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An Atlas of the Thoracic Ganglia in the Stick Insect, Carausius morosus

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An atlas of the thoracic ganglia in the stick insect, *Carausius morosus*

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SUMMARY

The present report describes the neuroanatomy of the three thoracic ganglia in the stick insect, *Carausius morosus*, the subject of numerous behavioural and neurobiological studies. The structure of the ganglia is summarized in an atlas of the major features. The results are compared with published descriptions of other insects and arthropods. Numerous similarities with locusts encourage the use of a common nomenclature even where minor differences make homology uncertain pending detailed investigation. Five out of the nine longitudinal tracts described in locusts can be readily identified in the stick insect. Three major tracts (LDT, DIT, VIT) and two smaller tracts (MDT, DMT) are compact and well defined. The VMT and MVT are also prominent but these two tracts are not clearly separated except near the rostral margin of the neuropile. An eighth tract, the VLT, is much less distinct: it is represented by scattered fibres in neuropile lateral to the DIT. The iLVT and oLVT, the two parts of the ninth tract, are quite inconspicuous: in some, but not all, preparations they can be identified as two thin bands running along the ventral and ventrolateral margins of the ganglion. As in locusts, six dorsal commissures (DCI–DCVI) and five ventral commissures (VCI, vVCII, dVCII, SMC, PVC) connecting the left and right hemiganglia have been named although the two most dorsal commissures, DCII and DCIV, are often subdivided. The VCII is retained as a single unit with dorsal and ventral parts. Of the dorsal-ventral tracts only the transverse tract (TT) and the circle tract (CT) are well-defined. Roots of lateral nerves are left unnamed pending more detailed study but several conspicuous branches are included in the drawings as guides to orientation in the lateral neuropile. The ventral association centre (VAC) and several other neuropile divisions are described. Pro- and mesothoracic ganglia derive from single neuromeres. The metathoracic ganglion results from the fusion of the third thoracic and the first abdominal neuromeres: each part contains its own set of commissures and dorsoventral tracts. The results underline the qualitative similarities of the thoracic ganglia in insects; they provide a basis for more precise descriptions of identified neurons and functional specialization within the ganglia of the stick insect.‡

1. INTRODUCTION

A necessary prerequisite for the study of neural integrative mechanisms is a description of the basic structure of the central nervous system. As physiological and anatomical methods for studying the nervous system become more sophisticated it is increasingly important to establish a basic anatomical framework to which specific results can be related. For the most frequently studied vertebrates, this need is filled by a standard neuroanatomical atlas. Such an atlas provides a set of landmarks defining regions of structural specialization. In turn, as structurally identified areas receive functional labels, an atlas can be used as a guide for further physiological, ultrastructural, and immunohistological investigations. An atlas also provides a necessary basis for comparison of different species.

The routine use of a standard atlas is not well-established in invertebrate neurobiology. Comprehensive neuroanatomical descriptions comparable to those common for vertebrate laboratory animals are largely restricted to the octopus (Young 1971) and other cephalopods (Young 1979). However, the cephalopods have been less commonly used for neurobiological studies than other molluscs, crustaceans and insects. With a few exceptions, anatomical descriptions of more frequently studied invertebrates are lacking or incomplete, although early neuroanatomists did provide detailed descriptions of neuron morphology and organization. More recently, Strausfeld (1976) provided an atlas of the brain of a fly and reviewed the older literature. Specific areas of the brain, in particular the

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‡ See page 119 for a complete list of abbreviations.

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mushroom bodies (Schürmann 1973; Mobbs 1982), the olfactory glomeruli (Ernst *et al.* 1977; Masson 1977; Schneiderman & Hildebrand 1985; Hildebrand & Montague 1986) and the optic lobes (Strausfeld 1970; Strausfeld & Blest 1970) have received intensive study.

The thoracic ganglia initially received less attention although they play a central role in many behaviours which have been extensively studied both behaviourally and physiologically. Whereas Bullock & Horridge (1965) in their encyclopedic review could draw upon abundant material concerning the structure of the brain in many different invertebrates, they found only scattered material on the thoracic ganglia. An early, gross anatomical description of the thoracic and suboesophageal ganglia in insects was provided by Pipa *et al.* (1959). In their description of the major longitudinal tracts and transverse commissures of the cockroach, they established a nomenclature that has been largely followed by later authors. Gregory provided more details for the mesothoracic ganglion of cockroaches and introduced nomenclatures for the roots of lateral nerves (Gregory 1974) and for groups of neurons (Gregory 1984). The gross neuroanatomy of locusts, a second orthopteromorph group commonly used in neurobiological research, remained undescribed until Tyrer & Gregory's 1982 publication.

The pace of gross neuroanatomical studies in invertebrates has accelerated in recent years. The ultimate goal of describing the complete complement of neurons and all their connections has been attained for a simple organism, the nematode *Caenorhabditis* (White *et al.* 1986). Skinner (1985*a*, *b*) provided a detailed account of the fourth abdominal ganglion in the crayfish, and Kondoh & Hisada (1986) added a description of the terminal ganglion. Babu & Barth (1984) studied the central nervous system of the spider, *Cupiennius salei*; Rehder (1988) described the prothoracic and suboesophageal ganglia of the honeybee.

A description of the gross neuroanatomy is an important aid because it creates a framework for organizing detailed anatomical and histochemical results. In the absence of such a description, neuroanatomical descriptions of individual neurons relied on coordinate grids (see, for example: Burrows & Hoyle (1973); Graham & Wendler (1981)) or simply showed drawings of the neuron within an empty outline of the ganglion (for a list of references, see Tyrer & Gregory (1982)). When an atlas is available, descriptions of identified neurons can be made more precise by including histological sections showing relevant landmarks (see, for example, Siegler & Burrows (1984); Edwards & Reddy (1986); Pflüger *et al.* (1986)). In parallel with these studies of identified neurons, a variety of immunohistological techniques have been developed to reveal regional differences within ganglia (Watson 1986; Bernard & Thomas 1988; review Nässel 1987).

Nevertheless, gross neuroanatomy remains relatively neglected in many invertebrates commonly used in physiological studies. The stick insect, *Carausius morosus*, is one such animal: both reflex physiology and locomotion have been intensively studied (reviews Bässler 1983; Graham 1985). A variety of interleg coordinating mechanisms and intraleg reflexes mediated primarily by the thoracic ganglia have been identified (Cruse 1990).

Marquardt's (1940) detailed description of the thoracic muscles and peripheral nerves in *Carausius* provided the necessary anatomical basis for the physiological investigation of peripheral elements in these reflex and motor mechanisms. As the focus of attention shifts to the neural mechanisms for integrating sensory information into motor patterns, the absence of a general neuroanatomical description of the thoracic ganglia is a major handicap. As a first step towards filling this need, the positions of the cell bodies and the primary neurites of motoneurons were mapped (Storrer *et al.* 1986), but this account was again restricted to plan drawings in a ganglion outline. Some information on the ultrastructure of the thoracic ganglia is also available. Lane (1974) published several electron microscope (EM) photos illustrating general features of the neuropile and synaptic structure in the stick insect. The neural sheath has received special attention in connection with the aberrant haemolymph constitution of this phytophagous insect (Lane 1974; Huddart 1971). However, the gross neuroanatomy of the central nervous system (CNS) has been neglected.

The goal of the present study is to describe the gross anatomical structure of the thoracic nervous system in the stick insect and to establish a standard terminology as a basis for subsequent physiological and neuroanatomical studies. The emphasis is on a pictorial presentation with a minimum of textual description. In a companion paper (Schmitz *et al.*, manuscript in preparation), this framework is used in describing the afferent projections from leg proprioceptors known to be important in leg reflexes and locomotion.

MATERIALS AND METHODS

Adult female stick insects, *Carausius morosus*, were taken from laboratory colonies in Bielefeld and Konstanz. The thoracic ganglia were stained according to the osmium/ethyl-gallate procedure of Wigglesworth (1957) as modified by Wohlers & Huber (1985). Animals were opened dorsally and the gut was removed to expose the ventral nerve cord. The fatty sheath surrounding each ganglion was removed to facilitate orientation of the ganglion during embedding. The nerve cord was fixed *in situ* overnight (18 h) in a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.2) at 4 °C. Then the individual thoracic ganglia were dissected free and rinsed in PB for 30 min. They were post-fixed in 1% OsO_4 in PB for 2 h, rinsed thoroughly in PB for 30 min, and stained with saturated ethyl-gallate solution (ethyl-3,4,5-trihydroxybenzoate, Merck) in PB for 2.5 h. After dehydration, the ganglia were embedded in soft Durcupan (Fluka). Serial sections were cut at either 10 or 16 μm in each of the three standard planes. Nine series were available for the mesothoracic ganglion, three for the prothoracic, and four for the metathoracic ganglion. Representative sections were drawn under ×250 magnification using a camera lucida.

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TERMINOLOGY

The numbering system applied to the lateral nerves differs in the cockroach (Pringle (1939), modified by Pipa & Cook (1959), summarized in Pipa & Delcomyn (1981)), the locust (Campbell 1961; Tyrer & Gregory 1982), and the stick insect (Marquardt 1940), three commonly studied orthopteromorph insects (see Bullock & Horridge 1965). As a further complication, the order in which the lateral nerves arise in the stick insect has led recent authors (see, for example, Bässler (1983)) to reverse the original designation of lateral nerves 3 and 4 (Marquardt 1940). Here we will continue this revised usage, so that the lateral nerve supplying sensory organs of the coxa and continuing to fuse with nervus cruris, the main leg nerve, is labelled n13.

The designation of structures in the ganglia follows the system originally applied by Pipa *et al.* (1959) to the cockroach and then extended with some modification to the locust (Tyrer & Gregory 1982; Pflüger *et al.* 1988) and to the abdominal ganglia of crayfish (Skinner 1985*a*, *b*). Structures are labelled according to their position and relation to other structures in the ganglion. For the sake of uniformity, the terminology used in the locust is followed as closely as possible, although differences in some structures make their homology uncertain.

RESULTS

(*a*) *General structure of the thoracic ganglia*

The ventral nerve cord consists of separate segmental ganglia linked by connectives. Each thoracic segment contains a bilaterally symmetrical ganglion located near the point at which the legs join the thorax. The prothoracic and mesothoracic ganglia derive from single neuromeres. The metathoracic ganglion arises from the fusion of the third thoracic neuromere and the first abdominal neuromere. The second abdominal neuromere forms the first free abdominal ganglion; it is connected to the metathoracic ganglion by short connectives. In some animals the fat body sheath around the ventral nerve cord does not separately enclose these connectives, giving the impression that the second abdominal ganglion is united with the metathoracic ganglion, but actual fusion does not occur.

The tissue structure of the ganglia (figure 1) follows the general arthropod plan (review Lane 1974). The core is a matrix of neural processes and associated glial processes. This matrix is designated as neuropile in the broad sense. The neural cell bodies (somata) are clustered around the neuropile. The largest somata have diameters of up to 65 μm. The major groups form caps on the ventrorostral and ventrocaudal aspects of the ganglion. Smaller groups of somata are located

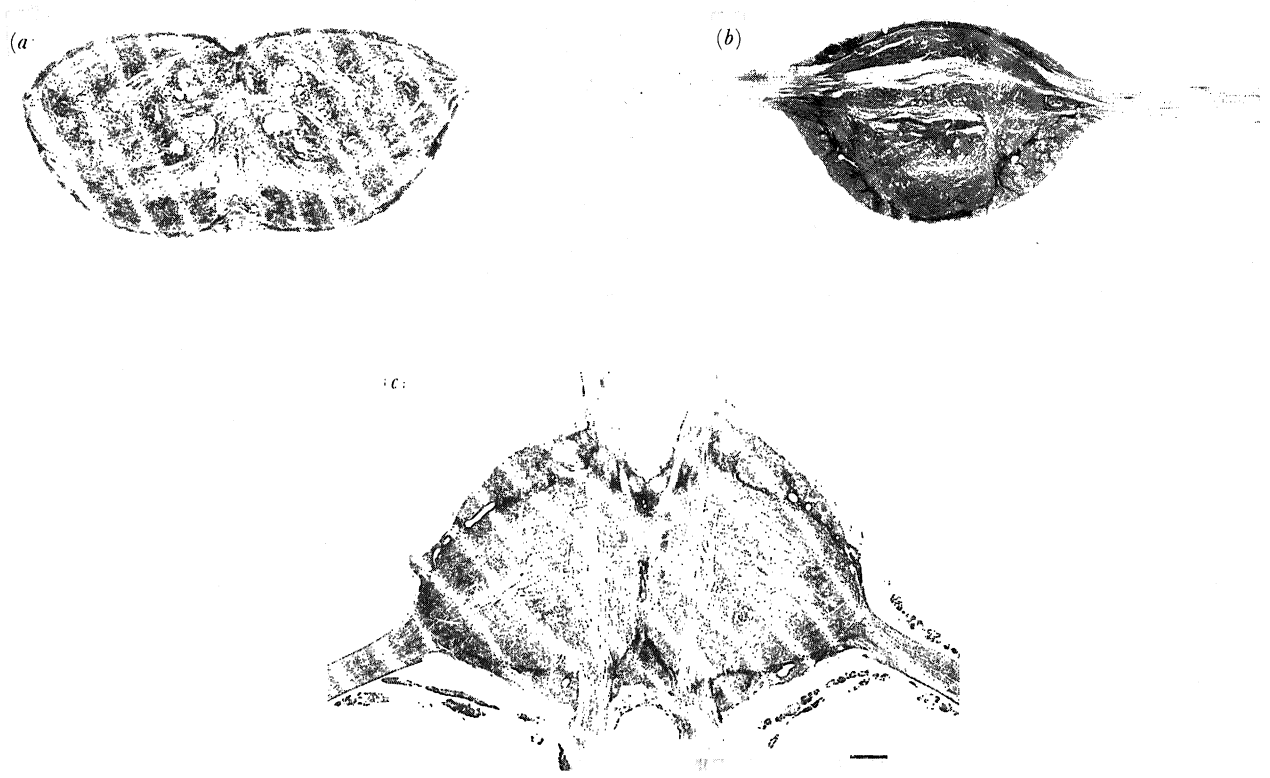

Figure 1. Representative photographs of sections from a mesothoracic ganglion stained with ethyl-gallate. The plane of the sections and the corresponding drawings are as follows: (*a*) transverse (figure 2*f*); (*b*) sagittal (figure 3*d*), and (*c*) horizontal (figure 4*e*). The length of the bar in this and the following figures corresponds to 100 μm.

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ventrally and dorsally along the midline. The centre of the dorsal and ventral surfaces of each hemiganglion is mostly free of somata. The entire ganglion is enclosed in a thin cellular layer, the perineurium, and bounded by a connective tissue membrane, the neurilemma (Lane 1974; see Gregory (1974) for a discussion of variant usages of these terms). Located outside the neurilemma is the neural fat body sheath. In the stick insect this sheath consists of a loosely attached, continuous layer of fat body cells (Lane 1974).

Structure is imparted to the neuropile by conspicuous bundles of nerve fibres (figure 1). These include longitudinal tracts, transverse commissures, dorsoventral tracts and oblique tracts. The longitudinal tracts are formed by fibres in the connectives as they traverse the ganglion. In the thorax of the stick insect, each connective contains about 1100 countable fibres with diameters larger than 0.5 μm. In passing through the ganglion, these fibres diverge into discrete bundles which maintain their relative dorsoventral and mediolateral positions. The commissures contain fibres crossing from one hemiganglion to the other. Prominent dorsoventral and oblique fibre bundles include groups of primary neurites ascending from somata at the margin of the ganglion and the roots of peripheral nerves. Besides these coherent fibre bundles, the neuropile contains areas in which the neural processes do not share a common orientation (figure 1). Such areas are referred to as neuropile in the narrow sense. These neuropile areas can be divided on the basis of their general appearance into areas of fine or coarse texture. The former contain mostly small dendrites and fine terminal processes whereas the coarse appearance of the latter is imparted by fibres of larger diameter. In the following sections these structural features will be described for each thoracic ganglion beginning with the mesothoracic ganglion.

(*b*) *Mesothoracic ganglion*

The basic anatomical pattern is most easily illustrated by using the mesothoracic ganglion because this ganglion is derived from a single neuromere and the lateral nerves are evenly arranged at the margin of the ganglion. The most prominent features in the neuropile are the longitudinal tracts and the commissures.

Longitudinal tracts

As in the locust (Tyrer & Gregory 1982) and cockroach (Gregory 1974) each connective divides to form nine longitudinal tracts passing through each thoracic hemiganglion. Their arrangement can be best seen in transverse sections (figure 2). The tracts are most easily introduced in terms of five dorsoventral levels defined by the commissures and a prominent ventral neuropile, the VAC.

The most dorsal level consists of the two dorsal tracts: the lateral dorsal tract (LDT) and the medial dorsal tract (MDT). These run along the dorsal margin of the ganglion and pass over all commissures. The LDT forms a well-defined, compact bundle almost immediately upon entry of the rostral connective into the ganglion. It contains a cluster of *ca.* 10 large fibres with diameters of up to 10 μm at its lateral margin and smaller fibres medially. The MDT is slower to separate from fibres destined for the DIT, DMT and VLT. Compared to the LDT, the MDT is a thinner, more diffuse band and the fibres are generally smaller in diameter. Several isolated fibres medial to the compact LDT have been included in the MDT. These two tracts lie adjacent to one another throughout much of their course through the ganglion.

The second dorsoventral level consists of two tracts, the dorsal medial tract (DMT) and the dorsal intermediate tract (DIT), which pass between the transverse commissures DCII and DCIII and then between DCV and DCIV (figure 2*f*, *h*). The DMT is a small, relatively compact bundle of medium-sized fibres located near the midline. The DIT, in contrast, is one of the largest and more prominent bundles in the ganglion, equalled only by the VIT and the VMT–MVT complex. The DIT is subdivided over much of its course by thin transverse fibre bundles. One subgroup lies dorsomedial and another lies ventrolateral to the main bundle. Because the separation is often minimal, particularly for the ventrolateral subgroup, it is drawn in only some of the figures. The DIT includes 20–25 large fibres with diameters exceeding 4 μm. The two largest, which lie on the ventrolateral margin, have diameters of about 20 μm.

The third dorsoventral level separates the dorsal and the ventral commissures at the midline. This horizontal plane contains two tracts. The ventral intermediate tract, VIT, is a large compact bundle similar to the DIT. It includes *ca.* 20 large fibres with diameters of 4–20 μm. Like the DIT, the VIT occasionally subdivides. In particular, several of the largest fibres may split off from the ventrolateral margin to form a separate bundle. The second tract in this level, the ventral lateral tract (VLT), is the most lateral tract within the neuropile of the thoracic ganglia. In stick insects, this tract is a poorly defined collection of scattered fibres; it includes 5–7 medium-sized fibres with diameters of up to 5 μm. The location of the VLT is indicated in the drawings as a dotted line to indicate scattered fibres passing through neuropile. The VLT passes above the descending arm of the DCIII (figure 2*f*) but it assumes a ventral position near the VIT in the posterior part of the ganglion (figure 2,*j*) and therefore is included here with the VIT in the third level.

Midway through the ganglion another longitudinal bundle appears at the midline below the DMT in an area of course-grained neuropile which is part of mcN (figure 2*f*, *g*). These scattered fibres are apparently derived from DCI and DCII and possibly from the TT. They coalesce to form fairly prominent bundles in passing the midline trachea (mtr) and then disperse between the levels of DCIV and DCVI. Hence they do not represent a longitudinal through tract and have not been included in the figures.

The fourth dorsoventral level contains tracts passing below the ventral commissures dVCII and SMC. The

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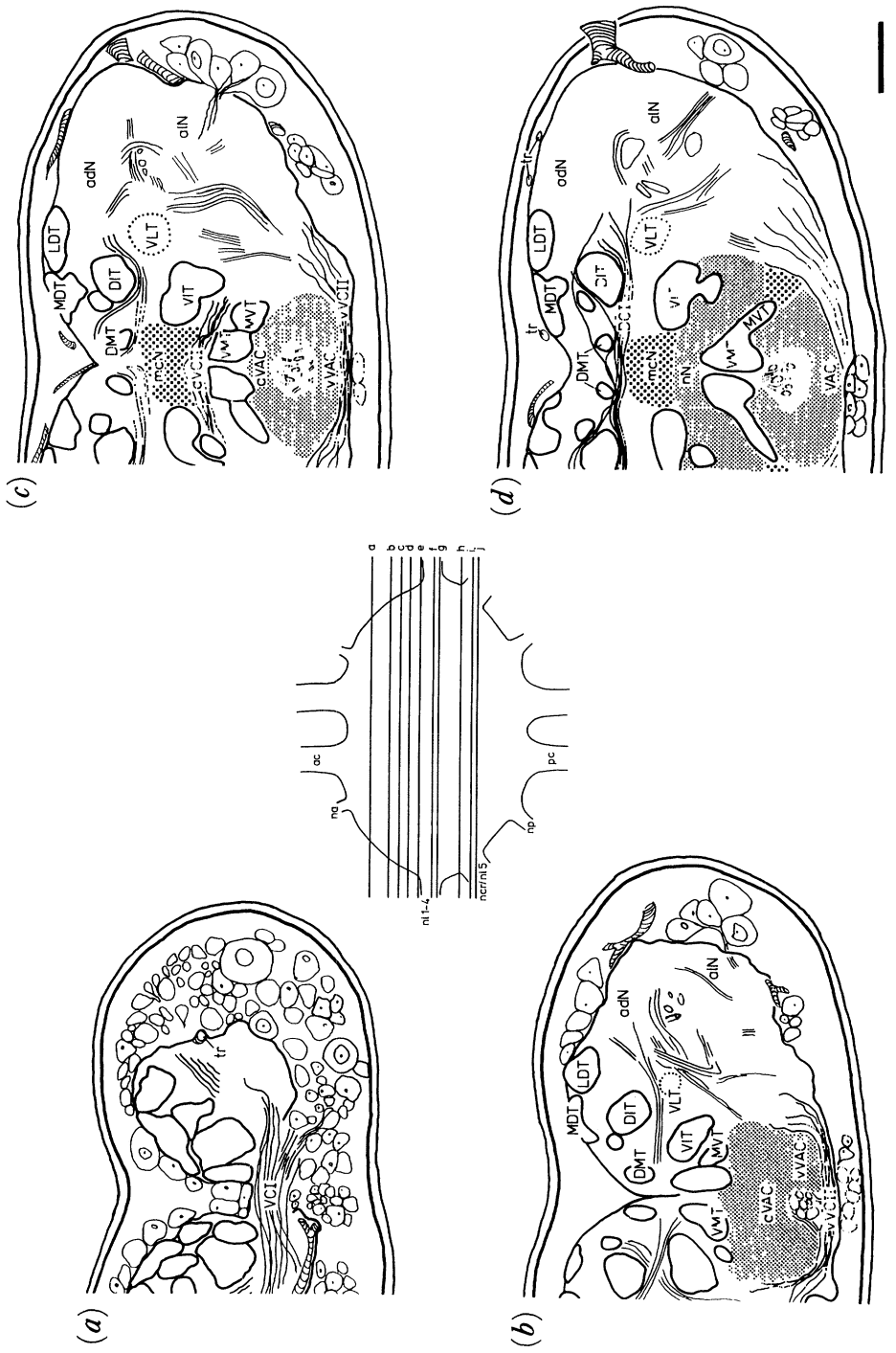

Figure 2 (*a–d*). For description see overleaf.

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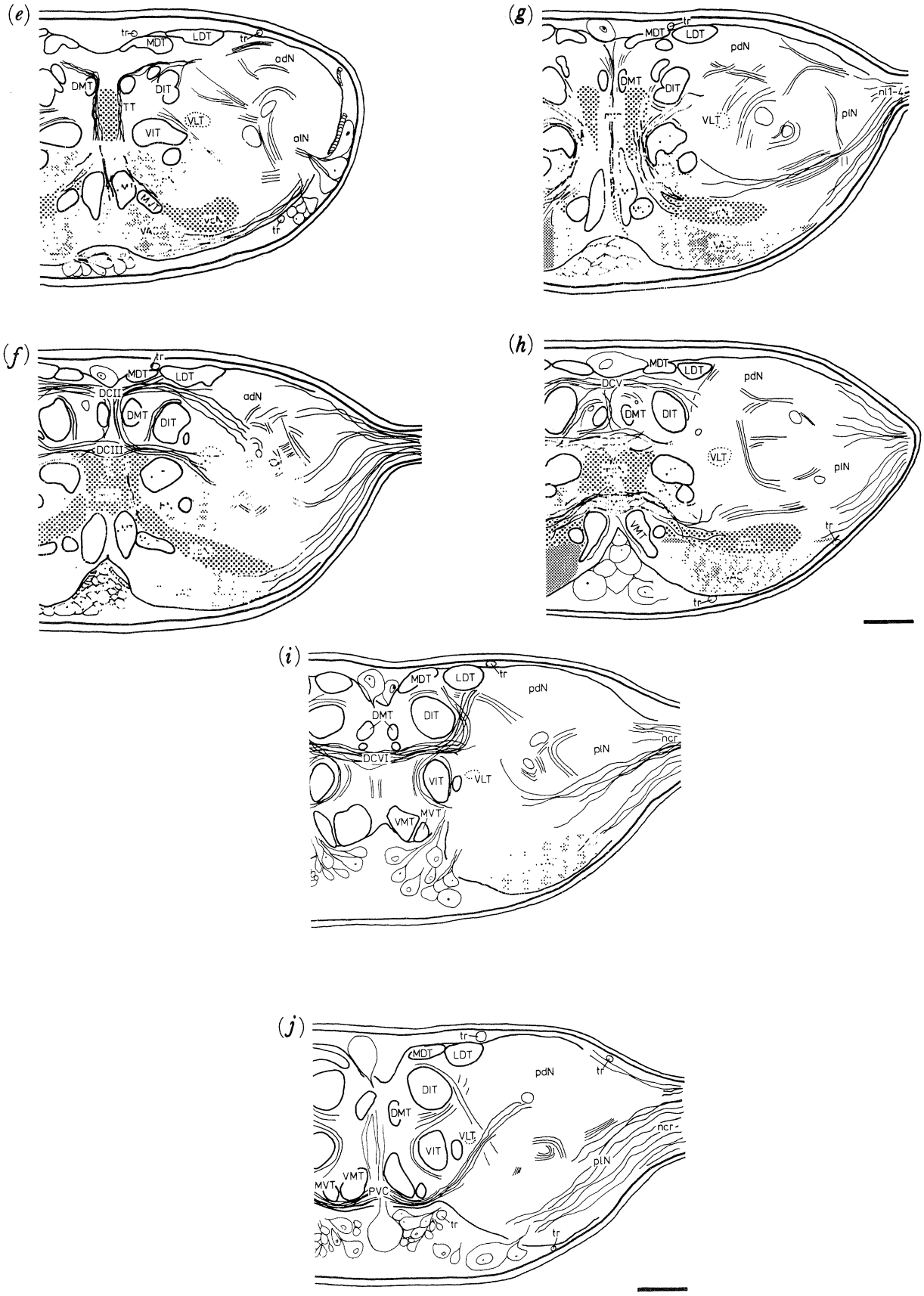

Figure 2. Transverse sections through the mesothoracic ganglion. The figure presents selected serial sections from a series cut at 10 μm and stained with ethyl-gallate. The inset shows the approximate location of the illustrated sections. To achieve a concise presentation, some of the drawings (*d*, *e*, *f*, *i*) are composites of two adjacent sections in the series. In this and the subsequent figures, bundles running perpendicular to the plane of section are outlined, neuropile regions with clear borders are shown by coarse and fine shading to denote coarse and fine texture. Due to limitations in space some labels are placed on the contralateral side. The somata layer surrounding the neuropile is indicated by a few exemplary somata; in fact, this layer is densely packed with cell bodies.

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most prominent tracts are the VMT and the MVT, both of which consist of compact fibre bundles. These two tracts are closely adjacent through much of the ganglion, making a clear distinction difficult. Together they include *ca.* 30 large fibres with diameters of 4–10 μm. Unlike the arrangement in the locust, the MVT, like the VMT, lies medial to the CT. The clearest separation occurs rostrally as these bundles descend shortly after entering the ganglion (figure 2*b*). In more caudal transverse sections, the two often form an oblique band or a deltoid extending ventrolaterally. The MVT occupies the lateral part of this band, but a clear separation of MVT and VMT is not always evident.

Associated with these two tracts is a loose collection of longitudinal fibres on the midline below VMT. These fibres diverge rostrally from the MVT–VMT; immediately caudal to VCI and in front of the aVAC they descend to a position on the medial surface of the ventral midline cleft (unlabelled profiles in figure 2*b* between the vVCII and the aVAC). They continue in this position as the cleft closes ventrally (figure 2*c*, *d*), coursing in a central cylinder between aVAC and vVAC. At the level of DCIII, the VMT descends into this region after passing dorsal to the aVAC. At this junction, fibres can be followed from close proximity to the VMT into the ventral passage. However, many of the fibres in this passage may enter the VAC rather than continuing to rejoin the VMT–MVT and some are clearly collaterals of fibres continuing in the VMT. Thus it is not clear that this group represents a longitudinal through tract. In the middle of the lVAC itself and posterior to the SMC, a compact bundle of small diameter fibres (not shown), presumably primary neurites from ventrocaudal somata, forms a distinctive longitudinal structure.

In locusts (Tyrer & Gregory 1982), the final dorsoventral level contains the two bundles of the lateral ventral longitudinal tract (LVT). In the stick insect, two thin, inconspicuous bands can be followed along the margin of the ganglion in some but not all preparations. The inner LVT (iLVT) is a single layer of dispersed fibres on the ventral surface of the VAC: it was more readily apparent in sagittal (figure 3*e*) than in transverse sections. The outer LVT (oLVT) is more compact than the iLVT. It occupies a position lateral to the VAC at approximately the dorsoventral level of the MVT–VMT. This tract was more readily followed in horizontal sections. A clear identification of the LVT is difficult because many fibres from the peripheral nerves also run along the margin of the neuropile. In particular, a loose bundle of longitudinal fibres is present between the ncr and the n11-4.

In passing through the ganglion, most fibres apparently remain within the same tract: there are no large bundles crossing from one tract to another. However, this pattern is not absolute. In one animal examined, a large distinctive fibre separated from the DIT at the level of DCIV and moved ventrally to join the VIT at the level of DCVI; on the contralateral side in the same animal, this fibre remained in the DIT for the entire passage through the ganglion.

Transverse commissures

Fibre bundles crossing the midline of the ganglion have been grouped into six dorsal commissures (DCI–DCVI) and five ventral commissures (VCI, dVCII, vVCII, SMC, PVC) in accord with the original locust nomenclature. As a group, the commissures are less distinct than the longitudinal tracts; the fibres are smaller in diameter (generally under 6 μm) and compact bundles are shorter in length. Furthermore, some commissures consist of several dispersed groups of fibres. The arrangement of the commissures is best seen in parasagittal sections near the midline (figure 3*a*, *b*) where the commissural fibres form relatively compact bundles crossing between the left and right hemiganglia. The commissures maintain their relative positions in more lateral sections; the larger bundles can be followed laterally almost to the level of the VLT in the middle of each hemiganglion (figures 3*e*, 4*d*). The division into dorsal and ventral commissures is at the level of the VIT. Numbering begins rostrally.

The dorsal commissures (figure 3*a*) are separated into a rostral and a caudal triplet by the midline trachea (mtr), which passes dorsoventrally through the centre of the ganglion (figures 2*g*, 3*a*, 4). In each triplet, the first and third commissures lie ventral and the second lies dorsal to the DIT.

In the rostral triplet, the DCI (figures 2*d*, 4*e*) contains thin fibres forming a small, compact bundle near the rostral margin of the neuropile. The majority of these fibres continue laterally to pass below the DIT and then turn dorsally; a smaller group passes through the dorsal margin of the DIT. The DCII and DCIII are found at the same rostrocaudal level (figure 2*f*), just rostral to the midline trachea. Near the midline the DCII is usually divided into several small, not very conspicuous bundles of mostly medium-sized fibres. In passing between the DIT and the MDT it becomes more compact. More laterally, these fibres descend into neuropile lateral to the VLT in the centre of the hemiganglion. At the midline, the DCIII is a large aggregation including compact bundles of large-diameter fibres (up to 6 μm) as well as medium and tightly packed small fibres. Rostrally, it is separated from the DCI by the descending transverse tract (TT, figure 3*b*). More laterally, this commissure is subdivided by neuropile; lateral to the VIT and the DIT the commissural fibres diverge into dorsal and ventral branches.

Caudal to the midline trachea, DCIV–VI repeat the triangular arrangement of DCI–III. Like DCIII, the DCIV lies below the DIT, presents a large circular profile at the midline, and contains bundles of large fibres (up to 6 μm in diameter). Like DCII, the DCV passes dorsal to the DIT (figure 2*h*) and consists of several small, scattered bundles of medium-sized fibres plus a compact bundle of small fibres. Finally, the DCVI is below the DIT and contains both large fibres and tightly packed, small fibres (figure 2*i*).

The ventral commissures contain mostly small and medium-sized fibres with diameters less than 3 μm. The VCI and the smaller vVCII and PVC, which lie

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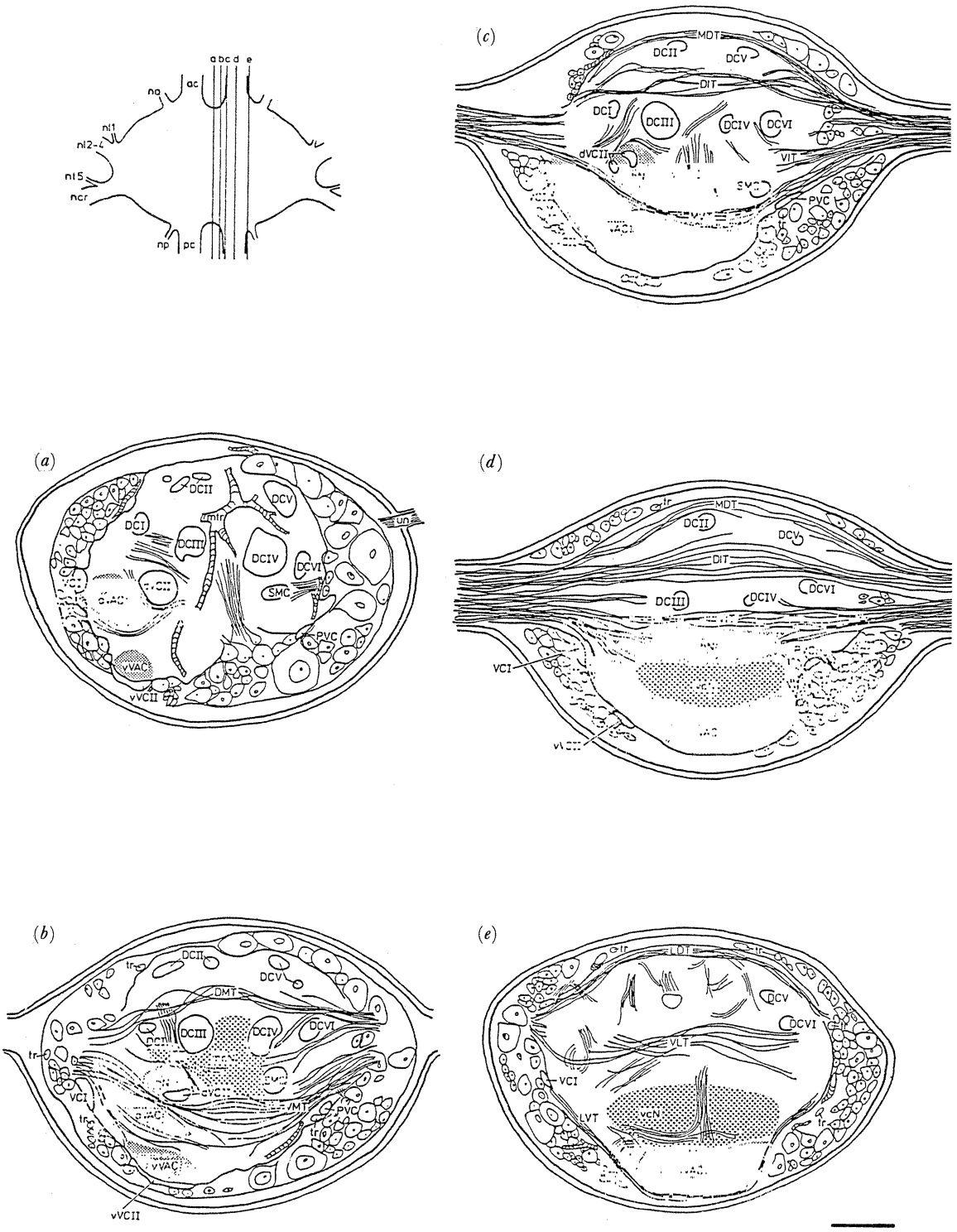

Figure 3. Sagittal sections through the mesothoracic ganglion.

at the ventral margin of the neuropile, form relatively compact bundles comparable to the longitudinal tracts; the two commissures within the neuropile, the dVCII and the SMC, are more similar to the dorsal commissures in that the fibres are more dispersed and their diameters are less uniform. The VCI is a thin band of medium-sized fibres at the rostroventral margin of the neuropile (figures 2*a*, 3*a*, *b*). In histological sections, the compactness of the VCI and the uniformity of the constituent fibres make it the most prominent ventral commissure in sagittal sections near the midline (figure 3*a*). The vVCII is the ventral part of what was originally termed the 'ventral commissural loop' in cockroaches and locusts (Pipa *et al.* 1959; Tyrer & Gregory 1982); it crosses beneath the vVAC as a thin sheet of mostly small diameter fibres. The dorsal part, the dVCII, passes dorsal to the aVAC and the VMT; it passes rostroventral to the VMT. It is partly surrounded by fine-grained neuropile (hN). The SMC forms a similar but slightly smaller structure in the caudal half of the neuropile. It is found in the same dorsoventral plane as DCIV and

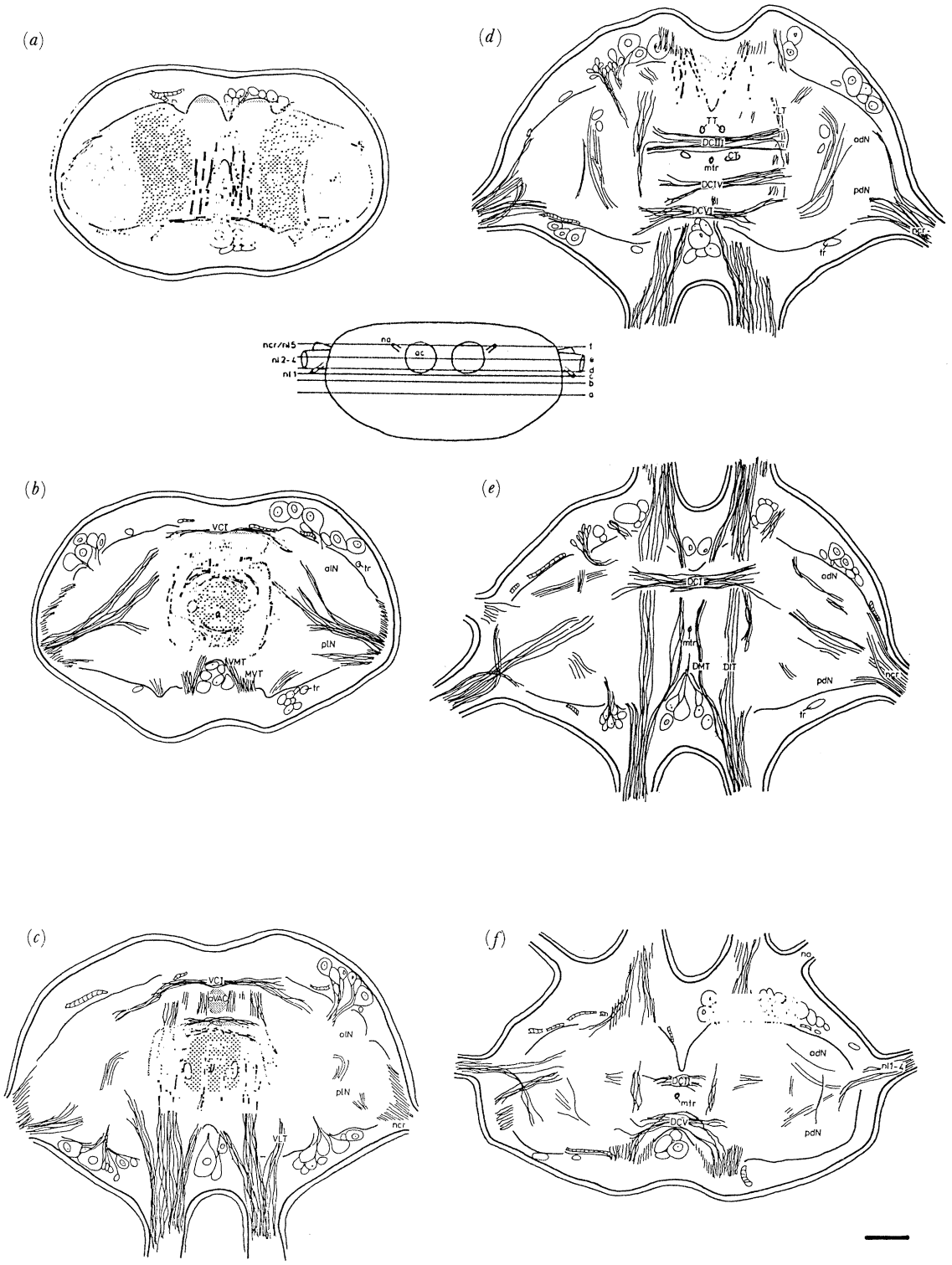

Figure 4. Horizontal sections through the mesothoracic ganglion.

DCV (figure 2*h*). Both these commissures, the dVCII and the SMC, contain a mixture of large and small fibres and may be subdivided. The final commissure, the PVC, is a small, compact bundle at the caudo-ventral margin of the neuropile (figure 2*j*).

Vertical tracts, oblique tracts and other landmarks

The most prominent features in this category are the transverse tract (TT) and the circle tract (CT). The TT is a thin bundle of small and medium diameter fibres which arises from ventral somata and passes between the aVAC and the vVAC. It rises behind the dVCII, continues between DCI and DCIII along the midline and then curves dorsolaterally around the DMT (figures 2*e*, 3*b*). The CT (figures 2*g*, 4*b–d*) forms an arc sweeping medially around the VIT. Ventrally, the arc enters the ventral band of coarse-grained neuropile (vcN) dorsal to the VAC. Dorsally, the main branch swings laterally between DIT and VIT; a smaller bundle is medial to DIT.

In the caudal half of the ganglion at the level of DCVI a compact band similar to the CT is found

medial to the VIT (figures 2*i*, *j*). At the same level, a bundle of fibres arising from ventral somata (figures 2*i*, 3*d*) ascends in a rostral direction, passing the lateral margin of the VIT and then the DIT at the level of DCVI. More laterally and slightly more rostrally between DCIV and DCVI, a short, compact bundle extends from the vcN in the direction of the VLT (figure 3*e*). This bundle does not appear to continue ventrally to the somata layer. Several smaller bundles ascending diagonally from ventrocaudal somata can be found in sagittal sections.

In horizontal sections, several of these vertical bundles can be identified slightly removed from both the rostral and the caudal margins of the neuropile. These smaller bundles are shown in some figures (e.g. 4*d*, 2*c*, *d*, *h*, *i*) but left unnamed.

The fibres from the lateral nerves follow several paths as they enter the ganglion, but the individual nerve roots are not easy to follow in the absence of specific staining. The discrimination between n15 and ncr and among n11-4 is particularly difficult because these nerves are fused as they enter the ganglion. A further difficulty is that the nerve roots do not generally follow the standard planes of section used here. Thus, only a qualitative description will be presented; a proper nomenclature must await further study.

The majority of the fibres from the lateral nerves continue either ventrally or dorsally along the margin of the ganglion. Some bundles turn and follow the lateral margin or penetrate into the neuropile. Two pairs of roots have been included in the figures as landmarks in the lateral neuropile. These roots follow a diagonal course towards the midline in the rostral (figures 2*e*, *f*, *g*; 4 *b*, *e*) and caudal (figures 2*i*, *j*; 4 *c*, *f*) halves of the neuropile. One member of each pair follows the ventral margin of the neuropile whereas the second follows a midventral course. The anterior, midventral bundle passes dorsal to the vcN towards the VIT.

In addition to these bundles, large diameter fibres following a course typical of motoneuron primary neurites can be followed in horizontal sections near the dorsal surface. Similar thick fibres evident at intermediate levels in sagittal sections (not illustrated) are part of a coarse-grained neuropile occupying the central core of each hemiganglion (e.g. figure 4*d*).

Neuropile regions

The appearance of the neuropile surrounding the tracts and commissures is not uniform (figure 1). Differences in appearance, together with boundaries provided by fibre bundles, allow several neuropile regions to be distinguished. Some have already been mentioned; these include the VAC, the hN, the mcN and the band of ventral coarse-grained neuropile (vcN).

The ventral association centre (VAC) is the largest and most distinctive neuropile. It appears as a uniform, fine-grained neuropile which occupies much of the ventral surface of the ganglion (figures 2*b–i*, 3*b–e*). The rostral portion is like a cylinder lying on its side and tilted slightly upward at its rostral end. In sagittal sections, the core of the cylinder is occupied by primary neurites from ventrally located somata and by fibres departing ventrally from the VMT either at the rostral margin of the ganglion or at a rostrocaudal level between DCIII and DCIV (figure 3*a*, *b*). It is not clear whether this bundle includes a significant number of through fibres. Some fibres can be seen to disperse laterally into the VAC, so this bundle may contain only fibres entering and leaving the VAC. It has not been included among the longitudinal tracts.

Following Tyrer & Gregory (1982), the aVAC is defined as the rostral area between the VCI and the TT. The ventral part of the cylinder corresponds to the vVAC of the locust, but in the stick insect there is no clear lateral separation from the aVAC. Posterior to the level of DCIII, the VAC continues caudally as two wedges (1VAC) which spread laterally on the ventral margin of the neuropile and caudally almost as far as the PVC. At the midline, the two wedges are separated by the VMT and by somata occupying the ventral cleft between the two hemiganglia. In addition to the fibres entering from the VMT, the 1VAC receives extensive bands of fibres entering laterally from the ventral nerve roots.

In transverse sections between the levels of DCI and the SMC (figures 2*d–h*, 4*a*), a band of neuropile with a coarse-grained appearance (vcN) lies lateral to the MVT above the fine-grained neuropile of the VAC. Whereas the uniform, fine texture of VAC shows a dense aggregation of thin terminal dendrites and axon endings, the coarse appearance of the vcN derives from tangles of fibres of both medium and small diameter. Among others, fibres from the TT, CT and SMC enter or pass through this region. As discussed above, this area also contains scattered longitudinal fibres, but these do not appear continuous between the connectives.

At the midline caudal to DCI (figure 2*d*), the central area medial and ventromedial to VIT is occupied ventrally by a fine-grained neuropile, the horseshoe neuropile (hN), and dorsally by the medial coarse-grained neuropile (mcN). Caudal to the TT, the coarse-grained neuropile occupies most of the medial area (figure 2*f–h*), while the hN assumes a more lateral position between the MVT-VMT and the VIT (figure 2*c–h*). The name given here to the latter derives from its appearance in horizontal sections (figure 4 *b*, *c*).

The central core of each hemiganglion is occupied by coarse-grained neuropile similar to the vcN in appearance; the outer margin of the neuropile is occupied primarily by fine-grained, darkly staining neuropile similar to the VAC. The boundaries of these areas are irregular and poorly defined, so they have not been included in the figures and the central core neuropile has not been formally named. However, the central core neuropile, together with other features, does allow a rough division of the marginal neuropile into four general areas: the anterior lateral neuropile (alN), the anterior dorsal neuropile (adN), the posterior lateral neuropile (plN) and the posterior dorsal neuropile (pdN). The division between anterior and posterior roughly follows a line between the mtr and the rostral margin of ncr. The division between dorsal

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and lateral is at the level of the ventral margin of the DIT; this also corresponds to the level at which the lateral nerves enter. In transverse sections taken midway through the ganglion, many small bundles of larger diameter processes, presumably from motoneurons, are evident in lateral parts of the dorsal neuropiles.

Guides for orientation

The following features provide useful landmarks for orientation in serial sections. The midline trachea can be used to find the separation between DCIII and DCIV in all planes. In transverse sections, the presence of a compact CT adjacent to the VIT also marks this level. Rostral to mtr, first the characteristic shape of the VAC and the arcs of DCII and DCIII and then the arc of DCI provide useful guides. Caudal to mtr, first the broad band of DCIV in the plane of the SMC and then the DCVI and the PVC on the ventral margin after the disappearance of the lVAC are prominent markers. In sagittal sections, it is easiest to start at or near the midline to locate DCIII and DCIV. In more lateral sections, the prominent DIT, VIT and the VAC/vcN are useful for determining the dorsoventral level in the ganglion. Still more laterally, useful markers are the two pairs of prominent lateral nerve roots composed of compact fibres of medium diameter which run diagonally on the ventral surface and at midventral levels. The larger fibres located at the dorsal margin and in the coarse-grained neuropile in the central core of the hemiganglion are also useful markers. In horizontal sections, the most prominent landmarks are again provided by the major longitudinal tracts, in particular the DIT, the VIT and the VMT–MVT. The DCIII, DCIV and DCVI appearing in one plane form a characteristic pattern. The mtr and the CT at the midline provide a clear separation between DCIII and DCIV. Several vertical bundles are compact and therefore distinctive when the staining is good, but because the fibres are often small, they may be easily overlooked. Other bundles closer to the rostral and the caudal margins of the neuropile are shorter but sometimes more distinctive because the fibres are larger. The two lateral nerve roots following a diagonal course at midventral levels in the horizontal plane are useful in differentiating areas of lateral neuropile. However, as described below, the length and course of these roots vary among the three ganglia depending upon where the nerves enter.

In physiological work, tracheae that are visible on the surface of the ganglion are helpful landmarks for positioning electrodes. In the stick insect, each hemiganglion is supplied by an anterior and a posterior tracheal trunk entering dorsolaterally. Within the ganglion, these trunks divide into several large, superficial tracheae which generally follow the border between neuropile and somata layer. These primary branches, in turn, split into numerous small branches which cannot easily be followed as far as the midline. The pattern varies from animal to animal, but as a rule, the following primary branches are found. From the rostral trunk, which enters just ahead of n11-4, one branch proceeds ventrally at the lateral margin of the neuropile and then resumes a medial course (figure 2*a*). A second, dorsal branch proceeds medially in a transverse plane; it roughly follows the dorsal border of the rostral somata cap. At about the middle of the hemiganglion it gives rise to a large branch which descends on the margin of the neuropile. The caudal tracheal trunk, which enters caudal to ncr, also divides into three primary branches. The pattern of branching is approximately the mirror image of that described for the rostral half, but the dorsal branch takes a more diagonal course toward the centre of the ganglion. Hence, the four dorsal branches, one from each of the four tracheal trunks, form a flat 'x' centred at the middle of the ganglion. The branches arriving at the ventral surface are usually more constant landmarks. The caudal branch is approximately below the PVC; the rostral branch is below VCI. Two identified somata, the fast extensor tibiae motoneuron and the fast depressor trochanteris motoneuron, lie just anterior to this rostral branch.

(*c*) *Prothoracic ganglion*

Like the mesothoracic ganglion, the prothoracic ganglion arises from a single neuromere. However, its shape is somewhat less symmetric in that the lateral nerves exit more anteriorly. The ncr exits at about the anterior–posterior level of DCI to DCIII instead of at the level of DCVI. As a result, the lateral neuropile is also drawn forward (figure 5*c*), so the rostral edge of the neuropile is more nearly at right angles to the longitudinal axis. This difference in the gross form of the lateral neuropile leads to differences in the course of some of the lateral roots, but the relative positions of the different neuropile areas remain unchanged. More medially, the structure is very similar to that of the mesothoracic ganglion. The longitudinal tracts and the commissures identified in the mesothoracic ganglion can all be found in the prothoracic ganglion in the same spatial relationships to one another (figure 5). The DMT appears slightly more prominent in the prothoracic ganglion; the MVT–VMT, MDT, and LDT, in contrast, appear less well-defined. Other differences are slight. The description given for the mesothoracic ganglion applies with little change to the prothoracic ganglion, so the details need not be repeated here.

(*d*) *Metathoracic ganglion*

The metathoracic ganglion contains two homologous neuropiles arising from the third thoracic neuromere (T3) and the first abdominal neuromere (A1). These neuropiles meet in an oblique angle (figure 7*b*). Laterally, the division is marked by the exit of the posterior nerve (np). Ventrally, the angle between the neuropiles becomes more acute, so that a cleft is formed. At the midline, this cleft becomes a channel filled with connective tissue which penetrates between the neuropiles of the two neuromeres. A large trachea passes dorsoventrally through this channel (figures 6*h*, 7*a*, *b*); this midline trachea is labelled Mtr in the

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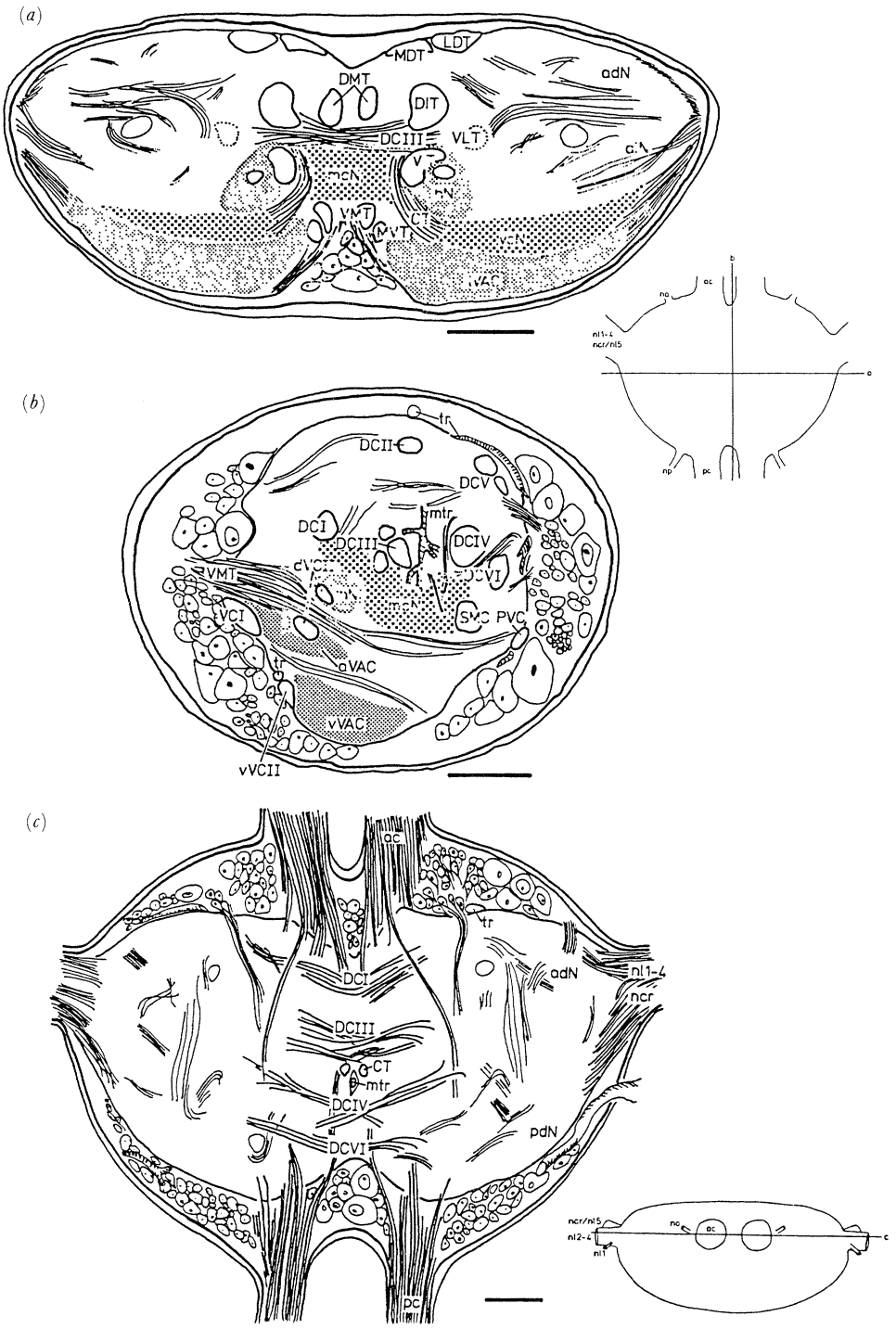

Figure 5. Representative sections through the prothoracic ganglion. The individual sections are from serial sections cut in the transverse (*a*), sagittal (*b*), and horizontal (*c*) planes. The insets indicate the location of the section illustrated.

drawings to avoid confusion with the midline trachea (mtr) passing through the centre of each neuromere. In addition, two trachea supply the caudal half of each hemiganglion: the second presumably corresponds to the trachea for A1.

As described by Marquardt (1940), each abdominal ganglion in the stick insect gives rise to an anterior, a lateral and a posterior nerve which generally exit from the ganglion in a common nerve trunk. In the metathoracic ganglion this trunk joins the posterior nerve (np) from the thoracic neuromere in a common sheath. In the serial sections, the np can be seen to spring from within the cleft between T3 and A1, whereas the A1 nerve trunk arises from the lateral margin of the A1 neuropile.

The fusion of two neuromeres is evident in the duplication of structures within the ganglion: each neuromere has its own mtr and a complete set of commissures and dorsoventral tracts. The longitudinal tracts continue their separate course through both neuropiles without reuniting between T3 and A1.

The T3 neuropile (figures 6*a–h*, 7) follows the

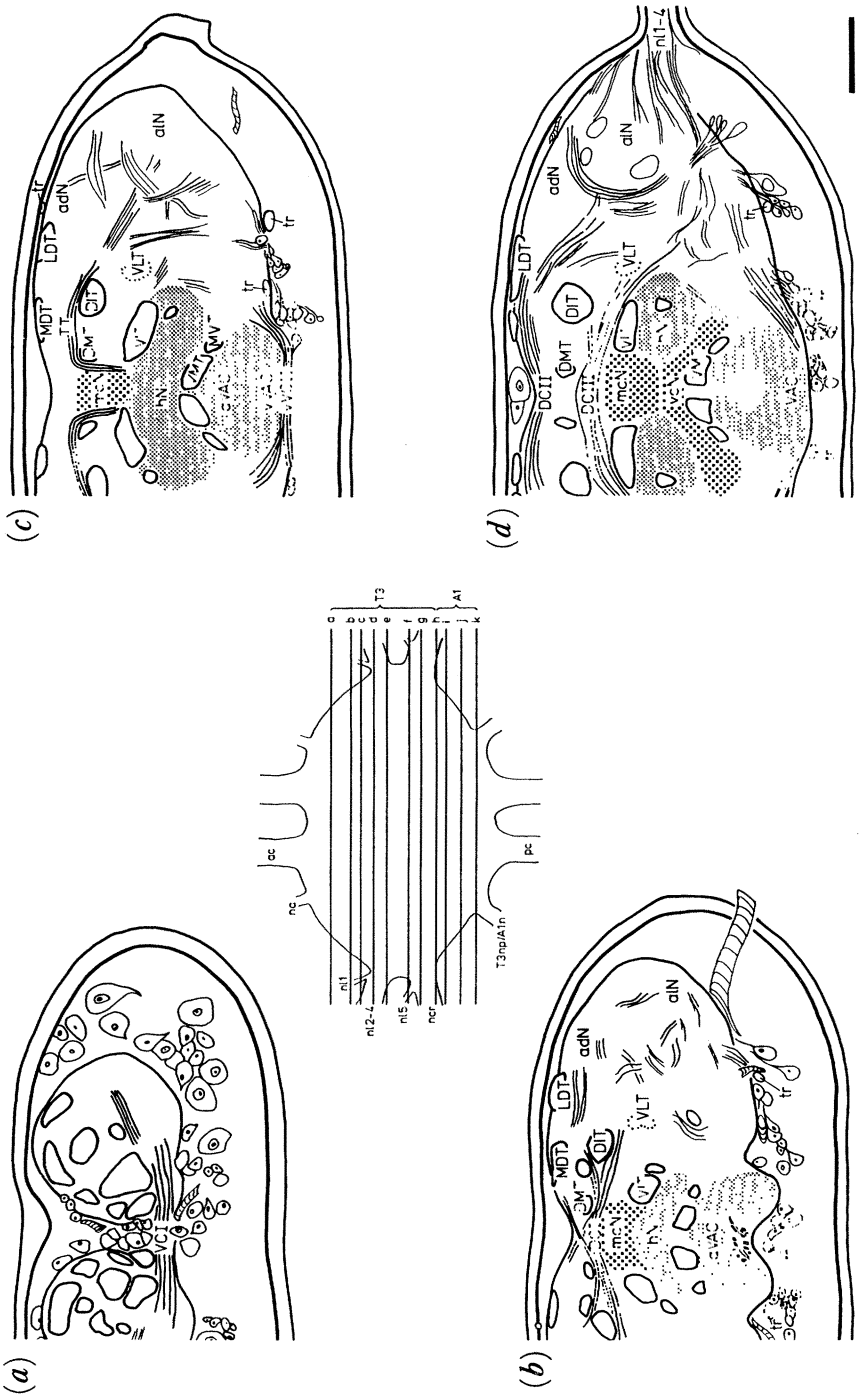

Figure 6. For description see page 114.

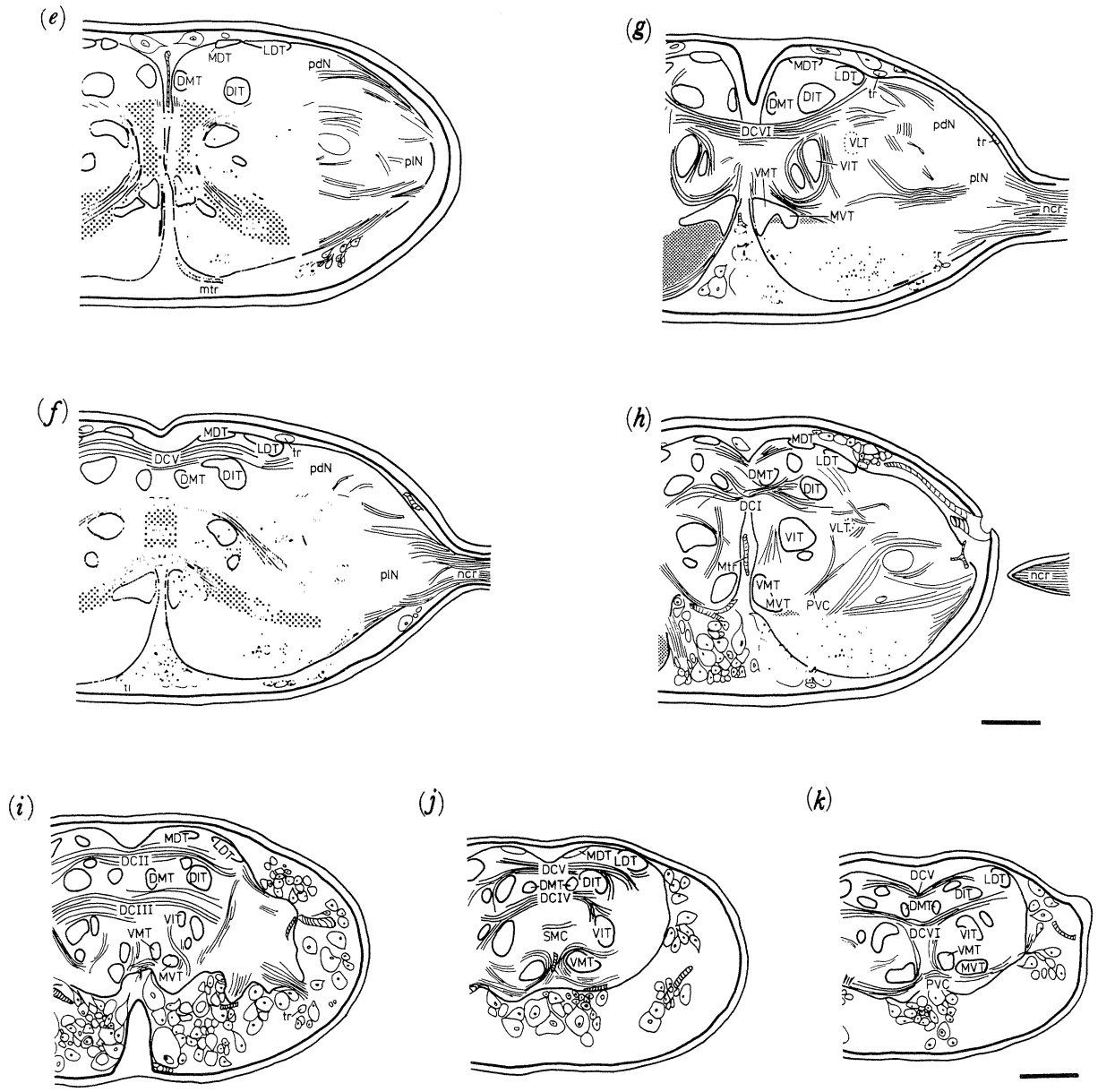

Figure 6 (*e–k*). Transverse sections through the metathoracic ganglion.

pattern already seen in the meso- and prothoracic ganglia. The A1 neuropile is considerably smaller than that of T3 (figure 6*h–k*). As a result, some structures in this neuropile are less well-defined; in other respects, the differences are slight. The LDT remains a prominent, compact bundle whereas the MDT is small and inconspicuous. The MVT and the VMT form a compact, common bundle. Because the VCI passes under the MVT–VMT and this tract retains its ventral position in passing between T3 and A1, the A1 VCI is pushed ventrally towards the vVCII and does not form such a large, distinctive bundle as in the thoracic ganglia. At the midline it is separate from the vVCII. The PVC lies within, rather than on the edge of the abdominal neuropile. On the ventral margin, small regions of fine-grained neuropile which would correspond to VAC are evident but not so distinctly stained as in the thoracic neuromeres.

DISCUSSION

The general structure of the thoracic ganglia in the stick insect resembles that of locusts (Tyrer & Gregory 1982) and cockroaches (Pipa *et al.* 1959; Gregory 1974). This similarity is perhaps not surprising since all three are members of the Orthopteromorpha (Hennig 1969). However, differences do occur at several levels of the organization and these will be considered in turn.

The gross neuroanatomy and the general form of the three thoracic ganglia vary in two respects. First, the external appearance of the thoracic ganglia varies from segment to segment. The three thoracic ganglia have the most similar appearance and the most evenly distributed arrangement of lateral nerves in cockroaches (Pipa & Cook 1959). In the stick insect, the two main lateral nerve trunks (n11-4 and n15+ncr) are symmetrically arranged at the margin of the mesothoracic ganglion. In the other two thoracic

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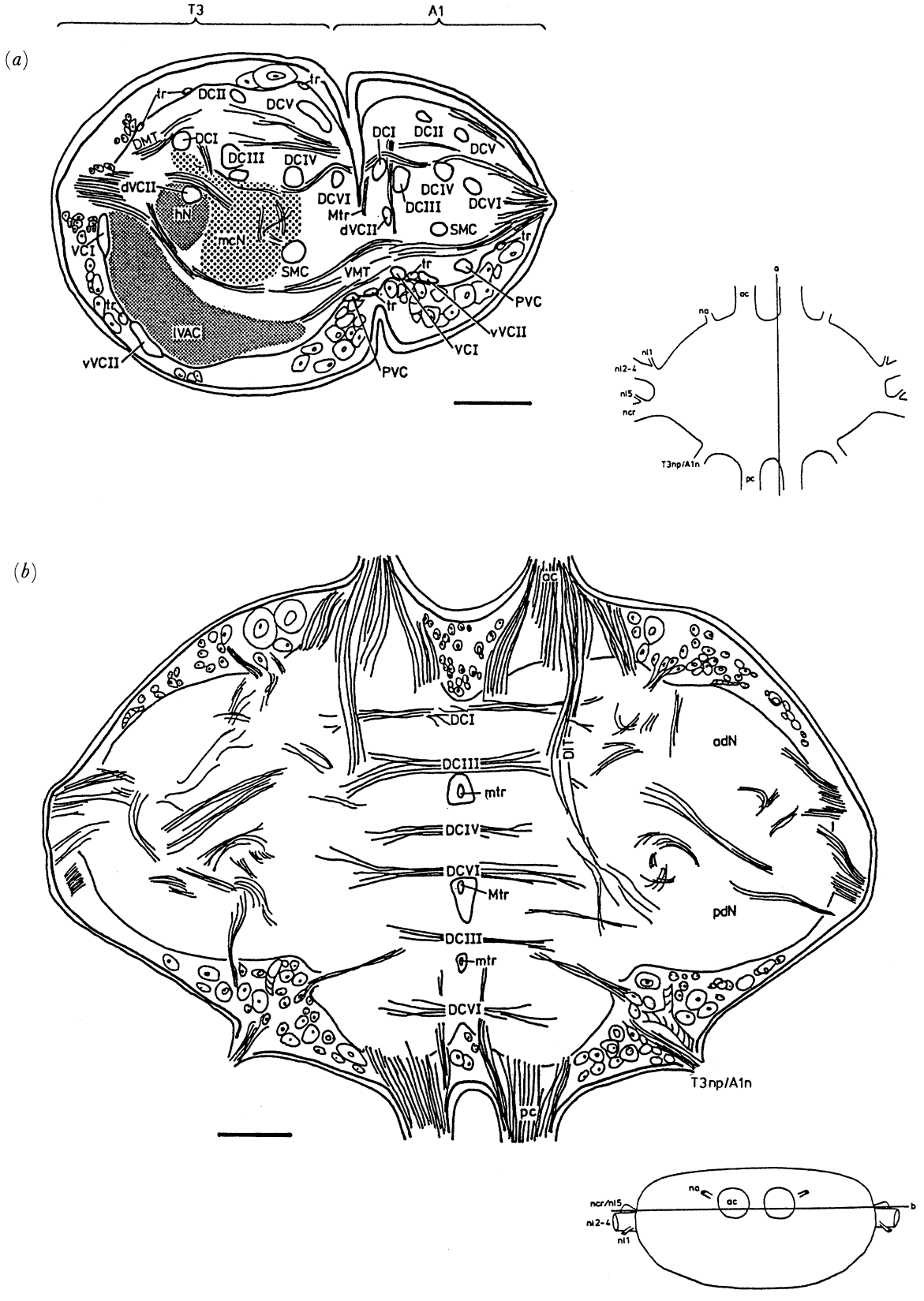

Figure 7. Representative sagittal (*a*) and horizontal (*b*) sections through the metathoracic ganglion.

ganglia, these two nerve trunks are closer together; they enter the prothoracic ganglion more rostrally. This difference in the arrangement of the lateral nerves is associated with differences in the gross shape of the lateral neuropile but does not affect more medial structures. Second, the metathoracic ganglion of the stick insect, like that of cockroaches, represents the fusion of the third thoracic neuromere and the first abdominal neuromere; in locusts, this ganglion includes the first three abdominal neuromeres. This

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difference in composition is reflected in the form of the metathoracic ganglion in the three species.

(*a*) *Tracts and commissures*

Although the tracts were presented above in terms of dorsoventral levels, the nomenclature groups the nine ipsilateral tracts into four pairs, two dorsal tracts (LDT and MDT), two medial tracts (DMT and VMT), two intermediate tracts (DIT and VIT), and two ventral tracts (MVT and LVT), and a single lateral tract (VLT). This grouping reflects the development of the nomenclature and therefore provides a useful framework for comparing the neuroanatomy of the three well-studied orthopteromorph species.

With the exception of the MVT and LVT, the other longitudinal tracts show a similar form and arrangement in *Carausius*, *Periplaneta* and *Locusta*. One minor difference concerns the LDT. In the stick insect, this tract is directly apposed to the neuropile margin throughout its passage through the ganglion, whereas in locusts, it is completely surrounded by neuropile at some levels. Whether this difference has any significance or is related to the wingless condition of *Carausius* can only be decided by comparative studies of other Phasmidae.

The descriptions of the ventral tracts, MVT and LVT, and the nomenclature applied to them are less consistent. In their original investigation of *Periplaneta*, Pipa *et al.* (1959) described and named a single 'ventral tract' consisting of an inconspicuous band of fibres spread along the ventromedial margin of the neuropile; they show this tract as a thin band below the VAC and continuous with the VMT medially. Pipa *et al.* (1959) also described a thin branch of the VMT which diverges from the main bundles to pass beneath the anterior VAC; they hypothesized that this branch contributes fibres to the VAC. In his reexamination of *Periplaneta*, Gregory (1974) divided this ventral tract into two lateral ventral tracts (iLVT and oLVT), which apparently are compact and well-defined in cockroaches, and three medial ventral tracts (MVT1-3). One of these bundles, the MVT1, resembles the ventral tract as originally described by Pipa *et al.* (1959): it forms a ventral continuation of the VMT which passes ventral to the VAC along the midline cleft. The second, larger component, the MVT2, is shown in the figures as an occasionally subdivided bundle located lateral to the VMT and dorsal to the VAC (Gregory 1974). (MVT3 appears in the list of abbreviations but not in the text or in the figures.) Subsequently Gregory (1984) retained the MVT1 for cockroaches but Tyrer & Gregory (1982) did not identify a corresponding structure in locusts. Pflüger *et al.* (1988) placed the iLVT in a position corresponding to the MVT1.

In *Carausius*, a group of fibres also leaves the MVT–VMT rostrally and passes under the aVAC. This group would correspond to the MVT1 in cockroaches. However, we have not included it among the longitudinal tracts or used the designation MVT1 because it is not certain that this bundle contains a significant number of through fibres. Caudally some of the fibres entering this bundle are clearly branches of fibres continuing in the VMT. As suggested by Pipa *et al.* (1959), this bundle may primarily contain fibres contributing to the VAC.

A precise localization of the MVT in *Carausius* faces two further difficulties. The first relates to the separation from the VMT. The name MVT has been applied to the tract in the position corresponding to the MVT in locusts and the MVT2 in cockroaches. In both species, these tracts are separated from the VMT by the ventral arm of the CT; in *Carausius*, both the VMT and the MVT pass medial to the CT. The proximity of the MVT and the VMT, the resulting difficulty in making a clear distinction at many levels, and the midventral location of MVT create the temptation to reserve the designation 'ventral tract' for those tracts lying on the ventral margin, i.e. oLVT, iLVT and possibly MVT1. This would be in accord with the original scheme of Pipa *et al.* (1959). Nevertheless, the MVT and VMT do form separate bundles in rostral transverse sections. This fact and the disadvantage inherent in revising an established usage has led us to apply MVT as defined by Gregory (1974, his MVT2) and Tyrer & Gregory (1982).

The second difficulty concerns the extent of the MVT. In all three species, the MVT is often subdivided in caudal sections, so the lateral separation from the ventral coarse-grained neuropile (vcN) is not always clear. Moreover, in cockroaches, fibres from nerve 2 occupy a similar position in rostral sections (Gregory 1984), so a final delimitation of the MVT in *Carausius* may require study of the nerve roots.

Difficulties in following the other components of the ventral tract, the iLVT and oLVT, have been mentioned above. Apparently these structures may also be variable or difficult to identify in locusts (Siegler & Burrows 1984; Pflüger *et al.* 1988). The corresponding fibre bundles evident in *Carausius*, like the tracts in locust (Tyrer & Gregory 1982), lie on the ventral margin of the neuropile and the iLVT courses ventrolaterally past VCII and the aVAC. More caudally, the iLVT lies beneath the lVAC rather than lateral to it, but in part this may be due to the greater lateral extent of the VAC in *Carausius*. In cockroaches, the iLVT and oLVT lie close together and at one point are separated only by one of the dorsoventral bundles of primary neurites; in *Carausius*, the corresponding structures are more widely separated.

In addition to the bundles of longitudinal fibres associated with the connectives, another longitudinal bundle was described among the tracts in cockroaches and in locusts (Gregory 1974; Tyrer & Gregory 1982). This tract, the ring tract, lies ventrolaterally against the VIT between dVCII and the SMC and lateral to the CT. We have not used this designation for *Carausius*. In horizontal sections, the dVCII, SMC and VIT do bound a ring-like area to which we have given the name mcN. This medial coarse-grained neuropile is characterized by numerous fibres of medium diameter and various orientations but these do not form a clearly defined tract. Small bundles of fibres can be found in transverse sections caudal to the mtr but they lie

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medial to the CT. Tyrer & Gregory (1982) show a bundle in approximately this position in locusts which is formed by the DMT dividing around DCIV. In the stick insect, these fibres do not appear to rejoin the DMT or to represent a clearly defined through tract. Instead, they seem to pursue an oblique course passing from the mcN under DCIV to the neighbourhood of DCVI.

In cockroaches, Gregory (1974) also identified a lateral extension of the ring tract ventral to the VIT. This area corresponds to the region of ventral coarse-grained neuropile (vcN) which extends caudally and laterally from the mcN. It includes a tangle of large fibres coursing laterally from the central commissures and longitudinal tracts.

The transverse commissures are arranged similarly in all three orthopteromorph species studied. This organization is most easily recognizable at the midline; more laterally, several commissures disperse into bundles which are less easily grouped. This separation may lead to difficulties in identification (e.g. Guthrie & Tindall 1968; Gregory 1974). The six dorsal commissures originally described in cockroaches and locusts are also apparent in *Carausius*. In the course of staining single neurons, Watson (1986) identified a seventh dorsal commissure (DCVII) lying dorsal to the DIT and even with or slightly caudal to DCVI. The corresponding position in the stick insect appears devoid of fibres crossing the midline. However, the DCV includes a small bundle of medium-sized fibres which cross slightly ventrocaudal to the main part of DCV and rostral to DCVI. Specific staining might show that this bundle corresponds to Watson's DCVII.

In early descriptions, the first and second ventral commissures were referred to as 'loops'. The description of these bundles, and the ring tract, creates an impression of circular pathways but this is probably not true for any of the constituent fibres. Therefore, a more useful long-term goal is a nomenclature both for the midlie bundles themselves and for their lateral branches analogous to that for lateral nerve roots (Gregory 1974). For the meantime, we have followed the more recent usage in referring to the bundles crossing the midline as 'ventral commissures'. We have also followed tradition in retaining the VCII as a single unit with dorsal and ventral parts.

Several individual fibres with large diameters can be followed within the longitudinal tracts in the ethyl-gallate material (e.g. figure 1 *a*, *c*). However, the largest nerve fibres in the stick insect are much smaller than the much studied giant interneurons in cockroaches. The absence of giant fibres is not unexpected in view of the diametrically opposed defensive responses: the cockroach relies on rapid avoidance running (review Camhi 1984, 1988) whereas the stick insect freezes and relies on camouflage. The giant fibres in cockroaches mediate avoidance responses elicited by cercal mechanoreceptors sensitive to touch and to wind currents. Edwards & Reddy (1986) have suggested that escape systems like that of cockroaches are characteristic of primitive insect groups. The stick insect does have large fibres in both the DIT and the VIT which might correspond to the set of seven giant fibres described in cockroaches, but more detailed investigation is required to establish homology.

(*b*) *Neuropile regions*

Several neuropile areas present a distinctive appearance in ethyl-gallate sections. One feature is the texture and this is correlated with the darkness of staining in ethyl-gallate sections. Different authors have used different terms for the distinction we have made between fine and coarse neuropile. For example, Strausfeld (1976) distinguishes between 'synaptic neuropile' and 'tract neuropile'.

The VAC is the best defined neuropile region. Pipa *et al.* (1959) proposed the name 'association centre' because this area is the target of several nerve roots from lateral nerves thought to be primarily sensory. Johnson & Murphey (1985) suggested replacing 'vVAC' (including the lVAC) with 'bristle neuropile', based on the finding that in crickets and locusts this area is the exclusive target of afferent fibres from tactile bristles, a class of mechanoreceptive hairs. Here, we have retained the term 'VAC' despite its functional connotation, because it is well established in the literature. The exact nature of the processing carried out is uncertain and, as other authors have pointed out, no analogy with cortical 'association areas' in mammalian cortex is implied.

In *Carausius*, the VAC has a somewhat different shape than that of locusts. In the latter, the rostral portion can be envisioned as a torus tipped upward rostrally with the TT ascending through the centre. In sagittal sections, the aVAC and vVAC present round profiles. In *Carausius*, this torus is stretched in a caudoventral direction, so that the VAC appears more like a cylinder with a bevelled end. In sagittal sections at the midline the aVAC and vVAC do present round profiles, but in slightly lateral sections these flatten and appear as wedges filling the rostroventral part of the ganglion. These wedges unite and continue in a laterocaudal direction (lVAC) on the ventral margin of the neuropile. The laterocaudal extent of this fine grained neuropile in *Carausius* exceeds that in locusts and cockroaches.

Besides the VAC, several other neuropile areas can be distinguished and named. In the present case, these suggestions arise from inspection of stained sections, but anatomical characterization of afferent fibres and initial physiological studies support the proposed distinctions and point to functional specializations. In the course of their study of sensory projections in locusts, Bräunig *et al.* (1981) and Pflüger *et al.* (1988) identified two additional neuropile areas (the medial Ventral Association Centre or mVAC and the Lateral Association Centre or LAC) which, in analogy with the VAC, could play an important integrative role for sensory input. The mVAC corresponds to the anterior portion of the fine-grained neuropile we have designated as hN. The lateral extensions surrounding the VIT are more prominent in *Carausius*; Pflüger *et al.* (1988) mention a halo of fine-grained neuropile surrounding major tracts but did not include this area in the mVAC. This midline neuropile has also been

designated as an 'anterior part of the ring tract' (Tyrer & Gregory 1982) and as acoustic or auditory neuropile (see for example Halex *et al.* (1988)). Murphey *et al.* (1985) also identified a medial intermediate neuropile in the abdominal and thoracic ganglia of crickets which is the target of non-bristle sensory fibres. They interpreted this pattern to support a suggestion of Zawarzin (1924) that the neuropile is organized into a ventral sensory area (VAC, bristle neuropile), an intermediate or associative area, and a dorsal motor area.

The lateral association centre defined by Pflüger *et al.* (1988) is divided into an anterior and a posterior part which correspond to the anterior–posterior division made here. These areas of more or less well delimited, fine-grained neuropiles form a shell around the more coarse-grained neuropile occupying the core of each hemiganglion. They are the targets of projections from leg proprioceptors (Schmitz *et al.*, in preparation). The dorsal areas also contain motoneuron dendrites.

That the core and lateral parts of each hemiganglion are not amorphous neuropile has been shown in some of the figures but it is beyond the bounds of the current study to propose a definitive nomenclature. The central core and the lateral areas do differ in appearance. The core contains coarse-grained neuropile, designated as central core neuropile but not drawn in the figures; the lateral areas generally contain fine-grained neuropile (adN, alN, pdN, plN). However, drawing a clear boundary between these areas would be misleading.

In general, vertical and oblique bundles in the lateral neuropile of *Carausius* are short and not well resolved in the standard planes of section. Nevertheless, both the alN and plN do include numerous compact bundles of various lengths representing either clusters of primary neurites rising from ventral cell bodies or roots of peripheral nerves. A detailed description of the latter must await study of material in which the lateral nerves are individually stained. In the present material, the tracing of nerve roots apart from the few prominent exceptions noted above is not feasible. The bundles of primary neurites are compact and prominent in horizontal sections from individual animals, but the available material does not yet allow an evaluation of the constancy of their pattern and location from one animal to another. An appropriate nomenclature in the stick insect would also benefit from a definition of somata groups, a task that Gregory (1984) performed for cockroaches. In locusts, two of these bundles in the rostroventral quadrant have been designated as the perpendicular tract and the anterior perpendicular tract: these include primary neurites from somata in the medial ventral cell group and the lateral ventral cell group, respectively (Watson 1986).

The I-tract (IT) is another oblique bundle away from the midline that is better defined in locusts than in *Carausius*. In locusts, the IT and the CT are equally prominent; the IT pursues an oblique, dorsoventral course lateral to the VIT. In *Carausius*, the prominent bundles with a similar orientation are slightly more caudal. The shorter, more lateral of the two mentioned above may correspond to the IT in locusts; the second, located closer to the VIT, corresponds to an unnamed bundle of initial segments also found in locusts at the level of DCVI (Tyrer & Gregory 1982).

Several features have been mentioned which appear reduced or modified in the neuropile of the wingless *Carausius* compared to that of locusts: e.g. I-tract, circle tract, apposition of LDT to the neuropile margin, LVT, VAC form, MVT and DCVII. Since these changes also are found in the prothoracic ganglion, it is not obvious that they relate to the presence or absence of wings on a particular segment. Moreover, it is known that loss of wings and associated muscles need not lead to loss of even the associated motoneurons (Arbas, 1983). A comparison with winged Phasmid species is required to decide whether these changes relate to the loss of wings or represent characteristic differences of the respective taxonomic groups. Comparative study of motoneurons innervating a bifunctional muscle acting on both wing and leg does indicate a reduction of the dendritic arborization in Phasmids with reduced or absent wings (Kittmann & Kutsch, in preparation), but the relationship to the gross anatomical details has not yet been characterized.

(*c*) *Comparison with other arthropods*

Many of the basic features found in orthopteromorph insects are also evident in other arthropods. Such features include the number and location of the major longitudinal tracts and their organization in four dorsoventral planes. These similarities were used by Skinner (1985*a*) as grounds for applying the orthopteromorph nomenclature to the fourth abdominal ganglion in the crayfish. The longitudinal tracts she identified correspond to those in insects with one exception: a ventral lateral tract not identified in insects was given the name VLT and the tract following a course similar to that of the VLT in insects was named the dorsal lateral tract or DLT and included in the second dorsoventral layer. In the stick insect, the VLT is more ventral for most of its course and it can be more readily grouped with the VIT. The number of the dorsal commissures and the organization of the lateral neuropile in the crayfish differs from that in orthopteromorph insects. Only three lateral nerves are present in the crayfish abdominal ganglion, so the arrangement of nerve roots also differ from those of the thoracic ganglia of insects. Skinner (1985*b*) characterized four neuropile areas in the crayfish, but these do not correspond in any obvious way to those of insects. In particular, the distinctive shape of one midline neuropile also led Skinner to use the name 'horseshoe neuropile', but this area does not appear in any way homologous to the area in the stick insect to which we have given the same name for similar reasons.

The basic structural similarities among the arthropod species studied encourage the use of similar nomenclature, despite the fact that further study is necessary to establish homologies. The desire to continue a common terminology explains some of the peculiarities in the naming. For example, the MVT and the VMT in locusts are shown as well-separated tracts throughout the ganglion, whereas in both the

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stick insect and the crayfish they run together for most of their length. Nevertheless, it seems appropriate to continue the distinction for the time being. On the other hand, some terms, such dVCII and vVCII, may imply a spurious relationship.

In conclusion, it is interesting to pose the question of why gross neuroanatomical studies of insects were neglected for some time despite the early work of neuroanatomists and why a standard atlas is not routinely used for the most common laboratory invertebrates. In part this neglect may reflect the impression expressed by some early neurobiologists that invertebrate neuropile is basically unstructured (Hughes 1952; Horridge 1968). Later, the emphasis on the unique shape of many identified neurons and the small size of invertebrate ganglia may have made a detailed atlas seem unnecessary. The finding that many identified neurons, in particular the motoneurons first studied, spread widely throughout the ganglion may have discouraged any hope of finding discrete, functionally specialized regions within the ganglion. In fact, as noted above, studies using specific labelling techniques have revealed considerable order both in the locations of major branches of identified neurons and in the organization of specific, functionally defined neuropiles.

Thus an atlas complements the study of individual elements in important ways. First, it allows a more precise specification of the morphology of identified neurons. Study of the anatomy combined with the staining of individual neurons can establish whether a particular neuron is unique or one of several neurons with similar morphology (Wilson 1981). Second, it provides a more exact means of determining whether pairs of neurons overlap than is possible through a comparison of their forms alone. Third, a knowledge of the basic neuroanatomy on a more global level is necessary for the application of autoradiographic, histochemical, ultrastructural and immunohistochemical methods aimed at identifying particular features of local areas within the neuropile.

In summary, the results presented here are intended as a guide to the gross neuroanatomy of the stick insect. They are meant to be used in interpreting detailed results obtained with more specific methods. The emphasis is on distinctive structures which can serve as landmarks for orientation within the thoracic neuropile. For the time being, the names carry only descriptive information; the next step will be to see to what extent functional systems follow these structural divisions. The companion paper (Schmitz *et al.*, in preparation) will present results for afferent sensory fibres.

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EXPLANATION OF ABBREVIATIONS USED IN FIGURES

A1	first abdominal neuromere
A1n	nerve trunk of first abdominal neuropile
ac	anterior connective
adN	anterior dorsal neuropile
alN	anterior lateral neuropile
CT	C-tract
DCI to DCVI	dorsal commissures I–VI
DIT	dorsal intermediate tract
DMT	dorsal medial tract
hN	horseshoe neuropile
LDT	lateral dorsal tract
LVT	lateral ventral tract
iLVT	inner lateral ventral tract
oLVT	outer lateral ventral tract
mcN	medial coarse neuropile
MDT	medial dorsal tract
mtr	midline trachea
Mtr	midline trachea between T3 and A1 neuropiles
MVT	medial ventral tract
na	nervus anterioris
ncr	nervus cruris (main leg nerve)
n11 to n15	lateral nerves 1–5
np	nervus posterioris
pc	posterior connective
pdN	posterior dorsal neuropile
plN	posterior lateral neuropile
PVC	posterior ventral commissure
SMC	supramedian commissure
T3	third thoracic neuromere
tr	trachea
TT	T-tract
un	unpaired nerve
VAC	ventral association centre
aVAC	anterior part of ventral association centre
lVAC	lateral part of ventral association centre
vVAC	ventral part of ventral association centre
VCI	ventral commissure I
VCII	ventral commissure II
dVCII	dorsal aspect of ventral commissure II
vVCII	ventral aspect of ventral commissure II
vcN	ventral coarse neuropile
VIT	ventral intermediate tract
VLT	ventral lateral tract
VMT	ventral medial tract

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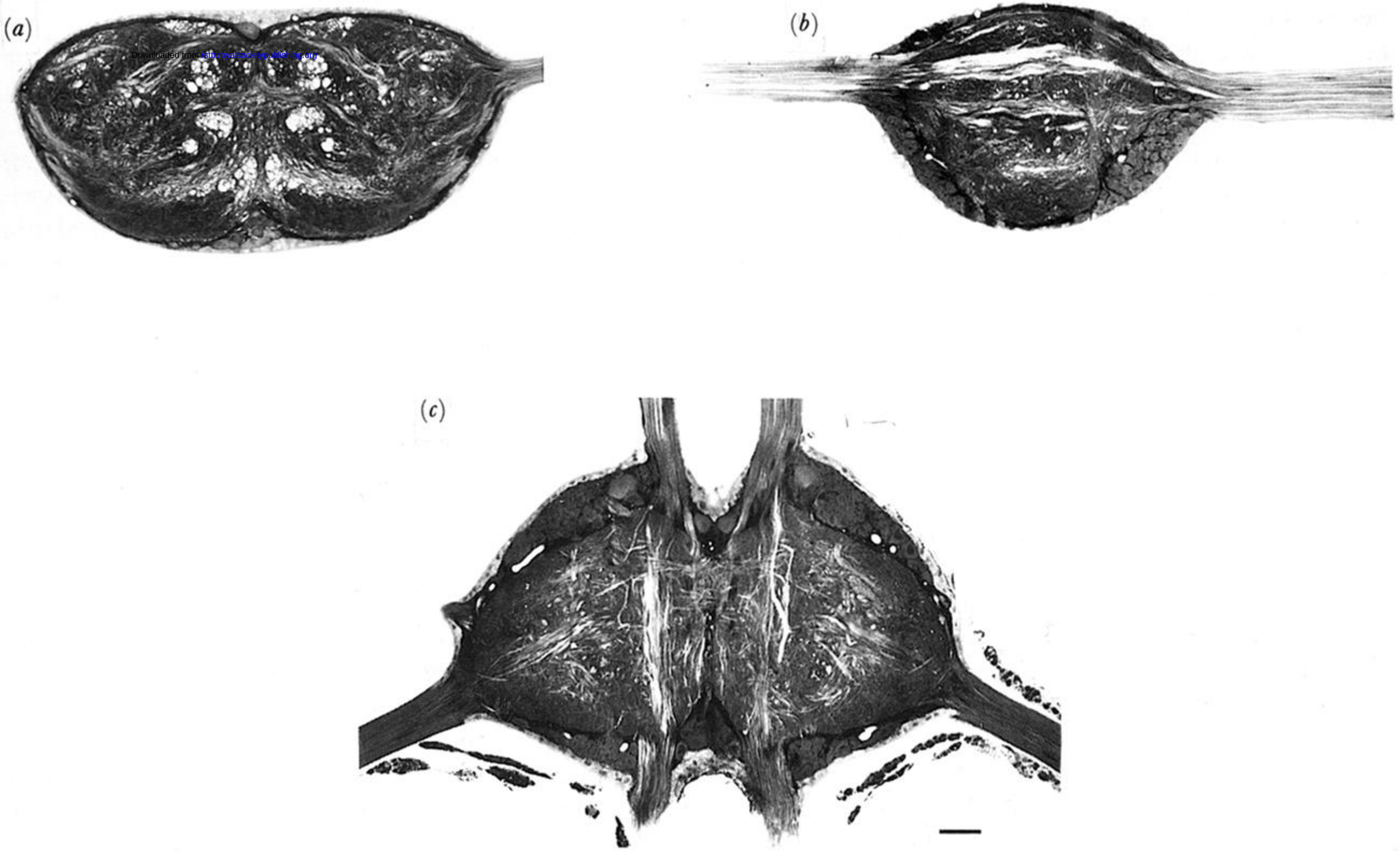

Figure 1. Representative photographs of sections from a mesothoracic ganglion stained with ethyl-gallate. The plane of the sections and the corresponding drawings are as follows: (*a*) transverse (figure 2*f*); (*b*) sagittal (figure 3*d*), and (*c*) horizontal (figure 4*e*). The length of the bar in this and the following figures corresponds to 100 μm.

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