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Neuromuscular systems in the fifth instar larva of silkworm *Bombyx mori* (Lepidoptera: Bombycidae): I - Cephalothoracic musculature and its innervation

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Abstract : The cephalo-thoracic musculature of the fifth instar larva of *Bombyx mori* comprises distinct groups of segmental muscle bands arranged in a stereotyped pattern. It includes dorsal, ventral, tergopleural, tergocoxal, lateral intersegmental, pleurosternal, sternocoxal, pleurocoxal and spiracular muscles. The cephalothoracic segments are innervated by the nerves of brain, suboesophageal ganglion (SG) and three thoracic ganglia (TG_1, TG_2, TG_3).The brain gives nerves for compound eyes, antennae, labrum, frontal ganglion and the integument in the head. The SG, TG_1, TG_2 and TG_3 give out a pair of lateral segmental nerves each, called the dorsal (DN) and ventral (VN) nerves. The DN of SG innervates muscles in the cephalic region, while its VN innervates muscles in the prothorax. The DN of thoracic ganglia innervates muscles in the dorsal, lateral and ventral regions of the hemi-segment while the VN innervates muscles in the ventral region. The innervation pattern indicates the presence of mixed nerves and multiple innervations that facilitate coordinated body movements and locomotion.

Keywords: *Bombyx mori*, Cephalothorax, Ganglionic nerves, Innervation, Musculature

INTRODUCTION

The cephalo-thorax of an insect is a composite structure formed by the union of head and thorax. The head, a sclerotised capsule formed by at least six sclerites, constitutes the major centre of sense organs and oral apparatus. The thorax comprises three segments, the prothorax, mesothorax and metathorax and represents the chief locomotory region of the body since it bears legs and wings (David and Ananthakrsihnan, 2006). Structurally and functionally the cephalothorax works like a single cohesive unit under the control of well articulated neuromuscular systems. The nerve-muscle integration in the region brings about coordinated action of all segments and facilitates the discharge of multiple functions such as the wing beat, body movement, respiration and circulation of body fluids, thermiogenesis (Sink, 2006). Following the pioneering studies of Snodgrass (1935,1958), the functional morphology, and physiology of muscles in various insects such as the locust (Usherwood, 1967, 1968), cockroach (Iles and Pearson, 1969) ,Drosophila (Sink, 2006), *Antheraea* (Lawrence and Truman, 1984), and *Heptagenia* (Deshpande and Pathan, 1982) leaf hopper(Burrows, 2007) and *Manduca sexta* (Eaton, 1982) received wide attention. Some attempts were made to elucidate the basic features of nerve-muscle coordination in insects (Libby, 1959 and Randall, 1968). The nervous control of gut mobility has been widely reported (Duve *et al*, 1999; Wolf and Harzsch, 2002 and Copenhaver, 2007).

Screening of available literature reveals the paucity of morphological studies on segmental musculature and its innervation by the ganglionic nerves in insects, more particularly in the lepidopteran larvae. Though, the outline morphology of muscles in the adult silkworm was examined (Kondoh and Obara, 1982), no such studies were made on larval stages. The neuromuscular anatomy of fifth instar larva of *Bombyx mori* assumes importance in view of its ability to spin the silk cocoon, the raw material of the sericulture industry. In our earlier report (Sivaprasad and Muralimohan, 1998), we have demonstrated that the metamorphic changes in the nervous system were accompanied by an increase in the complexity of peripheral nerves in the fifth instar larva of silkworm with possible increase in the number of synaptic contacts with the segmental muscles. The present investigation highlights the gross organisation of the cephalothoracic musculature, its innervations and its possible role in the larval body movements and locomotion in *Bombyx mori*.

MATERIALS AND METHODS

The fifth instar larva of multivoltine NB_4D_2 strains of *Bombyx mori*, reared in the laboratory as per Krishnaswami (1986), were fixed for 24 h in a fixative consisting of 25 ml of 40% formalin, 1.25 ml of acetic acid and 10 g of chloral hydrate in 100 ml of distilled water (Chauthani and Callahan, 1966). They were pinned dorsal side up on a wax block and dissected out in the mid-

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dorsal region from the last abdominal segment to the head. The gut was carefully removed along with the fatbody and attached tracheae.

The gross organization of the brain or cerebral ganglion (CG), suboesophageal ganglion (SG), and the three thoracic ganglia (TG_1, TG_2, TG_3) and their branching and innervation pattern and nerve-muscle anatomy of thoracic segments starting with SG were studied under a stereo-binocular microscope, by applying 1% methylene blue stain in distilled water. Occasionally alcoholic Bouin's fluid was added to the preparation to stain the nerve-muscle preparation blue-green. In such preparations the ramifications of the nerves and their finer branches could be clearly distinguished, counted and their innervation traced. Sketches of thoracic segmental musculature and its innervation pattern were made directly from the dissections. About 5 to 10 larvae were used to draw the sketches from each segment.

RESULTS

The results of the present investigation, presented in Figs. 1 to 8 and in Table 1, cover three aspects, vi.z, the nerves of the brain, thoracic musculature and its innervation by the segmental nerves emanating from SG and the three thoracic ganglia.

I. The Brain and its nerves: The central nervous system of silkworm consists of a nerve ring in the head region around the oesophagus and a longitudinal double ventral nerve cord below the gut. The nerve ring of silkworm consists of a bi-lobed cerebral ganglion (CG) or brain above the oesophagus, and another bi-lobed suboesophageal ganglion below the oesophagus, connected together by a pair of circum-oesophageal connectives (Sivaprasad and Muralimohan, 1990).

The principal nerves of the brain include those reaching the compound eyes, antennae, labrum and frontal ganglion, besides the tegumental nerve.

1.Optic nerves: A pair of optic nerves arises on the outer lobes of CG on either side, travel antero-laterally and each one of them bifurcates and proceeds towards the lateral stemmata that are destined to become future compound eyes (Fig. 1 (5)).

2.Antennal nerves: A pair of antennal nerves arises immediately anterior to the optic nerves, proceeds on

Table 1. Branching and innervation patterns of the dorsal (DN) and ventral (VN) nerves of the ganglionic nerves of the thoracic ganglia (TG_1, TG_2, TG_3) in the fifth instar larva of the silkworm, *Bombyx mori*. The number of motor and sensory branches, the thoracic muscles innervated by the motor branches and the special features of the nerve branches in each hemi-segment are also shown.

Ganglion	Nerve	No. of Motor branches	Number and Names of muscles innervated	No. of sensory branches	Area innervated by sensory branches	Special feature, if any
SG	DN	4 [M_1- M4]	33 HM	--	--	Motor Nerve
	VN	4 [M_1- M4]	7VM, 7DM	--	--	Motor Nerve
TG_1	DN	4 [M_1- M4]	12 VM, 32 LSM	2 [S_1, S_2]	VR, VLR, DR, DLR	Mixed Nerve
	VN	8 [M_1- M_8]	29 SCM, 5 TCM, 4 PSM	2 [S_1, S_2]	MVR, VR, VLR, LR	Mixed Nerve
TG_2	DN	6 [M_1- M_6]	48 DM, 8 SM,	1 [S_1]	DLR, MDR	Mixed nerve
	VN	6 [M_1- M_{M6}]	20 SCM, 2 PCM, 7 PSM, 17 TPM 4 DM	1 [S_1]	MVR, VR	Mixed nerve M_1 gives out a small MNC
TG_3	DN	10 [M_1- M_{10}]	21 VM, 12 SM, 78 DM	2 [S_1, S_2]	VR, VLR, MDR	Mixed nerve A branch of M_1 innervates 2 VM in the preceding segment i.e mesothorax, besides giving a small MNC.
	VN	4 [M_1- M4]	27 SCM, 2 PCM, 12 TCM, 4 TPM	1 [S_1]	MVR, VLR	Mixed nerve

DN: dorsal nerve; DM; dorsal muscles; DR; dorsal region; DLR; dorsolateral region; LR; lateral region; LSM: lateral intersegmental muscles; M_1 _ M_{10} : first to 10th motor nerves; MDR: mid-dorsal region; MVR: midventral region; MNC: median nerve connective; MVR: midventral region; PCM: Pleurocoxal muscles; PSM: Pleurosternal muscles; SG: suboesophageal ganglion; SCM: sternocoxal muscles; SM: spiracular muscles; TCM: Tergocoxal muscles; TG_1: prothoracic ganglion ; TG_2: mesothoracic ganglion; TG_3: metathoracic ganglion; TPM: tergopleural muscles; VLR: ventrolateral region; VM: ventral muscles; VN: ventral nerve; VLR: ventrolateral region; VR: ventral region.

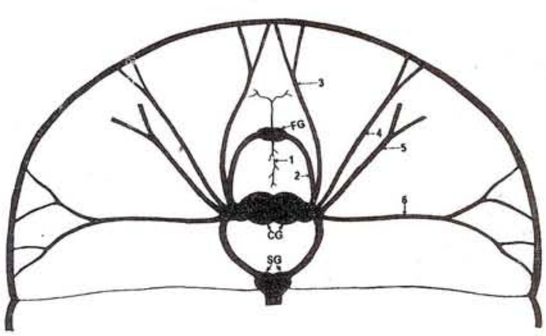

Fig. 1. *Distribution of brain nerves in the head capsule of the fifth instar larva of Bombyx mori. 1. recurrent nerve; 2. frontal ganglion connective; 3. labral nerve; 4. antennal nerve; 5. optic nerve; 6. tegumental nerve; CG. cerebral ganglion; FG. frontal ganglion; SG. suboesophageal ganglion.*

either side antero-laterally and innervates the antennal buds of the larva in the dorsal region of the head (Fig.1 (4)).

3.Labrofrontal nerves: These are a pair of nerves emerging anteriorly on either side of the brain. Each nerve bifurcates into two branches, namely a frontal ganglion connective and a labral nerve. The former proceeds antero-ventrally and medially and meets the frontal ganglion of the stomodeal nervous system, making a long anteroventral loop, while the latter bifurcates and proceeds to the labrum and mandibular regions. A small nerve, designated recurrent nerve emerges from the frontal ganglion medially (Fig.1 (1,2,3)).

4.Tegumental nerves: A pair of tegumental nerves arises from the brain, posterior to the antennal and optic nerves, extend into the dorsal region on either side, where they bifurcate and innervate the epidermis of cephalothorax (Fig.1 (6)).

II. The cephalothoracic musculature

The muscles in the cephalothorax of silkworm includes those present in the head and thorax. They are organized in the form of elongated bands in the fifth instar larval body and are designated based on the nomenclature used by Snodgrass (1935). The muscles that occur as separate bands throughout their length are considered individual muscles and are counted accordingly, while those fused partly or wholly are considered as branches of the same muscle.

The head muscles are arranged in five groups with varying number of muscle strips ranging from 2 to 6 in each half of the head capsule. Two groups of them have a dorso-lateral disposition, two groups have ventrolateral and one group has a ventral disposition (Fig. 2). The thoracic muscles appear in the form of distinct bands with specific sites of attachment and orientation and with a stereotyped pattern of arrangement in each hemi-segment. In all, the following nine types of muscles were discerned in the three thoracic segments of silkworm.

1.Dorsal muscles (DM): They are median longitudinal and obliquely dispositioned muscle strips of segmental length, lying under the dorsal body wall, with their

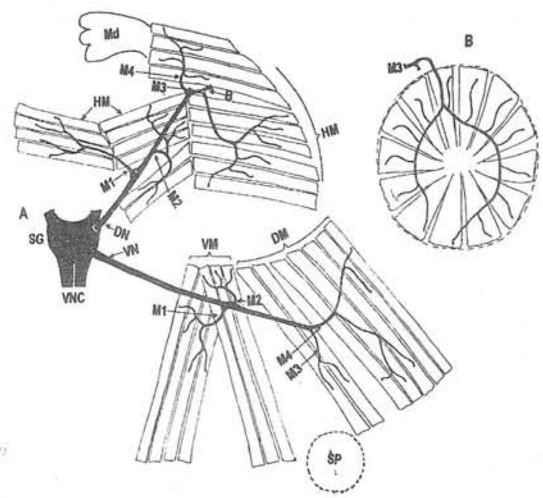

Fig. 2 *(A). Branching and innervation patterns of the dorsal (DN) and ventral (VN) nerves of the suboesophageal ganglion (SG) in the head capsule and prothorax of the fifth instar larva of Bombyx mori.* ***Inset B**: Distribution of the third motor branch (M 3), which could not be shown in the main figure (A). DM. dorsal muscles; M_1 to M_4 motor branches 1 to 4; Md. mandible; SP. spiracle; VM. ventral muscles.*

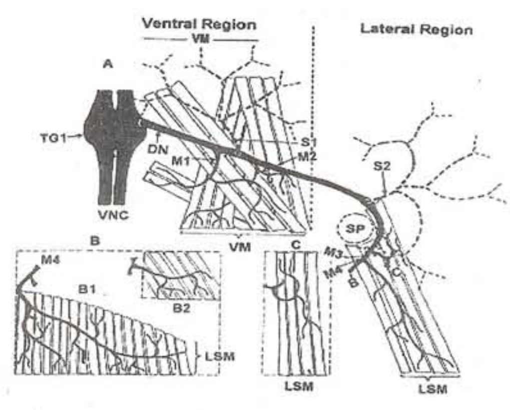

Fig. 3 *(A). Branching and innervation patterns of the dorsal nerve (DN) of the first thoracic ganglion (TG_1), in the prothorax of the fifth instar larva of Bombyx mori.* ***Insets B and C**: The distribution of motor branches M_4 and M_3 respectively. LSM. lateral intersegmental muscles; S_1 and S_2, sensory branches 1 and 2., VNC. ventral nerve cord. Remaining abbreviations are the same as in the legend for figure 2.* ***Note**: In all figures, the motor branches (M_1, M_2 etc.,) are shown in thick lines, while the sensory branches (S_1, S_2 etc.,) are shown in broken lines.*

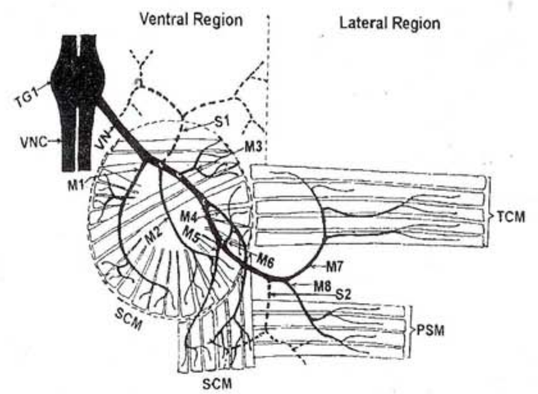

Fig. 4. *Branching and innervation patterns of the ventral nerve (VN) of the first thoracic ganglion (TG_1), in the prothorax of the fifth instar larva of Bombyx mori. PSM . pleurosternal muscles; SCM. sternocoxal muscles; TCM. tergocoxal muscles. Remaining abbreviations are the same as in the legend for figure 2.*

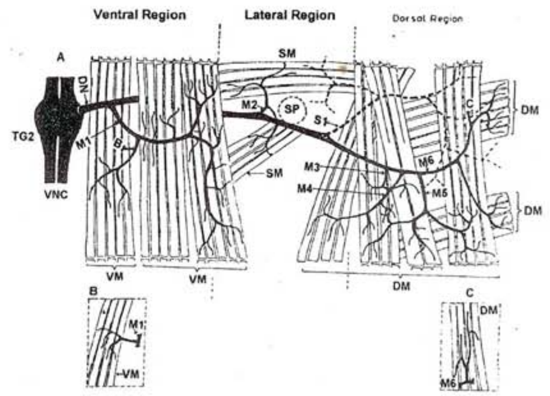

Fig. 5 *(A). Branching and innervation patterns of the dorsal nerve (DN) of the second thoracic ganglion (TG_2), in the mesothorax of the fifth instar larva of Bombyx mori.* ***Insets B and C :*** *Part of innervation by motor branches, M_1 and M_6, which could not be shown in the main figure (A). SM. Spiracular muscles. Remaining abbreviations are the same as in the legend for figure 2.*

attachments at the intersegmental folds. The principal DM are absent in the prothorax but they are prominently developed in the next two thoracic segments viz., mesothorax and metathorax. They vary greatly in their number, being 29 in mesothorax and 71 in metathorax. Their arrangement becomes complex due to the presence of external and internal layers (Figs. 2, 5,7)

2.Ventral muscles (VM): They are median longitudinal and obliquely dispositioned muscle strips of segmental length present under the ventral body wall. The extend into the inner and outer sternal region in all the three thoracic segments. In prothorax they are attached anteriorly to the head at the post-occipital ridge and posteriorly to the intersegmental wall. In mesothorax and metathorax, they lie in the sternal region with their attachments at the intersegmental walls. They display variation in number, being 12 in prothorax and 19 each in meso- and metathoracic segments (Figs. 2, 5,7).

3.Tergopleural muscles (TPM): They are lateral horizontal or oblique segmental muscle bands present in the external and peripheral area in meso- and metathoracic segments. They originate on the tergal body wall and are inserted on the pleura of the segment. They are absent in prothorax, but represented in the next two thoracic segments. In mesothorax 23 muscle bands are arranged in 6 groups, while in metathorax only 4 muscle bands are arranged in one group (Figs. 6, 8).

4.Tergocoxal muscles (TCM): They are horizontal muscle strips attached to the tergal wall at one end and to the coxal base at the other. They occur in the peripheral lateral area in both pro- and metathoracic segments, but absent in mesothorax. There are 5 TCM strips in prothorax and 12 in metathorax (Figs. 4, 8).

5.Lateral intersegmental Muscles (LSM): The longitudinal or oblique intersegmental muscle bundles lying in the lateral region of the body below the spiracle are designated lateral intersegmental muscles. Their intersegmental nature is evident as they occur in between pro-and mesothoracic segments. Anteriorly, they are attached to the pleural body wall and posteriorly to the dorsal tergum of the next segment (Fig.3).

6.Pleurosternal muscles (PSM): They are intersegmental muscles extending in between the lateral pleural the ventral sternal ridges in pro-and mesothoracic segments. Arranged either horizontally or vertically or obliquely, they occur as a group of 4 muscle strips. PSM are absent in metathorax (Figs.4, 6).

7.Sternocoxal muscles (SCM): They are horizontal or longitudinal or oblique intersegmental muscles lying in the sternal region, articulating the coxal base with the sternal wall. They are present in the leg-bearing meso- and metathoracic segments, with their insertions either within or outside the cone shaped coxa. They are essentially similar in arrangement in both the segments, except that a single group of muscles in one leg are represented by two or more groups in another (Fig.4, 6,8).

8.Pleurocoxal muscles (PCM): The horizontal intersegmental muscles articulating the episternum with the coxal base in the sternal area are designated as PCM. They occur as a pair of two strips both in mesothorax and metathorax but they are absent in prothorax (Fig. 6,8).

9.Spiracular muscles (SM): These are the horizontal or

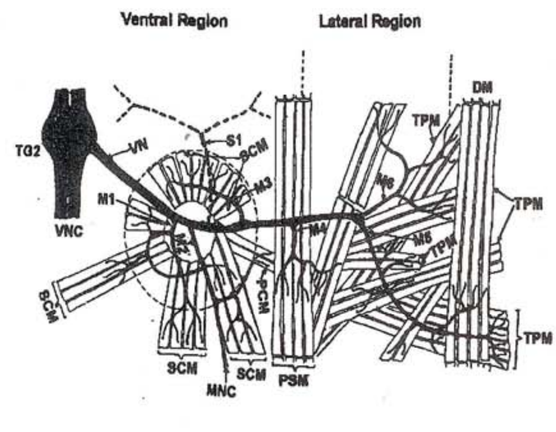

Fig. 6. *Branching and innervation patterns of the ventral nerve (VN) of the second thoracic ganglion (TG_2), in the mesothorax of the fifth instar larva of Bombyx mori. MNC. median nerve connective; TPM. tergopleural muscles; Remaining abbreviations are the same as in the legend for Fig. 4.*

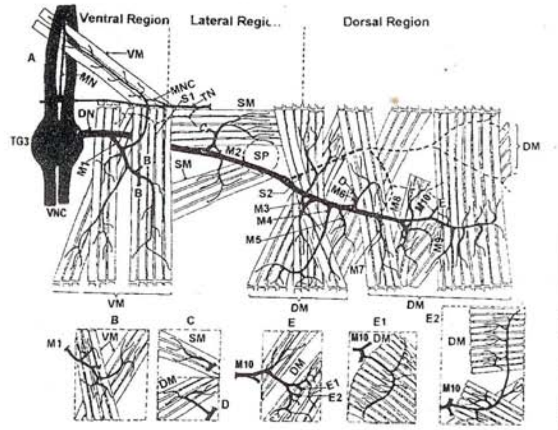

Fig. 7 ***(A).*** *Branching and innervation patterns of the dorsal nerve (DN) of the third thoracic ganglion (TG_3), in the metathorax of the fifth instar larva of Bombyx mori.* ***Insets B, C, D, E (E 1, E 2):*** *Part of innervation by motor branches, M_1, M_2, M_6 and M_{10} respectively, which could not be shown in the main Fig. (A). Abbreviations are the same as in the legend for Fig.4.*

oblique intrasegmental muscles lying in the inner lateral region of both meso- and metathoracic segments. They are arranged in two groups of 4 each in mesothorax and 5 + 4 in metathorax, with their attachments on the dorsum below the dorsal muscles at one end and on the ventrum below the ventral muscles at the other end (Figs. 5, 7).

III. Innervation patterns

The cephalothoracic muscles receive innervations from the segmental ganglionic nerves emanating from the suboesophageal (SG), prothoracic (TG_1), mesothoracic (TG_2) and metathoracic (TG_3) ganglia. These four ganglia give off two principal lateral segmental nerves on either side, designated as dorsal (DN) and ventral (VN) nerves. Both DN and VN give off a number of motor branches viz., M_1, M_2, M_3 etc., to the thoracic muscles and sensory branches viz., S_1, S_2, S_3 etc., to the epidermis of the body wall in each hemi-segment. Their number varies from 4 to 10 in DN and 3 to 8 in VN. The branching and innervation patterns of both DN and VN are presented in Figures 2 to 8 and in Table 1. Soon after their origin, they pass through the ventral and lateral regions and finally extend into the dorsal region of the segment in the larval body. They follow a specific course over the segmental muscles. The DN passes partly over the inner face of the ventral muscles, while the VN runs peripherally in between the external and internal muscle layers. The location of nerve branches is either midventral (MVR), ventral (VR), ventrolateral (VLR), dorsal (DR), dorsolateral (DLR) or middorsal (MDR) regions in the thoracic segments. They originate either on the dorsal aspect or ventral aspect of the nerve. Their course may be either anteroventral (AV), posteroventral (PV), anterolateral (AL), posterolateral (PL), anterodorsal (AD), posterodorsal (PD) or dorsolateral (DL).

1. Projections of dorsal nerve (DN): In the head region, the DN of SG gives off 4 motor branches (M_1 to M_4) which innervate about 33 head muscles (HM), probably comprising the cervical, mandibular, maxillary, labial and stomodeal muscles (Fig. 2). The DN of prothoracic ganglion (TG_1) sends 4 motor branches (M_1 to M_4), which innervate about 44 muscle strips including ventral (VM) and lateral intersegmental (LSM) muscles in prothorax (Fig.3 and Table 1). The DN of mesothoracic ganglion (TG_2) sends 6 motor branches (M_1 to M_6) to about 56 muscles including the spiracular (SM) and dorsal (DM) muscles in mesothorax (Fig. 5 and Table 1). The DN of metathoracic ganglion (TG_3) is the largest one and sends 10 motor branches (M_1 to M_{10}) and innervates about 111 muscle bundles in the metathorax including VM, SM and DM. The M_1 of TG_3, while innervating ventral muscles in the preceding mesothorax gives a small branch called median nerve connective (MNC) that links the DN with the median nerve (Fig.7 and Table 1). The DN of all the three thoracic ganglia (TG_1, TG_2, and TG_3) gives off 2, 1, and 2 sensory branches (S_1 and S_2) respectively in the ventral and dorsal regions. The S_1 innervates body wall in the ventral and ventrolateral regions, while the S_2 innervates the body wall in the dorsal and dorsolateral regions of the segmental wall (Figs.3, 5,7 and Table 1).

2. Projections of ventral nerve (VN): The VN of SG sends 4 branches (M_1 to M_4) and innervates about 14 muscle strips including VM and DM in the prothorax (Fig. 2; Table 1). The VN of TG_1 gives off 8 motor branches (M_1 to M_8) for about 38 muscles such as the sternocoxal (SCM), tergocoxal (TCM) and pleurosternal (PSM) muscles in the ventral region of the prothorax.(Fig.4 and Table 1). The VN of TG_2 sends 6 branches (M_1 to M_6) for about 50 external muscles strips in mesothorax that largely includes pleurocoxal (PCM), sternocoxal (SCM), pleurosternal (PSM), tergopleural (TPM) and dorsal (DM) muscles. Additionally, the M_2 of TG_2 gives out a small nerve called median nerve connective (MNC) that meets the transverse branch of the median nerve (MN) on either side (Fig.6 and Table 1). The VN of TG_3 sends 4 motor branches (M_1 to M4) for about 45 external muscles including sternocoxal (SCM), Pleurocoxal (PCM), Tergocoxal (TCM) and Tergopleural (TPM) muscles (Fig. 8; Table 1). Like the DN, the VN of all the three thoracic ganglia, viz., TG_1, TG_2, and TG_3 gives out 2, 1 and 1 sensory branches (S_1, S_2) respectively, which innervate the body wall in the ventral and ventrolateral regions of thoracic segments(Figs.4, 6, 8).

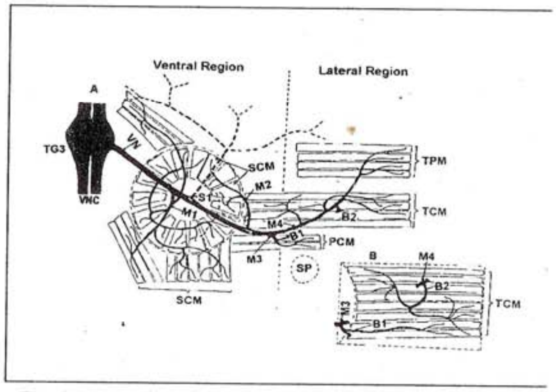

Fig. 8 *(A).* *Branching and innervation patterns of the ventral nerve (VN) of the third thoracic ganglion (TG_3), in the metathorax of the fifth instar larva of Bombyx mori.* ***Inset B:*** *Part of innervation by motor branches, M_3, and M_4 respectively shown in insets B_1 and B_2, which could not be shown in the main figure (A). Abbreviations are the same as in the legend for figure 4.*

DISCUSSION

The present investigation highlights the basic tenets of nerve muscle integration in the cephalothoracic segments of the silkworm larva.

1.Nerves of the brain and SG: The cerebral (CG) and suboesophageal (SG) ganglia together constitute a major regulatory centre for the movement of head, mouthparts and the antennae. The principal nerves of the CG such as the optic, antennal, labral and tegumental nerves show that they are predominantly sensory as evident from the distribution of their branches and terminal arborizations in the respective regions of the head. Obviously, they collect the information from the respective target organs and feed it into the brain. It is likely that the sensory perceptions from the mouth parts reach the brain through labral nerves and those from the antennae through the antennal nerves. The labral nerve probably controls the movement of mouthparts such as the labrum, labium, mandibles and maxillae. Obviously, the activities pertaining to touch, sight, smell etc., are assessed in the brain and necessary motor actions are initiated. Further, the CG of silkworm maintains contiguity with the frontal ganglion of the stomodeal nervous system through the frontal ganglion connective (Fig.1). The frontal ganglion (FG) contains sensory and motor associations of the stomodeal system and acts as the center of peristalsis (Snodgrass, 1935). It is known to controls gut mobility by producing two antagonistically working endogenous neuropeptides viz., allostatins and allatotropin (Duve *et al.*, 1999). Additionally, the FG-CG - SG complex has a significant role in controlling and modifying circadian rhythms in silkworm through peripheral clocks distributed in different organs of the body (Hasegawa and Shimizu,1987; Sehadova *et al.*, 2004) The frontal ganglion might have similar functional roles in the silkworm that needs elucidation.

The innervation pattern of SG suggests that it principally controls the movement of head and mouthparts. The levator, depressor, retractor and rotator functions of the cervical muscles and those of the mouthparts such as the mandibles, maxillae, labium and hypopharynx are under the influence of SG. Similarly, the innervation pattern of the nerves of SG suggests that it might have a regulatory action on the dilatory movement of cibarium i.e. the front portion of the oral cavity that could act as a powerful suction pump and facilitates food ingestion. Thus, in silkworm, the SG seems to act as the central organ of the gnathal and cervical regions of the head and probably functions as an inhibitory centre as advocated by Snodgrass (1935). Moreover, the SG does not seem to receive any sensory input from the body wall either through its DN or VN. It is likely that the sensory functions of the cephalothorax are shared by both CG and TG, while the SG exclusively controls the motor functions. Clearly, the CG seems to be the center of association between the major sense organs located on the head while the SG seems to act as the major motor center in the cephalothoracic region of silkworm. Further research involving electrophysiological, anatomical and behavioural techniques is needed to analyze the definitive functions of both CG and SG in *Bombyx mori.*

2.The musculature: The thoracic musculature in the silkworm is in accordance with the segmental nature of the body and conforms closely to the typical insect musculature with a stereotyped pattern of arrangement (Snodgrass, 1958 and Sink, 2006). The muscular arrangement has become more complex in the thorax due to the presence of leg muscles. The complements of thoracic muscles in *Bombyx mori* are grouped into three categories, namely the dorsal (DM), ventral (VM) and lateral (LM) muscles. They occur in two layers, external and internal and derive their names accordingly. Each group possesses a distinctive set of properties, size, shape, number, orientation and attachments to epidermis and synaptic innervation.

The internal groups of dorsal muscles (DM) occur as longitudinal bundles of segmental length and are attached to the epidermis in the thoracic intersegmental folds. The external dorsal muscles on the other hand are seldom of segmental length and appear as short intrasegmental bundles with oblique or transverse disposition. Functionally they are known to act as protractors, as their contraction lengthens the body by decreasing the overlapping of segments as suggested by Snodgrass (1935) and this function is coordinated by the DN. In contrast, the internal group of ventral muscles (VM) with a similar disposition is typically intersegmental and is known to function as the retractor, as its contraction shortens the length of the thoracic segments under the control of VN of each thoracic ganglion.

The internal group of ventral muscles (VM) occurs as short oblique intrasegmental muscle strips and is known to act as sternal protractor similar to that of the external ventral muscles. The integrated locomotory activity in the thorax is achieved by the simultaneous contraction and relaxation of DM and VM in which a wave of peristalsis passes over its thorax due to simultaneous contraction of DM and VM.. The contraction of the former results in the shortening of the segment followed by lifting its posterior end from the ground that enables forward leg movement. Conversely, the contraction of VM brings down the segment so that the legs are attached to the ground firmly during locomotion as demonstrated by Barth (1937). The lateral groups of muscles do not conform so closely to a general plan of arrangement and includes muscles such as the spiracular (SM), tergosternal (TSM), tergopleural (TPM), tergocoxal (TCM), lateral intersegmental (LSM) and pleurocoxal (PCM) muscles. Most of them are intrasegmental in position with an external horizontal disposition and lack the internal counter parts, with the sole exception of the spiracular muscles (SM), which occur in two groups, one above and the other below the spiracle (Fig. 5,7). Certain lateral muscles such as PSM and TPM lie on intersegmental folds and still others occur as oblique end-pieces in the pleural area. Many lateral muscles are known to sub-serve the functions of lifting the body above the substratum and the regulation of respiratory movements (Deshpande and Pathan, 1982).

Yet another group of muscles in silkworm larva includes the sternocoxal muscles (SCM) of the legs, represented as external and internal layers with oblique disposition in the leg or with vertical disposition at the coxa. The external layer is inserted at the rim of the coxal circumference and the internal layer is extended deep into the coxa. Probably, they act as antagonistic muscles and perform depressor and elevator functions in the leg movement as reported by Burrows (2007). The walking movement and other types of movements such as those related to raise, depress, extend or flex the body or its parts over the ground etc., are performed by the sternocoxal muscles with a set of antagonistic bundles of SCM (David and Ananthakrsihnan, 2006). Together with TCM and PCM, the sternocoxal muscles constitute a major group of contractile machinery of the leg movement in silkworm. According to Snodgrass (1935), the TCM and SCM with their attachments at the anterior coxal base act as the tergal remoter, causing the retardation of the leg movement. Obviously, in silkworm the internal group of TCM of pro- and mesothoracic segments, with its attachment to the anterior coxal base (Figs.4, 8), probably acts as the tergal promoters and accelerates the leg movement. Both tergal promoters and remoters are absent in the mesothorax of silkworm as evidenced by the absence of TCM in that segment. Likewise, the SCM of the mesothorax, attached to the anterior half of the coxal base (Fig.8), may act as sternal promoters and those attached at the posterior coxal base function as the sternal remoters of leg movement in silkworm. Similarly, the PCM of meso- and metathoracic segments of silkworm, together with SCM act as coxal promoters by functioning as the abductors of the coxa. Since, the PCM, TCM and SCM are innervated by the VN of the thoracic ganglia, it is presumed that the leg movement and the locomotion of both the larva and adult flies in the silkworm are coordinated by the VN in each thoracic segment, since the leg musculature represents a prototype of musculature for the development of adult musculature (Singh *et al.*, 2007). The abductor function of prothoracic coxa is retarded either partially or completely in silkworm, as evidenced by the absence of PCM (Snodgrass, 1935).

The presence of three groups of muscles viz., DM, VM and LM in each segment has evolutionary significance. As suggested by Snodgrass (1935), the most advanced feature of insect locomotion is the presence of a single group of PCM, which imparts free leg movement in longitudinal, transverse and vertical planes, while the addition of another group of muscles like SCM ensures hinge movement in transversely inclined axis. Further addition of TCM to these muscles retards the leg movement and thus represents a primitive condition. Obviously, within the thorax of a silkworm, the mesothorax with simple set of musculature of SCM and PCM represents an advanced locomotory feature over pro- and metathoracic segments in which the leg movement is primitive due to the addition of TCM. Accordingly, the pattern of thoracic musculature indicates the primitive nature of locomotion in silkworm, an evolutionary change probably caused by its domestication.

3. Specific features of innervation: The innervation pattern of motor branches in silkworm is similar to that observed in other insects (Randall, 1968; Konda and Obara, 1982 and Eaton, 1982) and displays the following features.

i. Most of the ganglionic nerves of TG are of mixed type as they contain both motor (M_1, M_2 etc) and sensory branches (S_1, S_2 etc.). The nerve branches that innervate muscles are considered motor nerves, while those innervate the epidermis as sensory nerves.

ii. The motor branches of the DN are extended into the ventral, lateral and dorsal regions of the hemi-segment, while the ventral nerve innervates ventral internal and lateral internal muscles in the ventral and lateral regions and both dorsal external and dorsal internal muscles in the dorsal region in each hemi-segment. On the other hand, the motor branches of VN are restricted to the ventral region of the thoracic segments and innervate ventral internal and ventral external muscles. The distribution of DN and VN indicates that the lengthening of the thoracic segments, brought about by the contraction of dorsal external, dorsal internal and ventral internal muscles is coordinated by the thoracic ganglia through their DN. Similarly, the innervation of external layer of ventral muscles by the VN indicates that the shortening of the thorax due to contraction of ventral external muscles is facilitated by the VN of thoracic ganglia.

iii. The potential number of motor branches of DN is the function of the number of muscles to be innervated. For instance, the DN of TG_1 with the four motor branches innervates 44 muscles in the prothorax while that of TG_3 with 10 motor branches innervates over 100 muscles in metathorax (Fig.2, 3, 7). However, the VN does not show such correlation between the number of motor branches and muscles. For instance, the VN of TG_3 (Fig.8) with 4 motor branches, innervates 45 muscles compared to that of TG_1 with 7 motor branches which innervates only 38 muscles in the prothorax (Fig. 4).

iv. The region-wise projections of motor branches in a segment seem to vary in accordance with the complexity of its musculature. For example, one motor branch is given out in the ventral and lateral regions where it innervates only internal layer of ventral muscles and 4 to 10 motor branches are given out in the dorsal region where they innervate both external and internal layers of dorsal muscles

v. The DN collects sensory perceptions from the lateral and dorsal regions, while the VN does so in the ventral region of the thoracic segments as evidenced by the distribution of sensory ramifications in the respective regions. Interestingly, in the silkworm some amount of overlapping occurs in the lateral region of the segment between the sensory branches of DN and VN as observed in *Manduca sexta* (Levine *et al*, 1985).

vi. Multiple innervations are the common feature of silkworm musculature. Such innervations may involve both excitatory and inhibitory synapses with antagonistic functions as reported in other insects (Usherwood, 1967; Iles and Pearson, 1969; Shepheard, 1970 and Wolf and Harzsch, 2002).

vii. The innervation pattern is both intersegmental and intra-segmental. While a vast majority of motor nerves show intrasegmental muscular innervations, a few of them show intersegmental innervations. For instance, a small branch of M_1 of the DN of TG_3 extends into the preceding mesothorax where it innervates two large X-shaped mid-ventral muscles, which extend in between the two cords of the ventral nerve cord (Fig.7).

The multiple roles of muscles, viz., body movements, maintenance of posture, stabilizing joints, thermiogenesis, and circulation of body fluids are effectively coordinated by well articulated neuro-muscular integration. The significant aspect of nerve-muscle integration in the cephalothoracic segments of silkworm is its crucial role in crawling movement and the spinning behaviour of the caterpillar. The crawling movement of the larva is facilitated by the undulating contractions of dorsal and ventral muscles lining the body wall. In this mechanism, the median nerve connectives (MNC) act as the intersegmental nerves (Figs. 6,7) between meso- and metathoracic segments and innervate the muscles present in other segments, forming an integrative and cohesive mechanism within the nervous system on one hand and between the nerve and muscle on the other. Such nerve-muscle integration expedites greater and efficient locomotory activity among the thoracic segments as opined by Barth (1937). Further, the neural integration among the DN, VN and MNC on one hand, and its connectivity with the X-shaped ventral muscle bundles of mesothorax that extends in between the two cords of the ventral nerve cord (Fig.7) on the other, may impart sufficient neuromuscular strength to the silkworm larva for spinning the cocoon at the end of fifth instar. The raising of the head of the silkworm above the ground and keeping the thorax in vertical position at right angles to the substratum during spinning activity is probably facilitated by such neuromuscular integration in the cephalothoracic region that needs to be ascertained. The functional cohesiveness achieved by neuromuscular integration in the cephalothorax of the silkworm, is in close conformity with those of certain orthoptern and lepidopteran insects (Libby, 1959), and this homology indicates that the holometabolous lepidopteran larvae, such as that of *Bombyx mori* are presumably evolved from certain immature forms of orthoptera.

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