water; all reagents must be of analar purity.

The animals were killed in one of two different ways:

1. By immersion in the prefixation solution;
2. By abdominal injection with the prefixation solution.

After killing, the insects were immersed in the fluid, the head, thorax and the abdomen opened, and the suboesophageal ganglion dissected out with the aid of a dissecting microscope; it was then left in this solution for 12 to 15 hours to give optimum results.

Different periods of prefixation, viz., 2, 4, 6, 12, 15 and 48 hours were tried. Strausfeld & Blest (1970), mentioned a range from 6 to 48 hours. Less than 12 hours or more than 15 hours gave very poor results in Acheta, with little detail visible in the sections, which were generally dark brown if left for more than 15 hours in the prefixation solution.

c. pH of prefixation:

Strausfeld & Blest (1970), mentioned that prefixation in glutaraldehyde buffered to pH 6.8 to 7.4 was subsequently used as the standard impregnation procedure, while Trujillo-Cenóz (1959) used, for specimens of Pholus labruscoe at the end of the larval stage, 1% Osmic acid solution in a veronal buffer at pH 7.9 as a fixative solution. Trujillo-Cenóz & Melamed (1963), added that with adult specimens of Musca domestica and Calliphora vomitoria, pH 7.4 was used.

Three values of pH were investigated in this study: 6.9, 7.2 and 7.3. pH 7.2 and 7.3 gave good results, while pH 6.9 gave poor conditions for dissection with a little subsequent shrinkage and darkening, so that not many details could be recognised in the final preparation. Strausfeld & Blest (1970), stated that the pH, molarity and duration of fixation in the buffered glutaraldehyde

could be varied to give different results. Generally the preparation was considered ideal if it contained many recognisable cells or axons and dendrites.

d. Fixation:

After removal, the ganglia were fixed in a solution of one part of 25% glutaraldehyde solution added to 4 parts of 2.5% Potassium dichromate solution in distilled water; this solution must be made up immediately before use.

The subeosophageal ganglia were fixed in this fluid for 36 hours in the dark at 20°C; four different periods were tried, viz., 24, 36, 48 and 72 hours. Strausfeld & Blest (1970), stated only that material is fixed in this fluid for 2 to 3 days in the dark at 20°C.

Generally, 36 hours fixation gave the best results, with bright, clear sections; other times resulted in darker sections which sometimes contained dark brown bundles of tracts.

e. Silver impregnation:

After fixation, the material was washed several times in 0.75% silver nitrate and left in that solution for 48 to 72 hours in darkness at 20°C.

The silver impregnation was tested for several different periods, viz., 24, 36, 48 and 72 hours. Sections impregnated for 2 - 3 days generally showed little difference in quality, but impregnation for less than 28 hours resulted in hazy, grey sections.

f. Temperature of fixation and silver impregnation:

This was always 20°C in the dark. Cajal (1933), emphasised the importance of controlling the temperature during the impregnation period and found the optimal range between 20°C and 26°C; below 15°C he found it was very difficult to obtain good results. Strausfeld & Blest (1970), reported that temperatures above or below 20°C can give very poor results.

g. Embedding:

Material was examined as thick Celloidin sections (8% Necoloidine solution as supplied by B. D. H. Chemicals Ltd., England).

The impregnated material was passed through:

1. Absolute alcohol
2. Equal parts of absolute alcohol and ether
3. 2% Celloidin in alcohol and ether, 4 days
4. 4% Celloidin in alcohol and ether, 4 days
5. 8% Celloidin in alcohol and ether, one week

The effect of varying the duration of embedding in Celloidin was tested at 4, 6 and 7 days for both 2% and 4% Celloidin, and 6 or 7 days for 8% Celloidin. Generally, 4 days for 2% and 4% Celloidin gave satisfactory results. One week for 8% Celloidin was enough to allow the celloidin solution to penetrate the ganglion tissue.

6. A paper ring, made from stiff card, reinforced with sellotape, was half filled with 8% Celloidin (Dennell, 1940; Pantin, 1964).

7. The half-block was hardened by placing it in a covered desiccator with cotton wool soaked in chloroform.

8. When the Celloidin had hardened its surface was softened with a drop of alcohol-ether, then the ring filled to the top with 8% Celloidin. The material was then inserted in the Celloidin, orientated and left in the desiccator for hardening.

During the above procedure any bubbles which appeared in the Celloidin solution were allowed to rise to the surface and burst by the cautious addition of a little ether.

h. The duration of hardening the blocks:

1. Various durations were tested for hardening the ring half-filled with 8% Celloidin by using the vapour of chloroform for 2, 3 or 4 hours and overnight. Between 3 and 4 hours was enough to give hard Celloidin ready for the next step; more than 4 hours made the half blocks too hard and could cause a fracture between its two halves when the block was ready for cutting. Less than 3 hours meant that the Celloidin was not hard enough and caused the object embedded to sink to the bottom.

2. The hardening of the whole Celloidin blocks by chloroform vapour after orientation was studied at 4 or 6 hours and overnight. 6 hours hardening enables the blocks to be kept in a good condition under cedar wood oil, soft and suitable for easy cutting after more than two weeks.

i. Clearing the blocks:

After hardening for 6 hours the blocks were removed and cleared in cedar wood oil + a few drops of absolute alcohol and

chloroform for not less than 12 hours; the material could then be stored for more than two weeks under cedar wood oil if necessary.

j. Cutting:

After trimming, the blocks were mounted with a few drops of 8% Celloidin on the wooden block holder of a sledge microtome. Chloroform vapour was used to harden the attachment of the block, and the material was cut as a set of transverse sections.

Experimental sections were cut at 5 different thicknesses, viz., 25, 40, 60, 80 and 100 µm; between 40 and 80 µm gave good results, but generally it was found that 60 µm was most suitable and gave good sections which were very easy to handle. Boycott & Dowling (1969), during their studies of the mammalian retina by Colonnier's method, cut at about 75 µm for horizontal sections and 95 µm for vertical sections. Strausfeld & Blest (1970), stated simply that in studies on insects by Colonnier's technique the brains were examined as thick (40 to 150 µm) Celloidin sections mounted serially under cover slips in Permount.

It should be noted that the cut sections must be kept in serial order. This was done by placing a few of such sections in a small separate dish containing some cedar wood oil for clearing the sections. The knife and the block were also moistened with cedar wood oil before cutting; this is very important to obtain good sections. Sections were cut as a transverse serial sections.

k. Mounting:

In all the available reported work for which Colonnier's modification has been used, the mounting medium employed was Permount, the synthetic proprietary material, as introduced by

Vaisamurat & Hess (1953), for sections treated with Golgi impregnation after formalin fixation. Permount was also used by Boycott & Dowling (1969) and Strausfeld & Blest (1970).

The mounting media tried in the present work were: D. P. X., Canada Balsam, Styrolite (as supplied by Raymond A. Lamb, London) and Permount (supplied by Fisher Scientific Company, New Jersey, U. S. A.). A number of preparations were mounted in serial order, on slides using cover slips. Sections mounted with D. P. X. did not clear but appeared opaque or milky due to an interaction between the mounting medium and the Celloidin. Such sections also became very dark, a brown coloration developing after one week, as was also the case for Celloidin sections mounted in Canada Balsam. Styrolite and Permount gave very good results, the sections remaining clear and undarkened.

Two of the reasons causing darkening of the sections mounted after silver impregnation sections are the use of an incompatible mounting medium, and exposure of the slides to light. For this reason the slides must be stored in darkness. Boycott & Dowling (1969), mentioned that, "for Golgi material no fading of the impregnation has been seen in two years, but darkening of the unstained background of some of the sections has occurred". They concluded, that it is probably due to exposure to the microscope light used in examining the sections. Guillery (1966), reported a similar problem during his studies of Golgi preparations. Such changes have also been observed in sections of the brain of Sphinx ligustri by Strausfeld & Blest (1970), who mentioned that some preparations suffered darkening of the unimpregnated background.

(B) The double-impregnation procedure:

a. The modified Kallius' procedure:

A modification of the double-impregnation procedure of Kallius (1910), was used by Strausfeld & Blest (1970), who stated that only a few components were clearly shown up against a deep brown background of unimpregnated tissue. In this present work the same procedure was tried with minor changes in duration.

For this method, material was fixed in buffered glutaraldehyde at pH 7.2 for 24 hours, then washed in distilled water for 4 hours prior to immersion for 4 days in a solution containing 5 ml of 1% osmium tetroxide and 20 ml of 2.5% potassium dichromate. The solution was renewed every day. The material was then washed for 4 hours in distilled water before immersion in 0.75% silver nitrate solution, for 2 days in darkness at 20°C, then embedded in Celloidin, and sectioned. The sections were mounted in Styrolite under cover slips.

Material prepared by this method showed only a few axons and their dendrites very clearly against a brown background. Nevertheless this method may have some useful applications (see Figs. 87, 105 and 106).

b. The mixed impregnation method of silver nitrate & osmium tetroxide followed by ethyl gallate:

The suboesophageal ganglion were dissected out in 70% alcohol. They were then fixed at low temperature (0 to 4°C for 1 hour in 1% osmium tetroxide solution which was regulated to pH 7.2 by the acodylate buffer in isotonic sodium chloride. The fixed material was then transferred to saturated solution of

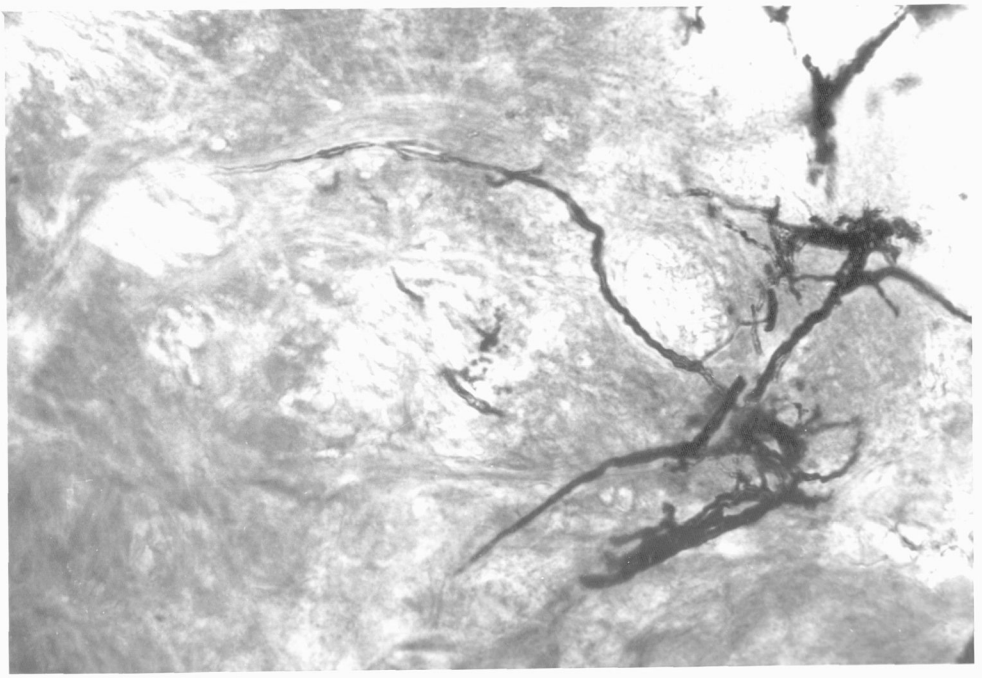
ethyl gallate in 0.25% cresol and stored for 2 hours.

A second fixation of the ganglia was performed by immersing them for 24 hours at 20°C in the dark, in a fresh solution of one part of crude 3% glutaraldehyde solution and 4 parts of 2.5% aqueous potassium dichromate.

After fixation the ganglionic material was washed several times with 0.75% silver nitrate and stored in it for 36 hours at 20°C in the dark. It was then dehydrated by alcohol or dioxan and cleared by cedar wood oil. Finally the ganglia were embedded in paraffin or Celloidin and cut into sections of 25 - 30 µm thickness. Such Celloidin sections are very difficult to handle and must be treated with care. 20 - 25 µm sections from paraffin embeding were easier to deal with (thick sections, see Figs. 77, 78, 127 and 128).

All drawings in this section were made with the aid of a camera lucida using the light-microscope at a magnification of X 100 and X 400. All photographs negative of Golgi-impregnated material have been taken from thick (60 - 80 µm) Celloidin sections viewed under a (Carl Zeiss) photomicroscope at magnifications of between X 50 and X 200. Owing to the thickness of the section and the nature of the material sectioned the definition of photograph reproductions is necessarily impaired.

Figs. 77 & 78. Some large diameter axons and their dendrites as shown in material stained by a mixed impregnation method (using silver nitrate & osmium tetroxide followed by ethyl gallate, X 320).



77



78

3. Results: Identification and characterisation of structural units based on Golgi impregnated material.

(A) The different types of dendritic endings:

Three main kinds of dendritic endings were found.

a. An ending branched into fine scattered fibrils or coarse scattered fibrils.

b. An ending separated into fragments and punctiform structures, lightly or heavily impregnated.

c. An ending terminating in long thread like or spiny fibrils.

(B) The various kinds of branched endings and their dendrites (structural units):

These structural units are shown in figures 79 to 82. They have been categorised in groups A, B, C, etc. Each different structure in each group has been given an identification number such as A_1, A_2...; C_1, C_2, C_3, etc., and a drawing made to show its appearance. The identification numbers do not relate to the sequence in which the type of structure was observed during examination of the serial sections, but form subdivisions of the major type.

These units are as follows:

Types A_1 & A_2 (Figs. 79, 83, 84 and 121): An agglomeration of large and small stellate endings, deep or shallow in extent, and occupying a wide transverse field. They arise from the main axon and collaterals, which are either straight or more or less twisted.

Type B_1 (Fig. 79): The fibre is branched laterally into fibrils, terminating in heavy punctuate endings.

Type B_2 (Figs. 79, 85 and 109): A relatively large-diameter collateral fibre which branches into finer fibres, themselves

bearing laterally branching fibrils with punctiform endings and occupying a deep and wide field. This structure normally arises from a motor neuron.

Type B_3 (Figs. 80 and 87): Large arborescent collaterals, terminating in fine long and short fibrils with punctiform endings.

Type B_4 (Fig. 80): A long axonal branch, covered with punctiform structures and occupying a small field.

Type C_1 (Figs. 79 and 107): A large structure, associated with a medium sized field axon, branching into long, straight, unbranched, thread-like fibrils (usually arising from a motor neuron).

Type C_2 (Figs. 81 and 106): An inverted Y-shaped fibre with no branching; the two bifurcations are equal in size.

Type C_3 (Figs. 81 and 86): As above but the two arms of the Y are unequal in length.

Type C_4 (Figs. 81 and 126): A more or less straight axon with a few fine punctiform structures along it.

Type C_5 (Fig. 81): A relatively straight axon ending in two branches, either smooth or spiny.

Type D_1 (Fig. 79): A dense, relatively large-diameter fibre terminating in a cluster of shallow, fine-branching fibrils and punctiform endings.

Type D_2 (Figs. 80 and 87): A densely impregnated large-diameter fibre, either straight or twisted, terminating in darkly impregnated bulbs with very short branching fibrils.

Type D_3 (Fig. 80): A dense large-diameter fibre, straight and terminating in darkly impregnated bulbs with long branching fibrils which carry punctiform endings.

Type E_1 (Figs. 80, 88 and 90): An axon branched into small diameter clearly impregnated fibres, which are then subdivided into very fine fibrils some of which carry a few very small fine punctiform endings. The axon terminates in a point covered with denser punctiform endings.

Type E_2 (Figs. 80, 89, 90 and 122): A large-diameter axon divided distally into two short branches; each division carries some uncondensed fibrils which may end in punctiform structures. This structure could, to judge by its size, be a compound structure resulting from two or more axons running together.

Types F_1 to F_6 (Figs. 81 and 82): A variety of long axons all terminating in very fine punctiform structures or fibrils. The axons are either in the form of continuously impregnated fibres or finely punctiform or condensed linear structures (see Figs. 91 and 110).

Type G_1 (Fig. 81): A narrow field occupied by a large-diameter axon with root-shaped endings, composed of scattered fibrils carrying some punctiform structures.

Type G_2 (Fig. 81): A wide field large axon forms typically root-shaped with very fine symmetrical fibrils.

Type G_3 (Fig. 81): A wide field, large-diameter axon with a root-shaped structural unit, branching terminally and carrying long punctuate asymmetrical fibrils.

Type G_4 (Fig. 82): A wide field axon, branching early into fibrils which themselves branch again into fine fibrils and punctiform endings.

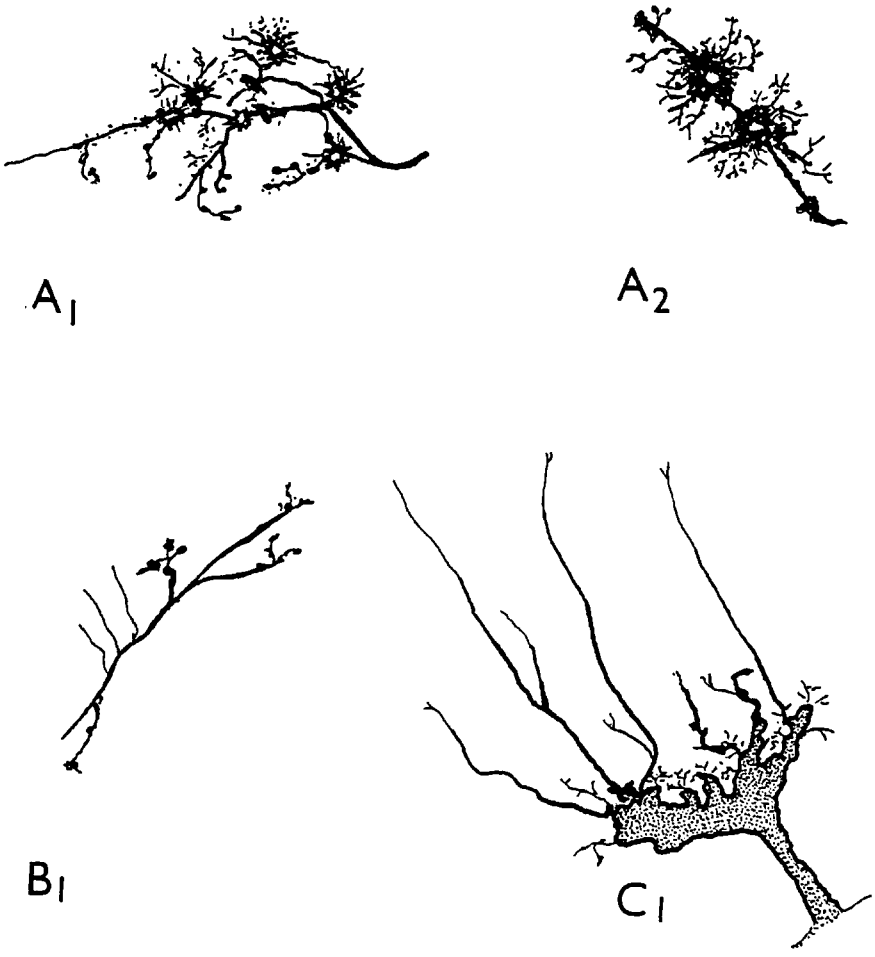
Type H (Fig. 81): A single long axon with lateral spines.

Type I (Fig. 82): A typical tree-like structural unit, cupressi-

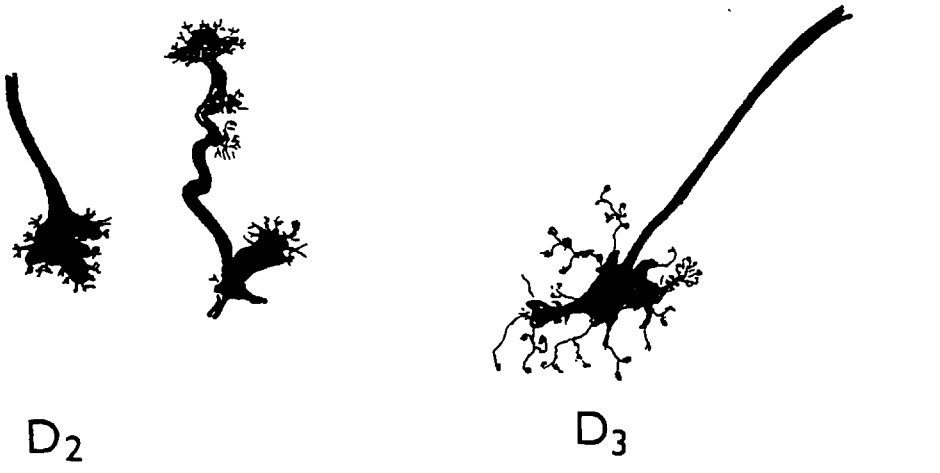
form in appearance.

Type K (Fig. 82): A large-diameter axon, branching into a rosette-like complex composed of very fine processes.

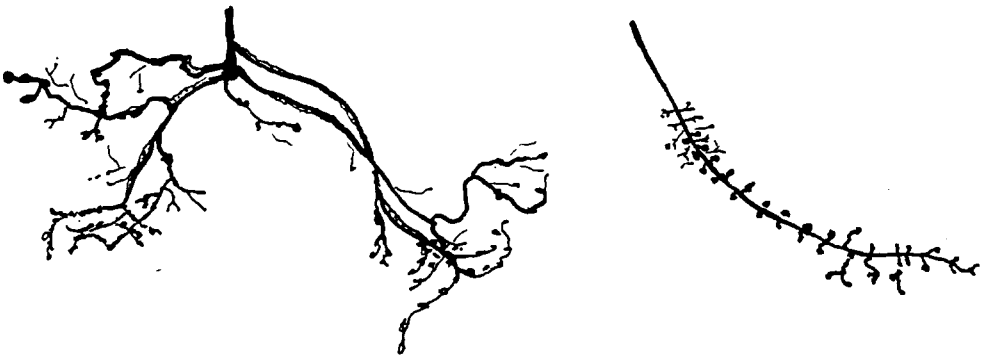
Figs. 79 to 82 show the various kinds of branched endings and their dendrites (silver impregnations).



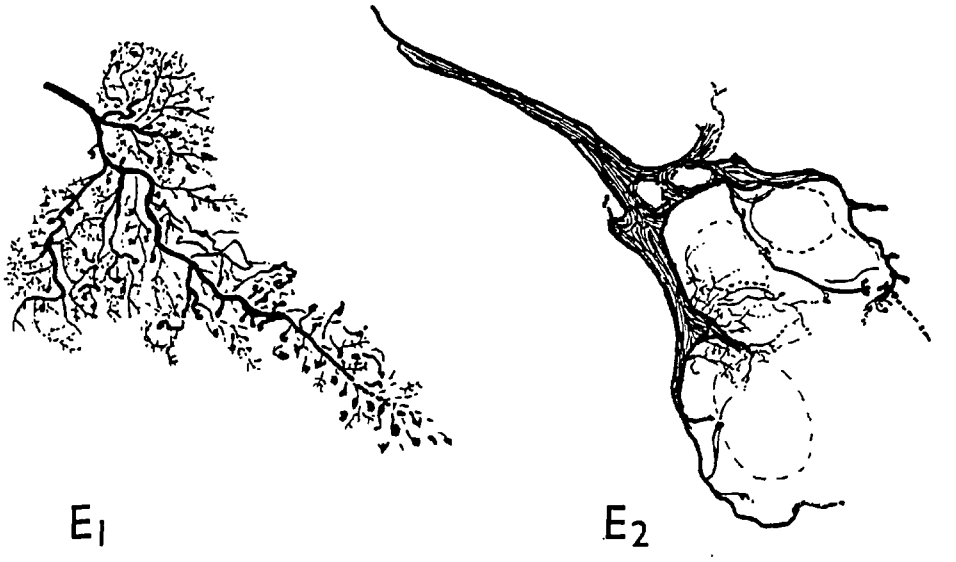
79



D_2 D_3



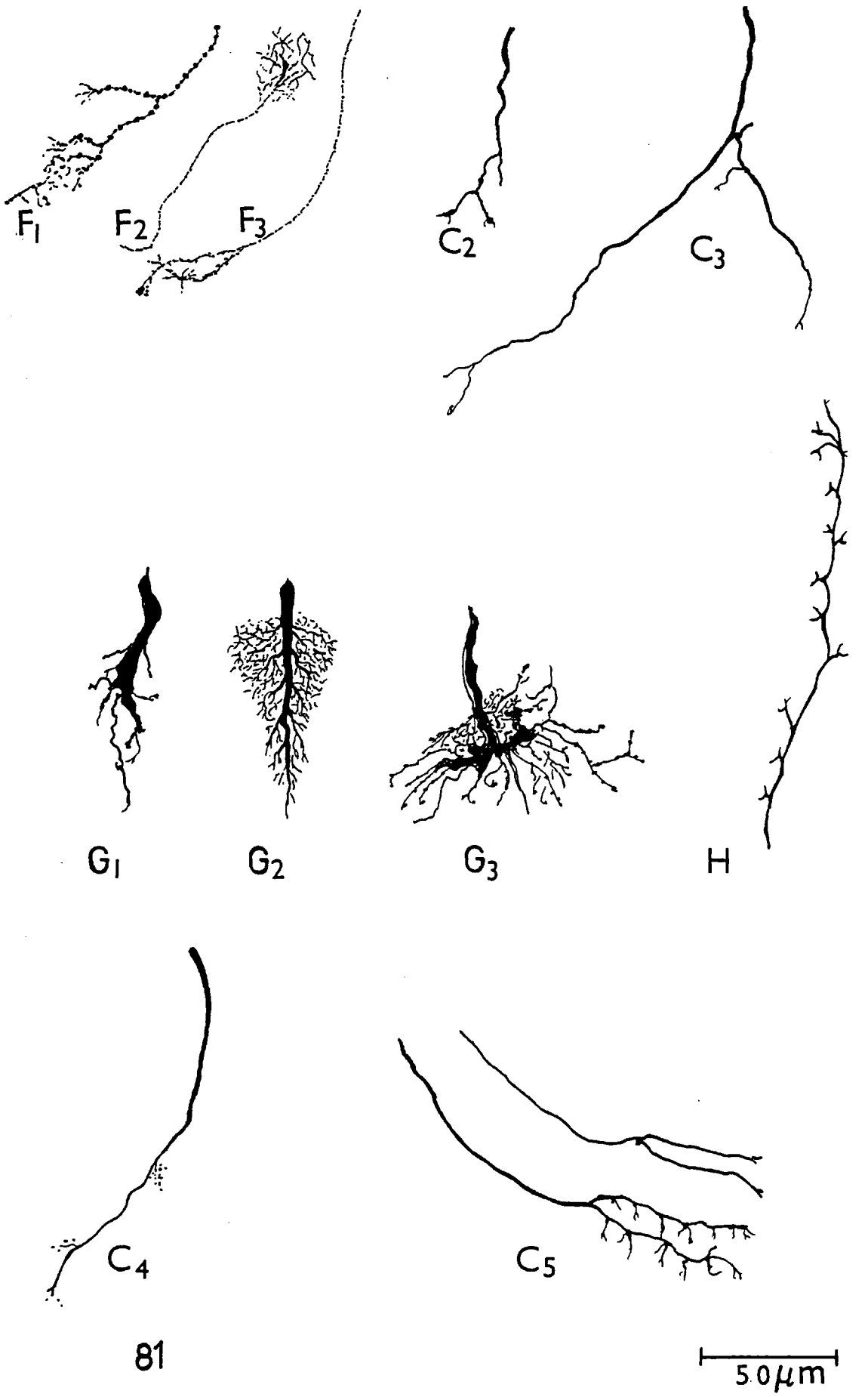
B_3 B_4

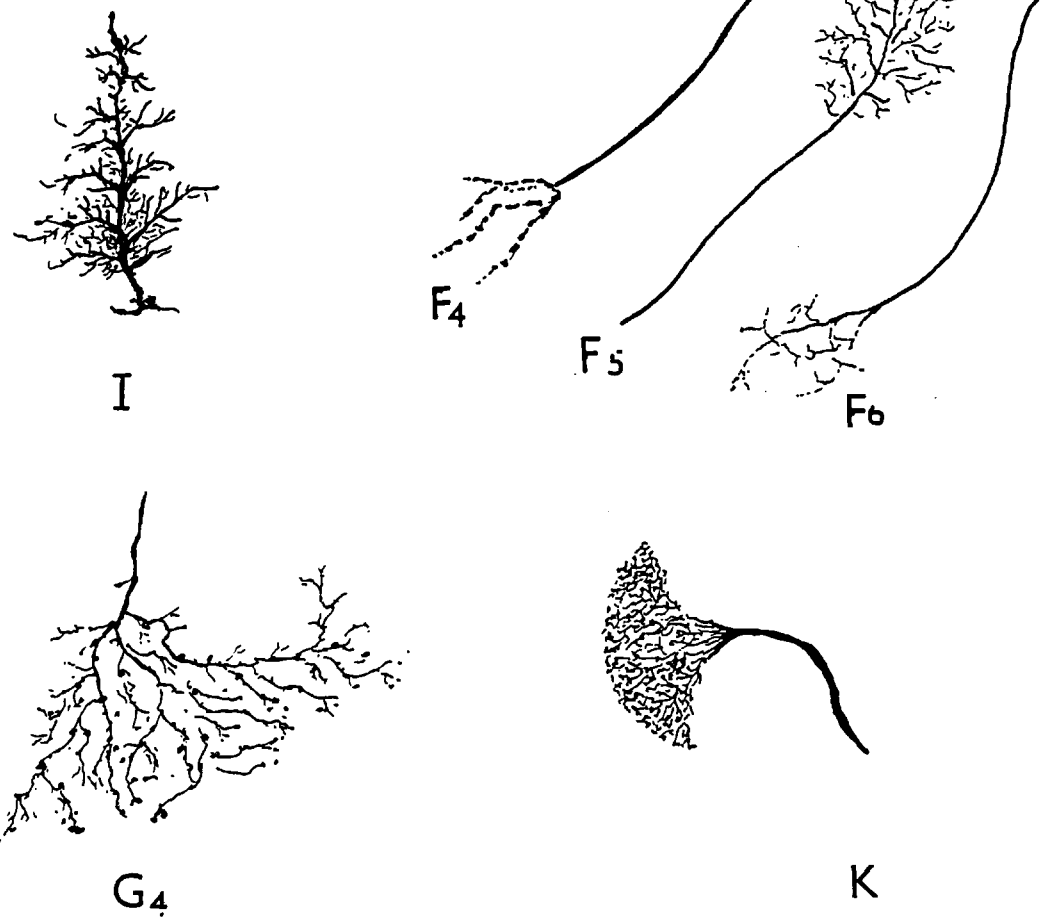


E_1 E_2

80

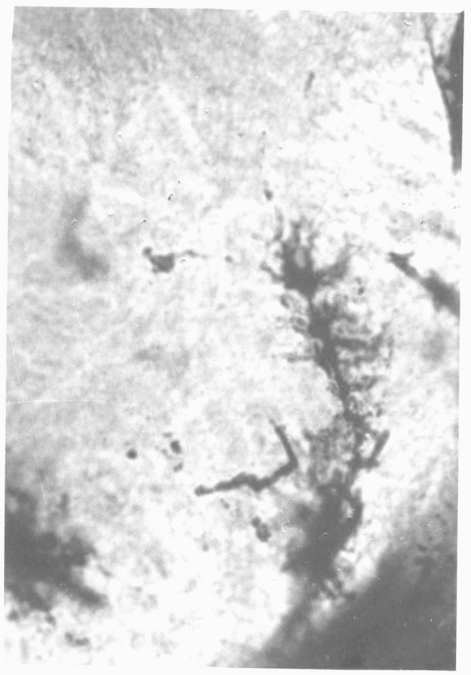
5.0 μm


F1
F2
F3
C2
C3
G1
G2
G3
H
C4
C5
81
5.0μm

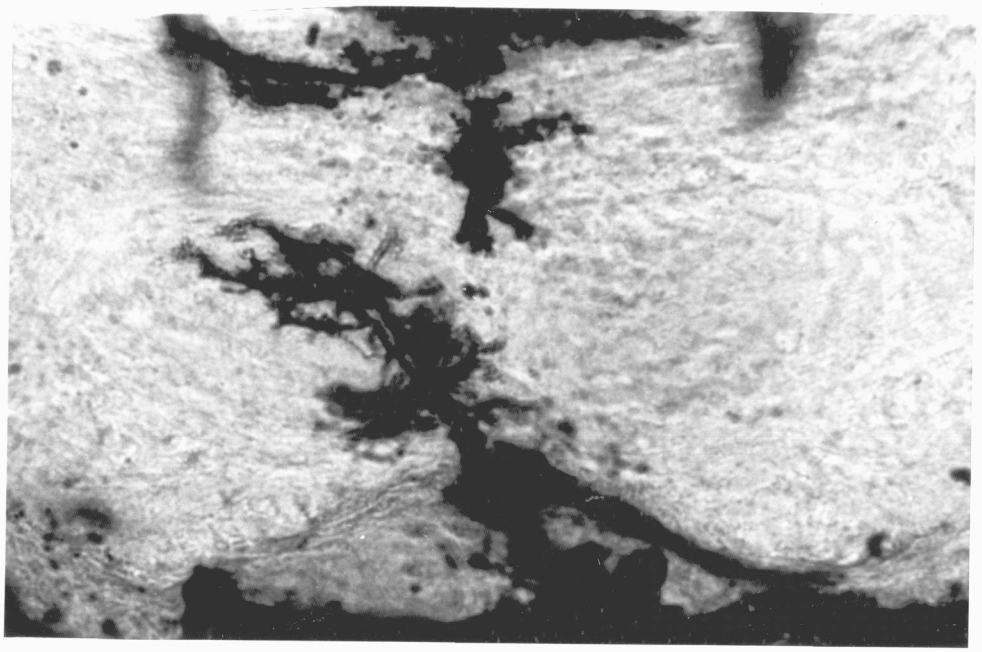


82

Fig. 83 & 84. Golgi preparations of two types of endings: A_1 (Fig. 83), the small stellate dendritic ending, and A_2 (Fig. 84), the large stellate dendritic ending. (X 480).



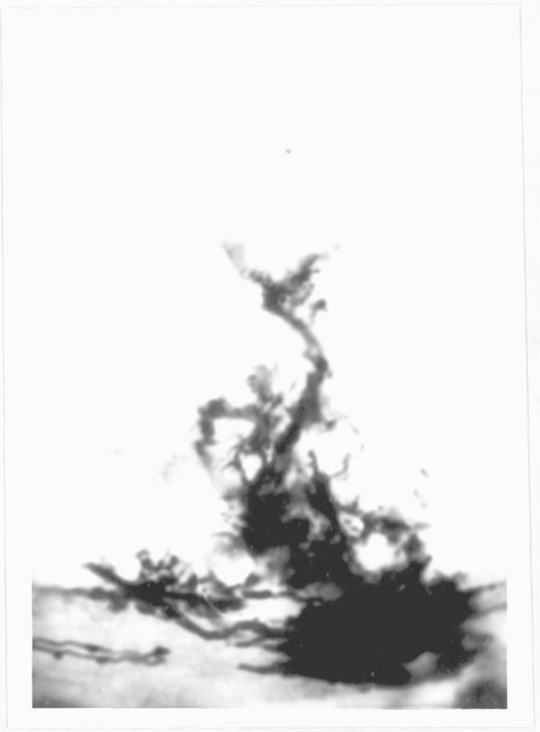
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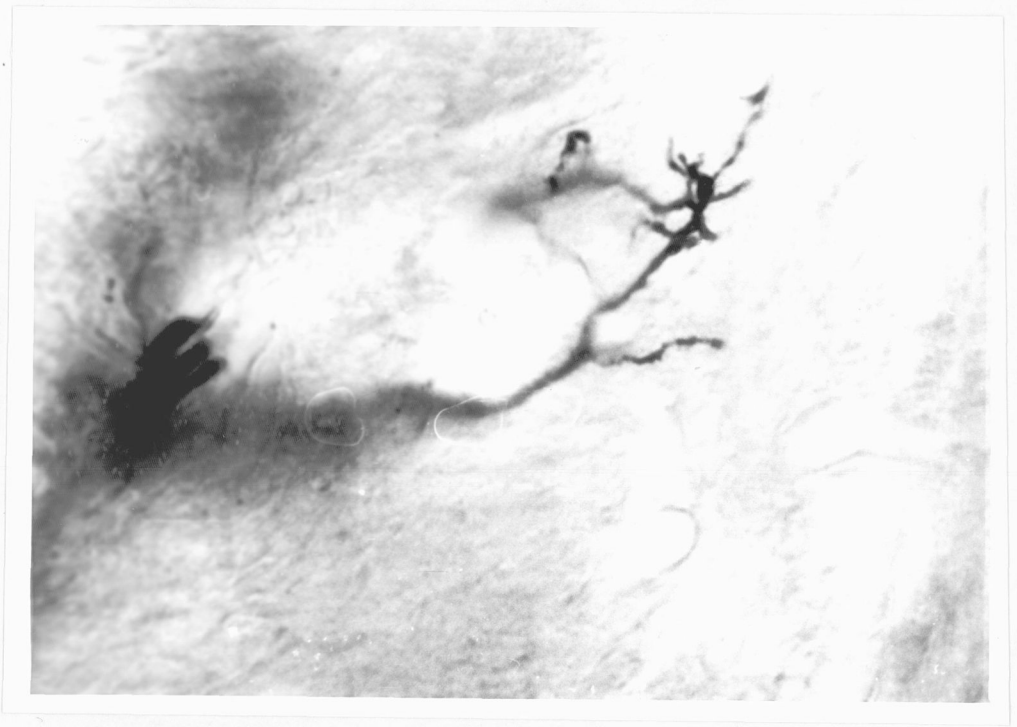
84

Fig. 85 Golgi preparation of motor neuron to show type B_2 dendritic ending (X 1100).

Fig. 86. Golgi preparation of type C_3 dendritic ending (X 1100).

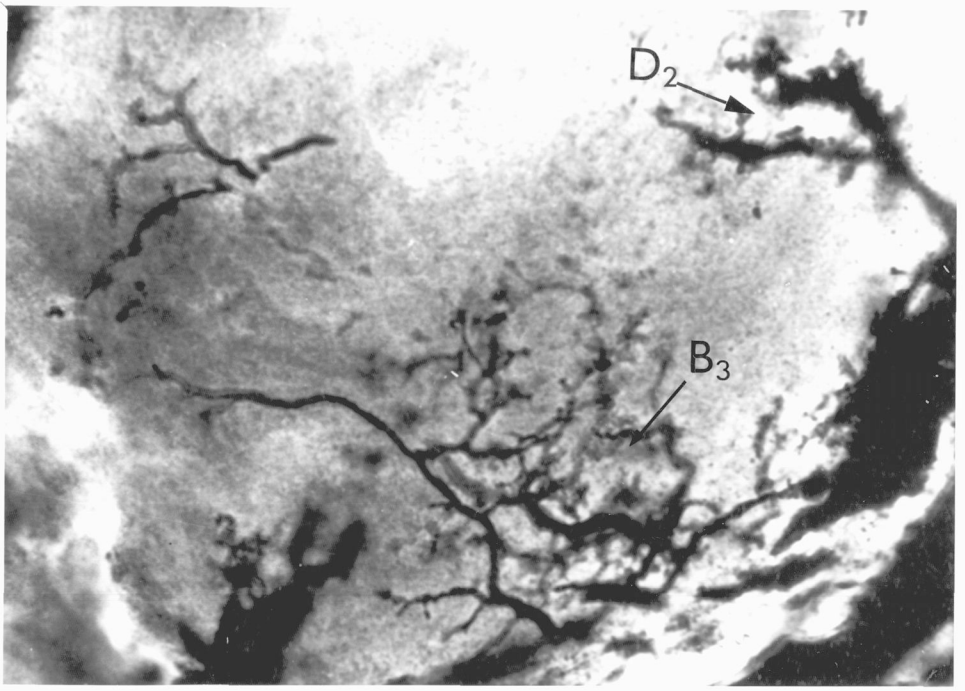


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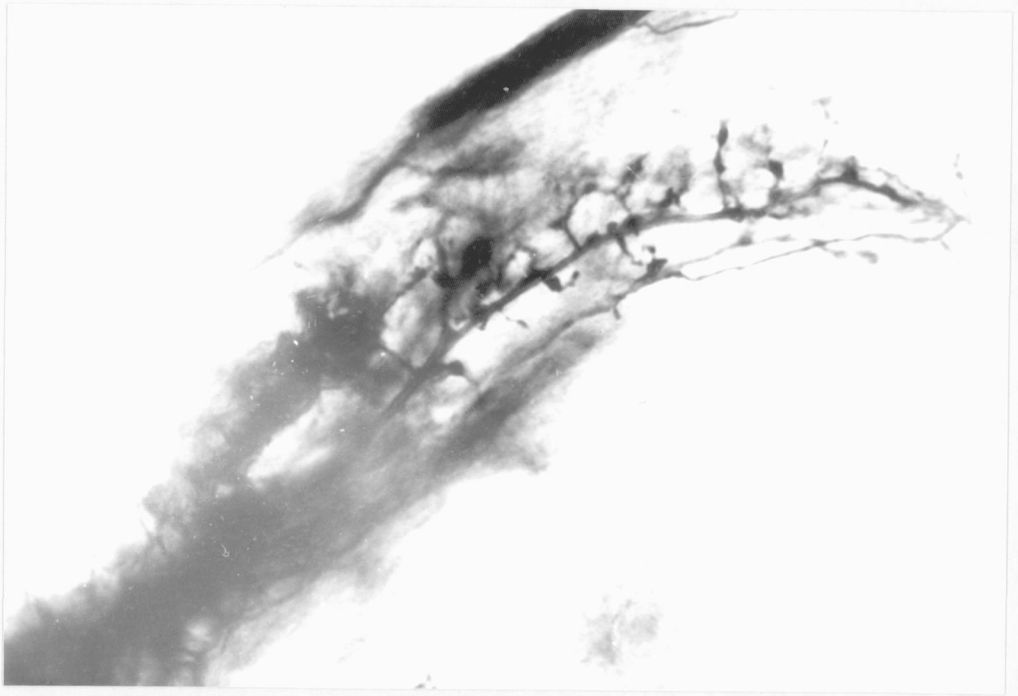
Fig. 87. Some dendritic endings as appearing in sections stained by a modified double-impregnation procedure (type B_3 and D_2, X 480).



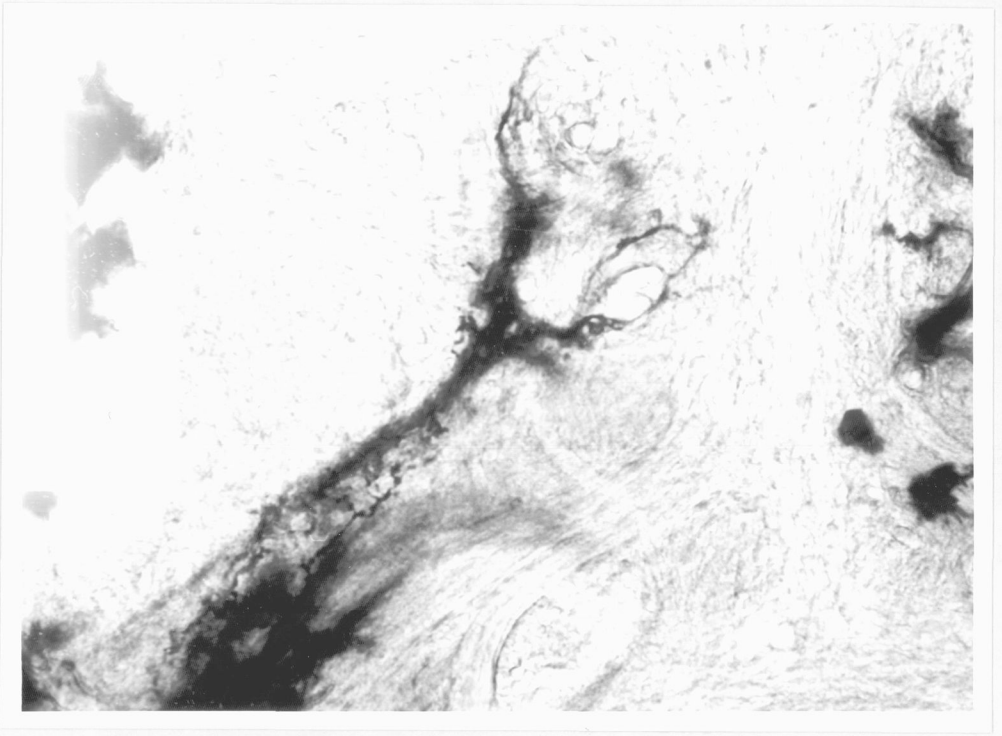
87

Fig. 88 Golgi preparation of type E_1 structural ending (X 920).

Fig 89. Golgi preparation of type E_2 dendritic ending (X 480).



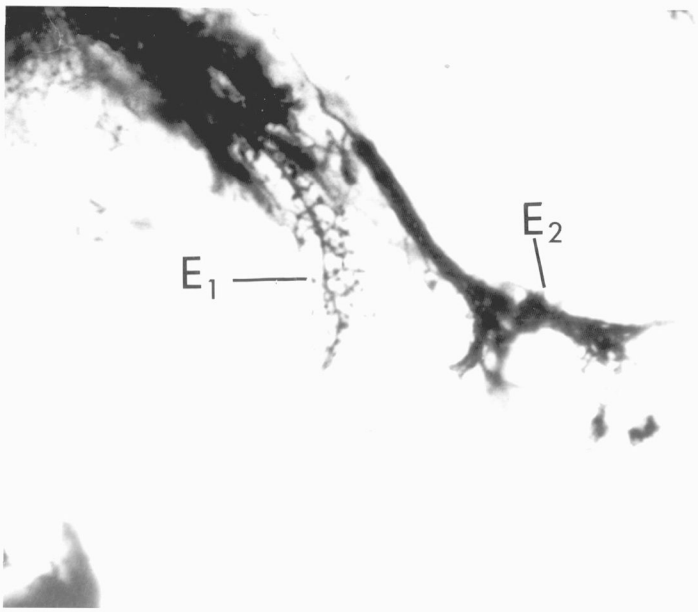
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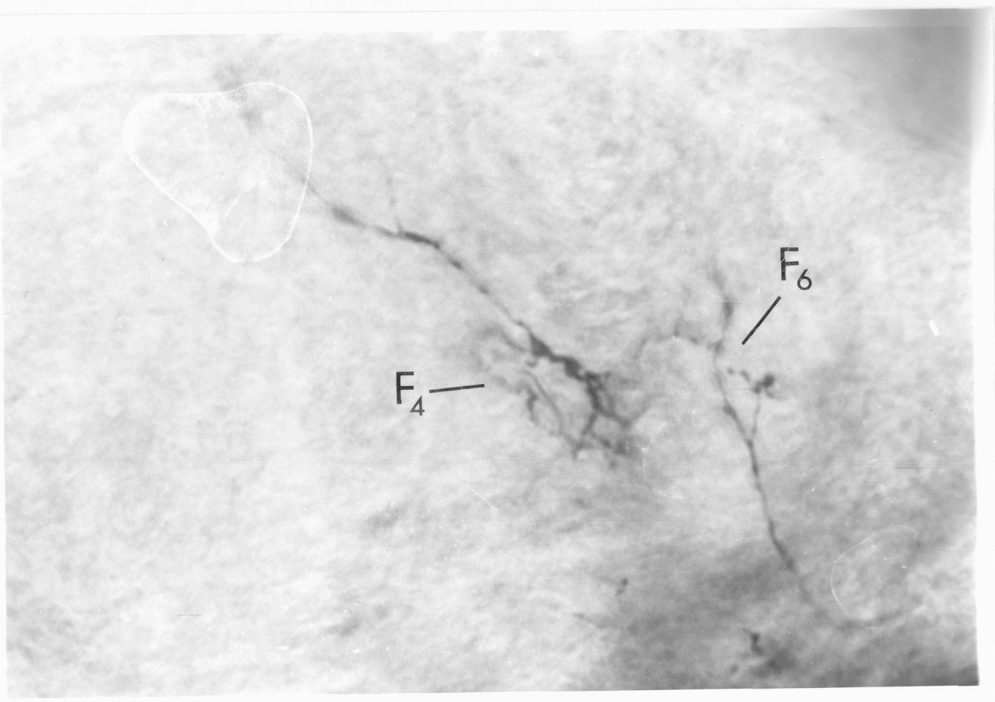
89

Fig. 90. Golgi preparation of types E_1 and E_2 structural endings. (X 480).

Fig. 91 Golgi preparations to show axons and dendritic endings of type F_4 and F_6 (X 1200).



90



91

(C) Axons and their branches and terminations in the suboesophageal ganglion:

The ganglion may be divided into three main parts; anterior, middle and posterior. All axons and their terminations located in each part were reconstructed from several sets of silver-impregnated material. Each part is easily delimited by tracing the nerves as they appear and disappear in their particular portion of the ganglion.

The first part is defined as the region extending from the fusion of the circum-oseophageal connectives to the maxillary nerves. The middle part is the region starting with the maxillary nerves and ending in the area from which the labial nerves arise. The third or posterior region occupies the remainder of the ganglion, including the origin of the labial nerve.

The various axonal units are numbered, i.e. 1, 2, 3, etc., according to their appearance when the ganglion is scanned from anterior to posterior limits.

a. Axons in the anterior part of the ganglion (units 1 to 24):

1. This axon runs from the dorsolateral region of the ganglion ventrally dividing into two collaterals (Figs. 92 and 97), which give off two combinations of dendritic branching; a short one spreads into the middle of the ipsilateral region, while a longer one distributes throughout the anteroventral region of the ganglion. Both collateral branches have type E_2 endings (Figs. 92 and 95). It is not clear whether this is derived from an axon or collateral of a motor neuron at the antero-dorsolateral region of the ganglion or (less probably) whether it represents the central termination of a sensory

axon in the mandibular nerve.

2. This axon runs a short distance from the lateral region of the ganglion (Fig. 92) to the ipsilateral neuropile, where the dendrites extend and arborize to join other dendrites from structural unit No. 3 (Figs. 92 and 95), whose axon could not be traced further than the mid-ipsiplateral region before the midline. Unit No. 2 presumably originates from a motor neuron of the anterolateral group. The axon runs via fibre tract st_5 (cf. the account of osmium-stained axonal tracts p. 69). In general units 2 & 3 have type B_3 endings.

3. This axon gives rise to the dendrites that join their homologues from unit 2, (Figs. 92 and 95).

4. This axon, of unknown origin, starts from the ventral region of the ganglion and extends dorsally with type C_5 structural endings near the dorsal region of the neuropile (Figs. 92 and 95). The axon runs through fibre tract st_8.

5. This axon starts from the ventrolateral area, running dorsally with type C_3 endings (Fig. 95). Its main axon terminates in the dorsal region, while another short collateral ends near the mid-line in the central region of the ganglion. This axon comes from the hypopharyngeal nerve and runs through fibre tract st_8.

6. An axon which comes from the dorsal region of the ganglion (Figs. 93 and 95); it is one of the single fibre structural types, and extends ventrally with type C_4 endings near the central region of the ganglion.

7. This axon runs slightly dorsally from the dorsolateral region of the ganglion, with type D_3 endings near the midline (Figs. 93, 95 and 197). Its collateral crosses the midline to the contralateral neuropile where it breaks up into dendrites (Fig. 95). The origin is not clear but the axon runs via fibre tract st_{2a}.

8. This axon and its dendrites run from the dorsolateral region of the ganglion, with endings of type C_1 structure located exactly near the centre of the ganglion (Figs. 93, 95 and 98).

9. In the same position as axon 8, running dorsally and with type C_4 endings (Figs. 93, 95 and 98).

10. This axon extends from the dorsolateral region of the ganglion (Fig. 95), to its ventral side with a type E_2 endings (Fig. 93); the origin is not clear but the axon runs through fibre tract st_6.

11. This is a bundle of four axons with differently formed endings, all situated in the ventral region of the anterior part of the ganglion (Figs. 93 and 95).

11.a. This axon leaves the lateroventral region of the ganglion and extends ventrally with a long fibre ending in type B_3 terminations near the midline. The other three axons follow the same course but consist of long fibres (11.b), terminating in type F_6 endings, while 11.c. finishes with a type F_4 ending and 11.d. with a type F_2. All the evidence indicates that these axons come from a lateral group of association and motor neurons (in terms of the correlation of the Golgi material with the osmium-stained

axonal tracts) and that they run through fibre tract st5. The dendrites of these axons terminate in the area which contains the ventral glomerular body.

12. This structure is situated in the middle of the antero-dorsal part of the ganglion. It extends ventrally from the dorsal region and has a type G_4 (root) ending, the arborizations of which spread over a large field on both sides of the midline. The dendrites which reach the contralateral side meet those coming from structural unit 17. (Figs. 93 and 95).

13. This axon and its collateral resembles a shallow tree. It is situated in the lateroventral region of the anterior part of the ganglion. It appears just behind the root of the groups of No. 11 axons (Figs. 93 and 96), and it extends shortly ventrad with a type I (tree-shaped) ending in the lateroventral region of the neuropile. It seems that the origins of this structure are situated among the lateral association and motor neurons and their dendrites spread into the area which contained the lateral glomerular body.

14. This structure is composed of three long fibres (14.a, 14.b & 14.c), which come from the mandibular nerve in the dorsolateral region of the ganglion. As seen in transverse sections of the ganglion the three fibres run to the middle part of the ipsilateral neuropile (Fig. 96), with type F_4 endings. Fibres 14.a. and 14.b. are linked together by two crossfibres (Fig. 94). The three fibres all run through fibre tract st_{2a}.

15. This axon and its dendrites lie close to the ending of structural unit 14. It joins fibre 14.b. with a type F_6 ending

(Fig. 94), and also extends dorsally to the dorsal region of the neuropile with its origin just along the midline in an area which contains a few association neurons (Fig. 96).

16. This axon originates from the dorsolateral region of the ganglion (Fig. 96), probably from the circum-oesophageal connectives, and runs ventrally. Its collaterals and dendrites (Fig. 92), arborize with type B_3 endings in the middle region of the ipsilateral neuropile. One collateral passes a short distance into the contralateral neuropile and disappears. Comparison with the osmium-stained material suggests that this unit is a component of fibre tract st_5.

17. This axon originates from the ventral region of the ganglion (Fig. 96), in which motor and association neurons occur. The axon extends a short distance dorsally, with a type B_1 ending (Fig. 93), in the area which contains the ventral glomerular body (Fig. 96). This axons probably originates from the ventral region of the ganglion occupied by association and motor neurons.

18. An axon coming from the ventrolateral region (Figs. 92 and 96), and extending dorsally into two collaterals, one ending shortly with a type G_3 termination in the lateral glomerular body, while the other extends, with type B_3 endings, in the middle of the ipsilateral region of the ganglion. The origin of this axon is not clear but it arises from a region which contains association and motor neurons; it probably runs in fibre tract st_1 and might originate from a motor neuron.

19. An axon coming from the ventral region of the ganglion and extending dorsally (Figs. 94 and 96) with a type B_3 ending in the area containing the ventral glomerular body. Comparing it with the

osmium stained material leaves no doubt that this axon comes from the hypopharyngeal nerve and runs through fibre tract st_8.

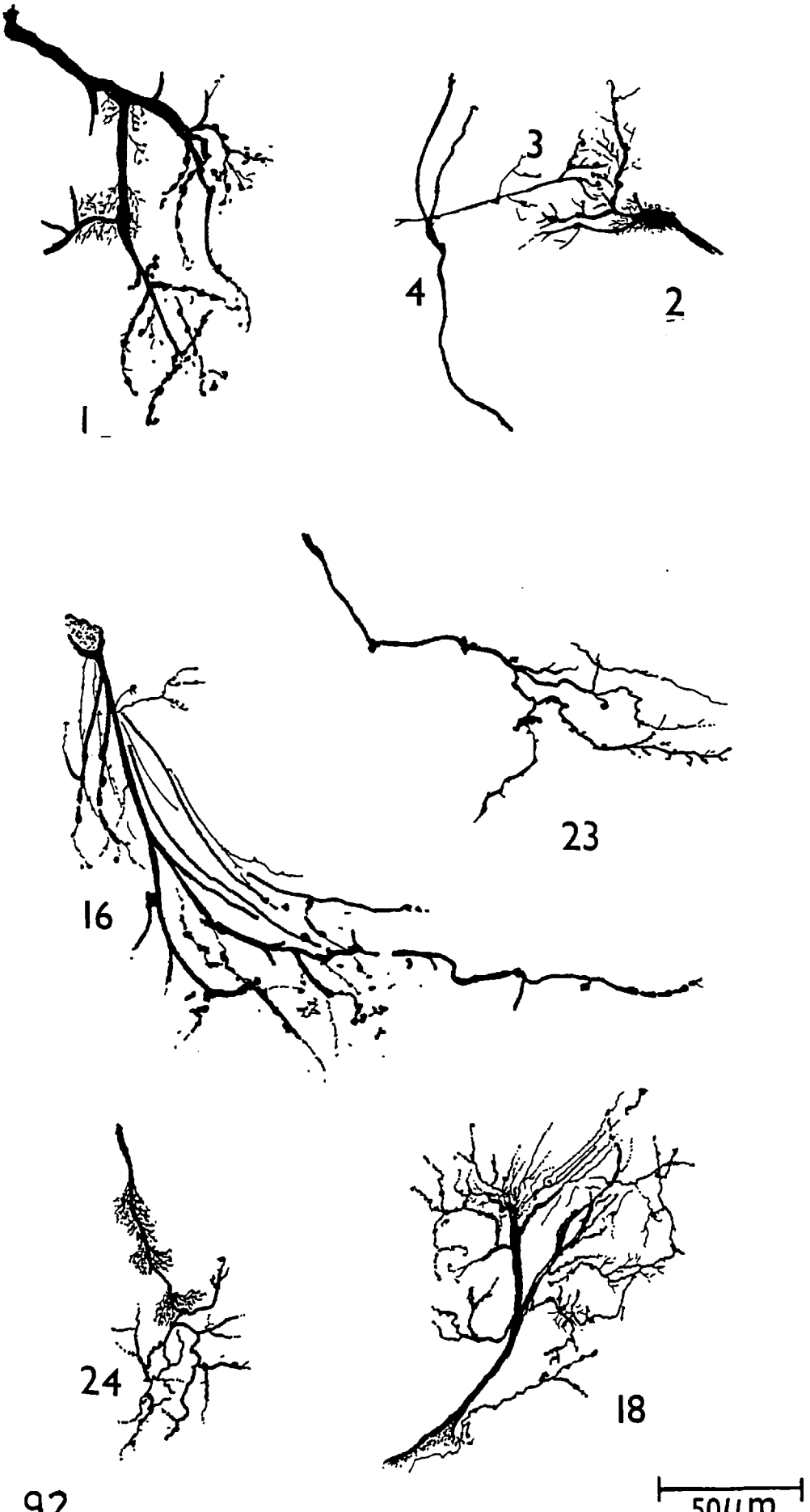
20, 21 & 22. These are axons from the ventral motor neuron. Unit 20 runs dorsally with a type C_3 ending, and unit 21 runs dorsalward with a type C_2 ending (Figs. 94 and 96). Axon 20 ends in the central area of the ganglion, while axon 21 extends dorsally and ends very shortly in the ventral region of the ganglion. It is likely that the dendrites of axons 20 and 21 join those coming from axon 19, whose main axon comes from the hypopharyngeal nerve. Their terminations also join another structural unit of unknown origin, i.e. the axon referred to here as No. 22 (Figs. 94 and 96).

23. This axon starts from the anterior cervical nerve and extends ventrally into a type B_3 ending in the central area, very near the midline where the central glomerular body lies (Figs. 92, 104, 105 and 106).

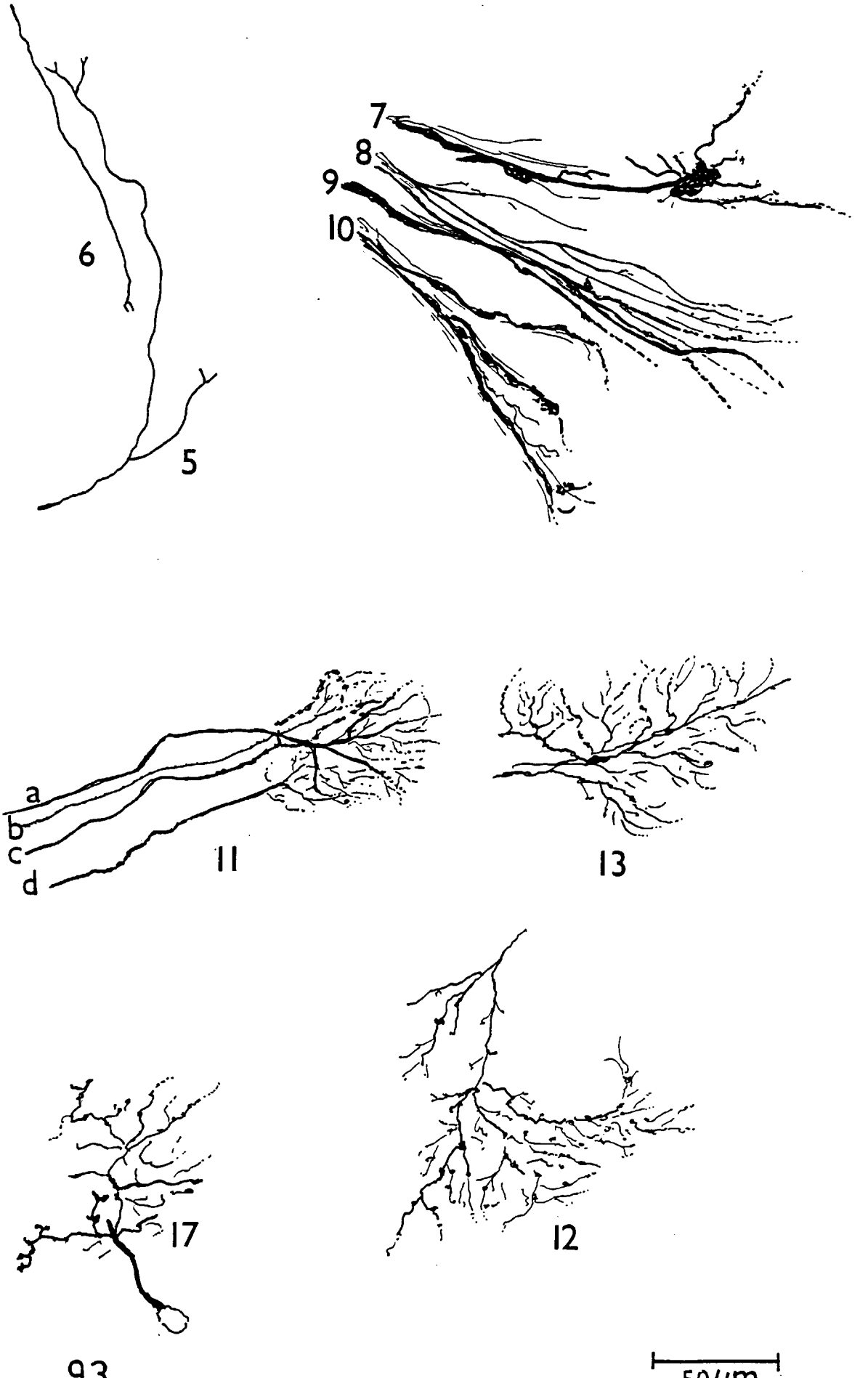
24. As above, except that the type B_3 endings terminate in the lateral area (Figs. 92 and 104). This axon runs through fibre tract st_{19}.

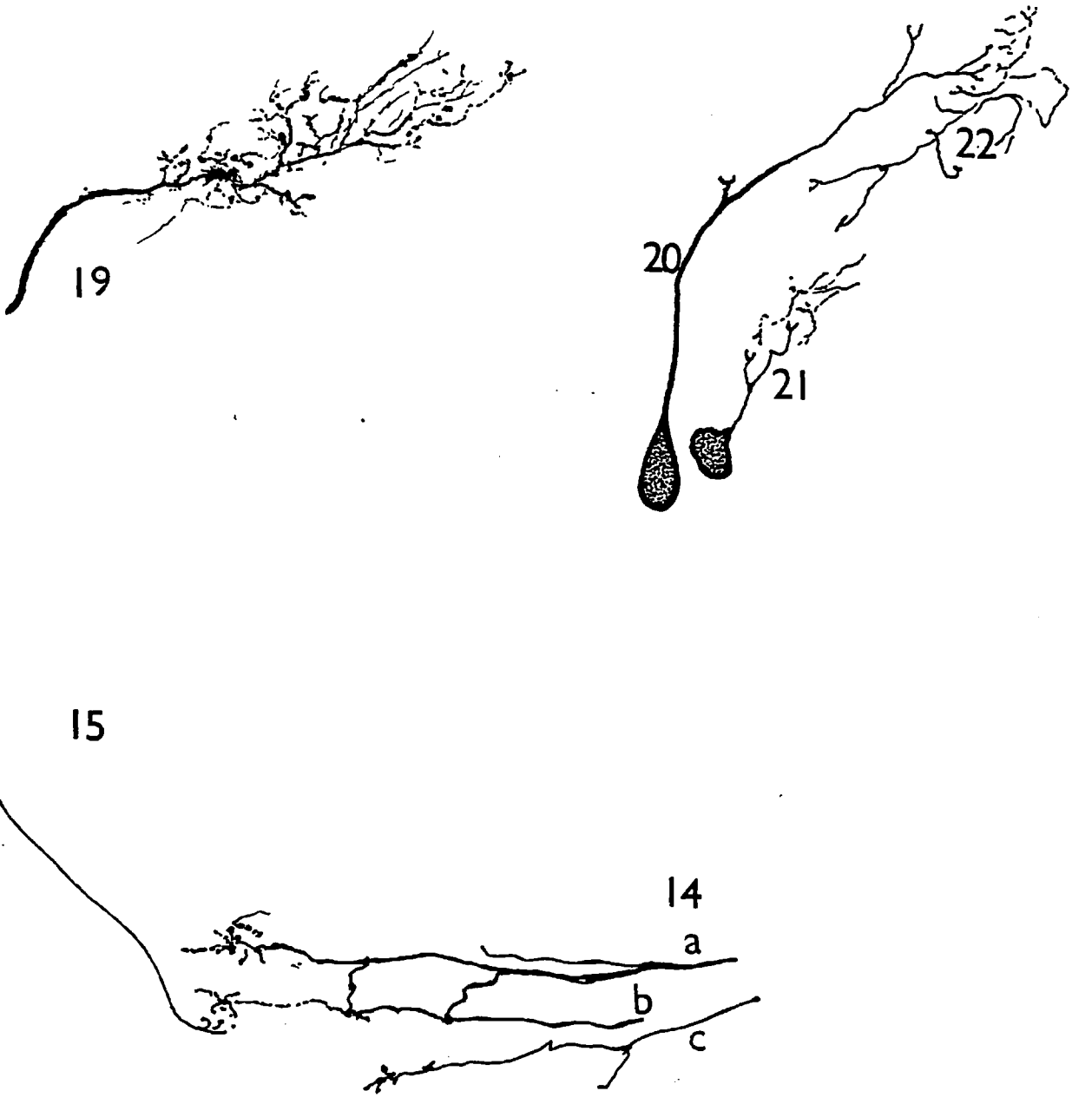
Note: Though units 23 and 24 appear in the end of the anterior part of the ganglion they are illustrated in sections appertaining to the beginning of the middle part of the ganglion.

Figs. 92 to 94 show the various axons and their branches and terminations, in the anterior part of the ganglion.



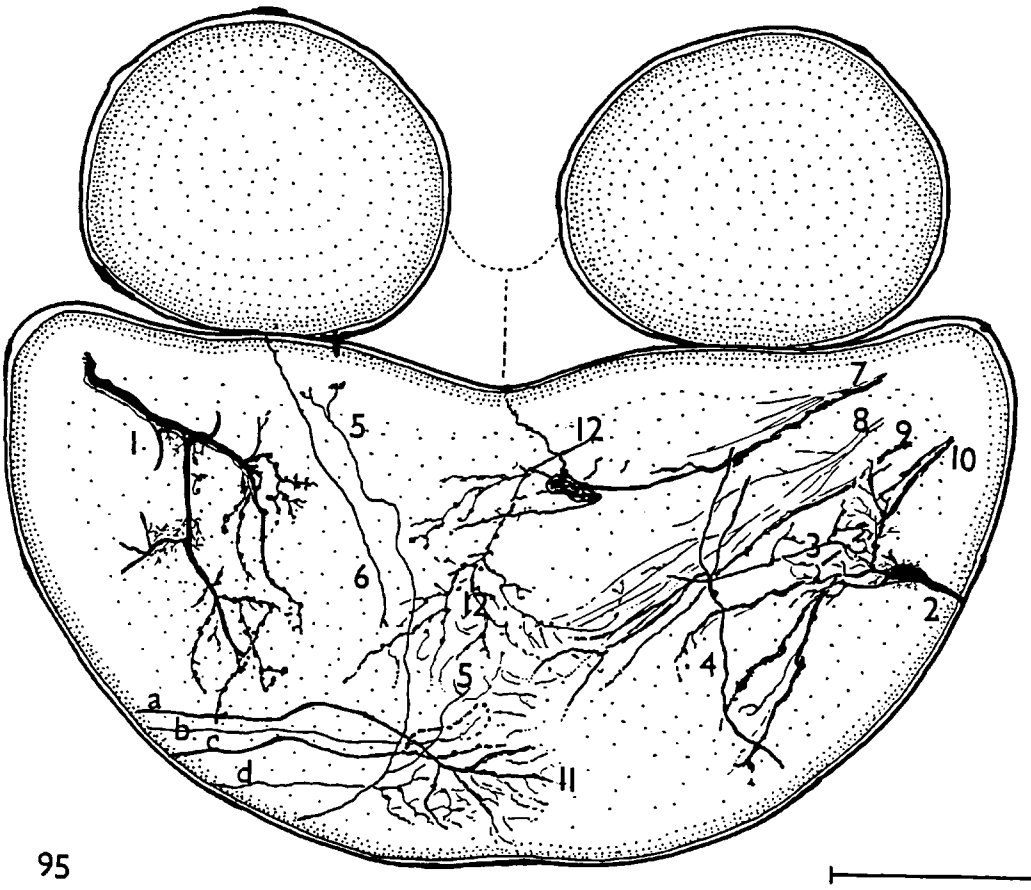
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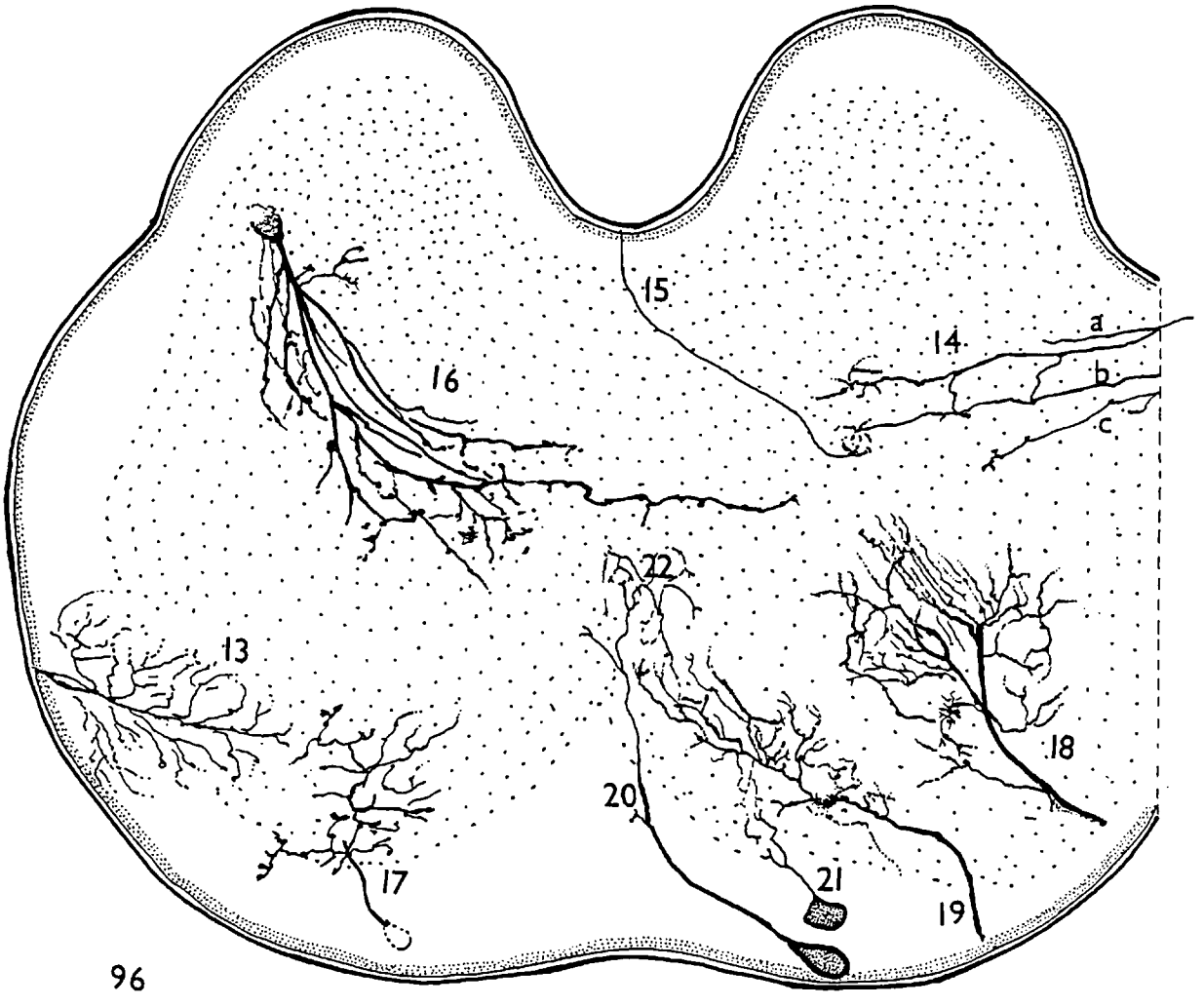
94

Figs. 95 & 96 show the various axons and their positions, as located in the anterior region of the ganglion.



95

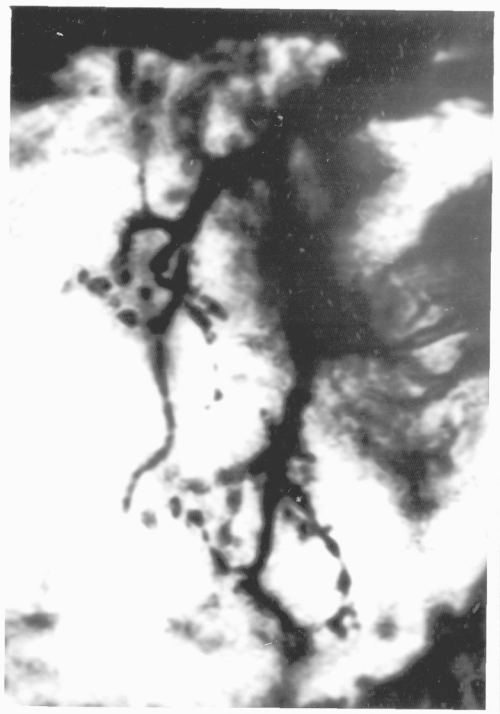
100 μm



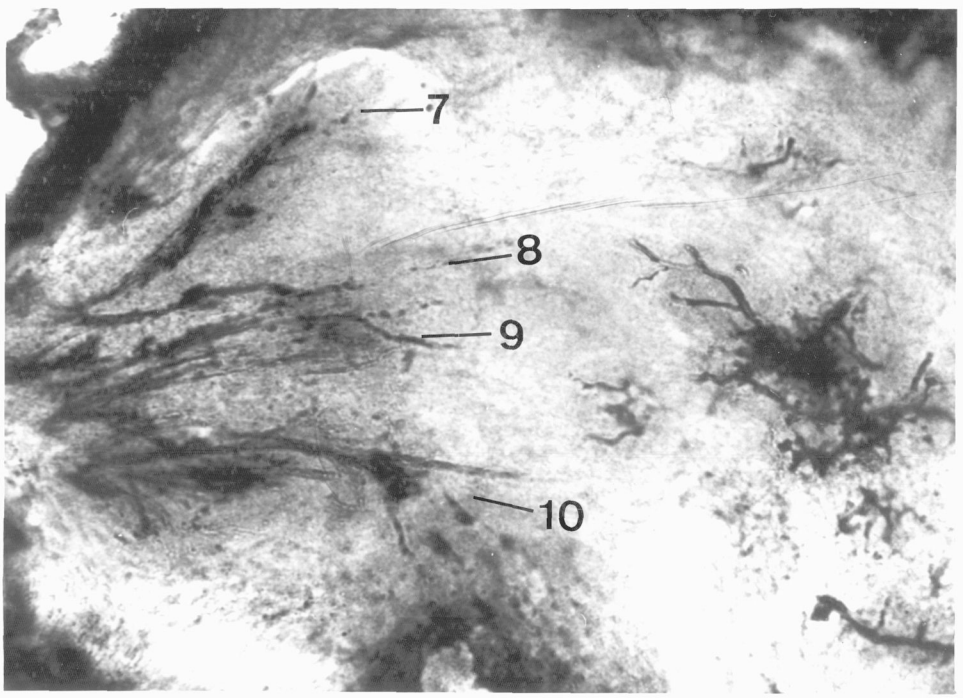
96

Fig. 97. Golgi preparation to show structural unit No. 1 from the anterior region of the suboesophageal ganglion (X 480).

Fig. 98. Golgi preparation to show anterior group of units, Nos. 7, 8, 9 and 10 (X 336).



97



98

b. Axons in the middle part of the ganglion (units 25 to 50):

25. This axon, of unknown origin, starts from the lateral region of the ganglion and runs ventrally with type B_1 endings in the ventral region of the neuropile occupied by the ventral glomerular body (Figs. 99 and 104).

26. This unit crosses almost the whole length of the ganglion. It comes from the dorsal part of the ganglion, runs ventralward in the middle of the ganglion, not far from the midline, with root-endings of type G_1 in the ventral region of the ganglion, where their dendrites arborize to join others coming from unit 27; this structure probably runs along fibre tract st_{11} (Figs. 100 and 104).

27. This unit occurs in the ventral region of the ganglion. It seems that the main axon runs through fibre tract st_{21}, ending with two type G_1 processes. The terminal dendrites join homologues from unit 26 in the area occupied by the ventral glomerular body (Figs. 100 and 104).

28. This unit is situated in the laterodorsal region of the ganglion (Figs. 99 and 104) and is composed of several long axons extending ventrally, with type C_1 endings (Fig. 107) in the middorsal ipsilateral area of the neuropile. This unit seems to derive from, or be closely associated with, some of the giant motor cells which are located in the laterodorsal region. It is not clear how many branches are derived from each cell, nor is it easy to identify the main axon among the several long fibres.

29. This structure derives from the motor neuron at the lateral region of the ganglion. The fibre branches run a short way

ventralwards with type B_2 endings (Figs. 99 and 104).

30. This axon runs from the lateroventral region of the ganglion dorsad for a short distance with a type B_2 ending. The main branch originates from a lateroventral motor neuron. Comparison with osmium-stained sections indicates that this main fibre runs through tract st_{12} while its dendrites arborize in the ventral glomerular body (Figs. 99 and 104).

31. An axon coming from the maxillary nerve and extending a short distance into the neuropile, with type C_5 endings in the area occupied by the lateral glomerular body (Figs. 99 and 104). Presumably this axon runs via fibre tract st_{15}.

32. This unit is situated in the lateral region of the ganglion, extending a short distance ventrally, with a type B_2 ending shortly before axon 25 in the area around the border of the lateral glomerular body (Figs. 99, 104 and 109). Comparing its position with that of axonal tracts stained with osmium ethyl gallate it is clear that the main branch comes from one of the large motor neurons which are located in the lateral region of the ganglion. The axon probably runs through fibre tract st_9.

33. This unit seems to derive from the maxillary nerve and runs a short distance dorsad, with a type A_2 ending. All the branches of the triple stellate radiation of dendrites terminate in the lateral glomerular body (Figs. 100 and 104).

34. This unit is situated in the ventrolateral region of the ganglion and runs a short distance dorsad, with a type A_1 ending. It appears to be the main axon of an association neuron.

35. A large diameter axon of unknown origin, this appears first in the lateral region of the ganglion and extends ventrally, with a type D_1 ending. Its dendrites terminate in the lateral area of the neuropile (Figs. 100 and 104).

36. A long bundle of axons run ventrally from the lateral part of the ganglion and are arborescent in the ventral region of the ganglion, with type F_5 endings (Figs. 100, 104 and 108). The origin of these fibres is not entirely clear but they are probably from the maxillary nerve.

37. This unit is situated in the lateroventral region of the ganglion. It extends dorsally from an area occupied by some motor and association neurons, but the precise origin is not clear. It has a typical type I tree ending (Figs. 101 and 104).

38. The main axon originates from the maxillary nerve in the lateral region of the ganglion. It is usually very close to the beginning of unit 36, and extends a short distance to the ipsilateral neuropile, with a type E_1 ending (Fig. 101). Its dendrites join their homologues from unit 39 in the area occupied by the lateral glomerular body (Fig. 104). The main axon runs a short way through fibre tract st_{15} then spreads out with a large, wide field of small terminations (type E_1) in the middle of the lateral glomerular body. This unit is almost invariably impregnated and very clear.

39. A long varicose axon which originates from the ventral region of the ganglion, extends dorsally and has two branches, carrying type F_1 endings. One branch runs to the middle of the

ipsilateral neuropile, its dendrites joining their homologues from unit 38. The other runs to the centre of the ganglion and ends just before the midline (Figs. 101, 104 and 108). The main axon may arise from an association neuron, and probably runs with fibre tract st_{14}; its dendrites arborize in the lateral glomerular body.

40. This unit originates from the mid-ventral region of the ganglion, extending dorsally (very close to the midline) with type G_1 endings just before the centre of the ganglion. The axon visible in Golgi preparation presumably comes from a motor neuron in the mid-ventral region of the ganglion, and it and its collateral run through fibre tract st_{22}; see Figs. 101 and 104.

41. This unit lies in the central region of the ganglion. It is of unknown origin, spreads centrally with two branches ending in type D_2 structures (Fig. 101). One branch ends very near the midline, while the other spreads laterally and terminates after a short distance in the neuropile (Fig. 104).

42. A very fine punctuate axon which originates from the lateroventral region of the ganglion, extending extending dorsally with two main long, fine punctiform branches. One ends in the centre of the ganglion, very near the midline, while the other ends in the middle area of the ipsilateral neuropile (Figs. 102, 104 and 110). These two main branches and all the other subsidiary ones terminate in type F_3 structures. The origin of this unit is uncertain.

43. A long fibrillar axon from the maxillary nerve which expands dorsally and ends with several branches (Fig. 102); some terminate after a short distance in the lateral region of the

ganglion, with type F_4 endings, while the far end runs to the middle region of the ipsilateral neuropile with a type C_3 ending (Fig. 104). The main axon runs through fibre tract st_{20}.

44. This long axon (Figs. 102, 104 and 111) arises from the maxillary nerve, and has a type C_3 ending. Its shorter collateral soon terminates in the lateroventral region of the ganglion, while the axon and its main collateral end in the centre of the ganglion very near to the midline. The main axon runs through fibre tract st_{15}.

45. This axon originates from the mid-ventral motor neuron (Figs. 102 and 104), and extends with one long fibre apparently ending after a short distance in the ventral region of the neuropile. It is very difficult to make out the termination and to ascertain how far it reaches. The axon runs through fibre tract st_{24}.

46. This long axon originates from a motor neuron in the mid-dorsal region of the ganglion (Figs. 102 and 104). The main axon extends ventrally along the midline, and spreads out with a type G_4 ending after a short distance in the mid-dorsal region of the neuropile. The main axon runs through fibre tract st_{22}.

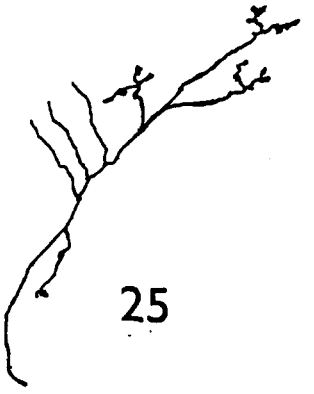
47. This axon, of unknown origin, runs from the latero-dorsal region of the ganglion and has a type G_4 ending near the midline in the mid-dorsal region of the neuropile, where its dendrites join their homologues from unit 46 (Figs. 103, 104 and 112). The main axon probably runs through fibre tract st_{17}.

48. This large-diameter axon, of unknown origin, is situated in the centre of the ganglion. A large branch extends dorsally with type K endings near the dorsal midline area - the area where the dendrites of unit 46 and 47 are arborescent. Another branch runs ventralward, ending in a type D_2 structure near the midline in the mid-ventral region of the neuropile (Figs. 103 and 104).

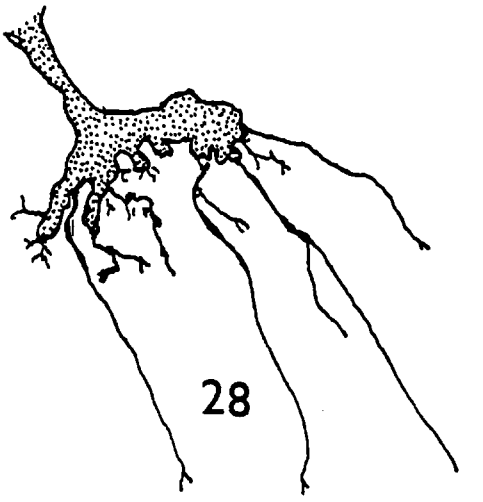
49. A very fine punctuate axon of unknown origin, which appears in the middle of the neuropile near the midline (Figs. 103 and 104).

50. An axon from the dorsal region of the ganglion (Fig. 103), which extends ventrally with wide field terminations in the laterodorsal region of the ipsilateral neuropile (type F_5 and C_4 structural endings, Fig. 104).

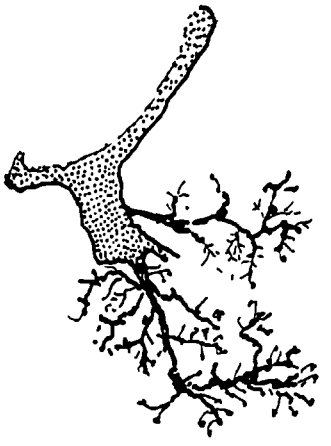
Figs. 99.25 to 103.50 show the various axons and their branches and terminations in the middle part of the ganglion.



25



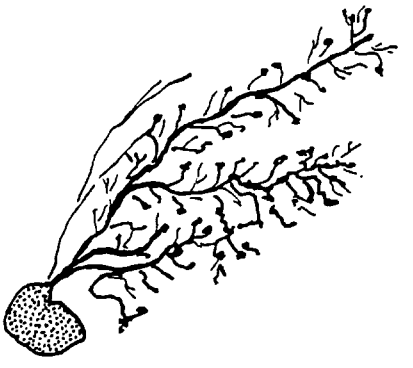
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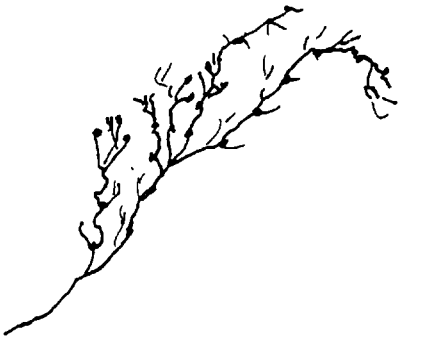
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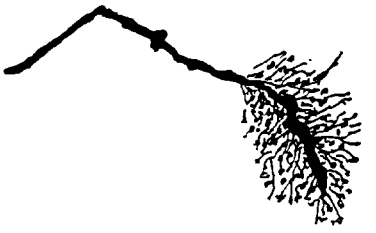
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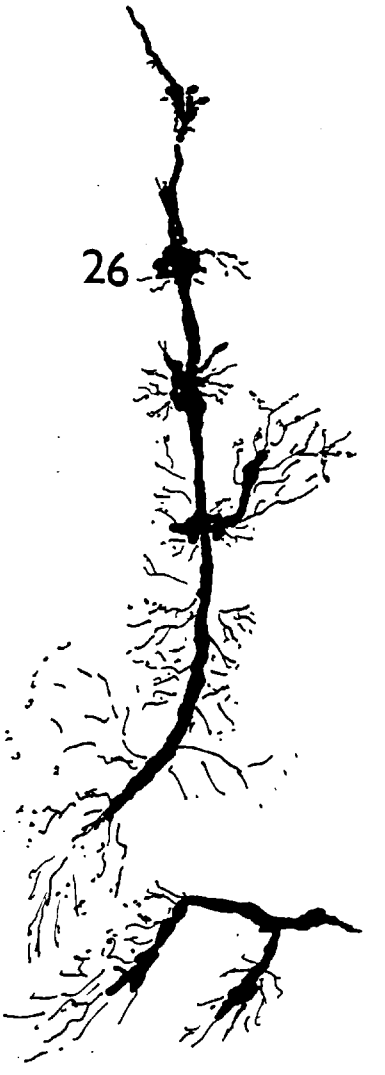
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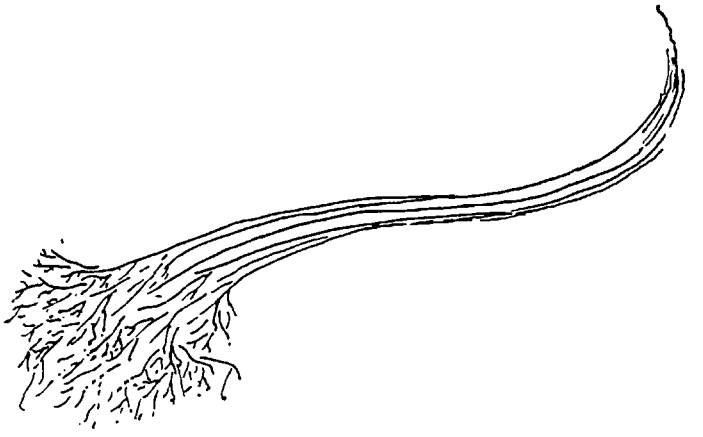
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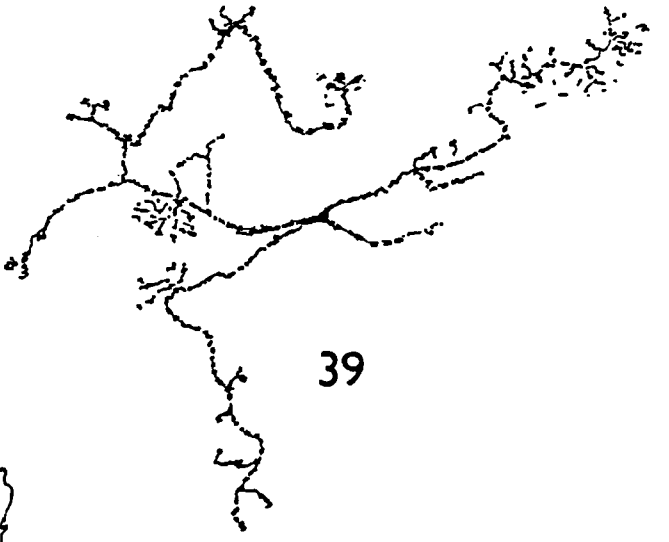
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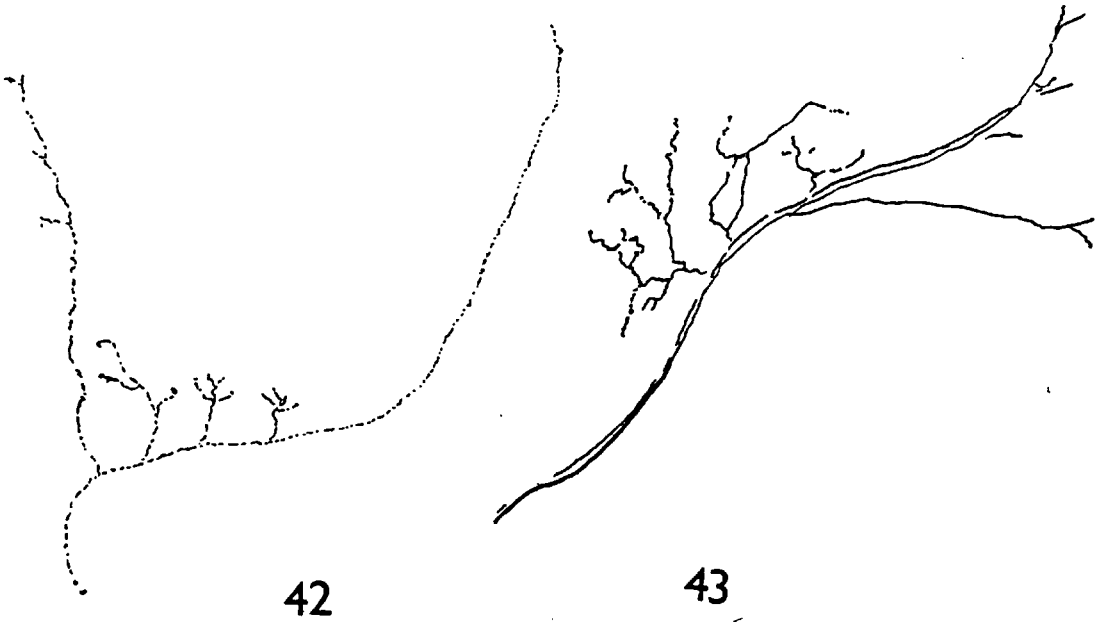


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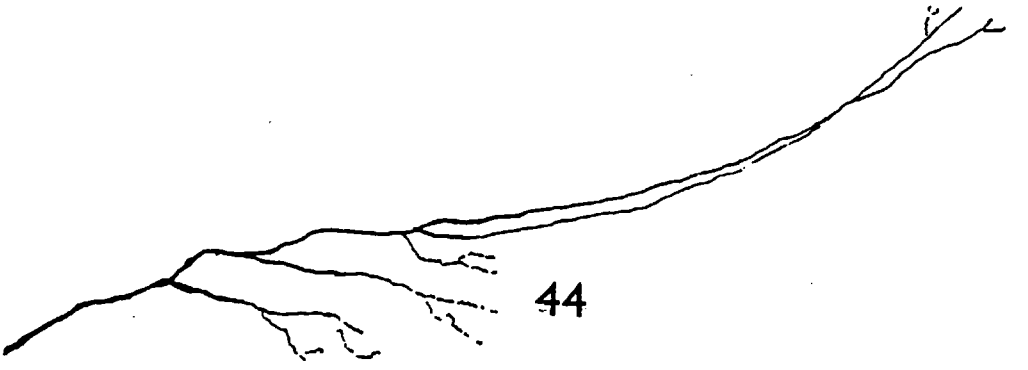


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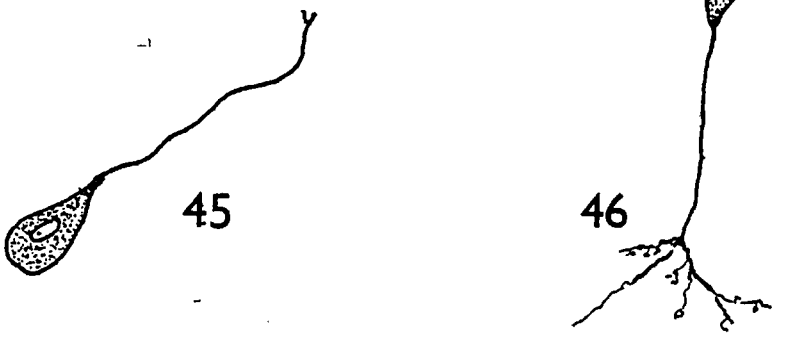
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42 43



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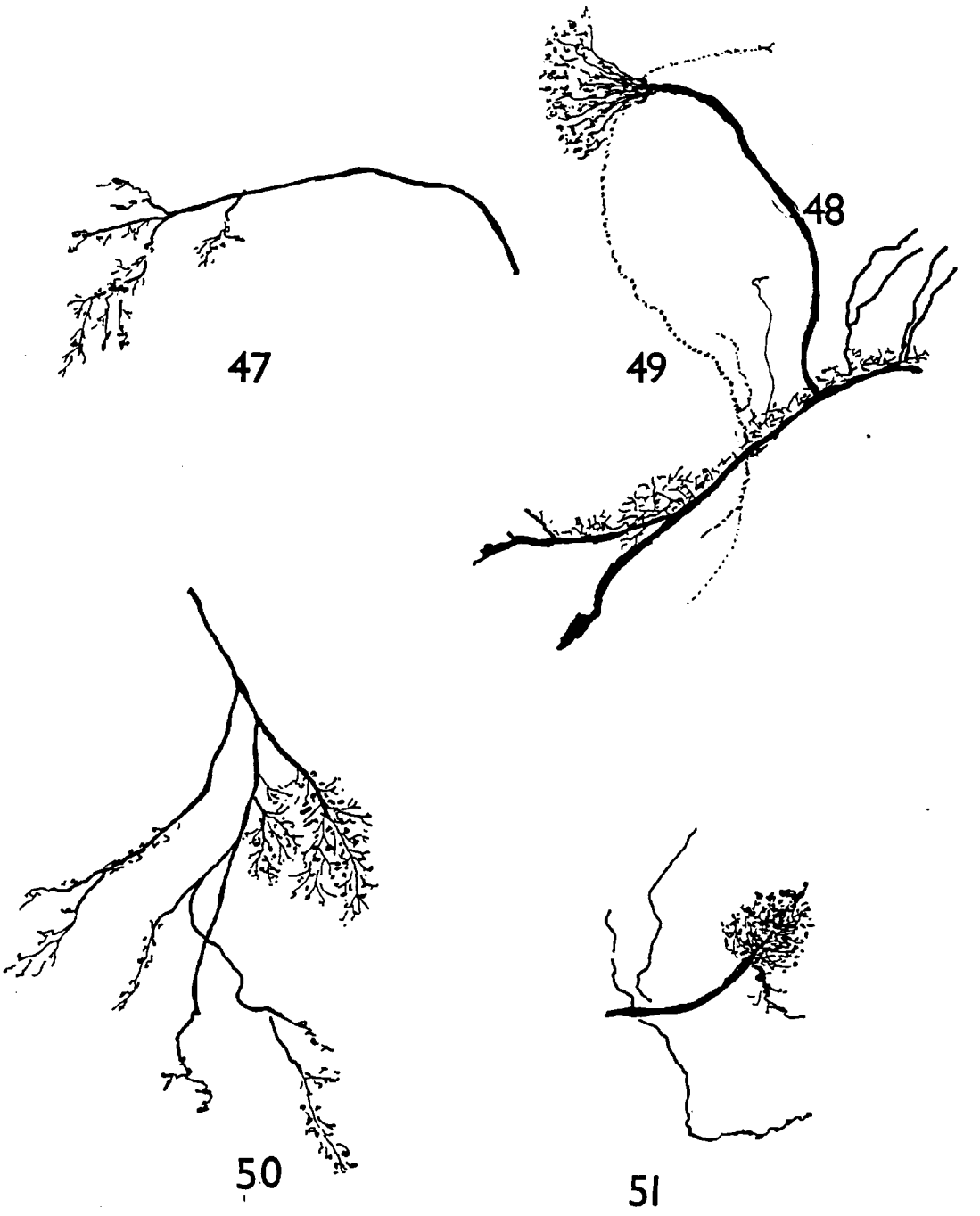
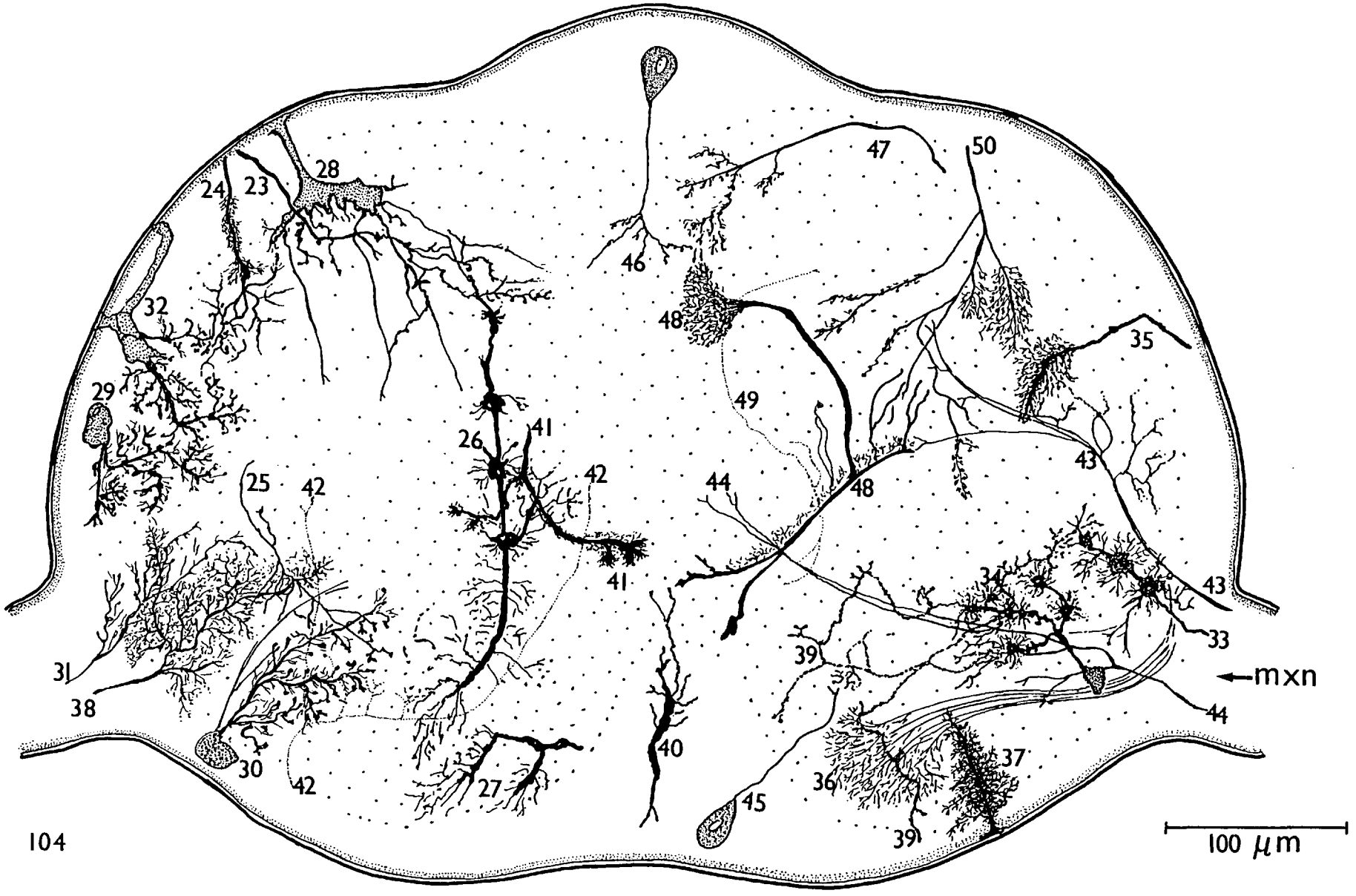

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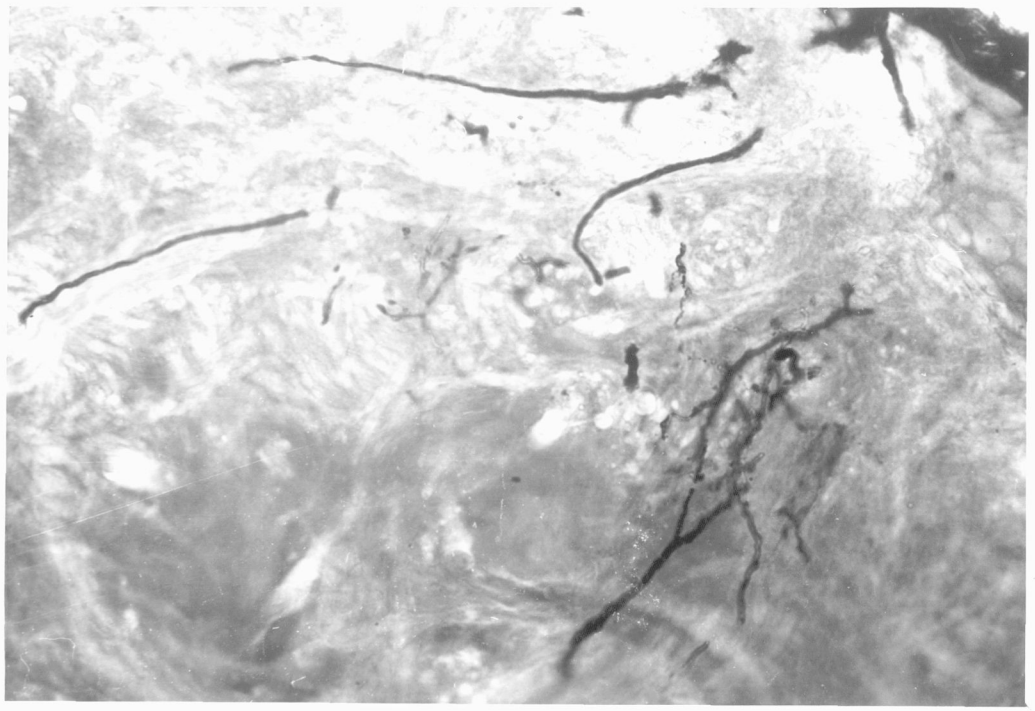
Fig. 104. Various axons and their position in the middle part of the ganglion.


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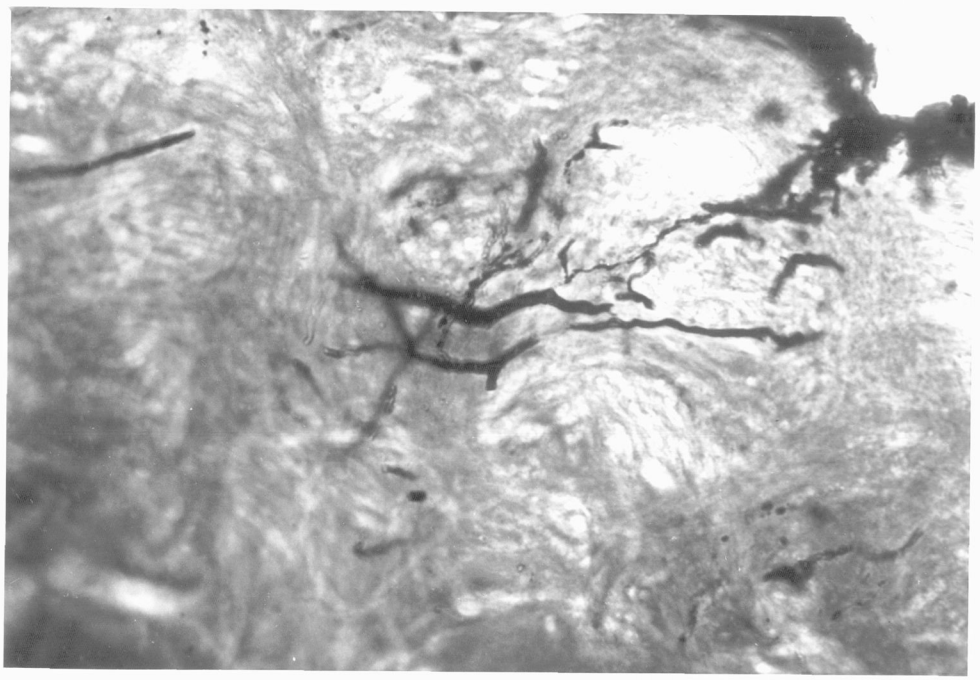
104

Fig. 105. Double-impregnation preparation of some large diameter axons as shown in material stained by the modification of Kallius' procedure (double impregnation procedure, X 350).

Fig. 106. Axon 23 (from the anterior cervical nerve) as shown in material stained by a modified double-impregnation procedure (X 350).



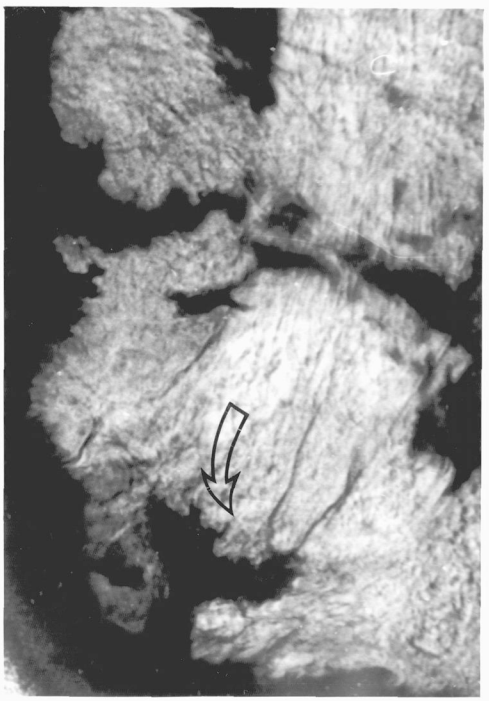
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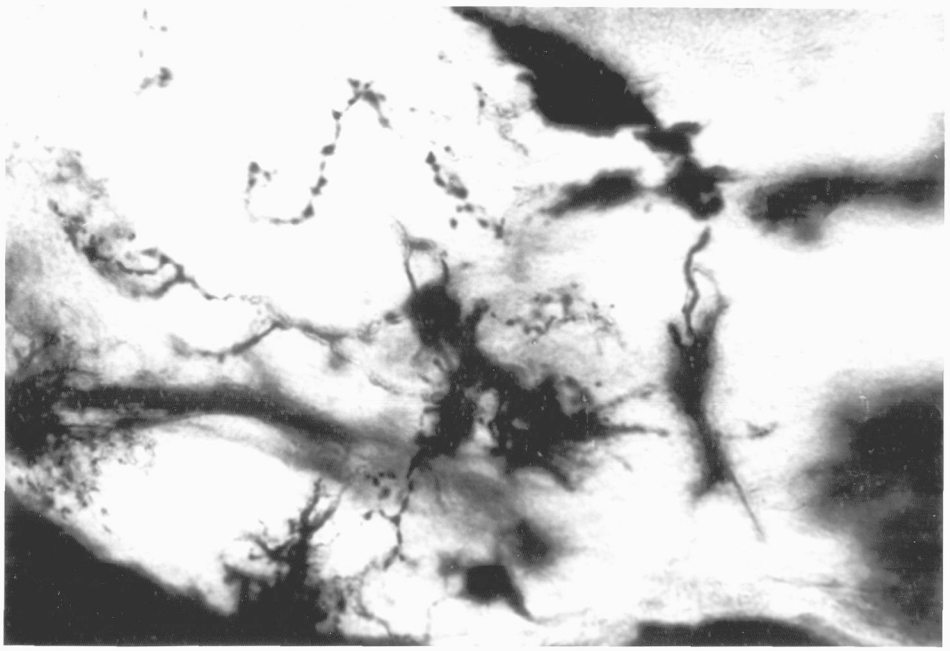
106

Fig. 107. Golgi preparation of unit 28 and type C_1 structural ending (X 480).

Fig. 108. Golgi preparation of units 36 and the long, condensed punctate fibrils of axon 39 (X 480).



107



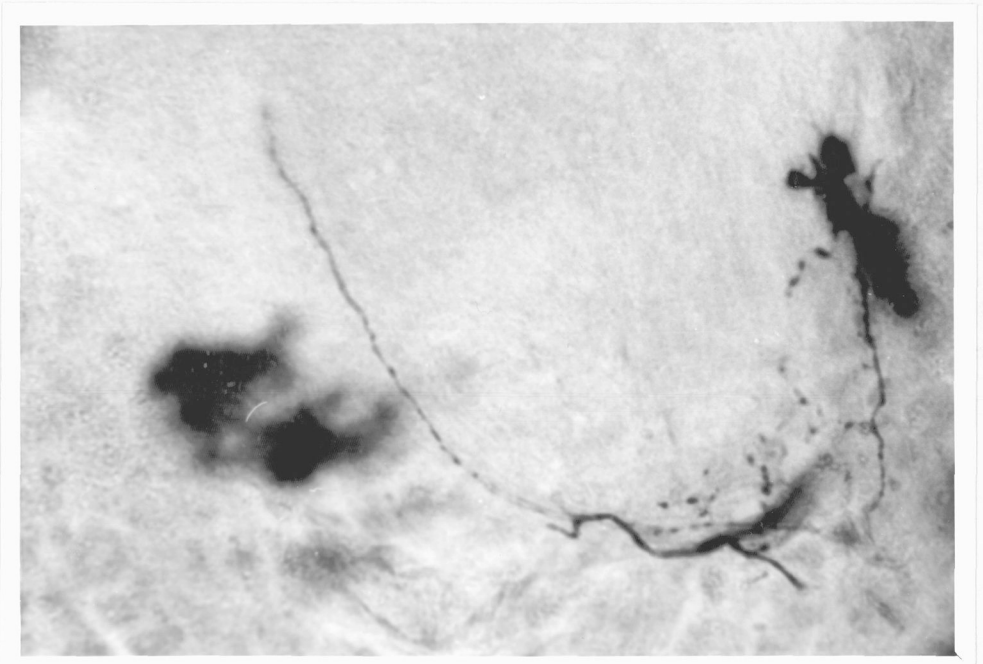
108

Fig. 109. Golgi preparations of motor neuron axon No. 32, to show type B_2 dendritic ending (X 1320).

Fig. 110. Golgi preparation of a fine punctate axon, No. 42, with type F_3 ending (X 480).



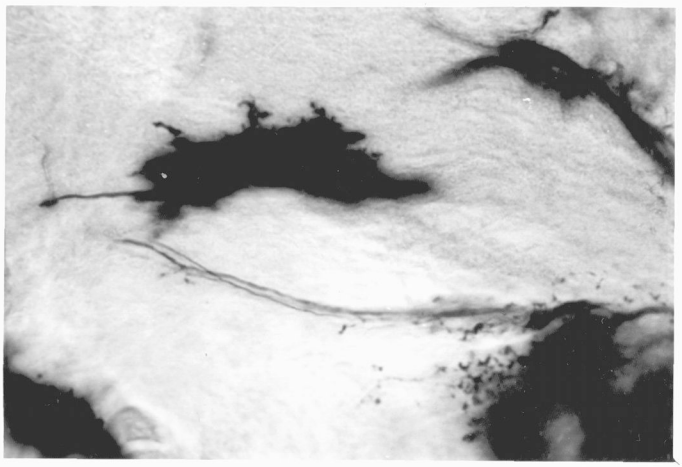
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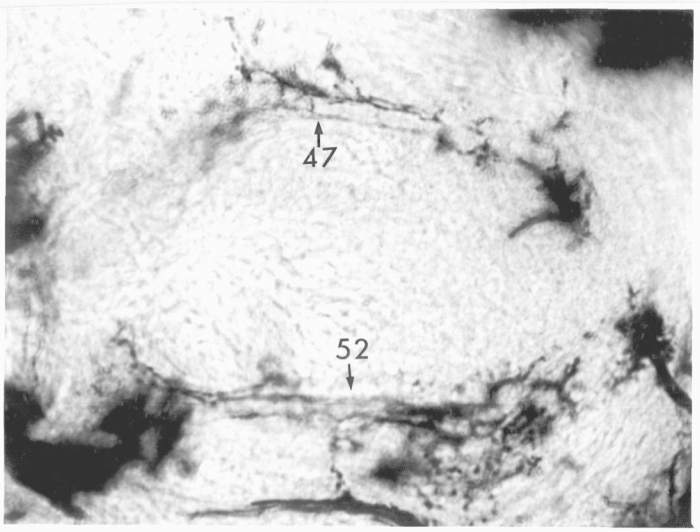
110

Fig. 111. Golgi preparation of unit 44, in the middle region (a long fibre axon, X 350).

Fig. 112. Golgi preparation of axons 47 and 52 (X 350).



111



112

c. Axons in the posterior part of the ganglion (units 51 to 69):

51. This axon from the lateral region of the ganglion runs a short distance to the lateral part of the neuropile, where it ends in type G_2 structures. It could be one of the axons from the maxillary nerve (Figs. 103 and 117).

52. This main axon runs from a point near the centre of the ganglion, with a collateral extending to the lateral region of the ganglion. The axon and collateral terminate as wide field endings, one with a type B_3 structure, the other with a type G_4 structure (Figs. 103, 117 and 118). This unit seems to receive some dendrites from the maxillary nerve and from the labial nerve. The main axon runs along fibre tract st_{25}.

53. This bundle of long axons from the maxillary nerve gives rise to a wide field termination from the lateral to the central parts of the neuropile with two different types of ending, F_5 and G_4. These axons run in fibre tract st_{15} (Figs. 113, 117 and 119).

54. A bundle of long axons from the maxillary nerve, with type B_3 endings just before the centre of the ganglion. These axons run through fibre tract st_{16} (Figs. 113, 117 and 120).

55. A group of long axons from the maxillary nerve, running to the ventral region of the neuropile, where their dendrites are arborescent (Figs. 113, 117, 119 and 120). The axons have type G_4 endings. This group of axons presumably runs through fibre tract st_{26}.

56. This axon originates from the maxillary nerve and runs dorsally to the middle region of the ipsilateral neuropile where its dendrites spread out with type A_1 endings (Figs. 114, 117 and 121). This axon apparently runs through fibre tract st_{15}.

57. A group of long axons which originate from the maxillary nerve and run dorsally, dividing into two groups which end in type B_4 and H structures. The first group ends after a short distance in the lateral border of the ganglion, while the second group extends a short way into the lateral ipsilateral region of the neuropile (Figs. 114 and 117).

58. A group of axons which originates from the labial nerve, extending dorsally with two main branches to the centre of the ganglion (Figs. 114, 117 and 112). The dendrites of these run with type E_2 endings around the area occupied by longitudinal tracts st_{28} and st_{30}. The axons run along fibre tract st_{36}.

59. A group of long axons which originate in the labial nerve, and extend near unit 58 with some branches curving slightly to the ventral region of the neuropile and having type E_1 dendritic endings (Figs. 114, 117 and 122).

60. This axon originates from the labial nerve and extends to the lateroventral region of the neuropile, where its branches and dendrites are arborescent, forming type G_4 endings (Figs. 115, 117 and 123).

61. This is a long axon from the labial nerve which expands dorsally, with type F_5 dendritic endings in the lateral region of the neuropile (Figs. 115 and 117). There is little doubt that this axon runs along fibre tract st_{36}.

62. This long axon appears in the ventral region of the ganglion and extends dorsally, giving off a short collateral ending in the lateral part of the neuropile, with the main axon running further dorsally and ending in the middle of the neuropile of the ipsilateral region (Figs. 115 and 117). Both endings are of type C_3.

63. This long, very fine axon appears in the middle of the neuropile (Figs. 115 and 117). It is of unknown origin, extending ventrally to the lateroventral region where it spreads out with type F_2 endings.

64. This long, fine axon of somewhat uncertain origin extends from the lateral region of the ganglion to the mid-ventral part of the ipsilateral neuropile with type F_3 endings (Figs. 115 and 117). This axon probably comes from the labial nerve and runs through fibre tract st_{36}.

65. This axon runs from the lateroventral region of the ganglion a short way dorsalwards to endings in the lateral region of the neuropile with type C_2 terminations (Figs. 115 and 117).

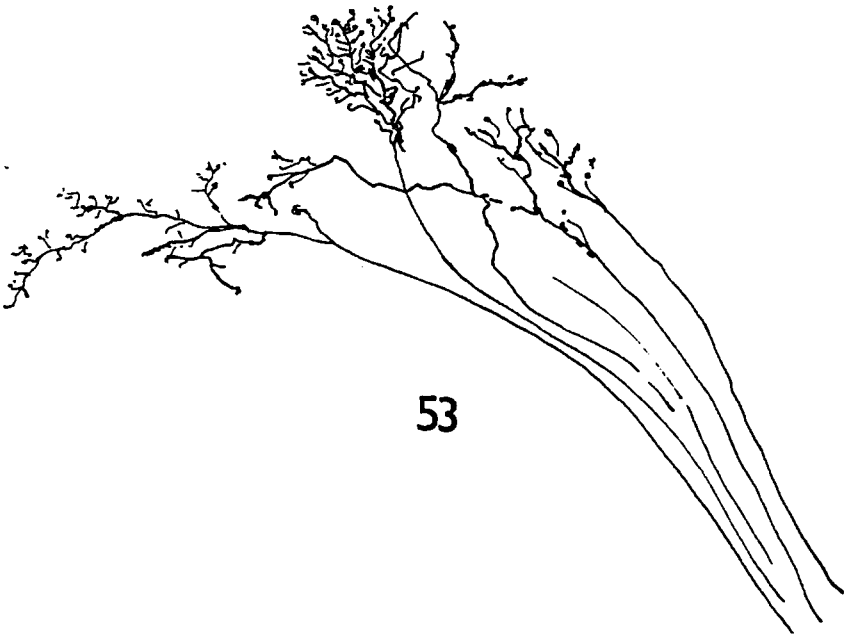
66. This axon, also of uncertain origin, runs from the centre of the ganglion ventrally to its lateroventral region with type G_2 endings, its dendrites arborizing to join their homologues from axon 60 (the latter coming from the labial nerve) (Figs. 116 and 117).

67. This large diameter axon runs from the dorsal region of the ganglion, ventrally to the middle of the ipsilateral neuropile, dividing into two branches with type D_3 endings (Figs. 116, 117 and 124). This unit possibly arises from the mid-dorsal motor neuron.

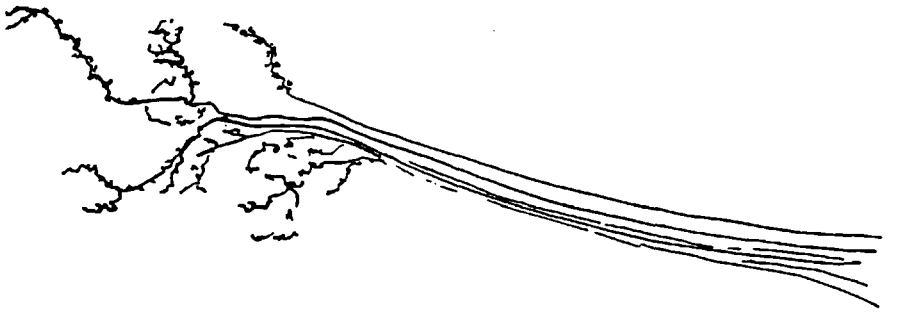
68. This structure is situated in the dorsal region of the ganglion. It originates near the midline, with a long axon curving slightly dorsad, and with type G_4 endings in the dorsal region of the neuropile (Figs. 116 and 117). This axon appears to arise from the mid-dorsal group of association and motor neurons and runs through fibre tract st33, curving around one of the longitudinal tracts which occupy this area.

69. This wide field structure is situated in the dorsal region of the ganglion. The large-diameter axon runs from the laterodorsal region to type B_2 endings in the dorsal area. Some branches end very near to the midline and others in the laterodorsal region and the middle of the ipsilateral neuropile (Figs. 116, 117 and 125). This structure presumably arises from the group of motor or association neurons in the dorsolateral region of the ganglion. It appears to run through fibre tract st37, and usually ends in the region which contains some of the longitudinal fibre tracts.

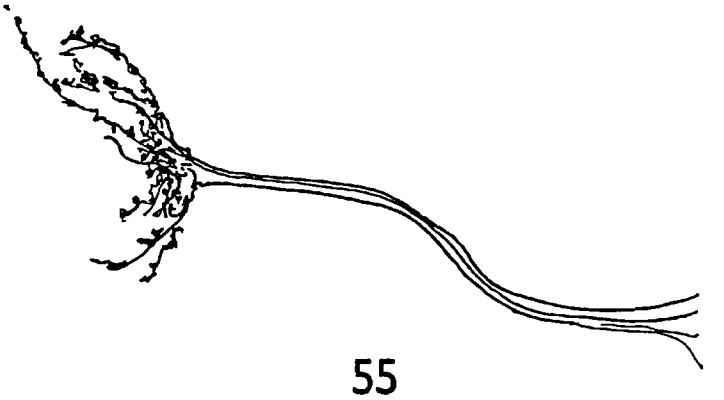
Figs. 103.51 & 103.52; 113.53 to 116.69. Showing the various axons and their branches and terminations, located in the posterior part of the ganglion.



53

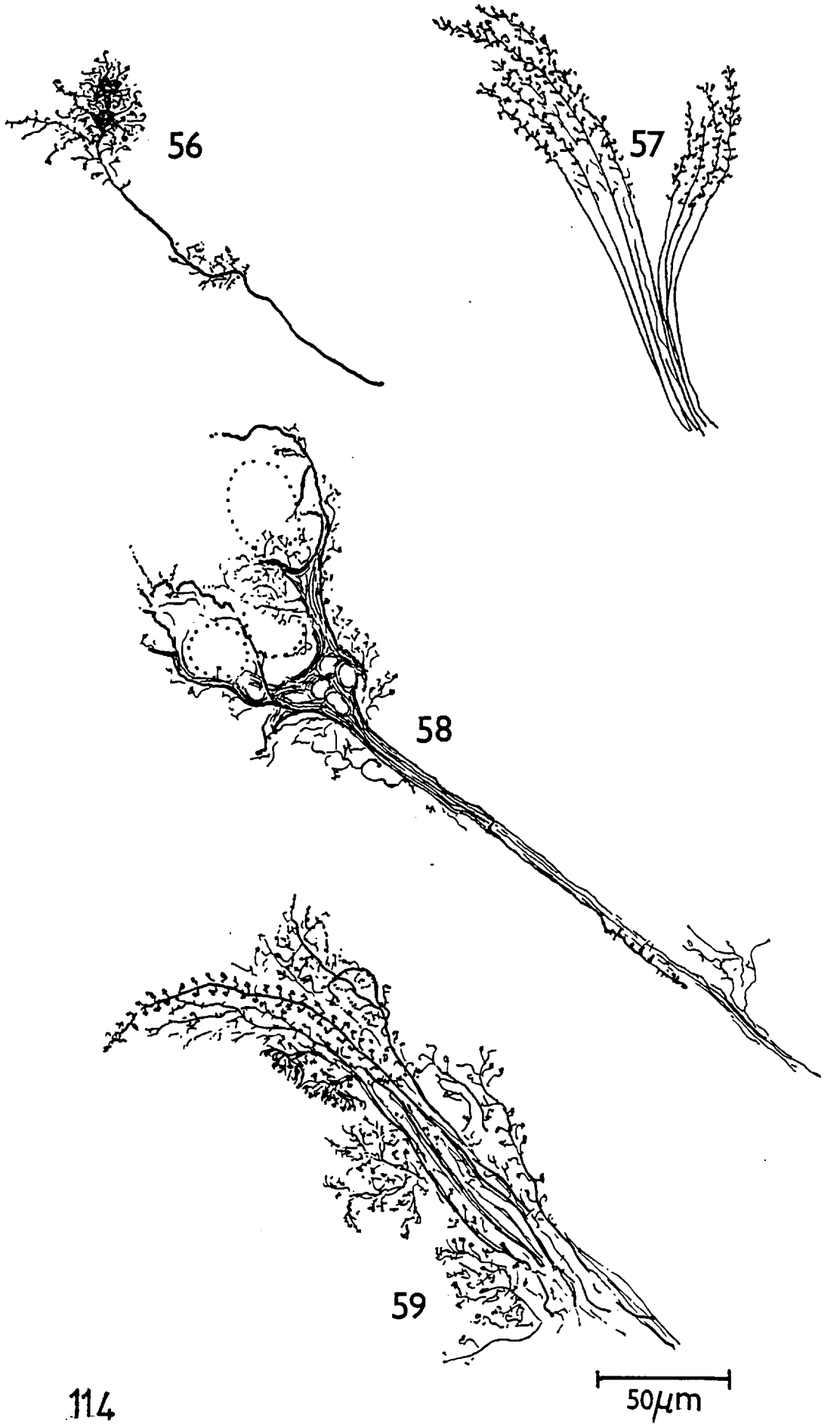


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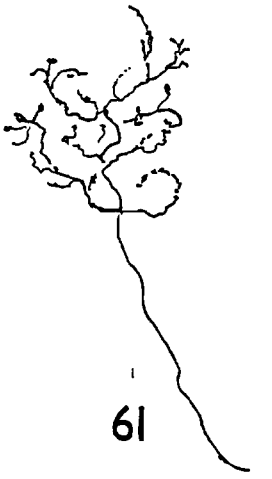
50μm



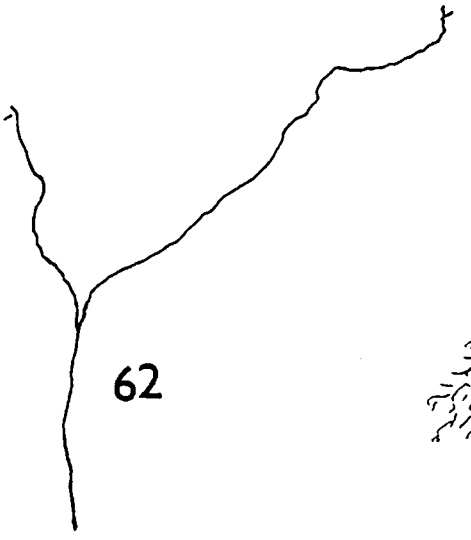
114



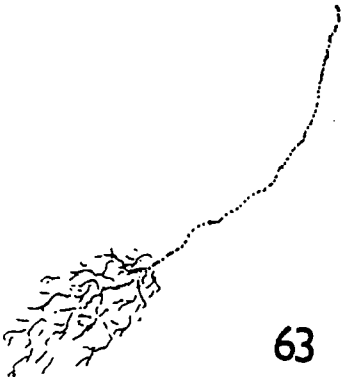
60



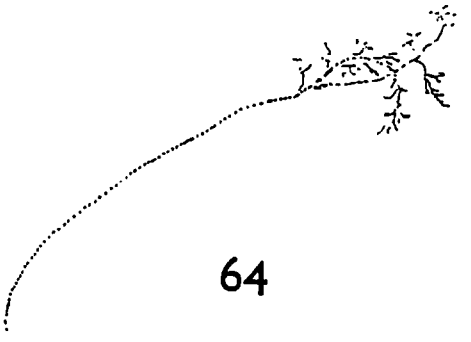
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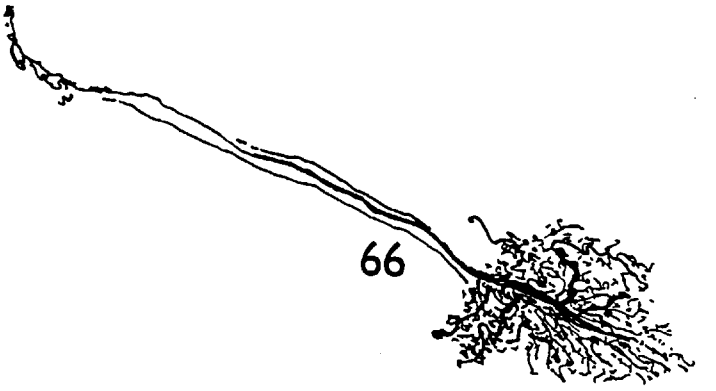


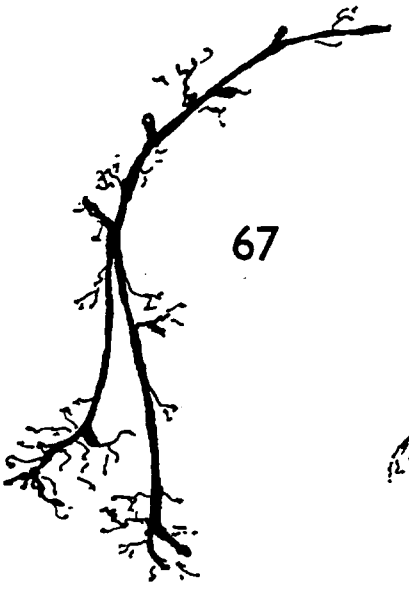
64

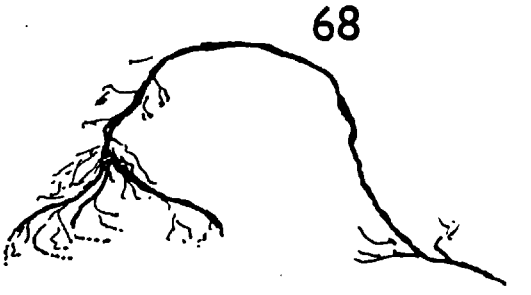


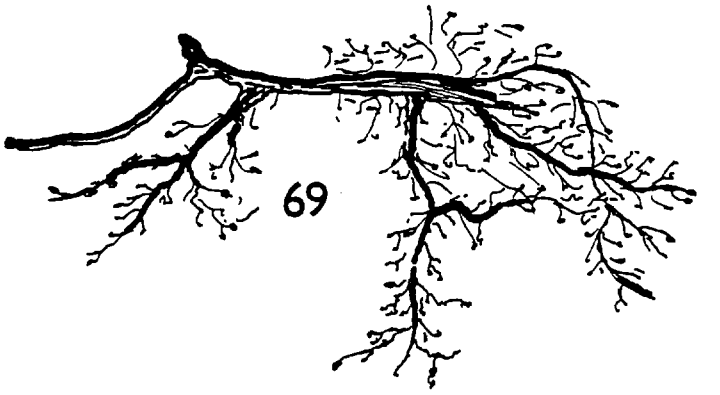
65

50µm









116

50μm

Fig. 117. Various axons and terminations, with their positions as located in the posterior part of the ganglion.

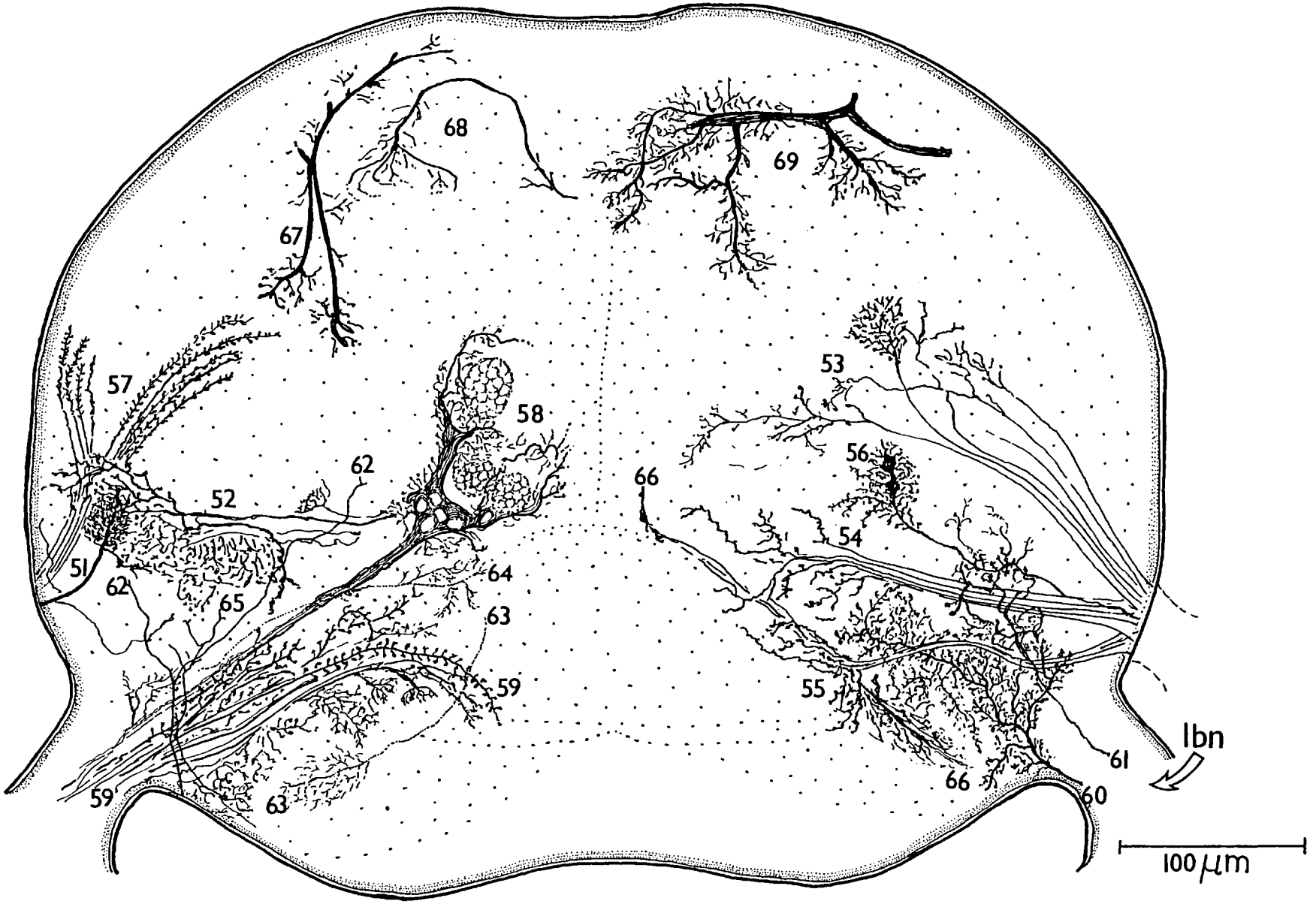
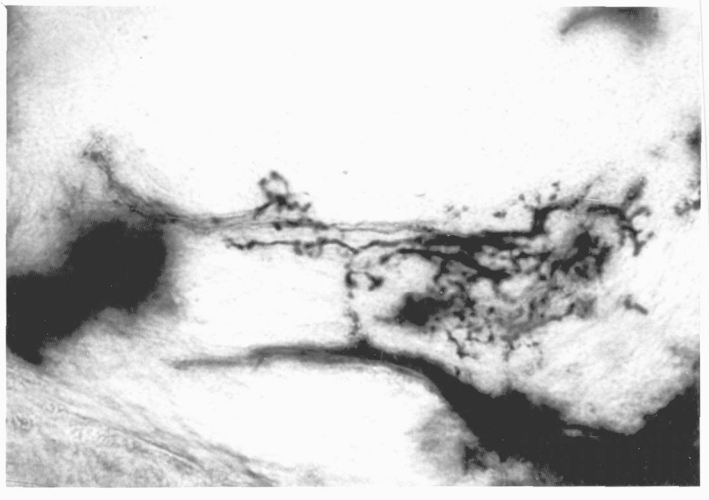

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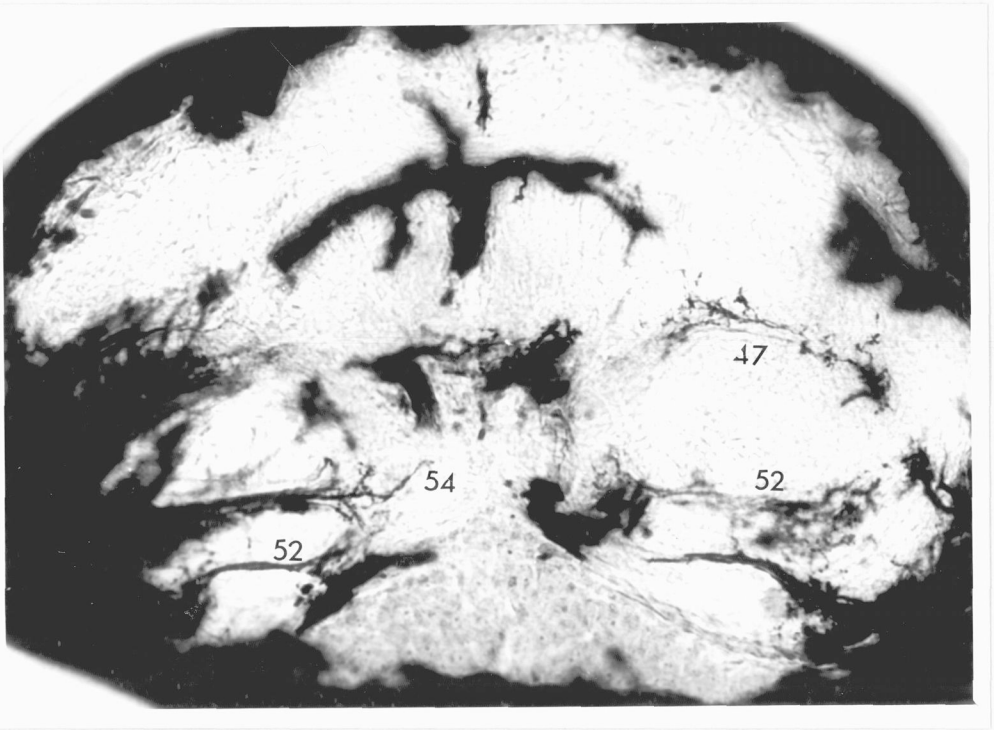
Fig. 118. Golgi preparation of unit 52 (X 480).

Fig. 119. Golgi preparation to show some units from the posterior region of the suboesophageal ganglion (units 52, 53, 54 and 55) and one unit from the middle region, No. 47 (X 264).

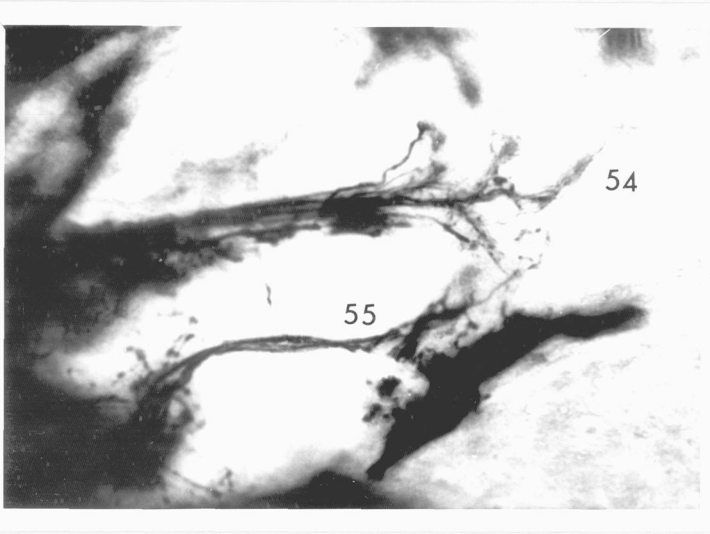
Fig. 120. Golgi preparation of units 54 and 55 (X 560).



118



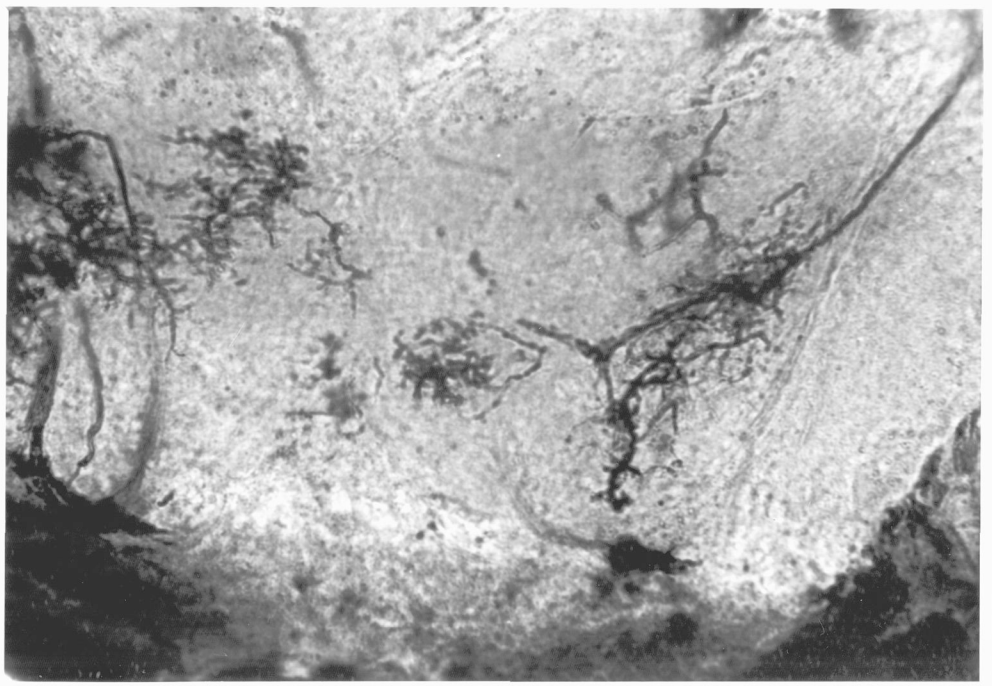
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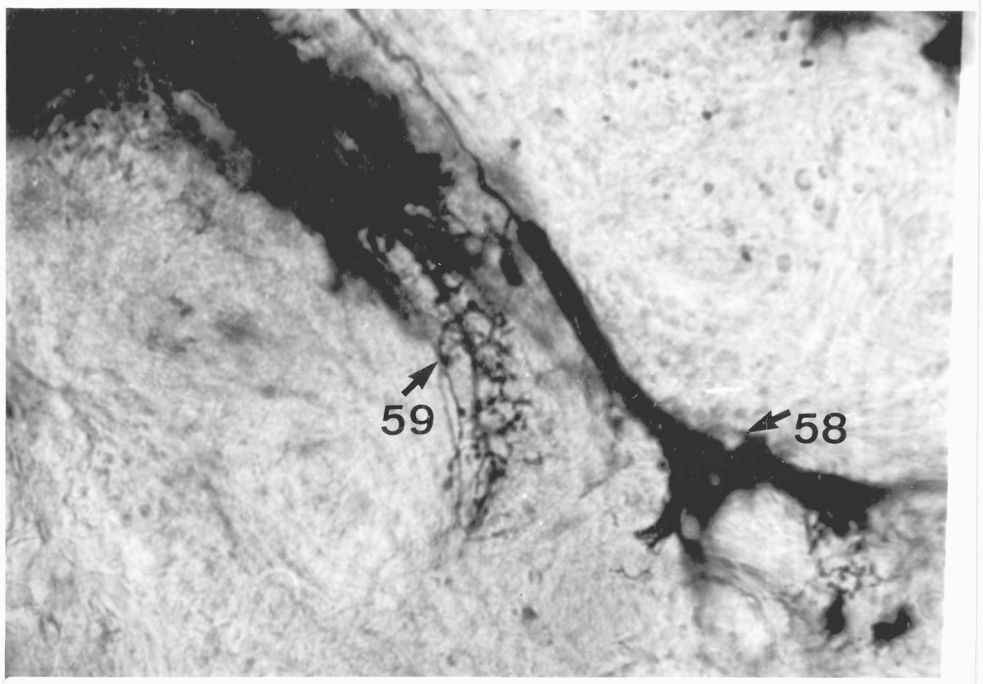
120

Fig. 121. Golgi preparation of axon 56 (right hand side) and some of the stellate endings on the left side (X 350).

Fig. 122. Golgi preparation of large diameter axon No. 58 and axon No. 59, with terminations (X 520).



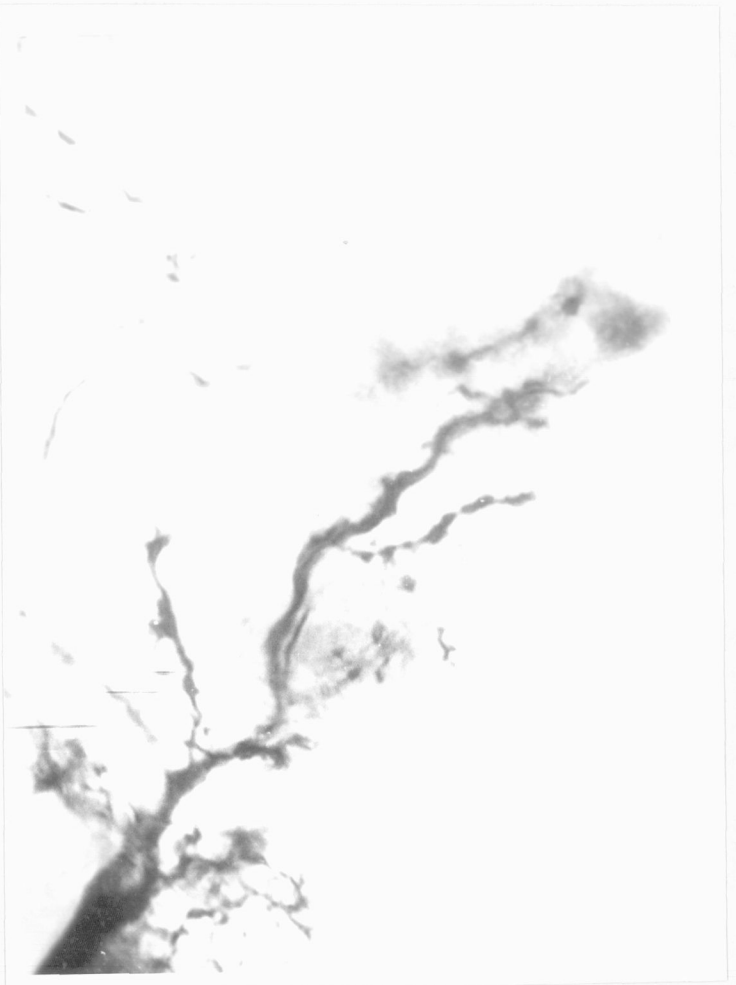
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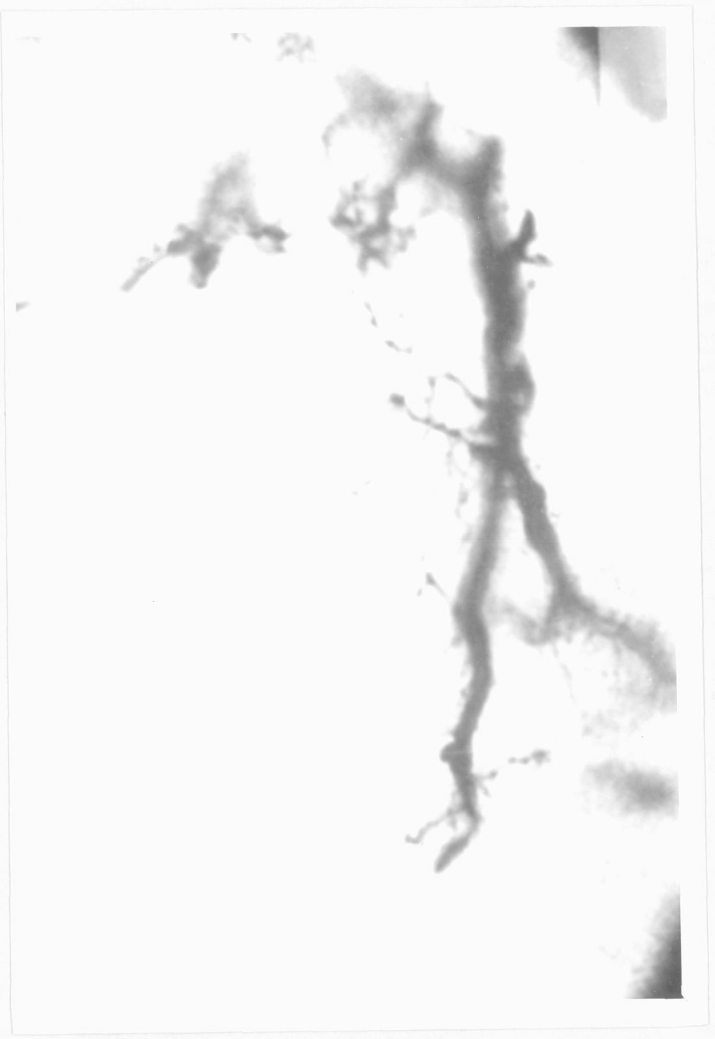
122

Fig. 123. Golgi preparation of the main axon and its branches of unit No. 60 (X 520).

Fig. 124. Golgi preparation of axon No. 67 (X 920).



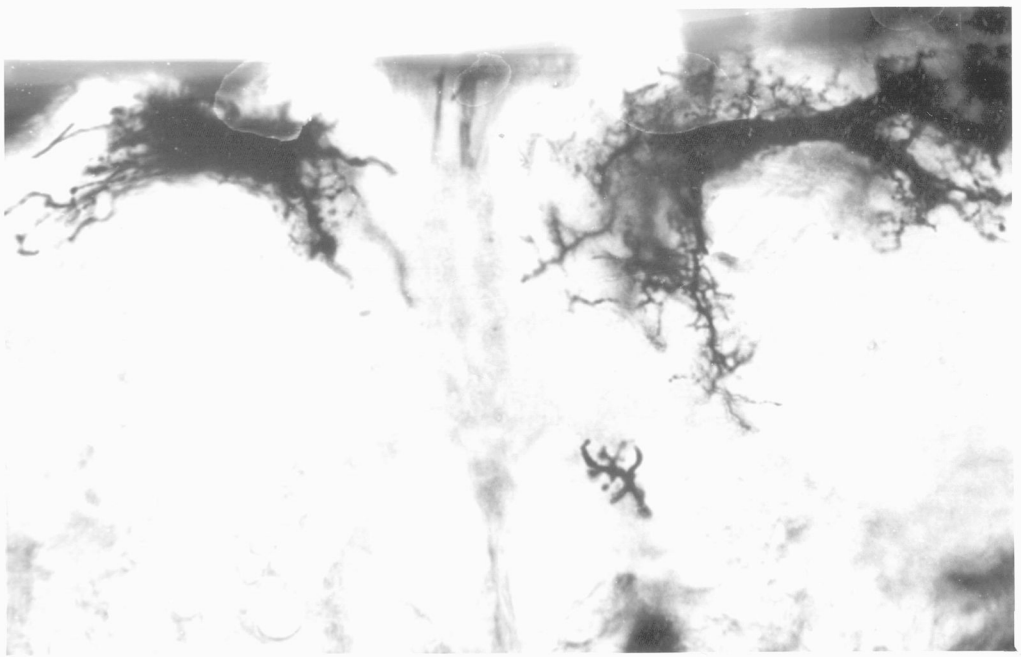
123



124

Fig. 125. Golgi preparation to show unit No. 69 in the posterior region of the suboesophageal ganglion (X 560).

Fig. 126. Golgi preparation of some types of dendritic ending and a large diameter axon with type C_4 ending (X 220).



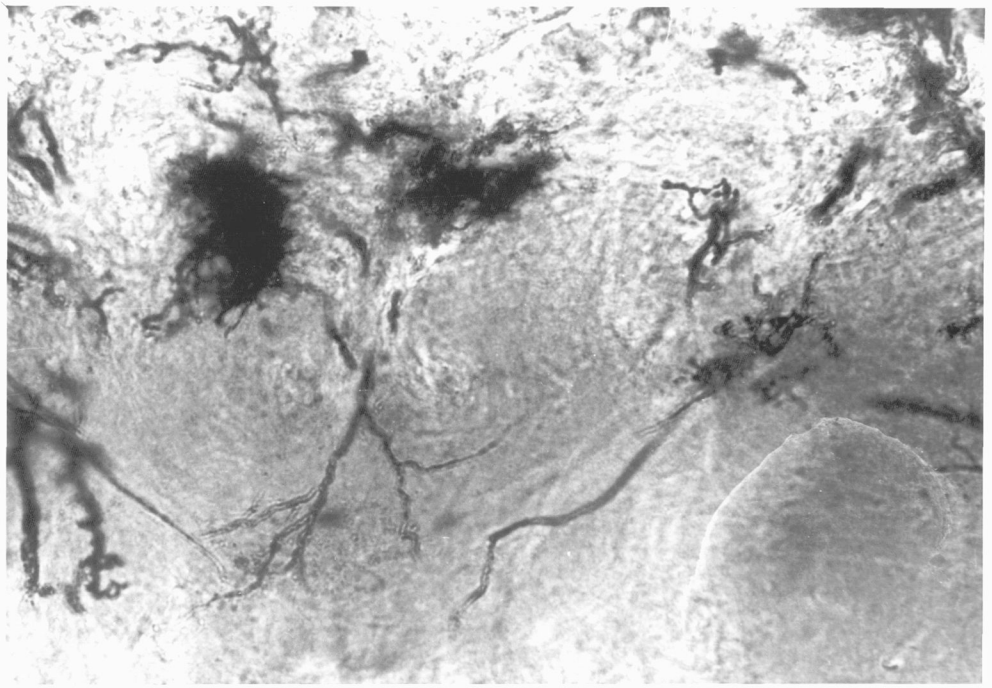
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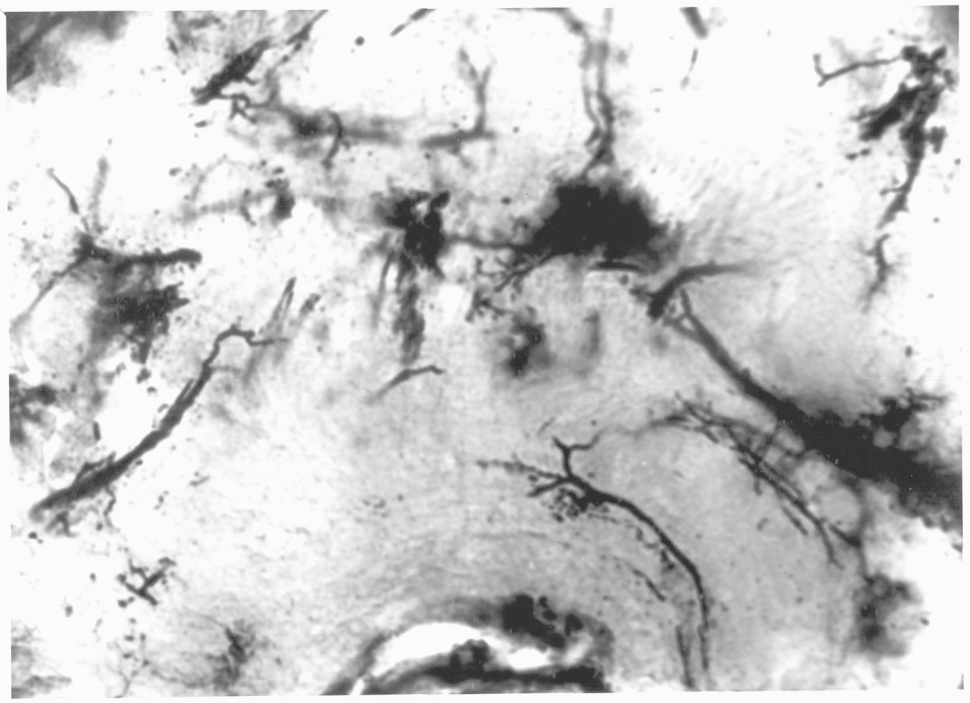
126

Fig. 127. Axon No. 66 and some other endings as stained by a mixed impregnation method (X 350).

Fig. 128. Some axons and dendrites as shown by the mixed impregnation method (X 350).



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128

VII GENERAL DISCUSSION

The present study was undertaken in order to investigate the detailed histological structure of the suboesophageal ganglion of Acheta domesticus (Linn.). The insect nervous system is extremely complex and only a few cases have been worked out in detail.

The suboesophageal ganglion is mentioned and described very briefly in insects by some investigators, but has otherwise received no detailed attention (Guthrie, 1961). Guthrie also pointed out that the suboesophageal neuromeres are so closely integrated in Gerris that only in sections passing through certain parts of the central nervous system can they be distinguished. The positions of the transverse tracts is the most reliable guide to their orientation, but the main fibre mass is essentially continuous, and it is difficult to decide the precise limits of the elements within it.

Several topics have been dealt with above which require discussion in the light of other work; of these, the following subjects have been selected as of special interest.

1. Rearing technique:

In the present work, the method followed for rearing the crickets was slightly different from that described by Ghouri & Mcfarlane (1958) and Kubra (1978). A mass culture rearing colony was established, so providing a very simple method for rearing large numbers of insects without elaborate facilities or equipment. Even the laboratory temperature (22°C to 28°C) was satisfactory, and humidity did not require special control after the colonies had been provided with sufficient water and moistened sand for

oviposition.

2. Dissection of the suboesophageal ganglion:

Many different methods of killing the adult insects anaesthetically before dissection and histological treatment were tried, but the best results were obtained after abdominal injection with the fixative solution, followed by dissecting the material after immersing the insects in 70% alcohol. This method helps the fixative solution to penetrate the tissues and gives good histological preservation.

3. General description:

In the general description of the suboesophageal ganglion of some insects most of the previous studies showed that the suboesophageal ganglion is the ventral ganglionic centre of the head and is composed of the united ganglia of the three primitive gnathal segments. It follows that though its components are fused, the ganglion continues to supply the three segments bearing the mouth parts with sensory and motor nerves. It therefore gives off three large pairs of nerves, the anterior to the mandibles, a middle pair to the maxillae, and the posterior ones to the labium (see Albrecht, 1953; Imms, 1957; Guthrie, 1961; Vasvary, 1966; Richards & Davies, 1977). In Gerris the intimate fusion of the three gnathal neuromeres occurs early in development with the result that the components are difficult to identify in the adult. Also the labial centre appears the most distinct in sections, due to its greater bulk and to the anteriorward orientation of its nerves, while the maxillary and mandibular centres have a common lateral nerve trunk and ganglion so that for purposes of topographical description they may be regarded as a single unit (Guthrie, 1961).

Nesbitt (1941), recognised that other smaller nerves are also present in Orthopteroid insects, sometimes arising separately, sometimes in conjunction with one of the larger nerves. These supply the hypopharynx, the salivary ducts and the muscles of the neck. Nesbitt mentioned only one pair of nerves leaving the ganglion to the neck muscles in Gryllus, at the extreme posterior part of the ganglion. He did not recognise the other pair which normally leaves from the aterodorsal surface of the ganglion and runs to the dorsal cervical muscles. Albrecht (1953), identified three very slender pairs extending to the muslces and membrane of the anterior part of the neck and the back of the head in the suboesophageal ganglion of Locusta migratoria. He mentioned that the first two pairs originate from the posteroventral surface of the ganglion, while the third pair arises from the posterolateral end of the ganglion, which receives a pair from the anterior margin of the first thoracic ganglion before it innervates the posterior region of the neck. Henry (1958), found that in Melanophus sp. (Orthoptera), the dorsal cervical muscle bundles are innervated from a nerve arising near the base of the nerve to the salivary (labial) gland. Vasvary (1966) recorded in the suboesophageal ganglion of Tibicen chloromera (Cicadidae) two pairs of nerves arising from the mediolateral and posterolateral areas and innervating the cervical muslces; these two form the first cervical nerve, while the second cervical nerve branches from the nerve innervating the salivary glands, which in turn arises from the posterior lateroventral surface of the ganglion. Vasvary follows Nesbitt (1941), who mentioned another pair of nerves arising from the posterior medioventral surfaces of the ganglion in close association with the interganglionic connectives and innervating the salivary ducts. I believe, however, that all the nerves described by Nesbitt from the extreme posterior part of the suboesophageal ganglion to the salivary ducts do in fact supply the salivary glands,

rather than their ducts. Innervation of the salivary glands by the suboesophageal ganglion is to be expected since the glands arise as embryonic invaginations of the labial segment.

In general in many species there is more than one pair of nerves innervating the cervical muscles (see Albrecht, 1953). Schmitt (1959), for example, found that in the grasshopper, Dissosteira carolina, there are two fine nerves (the cervical nerves) between the posterior lateral surface of the suboesophageal ganglion and the cervical muscles. The presence of these may indicate that the cervix of insects involves a labial component.

4. Cellular composition:

In the present study the perineurial cells are considered as type I glial cells. As mentioned above this view is shared by Wigglesworth (1959), Heywood (1965) and Ali (1967 & 1973). Pipa (1961), however, excludes the perineurium from the glial elements of the insect nervous system while Eastham (1930) and Ashhurst (1959), claim that it is very difficult to determine the origin of the neurilemma cells as they are small and few in number. Much more needs to be known of insect connective tissue and of glial cells before a firm decision can be reached (Ali, 1967).

Investigations carried out during the past few years indicate that the non-cellular neural lamella is certainly part of a continuum which resembles vertebrate basement membrane in its histochemical properties. The neural lamella or neurilemma of the suboesophageal ganglion of Acheta domesticus is more distinct in Mallory's connective tissue stain preparations, where it appears blue. It appears grey with Mallory's phosphotungstic haematoxylin,

and black in osmium ethyl-gallate preparations. The perineurial cells most usually appear red and grey respectively with Mallory's connective tissue stain and phosphotungstic haematoxylin, but this is hardly enough to characterise their relationship to glial tissue.

Type II and IV glial cells are similar to those reported by Wigglesworth (1959) and Ali (1973). Type IV cells always surround the neuropile and the glomerular bodies and are also sometimes seen individually scattered in the neuropile. Normally they occur in large numbers associated with those peripheral axons which run through or around the neuropile and with the axons running via the interganglionic connectives.

In the suboesophageal ganglion of Acheta, type III glial cells could not be found; these correspond to large, well defined cells described by Wigglesworth (1959), in Rhodnius and Ali (1967), in Pieris. Sánchez (1923), during studies of the nervous system and neuroglia of Pieris, also found cells which he thought were concerned with the breakdown of larval neurons, though in fact it is quite clear from his descriptions and figures that these correspond to the giant (Type III) glial cells or, more precisely, to their large, presumably endopolyploid, nuclei (Ali, 1967). Heywood (1965), claims in the absence of a perineurium, that type III glial cells of Pieris form an enclosing layer around the neurons and axons, and that this layer may have a selective permeability to the various ions within the blood. Ali (1967), however, found that type III glial cells were conspicuous only in the brain and parts of the thoracic nervous system of Pieris, and were not found in all regions of the central nervous system. They are present before the breakdown of the neural lamella, and Ali thinks it is possible that throughout the whole life of the insect these cells form part of

the barrier against toxic compounds entering the central nervous system (see Hoyle, 1952 & 1953; Twarog & Roeder, 1956; and Wigglesworth, 1959), though there does not seem too much evidence for this.

Ali (1973), says that the type III cells are the most conspicuous elements in the neuroglia; they are mainly recognisable by their giant nuclei. These are elongate, with somewhat irregular boundaries, and they sometimes reach 60 μm by 21 μm, though such large dimensions were only found in the prepupa and early pupa of Pieris brassicae. She added that in the larva their length does not exceed 50 μm and width 15 μm. They are few in number, not more than seven or eight in each abdominal ganglion, except in the first abdominal ganglion where there are nine or ten; in each thoracic ganglion there are ten to twelve and in the suboesophageal ganglion there are twelve to fourteen, while in the brain they are much more numerous, usually around sixty-five to seventy. Ali also found that their cytoplasm is not easily seen in the abdominal ganglia, but in the thoracic ganglia, suboesophageal ganglion and brain it is more distinct. Despite a careful search for such cells they are apparently absent from the suboesophageal ganglion of Acheta domesticus and it is, therefore, difficult to believe that they play a special functional role which cannot be taken on by other glial tissue.

5. Neurons (ganglion cells):

In Acheta, as in most other insects, the ganglion cells lie in the ganglionic cortex, i.e. between the neuropile and the perineurium. In many places they form a relatively thin layer of cells around the periphery of the ganglion, including all the posterior surface of the neuropile, but they are more strongly aggregated on the ventral region of the centre of the ganglion.

Guthrie (1961), compared the ventral ganglia of annelids and arthropods and pointed out that in both cases the cell body layer occupies predominently the lateral and ventral regions of the ganglion surrounding a neuropile. This is certainly the case in Gerris and many other insects, despite the extension of the cell body layer to the dorsal region of the ganglia. Mill (1964), reported that in the abdominal central nervous system of Aeshna nymphs, the cell bodies of the motor cells and interneurons lie around the periphery of the ganglion, the central part being occupied exclusively by neuropile crossed with transverse and longitudinal fibre tracts. Ali (1967), reported that in the neuron layer of the suboesophageal ganglion of Pieris, there are very few cells on the dorsal side of the ganglion, but on the ventral side there is a thick cellular pad. The neurons seem to be concentrated in the ventral and lateral regions, especially in the middle of the ganglion, where there are virtually no cells on the dorsal side. But at the anterior and posterior ends of the ganglion the distribution of the cells is more uniform. Ali considered that the majority of these peripheral cells are (to judge by their size), motor neurons and lie over the posterior region of the neuropile.

This general constancy of distribution persists through metamorphosis in Pieris, despite changes in the position of some cell groups and axonal tracts due to contraction of the ventral cord at pupation. In Acheta with its simple metamorphosis, such changes presumably do not occur.

6. Neuropile and its associated structures:

The neuropile is a very complex area, with axons of different diameters and various fibre tracts from groups of neurons passing into the central neuropilar zone and running in

different directions sometimes transversely or longitudinally. In some regions these tracts are easy to recognise but elsewhere they cannot be followed so readily. In Anax and other Anisopteran nymphs, the axons occupying the ventral part of peripheral nerves are difficult to trace after they enter the ventral neuropile (Fielden, 1963). The neuropile also contains some glomerular regions.

In the present work three major groups of fibre tracts have been described as the forty-two suboesophageal tracts include the following major kinds: transverse, longitudinal and oblique. It should be noted that little information has been given about the relations with the mandibular nerve and its axonal tracts; this is because this nerve always leaves the suboesophageal ganglion very close to the origin of the circum-oesophageal connectives. This region always becomes very heavily stained with osmium ethyl-gallate and it is, therefore, not easy to identify the structures there or to trace the fibres or axonal tracts in transverse sections, though elsewhere such connections can be followed easily. Thus, some sensory neurons have axons which run through fibre tract st_8 dorsally from the hypopharyngeal nerve to the centre of the neuropile, passing dorsally to the ventral glomerular body, where they break up. The axon of another sensory neuron runs dorsally and turns to enter longitudinal tract st_4, while that of a further sensory neuron runs dorsally to enter the central glomerular body.

By carefully studying the relations with the maxillary nerve and its axonal tracts, it becomes clear that many individually identified axons can be associated with identifiable fibre

e.g. st_9, st_{10}, st_{15}, st_{16} and st_{20}. These sometimes contain both sensory and motor axons; e.g. motor neuron 17 of the dorso-lateral region of the ganglion gives rise to an axon which passes ventrally to enter the maxillary nerve; another motor neuron, No. 18, located in the ventrolateral part of the ganglion has an axon which runs dorsally to enter the maxillary nerve, while yet another motor neuron of the mid-ventral group (No. 21), gives off an axon that passes dorsad then curves down to the maxillary nerve via fibre tract st_9. The axon of interneuron 20 of the median ventral group runs dorsally, then curves to enter the maxillary nerve. On the other hand, axons apparently from two sensory neurons (axons 22 and 23) of the maxillary nerve pass into the central glomerular body in the middle region of the ganglion through fibre tract st_9, while axon 23 runs dorsally to enter the second anterior dorsal commissure, with branches to the central glomerular body. This axon runs via fibre tract st_{15}.

In addition to the axons from sensory neurons mentioned above in connection with the maxillary nerve, there are also another three: the first (No. 31) enters longitudinal tract st_{29}, after passing along through fibre tract st_{16}. The second (No. 32) has an axon which enters longitudinal tract st_{28}, via fibre tract st_{15}. The third (No. 33) enters longitudinal tracts st_{32a} passing along fibre tract st_{20}.

It should be noted that the nerves leaving the suboesophageal ganglion supply structures such as the labium and maxilla which contain numerous muscles and sensory receptors. It is thus evident that such nerves must contain both sensory and motor tracts (Pringle, 1938; Albrecht, 1953). It is also clear from the above and from transverse sections which show the labial nerve in

Acheta that both types of tract can be recognised histologically. Thus, neuron 38 of the lateral group sends an axon into a motor component of the labial nerve; axon No. 40, also in the labial nerve, is a sensory fibre ending in the ventral region of the ipsilateral neuropile, and axon No. 39 of the labial nerve appears to arise from an interneuron in a contralateral lateral group.

The transverse sections also show what appear to be two sensory axons from the salivary nerve; the first (No. 44) enters longitudinal tract st_{27}, while the second runs to enter longitudinal tract st_{39}. In the case of the anterior and posterior cervical nerves, the transverse sections show two motor neurons whose processes pass into the anterior cervical nerve. The axon of No. 24 runs via fibre tract st_{19}, and that of No. 26 of the mid-dorsal group runs ventrally and curves to enter the anterior cervical nerve. Another motor neuron, No. 46 from the lateral group, has an axon which runs ventrally, passing into the posterior cervical nerve.

7. Giant or large fibres:

Transverse sections of Acheta show also some large fibres, especially those running through some of the longitudinal tracts, e.g. st_{28}, st_{30} and $st_{32a, b \text{ and } c}$. Through these tracts large fibres run along the periphery or the middle of the neuropile, traversing the whole length on both sides of the ganglion and joining the anterior and posterior connectives together. In the case of fibre tracts $st_{32a, b \text{ and } c}$, the three tracts usually join together before leaving or entering the connectives at the anterior and posterior ends of the ganglion. From parasagittal and frontal sections, it can be seen that most of these fibres pass through the whole length of the ganglion. Hilton (1911b), mentioned that in the central nervous system of larvae of Corydalis association fibres

probably pass through several ganglia without terminations. He added that although some motor fibres might pass from one ganglion to the next either above or below, there was little evidence of it. However, Mill (1964), recognised that in the abdominal nervous system of Aeshna nymphs, each connective includes two centrally located fibres which are markedly larger in cross-sectional diameter than the rest and he regards them as giant fibres.

In general, giant or large diameter fibres have been known in insects for many years; they have been described in the abdominal nerve-cord of Periplaneta (Pumphrey & Rawdon-Smith, 1937); in Locusta (Cook, 1951); in the dragonfly Anax imperator (Hughes, 1953); in the thoracico-abdominal nervous system of Drosophila (Power, 1948); in the thorax of Locusta, Aeshna, Apis and Calliphora (Satija, 1958a, b, c and d); and in the central nervous system of Gerris (Guthrie, 1961). Roeder (1948) has claimed that the abdominal giant fibres are involved in the escape mechanism of Periplaneta but the work of Cook (1951), suggested that the situation was somewhat different in Locusta. Generally these previous investigations show that the abdominal giant fibres pass through all of the abdominal ganglia and the present study of Acheta shows that comparable tracts run through the suboesophageal ganglion.

In Anax the giant fibres send off collaterals into the neuropile of each abdominal ganglion and in Periplaneta and Locusta they appear to pass without diversion through the abdominal nerve cord. The suboesophageal ganglion of Acheta shows both situations; large fibres pass through the ganglion without diversion, i.e. see figs. 50, 53, 54 and 56, but some of these fibres sometimes send off collaterals into the neuropile, for example, see

neuron 60, which runs with longitudinal tract st_{27} with its axon passing through the whole ganglion, sending off a collateral which passes to the anterior region of the dorsal contralateral neuropile (see also figs. 60, 61, 62 and 72). Mill (1964), recorded that in Aeshna only two giant fibres run on either side of the nerve cord; the cross-sectional diameters of the other fibres form a continuous size distribution but there are also four large, longitudinal fibres present on each side of the eighth ganglion. In fact the suboesophageal ganglion of Acheta shows more than those numbers of large-diameter longitudinal fibres.

8. Glomerular bodies:

Very few studies deal with the glomerular bodies of the suboesophageal ganglion in insects (see Ali, 1974). The glomerular regions of the neuropile are those where, using suitable techniques, the axons can be seen breaking into smaller branches and tiny dendrites. If the glomerular body is well developed, it appears histologically as very distinct, staining darkly in Mallory's and osmium preparations. If less well developed, the stain is paler, depending upon the degree of development of the glomerular body. Ali (1967 & 1974), recognised only traces of a ventral glomerular body in the thoracic ganglia of the adult Pieris brassicae whereas the lateral and dorsal pairs are prominent. She also found that in the larval stages, two pairs of glomerular bodies occur in the middle of the ventral and lateral regions. In the suboesophageal ganglion of the fifth instar larva there is a pair of large dorsal glomerular bodies extending from the middle of the ganglion to near the posterior end, as well as smaller lateral and ventral pairs of glomerular bodies. The lateral glomerular bodies lie towards the centre of the ganglion; the ventral pair is not very well

developed and ends near the posterior end of the ganglion.

As a result of the present studies, the suboesophageal ganglion of the adult of Acheta domesticus has been shown to have three conspicuous pairs of glomerular bodies (ventral, dorsal and lateral). There is also an additional unpaired glomerular structure near the anterior central region of the ganglion, which may be called the anterior central glomerular body or the central glomerular body (cgrb). This glomerular centre does not extend further behind the middle of the ganglion but is very distinct and well developed.

Some fibre tracts contribute to the glomerular bodies, e.g. st_{2b} (the anteroventral commissure) runs ventrally, crossing the midline and the ventral glomerular body, where I believe some axons terminate while others continue to the ventro lateral neuropile. The same situation applies in fibre tract st_8, from the hypopharyngeal nerve, some of whose axons terminate in the ventral glomerular body while others continue to the centre of the neuropile. The anterodorsal commissure st_3, has fibres which make a transverse commissure between the two circum-oesophageal connectives; some of these fibres run to the dorsal glomerular body.

Generally the axons which run through fibre tracts to enter a glomerular body may be from bipolar or multipolar motor neurons, (see p. 279), association neurons or sensory fibres entering the ganglion to arborize in the glomerular body. For example, from transverse serial sections of the suboesophageal ganglion of Acheta it was found that in motor neuron 5 of the anterolateral group, the axon runs ventrally to the ventral glomerular body where the dendrties arborize; this axon runs via

tract st_5 (Fig. 66). Sensory axon 8 from the hypopharyngeal nerve passes dorsally through fibre tract st_8 to the ventral glomerular body where its dendrites arborize. Interneuron 16 of the ventral group sends off an axon which passes through fibre tract st_6 (Fig. 66), to the laterodorsal region of the neuropile occupied by the lateral glomerular body.

In the central glomerular body, sensory axon 9 from the hypopharyngeal nerve runs dorsally (Fig. 67) through fibre tract st_8 to enter the central glomerular body while a collateral leaves to join the ventral glomerular body with dendrites arborizing in both. One of the axons of motor neuron 13 of the mid-ventral group (Fig. 67), passes dorsally to arborize in the central glomerular body and interneuron 14 (Fig. 67) of the mid-ventral group also gives off an axon which runs dorsally to enter the central glomerular body. An axon of motor neuron 13 and interneuron 14 runs through fibre tract st_7, while the axon of motor neuron 19 (Fig. 67) of the ventrolateral group runs dorsally through fibre tract st_{12} to enter the central glomerular body in which it arborizes. A sensory neuron, No. 22 (Fig. 67), which contributes to the maxillary nerve, gives off an axon which passes through fibre tract st to enter the central glomerular body, and sensory axon 23 (Fig. 67), which contributes to the maxillary nerve, runs dorsalward with one collateral branch to the central glomerular body, the main axon running through fibre tract st_{15}.

Some longitudinal fibres reconstructed from frontal sections of the median part of the ganglion contribute to the central glomerular body, e. g. the ascending axon 69 (Fig. 73) from the first thoracic ganglion, passing through longitudinal fibre tract st_{29} to enter the anterocentral glomerular body.

An axon of motor neuron 71, (Fig. 73) of the lateral group also runs dorsally to enter the anterocentral glomerular body in which dendrites arborize. Another ascending axon, No. 73, (Fig. 73) from the first thoracic ganglion, runs through longitudinal tract st27, which passes upwards crossing the midline to the contralateral side and continuing anteriorly, enters the anterior central glomerular body in which it arborizes. An axon of motor neuron 78 (Fig. 73), from the anterolateral part of the ganglion runs to the middle of the ganglion and enters the anterior central glomerular body.

In general these glomerular bodies are centres where the different axons break into smaller branches and tiny dendrites. In the examples given above, axons from motor neurons, association neurons and sensory neurons were all found arborizing in the glomerular bodies.

The terminal arborizations of the fine branching fibrils or dendrites of all three kinds of axons can be clearly seen in the anterior central glomerular body. I conclude that the glomerular bodies represent important association centres in the suboesophageal ganglion. They allow sensory neurons to transmit their impulses which can be received by motor neurons either by direct association of the fibrils or dendrites or through an intermediate neuron (association neuron). For this reason the glomerular bodies (association centres) consist of a dense mass of interconnecting terminal fibrils and synapses.

9. Detailed analysis of individual neurons:

(A) Metal impregnation techniques:

In order to study the fine structure of the nervous system, it

was necessary to employ the metal impregnation techniques outlined in the section on methods. These techniques often gave good results with both cells and fibres, but rarely stained elements in their entirety, due in part to the slight affinity for stains of the delicate polar filament or cell processes (Zawarzin, 1924, quoted from Guthrie, 1961). For this reason, some of the fibres figured in the illustrations are shown without cell bodies (Guthrie, 1961). Dowling & Boycott (1965 & 1966), did not give much information on the distribution of synapses or the relationships of the different types of nerve cells during their electron-microscopic observations on the synaptic contacts of various cell types in the primate retina. Three years later, in 1969, the same authors stated that for such information light microscopy is necessary, and they reported that good results were obtained by Colonnier's modification of the Golgi-kopsch method (Colonnier, 1964), after their initially inadequate results with the Golgi-Cox method (as described by Sholl, 1953), and the rapid Golgi methods (Gatenby & Painter, 1937). Strausfeld & Blest (1970), also failed to obtain satisfactory results with Golgi-Cox and rapid Golgi methods during their studies of the optic lobes of Lepidoptera. They also reported that the modification of Holmes' silver method (Holmes, 1943) and Rowell's modification (Rowell, 1963), gave very poor results when employed to reveal fibre tracts. They claimed that these non-selective silver impregnation techniques do not reveal sufficient fine detail in the Lepidopteran and Dipteran nervous systems and that several variants of Golgi techniques were tried without success until the technique finally adopted, i.e. that devised by Colonnier (1964), which gave good results. Strausfeld & Blest also mentioned that another modification of the double-impregnation procedure (Kallius, 1910), revealed only a few components clearly against a deep brown background of unimpregnated tissue. The silver-on-

the-slide methods of Holmes (1947) and Romanes (1950), were found less satisfactory than that of Palmgren (1948), which gave some excellent results (Guthrie, 1961). Guthrie also found that he rapid Golgi silver-bichromate process occasionally gave good results (5% success), but that the Golgi-Cox mercury bichromate method gave a much higher percentage of successful preparations (50 to 70% success).

All these findings on the fine structure of the nervous system, as investigated by previous authors, give a clear picture of how difficult and capricious the metal impregnation techniques are for such studies, and for tracing or illustrating the neuron fibres and their cell bodies together in a single preparation. For this reason the present work attempts to identify some of the Golgi elements in terms of the axonal tracts which stained much more consistently with osmium tetroxide.

Although Colonnier's method of Golgi impregnation is widely considered a very successful method, the present studies indicated that a number of different techniques needed to be tried, varying such factors as the age of insects, period of prefixation, pH, fixation period, duration of silver impregnation, etc. Such variations were repeatedly attempted until successful results were obtained. A modification of the double-impregnation procedure of Kallius (1910) which was introduced by Strausfeld & Blest (1970), was also tried with minor changes in duration. Material prepared by this method showed only a few axons and their dendrites, but they were revealed very clearly against a brown background and the method may have some useful applications with other insects; it perhaps needs further investigation. Another double-impregnation procedure was also tried in this work: the

mixed impregnation method of silver nitrate and osmium tetroxide followed by ehtyl gallate. The aim of this technique was to illustrate the neuron fibres and cell bodies together, but unfortunately only a few large axons appeared very clearly and I suggest that this technique needs further trial on a larger scale if one is to evolute it fully.

(B) Modern techniques:

There are some other recent developments in technique which have been developed for the structural investigation of the central nervous system, e.g. injection of cobalt salts or dye into the neuropile or selected cell bodies with radioactively labelled horseradish peroxidase and with amino acids. For these modern techniques see Heimer, 1970; La Vail & La Vail, 1972; Cowan et al., 1972; Pitman et al., 1972; Llinás, 1973; Goodman, 1974; Altman & Tyrer, 1974; Strausfeld & Obermayer, 1976. Gregory (1974), used two methods: Bodian silver-stained sections and Procion Yellow impregnation, to examine the general neuroanatomy and details of the nerve roots in the mesothoracic ganglion of adult males of Periplaneta americana. The basic neuroanatomy was determined from silver-stained sections, and these observations were verified and amplified by fluorescence studies of material stained with Procion Yellow. Gregory also attempted to cover a wider field at the less detailed level required for interpreting histochemical preparations of the central nervous system. This used his improved (Bodian) silver technique and a new use of Procion Yellow to display whole nerve tracts instead of single fibres. He used this method to examine four main points: the roots of the peripheral nerves; neuron cell body groups; and longitudinal and transverse nerve fibre tracts. Cobalt chloride can also be injected into an identified nerve cell body in an insect

ganglion and reacted there with ammonium sulphide to stain the soma and its branches with a black precipitate. The stained cell body and its branches throughout the neuropile are visible under both the light- and electron-microscope (Pitman et al, 1972). The same authors also claimed that the study of the correlations between neural structure and function were advanced by the technique of intracellular injection of the fluorescent dye and Procion Yellow. They added, however, that there are some disadvantages with this method. The Procion dye is not electron-opaque, a fact that precludes observations at the ultrastructural level. Therefore, the precise determination of synaptic contacts on the neurites of the identified cells has not been possible. They also found it is difficult to observe the detailed branching pattern of nerve processes in whole mounts of the insect central nervous system stained with Procion Yellow. All these modern techniques need further application to the insect nervous system.

In general one of the most useful techniques for observing individual neurons has been the Golgi method and its variations. This has been a powerful tool because a small proportion of a neuronal population is visualised and those neurons that have been impregnated are filled entirely. Although this method gives much cellular detail and has been extended from the light-microscopic to the ultrastructural level (Blackstad, 1965 & 1970), it has the major disadvantage that neurons are stained more or less randomly and may for this reason give capricious results (Pitman et al, 1972). In fact the Golgi-Colonnier method which was used by Strausfeld and his co-workers as a metallic impregnation technique for the insect nervous system proved one of the most helpful methods used in the present work.

(C) Some remarks on the metal impregnation results:

Although Golgi impregnation techniques give much cellular detail, the neurons and axons are impregnated randomly in this method, and not all the neurons with their axons can be seen in one set of preparation from one ganglion. It therefore requires several sets of sections from at least six to ten ganglia to cover a suitably large proportion of the neurons or axons and their branching, and also to know which units are repeatedly represented in different ganglia. In this way it is possible to avoid criticism that the Golgi method and its variations have the disadvantage that neurons are stained more or less randomly and that the results can be capricious.

In the different types of dendritic endings most of the punctiform structures are synaptic bulbs which form terminal contacts with other dendrites from different axons. Sometimes these punctiform structures were very distinct and numerous, while others have just a few between the dendrites; see axons Nos. 2 & 3, and their contacts (Figs. 92 & 96). Usherwood (1973), gives a photograph of nerve terminals forming synaptic bulbs on somata in an abdominal ganglion of a marine snail. The photograph taken with a scanning electron-microscope, gives an apparently three-dimensional view of the structures observed (cf. also Dyson, 1978, who gives a scanning electron-microscope photograph of nerve terminal bulbs from the mollusc Aplysia californica.

In terms of the description of the various kinds of branched endings and their dendrites (pp. 170 - 173), the terms deep, shallow, wide or narrow transverse field have the following meanings: deep or shallow describes the extension of the field through the ganglion, while the other terms denote that it occupies a wide (large area) or narrow (small area) part of the transverse field.

Axon No. 1 was found in the anterior part of the ganglion and runs from the dorsolateral region, with two collaterals, to the middle of the ipsilateral region and the anteroventral region of the ganglion, terminating in a type E_2 ending. Due to the heavy impregnation at the anterior and posterior parts of the ganglion, especially near or around the circum-oesophageal connectives, there is not much detail given about the mandibular nerve which enters the suboesophageal ganglion very near to the circum-oesophageal connectives and is situated in that heavily staining area. It is, therefore, not clear whether unit No. 1 is derived from an axon or collateral of a motor neuron at the anterodorsal lateral region of the ganglion or, more probably, whether it represents the central termination of a sensory axon in the mandibular nerve.

The motor neurons normally terminate in differently shaped endings, most of which occupy a wide or deep field. Their endings may be one of the types B_1, B_2, B_3, C_1, C_2, C_3 or G_4. For example, axon No. 2 presumably originates from a motor neuron of the anterolateral group and its axon runs through fibre tract st_5 with a wide field type B_3 ending. Axon No. 17 originates from the ventral region of the ganglion occupied by association and motor neurons and has a type B_2 ending which terminates in a few heavy punctuate structures. Axons Nos. 20 and 21 arise clearly from ventral motor neurons. In unit No. 20 (Fig. 94), the axon runs dorsally with a type C_3 ending composed of a Y-shaped fibre, the two arms of which are unequal in length. Unit 21 (Figs. 94 and 96), has a type C_2 ending which is again Y-shaped with no further branching, but the two bifurcations are equal in length. Unit No. 28 (Fig. 99), seems to derive from or is associated with some of the large, presumably motor cells

located in the laterodorsal region of the ganglion. This unit is found in two preparations and only occurs in a heavily impregnated area. It may possibly comprise two large motor neurons, each with several long axons extending ventrally, with a type C_1 ending (Fig. 104), or, since I consider that type C_1 endings always accompany multipolar neurons, this unit may be a single multipolar neuron. Dyson (1978; Fig. 8.2; p. 351), gave two good examples of multipolar neurons which appear to resemble those found here. Unit No. 29 (Fig. 99), apparently derives from the motor neuron at the lateral region of the ganglion. The fibre branches run ventrally and terminate in type B_2 endings, which are composed from a fibre branching into finer fibres, themselves bearing laterally branching fibrils with punctiform endings and occupying a wide, shallow field. In unit No. 30 it is clear that the main branch originates from a lateroventral motor neuron and runs dorsally for a short distance with a type B_2 ending (cf. p. 204). Unit No. 32 also has its main branch from one of the large motor neurons located in the lateral region of the ganglion and extending for a short distance ventrally with a wide field type B_2 ending (see Figs. 99, 104 and 109). The axon of unit 45 originates from the mid-ventral motor neuron (Figs. 102 and 104), and extends with one long fibre, but in this case it is very difficult to follow the termination. The long axon of Unit 46 (Fig. 102) originates from a motor neuron in the mid-dorsal region of the ganglion and its main axon extends ventrally along the midline and spreads out with a type G_4 ending which occupies a wide field. The axon branches early into fibrils which themselves branch again into fine fibrils with punctiform endings.

In general there are many axons whose origins are not identified and lie outside the suboesophageal ganglion. Their

neurons may be bipolar or multipolar. Also, in cases where association neurons lie close to motor neurons, the origins of adjacent axons may be from the former rather than the latter.

As mentioned above, although the metallic impregnation techniques provide much cellular detail, the neurons and axons become impregnated randomly. Each set of preparation, therefore, tends to show a somewhat different picture from one ganglion to another, but this should not obscure the fact that there are some units which clearly occur with regularity in almost all sets of sections, e.g. units 1, 16, 26, 36, 38, 46, 53, 54, 55, 58, 59 and 60.

10. Attempts at a functional analysis: grouping units into functional systems:

(A) Functional properties of the suboesophageal ganglion in relation to the brain and ventral nerve cord:

a. Transmission along ventral nerve cord (longitudinal connectives).

Motor neurons are almost absent from the insect brain, except for a few which innervate the antennae and perhaps others which control pigment migrations in the compound eye (Wigglesworth, 1965). Analogy with other insects suggests that there will be some descending axons to the suboesophageal ganglion from interneurons in the brain to the efferent neurons of lower centres as well as ascending axons to the brain from the interneurons of lower centres. Otherwise the brain receives only the afferent fibres of sense organs in the head.

The suboesophageal ganglion plays an important role as an

association centre. Longitudinal tracts pass through it from the lower ventral nerve cord to the brain or in the opposite direction, running along the periphery of the neuropile, while others terminate there in contact with individual neurons or in glomerular bodies.

In Acheta most of the longitudinal tracts in the suboesophageal ganglion which are revealed in sagittal and frontal sections (Figs. 49 - 65), contain axons from or to the brain. Through these longitudinal tracts there are many ascending sensory axons or fibre from association neurons to the brain, and also some descending pathways to the lower efferent system through interneurons and on to the motor neurons supplying muscles or other effectors. The longitudinal tracts containing these fibres in Acheta are mainly st_{28-30} and $st_{32a, b \text{ and } c}$, (Figs. 31, 44, 50 and 53 - 65). Some individual axons are also shown in Figs. 72 and 73), e.g. Nos. 56, 57, 60 and 68. Generally most of these bilateral fibre tracts from or to the brain run through the entire length of the suboesophageal ganglion in a dorso-lateral position near the periphery of the neuropile (Figs. 55 and 56), and they contain some large diameter axons. Guthrie (1964), found that in the descending tracts in the ventral nerve cord of the locust, the fibres composing the dorsal tegumentary nerve enter the central nervous system, and a number of fibres pass forward into the protocerebral level, but the greater proportion of larger fibres pass posteriorly through the longitudinal connectives into the suboesophageal ganglion. He added that within the posterior region of the suboesophageal ganglion, many of the larger fibres cross over to the opposite side and descend further posteriorly on this side. Some of these crossing fibres were also found in Acheta, especially in those running through fibre tract st_{27} (see Fig. 65), while some other longitudinal tracts contain large diameter axons, i.e. $st_{32a, b, \text{and } c}$.

Guthrie (1980), gave a photograph of a transverse section of the abdominal ganglion of the cockroach Periplaneta americana (see his Fig. 4.9A, p. 64), to show some ascending giant fibres, which is similar to some of the large fibres shown in Acheta in Figs. 30 and 42 - 48. His sections of the nerve cord reveal a group of large fibres near the centre of each longitudinal connective; these are the giant fibres and appear to be single, unicellular elements. At first it seemed as if these cells terminated in the metathoracic ganglion, and were linked synaptically to longitudinal fibres in more anterior segments. The variable transmission that occurred in this region was later shown to be due not to synapses but to isthmi or zones of narrowing of the fibres within the ganglia. Otherwise the fibres extend without interruption into the brain. Guthrie (1980), also mentioned that in the locust, a pair of large axons cross over in the brain and then descend to the level of metathoracic ganglion where they are in synaptic relation with the fast extensor tibial motor neurons which mediate the powerful jump which the insect can make. This is maybe the same as the situation in Acheta; the large fibres there responding to moving objects and leading to a jump. Guthrie added that the cockroach also has a pair of descending giant axons from the brain to the thoracic centres and mentioned that this is probably a reflection of the greater development of the antennae in the cockroach.

b. Input system to the suboesophageal ganglion from the brain:

These include descending axons from the brain to the suboesophageal ganglion where they end. Most of these axons are presumably from association neurons, though it is not excluded that some may be from sensory neurons whose axons descend to connect with association neuron in the suboesophageal ganglion.

Examples of higher central input to the suboesophageal ganglion include axon 54, which passes to the middle region of the ipsilateral dorsal neuropile with two branches that terminate in contact with endings from association neurons of the ventral association group, and thus transmit impulses to the dorsolateral motor neuron group from which efferent connections leave the ganglion. A collateral from axon 54 to the anterior and middle region of the contralateral neuropile probably passes through fibre tract st_{2a} which presumably connects with another axon from an interneuron of the ventrolateral group (its axon runs via fibre tract st1). Axon No. 55, also from higher centres of the nervous system, runs ventral to the postero-median end of the ganglion, where it apparently terminates near dendrites of cells in the ventral motor neuron group. This axon runs via longitudinal fibre tract st_7. Another descending axon (No. 59) passes posteriorly to just before the mid-lateral region of the ipsilateral neuropile and curves anteriorly again, perhaps to arborize in the anterior region of the ipsilateral dorsal neuropile, or possibly it turns to pass through fibre tract st_5 which terminates very near or in the ventral glomerular body. The axon of yet another descending association neuron (No. 61) passes to the mid-lateral region of the ipsilateral dorsal neuropile where it terminates in two branches, which appear to turn and enter the lateral glomerular body in that region.

c. Input system to the suboesophageal ganglion from the lower centres:

These axons or fibre tracts may be from the first thoracic ganglion or the lower centres; no attempt was made to trace their origin. Ascending axon (No. 58), emerges from the first thoracic ganglion and passes to the lateral posterior region of the suboesophageal neuropile, where it seems to connect with an axon from

the latero-posterior motor neuron group. Normally axon 58 runs through fibre tract st_{32}. The asecending axon 62 passes to the posterior region of the ipsilateral dorsal suboesophageal neuropile, and ascending axon 63 runs up and curves to the posterior region of the mid-dorsal ventral contralateral neuropile through fibre tract st_{37}. It connects there with some dendrites from an association neuron of the lateral group. Ascending axon (No. 70), from the first thoracic ganglion or a lower centre, passes anteriorly to the centre of the suboesophageal neuropile, where it presumably turns obliquely and runs through fibre tract st_{12} to connect either with a motor neuron of the lateroventral group or, more probably, with an association neuron which is in turn connected with dendrites of an efferent neuron of the mid-lateral group.

Another two axons from the first thoracic ganglion or lower centres ascend to the anterior central glomerular body of the suboesophageal ganglion. They are No. 69, which runs through fibre tract st_{29}, and No. 73 which runs via fibre tract st_{27}; they are probably then connected with a motor neuron of the mid-lateral group.

(B) Input system to the suboesophageal ganglion from the peripheral nerves:

These are afferent fibres from the bipolar or other sense cells of the sense organs. Their neurons are of course, situated near the periphery of the body and their afferent fibres constitute the initial part of a reflex arc system.

a. From mandibular nerve:

Afferent axon 75 of the mandibular nerve passes to the

anterolateral region of the contralateral neuropile where it enters the anterodorsal glomerular body and is connected to dendrites of interneurons which transmit impulses to the efferent neurons of the dorsolateral group. Another axon (No. 82), from the mandibular nerve passes to the middle region of the ipsilateral ventral neuropile where it probably joins an interneuron of the median anteroventral region, then connects with one of the motor neurons situated in the same position (median anteroventral region). A collateral is given off to the anterolateral region of the ipsilateral neuropile where I believe it enters the lateral glomerular body.

A third input axon (No. 83), has been traced from the mandibular nerve to enter the dorsal glomerular body with similar connections to axon 75.

b. From hypopharyngeal nerve:

Most of these sensory axons terminate in the glomerular bodies. For example, axon No. 8 passes dorsally to the ventral glomerular body, while axon No. 9 runs dorsally to enter the central glomerular body with a collateral to the ventral glomerular body. Normally the sensory axons which terminate in the ventral glomerular body connect with efferent neurons situated in the anterolateral group, while those which terminate in the central glomerular body normally connect either with ventrolateral efferent neurons or with the mid ventral efferent neurons. This means that some afferent axons of the hypopharyngeal sense organ (No. 8 and the collateral of No. 9), connect with efferent neurons of the anterolateral group, while others such as No. 9 link to efferent neurons of the ventrolateral or mid-ventral groups. Most of the connections to or from the hypopharynx run through fibre tract st_8.

c. From maxillary nerve:

All the more anterior sensory fibres of the maxillary nerve terminate in the central glomerular body. Afferent axons Nos. 22 and 23 run through fibre tract st_9 and st_{15} respectively and transmit impulses to the efferent neurons of the mid-ventral group. There are also some sensory fibres from the maxillary nerve, e.g. axons Nos. 31, 32 and 33, which enter longitudinal fibre tract st_{29}, st_{28} and st_{32a} respectively, but it is not very clear where they end or with which efferent neuron group they connect. From a series of frontal sections there appear to be some sensory axons of the maxillary nerve (e.g. Nos. 90 and 91) whose endings connect (presumably via one or more interneurons), with motor neurons of the lateral group. No. 90 from the main maxillary nerve passes to an efferent neuron in the lateral region of the ganglion, while No. 91 from the smaller branch of the maxillary nerve also eventually contacts motor neurons in the lateral region of the neuropile.

d. From labial nerve:

The afferent fibres in this nerve usually terminate shortly after passing dorsad to the ventral region of the neuropile, e.g., afferent axon 40 passes dorsally to the ventral region of the neuropile where it apparently connects via interneurons with efferent neurons in the ventral region of the ganglion. Axon 41 from the labial nerve enters longitudinal fibre tract st_4; it is not clear where it ends but it perhaps runs a short distance through fibre tract st_4, then turns obliquely and connects with one of the ventral efferent neurons in the middle or posterior part of the ganglion.

Afferent axon 87, also from the labial nerve, appears in frontal sections, passing to the posteroventral part of the neuropile, with a collateral to the lateral neuropile of the same side. The first branch which is given off in the posteroventral region presumably runs transversely and its efferent neuron is situated in the ventral region of the ganglion, while a collateral passes with two branches to the lateral neuropile of the same side; it is not clear whether it is connected with a lateral motor neuron or one of the ventral motor neurons. Another sensory axon (No. 88) from the labial nerve passes to the middle posteroventral region of the ipsilateral neuropile and perhaps runs transversely, then connects with the lateroventral group of motor neurons.

e. From the salivary nerve:

Two sensory axons have been traced into the posterior part of the suboesophageal ganglion. No. 44 runs to the mid-ventral part of the ganglion to enter longitudinal fibre tract st_{27}. This axon probably passes through fibre tract st_{27} and extends to the central glomerular body, where it arborizes and may thus connect with one of the ventral motor neurons dr, through fibre tract st_{27}, it turns obliquely to connect with the ventral motor neuron. In fibre tract st_{27} some fibres generally enter the central glomerular body, while others pass straight to the higher centres through the circum-oesophageal connectives. Sensory axon 45 runs from the salivary nerve to enter longitudinal tract st_{39} then passes shortly through this fibre tract, possibly terminating near a neuron of the lateroventral group. Other sensory axons from the salivary nerve were revealed in frontal sections, e.g., axons 76 and 77, which run to the middle region of the ipsilateral neuropile and eventually connect with efferent neurons of the mid-ventral group. Axon 79 also passes from the salivary nerve to the middle

region of the ipsilateral neuropile, then curves down and disappears shortly before the ventral connectives. I believe it turns obliquely to join one of the mid-ventral motor neuron group at the posterior end of the ganglion. Axon No. 80 from the salivary nerve runs to the midline and passes to the contralateral neuropile then disappears in the middle posterior region; presumably this axon is linked to some individual association or motor neurons in the mid-central area of the ganglion.

(C) Motor output systems:

Normally the large and medium sized unipolar motor neurons are in the peripheral region of the ganglion and connected to the neuropile; very few can be seen in the central region of the posterior part of the suboesophageal ganglion of Acheta. Centrally the endings and arborizations of dendrites or axons and collaterals of the motor neurons are connected with association neurons.

Most of the motor neurons which supply the anterior cervical nerve lie in the anterolateral group, e.g., motor neuron No. 24 (Fig. 67), while some others are found in the mid-dorsal efferent group (which normally contains very few neurons). No. 26 (Fig. 68) is a good example of an efferent neuron of the mid-dorsal group sending an axon to the anterior cervical nerve. The posterior cervical nerve also receives output axons from the lateral motor neuron group situated in the posterior part of the ganglion, e.g. motor axon No. 46 of the lateral group runs ventrally into the posterior cervical nerve.

Motor neurons of the maxillary nerve are normally situated either in the anterior or middle of the dorsolateral region of the ganglion, or in the anterior or middle of the ventrolateral region.

For example, the efferent axon of No. 17 passes ventrally from the dorsolateral region and curves around the lateral glomerular body to enter the maxillary nerve. The axon of motor neuron 18 which is located in the ventrolateral part of the ganglion runs dorsally then curves around the lateral glomerular body to enter the maxillary nerve.

In general very few motor neurons associated with the maxillary nerve were found in the mid-ventral group of the anterior or middle of the ganglion, but the axon of neuron 21 (Fig. 67, mid-ventral group), passes dorsally then curves down around the ventral glomerular body to enter the maxillary nerve. Motor neuron 92 (Fig. 74) from the anteroventral centre region of the ganglion has an axon which passes lateroventrally to enter the maxillary nerve.

Motor neurons associated with the labial nerve are situated in the contralateral region of the ganglion, e.g. the efferent neuron of the lateral group No. 38 (Fig. 68) sends its axon laterally, crossing the midline to the contralateral neuropile then curving ventrally to run into the labial nerve.

Motor neurons associated with the hypopharyngeal nerve are usually situated in the anteroventral region of the ganglion, e.g., neuron 85 of the anteroventral region, and neuron No. 65 (Fig. 72), which is located in the posterior lateral group, and send axons to the contralateral neuropile, curving down to enter the first thoracic connective to the lower centres.

(D) Intra-ganglionic relationships (the transverse commissures):

The first three transverse commissural fibre tracts appear in the anterior part of the ganglion. St_3 runs from the dorso-lateral region of the neuropile and its fibres form a commissure between the two circum-oesophageal connectives. Fibre tract st_{2a} from the lateral region of the neuropile runs in the dorsal part of the ganglion, forming the second anterodorsal commissure between the two halves of the neuropile in that area. Fibre tract st_{2b} contains fibres which run ventrally across the midline to form the anterior ventral transverse commissure.

Near the middle of the ganglion appears the dorsocentral commissure st_{17} (Fig. 25), whose fibres run towards either side of the midline of the dorsal region of the ganglion, forming a commissure between the two dorsolateral regions of the neuropile. Another transverse commissure is present shortly behind fibre tract st_{17}; this runs from the neuropile on the ventral border of the ganglion, close to the ventral boundary of the neuropile on either side of the midline and forms the ventrocentral commissure.

In the posterior part of the ganglion another two transverse commissures occur. The first dorsoposterior commissure, st_{23} (Fig. 28), in the dorsal part of the neuropile, runs across the midline and curves down ventrally to the middle of the ipsilateral neuropile of each side. The first ventroposterior commissure st_{25} in the neuropile on the ventral side of the ganglion, runs ventrally across the midline then curves very close to the boundary of st_{24}, (Fig. 28).

In the dorsoposterior part of the ganglion the second dorsoposterior commissure, st_{33} (Fig. 29) in the dorsal part of the neuropile, runs dorsally across the midline and laterally around st_{30} on either side of the ganglion.

In general very few axons and neurons were located clearly in relation to the transverse commissures. For example, interneurons Nos. 2 and 4 of the anterior ventrolateral and anteroventral group respectively (see Fig. 66), send their axons dorsally via fibre tract st_1, then curve and run through the second anteriodorsal commissure (st_{2a}), crossing the midline to the middle of the contralateral neuropile of the ganglion. The motor neuron (No. 26) of the mid-dorsal group sends an axon ventrally for a short distance via fibre tract st_{22}, then curves and runs through the dorsocentral commissure (st_{17}) to enter the anterior cervical nerve. The axon of motor neuron 38 of the lateral group, runs laterally, crossing the midline through the dorsocentral commissure to the contralateral neuropile of the ganglion, then curving ventralward to enter the labial nerve, while an axon of interneuron 39 of the lateral group also runs the same way as neuron 38.

(E) The co-ordination of glomerular bodies:

As mentioned above the glomerular bodies are centres where different kinds of axons break into small branches and dendrites. They contain the terminal parts of axons of efferent neurons, some from afferent neurons and others from association neurons, all arborizing in these centres. The dorsal glomerular body receives most of the axons from the dorsolateral region of the ganglion and those running through fibre tract st_3. Many axons arborize in the ventral glomerular body, some from the

hypopharyngeal nerve and others from the lateral and dorsal groups of neurons. The lateral glomerular body receives axons from the dorsolateral and ventrolateral groups, while the central glomerular body receives sensory axons from the maxillary nerve, from motor and association neurons of the lateroventral group and the mid-ventral group.

Seabrook (1968), has described the glomeruli of the seventh abdominal ganglion of Schistocerca gregaria; within the neuropile are numerous glomerular sensory fibres. These glomeruli are similar to the ventral association centres in the thoracic ganglia of Periplaneta (Pipa et al., 1959). In Schistocerca, sensory neurons arborize in the dorsal and ventral regions of the neuropile and glomeruli are found in both regions. Ali (1967), found that in the fifth instar larva of Pieris, some axons of motor and association neurons arborize in the glomerular bodies of the third abdominal ganglion.

11. Comparative analysis of axon form and endings in insects and other animals:

In the present study the identification and characterisation of structural units based on Golgi impregnated material was carried out under two main headings: the different types of dendritic endings and the various kinds of axons, branched endings and their dendrites. Generally three main kinds of dendritic endings were found and altogether twenty four distinct structural units were described. In this section of the discussion, an attempt is made to compare the endings and axonal units of Acheta with those of some other animals, mainly invertebrates.

Trujillo-Cenóz (1959), studied the fine structure of the central nervous system of Pholus labruscoe (Lepidoptera), and mentioned the morphological problems related to the fine structure of synapses. These problems appear very clearly in all his electron-micrographs, and I believe they are due to the single fixative technique which had been used (osmium tetroxide). For this reason in the present work, both the osmium tetroxide technique and Golgi impregnation procedures were employed so as to obtain clear pictures and correlate the two sets of results. Trujillo-Cenóz & Melamed (1963), studied the fine structure of the photoreceptor second optical neuron synapse in the insect retina of Musca domestica and Colliphora vomitoria, using both the osmium tetroxide and Golgi techniques but unfortunately they did not compare in detail the results given by the two methods for the synaptic terminations. Trujillo-Cenóz (1965), did, however, have some success with electron-micrographs of synaptic connections between second-order fibres and photoreceptor axons in the adults of Sarcophaga bullata and Lucilia sp. after using a Golgi impregnation method.

Boycott & Dowling (1969), gave light-microscopy studied of the organisation of the primate retina (Macaca mulatta), using Golgi impregnation, and provided photographs of some flat and midget bipolar cells showing different shapes of the axons, their terminals and some dendritic endings (see plate 34 p. 120). Also in plate 38 they gave an impression of the branching of an axon that ends in large terminals. Their illustration (fig. 48) was taken within the depth of the section so that the terminal knobs are not well resolved, but the axon and others out of focus in the field were drawn in detail in another figure (see Fig. 32B, p. 135). In fact many of the drawings made in the present work were executed

for the same reasons.

The photograph (Fig. 48), given by Boycott & Dowling (1969), is very similar to the type B_3 and D_2 endings of Acheta, shown in Fig. 87 and, for unit 60, in Fig. 123. The same authors also give some photographs taken after treatment with a rapid Golgi technique and Golgi-Colonnier methods of the amacrine cells from the human retina and their terminations; see, for example their Figs. 56, 58 and 60 (plate 39), p. 145. The terms wide-field and narrow-field used in their descriptions are very close to the same usages in the present study, and their figures also give some similarity of shape compared with motor neurons in the suboesophageal ganglion of Acheta (see my Figs. 99, units 29, 30; 102, unit 46; 104 and 109).

Strausfeld & Blest (1970), made some of the most successful Golgi studied in insects, and showed camera lucida drawings of processes, endings and their specialisations in the optic lobe neurons of the Lepidoptera. They recognised, among others, short varicose processes, spiny branching processes, unbranched spines and some simple endings such as forked, plug endings, etc. They also recognised three forms of retinula cell endings in the lamina ganglionaris of Pieris optic lobe (types 1, 2 and 3 retinula cell endings). Strausfeld & Blest also described in Sphinx and Pieris some distinct forms of long visual fibres recognisable in the lamina and three form-variants of long visual fibre ending in the medulla: the spiny long visual fibre, the smooth long visual fibre and the wide-field long visual fibre. Some of their descriptive terms, for example 'spiny', or 'smooth', 'wide-field', etc. have also been applied in the present study to appropriate structural units with almost the same meanings. Strausfeld & Blest also described endings derived from the retina of Pieris brassicae, including in

their Figure 27, p. 106, a pair of long visual fibre endings characteristically accompanied by a pair of monopolar cell terminals (the quad ending), with a camera lucida drawing of completely impregnated components of quad structures. These are very similar to unit 26 of Acheta from the middle part of the ganglion, with arborizing root-endings of type G_1 in the ventral region of the ganglion (Figs. 100 and 104). They do not, however, form the regular arrays of four quads seen by Strausfeld & Blest. Unit 26 in Acheta has the same quad structure shape along the axon.

Strausfeld & Blest (1970), also described 'T-cell' and 'Y-cell' endings in addition to the common descriptive terms shallow, wide-field or deep endings. All these terms can be applied equally in Acheta. Moreover, comparing some of Acheta structural units and endings with those illustrated by Strausfeld & Blest (1970), in their Fig. 25 (a summary diagram of the receptor elements in the optic lobe of Pieris brassicae), the two diffuse long visual fibre endings derived from spiny long visual fibre elements in the lamina, are very similar to B_4 endings in Acheta (Fig. 80), described here as long axonal branches covered with punctiform structures. The long fibre ending No. 5 of Strausfeld's drawing is very similar to type H endings in Acheta (Fig. 81), which was described here as a single long axon with lateral spines (see p. 172). Again their drawings of the shallow endings of monopolar cells and deep wide-field endings of wide-field radial monopolar cells, are similar to the types D_2 and D_3 endings in Acheta, respectively (see Figs. 80 and 87). Further examples of the similarities between endings and units in Acheta and those found by Strausfeld & Blest (1970), are given in Table 6.

TABLE 6

Comparison of nerve endings in
Lepidoptera optic lobes and in Acheta domestica

Lepidoptera (Strausfeld & Blest, 1970)	Acheta (present work)
1. Shallow ending No. 3 (Fig. 25)	Type B_1 (Fig. 79)
2. Long ending No. 4 (Fig. 25)	Type G_3 (Fig. 81)
3. Smooth long visual cell fibre (Fig. 26)	Type C_4 (Fig. 81) Unit 6 (Fig. 93)
4. Spiny long visual cell fibre (Fig. 26)	Type H (Fig. 81)
5. Wide-field, T-cell (Fig. 26)	Unit 38 (Fig. 101)
6. Large and medium-field, giant radial monopolar (Fig. 26)	Units 59, 60 (Figs. 114 and 115)
7. Long visual fibre ending (Fig. 26)	Root endings Types D_2, D_3, G_1, G_2, G_3 (Figs. 80 - 81)
8. Shallow monopolar cell endings	
9. Deep monopolar cell endings	
10. Y-cell (Fig. 40)	Types C group (Fig. 81) Unit 31 (Fig. 104).

From all these examples it appears that most of the endings found in Pieris and Sphinx have some similarity to those in the suboesophageal ganglion of Acheta, and which are also derived from interneurons or sensory neurons.

In the same year, 1970, Strausfeld provided another Golgi study on the optic lobes of some Diptera, which enables further comparison to be made. For example a spiny long visual fibre of Calliphora vomitoria (Plate 11, Fig. 6), is very similar to the type B_4 ending of Acheta and to the middle branch of units 57 and 59 (Figs. 80, 112, 114 and 117), in which the axonal branch is covered with punctiform structures occupying a small field. Again, in his Plate 12, Strausfeld gave two figures, the first (No. 14) of a type 3 (climbing) ending in the lamina of Syrphus elegans which is characterised by a unilateral arrangement of knobs along its length and is similar to unit 16 of Acheta in which the axon, probably from the circum-oesophageal connectives, and its collaterals and dendrites arborize with a type B_3 ending (Fig. 92). The second figure (No. 15) of Strausfeld's plate is described as a type 2 (capped) ending in the posterior median lamina of Eristalis tenax, and is very much like unit 39 of Acheta, which is a long varicose axon that terminates in a type F_1 ending (Figs. 101, 104 and 108). He also described the axon with punctiform linear structures as displaying blebbed processes (Strausfeld, 1970). The blebbed processes of the type 3 tangential component (Plate 13, figure 18) may be compared with the condition found in Acheta, for unit 39 which terminates with type F_1 endings. Strausfeld introduced other descriptive expressions during his study, for example the 'flower-like' endings, which correspond in the present work to type K (rosette-like) ending (cf. also Fig. No. 33, Plate 14 of Strausfeld), with the descriptions of unit 56 endings in Acheta;

Figs. 114, 117 and 121. Most of the structures which Strausfeld described as long visual fibre with forked endings are very similar to the axons with type A_1 or A_2 endings in the present work. Further, his group of types 1, 2, 3 and the wide-field variant of type 2 transmedullary cell endings (Plate 18) are very similar to units 53, 54 and 55 in Acheta (Figs. 113, 119 and 120).

Generally then, most of Strausfeld's types of fibre endings from the optic lobes of Diptera and Lepidoptera are found to resemble similarly shaped endings in the suboesophageal ganglion of Acheta. This tends to increase one's confidence in the Golgi impregnation method as a successful technique for studying the finer structural relationships and terminations in Acheta. It also suggests that there are not very great differences between the form of axonal endings in the nervous system in insects of widely different taxonomic position or in different regions of the nervous system. This in turn also perhaps implies that there are only a limited variety of functional relationships between neurons insofar as these depend on their terminations.

Not only is there this similarity in the form of endings and axons found in different insects, but also among different invertebrate groups. Young (1973), found some ending in the central nervous system of Loligo (Part I the optic lobe) very similar in shape to some of those occurring in the suboesophageal ganglion of Acheta. For example, his small dendritic field of a single complete second-order visual cell and recurrent axon terminating near the surface of the medulla (see Plate 11, Figs. 28 and 30), is similar to units No. 5 and 6 in the suboesophageal ganglion of Acheta. Further, Golgi preparations of his single circular dendritic fields of medium-sized second-order visual cells and

round dendritic fields (Figs. 34 and 35), show the same appearance as type E_2 (Fig. 80), and some other root endings (e.g., type G_3, Fig. 81), in Acheta. Young gave in his Fig. 81 (Plate 15), a dendrite of a giant horizontal cell showing lateral spiny processes which resemble those found in the present work e.g. types H and C_5 endings (see Fig. 81). When Young (1976), studied the suboesophageal centres of Loligo he gave a drawing of an oculomotor neuron and its dendrites and input fibres from the static nerve and median basal lobe (Fig. 27, p. 116), with structures similar to my type A_1 endings. He also gave in Plate 9 some endings resembling my type B_3 and some root endings also like those of Acheta (see Young, Figs. 91, 90 and 93). He also referred to some fine fibres in the subfrontal lobe of Octopus vulgaris (Fig. 8.40, p. 228 and Fig. 10.39a & b, p. 310), which are very similar to unit 42 in Acheta (Fig. 102 and 110) (Young, 1971).

Bullock & Horridge (1965) gave, after Cajal, some figures of Meshing synapses in the "deep retina" of the honey bee, showing the collateral arborizations of second-order neurons, all of which are not unlike those of root endings in Acheta (see Fig. 80, types D_2, D_3 and Fig. 81, types G_1, G_2, and G_3).

In contrast to the general similarity between axons and endings of various insect and invertebrate groups, one should note some recent investigations indicating possible causes of some differences in shapes of the same units and how they depend upon the technique of visualisation used.

Altman & Tyrer (1974), show for some Orthoptera that the results depend upon the method of filling and gave a good comparison of neuron morphology as demonstrated by Procion

Yellow and Cobalt filling methods for one of the motor neurons in the mesothoracic ganglion. Injection by Procion Yellow showed only the main branches and the cell body with the axon leading into a peripheral nerve, while cobalt filled neurons showed the branches more fully. The Cobalt filling method shows several subsidivisions of these branches, forming a large dendritic tree. The two methods therefore agreed only in showing the major features of the neuron, i.e.,the cell body, main axon and larger branches, and there were clear differences in the finer structure of the terminations. In fact something like this normally happens in Acheta, even when using a single technique (either the Golgi impregnation or the double impregnation procedures). The Golgi method sometimes shows a peripheral axon with all its branches and terminations while in another preparation the same main axon and all the large branches can be seen, but not the small branches and their terminations. This is also sometimes very clear in sections treated by the double impregnation method (see Figs. 77 and 78, stained by a mixed impregnation method, and Figs. 105 & 106 which show some large diameter axons stained by the modification of Kallius' procedure).

Altman & Tyrer (1974), also illustrated some sensory neurons terminating in the mesothoracic and metathoracic ganglion (see Figs. 2b, p. 162 and 8a, 8b,p. 169). The termination is similar to unit 26 in the suboesophageal ganglion of Acheta (Figs. 100 and 104), and the axon is situated in almost the same position as that found by Altman & Tyrer. It crosses almost the whole length of the ganglion, runs from the dorsal part to the ventralward in the middle of the ganglion, not far from the midline, with root-endings of type G_1. In general this shows strikingly that quite different techniques may reveal similar geometry of endings in

different ganglia of the same insect or in the nervous system of different insects.

Strausfeld & Obermayer (1976), used Cobalt chloride procedures either by diffusion into cut nerves or application via an electrode, in various Diptera. They found that this can only partially resolve nerve cells for light-microscopical observation. They found that enhanced resolution can be obtained by the addition of small quantities of serum albumin to the Cobalt chloride solution, thus yielding sufficient contrast in many nerve cells and enhancing the details of thin fibres and their appendages. They gave photographs of neurons from the thoracic ganglion of Calliphora vomitoria (their Figs. 1.A, 1.B, p. 5), and noted the blebbed appearance of the dendrites. Comparing their account of this unit with the present work shows that their Fig. 1.A is very similar to my unit 32 (Fig. 109), while 1.B is similar to the type E_2 endings and to unit 58 (Figs. 80, 89 and 114). Strausfeld shows further examples of axon form and endings in his book Atlas of an Insect Brain (1976), e.g. his Fig. 2.6 shows a typical motor neuron from the subeosophageal ganglion of Musca, with special emphasis on the densely branched spiny dendrite which emanate from the swollen pseudo-perikaryon. This agrees with most of the motor units and their endings given in the present work; for example see units 28, 29, 30 and 32 (Figs. 99, 104 and 109). An illustration in Strausfeld's Fig. 2.6 shows the terminal arborization of a sensory neuron in the suboesophageal ganglion of the same insects, very similar to unit 39 of Acheta (Figs. 101 and 108) in which, however, the axon arises from an association neuron. Plate 6.24 Figs. A and C of Strausfeld (1976), show a giant dendritic arborization of a descending neuron from the posterior optic foci, and part of the telodendritic arborization of

a large diameter ascending neuron from the contralateral half of the ventral nerve cord. These dendritic arborizations show a similar form to those of units 67 and 68, of types D_3 and G_4 respectively, in the suboesophageal ganglion of Acheta. Again Fig. 7.24 of Strausfeld (1976), shows terminal arborizations of recurrent elements in the calyx of Apis mellifica, and a dendritic tree in a protocerebral bridge-central body neuron (Fig. 7.27.A).

In appendix I Strausfeld (1976), gave a histological comparison of some different kinds of ending as revealed by different techniques, e.g. dendrites of the giant labula neuron in Calliphora vomitoria (Golgi method), arborizations of a neuron in the central body of Musca (Golgi-Cox method), and tract fibre processes in the inferior medical protocerebrum of Apis mellifera (Procion Yellow method), among others. Many of these endings are similar to those found in Acheta, which means that, at least under some 'conditions', successful Golgi or Procion Yellow methods give very similar results for the shape of the arborizations and endings which they reveal. Furthermore, Strausfeld & Housen (1977), reported that cobalt ions are incorporated by many interneurons of the insect central nervous system after injection of cobalt chloride or cobalt acetate into the neuropile, and they gave two planes of facus (Plate 1, Fig. 1 & 2), of the apical dendrites from a cobalt sulphide filled, calumnar neuron in the lobula of Calliphora erythrocephala, and another two planes of focus showing Golgi impregnated dendrites of the same species of columnar neuron. Profiles obtained by the cobalt sulphide method and Golgi impregnated technique are nearly identical, the differences relating only to the diameters of the main axon and some branches, due to the degree of successful filling by the cobalt salt and the success of the Golgi impregnation.

To conclude, from comparisons of the previous work mentioned above and the present work, it is clear that using different techniques, i.e. silver impregnation, or filling by cobalt salts, or Procion dyes to study the fine structures of the nervous system it is found that:

1. There is commonly a great similarity in the geometry and spatial relationships of the endings of the axons, units and their dendrites within the brain, the suboesophageal ganglion and the nerve cord of a variety of insects.

2. There is not much difference between terminations of axons of the nervous system in insects and some other invertebrates.

3. Good preparations of axons and their endings depend upon the success of the impregnation or of the filling methods used.

4. Despite slight differences in the diameters or thickness of some axons, there are normally different forms of many endings which may be revealed in essentially the same form by using the different available techniques.

From a purely histological standpoint the results of good Golgi procedures are, therefore, as valid as those obtained by other more recently developed methods.

VIII SUMMARY

1. A simple mass culture rearing technique for Acheta domesticus (Linn.) has been described, together with a simple method for dissection of the suboesophageal ganglion.

2. A general description of the ventral nerve cord of Acheta has been provided, with a detailed topographical description of the suboesophageal ganglion. It is situated above the mouth parts, below the beginning of the midgut and beneath the tentorium, while the middle portion of the ganglion is covered by the tentorial bridge.

3. The suboeosphageal ganglion gives off seven pairs of nerves, mandibular, hypopharyngeal, maxillary, labial, salivary and another two pairs (very small and slender), to supply the cervical muscles.

4. In the analysis of cellular composition, the cells found comprising the suboesophageal ganglion are of four main types: three of these are glial cells corresponding to the types I, II and IV of Wigglesworth (1959) and Ali (1973), while the fourth type of cell are neurons. Two layers of perilemma cells (ganglion sheath cells; perineurium; type I glial cells) are found. The perineurium is surrounded by the non-cellular neural lamella (neurilemma). Type II glial cells are simply scattered among the neurons that lie between the perineurium and the neuropile. Type IV glial cells surround the neuropile, separating it from the neurons, and may also surround some of the glomerular bodies. They occur in large numbers, especially when associated with the axons running through the interganglionic connectives, and the peripheral axons in the neuropile.

5. Three main types of neurons lie in the ganglionic cortex, and have been distinguished on the basis of their size. The largest (motor) neurons are around 30 - 40 μm in diameter (sometimes reaching 40 - 60 μm). The medium-sized cells are between 20 - 30 μm and the smallest cells (the association neurons) are around 10 - 20 μm.

6. The complex neuropile contains axons and various fibre tracts from the neurons and also some glomerular regions. The distribution of readily identifiable tracts and major pathways, as seen in transverse, frontal, parasagittal and sagittal sections, has been described. Forty-two suboesophageal tracts were recognised and classified into three major groups: transverse commissure, longitudinal connectives and oblique tracts.

7. Three conspicuous pairs of glomerular bodies are found in the neuropile of the suboesophageal ganglion in the adult of Acheta, in addition to an unpaired glomerular structure near the anterior central region of the ganglion. The three former pairs have been called the ventral, dorsal and lateral glomerular bodies, while the latter is here called the anterior central glomerular body. This glomerular centre is well developed; it appears very distinct before the middle of the ganglia and does not extend far behind the middle of the ganglia.

8. Some axonal pathways have been traced within the suboesophageal ganglion of Acheta and are described after staining by the osmium ethyle gallate technique. The neurons, axons and some of their probable interconnections have been illustrated, mainly from transverse and frontal sections, with some others from a series of vertical sections. Altogether one hundred and

twelve neurons and/or axons were observed in the present investigation. It is sometimes difficult to follow individual fibres throughout their length in preparations of this kind, and dendrite branches could not easily be located. For this reason many further axons, partially visible, could not be traced to cell bodies or to points of arborization within the neuropile. The fifty-three neurons which were located in the series of transverse section have been listed in figures as they appear in passing from the anterior to the posterior end of the ganglion.

The forty neurons (Nos. 54 to 93) which were located in a series of frontal sections, together with their axons, are also listed in the figures as they occur when passing gradually from dorsal to ventral sides of the ganglion. They represent three groups in the dorsal, central and ventral parts of the ganglion. Nineteen neurons (Nos. 94 to 112), which were identified in a series of vertical sections, have also been observed through the whole ganglion.

9. Detailed analysis of individual neurons has been made on sections of suboesophageal ganglion of Acheta domesticus (Linn.) impregnated mainly by a variation of the Golgi silver impregnation procedure (Colonnier, 1964). A number of different preparations were obtained after varying certain factors in the procedure. A modification of the double-impregnation procedure of Kallius (1910) was also tried with minor changes in duration. A new double-impregnation procedure involving silver nitrate and osmium tetroxide followed by ethyle gallate was also examined.

11. Identification and characterisation of structural units based on Golgi impregnated material showed three main kinds of

dendritic endings, which were described in detail. The axons and their branches and terminations in the anterior, middle and posterior parts of the ganglia were also located and reconstructed from several sets of silver impregnated material.

12. Twenty-four structural units were identified and described from Golgi preparations in the anterior part of the ganglion (extending from the junction of the circum-oesophageal connectives to the maxillary nerve). Twenty-six axons (units 25 to 50), were described from the middle part of the ganglion (starting with the maxillary nerve and ending in the area from which the labial nerve arises), while nineteen units (Nos. 51 to 69), were found in the posterior part of the ganglion (the remainder of the ganglion, including the origen of the labial nerve). The various axonal units were numbered from 1 to 69 according to their appearance when the ganglion is sectioned from anterior to posterior limits.

13. Correlation of Golgi and osmium tetroxide results.

It was found that tracing or illustrating the neuron fibres and their cell bodies together in a single preparation with any of the metal impregnation techniques was often too difficult and capricious. For this reason the present work attempts to identify some of the Golgi elements in terms of the axonal tracts which stained much more consistently with osmium tetroxide.

14. The functional properties of the suboesophageal ganglion in relation to the brain and ventral nerve cord was discussed in relation to transmission along the ventral nerve cord, input systems to the suboesophageal ganglion from the brain and lower centres, input system to the ganglion from the peripheral nerves, motor

output systems, intra-ganglionic relationships (the transverse commissures) and the co-ordination of the glomerular bodies.

15. An attempt at a comparative analysis was made in relation to axon forms and types of endings in Acheta, other insects and some invertebrates.

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X ABBREVIATIONS USED IN FIGURES

an	association neuron
br	brain
cc	circumoesophageal connectives
cgrb	central glomerular body
dgrb	dorsal glomerular body
g_1, g_2, g_3	thoracic ganglion
g_4 to g_8	abdominal ganglia
gl.I	type I glial cell
gl.II	type II glial cell
gl.IV	type IV glial cell
hpn	hypopharyngeal nerve
lbn	labial nerve
lgrb	lateral glomerular body
mdn	mandibular nerve
mln	median longitudinal nerve
mn	motor neuron
mxn	maxillary nerve
n_1, n_2	anterior and posterior cervical nerve
nl	neurilemma
np	neuropile
pm	perineurium
sg	suboesophageal ganglion
sgln	salivary nerve
st_1, st_2 ...	fibre tracts
tb	tentorial bridge
thc	first thoracic connectives
thcl	lateral nerve from the first thoracic connectives
vgrb	ventral glomerular body

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ADDENDUM

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