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

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**INTRODUCTION**

Ever since the studies of Snodgrass (1935, 1958), insect neuromuscular systems attracted the attention of several investigators. The morphological, physiological and anatomical studies over the years have contributed immensely to the existing knowledge on insect neuromuscular anatomy (Usherwood, 1967, 1968; Eaton, 1982; Yang, 1983; Bartos and Honegger, 1992; Brunn, 1998; Bullaro and Prete, 1999; Duve et al., 1999; Wolf and Harzsch, 2002; Kawasaki and Kita, 2004; Kent and Levine, 2004; Burrows, 2007; Copenhagen, 2007). Of late, extensive studies have been carried out on nerve topography and musculature of a variety of insects including the mosquito, *Culiseta inornata* (Owen, 2006), hymanopteran, *Nomia melanderi* (Yousef, 2005a, b), honey bee, *Apis mellifera* (Masuko, 2005; Shankland, 2005), *Drosophila* and *Calliphora* (Sink, 2006; Spieb et al., 2007), cricket, *Gryllus campestris* and cockroach, *Periplaneta americana* (Honegger et al., 2004; Alsop, 2005; Davis, 2005; Klass, 2008). Further, the embryonic development of coaxial muscles and their innervation in cockroach (Denburg and Fulop, 2005) and flight muscles in cricket (Ready and Josephson, 2005a, b) and the modulatory role of allatostatin on neuromuscular parameters (Kreiss et al., 1999) have also been reported. Although, insect musculature has been subjected to elaborate analysis and classification, the descriptions of the nervous system have been restricted to the examination of the peripheral distribution of abdominal and median nerves, the identification of motor and sensory neurons of the ganglia and tracing of the innervations of selected axons. Further, the available studies on neuromuscular systems of silkworm and other lepidopteran insects (Libby, 1959; Randall, 1968; Kondoh and Obara, 1982; Sivaprasad and Muralimohan, 1999), have not adequately focussed on detailed organisation of the segmental musculature and nerve-muscle integration in different segments of the insect body. Hopefully, such studies on *Bombyx mori* will have double advantage. Firstly, they help in analyzing the basic tenets of nerve-muscle integration in insects and its important role in body movements, locomotion, thermoregulation, respiration and the circulation of body fluids. Secondly, from economic point of view, it helps ascertain the mechanism underlying the cocoon spinning behaviour by this insect, which indirectly contributes to the sericultural industry in the form of quality silk. The present study examines the gross organisation of the abdominal musculature, and its innervation patterns in the fifth instar larva of *Bombyx mori*. Though, the study is not exhaustive, nevertheless provides insights for future investigations in this direction.
MATERIALS AND METHODS

The fifth instar larva of multivoltine NB. 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-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 abdominal musculature and the branching and innervation pattern of the nerves of the eight abdominal ganglia (AG 1 to AG 8) and the nerve-muscle anatomy of eight abdominal segments (AS 1 to AS 8) starting with AS 1, through AS 8 were studied under Labomed 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 distinguished well, counted and their innervation traced. Sketches of abdominal segmental musculature and its innervation pattern as seen in the right half of all abdominal segments were made directly from the dissections. At least five to ten larvae were used to draw the sketches from each hemi-segment.

RESULTS

The present report on silkworm neuromuscular systems, presented in Figs. 1 to 17 and in Tables 1 and 2, encompasses the abdominal musculature and its innervation by the segmental ganglionic nerves.

I. Abdominal musculature: In the silkworm larva the abdominal muscles are organized in the form of elongated bands that represent a stereotyped pattern of segmental arrangement. The muscles that occur in each segment as separate bands throughout their length were considered as individual muscles and were counted accordingly, while those fused partly or wholly were considered as branches of the same muscle. The muscles are designated based on the nomenclature used by Snodgrass (1935). In all, 12 types of muscles, arranged in two distinct layers viz., external and internal are represented in the abdomen. Depending on their site of location in the segment, the muscles are broadly categorized into three principal groups, viz., dorsal, ventral and lateral ones.

(A) Dorsal abdominal muscles

1) The musculi dorsales interni mediales (DIM): The longitudinal intersegmental muscle bands present in the mid-dorsal region of the segments are designated as the musculi dorsales interni mediales (DIM) or dorsal internal median muscles. They are of segmental length and are attached to the intersegmental folds in all the pregenital abdominal segments. Their number in each segment ranges from 8 to 16 (Figs. 1,3,5,7,9,11,13,15; Table 2), except for the last one, wherein only two muscle bands are represented (Fig. 15).

2) The musculi dorsales interni laterales (DIL): These are the longitudinal and oblique intersegmental muscles present in the dorsolateral regions of the segment. They are of segmental length and are arranged in the inner most area in all the abdominal segments and are attached to intersegmental folds at both the ends. Their number ranges from 4 to 8 in different abdominal segments (Figs. 1,3,5,7,9,11,13,15 and Table 2).

3) The musculi dorsales externi mediales (DEM): The short dorsal longitudinal external muscles lying in the posterior mesal part of the abdominal terga and occupying the exterior and peripheral position of the abdominal segment are designated as the musculi dorsales externi laterales (DEL) or dorsal external median muscles. They are slightly oblique in disposition with an anteriorly outward slant and with sites of attachment at the intersegmental walls. The number of DEM correspond to that of DIM and ranges from 8 to 16 in all segments except the last one where only two muscles are represented (Figs. 1,3,5,7,9,11,13,15 and Table 2).

4) The musculi dorsales externi laterales (DEL): These are the short dorsal longitudinal muscles occupying the exterior and peripheral portion in the postero-lateral part of all pregenital abdominal segments. Also called dorsal external lateral muscles, they exhibit a slight oblique disposition with an anteriorly outward slant, and are attached to the intersegmental walls at both the ends (Figs. 1,3,5,7,9,11,13,15 and Table 2).

B) Ventral abdominal muscles

5) The musculi ventrales interni mediales (VIM): The longitudinal intersegmental muscle strips present in the mid-ventral region of the segment are called musculi ventrales interni mediales (VIM) or ventral internal median muscles. They are of segmental length, lying in the inner segmental area in all the pregenital abdominal segments, with their attachment sites at intersegmental walls. Their number ranges from 4 to 16 in different abdominal segments (Figs. 1, 3, 5, 7, 9, 11, 13, 15 and Table 2).

6) The musculi ventrales interni laterales (VIL): These are longitudinal intersegmental muscle strips present in the ventral-lateral areas of the segment. Also called ventral internal lateral muscles, the VIL are attached to the intersegmental walls at both the ends and are located in the inner sternal area in all the pregenital abdominal segments. Their number in abdominal segments ranges from 7 to 20 (Figs. 1,3,5,7,9,11,13,15 and Table 2).

7) The musculi ventrales externi mediales (VEN): The short ventral longitudinal muscles lying in the posterior mesal part of the abdominal sterna in the exterior peripheral position of all abdominal segments are called musculi
The ventrales externi mediales (VEM) or ventral external median muscles. They arise on the posteromesal part of the sternum and are obliquely disposed with an anterior outward slant and are attached to intersegmental wall at one end and to the median ventrum at the other end. Their number is not constant, but shows great variation ranging from 3 and 17 in each segment of abdomen (Figs. 2, 4, 6, 8, 10, 12, 14 and Table 2).

8) The musculi ventrales externi laterales (VEL): The short ventral longitudinal muscle bundles occupying the exterior and peripheral position in the posterolateral part of the abdominal sterna are known as the musculi ventrales externi laterales (VEL) or ventral external lateral muscles. They are obliquely disposed with an anterior inward slant in all the pregenital abdominal segments. They arise on the posterolateral portions of the sterna.
and are inserted on the ventrum in the ventrolateral region of the hemic-section. Their number ranges from 3 to 10 in different abdominal segments (Figs. 2, 4, 6, 8, 10, 12, 14 and Table 2).

C) Lateral abdominal muscles

9) The musculi laterales interni (LI): These are oblique intrasegmental muscles lying in the inner lateral region near the spiracles in all abdominal segments, with their attachment sites on the dorsum below the dorsal muscles at one end and on the ventrum below the ventral muscles at the other end. They are arranged in two groups of 4 each in all abdominal segments (Figs. 1, 3, 5, 9, 11, 13 and 15) except the 7th abdominal segment (Fig. 7) in which 12 muscles are organized into two groups (8+4) near the spiracle.

10) The musculi laterales externi (LE): These are oblique and oblique groups of intrasegmental muscle bundles occupying the lateral exterior and peripheral portions of all pregenital abdominal segments are denoted as the lateral external muscles or musculi laterales externi (LE). These include tergopleural (TPM), tergosternal (TSM), pleurosternal (PSM), tergocoxal (TCM) and pleurocoxal (PCM) muscles. The TPM are attached to the tergum and pleural plates and occur in two or three horizontal and / or oblique groups in all abdominal segments except the eighth one, in which they are conspicuous by their absence. Their number ranges from 11 to 22 in each segment. TSM are represented in the form of horizontal groups in 1st, 2nd, 5th, 6th and 7th abdominal segments, and their number ranges from 4 to 11 with their attachments at the tergum and pleuron (Figs. 2, 4, 6, 8, 10, 12, 14). The TSM are present in 6 to 12 numbers in four abdominal segments (1st, 2nd, 3rd, 5th), in the form of horizontal bands connecting the pleuron with the sternum (Figs. 2, 4, 6, 10). The TCM are present only in the 4th abdominal segment in the form of a horizontal group comprising 8 muscle strips connecting the tergum with the pleuron. The TCM also occurs in the 4th abdominal segment in the form a horizontal group of 3 muscles that connects the pleuron with the coxal base of the pro-leg (Fig. 8).

11) Sternocoxal muscles (SCM): These are the horizontal or oblique peripheral intra segmental muscle bundles lying in the sternal region of the proleg-bearing third, fourth, fifth and sixth abdominal segments (Figs. 6, 8, 10, 12). They arise from the sternal wall and are attached to the coxal base at its rim or inserted on the coxal wall within the cone-shaped coxa. The SCM are essentially similar in arrangement in all prolegs and their number varies from 15 to 30 within the coxa. Additional groups, each consisting of 11 and 2 SCM occur outside the coxa in the third and fourth abdominal segments respectively (Figs. 6 and 8), where as they are absent in the fifth and sixth abdominal segments (Figs. 10, 12).

12) Cephalic muscles (CM): These are oblique intrasegmental muscles lying in the cephalic region of the eighth abdominal segment. They arise on the sternal wall and are attached to the postpedes (anal prolegs) at the rim. There are about 19 such muscles arranged in 3 groups in each postped of the segment (Fig. 6).

II. Innervation patterns: The abdominal musculature of silkworm is innervated by the segmental ganglionic nerves emerging from the eight abdominal ganglia (AG) to AG). In silkworm, they include a pair of lateral nerves, designated the dorsal (DN) and ventral (VN) nerves. The former originates on the doral aspect and the latter on the ventral aspect of the ganglion. Their branching and innervation patterns were traced and presented in Figs. 1 to 17 and in Tables 1 and 2. In their course, the ganglionic nerves give out two types of branches, the motor branches (M1, M2, M3, etc.) to the muscles and sensory branches (S1, S2, S3, etc.) to the body wall. Thence a nerve branch connected to the muscle was considered as the motor branch while that innervates the body wall as the sensory branch. In each hemi-segment, they follow a specific course from the point of their origin to the point of destination. Soon after its origin from the ganglion, the DN enters the midventral region and then passes into the lateral region, where it runs past the spiracle and finally reaches the dorsal region in all segments. On the other hand the VN is extended into the ventral and lateral regions of the segment, but does not reach the dorsal region. In its course, the DN passes over the inner face of the musculi ventrales interni mediales (VIM) and below the musculi ventrales interni laterals (VIL) in the ventral region, and on the inner face of the musculi dorsales interni laterals (DIL) in the dorsal region. The VN runs peripherally in between the external and internal layers of ventral muscles (VIM and VEM) in all abdominal segments. Their orientation in the abdominal hemi-segment may be anterovernal (AV), posterovernal (PV), anterolateral (AL), posterolateral (PL), anterodorsal (AD), and posterodorsal (PD) or dorso-lateral (DL).

A) Motor branches of dorsal and ventral nerves: The number of motor branches emanating from the DN ranges from 5 to 9, and that coming from the VN varies from 3 to 6 in each segment.

1) The DN of first abdominal ganglion (AG1) sends 9 motor branches (M1 to M9), which innervate about 79 muscle strips of the first abdominal hemi-segment, including 8 VIM in the ventral region, 12 VIL in the ventrolateral region, 8 LI in the lateral region, 8 DIL and 8 DEL in the dorsolateral region, 20 DEM and 15 DIM in the dorsal region. It also innervates two ventral muscles
Fig. 3 (A): Musculature of the second abdominal hemi-segment and its innervation by the dorsal nerve (DN) of the second abdominal ganglion (AG₂), M₁, M₃, M₅ and M₇ respectively. Insets B, C, D, E, F: The distribution of motor branches M₂, M₄, M₆ and M₈ respectively. Remaining notations are the same as in the legend for Fig. 1.

(i) The DN of second abdominal ganglion (AG₂) gives 8 motor branches (M₁ to M₈) in all the regions of the second abdominal hemi-segment, which innervates about 83 muscle bundles, comprising 11 VIM, 20 VIL, 8 LI, 7 DIL, 7 DEL, 14 DIM and 16 DEM. (Fig. 2 and Table 2). The VN gives out 6 motor branches in the ventral and ventrolateral regions of the hemi-segment which innervates about 55 muscle bundles, comprising 8 VEM, 10 VEL, 4 TSM, 16 TPM, 8 PSM (Fig. 4 and Table 2). Additionally, a branch of M₈ enters the next (third) abdominal segment in the lateral region, where it innervates 8 LE.

(ii) The DN of third abdominal ganglion (AG₃) gives 6 motor branches (M₁ to M₆) in the third abdominal hemi-segment, which innervate about 79 muscle strips including 16 VIM, 17 VIL, 8 LI, 7 DIL, 7 DEL, 16 DIM and 8 DEM. (Fig. 5 and Table 2). The VN gives out 4 motor branches in the ventral and ventrolateral regions of the hemi-segment which innervates about 69 muscle bundles, comprising 8 VEM, 4 VEL, 30 SCM, 5 PSM and 22 TPM (Fig. 6 and Table 2).

(iii) The DN of fourth abdominal ganglion (AG₄) gives 8 motor branches (M₁ to M₈) in the fourth abdominal hemi-segment, which innervate about 75 muscle strips including 13 VIM, 12 VIL, 12 LI, 7 DIL, 4 DEL, 16 DIM and 11 DEM. (Fig. 7 and Table 2). The VN gives out 4 motor branches in the ventral and ventrolateral regions of the hemi-segment which innervates about 58 muscle bundles, comprising 3 VEM, 20 VEL, 26 SCM, 8 TCM, 3 PCM, 15 TPM in the fourth abdominal segment, and 8 LE the fifth abdominal segment (Fig. 8 and Table 2).

(iv) The DN of fifth abdominal ganglion (AG₅) gives 8 motor branches (M₁ to M₈) in the sixth abdominal hemi-segment, which innervate about 86 muscle strips including 14 VIM, 19 VIL, 8 LI, 8 DIL, 5 DEL, 16 DIM and 16 DEM. (Fig. 9; Table 2). The VN gives out 6 motor branches in the ventral and ventrolateral regions of the fifth abdominal hemi-segment which innervates about 55 muscle bundles, comprising 7 VEM, 3 VEL, 17 SCM, 6 PSM, 4 TSM and 18 TPM. A branch from M₁ of VN crosses over to the next abdominal (sixth) segment and innervates 8 LE, additionally (Fig. 10 and Table 2).

(v) The DN of sixth abdominal ganglion (AG₆) gives 6 motor branches (M₁ to M₆) in the sixth abdominal hemi-segment, which innervate about 67 muscle strips including 10 VIM, 10 VIL, 6 LI, 6 DIL, 3 DEL, 14 DIM and 16 DEM. (Fig. 11 and Table 2). The VN gives out 4 motor branches in the ventral and ventrolateral regions of the sixth abdominal hemi-segment which innervates about 52 muscle bundles, comprising 7 VEM, 4 VEL, 18 SCM, 5 TSM, 4 PSM, 14 TPM in the sixth abdominal segment (Fig. 12; Table 2). In addition the M₁ of VN gives a small branch designated...
vii. The DN of seventh abdominal ganglion (AG₇) gives 6 motor branches (M₁ to M₆) in the seventh abdominal hemi-segment, which innervate about 49 muscle strips including 6 VIM, 12 VIL, 8 LI, 6 DIL, 8 DIM and 8 DEM. (Fig. 13 and Table 2). The VN gives out 3 motor branches (M₁ to M₃) in the ventral and ventrolateral regions of the hemi-segment which innervates about 34 muscle bundles, comprising 8 VEM, 4 VEL, 11 PSM and 11 TPM in the seventh abdominal segment (Fig. 14 and Table 2).

viii. Since, the eighth abdominal ganglion is confluent with the seventh abdominal ganglion and it is located in the seventh abdominal segment, the DN of eighth abdominal ganglion (AG₈) is a longer one and extends into the eighth abdominal segment where it gives 5 motor branches (M₁ to M₅) for about 29 muscle strips including 4 VIM, 7 VIL, 8 LI, 4 DIL, 2 DEL, 2 DIM and 2 DEM (Fig. 15 and Table 2). The VN follows similar course as DN and gives out 4 motor branches (M₁ to M₄) in the ventral and ventrolateral regions of the eighth abdominal segment, which innervates about 24 muscle bundles, comprising 6 pyloric muscles (PM) and 18 cercal muscles (CM) (Fig. 16 and Table 2).

B. Sensory branches of dorsal and ventral nerves

i. The sensory branches of both DN and VN of all the abdominal ganglia are shown in the form of dotted lines in Fig. 1 to 16. They always arise on the dorsal aspect of the nerves and their number varies from 1 to 3 (S₁ to S₃). If their number is 3 as in the case of DN of AG₁, AG₂, AG₃ and AG₈, the S₁ arises in the ventrolateral region and the S₂ and S₃ arise in the dorsolateral and dorsal regions respectively (Figs. 1, 3, 15 and Table 2). Conversely, if there are two branches as in the case of DN of AG₁, AG₃, AG₅, AG₆ and AG₇, the S₁ and S₂ arise separately in dorsolateral and middorsal regions of the segment (Figs. 5, 7, 9, 11, 13 and Table 2).

ii. The VN of all abdominal ganglia gives off only one sensory branch (S₁) in the ventral region of the segment. It innervates the body wall in the ventral and ventrolateral regions of the respective abdominal segments (Figs. 4, 6, 8, 10, 12, 14, 16 and Table 2).

iii. Projections of cercal nerve (CN): The peculiarity of the last abdominal ganglion (AG₈) is that it possesses a cercal nerve in addition to the paired DN and VN. It arises in between these two nerves and runs down posteriorly into the conical anal papilla that bears the cerci laterally and anus at its apex. The CN splits into three prominent branches and innervates the body wall in the midventral cercal and lateral regions of the last segment besides the anal papillae. Since, the CN is a sensory nerve; it bears no motor branches (Fig. 17).

DISCUSSION

The insect muscle is implicated in a multitude of functions such as respiration, thermogenesis, circulation of body fluids, body movements and locomotion (Kawasaki and Kita, 2004 and Sink, 2006). The muscle in silkworm has an additional function of cocoon spinning which indirectly influences the sericulture industry in terms of silk production and productivity.

I. Abdominal musculature: The fundamental pattern of
abdominal musculature and nerve topography in the silkworm larva is in consonance with the segmentation of the body, with a stereotyped pattern of arrangement as described in various other insects (Snodgrass, 1935, 1958; Deshpande and Pathan, 1982; Shankland, 2005; Youssef, 2005a,b and Sink, 2006). However, minor variations are detectable in the number and disposition of muscles in different segments of the abdomen.

The potential number of abdominal muscles displays variation segment-wise, region-wise and layer-wise (i.e. external and internal) within each abdominal hemi-segment (Table 1). The total number of muscles is 113 in the AS₁, 138 in AS₂, 148 in AS₃, 133 in AS₄, 141 in AS₅, 119 in AS₆, 83 in AS₇ and 53 in AS₈. Comparatively, less number of muscles are present both at anterior and posterior ends of the larval body. The number of muscles

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Fig. 5. (A) Musculature of the third abdominal hemi-segment and its innervation by the dorsal nerve (DN) of the third abdominal ganglion (AG₃), in the fifth instar larva of Bombyx mori. Insets B, C, D, E: The distribution of motor branches M₁, M₄ and M₅ and M₆ respectively. Remaining notations are the same as in the legend for Fig. 1.

Fig. 6. Musculature of the third abdominal hemi-segment and its innervation by the ventral nerve (VN) of the third abdominal ganglion (AG₃), in the fifth instar larva of Bombyx mori. Inset: Arrangement of sternocoxal muscles (SCM) in the coxal region of the abdominal proleg and its innervation by M₁ and M₃. SPM: sternopleural muscles. Remaining notations are the same as in the legends for Figs. 1 and 2.
distributed in different sectors of the larva appears to vary as a function of the size of its body, with more muscles occurring in the broader middle region of the abdomen and fewer muscles in the narrow tapering posterior segments. In all, each half of the abdomen possesses about 928 muscles in all abdominal segments and in both extrinsic and intrinsic layers. Considering the bilateral symmetrical nature of the larval body, about 1836 of muscles are present in the abdomen of silkworm when both left and right halves of the larval are taken into consideration (Table 1).

The number of muscles in each region of the abdominal hemi-segment is also not constant. It varied from 10 to 45 in the dorsal region, 31 to 58 in the ventral region and 8 to 38 in the lateral region. In the entire abdomen, there are about 305 muscles in ventral region, 250 in lateral region and 282 in the dorsal region. The layer-wise projections indicate that the external layer comprises more muscles (426) than the internal layer (411), indicating the more complex nature of the extrinsic musculature compared to its intrinsic counterpart.

The dorsal group of muscles comprises primarily the longitudinal bundles of segmental length attached to the intersegmental folds. The internal dorsals retain their longitudinal position and segmental length and include two separate groups of muscle bundles called musculi dorsales interni mediales (DIM) and musculi dorsales interni laterales (DIL). The external dorsals, on the other hand, are seldom of segmental length but occur typically as short oblique or transverse groups of muscle bundles lying predominantly in the posterior part of segments. They include musculi dorsales externi mediales (DEM) and musculi dorsales externi laterales (DEL). The number of dorsal muscles in silkworm ranges from 10 to 45 in different segments of the abdomen (Table 1).

Functionally, these muscles act as tergal protractors since their contraction lengthens the body by decreasing the overlap of segments (Snodgrass, 1935). The ventral group muscles of the abdomen displays similar disposition like that of dorsal group, and includes both external and internal layers of muscle bundles. This internal group includes musculi ventrales interni mediales (VIM) and musculi ventrales interni laterales (VIL), which are typically intersegmental and are known to act as retractors of ventral segments. The external group includes musculi ventrales externi mediales (VEM) and musculi ventrales externi laterales (VEL) and represented in the form of short and oblique strips in the posterior half of the segments. The total number of ventral muscles in the abdomen of silkworm is also not constant, but ranges from 31 to 58 in different segments. Both VEM and VEL act as sternal protractors as their contraction causes reversal in position of body segments.

The locomotion in all insects (Barth, 1937) and probably in the silkworm as well, is triggered by simultaneous contraction of both dorsal (DIM, DIL, DEM, DEL) and ventral (VIM, VIL, VEM, VEL) muscles. The crawling movements exhibited by the silkworm larva is accompanied by change in body shape, during which all the ventral and dorsal muscles lining the body wall contract in such a way that the body segments become rigid and as reported by David and Ananthakrishnan.
The musculature in silkworm larva becomes highly complex and elaborate due to the presence of sternocoxal muscles in all proleg-bearing 3rd, 4th, 5th, and 6th abdominal segments. This group, comprising 17 to 30 muscles in each hemi-segment which occurs externally with an oblique disposition in the pro-leg or in a vertical disposition near the coxa (Table 1). However, such muscles are absent in non-leg-bearing abdominal segments, which reflects the fact that the pro-leg is a true metamorphic character without any phylogenetic importance (Libby, 1959). However, this statement cannot be universally applied to all insects, since the abdominal pro-legs have an important role in causing crawling movements of caterpillars during which they firmly attach the ventral body surface to the substratum (David and Ananthakrshnan, 2006). In fact, Alsop (2005) identified 368 muscles controlling the leg movement in a cockroach and emphasized that neural control of leg musculature could have significant role in evolution of movement in insects. Though, the detailed neuromuscular architecture of pro-legs of silkworm has not been adequately examined in the present investigation, nevertheless, the projected data on abdominal musculature and its innervation throws light on some fundamental aspects of neuromuscular mechanism governing the leg movement.

Functionally, the SCM, in close association with TCM, PCM and SCM are known to effect the leg movement in insect larvae (Snodgrass, 1935). In silkworm, the pro-leg muscles help in causing walking, searching and dispersive movements during active feeding. Additionally, they act as anchorage to the substratum and provides firm grip to the silkworm larva at the time of cocoon-spinning activity, during which, the larva virtually stands on its abdominal pro-legs and lifts its entire thorax and part of abdomen above the substratum and freely rotates its anterior end. Accordingly, during these movements, the TCM attached to the anterior coxal base may act as tergal promoters of the legs and help in...
Fig. 9. (A). Musculature of the fifth abdominal hemi-segment and its innervation by the dorsal nerve (DN) of the fifth abdominal ganglion (AG 5), in the fifth instar larva of Bombyx mori. Insets B, C, D, E: The distribution of motor branches M1, M2, M6, and M8 respectively. Remaining notations are the same as in the legend for Fig. 1.

Lifting the anterior half of the body from the substratum, while the SCM with similar articulations may act as sternal promoters of the prolegs and facilitate their firm attachment to the substratum in the abdominal region. Further, PCM and PSM, in association with SCM may function as coxal promoters by acting as abductors of coxa as advocated by Snodgrass (1935). According to Snodgrass (1935) the most advanced feature of insect musculature is the presence of PCM or PSM that allows free leg movement in longitudinal, transverse and vertical planes, while the addition of TCM, TSM and TPM retards the leg movement and thus represents a very primitive feature of locomotion. Though, the presence of only two groups of muscles, viz., PCM or PSM and SCM in other pro-leg bearing segments (Figs. 6, 8, 10, 12) suggests an advanced feature of locomotion of the hinge in transversely inclined axis, the distribution of similarly disposed muscles such as TSM, TPM etc. in the non-leg bearing segments, indicates the most primitive nature of locomotion in the silkworm, with characteristic sluggish body movements when compared to other active insects.

**Innervation patterns:** The innervation pattern of abdominal musculature by the branches of DN and VN of abdominal ganglia of silkworm presents certain variations with in the framework of basic innervation patterns observed in other insects (Libby, 1959; Randall, 1968 and Taylor and Truman, 1974). The following are the specific features of muscular innervations in the silkworm.

i. All the three groups of muscles, viz., ventral internals (VIM and VIL), lateral internals (LI) and both dorsal externals (DEL and DEM) and dorsal internals (DIM and DIL) receive innervation exclusively from the DN in all the abdominal segments. The muscles such as the ventral externals (VEM and VEL) and lateral externals (TSM, PSM, TPM, TCM, SCM, PCM, SM) receive innervations from the VN only. The locomotory function caused by the contraction of dorsal and ventral muscles is obviously triggered by the collective action of DN and VN, while the ventilatory movements of spiracles are coordinated by the DN.

ii. The sternocoxal muscles of pro-legs in the 3rd, 4th, 5th and 6th abdominal segments and those involved directly or indirectly in larval movements, viz., PCM, SCM, SPM etc., receive innervations exclusively from the VN of all abdominal ganglia. Evidently, the central neural control mechanism of larval locomotion in silkworm is located in the ventral ganglia and it is executed through the ventral nerves of the segmental ganglia.

iii. Another noteworthy feature is the presence of multiple innervations, in which muscle bundles receive innervations from more than one motor branch of DN and VN. For example many dorsal, ventral and lateral internal muscles receive such multiple innervations from the motor branches of the DN in all abdominal segments. In such cases either different sub-branches of a motor nerve or different motor nerves innervate the same muscle bundles. Both these situations are common in silkworm (Figs. 1, 3, 4, 5, 6, 7, 9, 11, 13). They reflect the presence of fast or excitatory and slow or inhibitory axons in the nervous system of silkworm similar to that observed in a variety of insects (Usherwood, 1968; Iles and Pearson, 1969; Shepheard, 1970 and Stokes et al., 2005). However, it needs further elucidation in silkworm.
iv. The presence of intersegmental nerves is another interesting feature of silkworm nerve muscle coordination. As in the locust (Shepheard, 1970), the silkworm possesses certain intersegmental nerves which innervate muscles of adjacent segments. Such nerves emanate either from the DN or from the VN, either in the form of a motor branch or median nerve connective (MNC) and achieve intersegmental coordination in four different ways. The first intersegmental coordination is seen in between the thorax and abdomen and is facilitated by a small motor branch of DN of AG1, which runs in the anterior direction and extends into the preceding metathorax and courses along and innervates two large ventral muscles lying in midventral position in between two chords of the ventral nerve chord (Fig. 1). The second type of intersegmental coordination is achieved by the ventral nerves of AG2 (Fig. 4), AG4 (Fig. 8), AG5 (Fig. 10) which give an intersegmental motor branch that extends
Fig. 12. Musculature of the sixth abdominal hemi-segment and its innervation by the ventral nerve (VN) of the sixth abdominal ganglion (AG₆), in the fifth instar larva of Bombyx mori. Insets B : Arrangement of sternocoxal muscles (SCM) and their innervation by M₁ in the coxal region of the abdominal proleg. Inset C: Distribution of M₆. PSM: pleurosternal muscles; Remaining notations are the same as in the legends for Figs. 1 and 2. Note: An intersegmental nerve designated median nerve connective (MNC) arises from the M₁ which extends into the next segment.

into and innervates lateral external muscles or musculi lateralis externi (LE) in the third, fifth and sixth abdominal segments. The third type of intersegmental coordination is facilitated by the participation of entire nerves, viz., the DN, VN and CN of the 8th abdominal ganglion which originate actually in the seventh abdominal segment from the confluent AG₇, which then extend into the ganglion-less eighth abdominal segment and innervates all segmental muscles or body wall there (Figs. 15, 16, 17).

The fourth type of intersegmental coordination is facilitated by the median nerve connectives (MNC) originating either from the DN or VN of abdominal ganglia. For instance, a MNC arises from the motor branch of DN of AG₇ and joins the transverse nerve (TN) of the median nerve (MN) and innervates selected ventral muscles in the metathorax (Fig.1). Similarly, the VN of AG₇, AG₆, AG₅ and AG₄ give median nerve connectives that extend in between 1st and 2nd (Fig. 2), 3rd and 4th (Fig. 6), 5th and 6th (Fig. 10) and in between 6th and 7th (Fig. 12) abdominal segments where their motor branches innervate some lateral intersegmental muscles (LE) in association with the TN of respective median nerves (not shown in Fig.).

The significant aspect of neuromuscular integration in the silkworm obviously, is the presence of a bidirectional arrangement of intersegmental nerves in which they extend in opposite directions in the thorax and abdomen. For instance, one such intersegmental motor branch extends from the first abdominal segment into the metathorax (Fig.1). Conversely, such branches extend from a previous segment to the next segment in the abdomen (Figs. 4, 8). Such an arrangement could ensure approximation of adjacent segments in the thorax and abdomen during locomotion in which a wave of peristalsis passes over the larval body. Barth (1937), demonstrated that peristalsis in the insect larval body involves simultaneous contraction of at least three segments. But the arrangement of intersegmental nerves in silkworm suggests that the peristalsis involves the simultaneous contraction of muscles in at least two segments. Clearly, in silkworm the coordinated activity of thorax and abdomen and that of individual segments is monitored by the central nervous system through segmental and intersegmental nerve branches of DN and VN. The combined action of these two nerves could help in causing stiffness of abdominal segments due to contraction of dorsal group of muscles and relaxation of ventral group of muscles, that could provide the tensile strength required for pumping of haemolymph and for opening and closing of the spiracles for effective ventilation during respiration as reported in certain locusts (Ramirez and Pearson, 1989; Youssef, 2005 a, b).

Such an integrated mechanism enhances physical strength of the body for effective cocoon-spinning activity by the silkworm larvae at the end fifth instar.

The number of motor branches of DN and VN seems
Fig. 13. (A) Musculature of the seventh abdominal hemi-segment and its innervation by the dorsal nerve (DN) of the seventh abdominal ganglion (AG7), in the fifth instar larva of Bombyx mori. Inset B: The distribution of motor branch M6. Remaining notations are the same as in the legend for Fig. 1.

Table 1. Distribution of Muscles in the eight abdominal ganglia (AG1 to AG8) of the fifth instar larva of the silkworm, Bombyx mori. The number of abdominal muscles were shown region-wise, layer-wise (external / internal) and segment-wise separately. The number of SCM in the leg-bearing segments is also shown.

<table>
<thead>
<tr>
<th>Segment</th>
<th>No of muscles: region-wise</th>
<th>No of muscles: layer-wise</th>
<th>No of SCM in leg-bearing segments</th>
<th>No of muscles: segment-wise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ventral</td>
<td>lateral</td>
<td>dorsal</td>
<td>internal</td>
</tr>
<tr>
<td>AS - 1</td>
<td>34</td>
<td>36</td>
<td>43</td>
<td>51</td>
</tr>
<tr>
<td>AS - 2</td>
<td>58</td>
<td>36</td>
<td>44</td>
<td>60</td>
</tr>
<tr>
<td>AS - 3</td>
<td>45</td>
<td>35</td>
<td>38</td>
<td>64</td>
</tr>
<tr>
<td>AS - 4</td>
<td>31</td>
<td>38</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>AS - 5</td>
<td>43</td>
<td>36</td>
<td>45</td>
<td>65</td>
</tr>
<tr>
<td>AS - 6</td>
<td>31</td>
<td>31</td>
<td>39</td>
<td>48</td>
</tr>
<tr>
<td>AS - 7</td>
<td>28</td>
<td>30</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>AS - 8</td>
<td>35*</td>
<td>08</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>305</td>
<td>250</td>
<td>282</td>
<td>411</td>
</tr>
</tbody>
</table>

* Includes 6 pyloric muscles and 18 cercal muscles, counted as ventral externals.
AS1 to AS8 = Abdominal segments 1 to 8.
to vary as a function of number of muscles innervated and the complexity of musculature. With a few exceptions (VN of AG\textsubscript{2}, AG\textsubscript{4} and AG\textsubscript{5}), the greater the number of muscles present in the given area of the segment, the greater will be the number of branches and vice versa. Thus, the DN sends 4 to 9 motor branches in the dorsal region where they innervate both internal and external layers of muscles, and four branches in the ventral region where it innervates only the external layers.

vii. The origin and distribution of sensory branches follow a specific rule in silkworm. The sensory branches of DN arise only in the lateral and dorsal regions of the segment and they innervate those two regions only. On the other hand the sensory branches of VN originate only in the ventral region of the segment and innervates that particular region.
sensory input either through the DN or VN. Hence, it facilitates the entry of the branches of VN and DN in the lateral region of the segment. Such an arrangement facilitates the entry of sensory inputs from ventrolateral and lateral regions in addition to dorsal and ventral regions. Likewise, those of VN receive sensory information also from the ventrolateral region of the segment in addition to dorsal and ventral regions. Though, the territory of the sensory branches is restricted, often there exists a sort of overlap between the branches of VN and DN in the lateral region of the segment. Such an arrangement facilitates the entry of sensory input either through the DN or VN. Hence, it is appropriate to suggest that, the sensory branches of DN receive sensory information also from the ventrolateral region of the segment in addition to dorsal and dorsolateral regions. Likewise, those of VN receive sensory inputs from ventrolateral and lateral regions in

### Table 2. Branching and innervation patterns of the dorsal (DN) and ventral (VN) nerves of the ganglionic nerves of the eight abdominal ganglia (AG1 to AG8) in the fifth instar larva of the silkworm, Bombyx mori. The number of motor and sensory branches, the muscles innervated by the motor branches , the area innervated by the sensory branches in each hemi-segment and the nature of the nerve are also shown .

<table>
<thead>
<tr>
<th>Ganglion</th>
<th>Nerve</th>
<th>Motor branches</th>
<th>Sensory branches</th>
<th>Nature of the nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Muscles innervated</td>
<td>Number</td>
</tr>
<tr>
<td>AG1</td>
<td>DN</td>
<td>9 [M, M]</td>
<td>8 VIM, 12 VIL, 8 DIL, 8 LI, 8 DEL, 20 DEM, 15 DIM &amp; 2 VM in metathorax</td>
<td>3 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>4 [M, M]</td>
<td>8 VEM, 6 VEL, 1 TSM, 6 PSM, 11 TPM</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG2</td>
<td>DN</td>
<td>8 [M, M]</td>
<td>11 VIM, 20 VIL, 8 LI, 7 DIL, 7 DEL, 14 DIM, 16 DEM</td>
<td>3 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>6 [M, M]</td>
<td>17 VEM, 10 VEL, 4 TSM, 8 PSM, 16 TPM, and 7 PCM &amp; 8 LE in AS -3</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG3</td>
<td>DN</td>
<td>6 [M, M]</td>
<td>16 VIM, 17 VIL, 8 LI, 7 DIL, 7 DEL, 16 DIM, 8 DEM</td>
<td>2 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>4 [M, M]</td>
<td>8 VEM, 4 VEL, 30 SCM, 5 PSM, 22 TPM</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG4</td>
<td>DN</td>
<td>8 [M, M]</td>
<td>13 VIM, 12 VIL, 12 LI, 4 DEL, 7 DIL, 11 DEM, 16 DIM</td>
<td>2 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>4 [M, M]</td>
<td>3 VEL, 3 VEM, 26 SCM, 8 TCM, 3 PCM, 15 TPM &amp; 8 LE in AS -5</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG5</td>
<td>DN</td>
<td>8 [M, M]</td>
<td>14 VIM, 19 VIL, 8 LI, 8 DIL, 5 DEL, 16 DEM, 16 DIM</td>
<td>2 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>6 [M, M]</td>
<td>7 VEM, 3 VEL, 17 SCM, 6 PSM, 18 TPM, 4 TSM &amp; 8 LE in AS -6</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG6</td>
<td>DN</td>
<td>6 [M, M]</td>
<td>10 VIM, 10 VIL, 8 LI, 3 DEL, 6 DIL, 16 DEM, 14 DIM</td>
<td>2 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>4 [M, M]</td>
<td>7 VEM, 4 VEL, 18 SCM, 5 TSM, 4 PSM, 14 TPM</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG7</td>
<td>DN</td>
<td>6 [M, M]</td>
<td>4 VIM, 12 VIL, 8 LI, 6 DIL, 3 DEL, 8 DEM, 8 DIM</td>
<td>2 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>3 [M, M]</td>
<td>8 VEM, 4 VEL, 11 TPM, 11 PSM</td>
<td>1 [S]</td>
</tr>
<tr>
<td>AG8</td>
<td>DN</td>
<td>5 [M, M]</td>
<td>4 VIM, 7 VIL, 8 LI, 4 DIL, 2 DEL, 2 DIM, 2 DEM</td>
<td>3 [S, S]</td>
</tr>
<tr>
<td></td>
<td>VN</td>
<td>4 [M, M]</td>
<td>6 PIM, 18 CM</td>
<td>1 [S]</td>
</tr>
</tbody>
</table>

AG1 TO AG8. 1. First to eighth abdominal ganglia; AS: abdominal segment; CM: cercal muscles; CN: cercal nerve; DEL: musculi dorsales externi laterales; DEM: musculi dorsales externi mediades; DIL: musculi dorsales interni laterales; DIM: musculi dorsales interni mediades; DLR: dorsolateral region; DM: dorsal muscles; DN: dorsal nerve; DR: dorsal region; LE: musculi externi laterales; LI: musculi interni laterales; LR: lateral region; M 1 - M 9: 1st to 9th motor nerves; MDR: mid-dorsal region; MNC: median nerve connective; MVR: midventral region; PCM: pleurocoxal muscles; PM: pyloric muscles; PSM: pleurosternal muscles; SCM: sternocoxal muscles; SM: spiracular muscles; TCM: tergocoxal muscles; TPM: tergopleural muscles; TSM: tergesternal muscles; VEL: musculi ventrales externi laterales; VEM: musculi ventrales externi mediades; VIL: musculi ventrales interni laterales; VIM: musculi ventrales interni mediades; VLR: ventrolateral region; VM: ventral muscles; VN: ventral nerve; VR: ventral region.

viii. Though, the territory of the sensory branches is restricted, often there exists a sort of overlap between the branches of VN and DN in the lateral region of the segment. Such an arrangement facilitates the entry of sensory input either through the DN or VN. Hence, it
addition to the ventral region of the segment. The morphological and physiological studies on Manduca sexta (Taylor and Truman, 1974) substantiate this point of view.

Further, the cercal nerve with its sensory ramifications in the eighth abdominal segment collects sensory inputs throughout the segment. The important role of sensory fibres of DN, VN or CN is not clearly established. However, it may be presumed that they perceive sensory inputs pertaining to chordotonal organs or mechanoreceptors which are distributed throughout the body. More significantly, the sensory nerves play a key role in sound reception and transduction through the chordotonal organs (Yack, 2004).

It may be concluded that, the neuromuscular integrative mechanism observed in Bombyx mori is in agreement with similar studies made on the Cecropia silkworm and that present in other hexapod insects (Libby, 1959). The homology in neuromuscular patterns suggest that the silkworm and other holometabolous lepidopteran insects have a common phylogenetic origin from the stock of immatures forms of hemimetabolous orthopterans as presumed by Libby (1959).

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