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Morphogenesis of the branching pattern of a group of spiking local interneurons in relation to the organization of embryonic sensory neuropils in locust

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SUMMARY

The embryonic development of the principal tracts, commissures and neuropils in the thoracic ganglia of the locust *Schistocerca gregaria* are described. We show that the major tracts and commissures are generated during the earliest stages of axon outgrowth. Some longitudinal tracts can be identified as early as 42 % of embryonic development and by 55 % all tracts except the dorsal median tract (DMT) and median dorsal tract (MDT) can be recognized. DMT and MDT cannot be reliably identified until 65 %. The major neuropilar regions, in contrast, are identifiable relatively late in embryogenesis. They are first evident at 65–70 %, but do not become fully distinct until 70–75 %. This coincides with the developmental timing of synaptogenesis.

Onto this developmental groundplan we have mapped the growth of an identified group of local interneurons. The early growth of these interneurons (50–65 %) is characterized by slow and directed axon outgrowth which assembles the basic skeletal structure of the interneurons without aberrant growth. This is followed by a period of extensive growth (65–80 %) during which the basic scaffold is elaborated. Finally there is a maturation phase during which branches are pruned away to produce the mature interneuron structure. We show that, despite initial extensive overgrowth of branches, there is no branching into inappropriate neuropil regions in the embryo. The development of arborizations within specific neuropils appears to be tightly controlled. By using this information on interneuron growth and neuropil development it is now possible to begin to understand the developmental mechanisms that shape the neuronal architecture of the locust central nervous system.

1. INTRODUCTION

The organization of the thoracic ganglia of adult locusts is known in great detail so that the major tracts which merge to form connectives, the commissures (Tyrer & Gregory 1982) and the major areas of neuropil are described (Pflüger *et al.* 1988). This precise description of neuronal architecture has allowed detailed analyses of the projections from sensory neurons (Pflüger *et al.* 1988; Newland 1991) and the branches of spiking local interneurons (Burrows & Newland 1993) within the thoracic ganglia of adult locusts. Nevertheless, the development of the major anatomical features of the thoracic ganglia remains undescribed. Bastiani *et al.* (1984) described the early development of the neuropil and showed that some of the major tracts were identifiable as early as 45 % embryonic development, but did not extend their study to later stages of development.

The aim of this paper, therefore, is to describe the later embryonic development of the major anatomical

features of the thoracic ganglia and provide a framework for the analysis of the development of the many neurons known from studies of the adult central nervous system (CNS).

Onto this background we have also mapped the development of an identified group of local interneurons to help elucidate some of the developmental processes that shape the structure of the ganglionic neuropils. These interneurons belong to the midline group of spiking local interneurons (Burrows & Siegler 1984; Siegler & Burrows 1984) and receive mono-synaptic inputs from particular arrays of sensory neurons on a leg which define their receptive field (Burrows 1985; Burrows & Siegler 1985). Each interneuron has a distinctive receptive field that partly overlaps the receptive fields of other interneurons in the group, so that the surface of a leg is mapped into the CNS as a series of overlapping receptive fields (Siegler & Burrows 1984). All the interneurons in this group are derived from a single neuroblast during embryonic development (Shepherd & Laurent 1992)

and are fully differentiated and functional at hatching (Leitch *et al.* 1992). There are three distinct but successive phases in their development: an initial period of slow growth during which the basic scaffold of the interneuron is assembled (50–70%); a short period of rapid and expansive branch growth (70–80%); and finally a period of maturation during which some of the initially exuberant branch growth is pruned (80% to hatching) (Shepherd & Laurent 1992). Here we analyse the development of these interneurons in relation to the organization of the major structural features of the ganglionic neuropil. The work shows that the organizational groundplan of these interneurons, i.e. the location of the major axonal and dendritic branches, is established early in development by direct outgrowth of the primary growth processes without extensive growth into inappropriate regions of neuropil. Once the basic scaffold is established there is extensive elaboration of new branches which serve to establish the complex branching patterns of the functional neuron. Following this extensive growth there is a period of remodelling during which many branches formed earlier in development are apparently pruned away to produce the structure typical of these neurons in the adult.

By combining information from the studies on neuropil development and axon growth it is possible to visualize more clearly the processes that shape the organization of the developing CNS. It is intended that this work will provide new insight into the early development of the insect CNS and thus the developmental mechanisms that shape the structure and function of insect central neurons.

2. MATERIALS AND METHODS

Embryos were dissected from eggs obtained from our crowded colony of locusts (*Schistocerca gregaria* Forskål). The eggs were removed from tubes of moist sand in which they had been laid and were cultured on damp filter paper at a constant temperature of 30 °C. Embryos were removed from the eggs as required and staged according to the developmental timetable of Bentley *et al.* (1979).

The developing tracts, commissures and neuropils were analysed by staining pro- and mesothoracic ganglia by the osmium and ethyl gallate method of Wigglesworth (1957, 1959). Ganglia from staged embryos were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at room temperature and then in 1% osmium tetroxide in 0.1 M phosphate buffer for a further 1–2 h at 4 °C. They were then transferred to a saturated solution of ethyl gallate for 2–3 h and subsequently dehydrated and embedded in a soft mixture of Araldite. Serial sections (5 µm) were cut on an LKB V ultratome and drawn and photographed with a Zeiss Axiophot microscope. The nomenclature of the commissures, tracts and neuropils is that used by Tyrer & Gregory (1982) and Pflüger *et al.* (1988).

Spiking local interneurons of the midline group were identified in isolated nervous systems from embryonic locust as described in Leitch *et al.* (1992) and Shepherd & Laurent (1992). Thoracic ganglia were dissected from staged embryos and restrained ventral side upmost in a Sylgard 'coffin slide' filled with embryonic locust saline (Raper *et al.* (1983*a*). The soma of an interneuron at the ventral midline of the pro- or mesothoracic ganglion was impaled with a glass

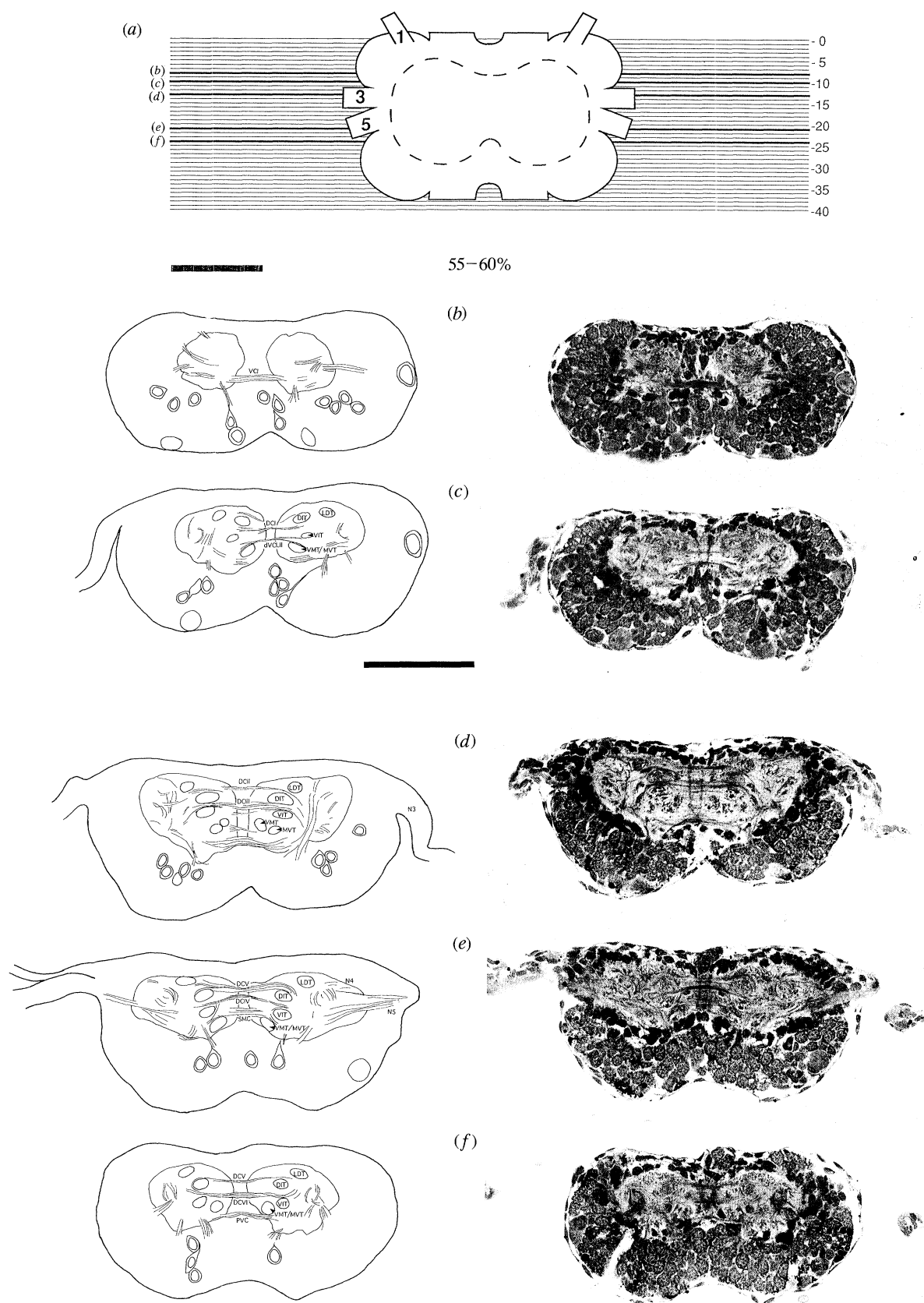
electrode filled with a 6% solution of hexaminecobalt chloride. In early embryos (before 70%), the somata were observed in a fixed stage microscope with Nomarski optics and a ×40 water immersion objective. In later stages (after 70%), somata were impaled under a binocular microscope and successful penetration was confirmed by measurements of resting potential and action potentials. In embryos older than 75%, the ganglionic sheath was treated for approximately 1 min with a 1% solution of protease (Sigma type XIV) in saline. The cobalt was injected by using 0.5 s pulses of positive current (1–2 nA) at 1 Hz for 2–5 min and the ganglia were processed for histology and Timm's silver intensification (Pitman *et al.* 1972; Bacon & Altman 1977). The morphology of the stained interneuron was checked by using a binocular microscope to confirm that it conformed to the basic structure of a midline spiking local interneuron (Burrows & Siegler 1984; Burrows & Watkins 1986). The fixed ganglia were then dehydrated and embedded in soft Araldite. Serial sections (5 µm) were cut on an LKB V ultratome and examined with a Zeiss Axiophot microscope. Sections of representative interneurons from five different embryonic stages (between 50 and 100% development) were then drawn in detail with a camera lucida at ×400. All ganglia are drawn to the same scale for direct comparison and the levels at which sections selected for display were taken are indicated by the thick horizontal lines in figures 1*a*–5*a*.

3. RESULTS

(*a*) Identification of commissures and tracts in the embryo

(i) Transverse commissures

The six major dorsal commissures DCI–DCVI of adult ganglia (Tyrer & Gregory 1982; Pflüger *et al.* 1988) are present in the earliest (55%) and the latest (95%) embryonic stages that were examined (figures 1–5). DCI is the most anterior dorsal commissure and in adult locusts is composed of two bundles of fibres that cross the midline, dorsal (dDCI) and ventral (vDCI) to the dorsal median tract (DMT), and a second pair of fibres that cross diagonally between the DMTs (Pflüger *et al.* 1988). Both the dorsal and ventral bundles of DCI can be identified in later embryonic stages (> 65%) (figures 4*c*, 5*c*), but in earlier stages the distinction is less clear. The T-tract separates DCI from the more posterior dorsal transverse tracts as it runs along the midline between DCI and DCIII and turns laterally, ventral to DCII. DCII and DCIII occur at the same level in the ganglia and can be identified in all embryonic stages older than 50% (figures 1*d*, 2*d*, 3*d*, 4*d*, 5*d*). DCII is dorsal and DCIII is ventral to DMT and the dorsal intermediate tract (DIT). DCIV is separated from DCII and DCIII by a midline division of the two halves of the neuropil with glial cells and tracheae. At this level the C-tracts which are prominent in adult ganglia are also evident in the embryonic ganglia. DCIV comprises a large bell-shaped bundle of fibres which splits laterally into a ventral group that is median to the ventral intermediate tract (VIT) and which runs laterally between VIT and the ventral median tract/median ventral tract (VMT/MVT) group. The more dorsal bundle of fibres runs laterally between DIT and VIT. This bell-shaped arrangement of fibres in DCIV becomes



Figures 1–5. Embryonic developmental stages 55–60%, 60–65%, 65–70%, 70–75% and 90–95% respectively. (a) Drawings of the ventral view of mesothoracic ganglia from 55–60%, 60–65%, 65–70%, 70–75% and 90–95% development embryos, cut into 5 µm transverse sections. The levels within the ganglia at which sections (b)–(f)/(g) were taken are indicated by the thick horizontal lines. All ganglia are drawn to the same scale for direct comparison. The remaining parts of the figures show camera lucida drawings (left) and photographs (right) of selected sections (shown in (a)) stained with osmium and ethyl gallate. Sections are taken at the equivalent levels in the ganglia for each developmental stage. Scale bars, 100 µm; abbreviations used are listed in table 1.

Table 1.

areas of neuropil	LAC	lateral association centre
	aLAC	anterior part of lateral association centre
	pLAC	posterior part of lateral association centre
	VAC	ventral association centre
	aVAC	anterior part of ventral association centre
	IVAC	lateral part of ventral association centre
	mVAC	median part of ventral association centre
	vVAC	ventral part of ventral association centre
commissures	DCI–VI	dorsal commissures I–VI
	dDCI	dorsal part of dorsal commissures I
	vDCI	ventral part of dorsal commissures I
	PVC	posterior ventral commissure
	SMC	supramedian commissure
	VCI	ventral commissure I
	VCII	ventral commissure II
	VCLII	ventral commissural loop II
	dVCLII	dorsal part of ventral commissural loop II
	vVCLII	ventral part of ventral commissural loop II
longitudinal tracts	DIT	dorsal intermediate tract
	DMT	dorsal median tract
	dDMT	dorsal part of dorsal median tract
	vDMT	ventral part of dorsal median tract
	LDT	lateral dorsal tract
	iLVT	inner lateral ventral tract
	oLVT	outer lateral ventral tract
	MDT	median dorsal tract
	MVT	median ventral tract
	VIT	ventral intermediate tract
	VLT	ventral lateral tract
	VMT	ventral median tract
nerve roots	N1	nerve 1
	N1ii	nerve 1, root ii
	N2	nerve 2
	N2ii	nerve 2, root ii
	N3	nerve 3
	N3i–iv	nerve 3, roots i–iv
	N4	nerve 4
	N5	nerve 5
	N5i–iv	nerve 5, roots i–iv

distinctive only in embryos > 70% (figures 4*e*, 5*e*) although the lateral split in the DCIV fibre bundle is present in 55–70% embryos (figures 1*e*, 2*e*, 3*e*). DCV is dorsal and posterior to DCIV and passes laterally between DIT and the median dorsal tract (MDT) (figures 4*f*, 5*f*, *g*). In embryos < 70%, MDT is not well developed but DCV can still be identified by its position dorsal to DIT (figures 1*e*, *f*, 2*e*, *f*, 3*e*). DCVI is ventral and posterior to DCV and passes between DIT and VIT (figures 1*f*, 2*f*, 3*f*, 4*f*, 5*g*).

The four ventral transverse commissures of the adult ganglia (ventral commissure I (VCI), ventral commissure II (VCII), supramedian commissure (SMC) and posterior ventral commissure (PVC)) are also identifiable in embryos older than 50%. VCI is the most anterior (figures 1*b*, 2*b*, 3*b*, 4*b*, 5*b*). VCII is just posterior to VCI at the same level as DCI (figures 1*c*, 2*c*, 3*c*, 4*c*, 5*c*). It forms a more or less vertical ring with two commissural components, dorsal and ventral (dVCII, vVCII). SMC is at the same level as DCIV but ventral to it, and dorsal to VMT/MVT (figures 1*e*, 2*e*, 3*e*, 4*e*, 5*e*, *f*). PVC is at the same level as DCVI and runs ventral to VMT/MVT (figures 1*f*, 3*f*, 4*f*, 5*g*).

(ii) *Longitudinal tracts*

In adult locusts four large and five smaller longitudinal tracts run through each half of the ganglion (see table 1). In early (55–65%) embryonic ganglia only DIT, VIT, the lateral dorsal tract (LDT) and the VMT/MVT group of tracts are clearly distinguishable (figures 1 and 2). In 65–70% embryos, DMT is also present but poorly defined (figure 3). By 70% all the major longitudinal tracts can be readily identified plus DMT, VMT, MVT and the ventral lateral tract (VLT) (figure 4). The lateral ventral tract (LVT), however, is difficult to distinguish. All of the longitudinal tracts are readily recognizable in the ganglia of 95% embryos (figure 5).

(b) *Identification of the principal neuropils in the embryo*

The fine fibrous areas of the ganglion, or neuropils, are regions where synaptic interactions between neurons take place. Morphological criteria can be used to subdivide the various neuropil regions in adult locust (Pflüger *et al.* 1988) and we adopt this terminology here.

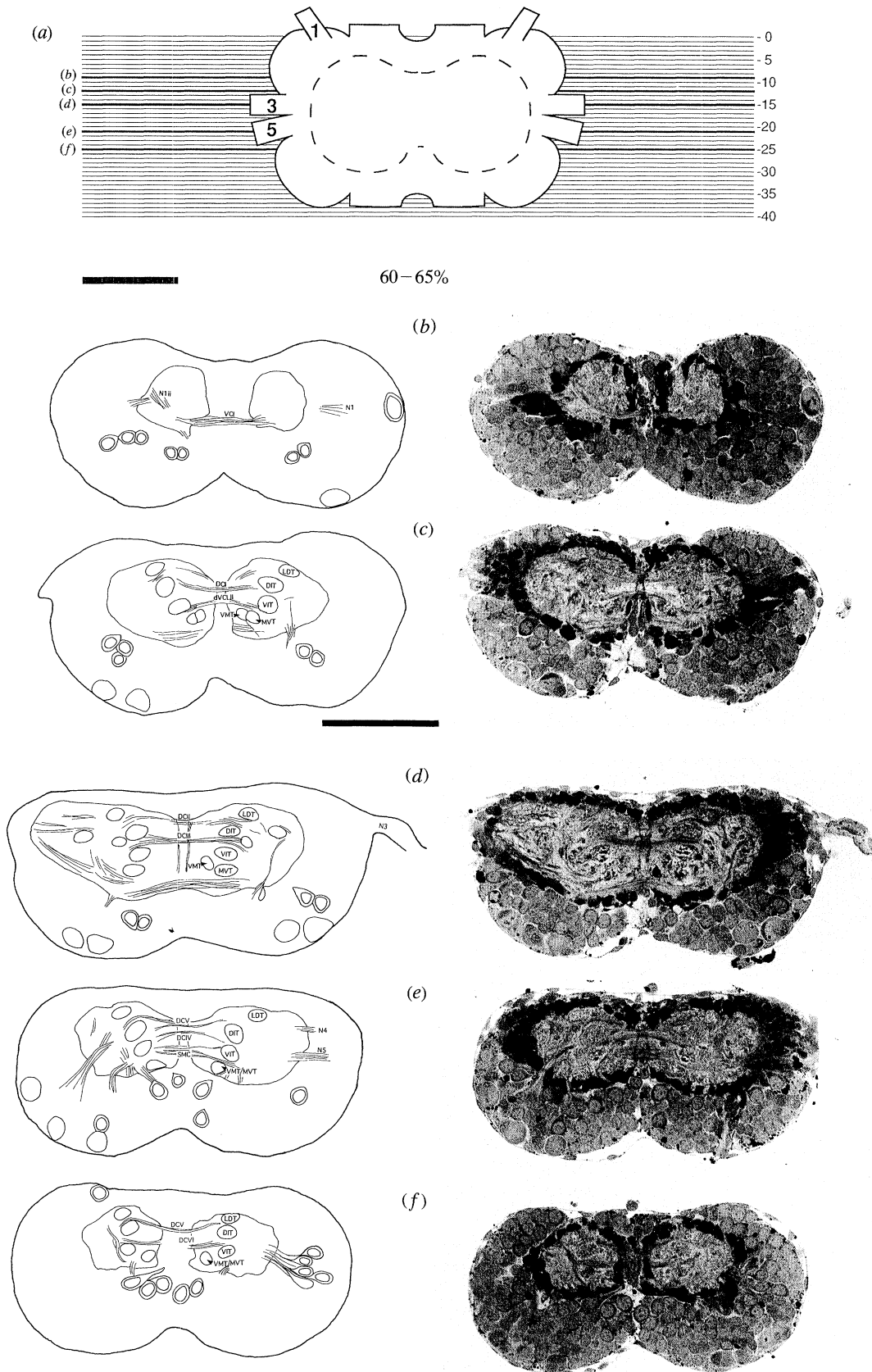


Figure 2. For description see figure 1.

(i) *Ventral association centre (VAC)*

The ventral neuropils are not clearly formed in embryos < 65 % (figures 1, 2). They are first evident at 65–70 % development but even at this stage are poorly defined (figure 3). By 70 %, however, their borders are more easily identified. In 70–95 % embryos, aVAC is

ventral to VMT/MVT, is bordered in part by VCI and VCII (figures 3*c*, 4*c*, 5*b*, *c*) and extends posteriorly as far as the T-tract. At the level of DCI it merges laterally with IVAC (figure 5*c*). mVAC is dorsal to aVAC, between VIT and VMT/MVT (figures 3*c*, 4*c*, 5*c*) and extends from the level of DCI to

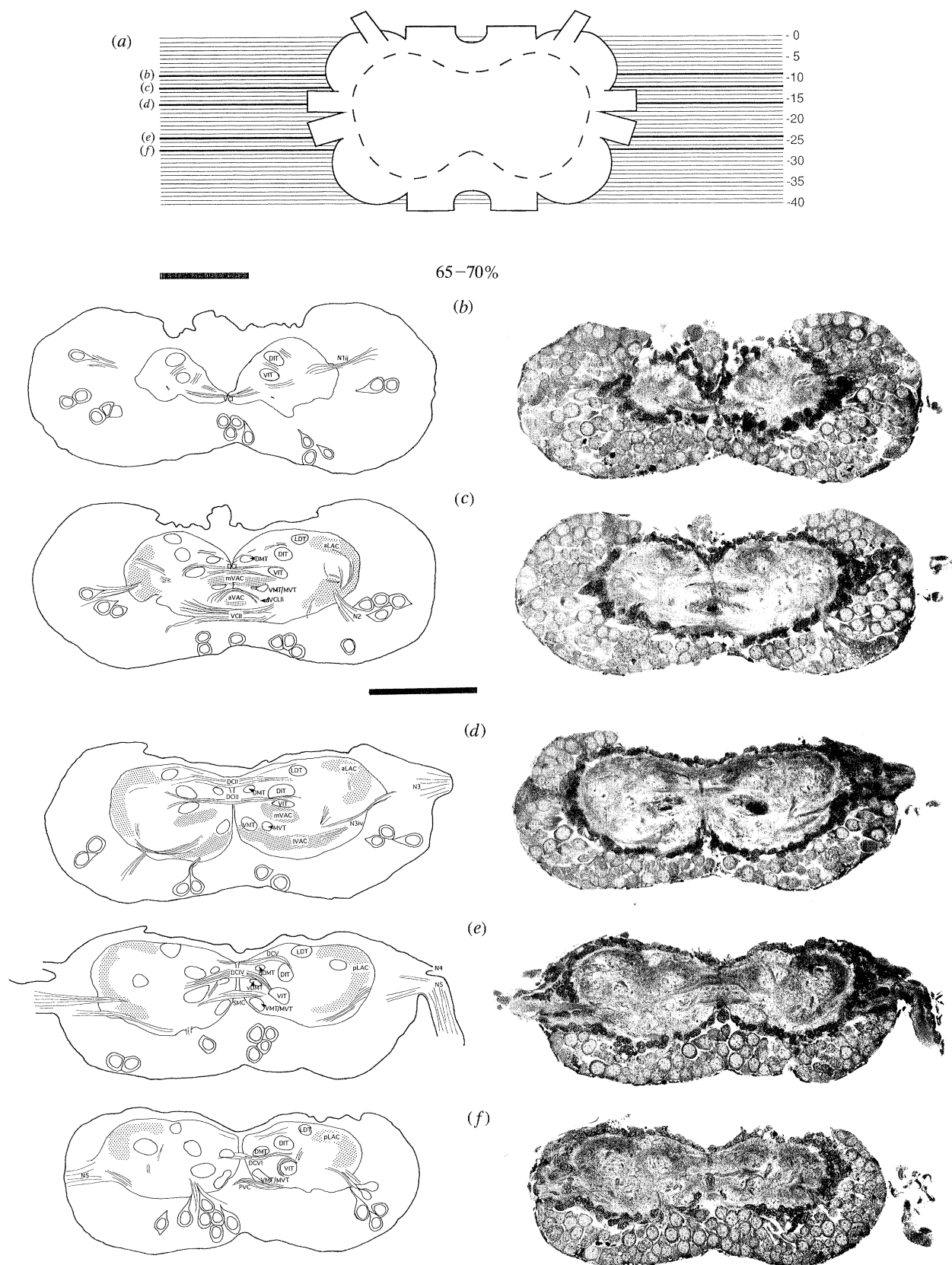
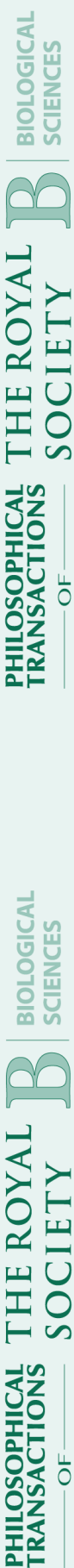


Figure 3. For description see figure 1.

DCII/DCIII (figures 3c, d, 4c, d, 5c, d). vVAC is ventral to aVAC and VCLII (figure 5c) and extends from the same level as DCI to DCII/III (figure 5c, d). Its medial, dorsal edge is bordered by iLVT and its ventral, lateral edge by oLVT. IVAC, which merges

with the lateral edges of aVAC and vVAC, extends laterally to the edge of the neuropil (figures 4c, d, 5c, d). In the embryonic ganglion it continues posteriorly to the level of nerve 3.



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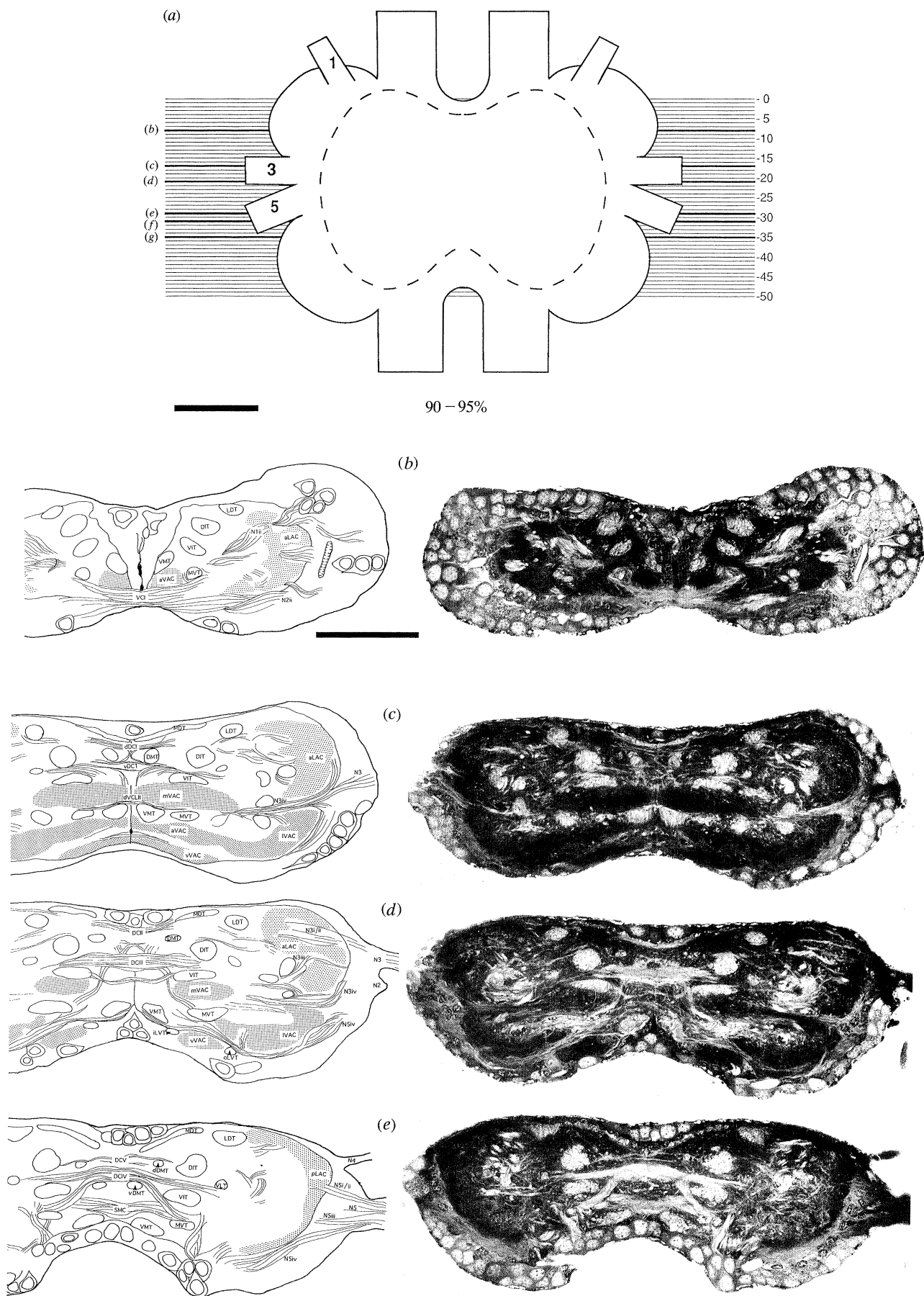


Figure 5. For description see figure 1.

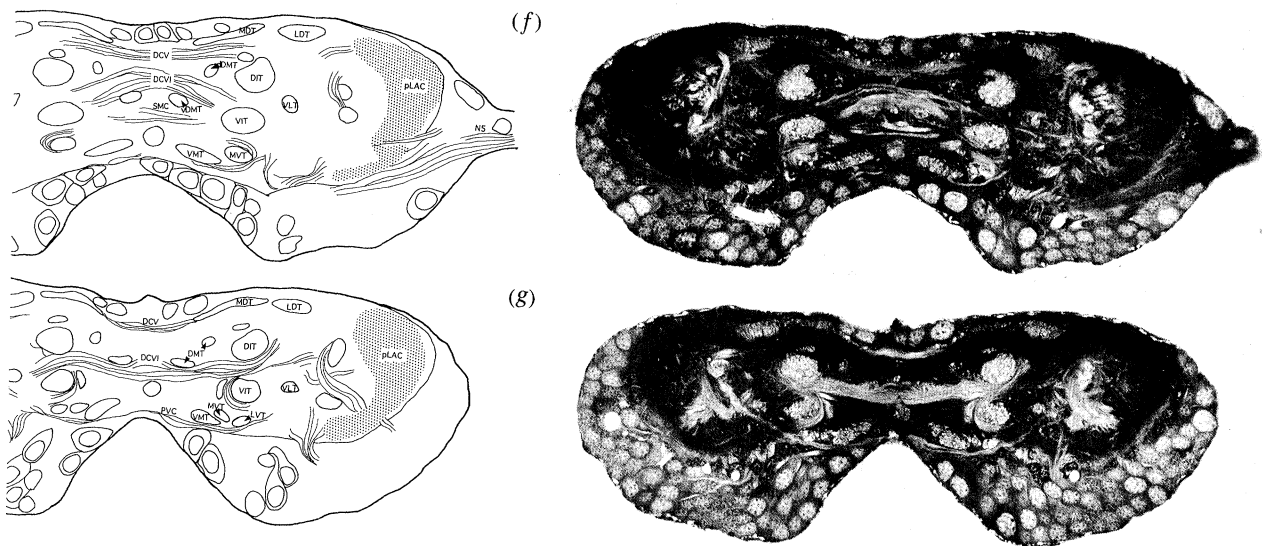


Figure 5. Continued

(ii) *Lateral association centre (LAC)*

The most lateral areas of dorsal neuropil (LAC) (Pflüger *et al.* 1988) in adults are not as densely packed and uniform and hence not as clearly defined as the ventral neuropils. LAC is distinguishable in embryos > 65% (figures 3, 4, 5). aLAC is evident in sections anterior and level with nerve 3 (figures 3*c*, *d*, 4*b–d*, 5*b–d*). pLAC starts at the same level as nerves 4 and 5 and continues posteriorly to the margins of the ganglionic core (figures 3*e*, *f*, 4*e*, *f*, 5*e–g*).

(c) *Morphogenesis of the spiking local interneuron branches in relation to the embryonic neuropils*

In adults, the midline spiking local interneurons can be identified by their cell bodies at the ventral midline, primary neurites that cross the midline in VCI and their distinct ventral and dorsal fields of branches.

(i) 60–65%

In 60–65% embryos (figure 6*a–e*), distinct dorsal and ventral fields of branches are already evident, with little or no overlap (figure 6*c*). The ventral field is small, consists of a large number of short unbranched processes emanating from the primary neurite as secondary processes and extends anteriorly to DCI and posteriorly to DCIV (figures 6*b–d*). The dorsal field is more extensive, extending anteriorly to DCI (figure 6*b*) with a few branches reaching as far as VCI (figure 6*a*), and posteriorly to DCVI (figure 6*e*). The branches cannot be referred to particular neuropils as these are not yet recognizable.

(ii) 65–70%

By 65–70% development, the embryonic neuropils can be identified (figure 7*a–e*). The dorsal and ventral fields are more extensive now owing to an increase in the number and length of the branches, but are still distinct and do not overlap. The branches of the ventral field extend anteriorly as far as DCI and

posteriorly to DCV and are predominantly in IVAC (figure 6*a–d*). The branches of the dorsal field are mainly within aLAC (figure 7*a–c*) although a few extend into pLAC (figure 7*d–e*).

(iii) 70–75%

Between 70 and 75% development there is an enormous expansion of the dorsal and ventral fields of branches (figure 8*a–e*). The distinction between the dorsal and ventral fields of branches becomes difficult to resolve because both fields fill almost the entire ganglion. The ventral field is particularly dense and extends from VCI (figure 8*a*) to DCVI (figure 8*e*) with branches in IVAC and pLAC. The dorsal branches extend to the outer dorsolateral limits of aLAC and pLAC neuropils (figure 8*b–d*). Such extreme branching is never seen in adults or later embryos.

(iv) 80–85%

By 80–85% development, there is a marked reduction in the number of branches (figure 9*a–e*) and the dorsal and ventral fields can again be resolved. The dorsal field still extends anteriorly to VCI (figure 9*a*) with most branches in aLAC and a few in pLAC. The ventral field, however, only extends as far as DCI (figure 9*b*), indicating that the most anterior branches have been withdrawn. The ventral branches are exclusively located in IVAC and vVAC (figure 9*b*). At this stage in development, variation in the relative sizes of the different branches is evident. It is also at this time that the dorsal branches begin to show the beaded appearance indicative of the synaptic varicosities that typify the adult dorsal branches.

(v) 90–95%

The reduction in ventral and dorsal branches is very obvious now (figure 10*a–e*). The dorsal field of

branches is restricted to aLAC (figure 10*a–c*) and the ventral branches to IVAC and vVAC (figure 10*b–d*).

4. DISCUSSION

In this paper we have described the embryonic development of the major tracts, commissures and neuropils of the thoracic ganglia of the locust.

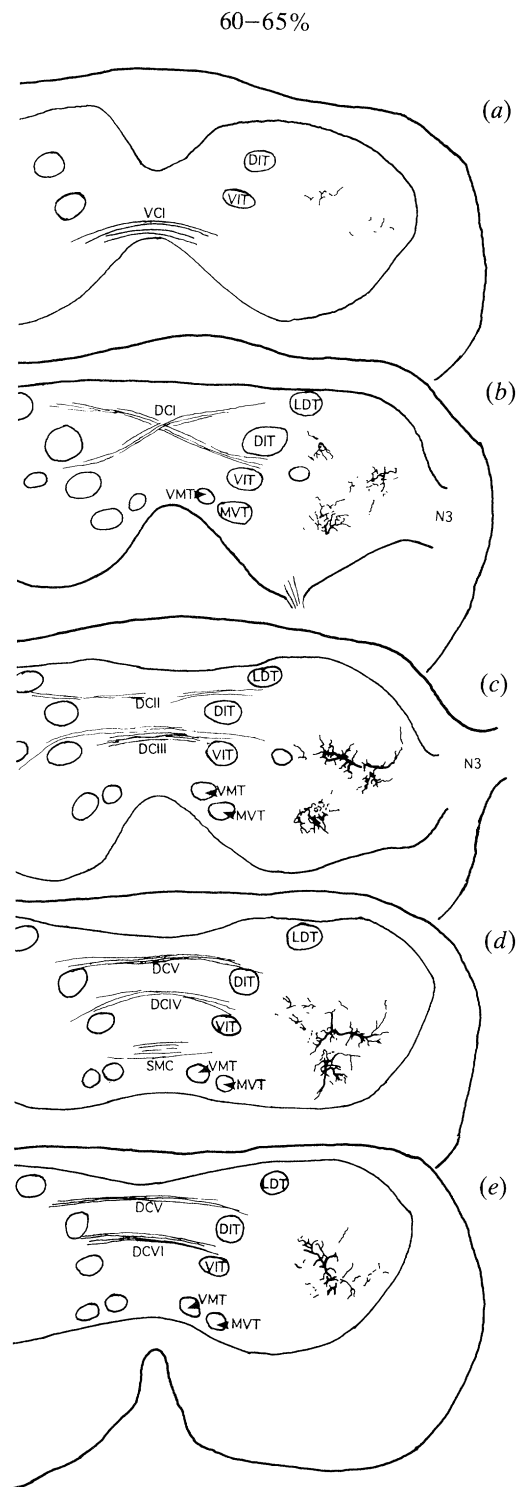
(a) *Development of the tracts and commissures*

The major axon pathways are assembled early in embryonic development. All dorsal and ventral commissures and all longitudinal tracts, with the exception of DMT and MDT, can be recognized by 55% development. The longitudinal tracts can even be seen as early as 42% development (Bastiani *et al.* 1984). They were able to recognize MDT at 42%, but we were unable to detect this tract or DMT clearly before 65%. The difference may not reflect any developmental process but may result from the different species of locust used or possibly the staining methods employed.

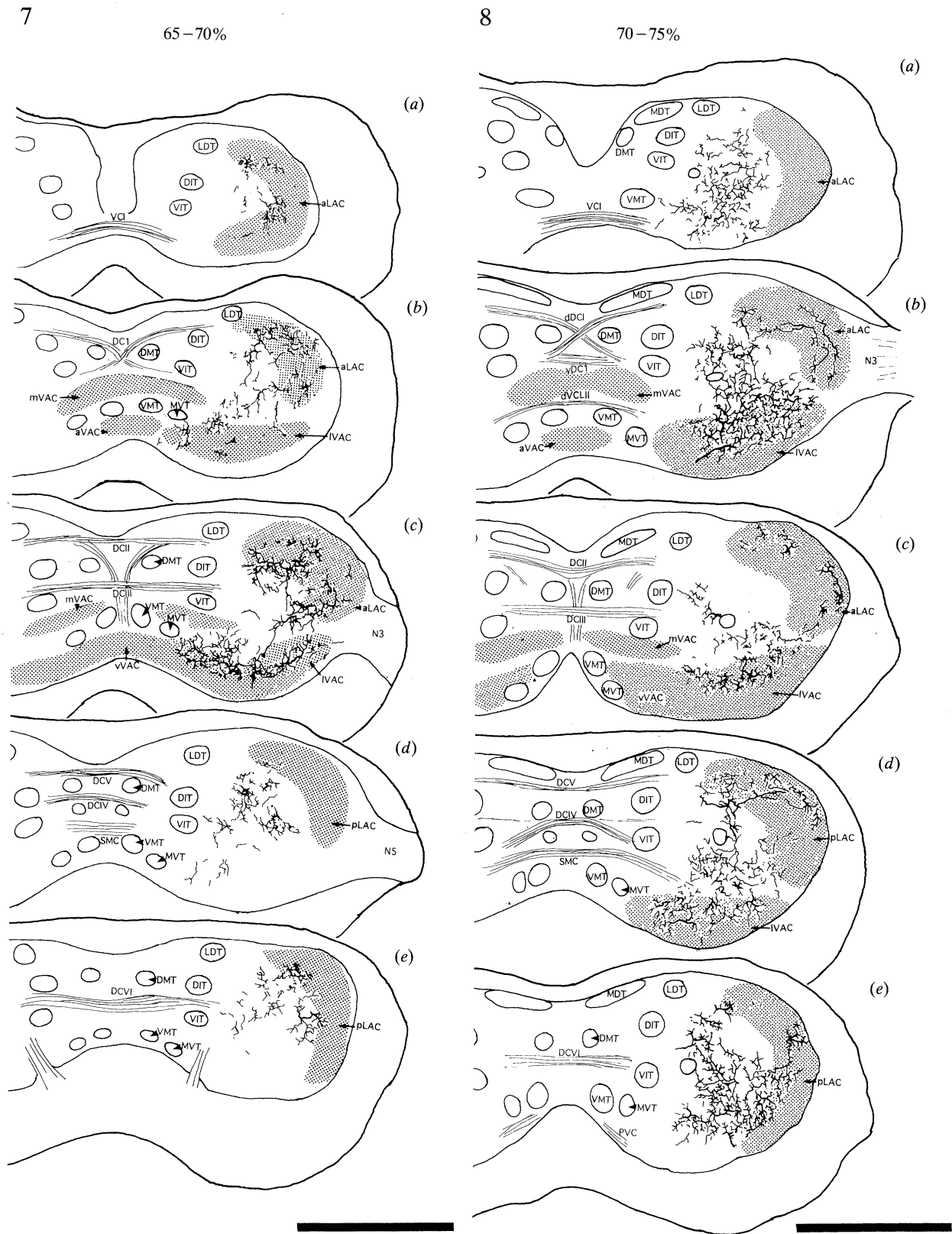
It seems likely that these tracts are founded by the growth of early developing pioneer axons of the CNS which establish a simple axonal scaffold during the earliest stages of axon growth (30–50%) (Raper *et al.* 1983*a, b, c*; Jacobs & Goodman 1989*a, b*). The scaffolding is used by the later developing axons for guidance to their target area. This process results in the bundling together of axons to form fascicles, whose pattern in early embryos is highly invariant (Raper *et al.* 1983*a, b*; Bastiani *et al.* 1984). These fascicles not only act as guidance cues for growing axons but also prefigure most of the known tracts and commissures and, therefore, the fascicles eventually expand to become the tracts. Since there are many more fascicles than tracts in the adults CNS, one must assume that fascicles fuse. Despite the possibility of a direct association between identified fascicles and the establishment of the tracts and commissures, remarkably little is known about any possible relation; the A/P fascicle forms part of the adult LDT, the vMP2 fascicle forms part of the VMT and the MP1/dMP2 fascicle forms part of the VIT Bastiani *et al.* (1984). Clearly it would prove a valuable exercise to examine the emergence of the major tracts.

(b) *Development of the neuropils*

In contrast to the major tracts and commissures the emergence of the major neuropil regions is a relatively late event. Even by 65–70% they are only barely distinguishable and do not become distinct until 70–75%. Whereas the establishment of tracts and commissures is a product of early axon growth, the neuropils are regions of synaptic interactions and their emergence is, therefore, likely to be linked to the development of synaptic structures and interactions. We know from previous studies (Leitch *et al.* 1992) that the development of anatomical synapses to and from these local interneurons occurs relatively late in embryonic development. Although immature synapses are seen at 65–70% development, the earliest mature



Figures 6–10. Embryonic developmental stages 60–65%, 65–70%, 70–75%, 80–85% and 90–95% respectively. Camera lucida drawings of selected sections through the mesothoracic ganglia from the different developmental stages in which a midline spiking local interneuron has been filled with cobalt. Sections have been selected at the levels of the major dorsal commissures DCI–DCVI. All sections are taken at the equivalent levels in the ganglia for each developmental stage. Scale bar, 100 μ m; abbreviations used are listed in table 1.



Figures 7 and 8. For description see figure 6.

synaptic contacts, with vesicles, presynaptic bar and postsynaptic density, are not seen until 70–75% and then only rarely. The emergence of the neuropils is also coincident with a period of massive neuron growth as seen both in the development of the local interneurons (Shepherd & Laurent 1992) and also in the arrival in the CNS of most of the sensory axons from the legs (D. Shepherd, unpublished observation).

(e) *Development of the local interneurons*

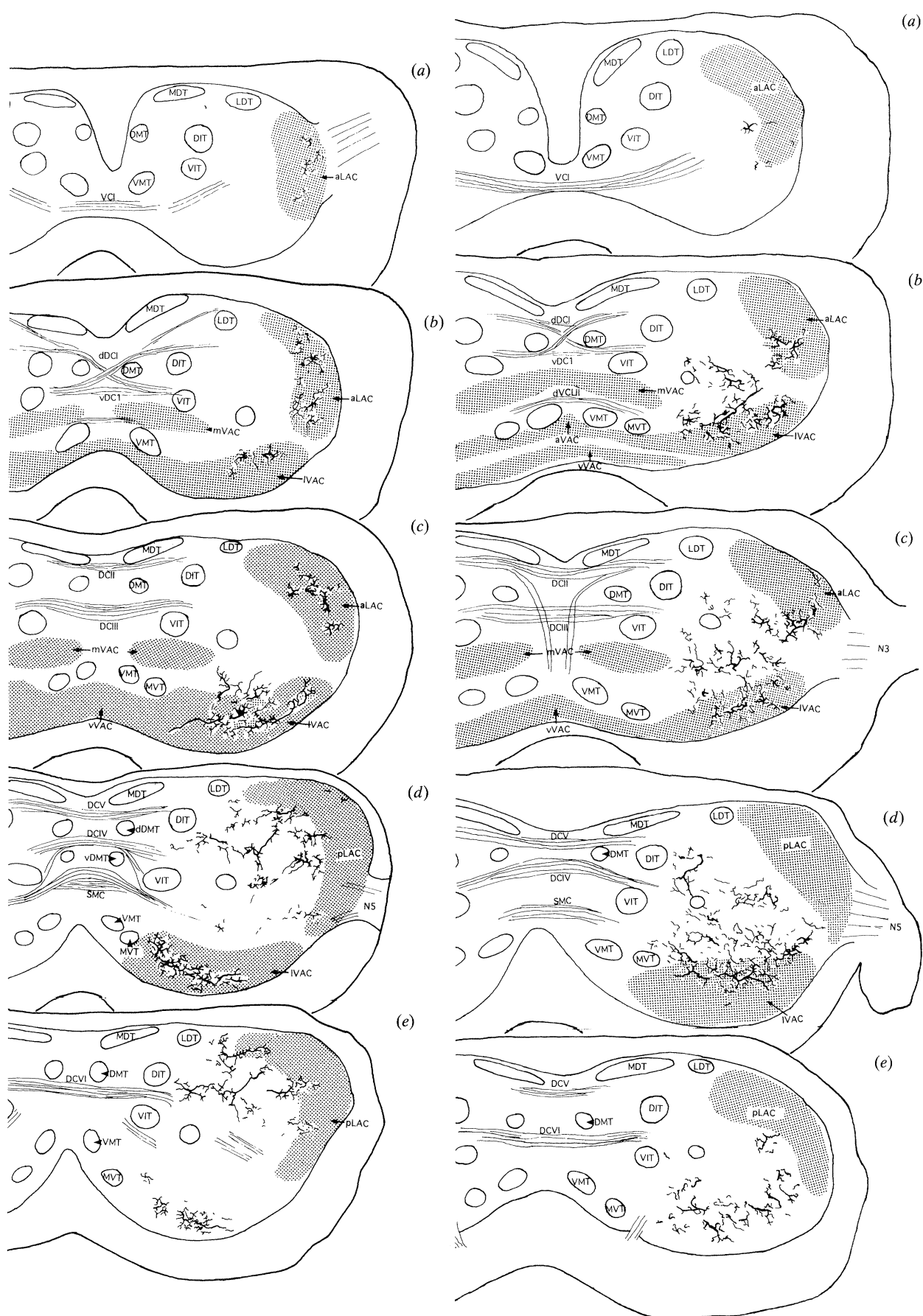
The observations of the growth of the local interneurons complements the previous studies of their development in wholemount ganglia (Shepherd & Laurent 1992). This work showed that all members of the group undergo exactly the same sequence of development and identified three distinct periods of

9

80–85%

10

90–95%



Figures 9 and 10. For description see figure 6.

local interneuron development: an early phase (50–65%) in which the basic scaffolding of the neuron's structure is assembled; a second shorter period (70–80%) of rapid growth during which there is a large and rapid expansion of the initial structure by multiple secondary and tertiary neurites; and finally a period of maturation (85%-hatching) during which excess branches are apparently pruned away just before hatching to produce a mature looking structure.

In the sectioned material presented here, we gain a new insight into some of the details of this growth process. The material illustrates clearly that the separate dorsal and ventral fields of branches arise very early in development during the phase of building the basic structural skeleton of the neurons. We also see that the two fields of arborizations grow directly into the regions of neuropil typical of the adult, i.e. ventral branches are predominantly located in the IVAC and the dorsal branches located within the aLAC and pLAC (Sieglar & Burrows 1984; Newland & Burrows 1993). There is little evidence of branching into inappropriate regions. We see, therefore, that the development of arborizations within specific neuropils is tightly controlled suggesting that the same mechanisms of selective adhesion that guide axons into the correct fascicles during early outgrowth (Raper *et al.* 1983*a, b, c*) may also guide growth directly to the appropriate neuropil regions. Since the region of neuropil in which a neuron terminates has an important role in determining the synaptic connections that the neuron can receive and make (Burrows & Newland 1993), these observations suggest selective adhesion may serve an important role in determining a neuron's synaptic connections.

The sectioned material also clearly illustrates the phases of rapid growth and the subsequent retraction of branches. During the period 70–75% excess branching is clear, particularly in the ventral field where the branching is denser than that seen at any other stage of development, both before and after. There is also considerable overlap of branches from dorsal and ventral fields such that it is no longer possible to resolve the two separate fields, as seen earlier in development. This is certainly not typical of these neurons when observed in the adult. The re-emergence of distinct dorsal and ventral fields later in embryonic development (post 80%) is a clear indication that there is a retraction of the earlier profuse growth. These local interneurons are not the first insect neurons to have their embryonic development described; there have been a number of comparable studies (Bentley & Toroian-Raymond 1981; Shankland & Bentley 1981; Raper *et al.* 1983*a*). Most of these studies concentrated on the development of the intersegmental interneurons and showed that the basic details and timing of the local interneuron development are comparable in many ways to these other neurons. The local interneurons do, however, differ in one important aspect. The profuse overgrowth and subsequent retraction of supernumary branches described for the local interneurons is not so obviously evident in developing intersegmental interneurons; indeed Shankland & Bentley (1981) specifically exclude this

possibility in their study of the development of the medial giant interneuron in locust. While this might suggest that the overgrowth and retraction of branches is a unique feature of local interneuron development it is likely that similar processes occur in the intersegmental interneurons but not on such a large or detectable scale. Either way what is clear is that the studies of the local interneurons have revealed the presence of a mechanism that may play an important role in shaping neuronal connectivity in the CNS of insects.

By looking at the development of the neuropil and the growth of the local interneurons in combination we can see how the two sets of information serve to enhance our understanding of the processes that shape the CNS. For instance the early phase of CNS development sees the assembly of the key structural elements of the nervous system: in the neuropil this is the emergence of the major tracts and commissures. At this time the interneurons assemble their basic skeletal structure of axon pathways and arborizations into the appropriate neuropil region, in preparation for synaptogenesis. Later we see the elaboration of this basic structure with the emergence of the major neuropils, the areas of synaptic interactions. This is indicative of the assembly of the synaptic machinery required for the integrative functions of the CNS. This period coincides with the rapid expansion of the local interneuron arborizations and it is presumably this growth, along with that of other neurons, that is the causal factor in the emergence of the neuropil regions. Thus, it is possible to see that the emergence of the neuropils is the product of the rapid and expansive growth of neurons within the CNS. This proposition is supported by the fact that at this time the local interneurons begin the major phase of synapse formation (Leitch *et al.* 1992). The separate sets of data therefore serve to reinforce our understanding of the developmental processes.

(d) *Future prospects*

The observed rapid expansion of neuron structure and its subsequent retraction are comparable with observations of developing neurons in the CNS of vertebrates (reviewed in Constantine-Paton *et al.* (1990) and Shatz (1990)). In vertebrates this overgrowth and retraction has been associated with the process of synapse formation. In these systems, neurons are initially seen to form a widespread and diffuse pattern of synaptic connections which is subsequently refined by an activity dependent process to produce the more focused patterns of connections typical of the adult (Schmidt & Edwards 1983; Fawcett & O'Leary 1985; Kobayashi *et al.* 1990). We already know that, in locust, the period of rapid growth is coincident with the onset of synapse formation in these interneurons (Leitch *et al.* 1992) and that synapse formation continues throughout the period of maturation as excess branches are pruned away. Furthermore during the maturation phase it is also evident that there are changes in both the distribution and the ratio of input to output synapses on branches in the dorsal and

ventral fields (Leitch *et al.* 1992). Clearly, therefore, there are changes in connectivity going on during this phase and it is intriguing to wonder whether a process comparable with that in vertebrate CNS is occurring in insects. There has been a number of attempts to study this phenomenon in insects (for review see Lnenicka & Murphey 1989), most of which have involved surgical removal of presynaptic sensory neurons. These studies, while not proving activity dependence, show that development of the branching pattern of central neurons is dependent upon the physical presence of the presynaptic neurons for normal sensory innervation (Shankland *et al.* 1982). Other experiments (Chiba *et al.* 1988) have shown that synaptic connectivity of the cricket cercal sensory system undergoes considerable modification during postembryonic life. While this evidence indicates a possible involvement of activity dependent mechanisms it is not proof. Recently, however, Pflüger *et al.* (1994) have provided direct evidence of activity dependent changes in synaptic connectivity in postembryonic development of synaptic connections in the locust CNS. Given this precedent it is likely that a study of the role of activity in shaping the receptive fields of these local interneurons may prove profitable. After all, these neurons present an interesting case; all 30+ members of this group of local interneurons are clonally derived from a single neuroblast (Shepherd & Laurent 1992) and are sometimes difficult to identify as individuals in the classic sense of insect neurons and are thus recognized only as a class of interneuron. Yet each interneuron has its own unique receptive field of inputs. This raises the question as to whether each neuron's receptive field is defined by its position in the lineage of its parental neuroblast according to the general theory of insect neurogenesis (Doe *et al.* 1985) or whether all members of the group are born equivalent and their fate (i.e. receptive field) determined by an interactive process, perhaps activity dependent, during the period of rapid growth and subsequent restructuring?

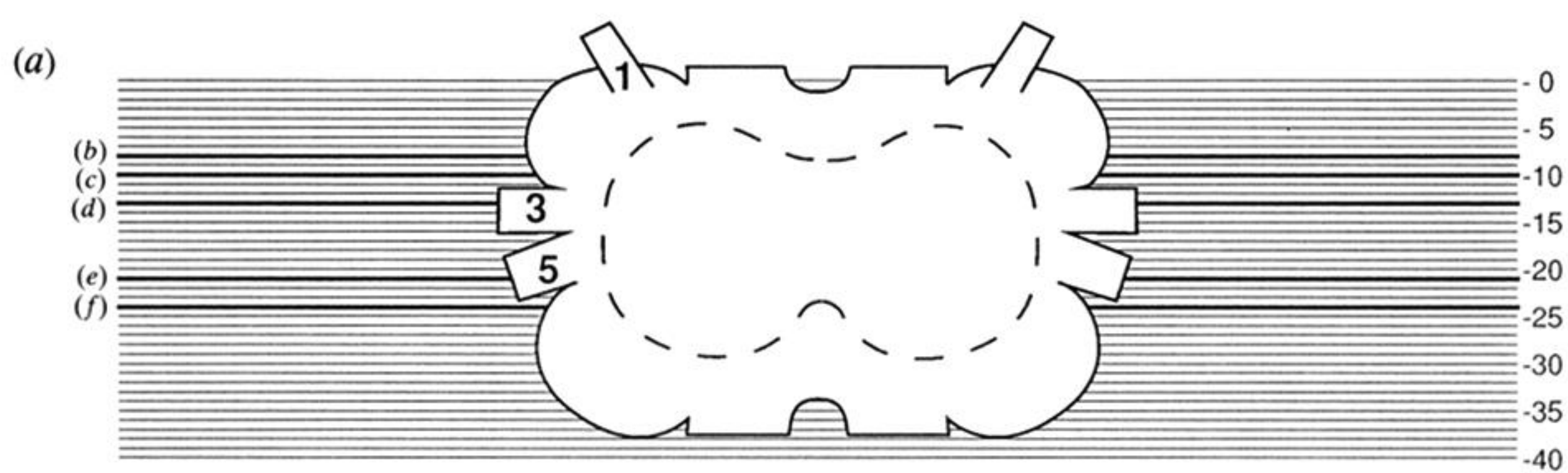
The main part of this work was done at the University of Cambridge Department of Zoology and was supported by a grant from the S.E.R.C. U.K. We are indebted to Professor Burrows for his advice and comments on this paper and we thank N. Maskell and F. Wei, Department of Zoology, University of Cambridge, for printing the photographs.

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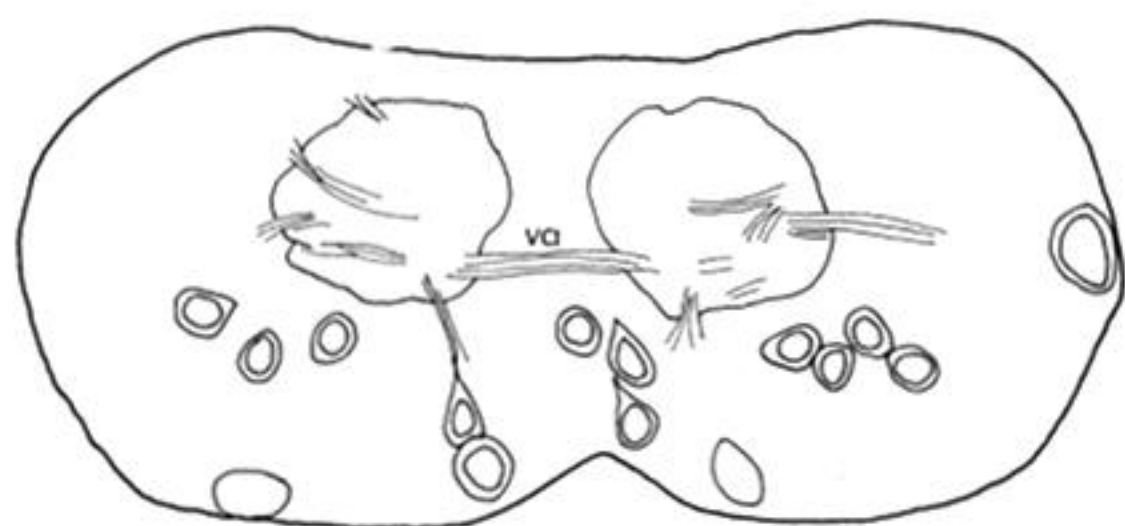
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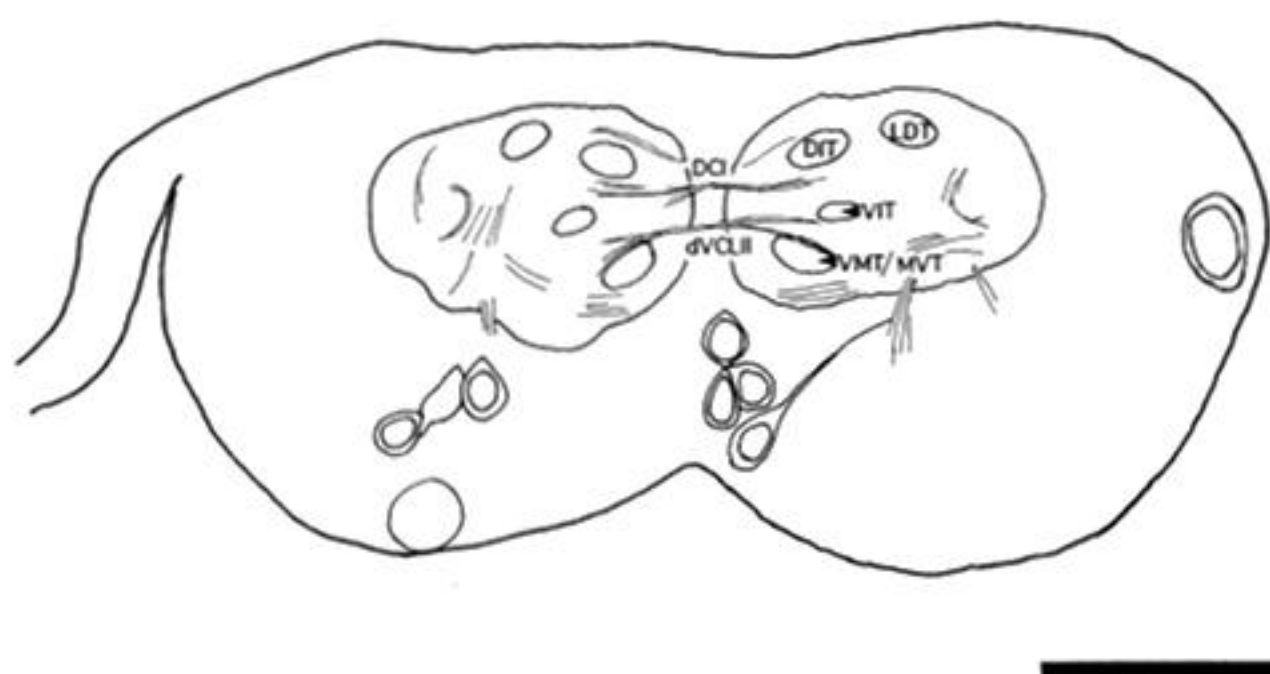
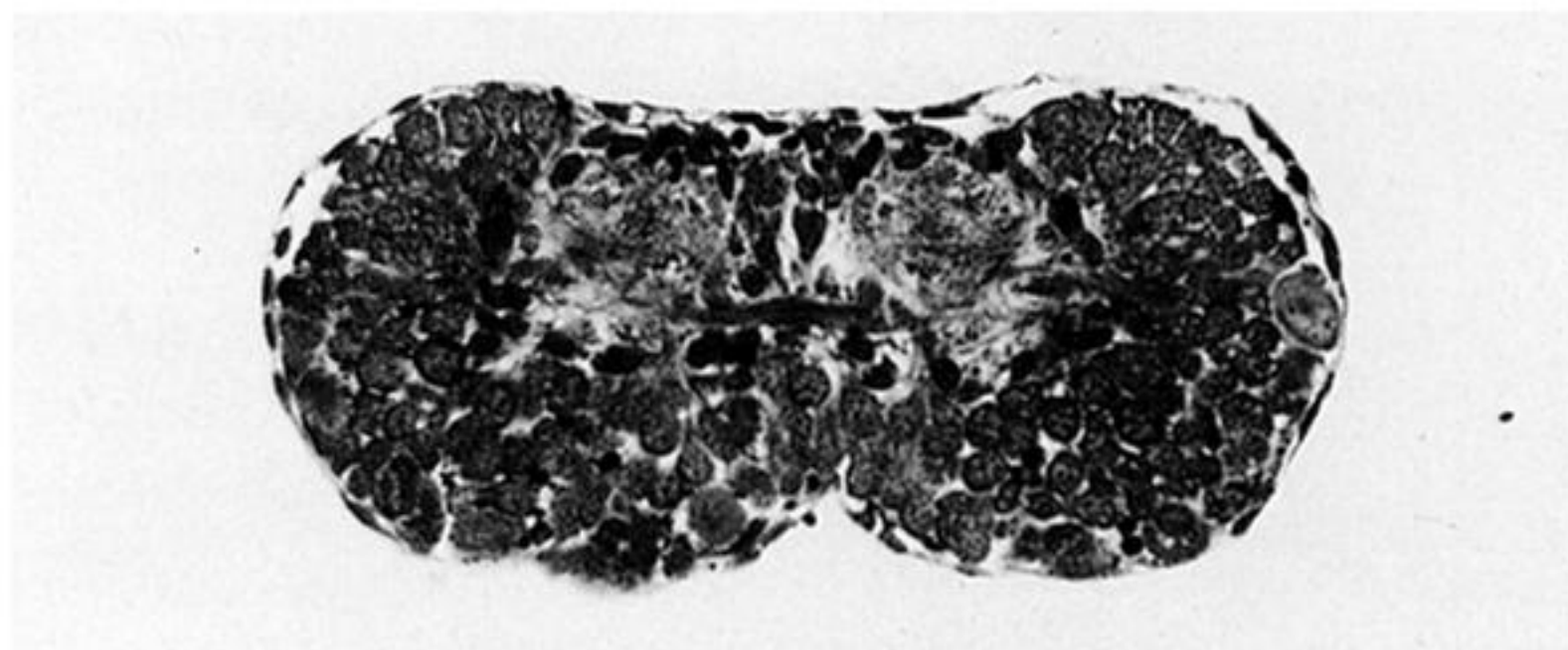


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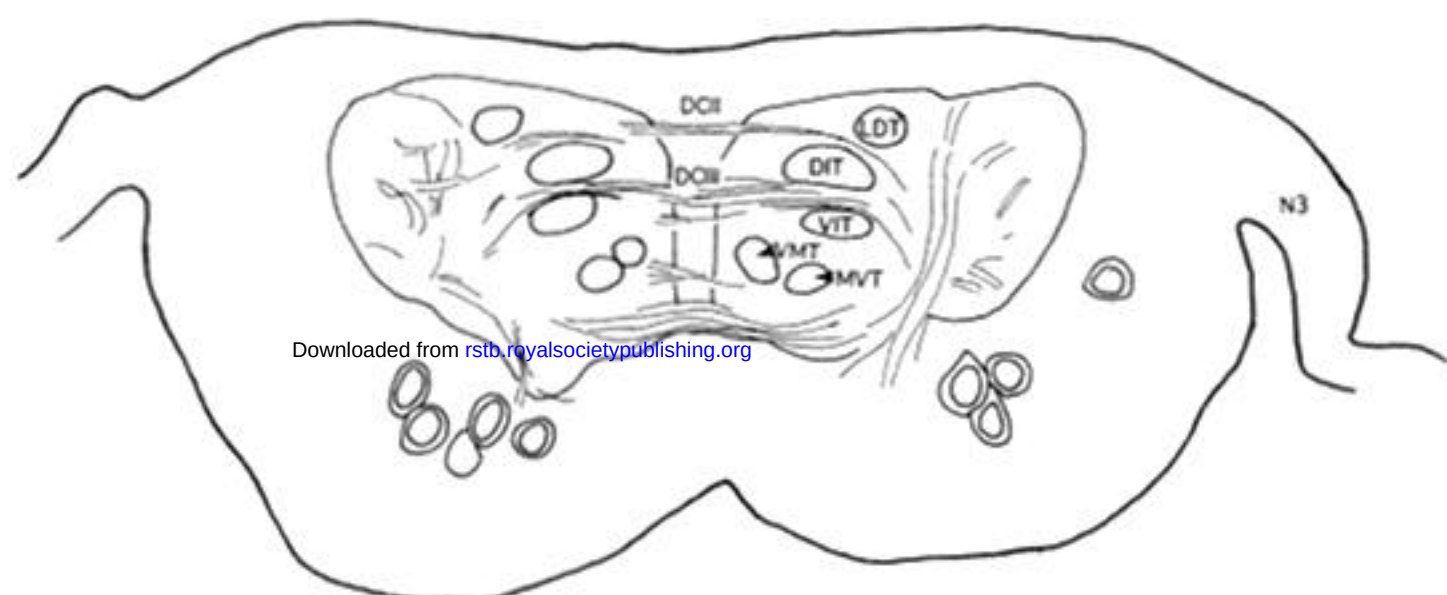
55–60%



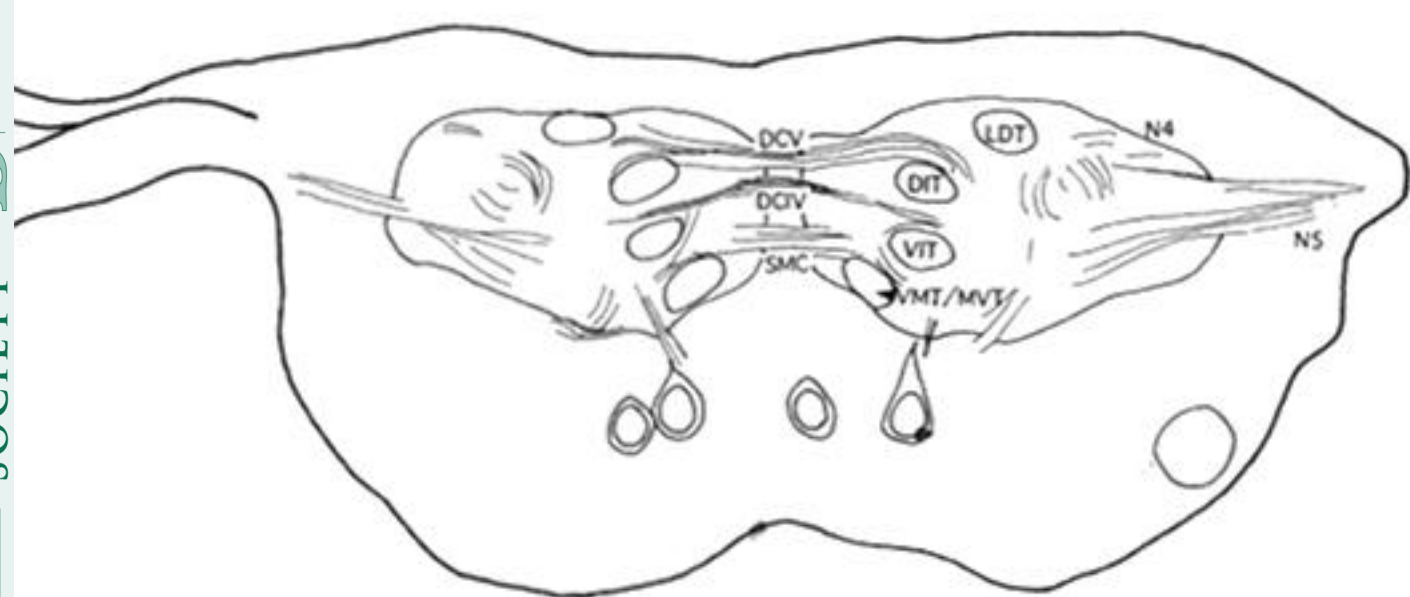
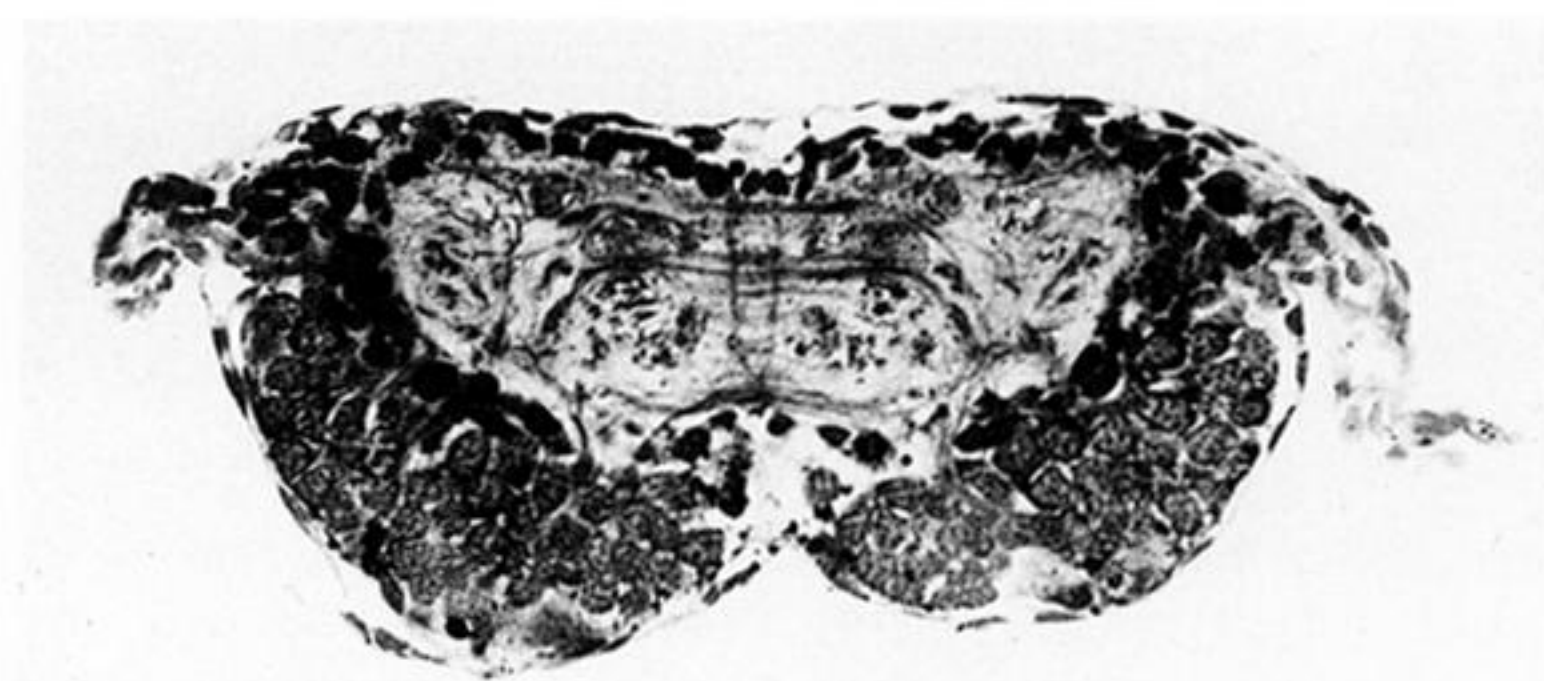
(b)



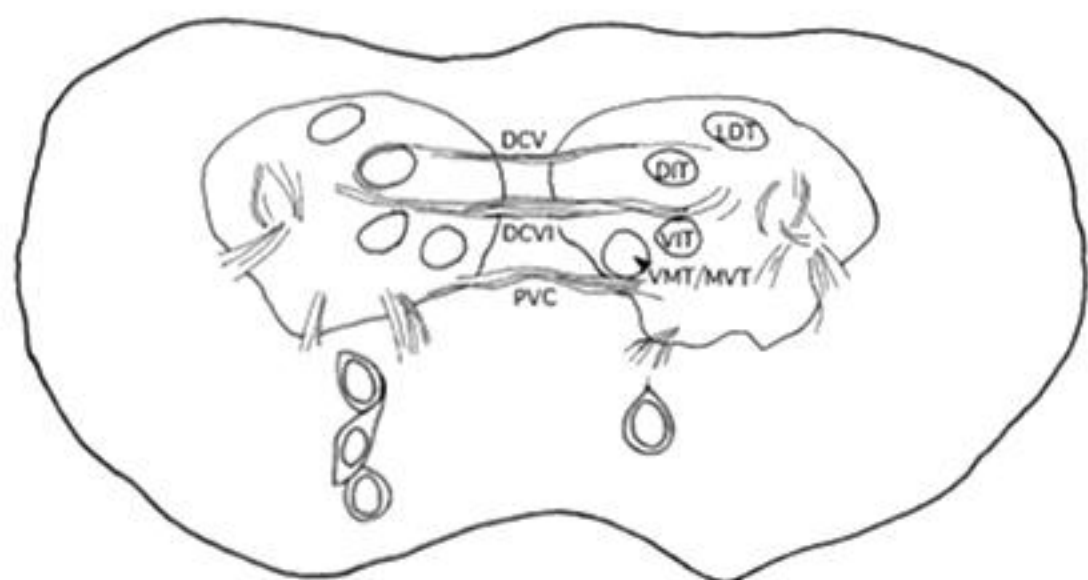
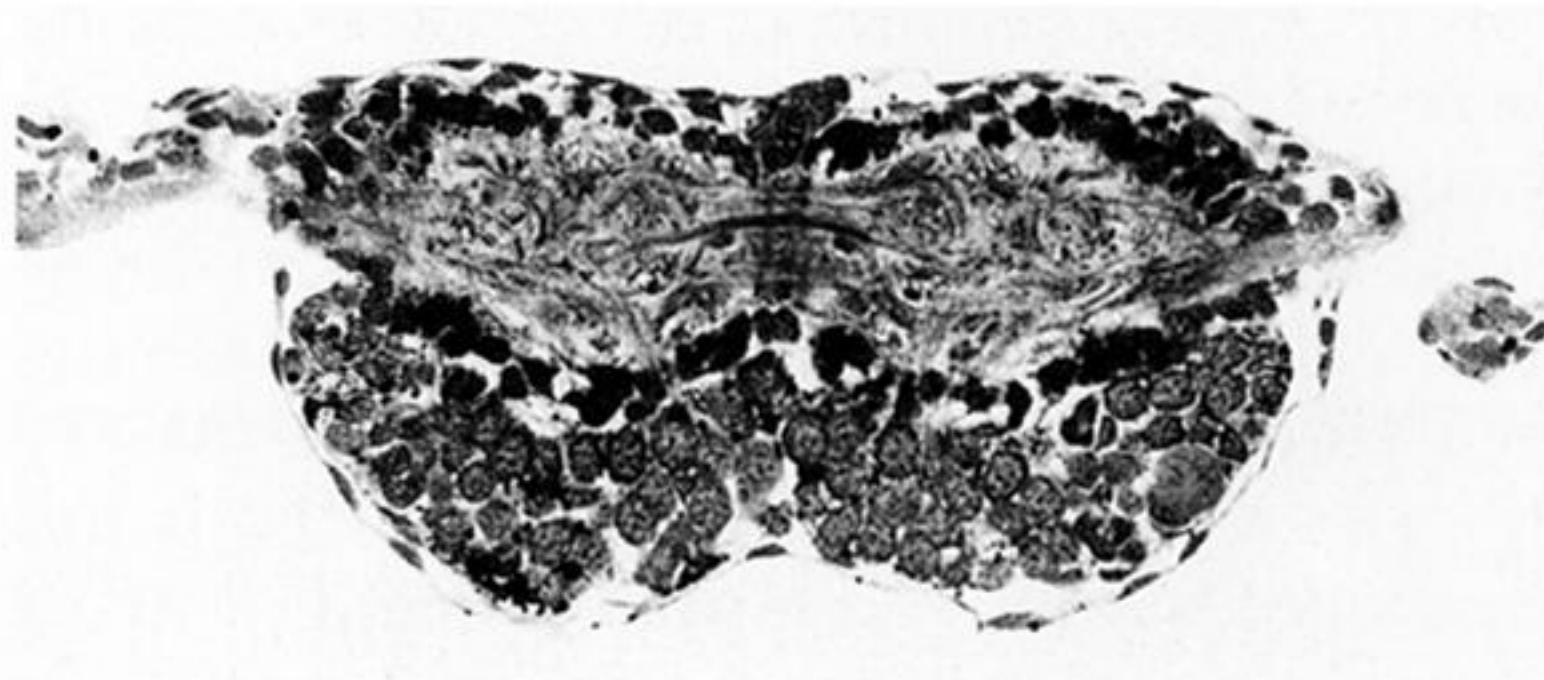
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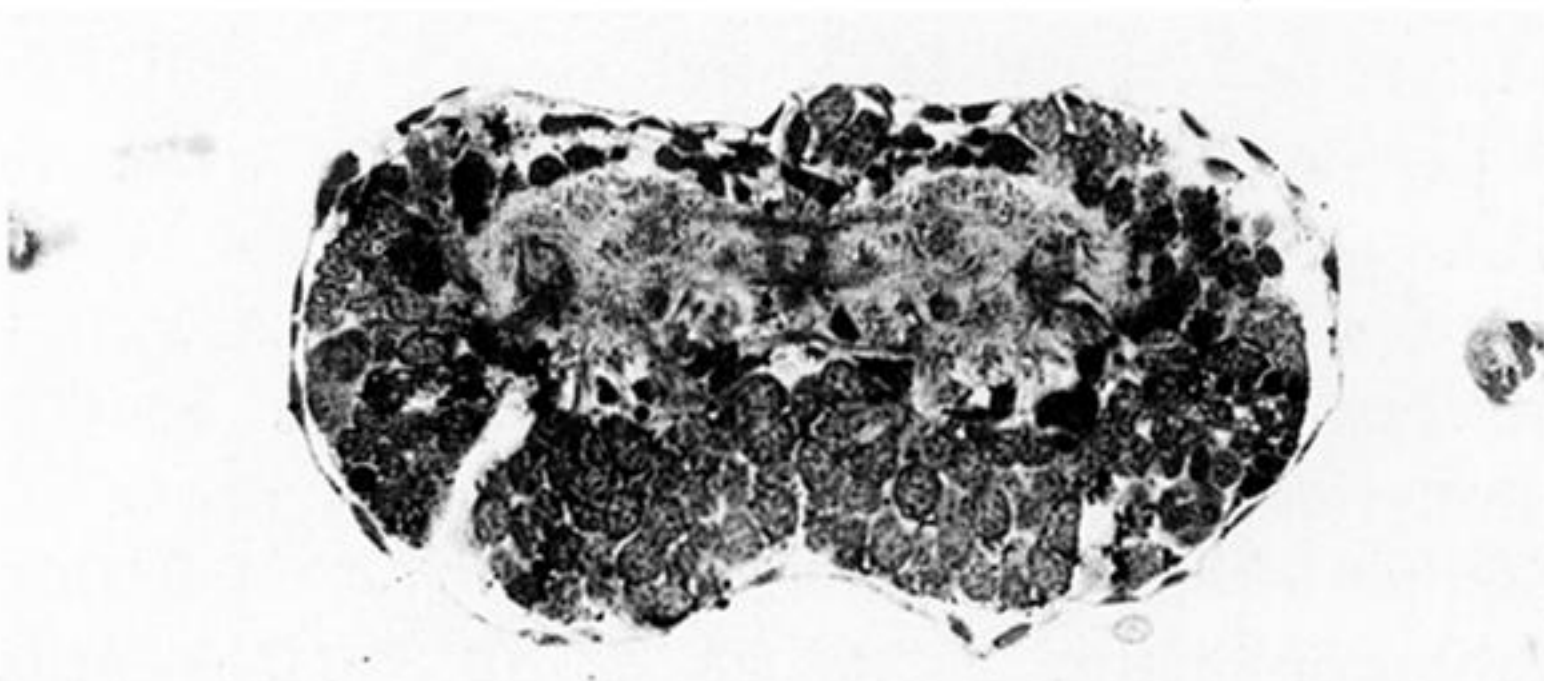
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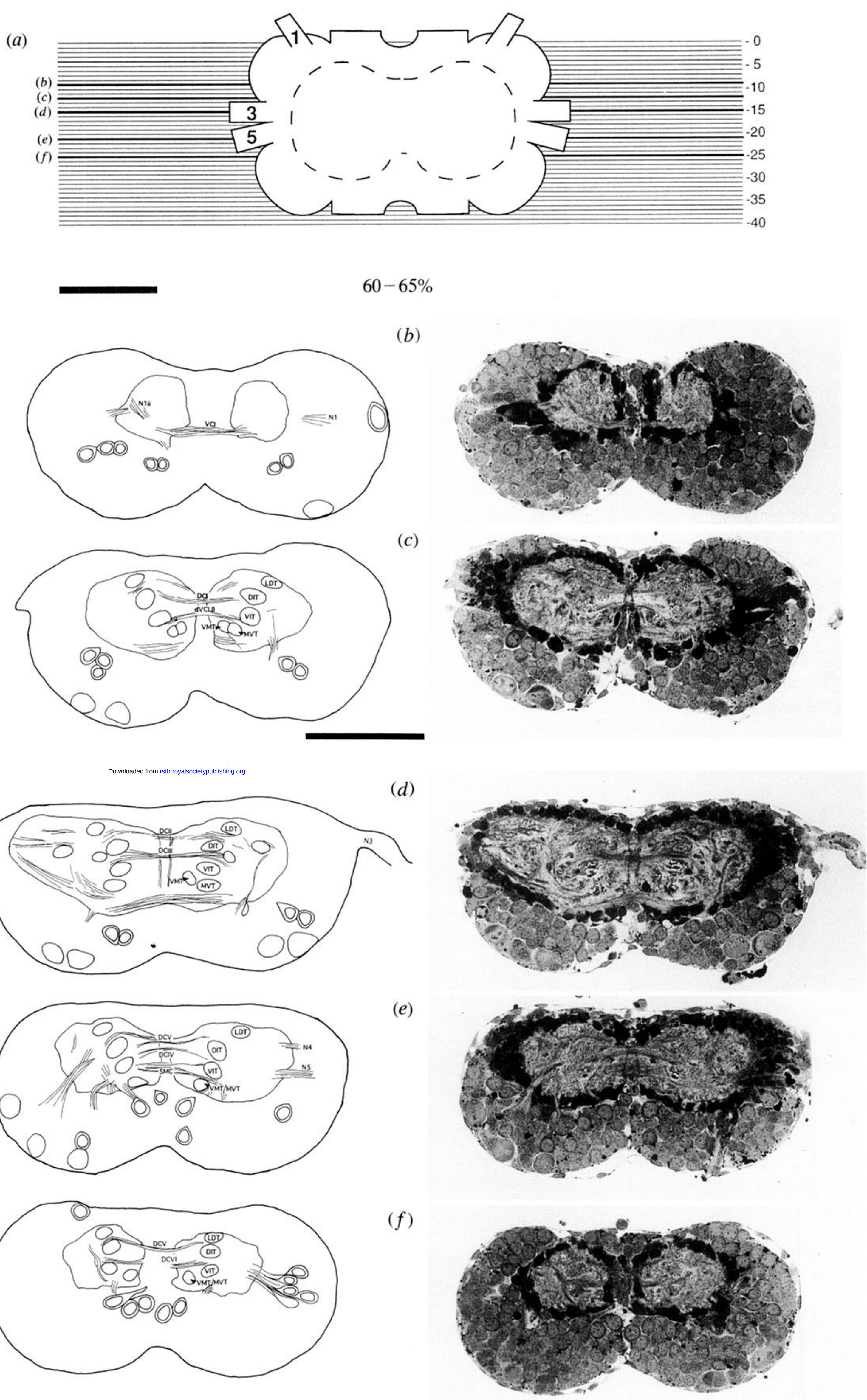
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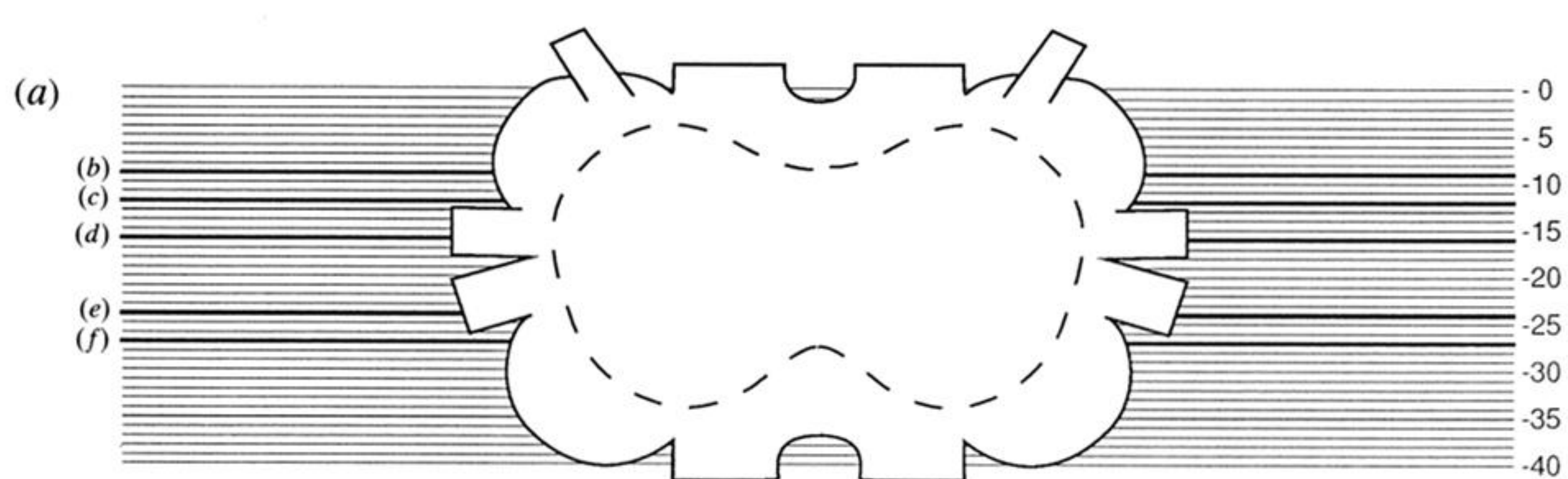


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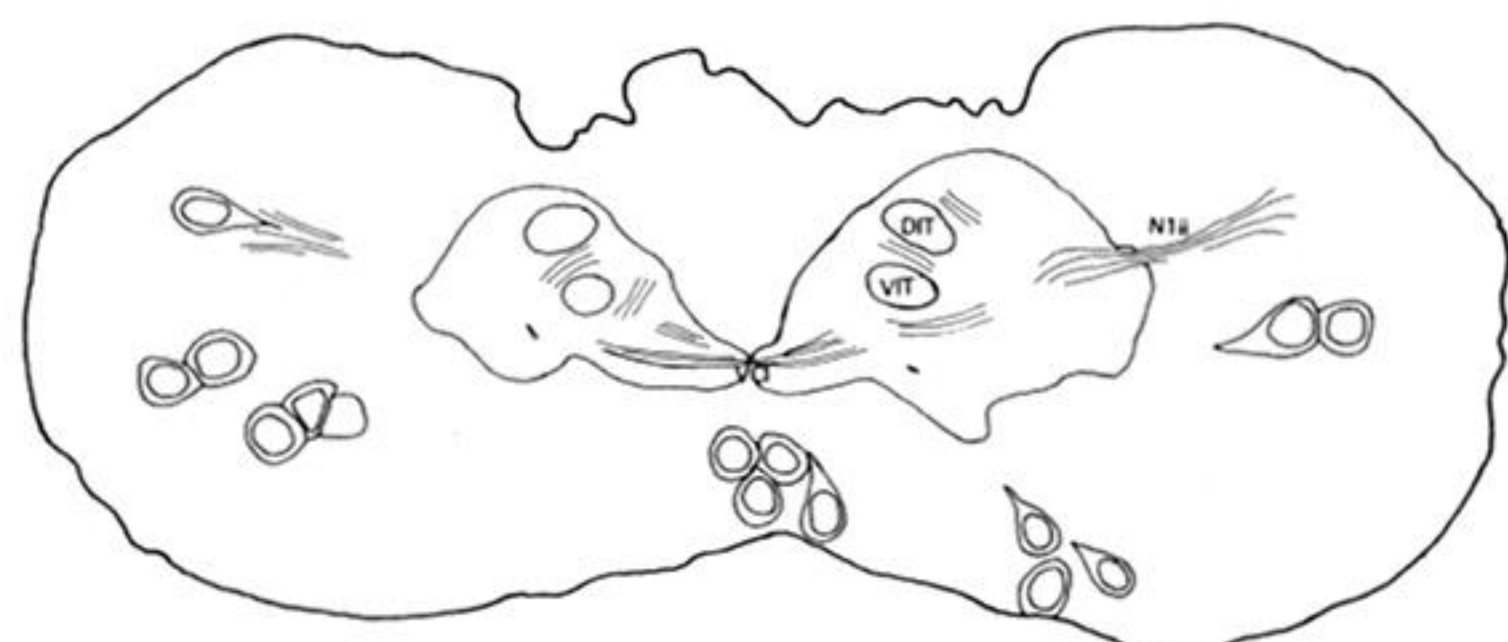


Figures 1–5. Embryonic developmental stages 55–60%, 60–65%, 65–70%, 70–75% and 90–95% respectively. (a) Drawings of the ventral view of mesothoracic ganglia from 55–60%, 60–65%, 65–70%, 70–75% and 90–95% development embryos, cut into 5 µm transverse sections. The levels within the ganglia at which sections (b)–(f)/(g) were taken are indicated by the thick horizontal lines. All ganglia are drawn to the same scale for direct comparison. The remaining parts of the figures show camera lucida drawings (left) and photographs (right) of selected sections shown in (a)) stained with osmium and ethyl gallate. Sections are taken at the equivalent levels in the ganglia for each developmental stage. Scale bars, 100 µm; abbreviations used are listed in table 1.

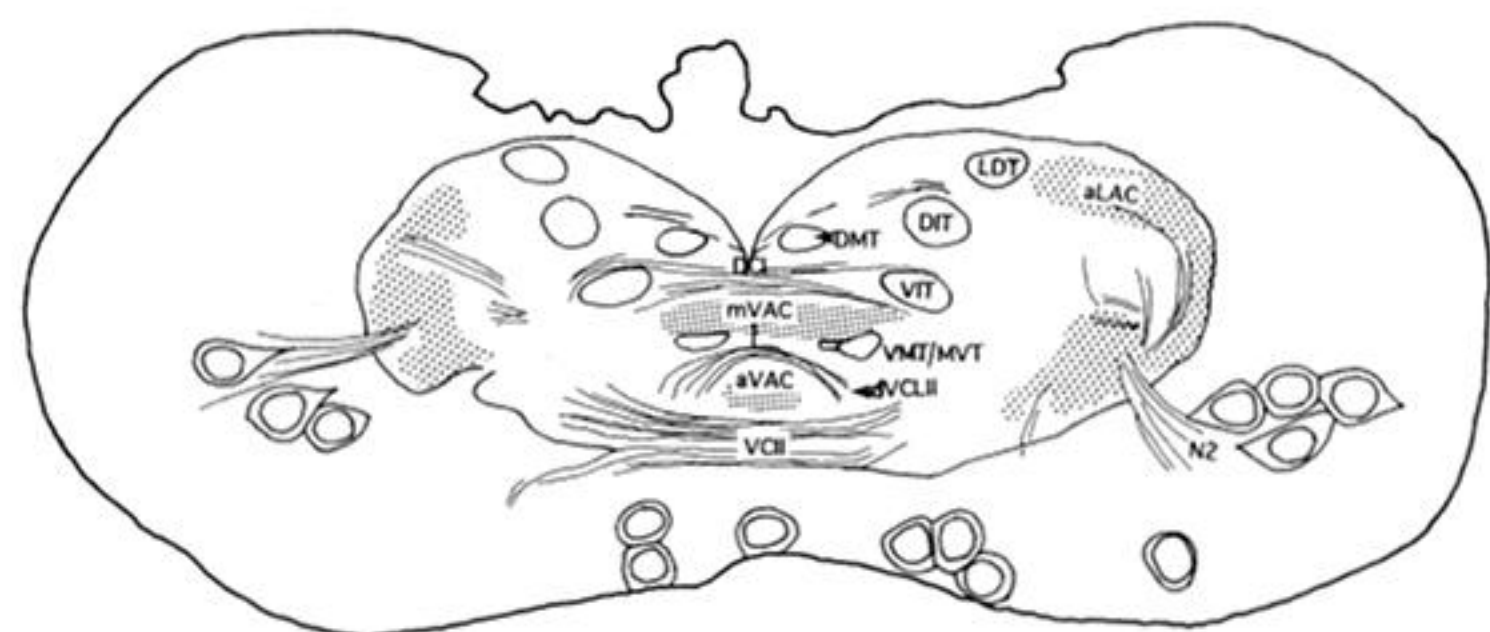
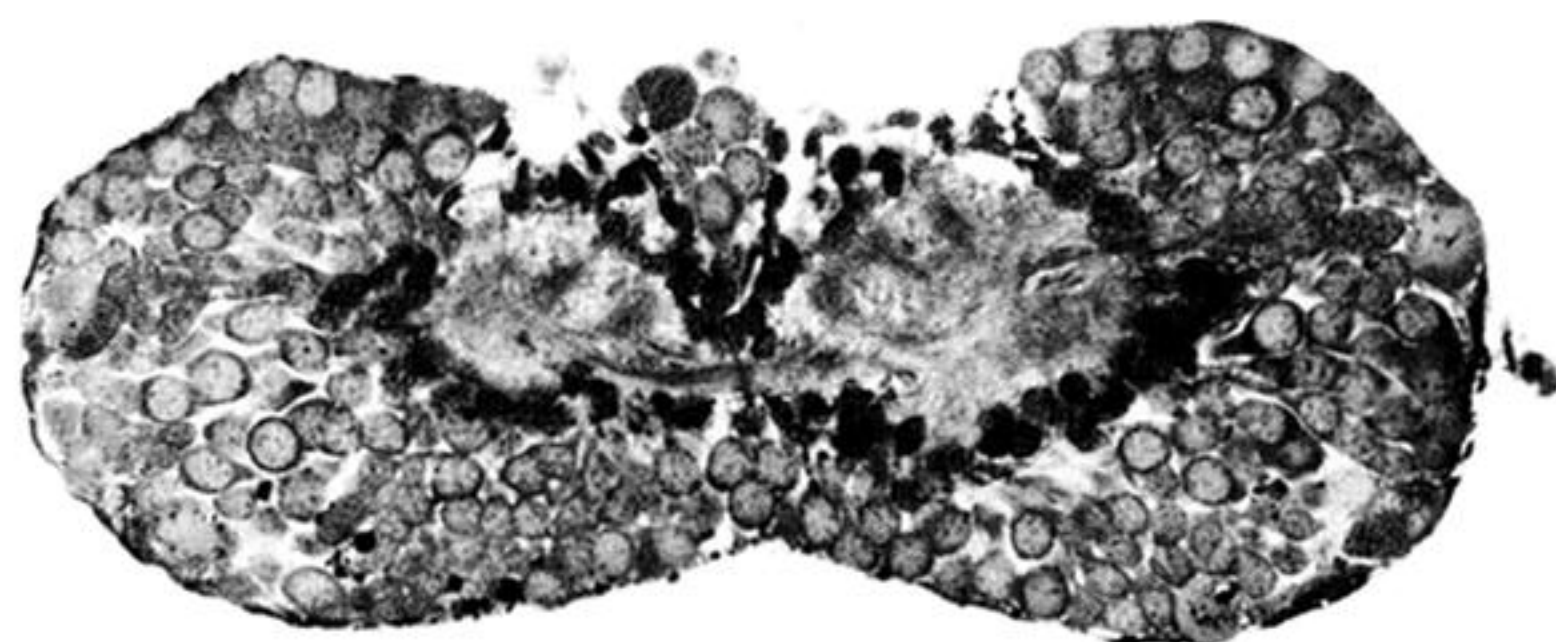




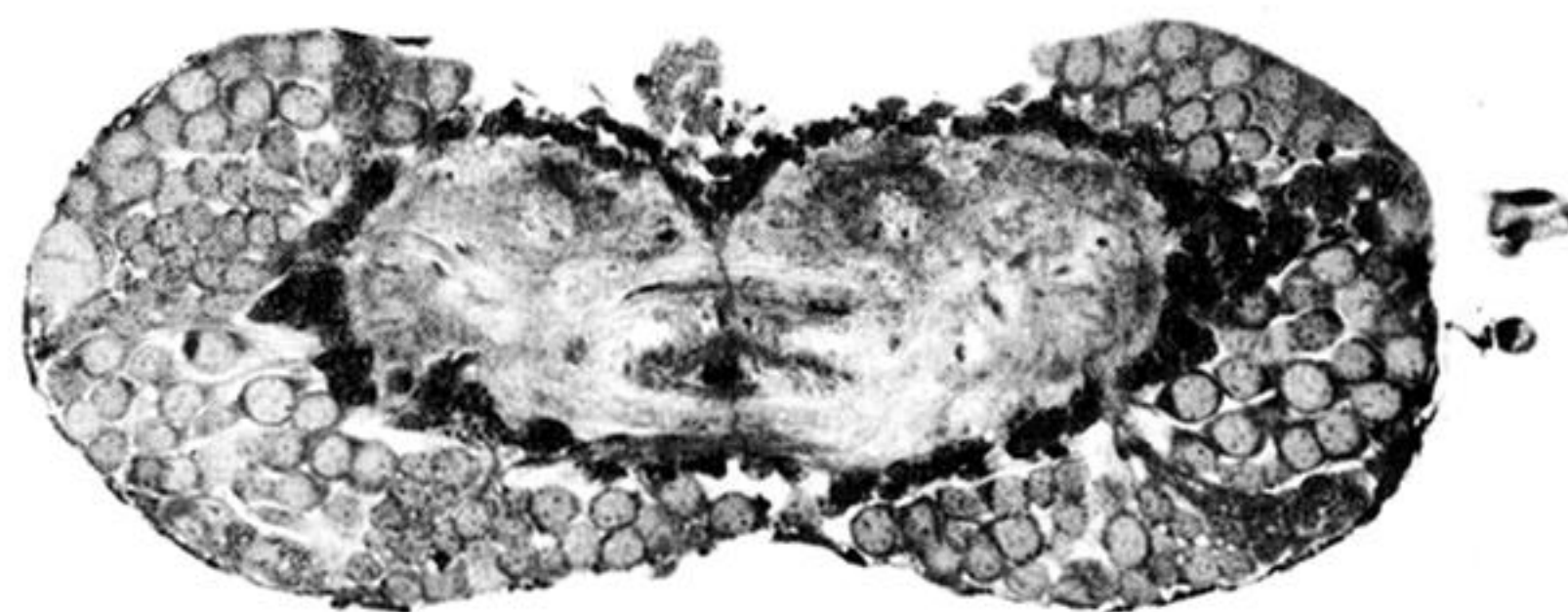
65–70%



(b)

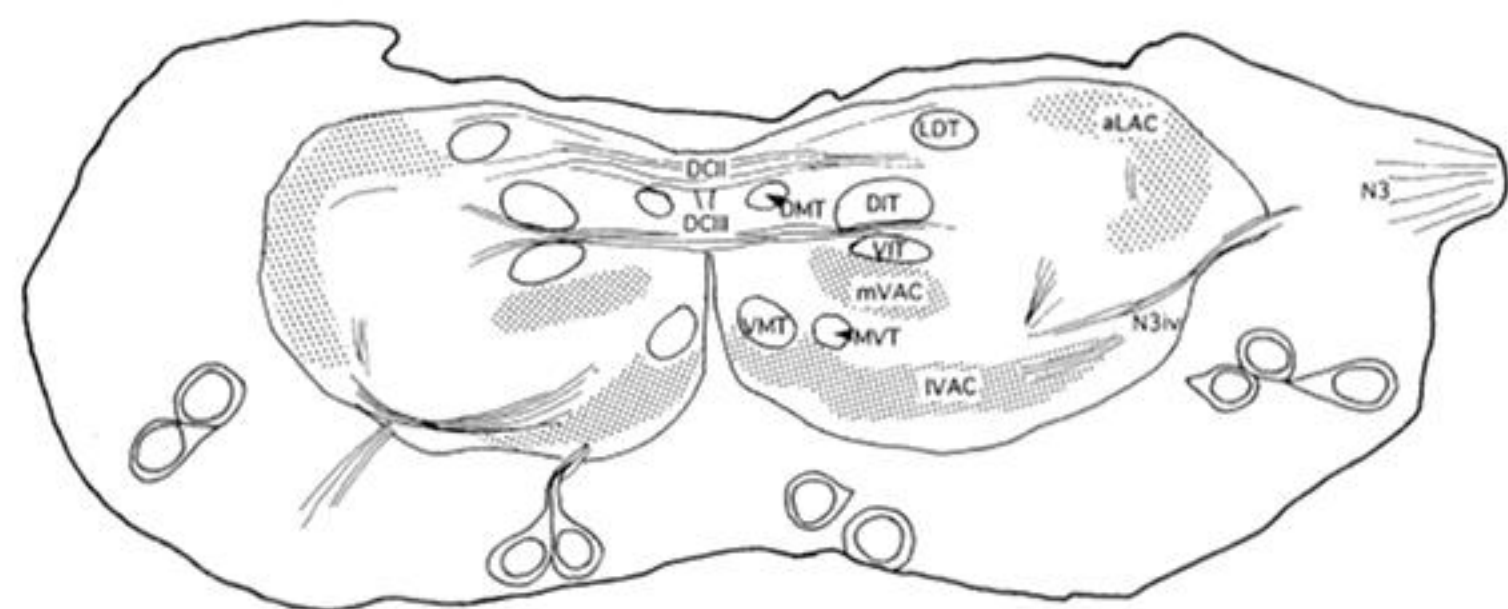


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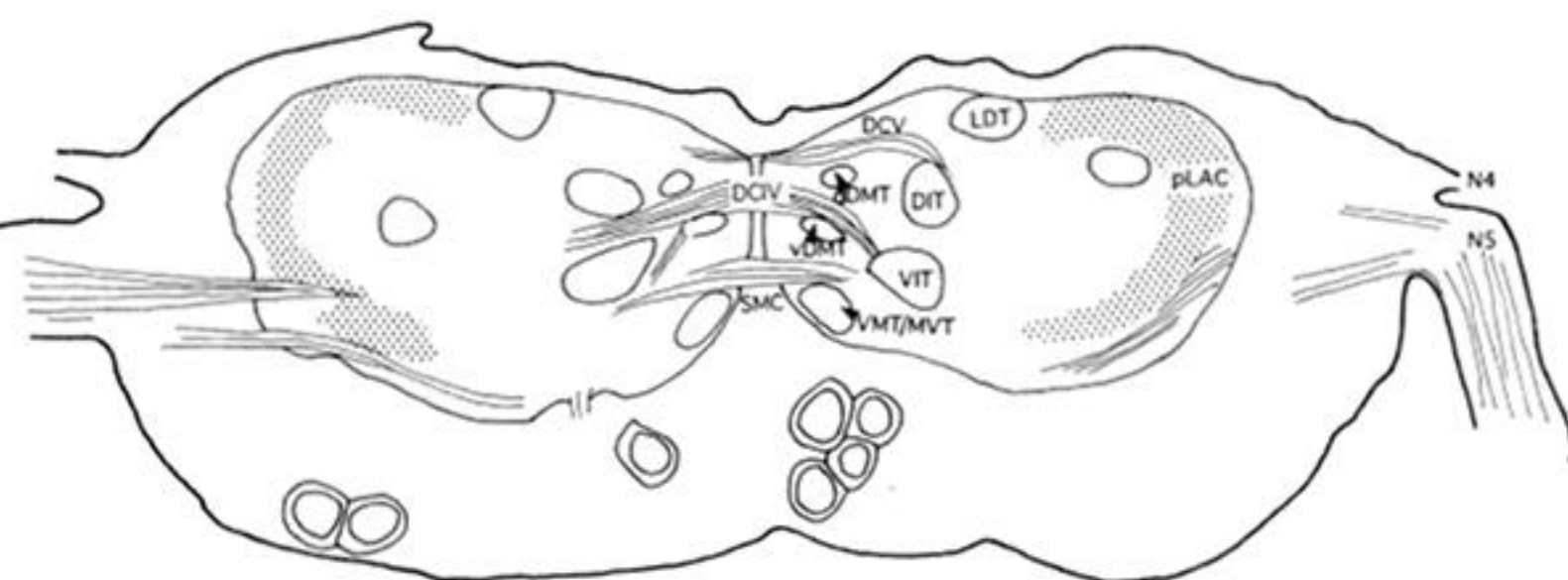


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(d)



(e)



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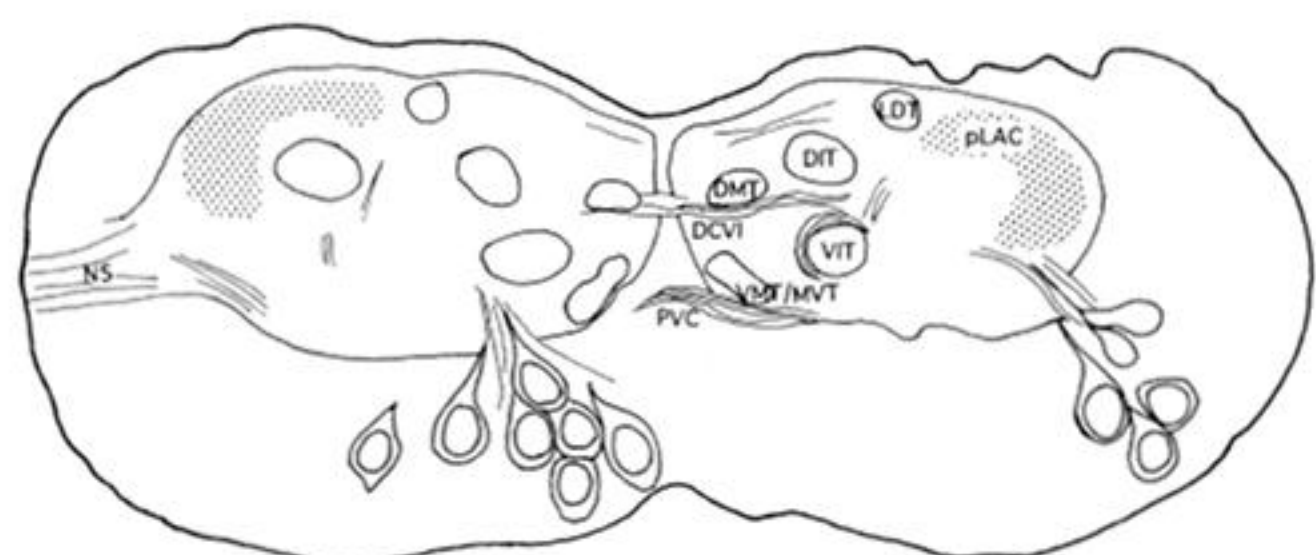
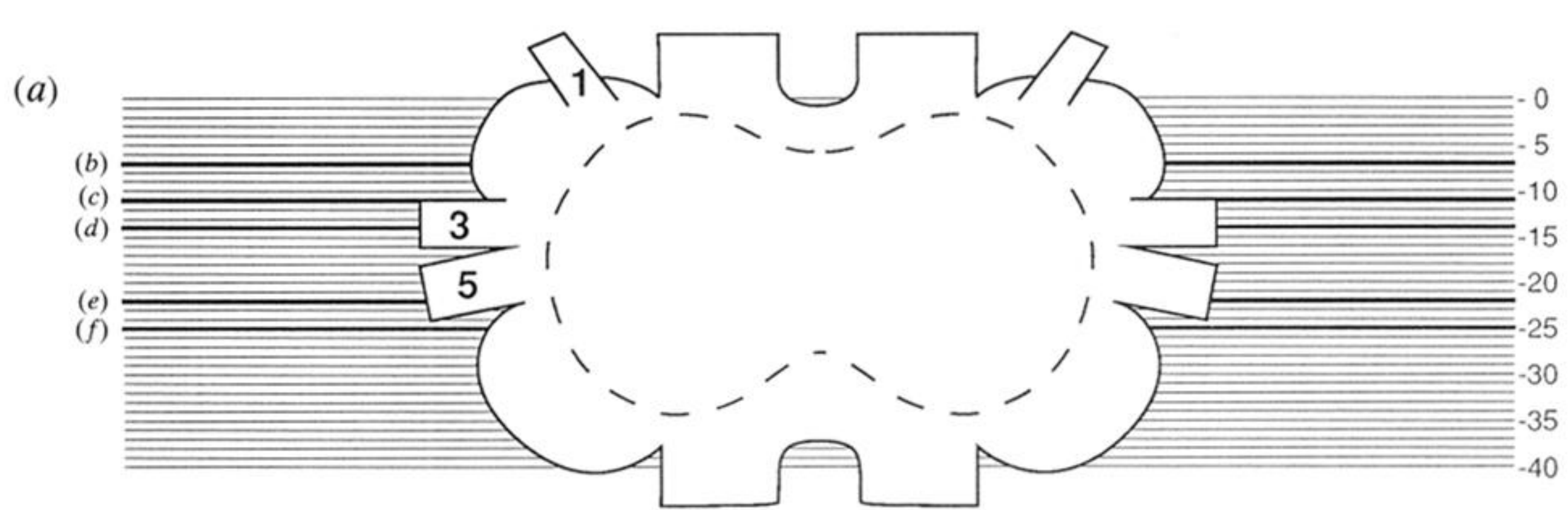
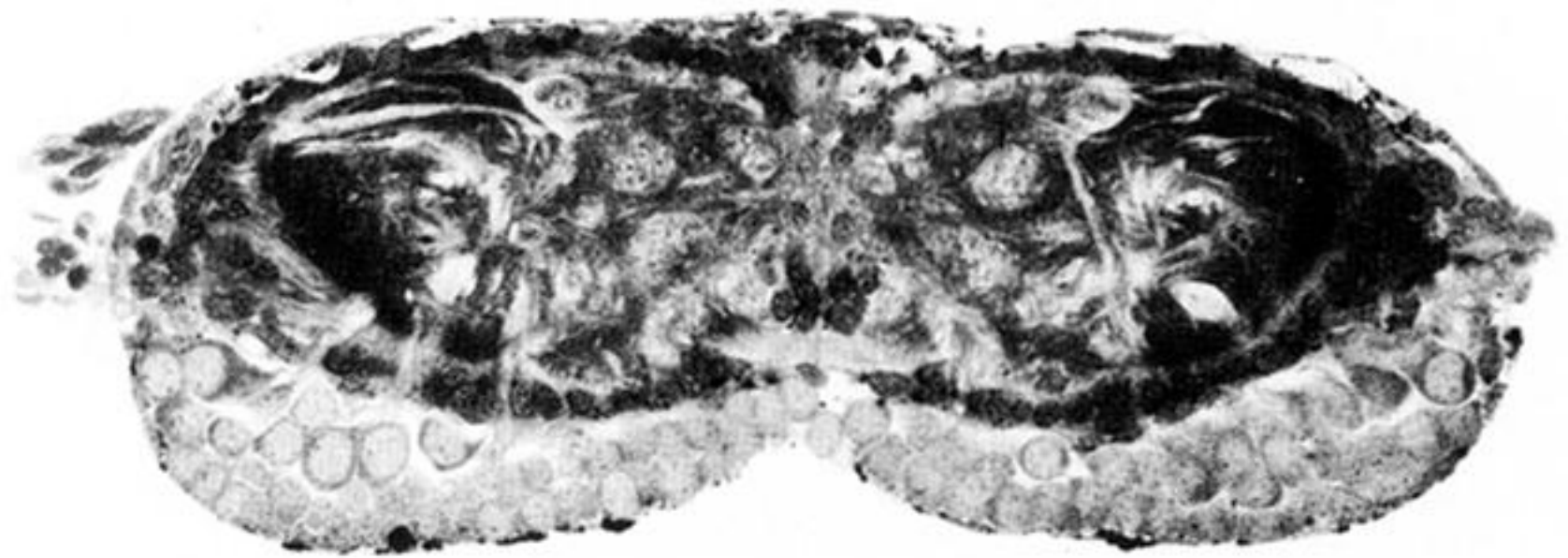


Figure 3. For description see figure 1.



70–75%

(b)



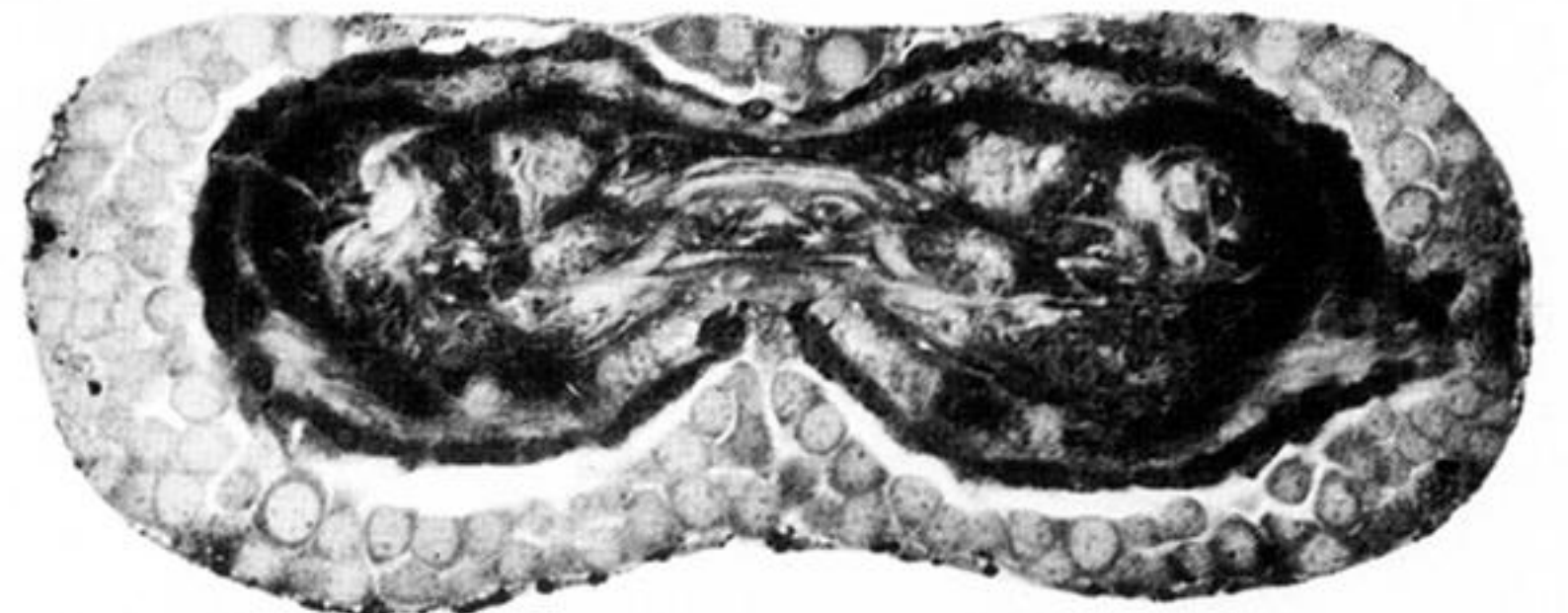
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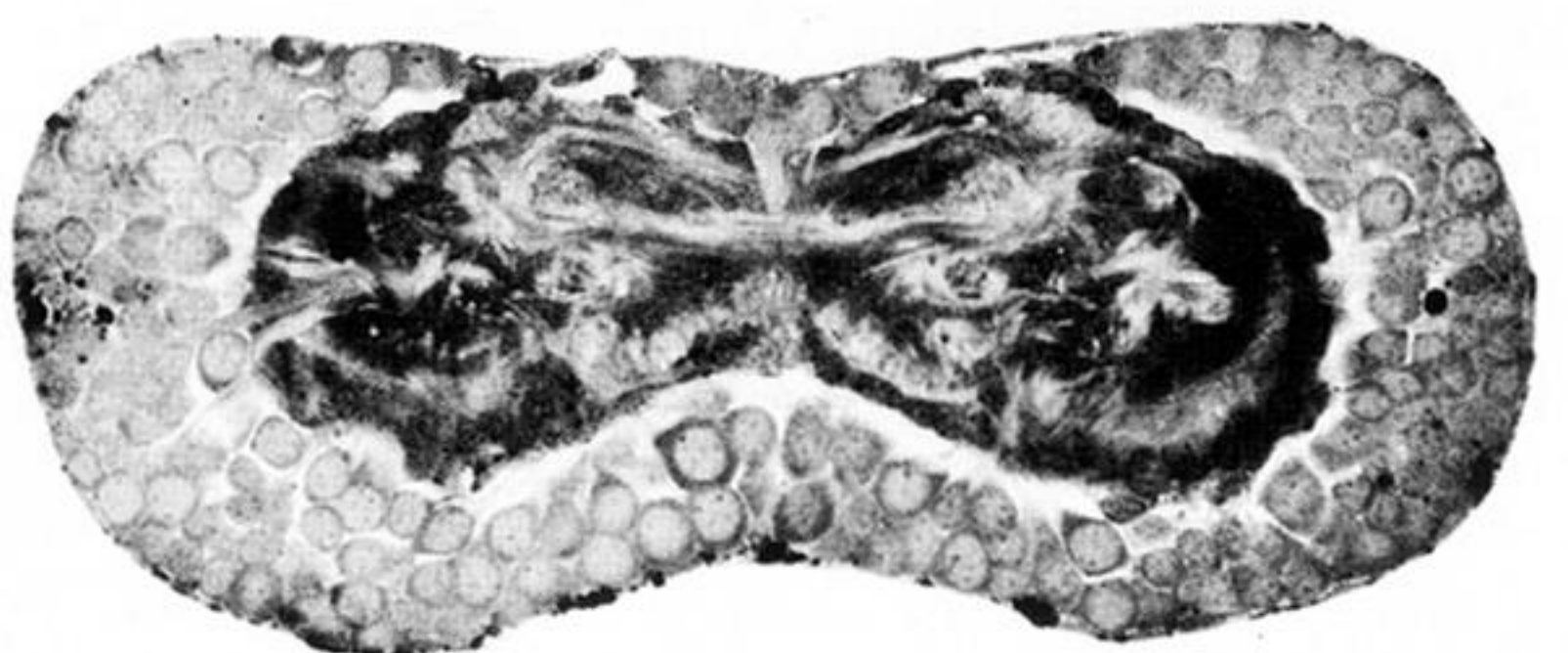


Figure 4. For description see figure 1.

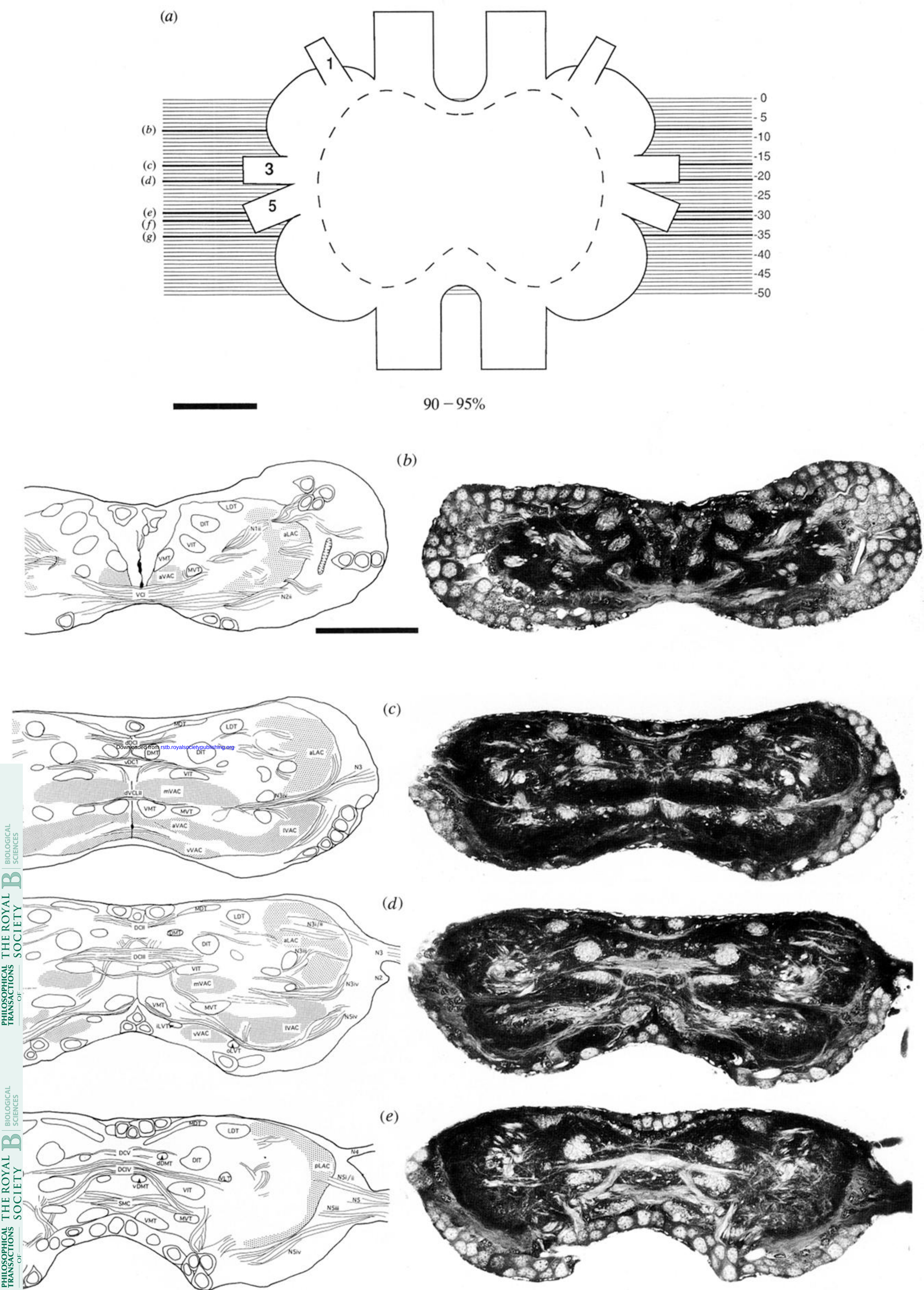


Figure 5. For description see figure 1.

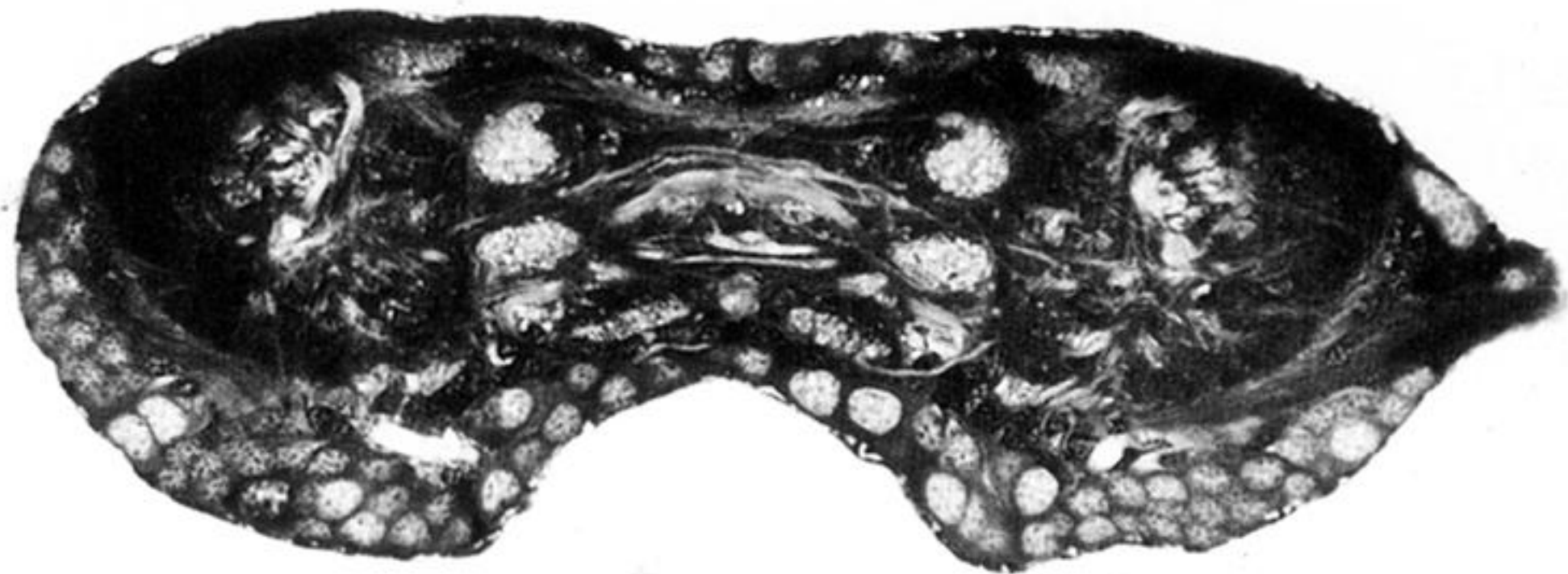
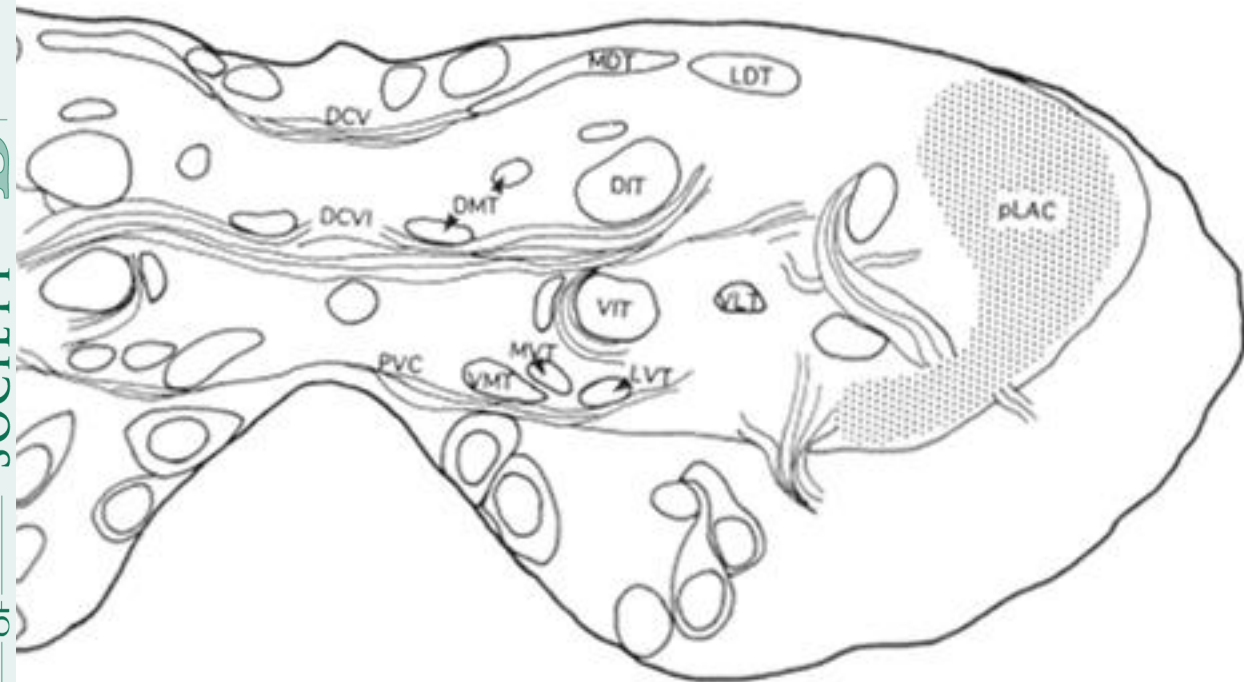
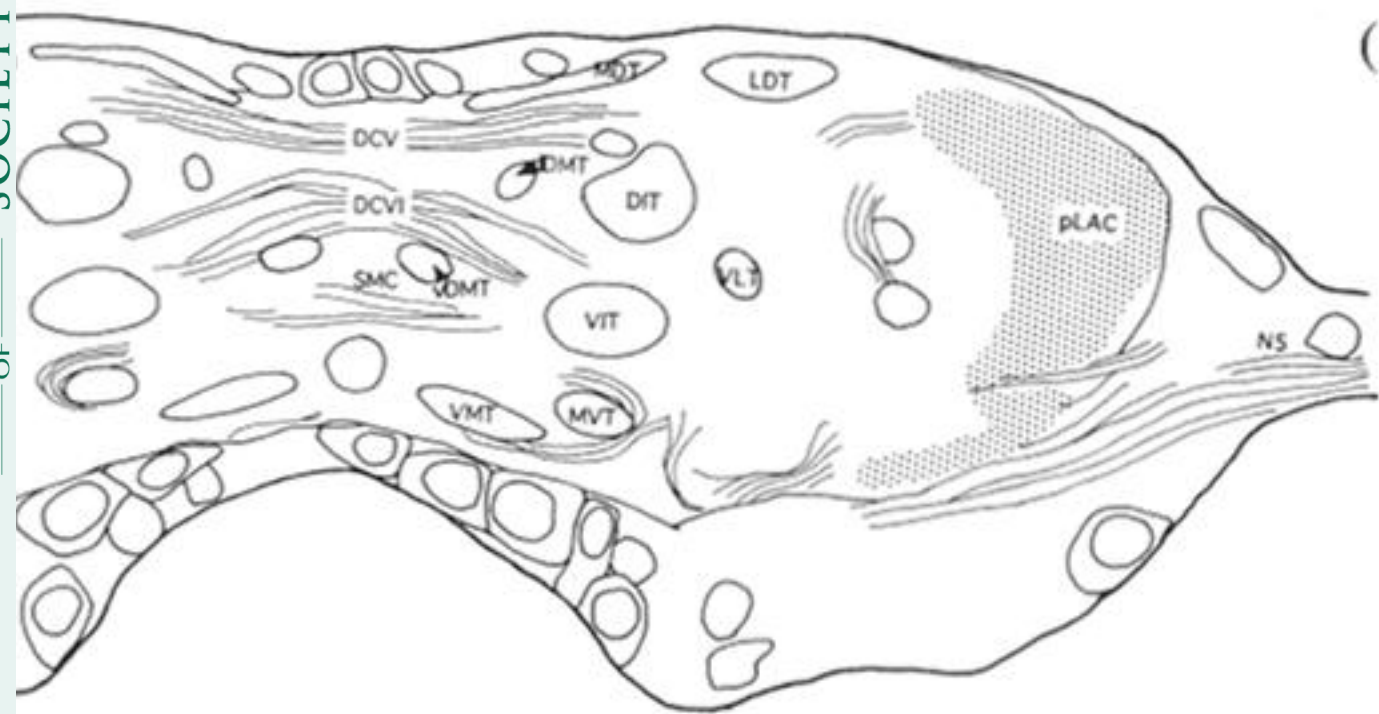


Figure 5. Continued