PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

# A Guide to the Neuroanatomy of Locust Suboesophageal and Thoracic Ganglia

N. M. Tyrer and G. E. Gregory

*Phil. Trans. R. Soc. Lond. B* 1982 **297**, 91-123
doi: 10.1098/rstb.1982.0035

*Phil. Trans. R. Soc. Lond.* B **297**, 91–123 (1982) [ 91 ]
*Printed in Great Britain*

# A GUIDE TO THE NEUROANATOMY OF LOCUST SUBOESOPHAGEAL AND THORACIC GANGLIA

By N. M. TYRER and G. E. GREGORY
*Department of Biochemistry, University of Manchester Institute of Science and Technology, P.O. Box* 88, *Manchester M*60 1*QD, U.K.*
*Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden AL*5 2*JQ, Hertfordshire, U.K.*

(*Communicated by J. Z. Young, F.R.S. – Received* 2 *April* 1981)

[Plate 1]

## CONTENTS



The organization of the thoracic and suboesophageal ganglia in the locust is presented to provide a framework into which details of individual neurons can be inserted as information becomes available. Three species were examined, *Chortoicetes terminifera* (Walker), *Schistocerca gregaria* (Forskål) and *Locusta migratoria migratorioides* (Reiche and Fairmaire). The basic plan of the ganglia is similar in all three species. Series

of selected sections in transverse, horizontal and sagittal planes are illustrated to show the arrangement of the main nerve fibre tracts and areas of neuropil, and these are described briefly. A guide is given to prominent features that assist in the interpretation of sections in each plane. In the simpler mesothoracic and prothoracic ganglia nine longitudinal tracts are present in each half of the neuromere, and six dorsal and four ventral transverse tracts (commissures) link the two halves. Four vertical or oblique tracts are conspicuous, the T-tract, ring tract, C-tract and I-tract. Major roots of each peripheral nerve useful as landmarks are numbered from anterior to posterior. Two regions of fine fibrous neuropil are prominent, the ventral association centre and an area associated with the ring tract, a little above it. In the metathoracic ganglion three abdominal neuromeres are fused posteriorly to the true metathoracic neuromere. All four neuromeres show modification of the basic framework chiefly in the arrangement of the ventral commissures and the degree of development of the ventral association centre. In the suboesophageal ganglion three neuromeres, mandibular, maxillary and labial, are fused together from anterior to posterior. They show increasing modification of the basic plan anteriorly. Additional anterior longitudinal tracts are present, which connect with the brain, the dorsal commissures are much reduced and compressed, particularly in the mandibular neuromere, and the ventral commissures of all three neuromeres differ considerably from those of the thoracic ganglia.

## Introduction

The present popularity of intracellular neuron staining techniques, such as use of Procion yellow (Kravitz *et al.* 1968; Stretton & Kravitz 1968), Lucifer yellow (Stewart 1978) and cobalt (Pitman *et al.* 1972) and its silver intensified version (Tyrer & Bell 1974; Bacon & Altman 1977), is resulting in a growing catalogue of physiologically identified nerve cells of the locust central nervous system (CNS), the individual branching patterns of which are known in detail. Examples are to be found in papers by Bentley (1970), Burrows (1973*a*, *b*, 1975), Burrows & Hoyle (1973), Altman & Tyrer (1974, 1977*a*, *b*), Hoyle *et al.* (1974), O'Shea *et al.* (1974), Rehbein *et al.* (1974), Tyrer & Altman (1974), Burrows & Siegler (1976), Bacon & Tyrer (1978, 1979), Altman & Kien (1979) and Tyrer *et al.* (1979). However, in many papers the neurons are described in isolation, the dye-filled cell usually being illustrated in a plan or side view of the otherwise unstained ganglion as though floating in a featureless bubble. Relationships of the cells to one another and to the overall organization of the ganglion, which are crucial to a better understanding of CNS function, remain little known. However, if the morphology of each neuron were related to identified nerve tracts and regions of neuropil in the ganglion, cell relationships could be established without the use of difficult multiple dye injecting techniques. Closing down the microscope substage diaphragm or use of Nomarski optics allows some of the more obvious features of ganglia to be seen in wholemounts, and far more is visible if ganglia containing filled cells are embedded and sectioned. Neuron branching can then be related to other anatomical features. This paper seeks to provide a framework for the interpretation of sectioned ganglia so that, as individual neurons are identified functionally, their branching can be related to other anatomical features.

Ideally, to interpret such material, comparable sections stained to reveal the neuroanatomy more completely by methods such as the reduced silver techniques of Bodian (1936) or Rowell (1963) should be available for reference.

This account is intended primarily as a pictorial representation and verbal description is

kept to a minimum. The basic structure of four important ganglia is illustrated by drawings to display the arrangement of the main nerve fibre tracts and areas of neuropil. The drawings are of selected sections from series in each of the usual planes of sectioning. As neurons become identified, whether by intracellular staining, Golgi–silver block-impregnation techniques or specific histochemical methods, it should be possible to relate them to this anatomical framework and so to each other. The account should also enable regions being studied by electron microscopy to be more precisely defined.

The maps given here are necessarily simplified. Detailed description of neuroanatomy is time-consuming and this account is intended as an interim guide, upon which more detailed studies (Gregory 1982) will be based. It will meanwhile help to fill an obvious gap in knowledge about the insect nervous system.

## Materials and methods

Suboesophageal and thoracic ganglia of mature adult males and females of *Chortoicetes terminifera* (Walker), *Schistocerca gregaria* (Forskål) and *Locusta migratoria migratorioides* (Reiche & Fairmaire) were examined. Serial paraffin wax sections were stained with reduced silver by either the Rowell (1963) silver nitrate method (plate 1) or the Power (1943) double impregnation modification of the Bodian (1936) protargol (silver–protein) technique.

Ganglia to be stained by the Rowell method were removed under saline (Usherwood 1968), fixed in alcoholic Bouin solution (Duboscq-Brasil) (Gray 1954) for 2 h at room temperature (20–25 °C), dehydrated in an ethanol series and embedded in Paraplast, melting point 56 °C (BDH Chemicals Ltd, Poole, U.K.). Sections were cut at 10 μm and impregnated at 60 °C and pH 7.6–7.8 for 20 h, followed by development for 8 min.

For Bodian staining, ganglia were dissected from $CO_2$-narcotized locusts under saline (Hoyle 1953) and fixed for 16–24 h in alcoholic Bouin solution previously 'aged' for about 40 days at 60 °C (Gregory 1970) or in synthetic substitutes (Gregory *et al.* 1980; Gregory 1980*a*). Ganglia were coloured with eosin during dehydration to aid orientation for sectioning, and embedded in Gurr's Paramat, melting point 56–57 °C (Searle Diagnostic, High Wycombe, U.K.). Sections were cut at 10–20 μm and stained by the procedure described previously (Gregory 1970). Fuller practical details are given elsewhere (Gregory 1980*b*). The copper content of the protargol impregnating solutions was 1.3 or 2.6 g per 65 ml, depending on section thickness, and developers contained 1 g of hydroquinone and 4–10 g of sodium sulphite ($Na_2SO_3.7H_2O$) in 100 ml. Only sulphite pure enough to yield the desired clear red stain was used (Gregory 1974*a*).

Definitive series of sections in the three most usual planes, transverse, horizontal and sagittal (vertical longitudinal) (see plate 1), and including examples of each of the three species studied, were selected, from a total of over 140 specimens, for clarity of staining and correct orientation. Individual sections that best illustrated the chief neuroanatomical features were then drawn with the aid of a ×20 objective and camera lucida drawing tube.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

## General structure of the ganglia

The ventral nerve cord ganglia are bilaterally paired structures linked by longitudinal, paired interganglionic connectives. The unit structure is a segmental neuromere which has a basic organization repeated throughout the nerve cord. The mesothoracic and prothoracic ganglia consist of only one neuromere each, while in others, such as the suboesophageal and metathoracic, neuromeres are fused to form a composite ganglionic mass with repetitive structures.

The general structure of each ganglion resembles that in cockroach (summarized by Gregory 1974*b*). Lane (1974) has reviewed the tissue components in detail. Each ganglion is bounded by a connective tissue sheath, the neural lamella (neurilemma) (NL, figure 1A), under which lies a thin cellular perineurium (PN). Beneath this is the glial cell layer (GL), in which lie the groups of cell bodies (somata) (CBG) of the neurons, mostly ventrally and laterally but with a few dorsally. The central core of the ganglion (GC) (neuropil *sensu lato*) is composed mainly of the neuron processes (nerve fibres) and the processes of the glial cells that envelop them.

The peripheral nerves of the thoracic ganglia are numbered according to the system of Campbell (1961), based on the mesothoracic ganglion of *Locusta*. This system differs from that used for cockroach (Pipa & Cook 1959; derived from Pringle 1939), in counting the most anterior paired peripheral nerve, rather than the anterior connective, as nerve 1. The names given to the nerves of the suboesophageal ganglion (Altman & Kien 1979) are derived from those employed in cockroaches (Willey 1961; Guthrie & Tindall 1968).

The tracheal supply to all four ganglia, and to the other ganglia of the CNS, has been described by Burrows (1980).

At the level of organization that we are considering we could find no important differences between the three species examined, except for a difference in size (see Altman & Tyrer (1977*a*) for comparative illustration). There were no differences in ganglia from males and females. We have therefore selected the best stained specimens for illustration, irrespective of species and sex.

## Organization of the ganglion core

The central core of the ganglion has a clear basic framework, provided by the system of nerve tracts, formed by groups of fibres running together in bundles (figure 1). Between the tracts lie finer fibrous areas, neuropil *sensu stricto*. The most useful landmarks are the longitudinal tracts (figure 1A), which run between anterior and posterior interganglionic connectives of the same side, and the transverse tracts (commissures), which link the two halves of the ganglion core across the midline (figure 1B). Other, generally smaller, bundles of fibres run vertically and obliquely, and consist chiefly of the roots of peripheral nerves and fibre bundles from the groups of neuron cell bodies. The terminology employed for the tracts is based on that devised for cockroach by Pipa *et al.* (1959) and amplified by Gregory (1974*b*).

## Description of plate 1

Sections of the metathoracic ganglion in *Chortoicetes terminifera* stained by the Fraser Rowell reduced silver method. A, transverse (cf. figures 8D, E); B, horizontal (cf. figure 9C); C, sagittal (cf. figure 10A, B). Note the serial repetition of homologous structures in B and C.


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

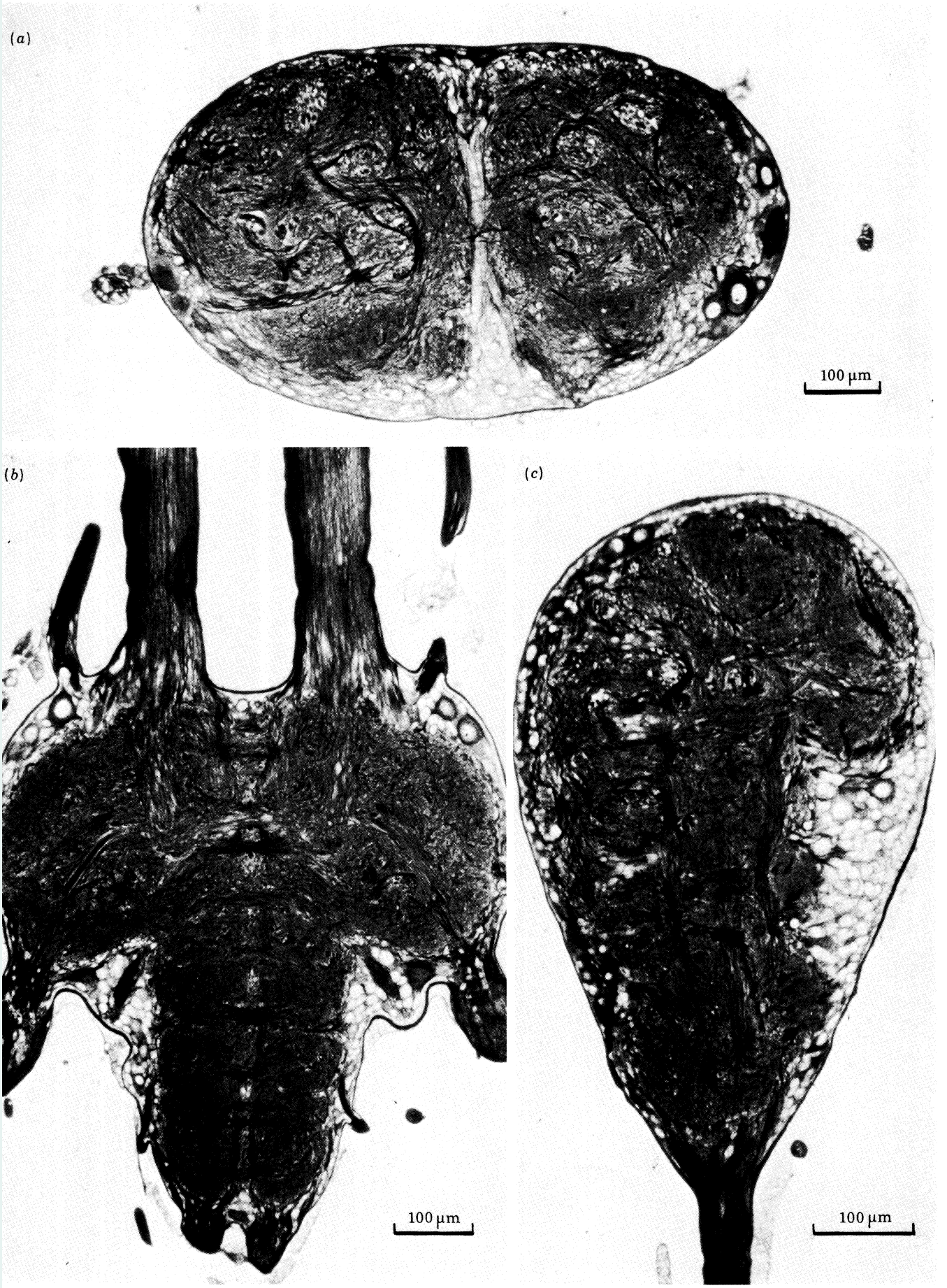



PLATE 1. For description see opposite.

(*Facing p.* 94)



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

For brevity, after the first mention tracts and other structures will generally be referred to by their initials. All abbreviations are listed at the end of the paper.

Single neuromere ganglia such as the mesothoracic and prothoracic display the basic plan most clearly. Where several neuromeres are fused, as in the suboesophageal and metathoracic

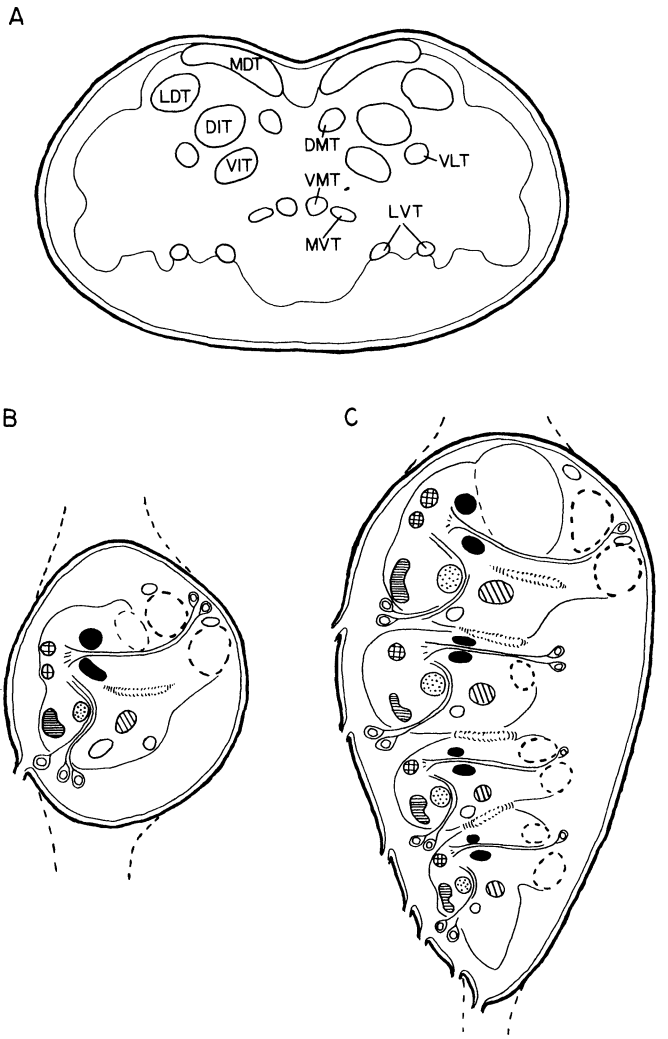



FIGURE 1. The basic framework of tracts and commissures in the ventral cord ganglia. A, simplified diagram of a transverse section of the mesothoracic ganglion to show the main longitudinal tracts. These tracts are present in all of the ventral cord ganglia although their spatial relationship in the ganglion core varies according to the ganglion and the level of section (see figures 2–13). B, the main dorsal commissures in a midline sagittal section of the mesothoracic ganglion (for labelling see figure 4), to be compared with C, the dorsal commissures in a midline sagittal section of the metathoracic ganglion (for labelling see figure 10) to show the serial repetition of these structures in the four fused neuromeres of the metathoracic ganglion.

ganglia, the structures characteristic of a single neuromere are repeated several times (figure 1C). We give first a detailed description of the mesothoracic ganglion which shows the unitary arrangement most clearly and follow this with descriptions of the other ganglia to show the modifications on the basic theme. For each ganglion we have drawn a series of sections in the transverse, horizontal and sagittal planes. The verbal description concentrates

on giving a guide to the features most likely to be useful to workers approaching the anatomy of the ganglia for the first time, particularly when using material in which the general structure does not show up ideally (e.g. radioautographs or material viewed with fluorescence optics).

## Mesothoracic ganglion

Sections showing the principal neuroanatomical features of the mesothoracic ganglion are illustrated in figures 2–4. The ganglion has six paired peripheral nerves (NV1–NV6) and one unpaired, posterior median nerve (MNV). The chief nerve fibre tracts and other structures are as follows.

### *Longitudinal tracts*

Nine longitudinal tracts run through each half of the ganglion core and are named according to their positions. The largest tracts are: the *median dorsal tract* (MDT) and the *lateral dorsal tract* (LDT); the *dorsal intermediate tract* (DIT); and the *ventral intermediate tract* (VIT). The smaller tracts are: the *dorsal median tract* (DMT) and the *ventral median tract* (VMT) close to the midline; the *ventral lateral tract* (VLT) lateral to the VIT; the *median ventral tract* (MVT) lateral to the VMT; and the *lateral ventral tract* (LVT) at the ventral margin of the core. The MVT is a diffuse grouping of fibres normally divisible into several bundles and the LVT separates into two bundles, the outer and inner LVTs, for most of its course.

### *Transverse tracts*

There are six dorsal commissures, numbered from anterior to posterior (DCI to DCVI), and four ventral commissures, ventral commissure I (VCI), ventral commissural loop II (VCLII), the supramedian commissure (SMC) and the posterior ventral commissure (PVC). VCLII, unlike the other commissures, forms a more or less vertical ring, with two commissural components, dorsal and ventral (d VCLII, v VCLII).

### *Vertical and oblique tracts*

Many of these are more complex and difficult to interpret than the foregoing tracts, and are either not included or not named in this account. However four are distinctive and form useful landmarks. These are named, from their shapes, the T-tract, ring tract, C-tract and I-tract (Gregory 1974*b*). The *T-tract* (TT, figures 2C, 3A–E, 4A–F) appears T-shaped in transverse sections of the ganglion. It ascends as two closely associated vertical bundles in the midline between DCI and DCIII. Dorsally the bundles turn laterally below DCII to pass above the DITs and towards the LDTs. The *ring tract* (RT, figure 3D) is a horizontal ring of fibres composed anteriorly of d VCLII and posteriorly of components of the SMC. The *C-tract* (CT, figures 2D, 3C–E) is a paired vertical tract, crescent-shaped in transverse sections, in the midpart of each half of the ganglion core at the transverse level of a midline trachea (m tr, figures 2D, 3A, B, F, 4A). The C-tract runs just medial to the lateral part of the ring tract (figure 3D) and curves between the VMT and MVT below and the DIT and VIT above (figure 2D). The *I-tract* (IT, figures 2D, 3C–E) runs up more or less vertically just behind and a little lateral to the C-tract.

The roots of the peripheral nerves form complex tracts that generally run obliquely and change direction along their courses, and so are often difficult to identify in sections cut in

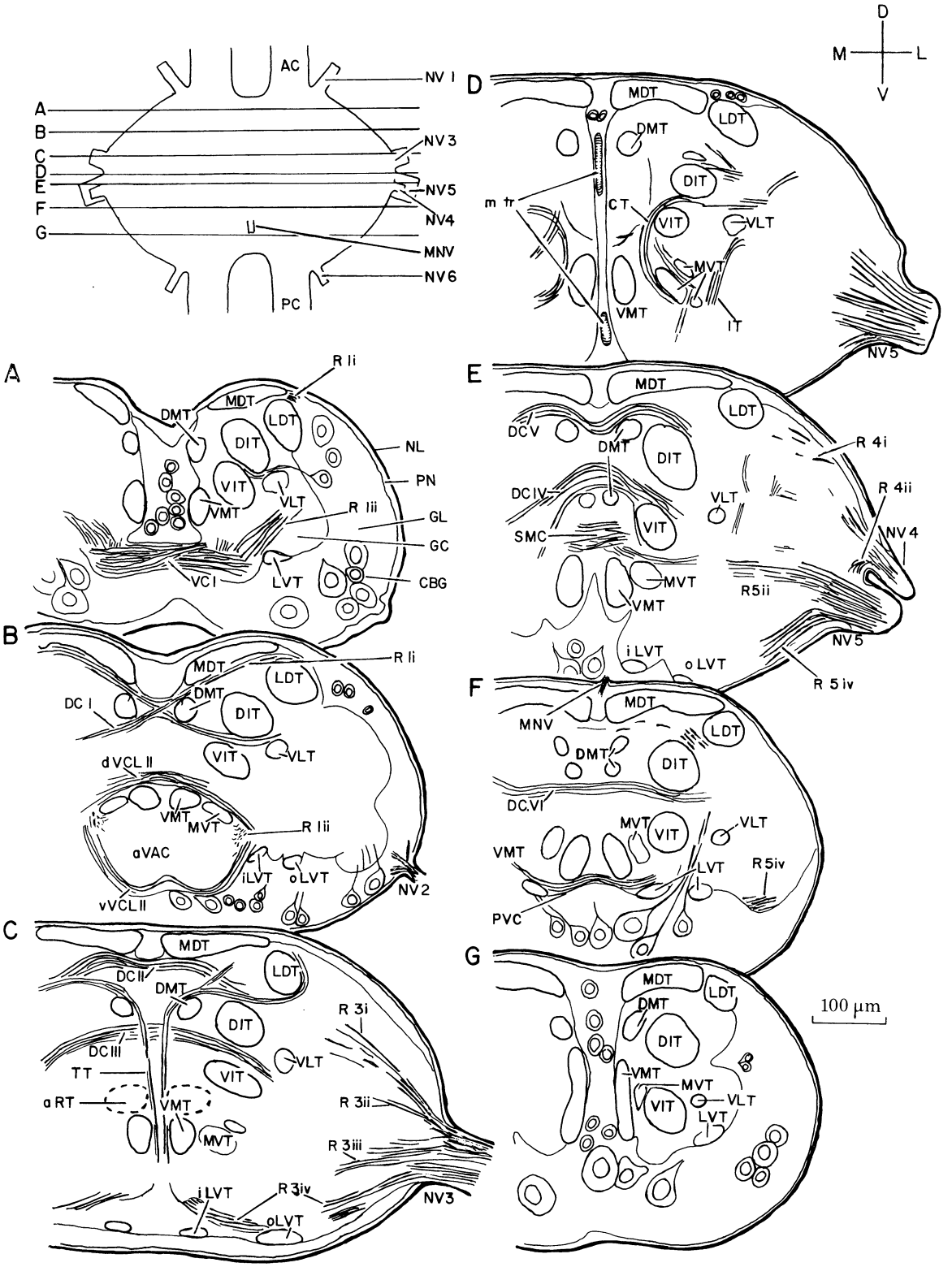



FIGURE 2. *Mesothoracic ganglion*, transverse sections showing chief neuroanatomical features. Section planes (A–G indicated in dorsal view of ganglion shown at reduced scale at top left. (*Chortoicetes terminifera*.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

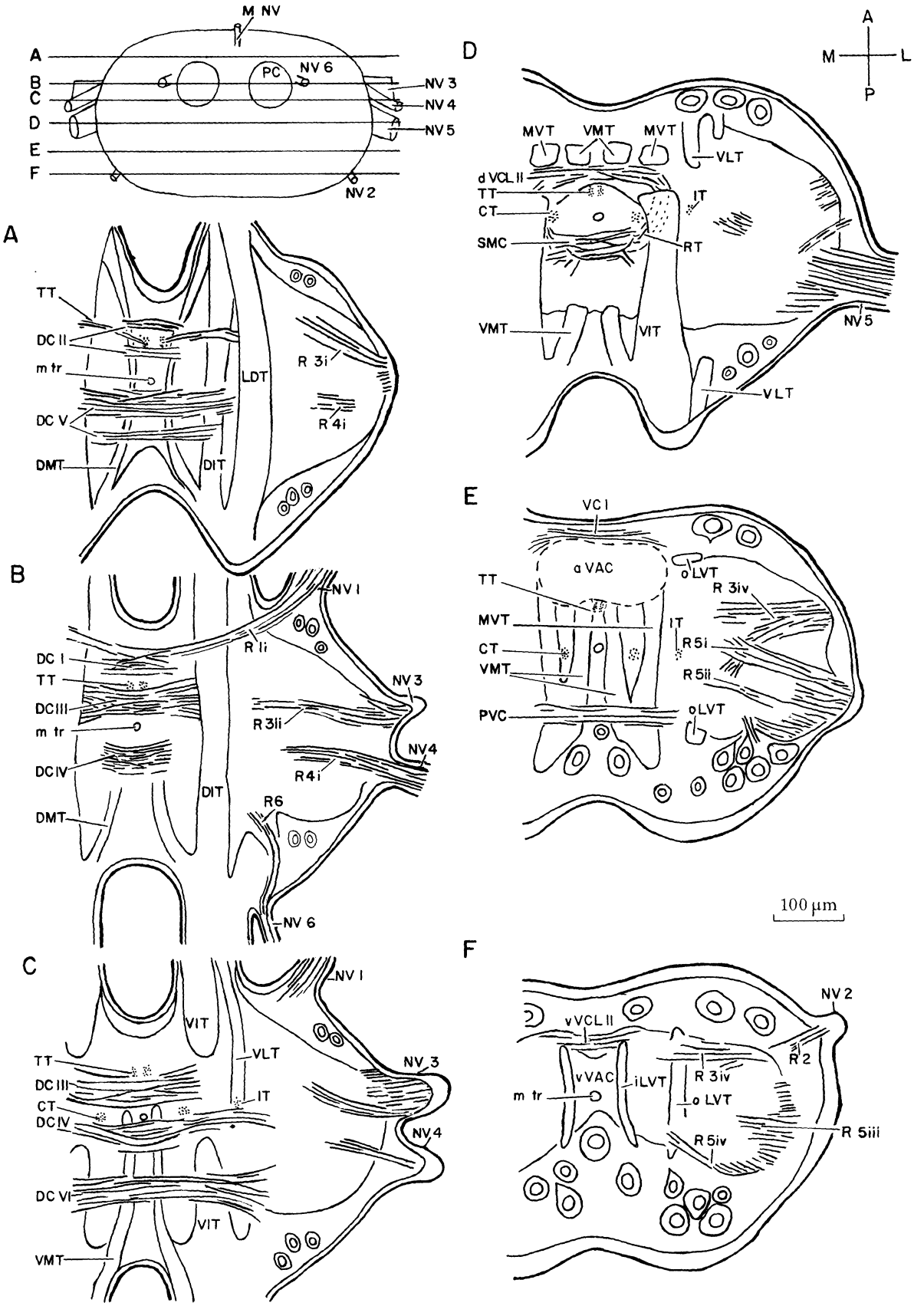



FIGURE 3. *Mesothoracic ganglion*, horizontal sections in planes A–F shown in posterior view of ganglion at top left. (*Chortoicetes terminifera*.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the usual planes. Dye-filling is generally necessary for their unequivocal analysis and they have yet to be examined as fully as in cockroach (Gregory 1974*b*). Here only major groupings of fibres that may be useful as landmarks are distinguished. Those of each nerve are for the time being simply numbered from dorsal to ventral, e.g. nerve 3 roots are designated R3i to R3iv.

*Other conspicuous structures*

A prominent region of particularly fine fibrous neuropil lies across the midline in the ventral part of the ganglion core. It appears similar to that termed the ventral association centre in cockroach (Pipa *et al.* 1959; Gregory 1974*b*) and this term will be retained here, though it implies a function for which physiological evidence is so far lacking. The anterior region of the ventral association centre (a VAC, figures 2B, 3E, 4A) lies between VCI and the T-tract. The VAC divides around the T-tract and behind this is composed of two cylindrical halves, one each side of the midline, and a ventralmost fused region across the midline (v VAC, figures 3F, 4A). Another conspicuous area of fine neuropil extends across the midline above a VAC and separated from it by the VMTs. It lies close behind d VCLII and seems associated with the ring tract, and so is provisionally labelled as 'anterior part of ring tract' (a RT, figures 2C, 4A, B).

*Guiding features*

If difficulties are encountered in distinguishing the above structures the following indicators in the various planes of section should help.

*Transverse sections* (figure 2)

Longitudinal tracts show most clearly in the posterior part of the ganglion. Most prominent are the LDT, DIT and VIT (tracts a, b and c of Tyrer & Altman 1974), which are arranged diagonally across each half of the ganglion core (figure 2F, G). The smaller DMT and VMT (dorsal and ventral wing sensory tracts (dwst and vwst of Altman & Tyrer 1977*a*) are also usually easy to distinguish. Often more difficult are the more diffuse MVT, and the MDT and LVT, because they abut on the glial layer and their axons may be mistaken for small neuron cell bodies.

One of the most useful landmarks for identifying the commissures is the midline trachea (m tr, figure 2D) which ascends centrally in the ganglion. In front of it lies DCIII and behind it is the characteristic inverted U of DCIV, beneath which run small ventral limbs of the DMTs (figure 2E). In front of DCIII run the vertical bundles of the T-tract, and immediately in front of these is the dorsal part of the ring of VCLII that surrounds the VAC. Dorsally, above where the arms of the T-tract turn laterally, is DCII. Posteriorly, DCIV appears as a large commissure close to the dorsal core margin, with its anterior part in the same vertical plane as DCIV and the SMC (see figure 2E). Near the posterior margin of the core, DCVI forms a coherent bundle extending for a considerable distance laterally. The PVC runs across some way below it.

*Horizontal sections* (figure 3)

The key to interpreting structures in more dorsal sections is first to identify the dors commissures by locating the midline trachea, between DCIII and DCIV, and the ascendi limbs of the T-tract, which pass between DCI and DCIII. DCIV also has a promine




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

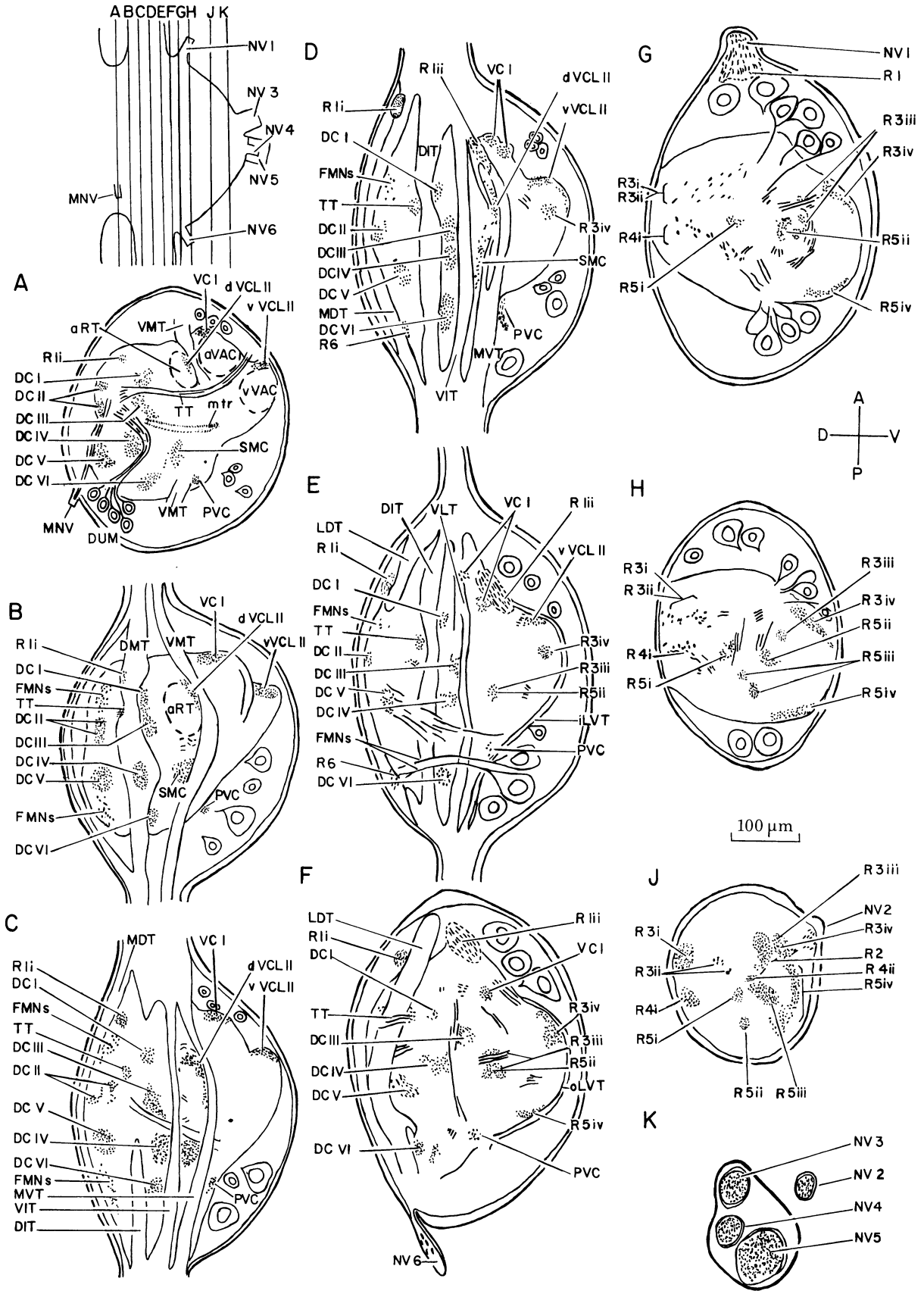



FIGURE 4. *Mesothoracic ganglion*, sagittal and parasagittal sections in planes A–K shown in dorsal view of half ganglion at top left. (*Chortoicetes terminifera*.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

midline fibre bundle dipping just beneath it from a conspicuous posterior dorsal unpaired median group of cell bodies (DUM, figure 4A). Once DCI, DCIII and DCIV are recognized, the other commissures should present no problems. In more ventral sections the ring tract is prominent, the large SMC fibres that make it up posteriorly being particularly characteristic (see figure 3D). Further ventrally a VAC is easily identifiable, with VCI in front of it (figure 3E) and the v VCLII below, in front of the most ventral region of the VAC (figure 3F).

*Sagittal sections* (figure 4)

Start with sections in or near the midline. Locate the midline trachea to identify DCIII and DCIV as above. If slight obliqueness of sections makes this difficult, DCIV can be recognized by the fibre bundle of the posterior dorsal unpaired median cell group (DUM, figure 4A) dipping beneath it. The anterior and ventral regions of the VAC help to indicate the ascending limbs of the T-tract passing between them. Once the T-tract is located DCI can be found in front of it, and DCIII behind it.

To find the nerve roots start laterally where the separate nerves are easy to distinguish and then follow them into the more difficult medial regions.

## Prothoracic ganglion

Sections illustrating the chief structural features of this ganglion are shown in figures 5–7. It has six pairs of peripheral nerves (NV1–NV6) as in the mesothoracic ganglion but differs from it in having an anterior as well as a posterior unpaired median nerve. Otherwise its organization is very similar to that of the mesothoracic ganglion and many features can be identified with the aid of the same landmarks. However, there are no large flight motoneurons in the prothoracic ganglion, apart from two groups of four motoneurons to dorsal longitudinal muscle 81 (Tyrer & Altman 1974), so that it is less rounded than the mesothoracic ganglion and its anterior quadrants are less massive. As there is no large sensory component from the wings in nerve 1, nerve 1 is much smaller. The area of fine neuropil associated with the anterior region of the ring tract (a RT, figures 5C, 6C, 7A–C) is more developed than in the mesothoracic ganglion.

## Metathoracic ganglion

Sections of the metathoracic ganglion are shown in figures 8–10. It consists of the true metathoracic ganglion (T3) with the neuromeres of the first three abdominal segments (Ab1–3) fused on to it posteriorly. In the labelling of the figures, where confusion might arise, structures in the various neuromeres are generally prefixed T3, Ab1, Ab2 or Ab3. The metathoracic neuromere possesses six paired peripheral nerves and one, posterior, median nerve. Nerves 1–5 are similar to their mesothoracic counterparts but nerve 6 is larger because it carries the input from the tympanum (Rehbein *et al.* 1974). The first abdominal neuromere has only one pair of (ventral) peripheral nerves but Ab2 and Ab3 have two paired nerves each, one dorsal (DNV, figure 9D, E), the other ventral (VNV, figures 8N, 9D–F). All three abdominal neuromeres have both an anterior and a posterior median nerve. The organization of each of the four neuromeres follows fairly closely the pattern of the mesothoracic ganglion, and with this understood the general framework of the metathoracic ganglion can be reasonably easily interpreted.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

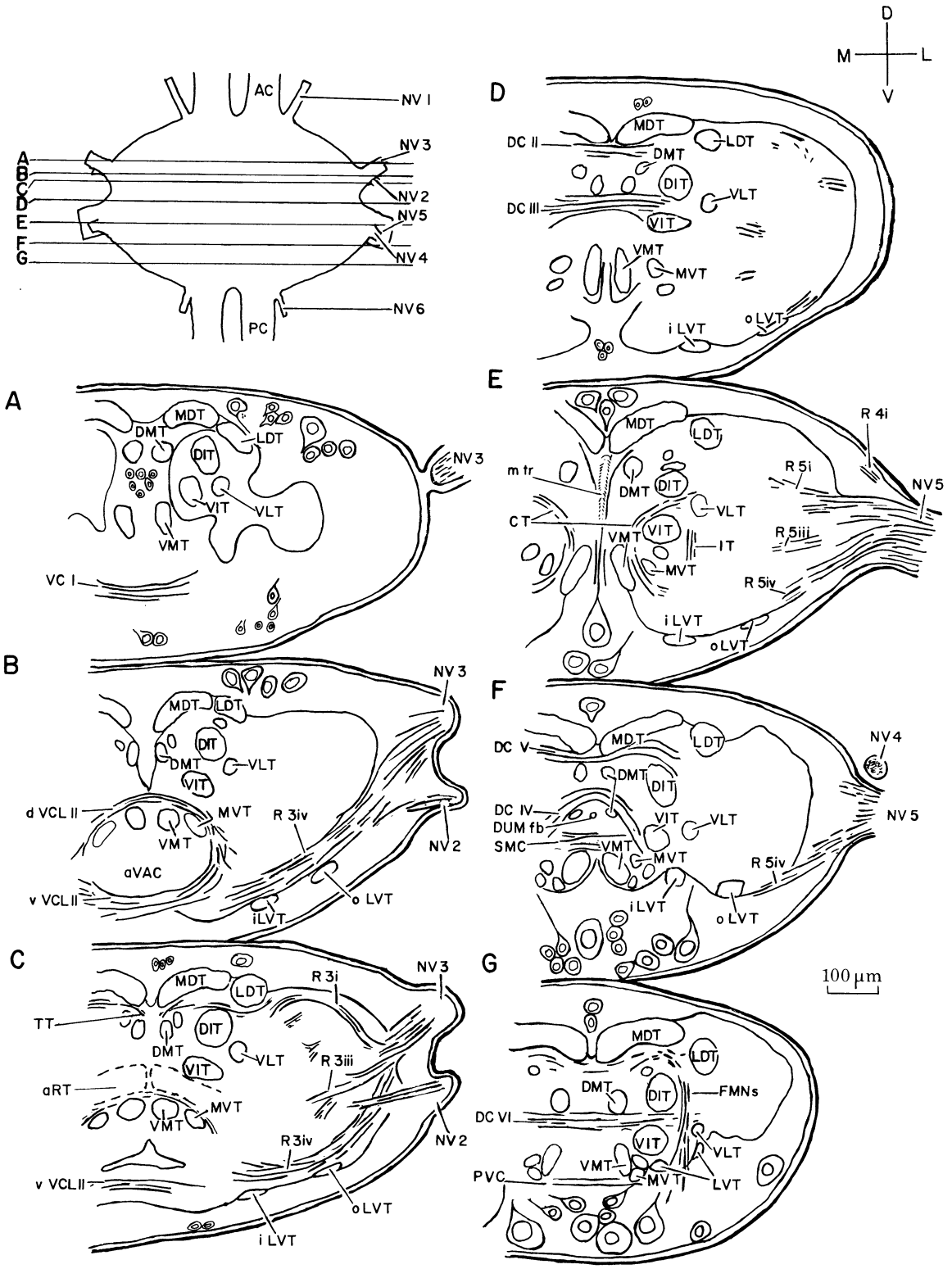



FIGURE 5. *Prothoracic ganglion*, transverse sections in planes A–G shown in dorsal view of ganglion at top left. (*Locusta migratoria migratorioides*.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

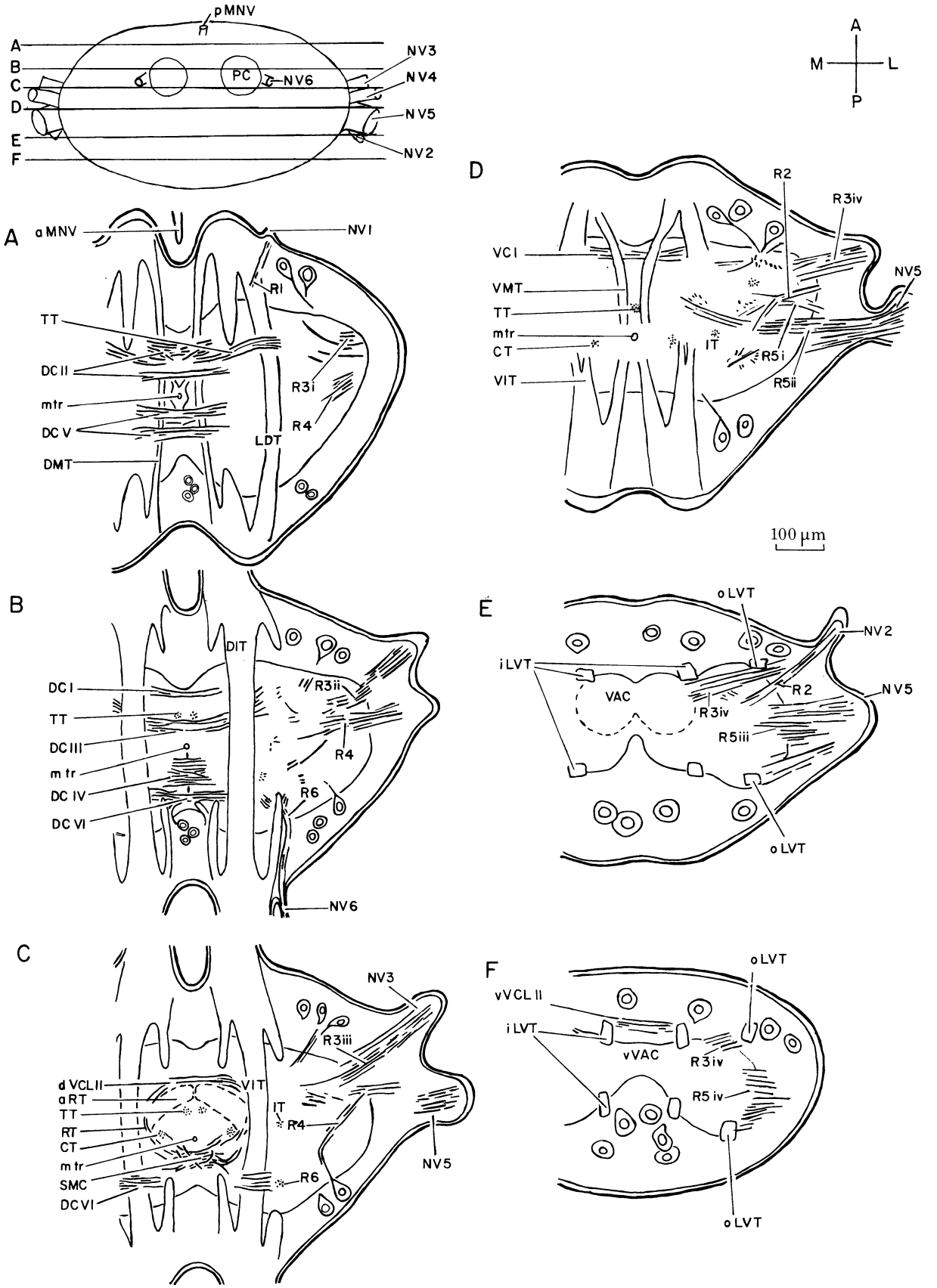



FIGURE 6. *Prothoracic ganglion*, horizontal sections in planes A–F shown in posterior view of ganglion at top left. (*Locusta migratoria migratorioides*.)

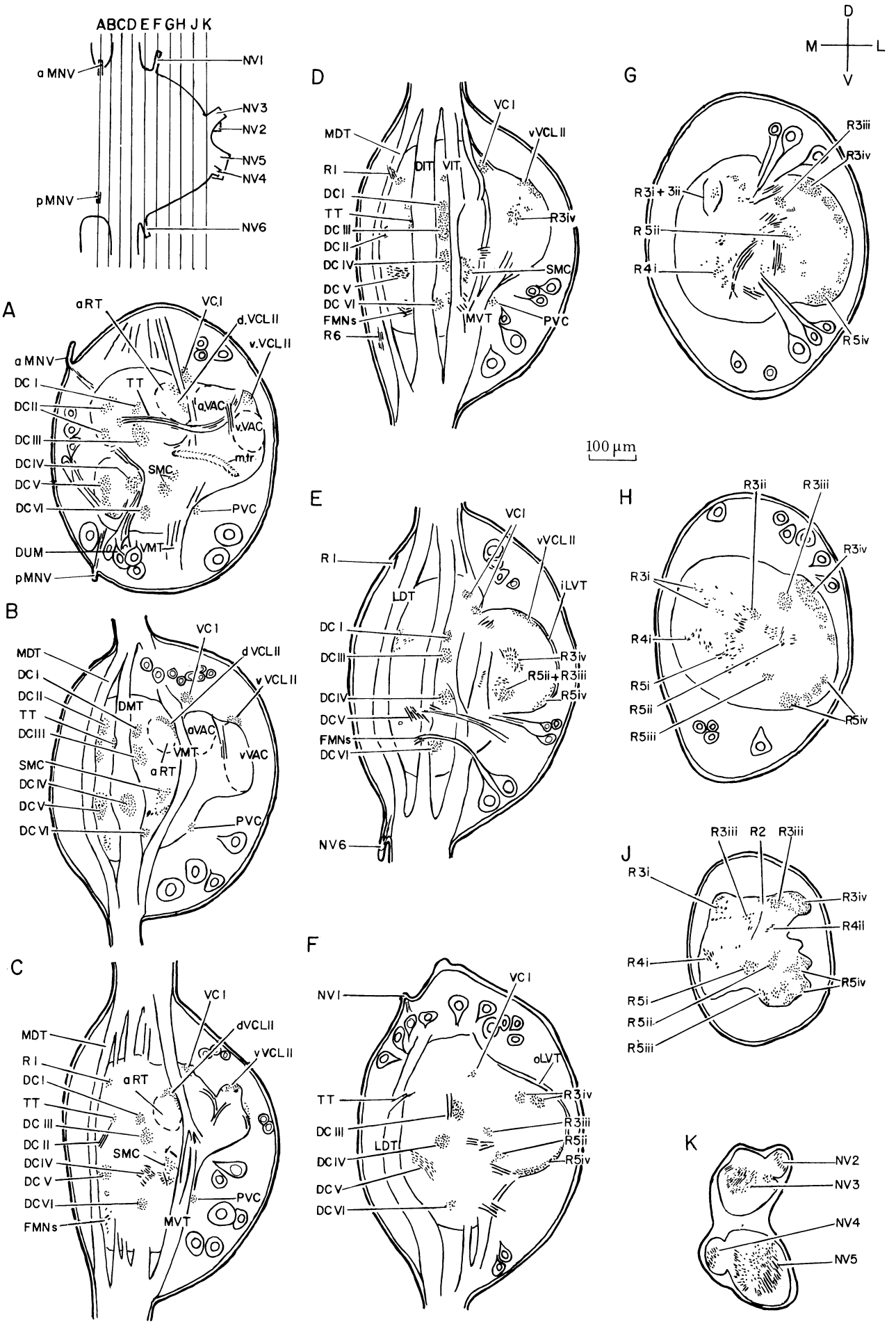



FIGURE 7. *Prothoracic ganglion*, sagittal and parasagittal sections in planes A–K shown in dorsal view of half ganglion at top left. (*Locusta migratoria migratorioides*.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The longitudinal tracts bear the same relationship to each other as in the mesothoracic ganglion and identification should present no problems. The arrangement of commissures is fairly similar in all four neuromeres (plate 1; figure 1), the six dorsal commissures of T3 being repeated in miniature in Ab1–Ab3. The ventral commissures differ somewhat from those of the mesothoracic ganglion. In T3, VCI, the SMC and the PVC are straightforward but VCLII lacks the dorsal part in front of the T-tract. An anterior ventral commissure (T3 AVC, figures 8A, 9E, 10A, B) above VCI at the anterior margin of the ganglion core may represent the missing dorsal part of VCII, displaced forward by the expansion of the fine neuropil above the VMTs and a VAC. This is probably equivalent to the a RT in other ganglia, enlarged because of the input from the tympanum (Rehbein *et al.* 1974). In the abdominal neuromeres only the SMC is clearly identifiable. An additional ventral commissure is present in Ab1 at the same horizontal level as the SMC but lying below DCIII, behind the T-tract, and is referred to simply as a ventral commissure (Ab1 VC, figures 8G, 10A) as are the other, more ventral commissures of the abdominal neuromeres (figures 8H, J, K, O, 9F, G). The VAC is much reduced in Ab1, but appears well developed in T3, Ab2, and especially Ab3.

### *Guiding features*

The best guide to the limits of the four neuromeres is four midline tracheae that ascend vertically in narrow columns of glial cells. The most anterior occupies its typical position between DCIII and DCIV of T3. The other three run *between* T3, Ab1, Ab2 and Ab3, clearly marking the boundaries between them. Ab1–Ab3 are also characterized by the central bulges of the VACs, especially in Ab2 and Ab3.

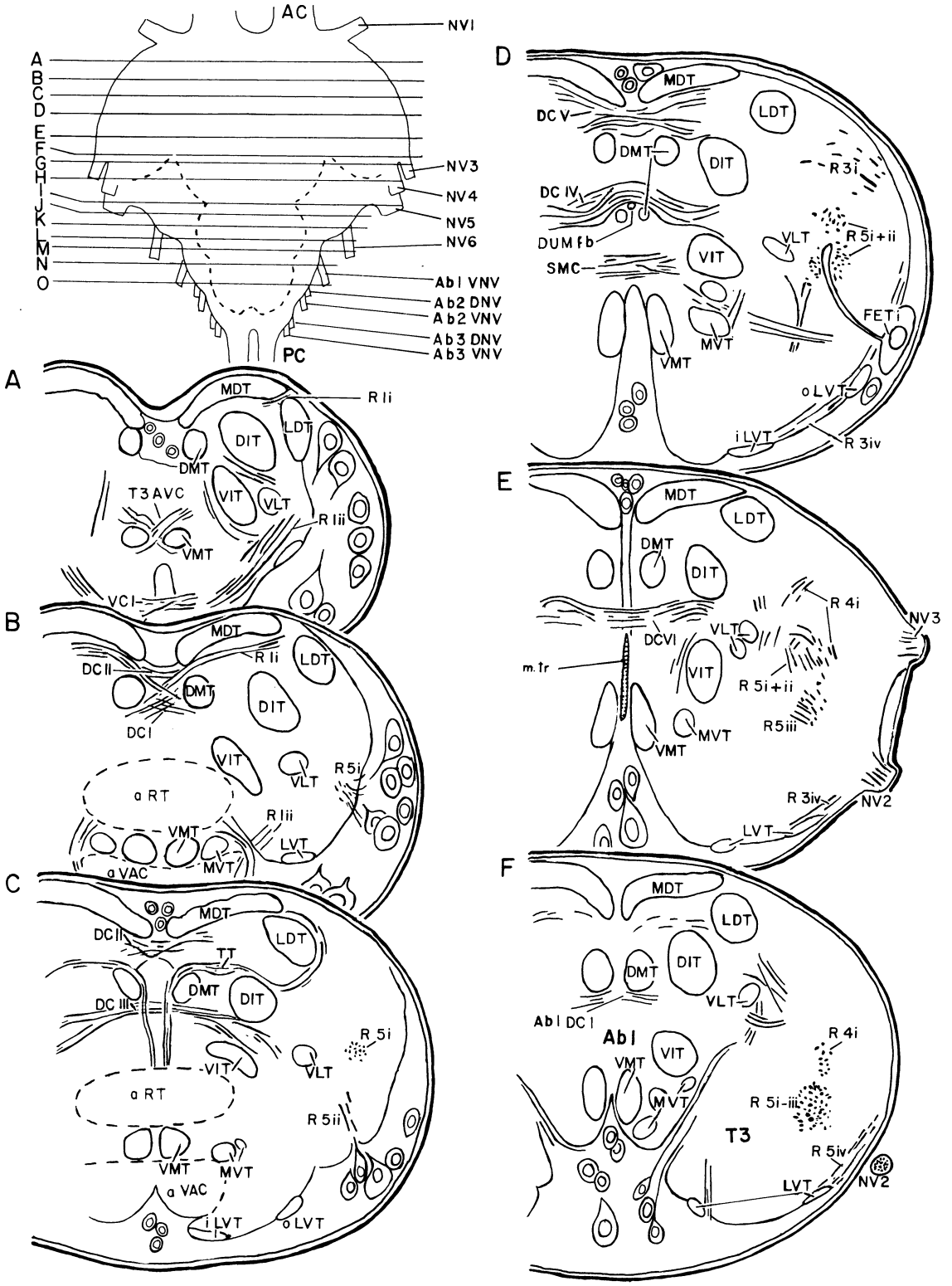
*Transverse sections* (figure 8)

The T3 neuromere is similar to the mesothoracic ganglion and presents no great difficulty. Once the individual abdominal neuromeres are delimited the dorsal commissures immediately in front of and behind each trachea can be identified as DCVI of the preceding neuromere and DCI of the succeeding one. In Ab1 interpretation of the other tracts is then relatively straightforward, though the T-tract, between DCI and DCIII, is the only prominent feature that divides up the dorsal commissures in the absence of an obvious midline trachea between DCIII and DCIV. Interpretation of Ab2 and Ab3 is rather less easy because the tracts are smaller and more compressed, particularly in Ab3, but they retain their usual shapes and relationships and the same guiding features can be used.

*Horizontal sections* (figure 9)

Use the four midline tracheae amid their neighbouring glial cells as landmarks. The paired ascending limbs of the T-tracts, though small in the abdominal neuromeres, are also useful and deeper in the ganglion. The transversely cut C-tracts can be useful landmarks in the T3 neuromere (see figure 9C–F). In the abdominal neuromeres clearly defined C-tracts are absent. The SMC is the most prominent landmark at this level. Fibres that curve forward from it along the medial sides of the VITs form what appear as partial ring tracts, which are particularly conspicuous in T3 and Ab1.


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



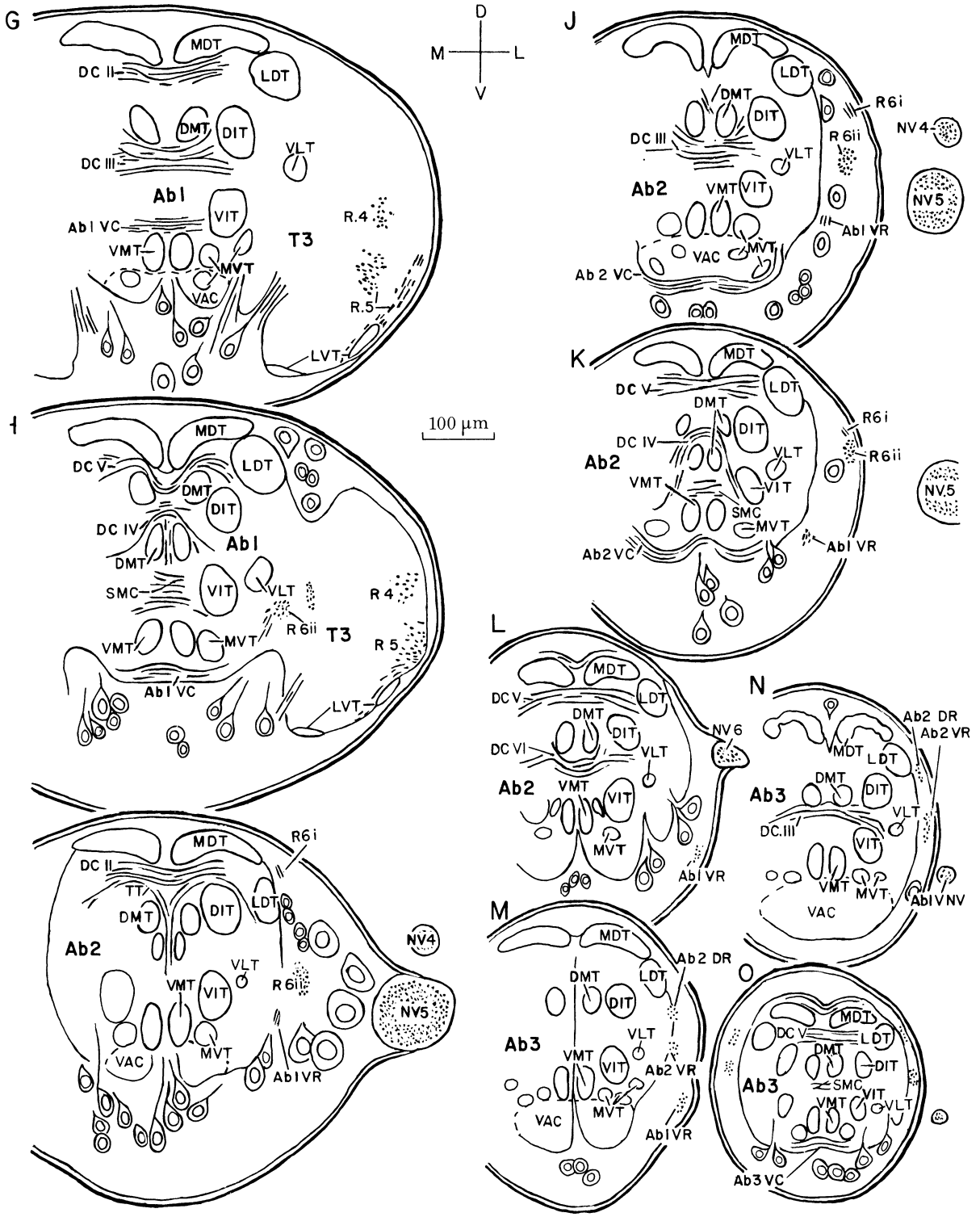
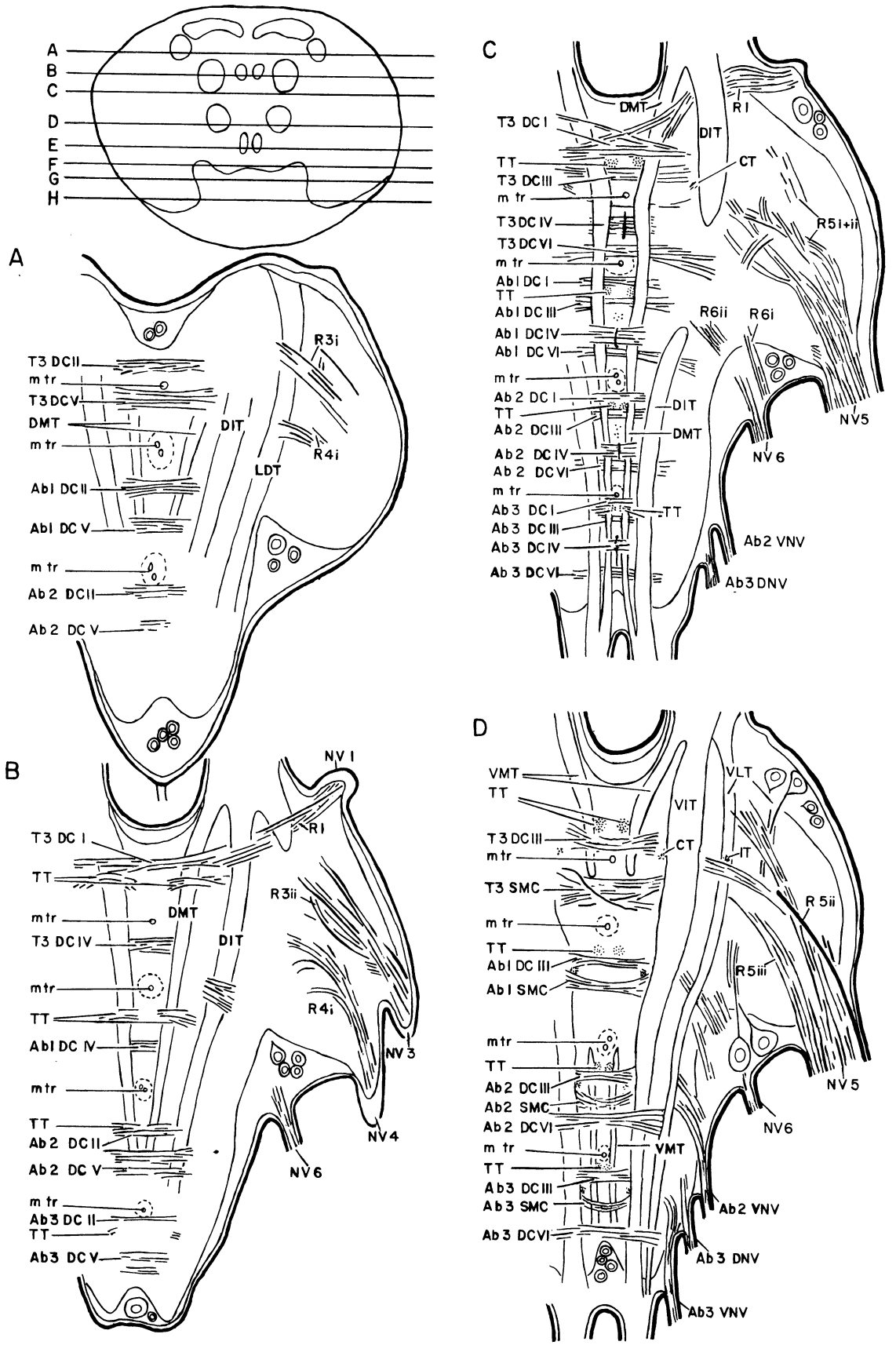



FIGURE 8. *Metathoracic ganglion*, transverse sections in planes A–O shown in dorsal view of ganglion at top left. (*Chortoicetes terminifera*.)




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



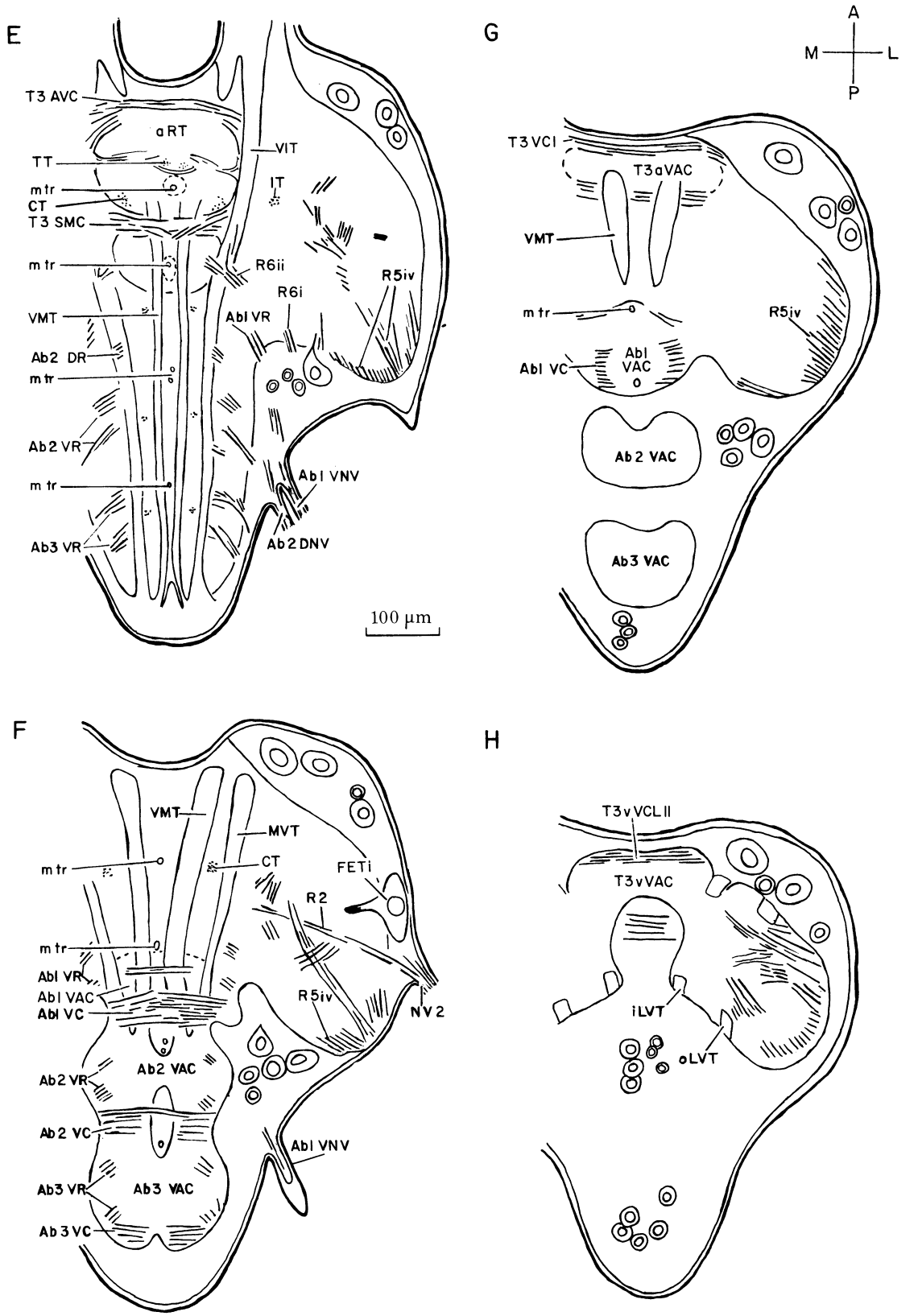
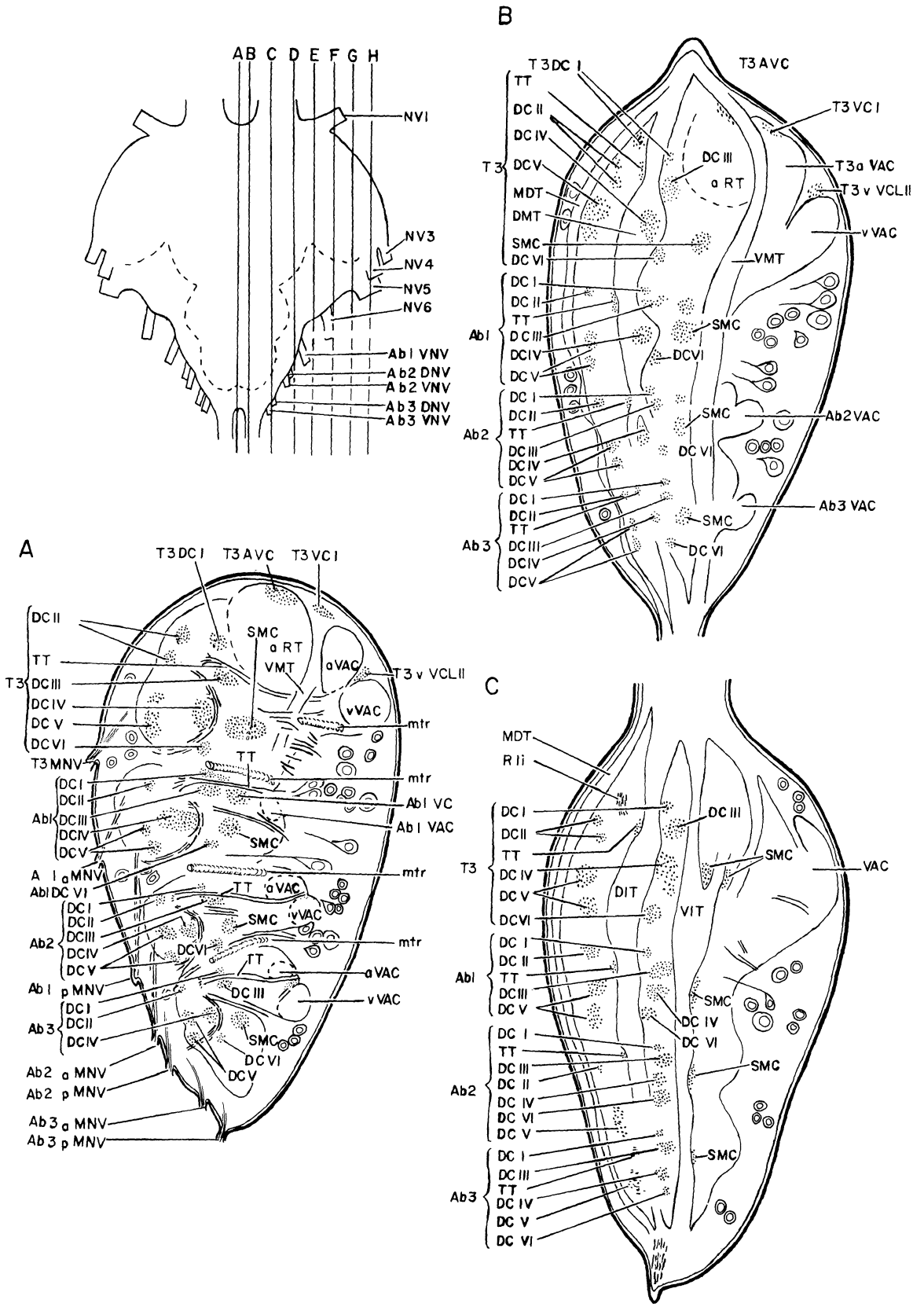



FIGURE 9. *Metathoracic ganglion*, horizontal sections in planes A–H shown in transverse section of ganglion at top left. (*Chortoicetes terminifera*.)




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

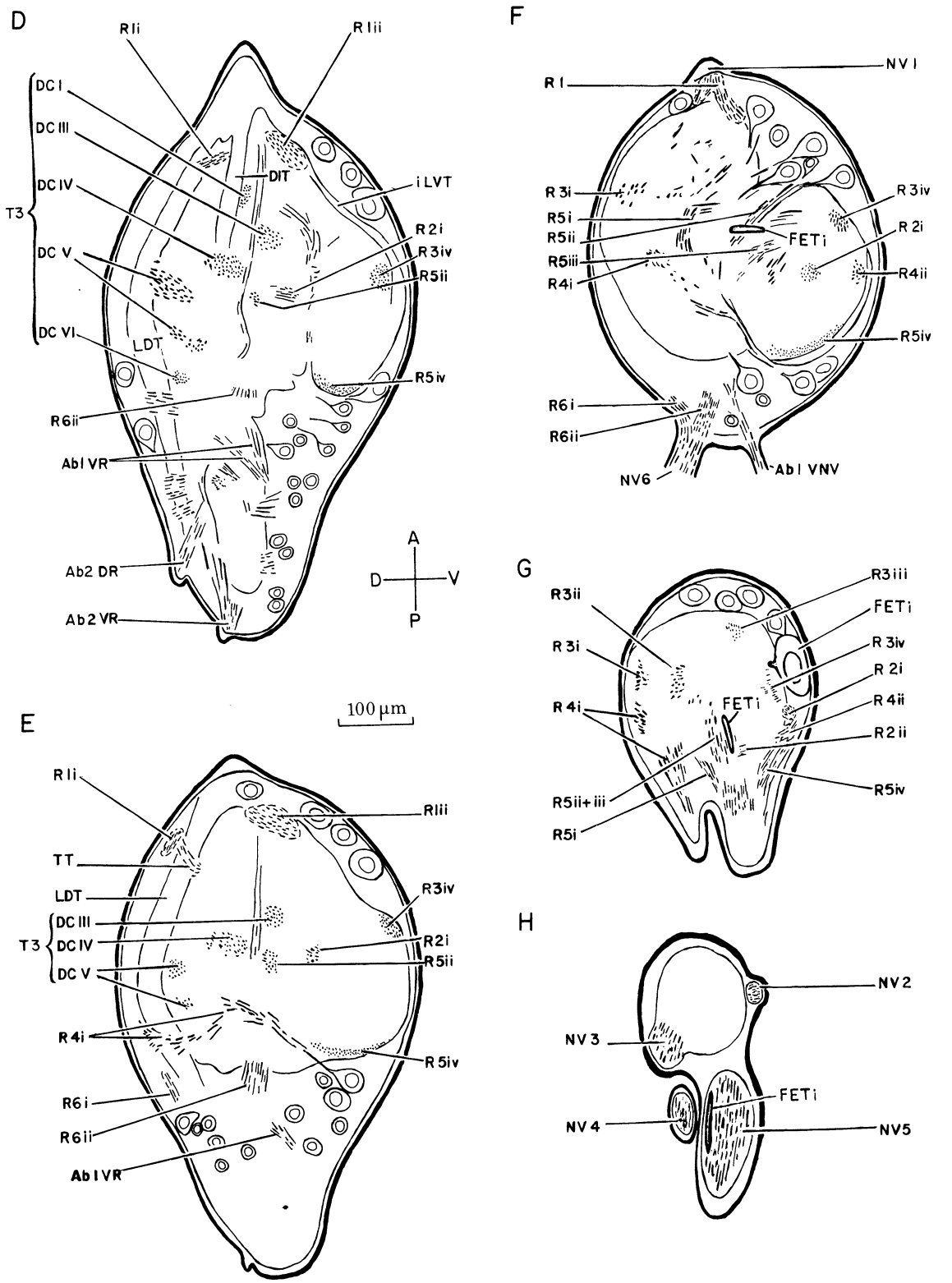



FIGURE 10. *Metathoracic ganglion*, sagittal and parasagittal sections in planes A–H shown in dorsal view of ganglion at top left. (*Chortoicetes terminifera*.)

*Sagittal sections* (figure 10)

In the midline the component neuromeres show particularly well (plate 1; figures 1C, 10A). The tracheae and T-tracts, and the fibre bundles of the dorsal unpaired median cells curving beneath DCIV of each neuromere are as characteristic as for the mesothoracic ganglion.

### Suboesophageal ganglion

Sections of this ganglion are illustrated in figures 11–13. It consists of three fused neuromeres, from anterior to posterior the mandibular (Md), maxillary (Mx) and labial (Lb) neuromeres. There are eight paired peripheral nerves, which are here numbered 1–8 from anterior to posterior, though this does not imply any homology with those of the thoracic ganglia. The order of numbering is that adopted by Altman & Kien (1979) and differs from the order used in cockroach by Guthrie & Tindall (1968). Each neuromere has one large nerve innervating the mouthparts: mandibular (N1), maxillary (N4) and labial (N5). The hypopharyngeal nerve (N2), is located ventrally on the mandibular neuromere. Nerve 3 is a small dorsal nerve to the corpus allatum. Nerves 6 and 7 are smaller, more lateral nerves of the labial neuromere to the posterior head, the neck and the salivary glands (Altman & Kien 1979). Nerve 8 usually runs together with the posterior connective for a short distance before branching from it. There is one, unpaired, posterior median nerve.

The basic organization of the more dorsal part of the core of each neuromere resembles that in the mesothoracic ganglion, though in the maxillary and particularly in the mandibular neuromere there is considerable reduction and compression. The system of longitudinal tracts running through all three neuromeres is largely similar in appearance and arrangement to that in the thoracic ganglia, but several additional tracts are present, provisionally labelled as MVTs, which run ventral and lateral to the VMTs. Most are not typical longitudinal tracts as they do not run throughout the ganglion. They include tracts that link the ganglion to the brain anteriorly, and roots of the maxillary and labial nerves that also run through to the brain.

Ventrally the neuromeres are rather less typical. Many of the commissures are not clearly homologous with thoracic ones and are here labelled simply as ventral commissures (VC, figures 11C–E, 12D, F, 13A, B). There is little trace of areas of neuropil corresponding to the thoracic VACs, but well developed ventral neuropils are present in each neuromere, associated with the bases of the three major paired peripheral nerves.

The labial neuromere is the largest and most similar to a thoracic one, with six dorsal commissures that plainly resemble the thoracic DCI to DCVI. Among the ventral commissures only an SMC and a PVC are clearly recognizable. A region where projections from wind sensory head hairs cross the midline between MVTs (Tyrer *et al.* 1979) is here termed the MVT commissure (MVTC, figures 11E, 12D, 13A).

In the maxillary neuromere the dorsal commissures are smaller. Tracts that seem to represent DCII and DCIV to DCVI can be recognized but those in the positions of DCI and DCIII are compressed together and difficult to delimit. Among ventral commissures, only a presumed SMC can yet be identified.

The dorsal part of the mandibular neuromere is very small and the commissures are extremely reduced. Small tracts that resemble DCIV and DCV are present posteriorly, and

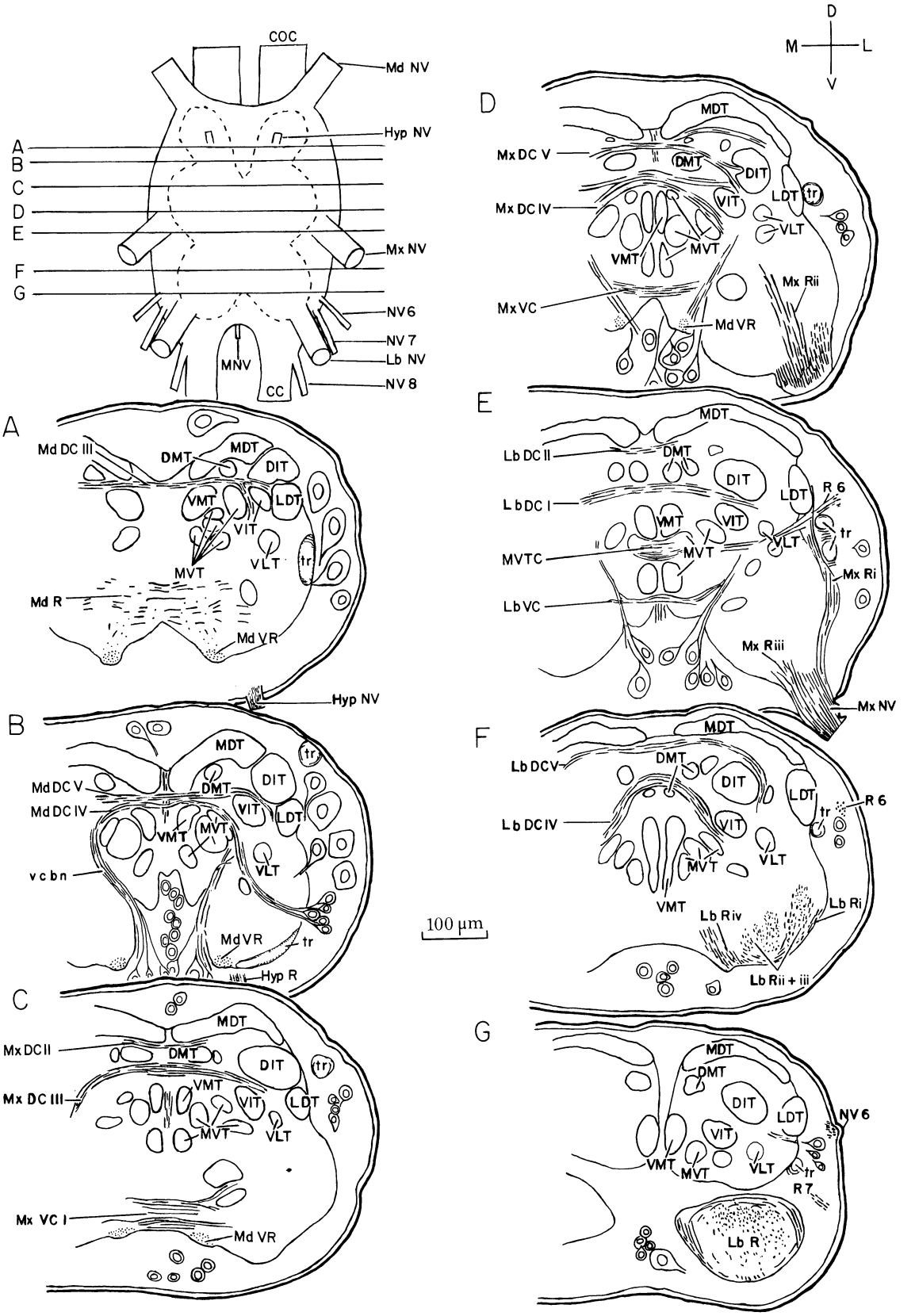



FIGURE 11. *Suboesophageal ganglion*, transverse sections in planes A–G shown in ventral view of ganglion at top left. (*Schistocerca gregaria*.)


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in front of them lie a few other transverse fibres, which are here tentatively labelled as DCIII. The neuromere is dominated by the ventral neuropil composed mostly of roots of the mandibular nerves, many fibres of which cross the midline in this region. The prominent ventral root of each mandibular nerve (Md VR, figures 11A–D) contains sensory projections from receptors on the mandibles and mandibular articulations (P. G. Mobbs, personal communication).

*Guiding features*

First identify the limits of the component neuromeres. They are not so easily separable as in the metathoracic ganglion, as midline tracheae are usually too small to be helpful landmarks and the neuromeres are more tightly fused together dorsally. Often the best indication of neuromere boundaries are the ventral bulges of the well developed neuropils associated with the mandibular, maxillary and labial nerves, together with the prominent groups of ventral cell bodies that intrude between them. The best starting point is the labial neuromere since it shows the chief anatomical features most clearly. No single landmark is available for identifying the commissures, and various combinations of features must be used.

*Transverse sections* (figure 11)

As well as the intrusions of the ventral cell bodies between the ventral neuropils (see figures 11B, E), the neuromere boundaries are best established by the root of the hypopharyngeal nerve (Hyp R, figure 11B), marking the mandibular–maxillary boundary, and the base of the maxillary nerve (Mx Nv, figure 11E) lying just in front of the maxillary–labial boundary.

The longitudinal tracts are most readily recognizable in sections just anterior to the bases of the labial nerves (figures 11F, G). Once identified here they can be followed anteriorly through the sections of the other neuromeres. Additional tracts (MVTs) appear in more anterior sections.

The most easily identifiable dorsal commissures in the labial neuromere are DCIV and DCV, lying in the same transverse plane. DCIV has the same inverted U-shape as in thoracic ganglia, with small ventral limbs of the DMTs passing beneath it and with DCV forming a shallow arch above it near the dorsal margin of the core (figure 11F). A prominent midline group of fibres that ascends anterodorsally from ventral cell bodies (see figure 13A), somewhat resembling the T-tract of the thoracic ganglia, separates DCIV from DCIII. DCII lies above and just in front of these fibres. DCIII is separated from DCI by some smaller vertical midline fibres. DCVI can be recognized as the most posterior dorsal commissure in the whole ganglion with considerable lateral extensions. Relate the ventral commissures to the dorsal ones. Thus the PVC can be found a little below DCVI in much the same transverse plane.

In the maxillary neuromere DCIV and DCV are the most easily recognized commissures (figure 11D). With these located, identify the closely associated DCI and DCIII with DCII above (figure 11C) and a little in front of them and DCVI close behind them.

The only dorsal commissures prominent in the mandibular neuromere are those provisionally labelled as DCIV and DCV (figure 11B). These lie in the same transverse plane, in the typical position close to the posterior margin of the neuromere but do not have the characteristic shapes seen in the other neuromeres. The tentatively designated DCIII in front of them is very small (figure 11A).


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

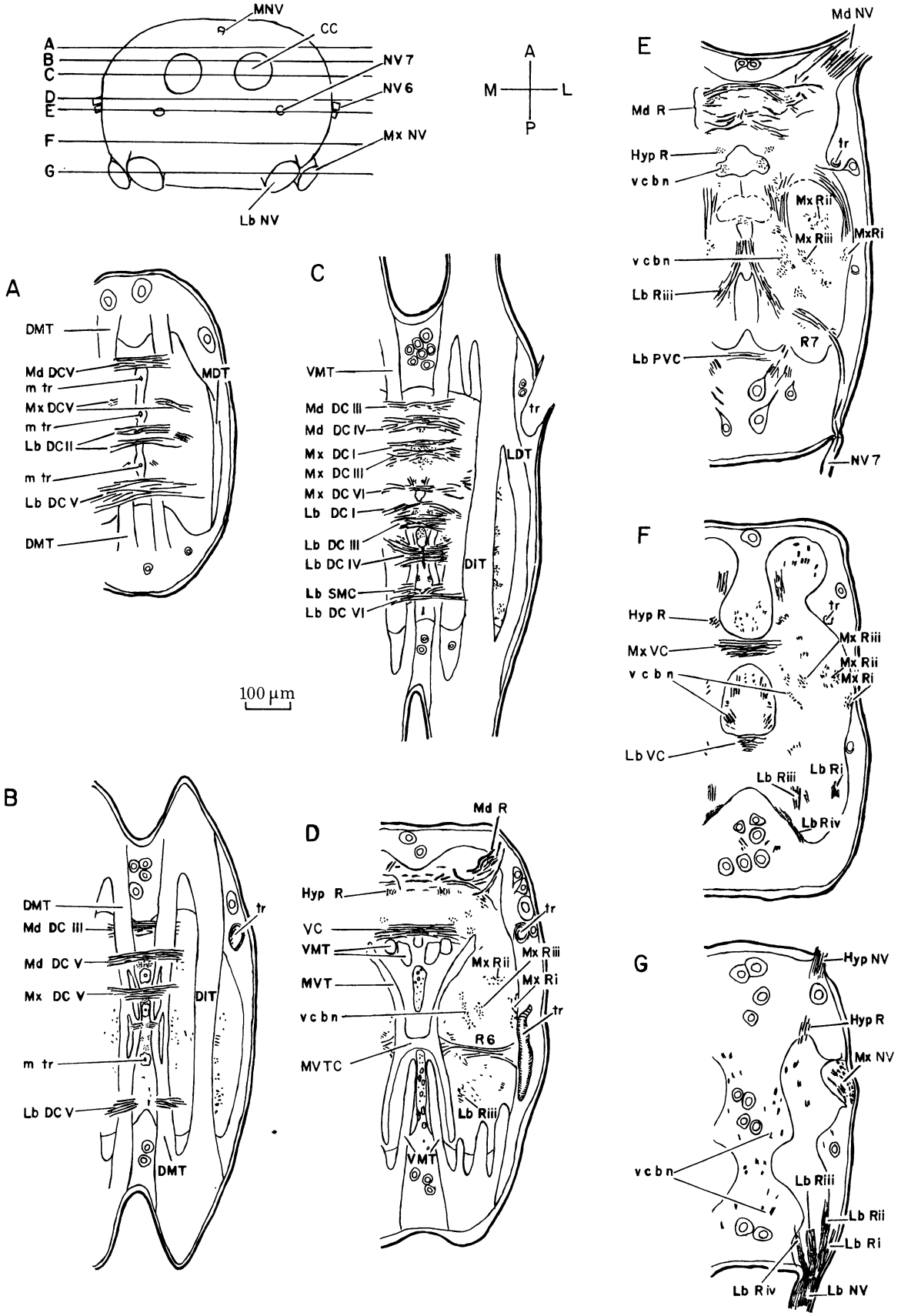



FIGURE 12. *Suboesophageal ganglion*, horizontal sections in planes A–G shown in posterior view of ganglion at top left. (*Schistocerca gregaria*.)




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

*Horizontal sections* (figure 12)

No simple guides can be used in the interpretation of horizontal sections because there is no particularly conspicuous feature in the dorsal part of the ganglion. First locate the boundary of the labial neuromere ventrally, shown by the ventral neuropils, intrusion of ventral cell bodies, and the entry points of the maxillary nerves just in front of it. More dorsally, the MVTC (figure 12D) lies close to the anterior boundary of the neuromere, which is also marked by an inconspicuous midline trachea (figures 12A, B).

Three groups of labial dorsal commissures lie just above the level of the MVTC (see figure 12C). DCI and DCIII are closely associated but can be separated by the small vertical midline fibres between them. DCIV lies behind DCIII, divided from it by a larger group of ascending midline fibres (see above) and an inconspicuous midline trachea. It can be recognized by its characteristic association with the midline bundle of fibres of dorsal unpaired median cells. Most posterior of all are DCVI and the SMC, which are closer together than in the thoracic ganglia. With these features located, the other labial commissures should be readily identifiable.

The boundary of the maxillary and mandibular neuromeres can be established similarly, aided by the points of entry of the hypopharyngeal nerves (as for transverse sections see above). The ventral mandibular neuropil is quite distinct in appearance because many of its mandibular nerve root fibres cross the midline (figure 12E). To identify the dorsal commissures of both neuromeres first locate the respective DCVs, which are conspicuous dorsally. More ventrally, the complex of the maxillary DCI and DCIII, and the mandibular DCIII, should be recognizable. The maxillary DCIV and DCVI are difficult to separate in horizontal sections.

*Sagittal sections* (figure 13).

As usual sections in or near the midline (figure 13A) are the easiest to interpret. The neuromere boundaries are clearly shown ventrally by the groups of cell bodies that intrude between the bulges of the ventral neuropils. Dorsally the characteristic dipping fibre bundles of the dorsal unpaired median cell groups below each DCIV plainly indicate each neuromere. Identification of most other features should then be straightforward. Structures in more lateral sections are less easy to identify and often need to be traced out from the midline area. Nerve roots are best traced inward from the nerve bases.

## Discussion

All the locust ganglia studied here appear to be constructed according to a common plan, albeit with various modifications, and the abdominal ganglia can be expected to conform to much the same pattern, although earlier studies by Cheze (1967) and osmium–ethyl gallate (Wigglesworth 1957) preparations by Seabrook (1968, 1970) only partly confirm this. In cockroach the basic framework of the mesothoracic ganglion (Pipa *et al.* 1959; Gregory 1974*b*) is similar, as is that of the other ventral nerve cord ganglia (Gregory 1982). Possibly ganglia of other insect species will be found to have a similar pattern of organization, which would extend the significance of the present work.

The value of invertebrate preparations in the study of the neural basis of behaviour is well recognized (Hoyle 1970; Fentress 1976) but to date most investigations have been physiological


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

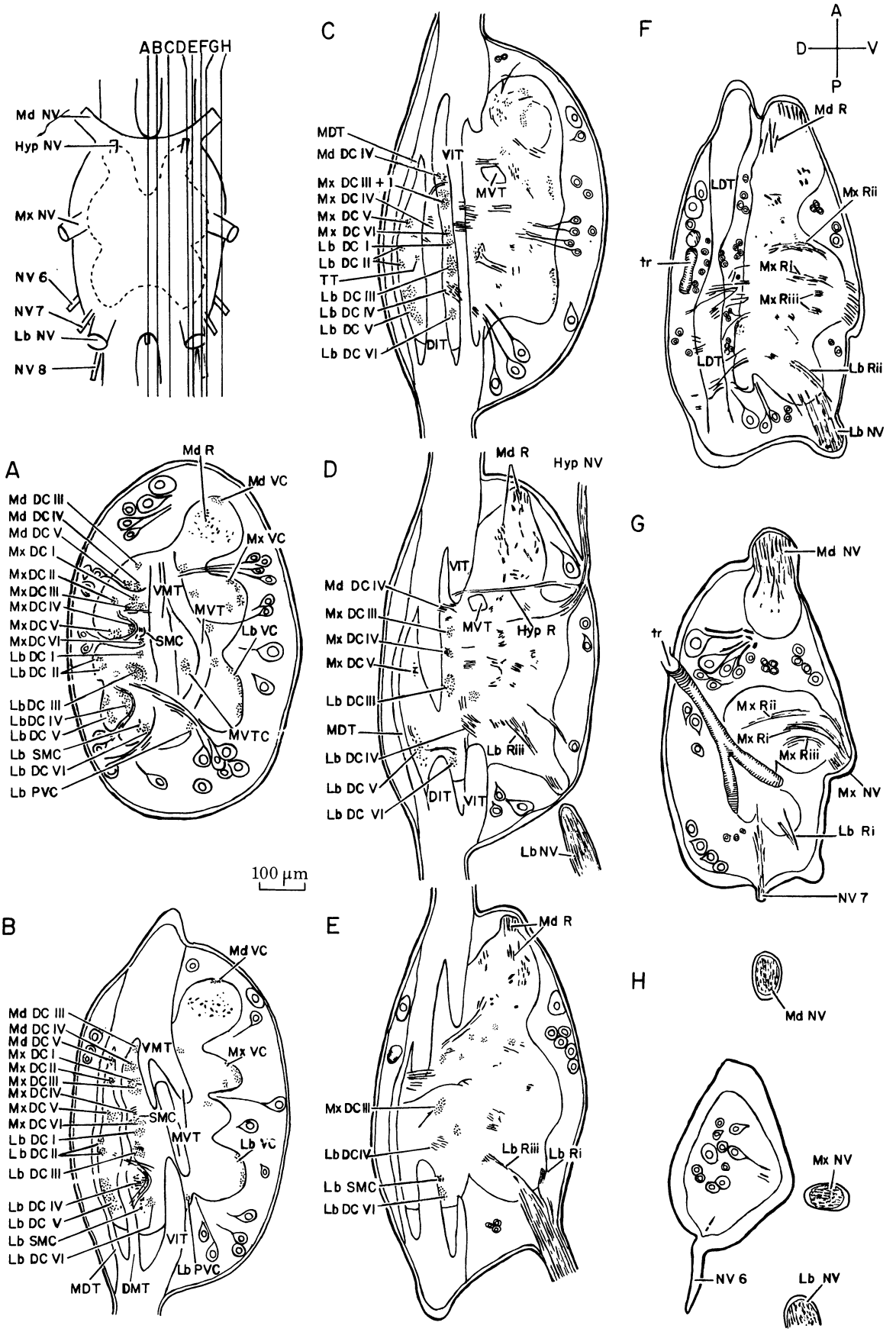



FIGURE 13. *Suboesophageal ganglion*, sagittal and parasagittal sections in planes A–H shown in ventral view of ganglion at top left. (*Schistocerca gregaria*.)




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

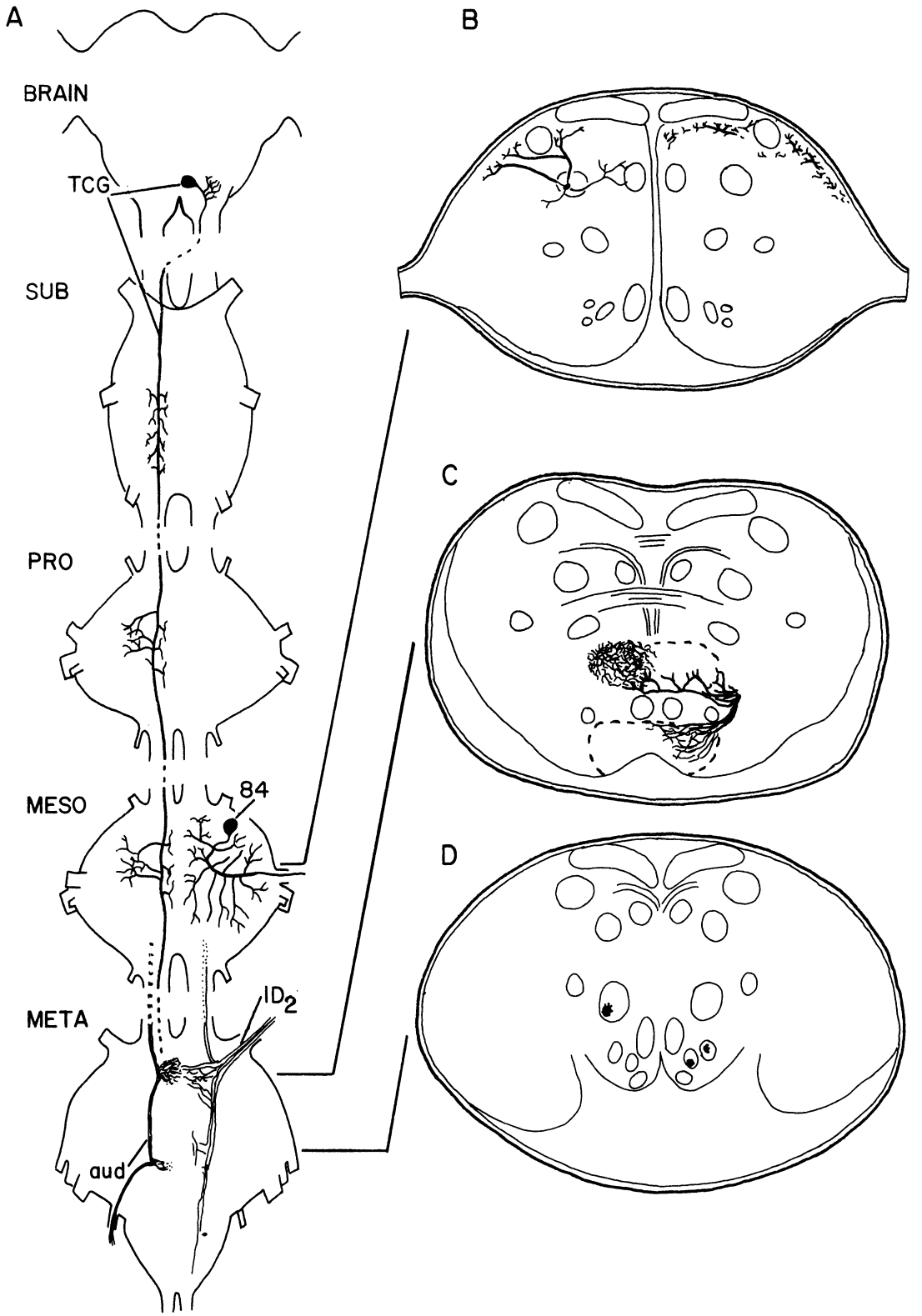



FIGURE 14. A, the relationship of the brain and the four ventral cord ganglia described in this paper. The course of the tritocerebral commissure giant interneuron (TCG) (Bacon & Tyrer 1978) through the ventral cord ganglia is shown, together with a flight motor neuron, a wing elevator (84) in the mesothoracic ganglion (Tyrer & Altman 1974), a group of wing hinge sensory neurons in the metathoracic ganglion ($1D_2$) (Tyrer


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

or behavioural. With the exception of the cephalopod nervous system (see Young 1971, 1979), few invertebrate nervous systems have been subjected to systematic anatomical analysis. There is minimal information about the organization of ganglia in preparations of molluscs such as *Aplysia*, *Tritonia* and *Helix*, for which there is a great body of neurophysiological and behavioural data. Similarly in leech and crustacean preparations knowledge of neurophysiological pathways far outstrips anatomical information.

The anatomical organization of the insect nervous system is better documented. A considerable body of information is available about specific areas of the brain (supraoesophageal ganglia) in numerous species, including locusts, grasshoppers and crickets (Jawlowski 1954; Satija 1958; Huber 1960; Goodman 1974; Williams 1975; Aubele & Klemm 1977; Ernst *et al.* 1977), cockroaches (Bretschneider 1914; Hanstrom 1928; Satija & Singla 1967; Guthrie & Tindall 1968; Boeckh *et al.* 1970; Frontali & Mancini 1970; Weiss 1974; Ernst *et al.* 1977) and various Lepidoptera (Pearson 1971), Hymenoptera (Vowles 1955) and Diptera (Power 1943, 1946; Boeckh *et al.* 1970; Groth 1971; Strausfeld 1976), and the optic neuropils have been well studied (Strausfeld 1970; Strausfeld & Blest 1970). Much insect neurophysiological work concerns the ventral cord ganglia, however, and about the anatomy of these less is known. Since the pioneering work of Zawarzin (1924) on *Aeschna* only a few accounts have been given, such as those by Pyle (1941) (*Ephestia*), Power (1948) (*Drosophila*), Pipa *et al.* (1959) and Guthrie & Tindall (1968) (cockroach), Guthrie (1961) (*Gerris*), Mill (1964) (*Aeschna*), Cheze (1967) and Seabrook (1968, 1970) (locusts) and Cloarec (1968) (*Mantis*). None deals with the locust suboesophageal and thoracic ganglia. In cockroach an attempt to provide further detail of the type required is being made in studies of the peripheral nerve roots of the mesothoracic ganglion (Gregory 1974*b*) and other aspects of the neuroanatomy (Gregory 1982).

The present account tries to present information on the basic structure of locust ganglia in a way easily accessible for physiological studies, rather than to describe individual neurons and their location. This method of presentation takes into account variations in ganglion topography and thus offers advantages over a system of arbitrary grid lines for locating structures, such as that used by, for example, Burrows & Hoyle (1973).

If individual neurons can be related to other structures in a ganglion further progress is possible in two ways. First, relationships can be established between intracellularly stained neurons in different preparations (see figure 14, and for example, Bacon & Tyrer 1979).

---

& Altman 1974) and the auditory neurons from the tympanum (aud) (Rehbein *et al.* 1974). These are drawn from cobalt fills.

B, a section through the mesothoracic ganglion (corresponding to figure 2) shows branches of the TCG in relation to the structures of the ganglion core on the left side, while the branching of the flight motor neuron is shown on the right. It is apparent that the branches of the TCG enter the same areas of neuropil as the flight motor neuron branches, and these regions could be examined for contacts between the two neurons.

C, a section through the metathoracic ganglion (corresponding to figure 8C) shows the projections from the auditory neurons on the left and projections from the $1D_2$ wing hinge sensory neurons on the right. Examination of these neurons in sections shows clearly that they occupy two quite distinct regions of neuropil, the auditory neurons projecting to the aRT and the $1D_2$ wing sensory neurons to the VAC. However, some branches of the $1D_2$ neurons extend into the aRT, suggesting functional association.

D, a section through the metathoracic ganglion (corresponding to figure 8F) showing that the auditory neurons and the wing hinge sensory run in different tracts.

Potentially the relationships of every neuron in these ganglia, once identified individually, could be analysed in this way.

Abbreviations not included in the general list: SUB, suboesophageal ganglion; PRO, prothoracic ganglion; MESO, mesothoracic ganglion; META, metathoracic ganglion.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Secondly, showing the location of the branches of a filled neuron in the context of the rest of the ganglion can suggest physiological experiments to explore other neurons close to it. The neuroanatomical framework can in this way be used for prediction as well as for interpretation. Furthermore, its use is not limited to intracellularly stained neurons but is equally applicable to Golgi, toluidine blue or osmium–ethyl gallate preparations, and also to the histochemical localization of neurotransmitters and receptor sites. Dopamine and serotonin distribution has already been defined in locust brain by fluorescence microscopy (Klemm & Axelson 1973) and the technique is now being applied to ventral nerve cord ganglia to map the positions of neurons containing these amines (Tyrer & Altman unpublished). Similarly, radioautographic and immunofluorescence methods for locating putative transmitters should add important data to the assemblage of anatomical and physiological information.

Once areas of neuropil have been clearly defined at the light microscope level, the higher resolution of the electron microscope can be used to examine synaptic detail. Easily recognizable landmarks should make it possible to identify accurately the regions being studied.

Anatomical information of the kind presented here is not difficult to obtain, and once available it can open up many lines of enquiry. An understanding of fundamental neuroanatomy is essential for rapid progress in all the related fields of invertebrate neurobiology. Without it many opportunities provided by the techniques of intracellular staining may be lost and many of the advantages offered by invertebrates in the study of nerve function neglected.

We thank Dr J. S. Altman and Dr C. M. Hackney for helpful discussion and Dr P. G. Mobbs for allowing us to see unpublished results. We wish to thank the Department of Biological Sciences, Portsmouth Polytechnic, for supplying some of the locusts used.

## References

Altman, J. S. & Kien, J. 1979 Suboesophageal neurons involved in head movements and feeding in locusts. *Proc. R. Soc. Lond.* B **205**, 209–227.

Altman, J. S. & Tyrer, N. M. 1974 Insect flight as a system for the study of the development of neuronal connections. In *Experimental analysis of insect behaviour* (ed. L. Barton Browne), pp. 159–179. Berlin: Springer-Verlag.

Altman, J. S. & Tyrer, N. M. 1977*a* The locust wing hinge stretch receptors. I. Primary sensory neurons with enormous central arborizations. *J. comp. Neurol.* **172**, 409–430.

Altman, J. S. & Tyrer, N. M. 1977*b* The locust wing hinge stretch receptors. II. Variation, alternative pathways and 'mistakes' in the central arborizations. *J. comp. Neurol.* **172**, 431–439.

Aubele, E. & Klemm, N. 1977 Origin, destination and mapping of tritocerebral neurons of locust. *Cell Tiss. Res.* **178**, 199–219.

Bacon, J. P. & Altman, J. S. 1977 A silver intensification method for cobalt-filled neurones in wholemount preparations. *Brain Res.* **138**, 359–363.

Bacon, J. & Tyrer, M. 1978 The tritocerebral commissure giant (TCG): a bimodal interneurone in the locust, *Schistocerca gregaria*. *J. comp. Physiol.* **126**, 317–325.

Bacon, J. & Tyrer, M. 1979 Wind interneurone input to flight motor neurones in the locust, *Schistocerca gregaria*. *Naturwissenschaften* **66**, 116.

Bentley, D. R. 1970 A topological map of the locust flight system motor neurons. *J. Insect Physiol.* **16**, 905–918.

Bodian, D. 1936 A new method for staining nerve fibres and nerve endings in mounted paraffin sections. *Anat. Rec.* **65**, 89–97.

Boeckh, J., Sandri, C. & Akert, K. 1970 Sensorische Eingange und synaptische Verbindungen im Zentralnervensystem von Insekten. Experimentelle Degeneration in der antennalen Sinnesbahn im Oberschlundganglion von Fliegen und Schaben. *Z. Zellforsch. mikrosk. Anat.* **103**, 429–446.

Bretschneider, F. 1914 Über die Gehirne der Kuchenschabe und des Mehklafers. *Jena. Z. Naturw.* **52**, 269–362.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Burrows, M. 1973*a* Physiological and morphological properties of the metathoracic common inhibitory neuron of the locust. *J. comp. Physiol.* **82**, 59–78.

Burrows, M. 1973*b* The morphology of an elevator and a depressor motoneuron of the hindwing of a locust. *J. comp. Physiol.* **83**, 165–178.

Burrows, M. 1975 Monosynaptic connexions between wing stretch receptors and flight motoneurones of the locust. *J. exp. Biol.* **62**, 189–219.

Burrows, M. 1980 The tracheal supply to the central nervous system of the locust. *Proc. R. Soc. Lond.* B **207**, 63–78.

Burrows, M. & Hoyle, G. 1973 Neural mechanisms underlying behavior in the locust *Schistocerca gregaria*. III. Topography of limb motoneurons in the metathoracic ganglion. *J. Neurobiol.* **4**, 167–186.

Burrows, M. & Siegler, M. V. S. 1976 Transmission without spikes between locust interneurones and motoneurones. *Nature, Lond.* **262**, 222–224.

Campbell, J. I. 1961 The anatomy of the nervous system of the mesothorax of *Locusta migratoria migratorioides* R. & F. *Proc. Zool. Soc. Lond.* **137**, 403–432.

Cheze, G. 1967 Étude structurale de la dernière masse nerveuse abdominale chez *Locusta migratoria migratorioides* (R. et F.). *Bull. Soc. Zool. Fr.* **92**, 749–757.

Cloarec, A. 1968 Étude structurale de la dernière masse ganglionnaire de la chaîne nerveuse abdominale de *Mantis religiosa* L. *Bull. Soc. Zool. Fr.* **93**, 331–346.

Ernst, K.-D., Boeckh, J. & Boeckh, V. 1977 A neuroanatomical study on the organization of the central antennal pathways in insects. II. Deutocerebral connections in *Locusta migratoria* and *Periplaneta americana*. *Cell Tiss. Res.* **176**, 285–308.

Fentress, J. C. (ed.) 1976 *Simpler networks and behaviour*. Sunderland, Massachusetts: Sinauer Associates.

Frontali, N. & Mancini, G. 1970 Studies on the neuronal organization of cockroach corpora pedunculata. *J. Insect Physiol.* **16**, 2293–2301.

Goodman, C. 1974 Anatomy of locust ocellar interneurons: constancy and variability. *J. comp. Physiol.* **95**, 185–201.

Gray, P. 1954 *The microtomist's formulary and guide*. London: Constable.

Gregory, G. E. 1970 Silver staining of insect central nervous systems by the Bodian protargol method. *Acta zool., Stockh.* **51**, 169–178.

Gregory, G. E. 1974*a* Effect of sodium sulfite purity on colour of Bodian silver staining of insect central nervous systems. *Stain Technol.* **49**, 401–405.

Gregory, G. E. 1974*b* Neuroanatomy of the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.). I. The roots of the peripheral nerves. *Phil. Trans. R. Soc. Lond.* B **267**, 421–465.

Gregory, G. E. 1980*a* Alcoholic Bouin fixation of insect nervous systems for Bodian silver staining. II. Modified solutions. *Stain Technol.* **55**, 151–160.

Gregory, G. E. 1980*b* The Bodian protargol technique. In *Neuroanatomical techniques. Experimental entomology*, vol. 3 (ed. N. J. Strausfeld & T. A. Miller), pp. 75–95. New York: Springer-Verlag.

Gregory, G. E. 1982 Neuroanatomy of the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.). II. Median neuron cell body groups. (In preparation.)

Gregory, G. E., Greenway, A. R. & Lord, K. A. 1980 Alcoholic Bouin fixation of insect nervous systems for Bodian silver staining. I. Composition of 'aged' fixative. *Stain Technol.* **55**, 143–149.

Groth, U. 1971 Vergleichende Untersuchungen über die Topographie und Histologie des Gehirns der Dipteren. *Zool. Jb.* (*Anat.*) **88**, 203–319.

Guthrie, D. M. 1961 The anatomy of the nervous system in the genus *Gerris* (Hemiptera–Heteroptera). *Phil. Trans. R. Soc. Lond.* B **244**, 65–102.

Guthrie, D. M. & Tindall, A. R. 1968 *The biology of the cockroach*. London: Edward Arnold.

Hanstrom, B. 1928 *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere unter Beruchsicktigung seiner Funktion*. Berlin: Springer-Verlag.

Hoyle, G. 1953 Potassium ions and insect nerve muscle. *J. exp. Biol.* **30**, 121–135.

Hoyle, G. 1970 Cellular mechanisms underlying behaviour – neuroethology. *Adv. Insect Physiol.* **7**, 349–444.

Hoyle, G., Dagan, D., Moberly, B. & Colquhoun, W. 1974 Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscle. *J. exp. Zool.* **187**, 159–165.

Huber, F. 1960 Untersuchungen über die Funktion des Zentralnervensystems und insbesondere des Gehirnes bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. vergl. Physiol.* **44**, 60–132.

Jawlowski, H. 1954 Über die Struktur des Gehirnes bei *Saltatoria*. *Annls Univ. Mariae Curie-Sklodowska* C **8**, 403–434.

Klemm, N. & Axelsson, S. 1973 Determination of dopamine, noradrenaline, 5-hydroxytryptamine in the cerebral ganglion of the desert locust, *Schistocerca gregaria* Forsk. (Insecta, Orthoptera). *Brain Res.* **57**, 289–298.

Kravitz, E. A., Stretton, A. O. W., Alvarez, J. & Furshpan, E. J. 1968 Determination of neuronal geometry using an intracellular dye injection technique. *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**, 749.

Lane, N. J. 1974 The organization of insect nervous systems. In *Insect neurobiology* (ed. J. E. Treherne), pp. 1–71. Amsterdam: North-Holland Publishing Co.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Mill, P. J. 1964 The structure of the abdominal nervous system of aeschnid nymphs. *J. comp. Neurol.* **122**, 157–171.

O'Shea, M., Rowell, C. H. F. & Williams, J. L. D. 1974 The anatomy of a locust visual interneurone; the descending contralateral movement detector. *J. exp. Biol.* **60**, 1–12.

Pearson, L. 1971 The corpora pedunculata of *Sphinx ligustri* L. and other Lepidoptera: an anatomical study. *Phil. Trans. R. Soc. Lond.* B **259**, 477–516.

Pipa, R. L. & Cook, E. F. 1959 Studies on the hexapod nervous system. I. The peripheral distribution of the thoracic nerves of the adult cockroach. *Periplaneta americana*. *Ann. ent. Soc. Am.* **52**, 695–710.

Pipa, R. L., Cook, E. F. & Richards, A. G. 1959 Studies on the hexapod nervous system. II. The histology of the thoracic ganglia of the adult cockroach, *Periplaneta americana* (L.). *J. comp. Neurol.* **113**, 401–433.

Pitman, R. M., Tweedle, C. D. & Cohen, M. J. 1972 Branching of central neurons: intracellular cobalt injection for light and electron microscopy. *Science, Wash.* **176**, 412–414.

Power, M. E. 1943 The brain of *Drosophila melanogaster*. *J. Morph.* **72**, 517–559.

Power, M. E. 1946 The antennal centers and their connections within the brain of *Drosophila melanogaster*. *J. comp. Neurol.* **85**, 485–517.

Power, M. E. 1948 The thoracico-abdominal nervous system of an adult insect, *Drosophila melanogaster*. *J. comp. Neurol.* **88**, 347–409.

Pringle, J. W. S. 1939 The motor mechanism of the insect leg. *J. exp. Biol.* **16**, 220–231.

Pyle, R. W. 1941 The fibre tracts of the fused thoracic ganglia of the adult *Ephestia kuehniella* Zeller (Lepodoptera: Pyralidae). *Psyche* **48**, 123–128.

Rehbein, H., Kalmring, K. & Romer, H. 1974 Structure and functions of acoustic neurons in the thoracic ventral nerve cord of *Locusta migratoria* (Acrididae). *J. comp. Physiol.* **95**, 263–280.

Rowell, C. H. F. 1963 A general method for silvering invertebrate central nervous systems. *Q. Jl microsc. Sci.* **104**, 81–87.

Satija, R. C. 1958 A histological and experimental study of nervous pathways in the brain and thoracic nerve cord of *Locusta migratoria migratorioides* (R. & F.). *Res. Bull. Panjab Univ. Sci.* (*Zool.*) **137**, 13–32.

Satija, R. C. & Singla, O. P. 1967 Structure of the brain of *Periplaneta americana* L. (Dictyoptera). *Res. Bull. Panjab Univ. Sci.* (n.s.) **18**, 297–302.

Seabrook, W. D. 1968 The structure of a pregenital abdominal ganglion of the desert locust *Schistocerca gregaria* (Forskål). *Can. J. Zool.* **46**, 965–980.

Seabrook, W. D. 1970 The structure of the terminal ganglionic mass of the locust, *Schistocerca gregaria* (Forskål). *J. comp. Neurol.* **138**, 63–85.

Stewart, W. W. 1978 Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalimide tracer. *Cell* **14**, 741–759.

Strausfeld, N. J. 1970 Golgi studies on insects. II. The optic lobes of Diptera. *Phil. Trans. R. Soc. Lond.* B **258**, 135–223.

Strausfeld, N. J. 1976 *Atlas of an insect brain*. Berlin: Springer-Verlag.

Strausfeld, N. J. & Blest, A. D. 1970 Golgi studies on insects. I. The optic lobes of Lepidoptera. *Phil. Trans. R. Soc. Lond.* B **258**, 81–134.

Stretton, A. O. W. & Kravitz, E. A. 1968 Neuronal geometry: determination with a technique of intracellular dye injection. *Science, Wash.* **162**, 132–134.

Tyrer, N. M. & Altman, J. S. 1974 Motor and sensory flight neurones in a locust demonstrated using cobalt chloride. *J. comp. Neurol.* **157**, 117–138.

Tyrer, N. M., Bacon, J. P. & Davies, C. A. 1979 Sensory projections from the wind-sensitive head hairs of the locust *Schistocerca gregaria*. Distribution in the central nervous system. *Cell Tiss. Res.* **203**, 79–92.

Tyrer, N. M. & Bell, E. M. 1974 The intensification of cobalt-filled neurone profiles using a modification of Timm's sulphide-silver method. *Brain Res.* **73**, 151–155.

Usherwood, P. N. R. 1968 A critical study of the evidence for peripheral inhibitory axons in insects. *J. exp. Biol.* **49**, 201–222.

Vowles, D. M. 1955 The structure and connexions of the corpora pedunculata in bees and ants. *Q. Jl microsc. Sci.* **96**, 239–255.

Weiss, M. J. 1974 Neuronal connections and the function of the corpora pedunculata in the brain of the American cockroach, *Periplaneta americana* (L.). *J. Morph.* **142**, 21–69.

Wigglesworth, V. B. 1957 The use of osmium in the fixation and staining of tissues. *Proc. R. Soc. Lond.* B **147** 185–199.

Willey, R. B. 1961 The morphology of the stomodeal nervous system in *Periplaneta americana* (L.) and other Blattaria. *J. Morph.* **108**, 219–261.

Williams, J. L. D. 1975 Anatomical studies of the insect central nervous system: a ground-plan of the midbrain and an introduction to the central complex in the locust, *Schistocera gregaria* (Orthoptera). *J. Zool.* **176**, 67–86.

Young, J. Z. 1971 *The anatomy of the nervous system of Octopus vulgaris*. Oxford: Clarendon Press.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

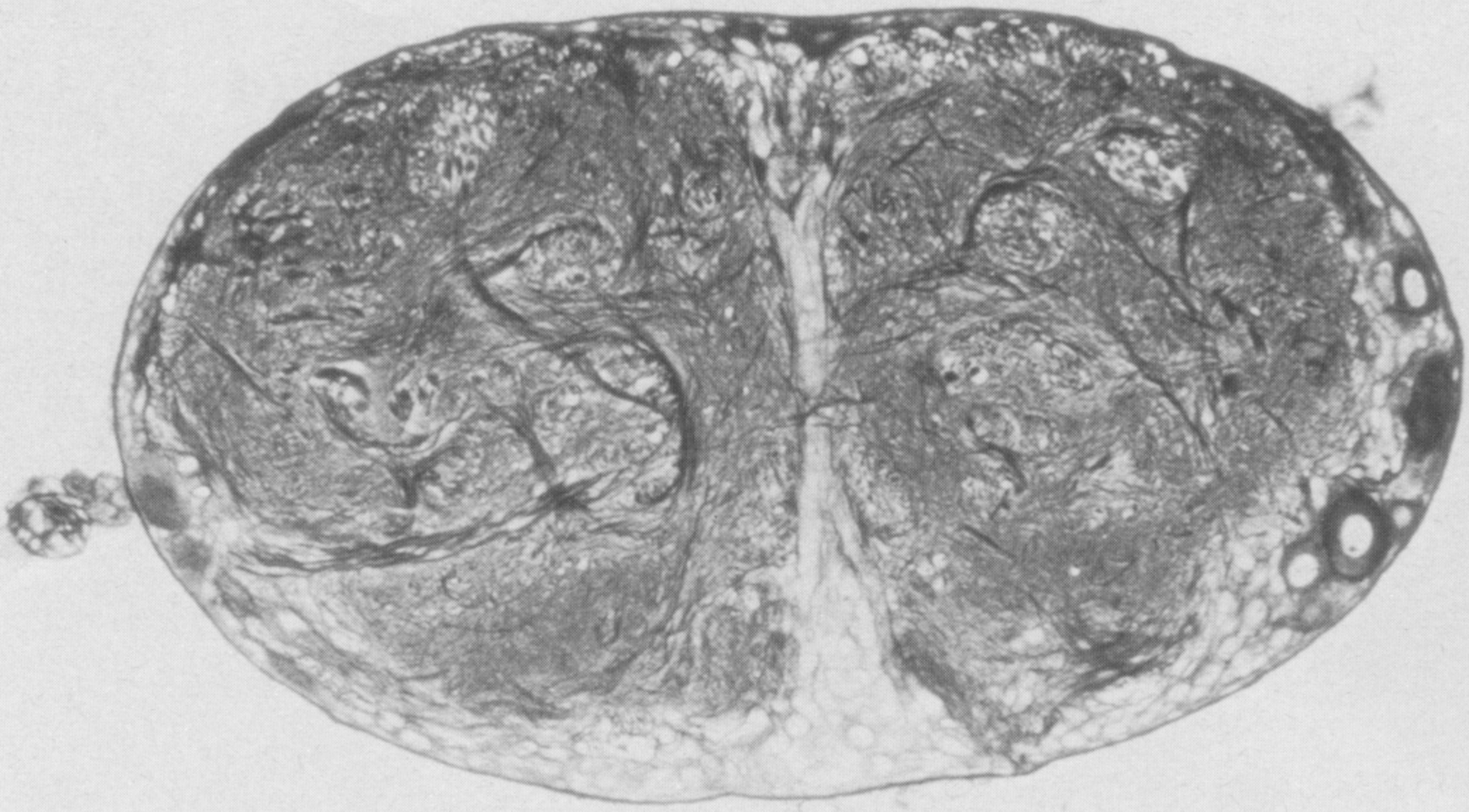
Young, J. Z. 1979 The nervous system of *Loligo*. V. The vertical lobe complex. *Phil. Trans. R. Soc. Lond.* B **285**, 311–354.

Zawarzin, A. 1924 Zur Morphologie der Nervenzentren. Das Bauchmark der Insekten. Ein Beitrag zur vergleichenden Histologie (Histologische Studien über Insekten VI). *Z. wiss. Zool.* **122**, 323–424.



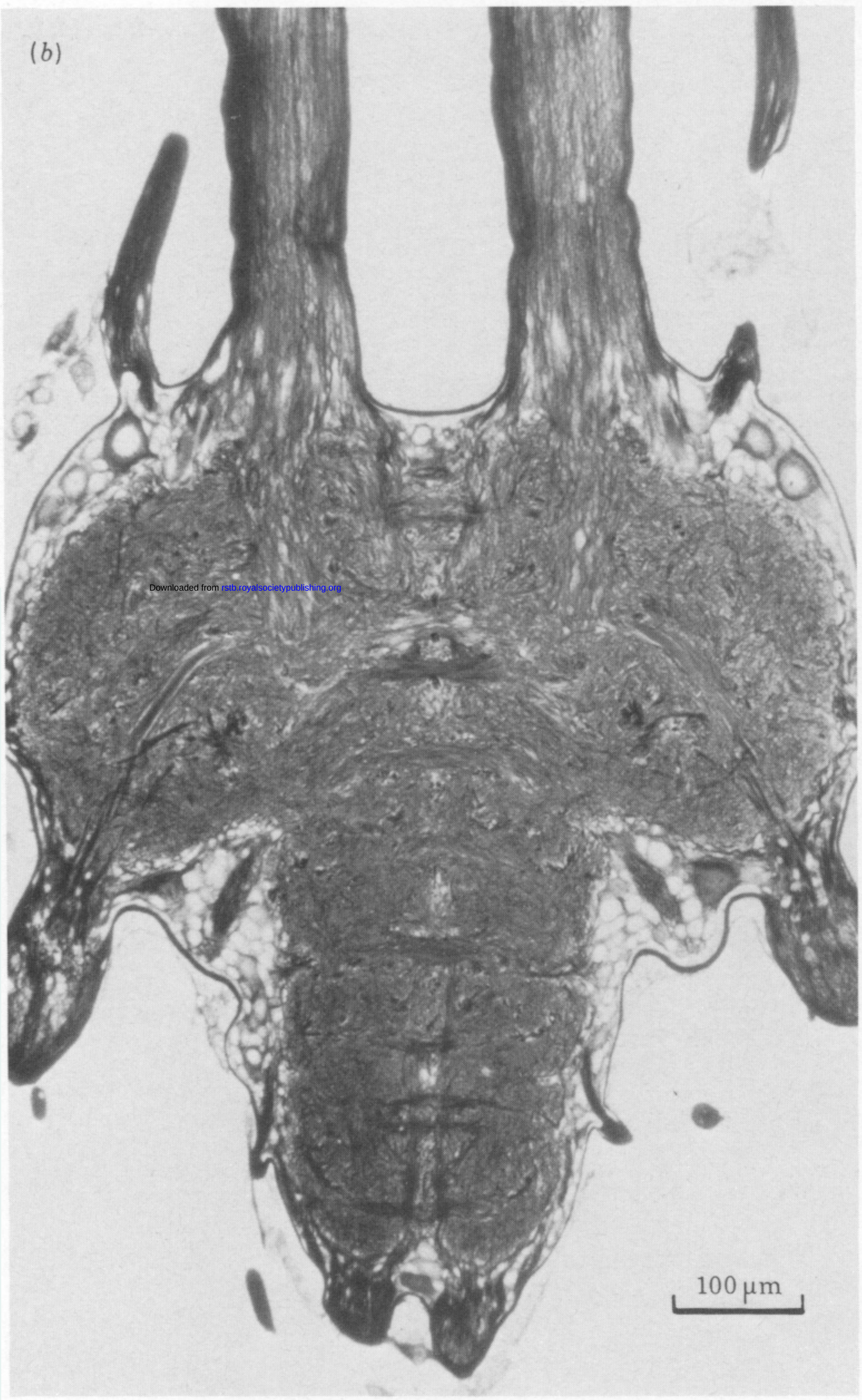
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(a)



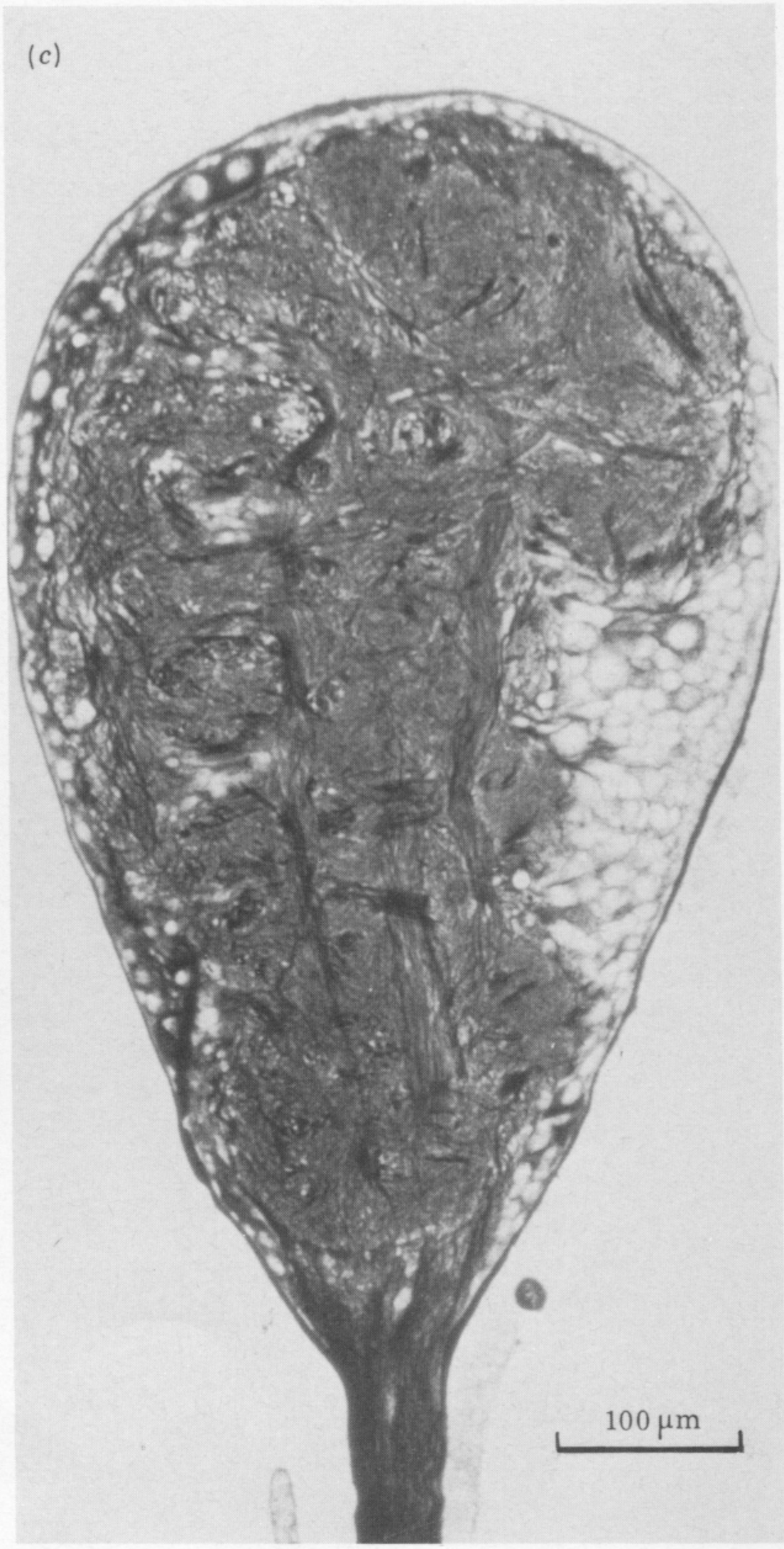
100 μm

(b)



100 μm

(c)



100 μm





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PLATE 1. For description see opposite.