Punjab Univ. J. Zool., Vol. 8, pp. 21-36, 1993.

HISTOLOGICAL STUDIES OF THE THORACIC GANGLIA OF *PIERIS BRASSICAE* DURING METAMORPHOSIS (PIERIDAE: LEPIDOPTERA)

FIRDAUSIA AZAM ALI

Department of Zoology, University of the Punjab, Lahore, 54590, Pakistan.

Abstract: A pair of peripheral nerves is given off from the prothoracic ganglion of the different developmental stages and the adult. In the larvae the most abundant among the neurones are the medium-sized motor neurones, while in the late pupae and adult the large motor neurones are the most prominent and numerous. The cellular contents, on the whole, are more abundant as compared to the abdominal ganglia. The neuropile is also that of the late pupae and adult when compared to the early stages or the abdominal ganglia. Thirty two fibre tracts in the larval prothoracic ganglia and 39 in that of the adult could be traced fully. These are given off by paired groups of neurones.

Key Words: Thoracic ganglia, Pieridae, neuropile.

INTRODUCTION

Arthropod bodies are specialized into functional regions or taga. The function of locomotion has been taken over almost exclusively by three body segments, collectively referred to as thorax. These are called pro —, meso — and metathorax respectively. Most of the immature insect forms bear paired appendages on these segments. Each segment is usually well sclerotized to maintain a rigid position so as to prevent the body wall from flexing during movement of the appendages (Elzinga, 1988). Each of these appendages receives a pair of peripheral nerves from the corresponding segment. The coordination among the appendages is due to the nervous ganglia via the synaptic junctions. Medulla or the neuropile which is surrounded by the cellular cortex is the seat of these synapsis (Richard and Davies, 1977; Blum, 1985).

Polypod lepidopteran larvae and the adults also have the same general structure where each thoracic segment has a pair of legs, each receiving a pair of peripheral nerves from the ganglion pf its segment. The present work on the histology of the prothoracic ganglia of *Pieris brassicae* was undertaken to provide the basic structure of these ganglia so as to get an initial understanding of the involvement of nervous system in locomotion.

MATERIAL AND METHODS

The different developmental stages used during the present work were taken from a colony. They were fed on cabbage leaves and kept constantly at 20-23 °C. Various larvae were killed for dissection and histological treatment in the middle of the instar. The ventral nerve cord was removed by dissection in all cases except in the 1st and 2nd instar larvae where the size was too small to do this satisfactorily. Material was fixed in Bouin's, Zenker's or Gilson's fixatives and stained in Heidenhain's iron Haematoxylin or Mallory's Triple Stain. It was embedded in paraffin wax and serially sectioned at 5-6 μm. Wigglesworth's (1957, 1959) method of osmium tetraoxide fixation followed by ethyl gallate treatment was used to study the finer histology. These sections were

mounted in D.P.X.

RESULTS AND DISCUSSION

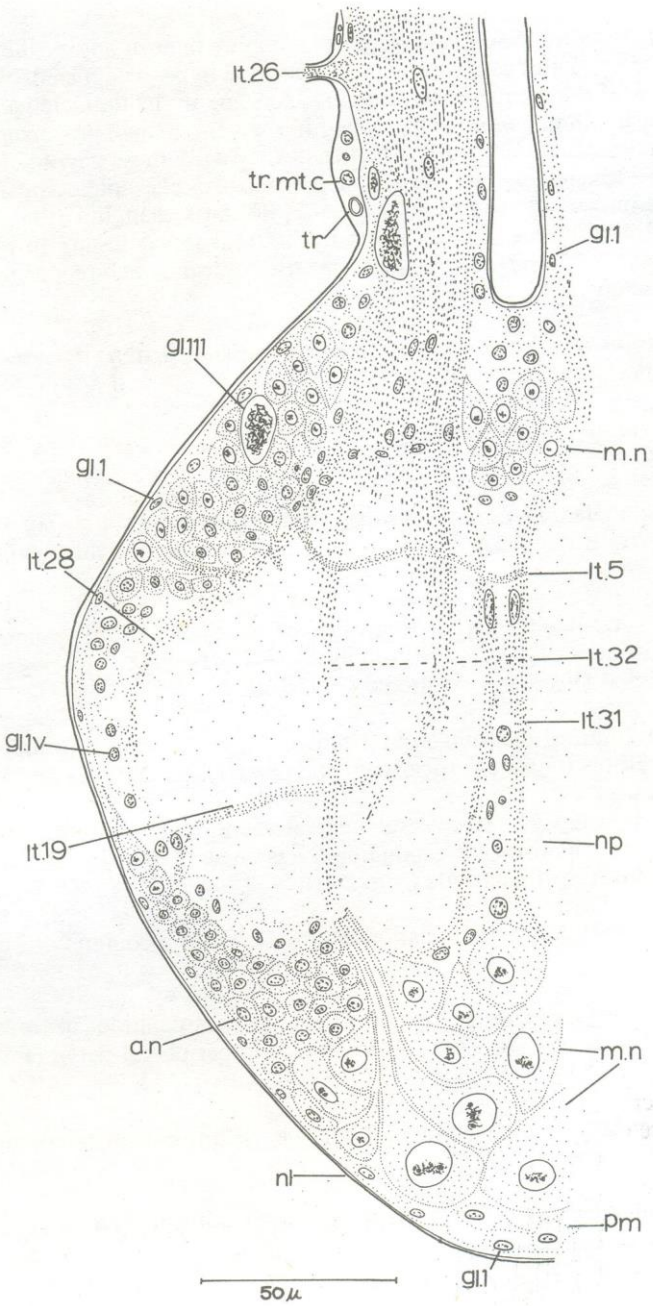
Peripheral nerves

Two main peripheral nerves are given off from each ganglion of the larvae. While in the abdominal ganglia both nerves come from the main body of the ganglion (Ali, 1991), in the thorax the case is somewhat different. The anterior or transverse nerve is very feebly developed and comes from the interganglionic connective just in front of the ganglion. Histological sections (Fig. 1; 1t. 26) show, however, that these fibre tracts originate in the main body of the ganglion, though it is not always possible to specify the neurones in a more anteriorly placed ganglion. In the late pupal stages and the adult two peripheral nerves are given off from the prothoracic ganglion. One is at the extreme anterior end and the other at the extreme posterior end, actually arising from thr mesothoracic part of the connective.

Cellular composition

The cells comprising the cortex of the prothoracic ganglion are of the same kind as described for the abdominal ganglia (Ali, 1993). The distribution of the different types of glial cells and the size of their nuclei is also similar. In the thoracic region a ventral diaphragm is not developed. The number of the type III glial cells is about 10-12 in the prothoracic ganglion instead of 7-8 as in the third or more posterior abdominal ganglia. Similarly the number of association nrurones is also much greater as compared to the abdominal ganglia (Ali, 1980). They are mostly in paired groups, one on each side of the ganglion. These groups give rise to many of the fibre tracts which run in the neuropile or are given off to the peripheral nerves. There are about 10 groups of these neurones on the prothoracic ganglion. In addition, association neurones are scattered among the motor neurones at the anterior and posterior end of the ganglion. The number of large motor neurones is also much greater compared with the number found in the abdominal ganglia and the number also increases during the pupal stage. But the most abundant among the neurones are the medium-sized motor neurones in the larva but in the adult the large ones are the more prominent and numerous. These groups of cells are described in detail in relation to their respective fibre tracts in the next section.

Fig. I. Frontal section through prothoracic ganglion of 5th instar larva shoeing neurones with their fibre tracts and other features. Cells with dark nuclei and dotted cytoplasm represent large motor neurones; cells with dark nuclei and white cytoplasm represent medium sized neurones; cells with dark cytoplasm represent association neurones; small clusters of circles represent axons cut teansversely and heavily shaded area represent glomerular bodies.


lt.26
tr. mt.c
tr
gl.1
gl.111
gl.1
m.n
lt.28
lt.5
lt.32
lt.31
gl.1v
lt.19
np
a.n
m.n
nl
pm
gl.1
50 μ

Neuropile and axonal tracts

The neuropile of the thoracic centre is more complex than in any of the abdominal ganglia, though it is of the same structured stratified type recognized by Maynard (1962). The pattern of the neuropile is generally the same in the thoracic ganglia of the 5th instar larva but it is much more complicated in the adult. There are some new tracts in the adult which are not found in the larval stages. Usually these new tracts are given off by motor neurones whose differentiation presumably takes place during the late pupal stages of the insect. The pattern of the neuropile starts changing after 48 hours of pupal life. Figs. 2-4 show the various tracts in different larval instars in the thoracic ganglia, while Figs. 5-9 and 10-11 show the histological structure during different pupal periods and adult respectively.

The differenciation of motor neurones and their associated fibre tracts is correlated with the development of the power of flight in the adult.

The following are the paired tracts and other features of the prothoracic ganglion in the 5th instar larvae:

Tract It.1: From dorsolateral motor neurones at anterior end of the ganglion. Runs ventrally around periphery of neuropile. Forms fine fibres along which glial IV cells are scattered (Fig. 2A).

Tract It.2: From ventrolateral motor neurones at anterior end of ganglion. Runs dorsally around periphery of neuropile forming a sort of boundary for it. Forms fine fibres along which glial IV cells are scattered (Fig. 2A).

Tract It. 3: From mid-dorsal and mid-ventral motor and association neurones. Forms a median partition of fibres running vertically up and down (Fig. 2A).

Tract It. 4: From a group of dorsolateral and lateral motor neurones. Runs across middle of ganglion, two opposite corresponding tracts cross in mid-dorsal line and then pass along dorsal boundary of neuropile (Fig. 2 A).

Tract It. 5: From lateral group of association neurones. From a commissure just below the chiasma formed by tract It.4 (Fig. 2A).

Tract It. 6: From ventrolateral group of motor and association neurones. Runs dorsalwords, curves towards outer side and joins second peripheral nerve (Fig. 2B and C)

Tract It. 7: From ventral group of association neurones. Runs dorsomedially and passes midline in the centre (Fig. 2B).

Tract It. 8: From ventral group of motor neurones. Runs dorsomedially close to tract It 7 and forms a commissure (Fig. 2C).

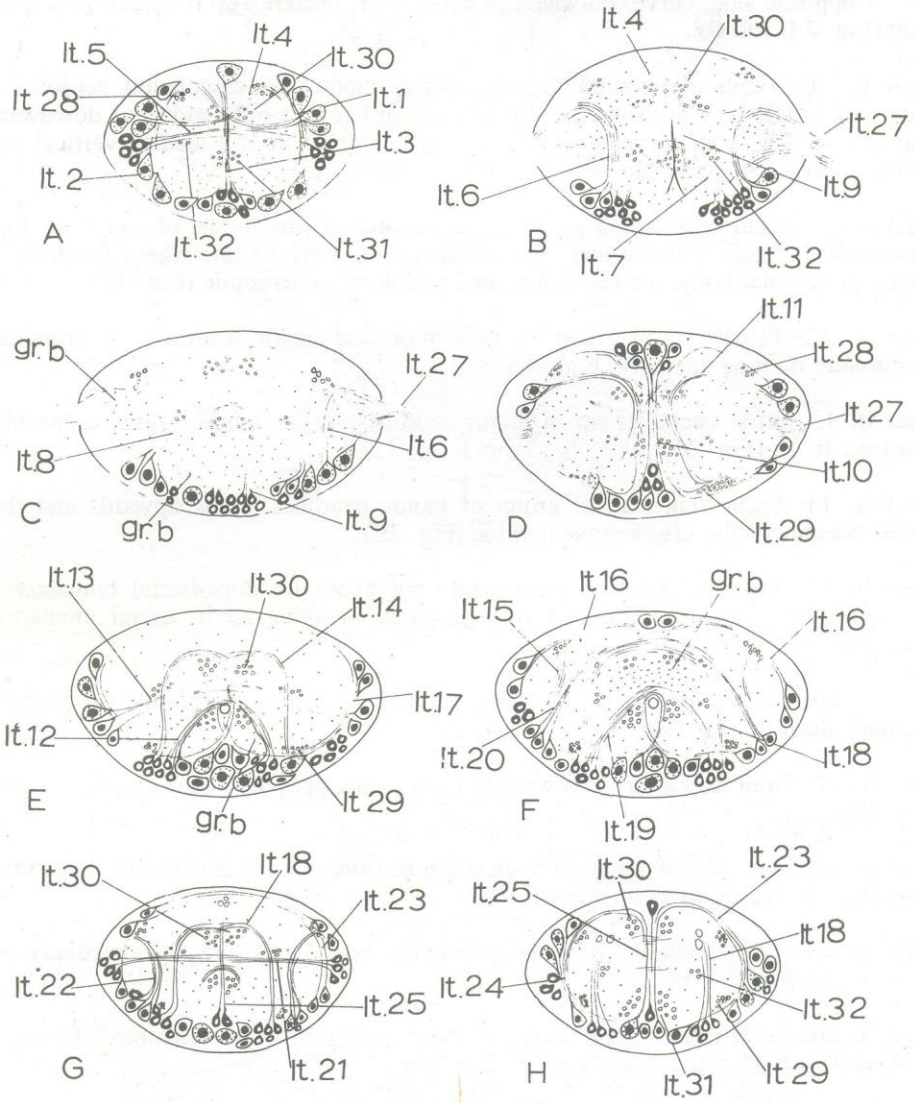


Fig. 2. Diagrammatic serial drawings of transverse sections of prothoracic ganglion of 5th instar larva showing the major fibre tracts with neurones. (Explanation same as in Fig. 1).

Tract It. 9: From ventral group of association neurones.Runs parallel to corresponding tract of opposite side; curves outwards in dorsal part, breaking up in lateral glomerular body (Fig. 2 B and C).

Tract It. 10: From mid-ventral and mid-dorsal motor and association neurones in middle of ganglion. The axons run dorsally upward from ventral side and downwards from dorsal side, then curves towards sides of neuropile. It is a median vertical tract dividing ganglion into a right and left half (fig. 2D).

Tract It. 11: From dorsolateral group of motor neurones in middle of ganglion. Runs dorsomedially, curves down and runs obliquely outwards. Some fibres break up in ventral glomerular body, the rest run around periphery of neuropile (Fig. 2D)

Tract It. 12: From ventral group of association and motor neurones. A prominent commissure running medially (Fig. 2E).

Tract It. 13: From lateral group of motor neurones. A commissure running medially near tract It. 12 (Fig. 2E).

Tract It. 14: From ventrolateral group of motor neurones. Runs upwards and then curves dorsomedially, crossing the midline (Fig. 2E).

Tract It. 15: From a group of transversely cut axons on dorsolateral boundary of neuropile. Runs downwards at sides of ganglion, breaking up in lateral glomarular body (Fig. 2F).

Tract It. 16: From dorsolateral and lateral motor neurones. Runs around periphery of neuropile dorsally (Fig. 2E).

Tract It. 17: From lateral motor neurones. Runs round periphery of neuropile ventrally (Fig. 2E).

Tract It. 18: from ventral group of association neurones. Runs dorsalward then curves medially and crosses midline (Fig. 2F-H).

Tract It. 19: From ventral group of association neurones. Runs dorsomedially and forms a commissure (Fig. 2F).

Tract It. 20: From lateroventral group of motor neurones. Runs dorsomedially and is associated with the fibres of tract It. 18 (Fig. 2F).

Tract It. 21: From lateral group of association neurones. Forms a median commissure (Fig. 2G).

Tract It. 22: From ventrolateral motor and association neurones. Runs upward and then curves outwards. Probably continues in the preceeding interganglionic connective and gives fibres to the anterior peripheral nerve of mesothoracic ganglion (Fig. 2G).

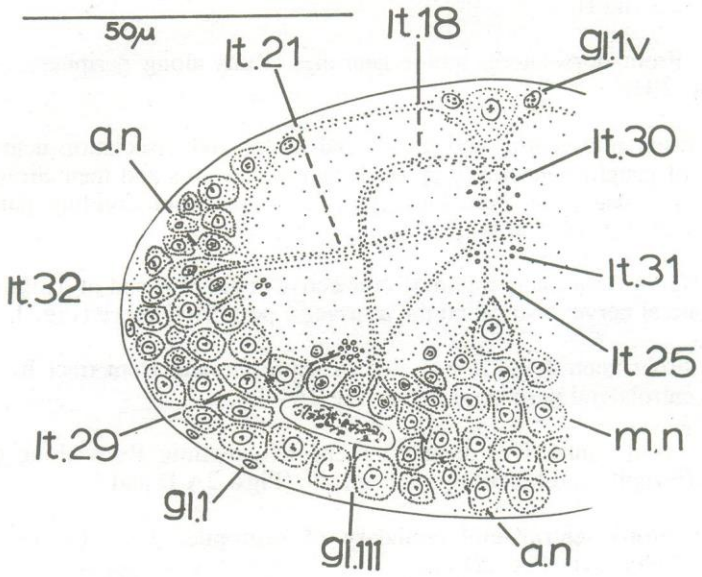


Fig. 3. Cross-section through prothoracic ganglion of 1st instar larva showing neurones, fibre tracts and glial cells.

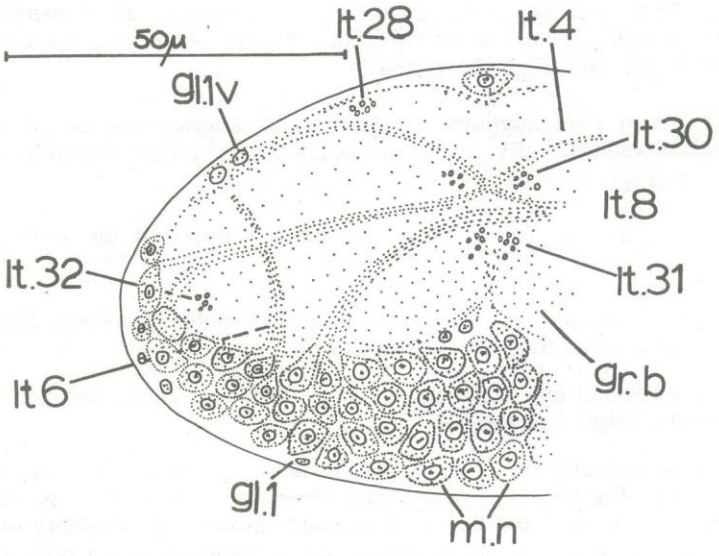


Fig. 4. Cross-section through prothoracic ganglion of 1st instar larva showing same features as in Fig. 3.

Tract It. 23: From lateroventral motor neurones. Runs along periphery of neuropile dorsally (Fig. 2G and H.

Tract It. 24: From dorsolateral motor neurones. Runs along periphery of neuropile ventrally (Fig. 2 H).

Tract It 25: From mid-ventral and mid-dorsal motor and association neurones. Runs along middle of ganglion vertically upwards and downwards and then curves outwards along periphery of neuropile. Forms a vertical median partition dividing ganglion into a right and left half (fig. 2G and H).

Tract It. 26: Nerve from interganglionic connective at anterior end of ganglion. It is the anterior peripheral nerve; also called the transverse peripheral nerve (Fig. 12).

Tract It. 27: From ventral and dorsal side of neuropile and from tract It. 6. It is the posterior or ventrolateral peripheral nerve (Fig 2A - D).

Tract It. 28: From connectives at anterior end of ganglion. Runs along dorsolateral periphery of neuropile and breaks up in neuropile (Figs. 2A-D and 1).

Tract It. 29: From ventrolateral boundary of neuropile. Joins the connectives at posterior end of ganglion (Fig. 2D-H).

Tract It. 30: From connectives at anterior end of ganglion. Runs through ganglion longitudinally and joins the connectives at posterior end of ganglion. The two opposite corresponding tracts run parallel to each other (Fig. 2A-H).

Tract It. 31: From interganglionic connectives at anterior end of ganglion. Passes through whole length of ganglion, on either side of midline and joins the connectives at posterior end of ganglion (Figs. 2A-H and 1).

Tract It. 32: From interganglionic connectives at anterior end of ganglion. Runs through ganglion longitudinally, lateral to tract It. 31 and joins connectives at posterior end of ganglion (Figs. 2 A-H and 1).

The following are the paired tracts and other features of the adult prothoracic ganglion:

Tract t. 1: From lateral and dorsolateral group of motor neurones. Passes around boundary of neuropile ventrally (Figs. 7 A and B).

Tract t. 2: From lateral and dorsolateral motor neurones. Passes around boundary of neuropile dorsally (Figs. 7 A and B).

Tract t. 3: From ventrally and dorsally placed motor and association neurones. Passes around boundary of neuropile dorsally. The fibres from ventral side go up vertically forming a median partition and then pass outwards around the periphery of neuropile. The fibres from dorsal side go down and divide the ganglion into a right and left half (Fig. 7 A and B).

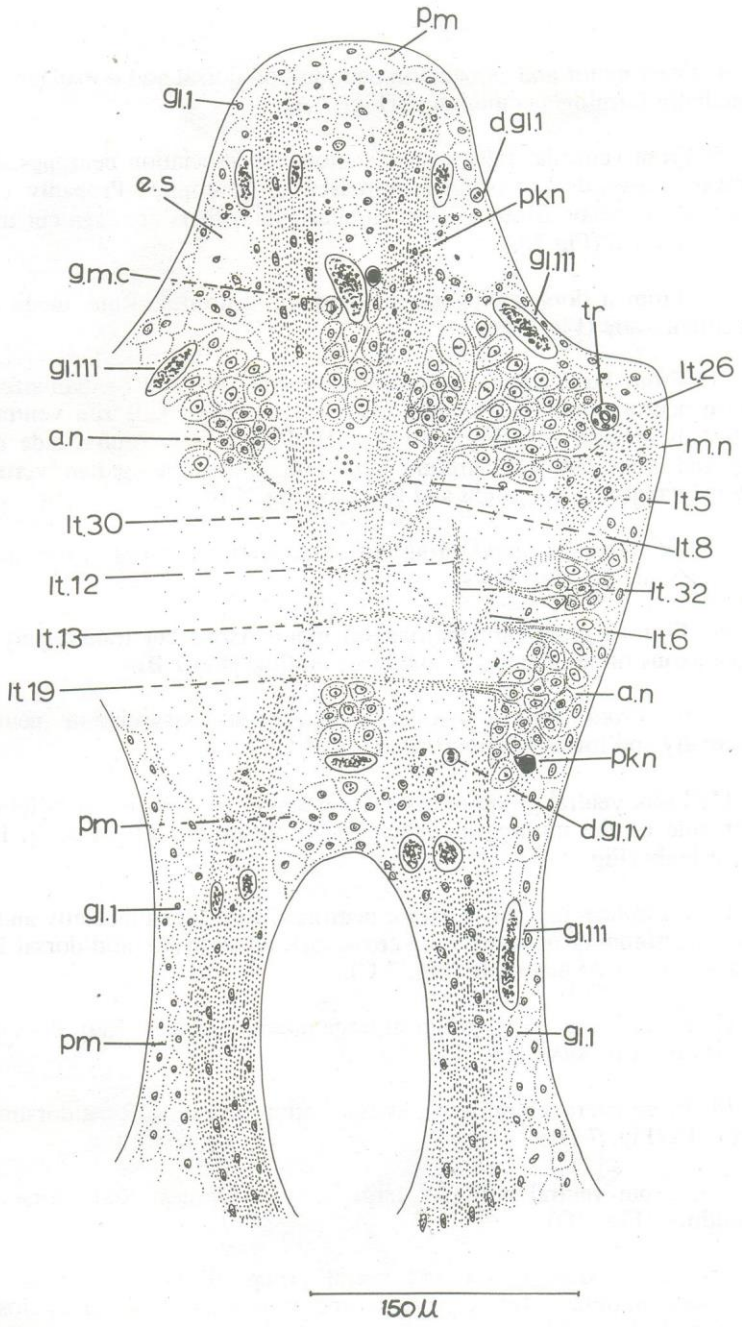


Fig. 5. Frontal section through prothoracic ganglion of 48 hours pupa showing neurones, fibre tracts, pyknosis, mitosis and other features.

Tract t. 4: From motor and association neurones on dorsal and dorsolateral side. Passes ventromedially forming a commissure (Fig. 7 A).

Tract t. 5: From ventrolateral group of motor and association neurones. Runs upward and outwards towards dorsolateral boundary of neuropile. Probably contributes to peripheral nerve. Some fibres are lost in neuropile, others are seen cut transversely in the sections studied (Fig 7A).

Tract t. 6: From a dorsolateral group of motor neurones. Runs medioventrally and forms a commissure (Fig 11B).

Tract t. 7: From large motor neurones on dorsal side and median-sized motor and association neurones on ventral side. Fibres from dorsal side run ventrally and then pass outwards around periphery of neuropile. Frbres from ventral side run vertically upwards and then outwards around neuropile. Forms a median vertical partition dividing the ganglion into a right and left half (Fig. 7 B).

Tract t. 8: From ventrolateral group of association neurones. Runs dorsomedially, forming a commissure (Fig 11B).

Tract t. 9: From a group of ventrolateral motor axons cut transversely in sections. Prominent axons running medially and cross midline (Fig 7 B).

Tract t. 10: From some dorsolateral motor and association neurones. Runs ventromedially and forms a commissure (Fig, 7 C).

Tract t. 11: From ventral group of association neurones. Both run parallel to each other on either side of the midline and then curve outwards and break up in the lateral glomerular body (Fig. 7 c).

Tract t. 12: From laterally placed motor neurones. Runs dorsomedially and some of the fibres of correspinding opposite tract cross each other in the mid-dorsal line and pass the dorsal boundary of neuropile (Fig. 7 C).

Tract t. 13: From lateroventral group of large motor neurones. Runs dorsomedially and crosses midline near dorsal side (Fig. 7 D).

Tract t. 14: From lateroventrally placed association neurones. Runs dorsimedially close to tract t. 13 (Fig. 7 D).

Tract t. 15: From ventral group of large motor neurones. Runs dorsomedially and crosses midline (Fig. 7D).

Tract t. 16: From dorsolateral and lateral group of large motor neurones. Runs medially, near midline, curves inwards and runs ventrally, finally lost in ventral glomerular body (Fig. 7 D).

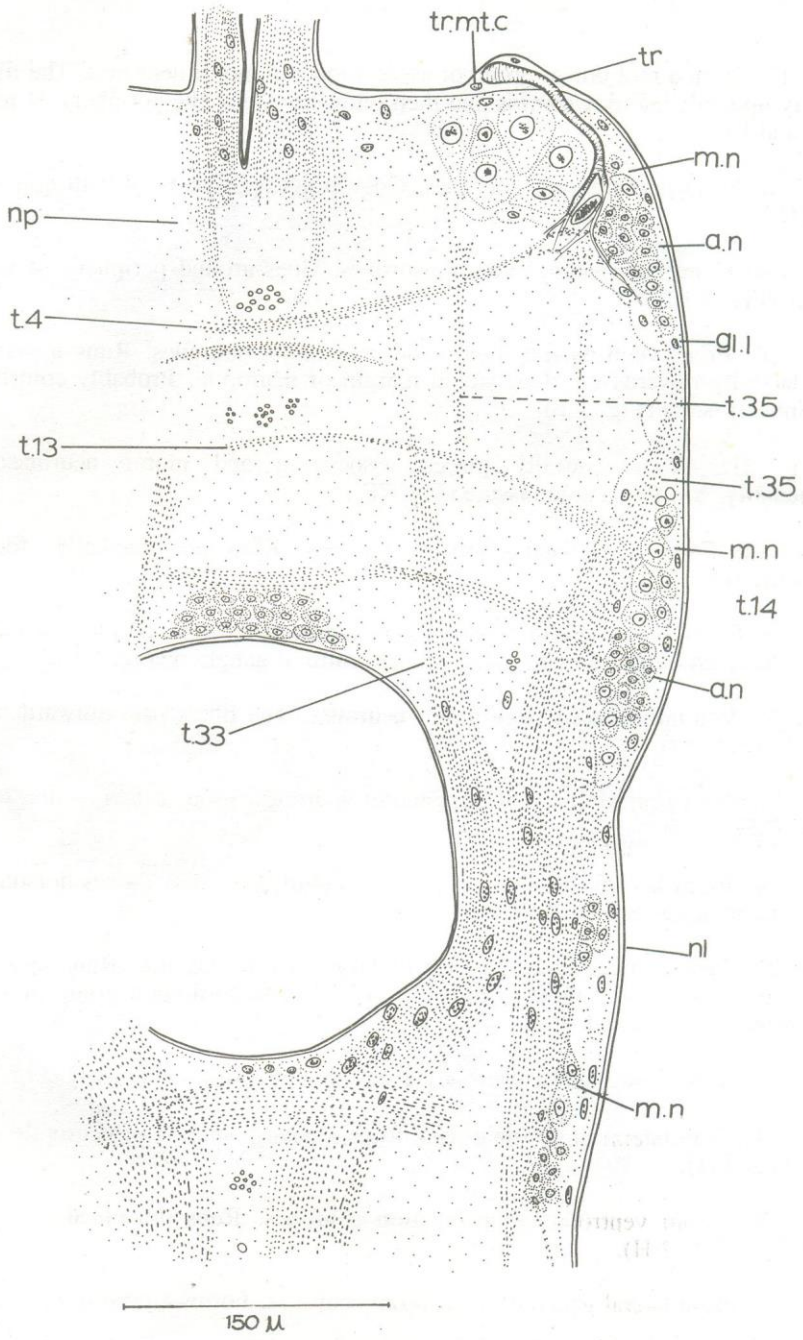


Fig. 6. Frontal section through adult prothoracic ganglion, as in Fig. 1.

Tract t. 17: From a mid-dorsal group of association and motor neurones. The fibres run vertically upwards and downwards and then outwards along the periphery of neuropile (Fig. 7 and E).

Tract t. 18: From lateral motor neurones. Goes around periphery of neuropile dorsally (Fig. 7 D).

Tract t. 19: From lateroventral motor neurones. Goes around periphery pf neuropile ventrally (Fig. 7 E).

Tract t. 20: From ventrolateral motor and association neurones. Runs upwards then curves laterally towards the dorsolateral margin of neuropile. Probably contributes to the peripheral nerve (Fig. 7 F).

Tract t. 21: From ventrally placed association and motor neurones. Runs dorsomedially, forming a commissure (Fig. 7E).

Tract t. 22: From ventrolateral motor neurones. Runs dorsomedially, forming a commissure (Fig. 7 E).

Tract t. 23: From ventral group of association and possibly some motor neurones. Runs dorsomedially and forms a commissure in the centre of ganglion (Fig. 7 E).

Tract t. 24: Ventrally placed large motor neurones. The fibres pass outwards and join tract t. 20 (Fig. 7 f).

Tract t. 25: From ventral group of large motor neurones. From a tract similar to tract t. 21 (Fig. 7 F).

Tract t. 26: From lateroventral group of large motor neurones. Passes dorsomedially, forming a commissure (Fig. 7 F).

Tract t. 27: From a ventrolateral group of large motor neurones. Runs upwards and then curves outwards and turns to run longitudinally, so forming a group of axons cut transversely (Fig. 7 G and H).

Tract t. 28: From lateral motor neurones. Runs around neuropile dorsally (Fig. 7 G).

Tract t. 29: From lateral motor neurones. Runs around boundary of neuropile ventrally (Fig. 7 G and H).

Tract t. 30: From ventrolateral association neurones. Runs dorsomedially, forming commissure (Fig. 7 H).

Tract t. 31: From lateral group of association neurones. Forms a prominrnt commissure (Fig. 7 H).

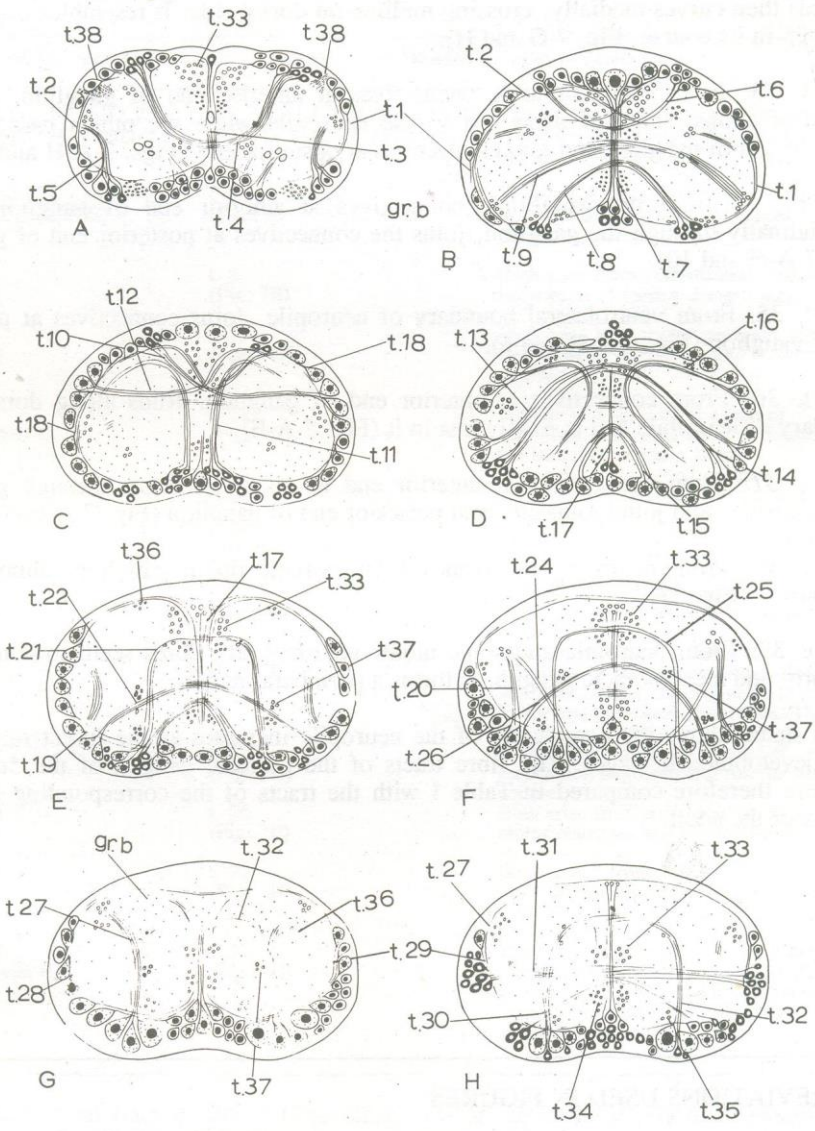


Fig 7. Diagrammatic serial drawings of adult prothoracic ganglion cut transversely with the same explanations as Fig. 1.

Tract t. 32: From lateroventral motor and association neurones. Runs vertically upwards then curves medially, crossing midline on dorsal side. It resembles tracts t. 21 and t. 25 in its course (Fig. 7 G and H).

Tract t. 33: From interganglionic connectives at anterior end of ganglion. A thick bundle of axons, some of them lost in the neuropile while the others pass through whole length of ganglion and join connectives at posterior end (Figs. 2 A-H and 10).

Tract t. 34: From interganglionic connectives at anterior end of ganglion. Runs longitudinally through the ganglion, joins the connectives at posterior end of ganglion (Fig. 7 A-H and 10).

Tract t. 35: From ventrolateral boundary of neuropile. Joins connectives at posterior end of ganglion (Fig. 7 A-H and 6).

Tract t. 36: From connectives at anterior end of ganglion. Runs along dorsolateral boundary of neuropile and is finally lost in it (Fig. 7 A-E).

Tract t. 37: From connectives at anterior end of ganglion. Runs through ganglion longitudinally, and joins connectives at posterior end of ganglion (Fig. 7 A-H).

Tract t. 38: From neuropile and comes from extreme tip of ganglion, almost from connectives (Fig. 7A).

Tract t. 39: From neuropile and some motor neurones. Its fibres seem to come from both pro- and mesothoracic ganglia. It forms a peripheral nerve.

As stated above the complexity of the neuropile increases as the insect reaches its final developmental stages. The fibre tracts of the thoracic ganglia of the 5th instar larva are therefore compared in Table I with the tracts of the corresponding thoracic ganglia of the adult.

ABBREVIATIONS USED IN FIGURES

a.n., Association neurone; d.gl., Dividing glial cell; gv.b., Glomerular body g.m.c., Ganglion motor cell; gl.I., Type I glial cell; gl.II., Type II glial cell; gl.III., Type III glial cell; gl.IV., Type IV glial cell; It. 1, It. 2, Larval thoracic fibre tract; m.n., Motor neuron; nl., Neurolamma; np.,Neuropile; pkn., Pyknotic cell; pm., Perineurium; t. 1, t. 2, adult prothoracic ganglion fibre tracts; tr., Trachea; tr. mt., Tracheal matrix; tr. mt. c., Tracheal matrix cell.

Table 1. Comparison of fibre tracts of the prothoracic ganglion of 5th instar larva and adult.

Tracts in the 5th instar larvae	Comparable tracts in the adult	Points of similarity
1. It. 1,2,3,4, 10,16,17,23, and 24 (Fig. 2 A, D and E-H)	t. 1,2,3,12,17,18,19,28 and 29 (Fig. 11 A,B,D,E,G and H)	These arise from corresponding groups of neurones and run the same course in the two stages.
2. It. 2 and 22 (Fig 2 B,C,E, and G)	t. 5 and 27 (Fig 7B)	These arise from corresponding groups of neurones and probably all contribute to the peripheral nerves.
3. It. 7 (Fig. 2B)	t. 8 (Fig. 7B)	Both arise from ventrolateral association neurones and form a commissure.
4. It. 8 (Fig. 2G)	t. 15 (Fig. 7D)	Both arise from motor neurones somewhat ventrolateral in position and run the same course.
5. It. 9 (Fig. 2B and C)	t. 11 (Fig. 7C)	Both arise from ventral group of association neurones and then break up in the lateral glomerular body.
6. It. 11 (Fig 2D)	t. 16 (Fig. 7D)	Both arise from lateral group of motor neruones and run the samse course.
7. It. 12 (Fig. 2E)	t. 23 (Fig. 7E)	Both arise from corresponding groups of neurones and form a commissure.
8. It. 13 (Fig. 2E)	t. 22 (Fig. 7E)	Both arise from lateroventral groups of motor neurones and form a commissure.
9. It. 14 (Fig. 2E)	t. 21 (Fig. 7E)	It. 14 might be comparable to t. 21 but where It. 14 arises from association neurones only, t. 21 originates from both motor and association neurones. The tracts run the same course.
10. It. 21 (Fig. 2G)	t. 31 (Fig. 7H)	Both arise from lateral groups of association neurones and run the same course.
11. It. 20 (Fig. 2F)	t. 26 (Fig. 7F)	Both arise from correspinding groups of motor neurones and run the same course.
12. It. 19 (Fig. 2F)	t. 30 (Fig. 7H)	Both arise from ventrolateral groups of association neurones and run the same course.
13. It. 18 (Fig. 2G and H)	t. 32 (Fig. 7H)	These two might be comparable but It. 18 originates only from association neurones whereas t. 32 arises from both motor and association neurones. Their course is the same in both stages.

The longitudinal tract It. 28-32 (Fig. 22A - H) of the larval prothoracic ganglion are similar to t. 33-37 (Fig. 23A-H) of the adult.

It. 5, 15 and 25 of the larva (Fig. 7A F-H) have no counterparts in the adult protheracic ganglion, while t. 4, 6, 7, 9, 10, 13, 14, 20, 24 and 25 (Fig. 7 A-D and F) have no equivalents in the larval prothoracic ganglion.

REFERENCES

ALI, F. A. 1973. Postembryonic changes in the central nervous system of *Pieris brassicae* (L) (Lepidoptera: Pieridae) *Trans. R. Ent. Soc. Lond,* **124**: 463-498.

ALI, F. A. 1980. Some quantitative aspects of the metamorphosis of the central nervous system of *Pieris brassicae* (L) *Pakistan J. Zool*; **12**: 99-114.

ALI, F. A. 1991. Histological changes occuring in some of the ganglia of the ventral nerve cord of *Pieris brassicae* (Linn.) (Pieridae: Lapidoptera). *Punjab Univ. J. Zool*; **6**: 19-29.

SHANKLAND, D. L. AND FRAZIER, J. L. 1985. In: Fundamentals of Insect Physiology ED. (M. S. Blum). 253-286. John Wily and Sons.

ELZINGA, R. A. 1988. Fundamentals of Entomology. Prentice-Hall, New Jersey. pp. 442.

MAYNARD, D. M. 1962. Organization of neuropile. *Am. Zoologist,* **2**: 79-96.

RICHARDS, O. W, AND DAVIES, R. G. 1977. Imm's General Textbook of Entomology. Vol. 1. (10th Ed.) Chapman & Hall Ltd. London.

WIGGLESWORTH, V. B. 1957. The use of Osmium in the fixation and staining of tissues. *Proc. Royl. Soc. Lond.* **147**: 185-199.

WIGGLESWORTH, V. B. 1959. The histology of the nervous system of an insect, (Hemiptera). i. The peripheral nervous system. ii. The central ganglia. *Q. J. Microse, Sci.*, **100**: 285-296; 299-313.

(Received: January 16, 1993)