Origin and peripheral distribution of the thoracic and abdominal median nerves in the fifth instar larva of the silkworm, Bombyx mori (Lepidoptera: Bombycidae)

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Abstract: The origin and peripheral distribution of the median nerves of the prothoracic and sixth abdominal ganglia have been traced using methylene blue staining technique. Each median nerve (MN), after originating from the posterior part of the ganglion, bifurcates into two transverse nerves (TN) that extend laterally into the right and left halves of the hemi-segment. The TN of thoracic median nerve bears two motor branches that innervate spiracular muscles and dorsolateral muscles, while the TN of sixth abdominal ganglion bears five motor branches and two sensory branches. Its motor branches innervate three groups of muscles, namely, musculi laterales interni, musculi dorsales interni laterales and musculi dorsales interni mediales, while its sensory branches extend over the body wall in the ventrolateral and dorsolateral areas of the hemi-segment. The median nerves are connected to ganglionic nerves by median nerve connectives that facilitate intersegmental coordination. The functional role of median nerves in respiration, blood circulation and intersegmental coordination is discussed keeping in view their projections and innervations.

Keywords: Bombyx mori, Innervation, Median nerve, Silkworm, Transverse nerve

INTRODUCTION

The segmental ventral ganglionic nerves of an insect include paired lateral nerves and an unpaired median nerve that collectively constituted the ‘ventral sympathetic system’ (Schmitt, 1965). The investigations pertaining to the origin, peripheral distribution and innervation patterns of these nerves will contribute to our understanding of the basic tenets of the nerve-muscle anatomy and insect physiology. In the last decade, significant efforts have been made to map the nerve topography and segmental musculature of a variety of insects such as Bombyx mori (Sivaprasad and Muralimohan, 1998), Culiseta inornata (Owen, 2006), Nomia melanderi (Youssef, 2005a, b), Apis mellifera (Masuko, 2005; Shankland, 2005), Drosophila and Calliphora (Sink, 2006; Spieb et al., 2007), Gryllus campestris and Periplaneta americana (Honegger et al., 2004 and Alsp, 2005, Denburg and Fulp, 2005, Davis, 2005, Ready and Josephson, 2005a, b and Klass, 2008). More significantly, some earlier investigations (Hoyle, 1959; Miller, 1960 and Lewis et al., 1973), devoted to trace the pathways and innervations of unpaired median nerves and to establish their physical association with the spiracular muscles that control tracheal respiration through their regulatory influence on the opening and closing movements of the spiracles in insects. Some in-depth studies focussed on understanding the anatomical and physiological aspects of median nerves in insects. Prominent among them were the pioneering studies of Burrows (1982 a, b), which identified the motoneurons responsible for the control of the expiratory and inspiratory movements of the spiracles in a locust and demonstrated their role in tracheal respiration through morphological and electrophysiological investigations. Of late, Myers and Evans (2005) described the peripheral neurosecretory cells of the thoracic median nerve in a locust, Schistocerca gregaria through cobalt chloride back-filling technique.

More recently, we (Sivaprasad and Muralimohan, 2009a, b) provided a comprehensive account on the branching and innervation patterns of the dorsal and ventral nerves of the ventral ganglia in the thoracic and abdominal segments of Bombyx mori, together with the neuromuscular integrative mechanism underlying the locomotory behaviour of the silkworm larva. Similar studies on the unpaired median nerves are wanting. The present study aims at achieving this objective in this economically important insect of sericulture.

MATERIALS AND METHODS

The fifth instar larva 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 ganglia, its branching and innervation patterns and the nerve-muscle anatomy of segments were studied under a stereobinocular microscope, after staining the preparation with 1% methylene blue 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 median and transverse nerves and their finer branches could be clearly distinguished, counted and their innervation traced. Sketches of the branches of median nerve, segmental musculature and its innervation were made directly from the dissections. About 5 to 10 larvae were used to draw the sketches from each segment.

RESULTS

General Organisation of Median Nerves: The peripheral nervous system (PNS) of B. mori includes nine unpaired longitudinal median nerves, three of which are present in the thoracic segments and six in the abdominal segments (Fig.1). Originating from the posterior part of the ventral ganglion, each median nerve (MN) runs in between the two interganglionic connectives of the ventral nerve cord (VNC) up to the intersegmental wall, where it enlarges to form a triangular swelling and bifurcates into a pair of lateral nerves, designated transverse nerves (TNs), that extend laterally into the right and left halves of the body, each covering a hemi-segment, both in the thorax and abdomen. The TNs of all median nerves, pass along the intersegmental wall or within the intersegmental fold and extend into the lateral region of the hemi-segment into the vicinity of spiracles (SP) or even beyond into the dorsal area of the hemi-segment. Of the nine median nerves, one each arises from the three thoracic ganglia (TG1, TG2, and TG3) and one each from the first to sixth abdominal ganglia (AG1 to AG6). However, the suboesophageal ganglion (SG) and the seventh abdominal ganglion (AG7) do not have median nerves of their own. Each pair of TN reaches particular target area in the segment. The TN of TG1 reaches first pair of spiracles (SP1) in front of the spiracle in the lateral region (Fig. 2). Its TN passes over the inner face of the ventral and lateral muscles of the first thoracic hemi-segment and bifurcates into two motor branches (M1 and M2) in front of the spiracle in the lateral region (Fig. 2). The M1 proceeds over the spiral, in the antero-lateral direction and divides into two small branches, one of which innervates 3 spiracular muscles (SM) in the prothorax, while the other loops round the spiracle and innervates two dorsal muscles in the dorsolateral region of the mesothoracic hemi-segment. The M2 moves in the postero-lateral direction, just below the spiral and innervates three SM, in metathorax. In all, each TN of TG1 innervates 8 muscles (6 SM + 2 DM) in each hemi-segment and 16 muscles (12 SM + 4 DM) in the whole segment. The MN of thoracic segments does not contain sensory branches and hence appears to be purely motor in function. Further, the TN on each side receives a median nerve connective (MNC) from the dorsal nerve (DN) of the second thoracic ganglion in the ventral region of the hemi-segment (Fig.2).

The Median Nerve of TG2: It originates from the posterior end of TG2. Its TN passes over the inner face of the ventral and lateral muscles of the first thoracic hemi-segment and bifurcates into two motor branches (M1 and M2) in front of the spiracle in the lateral region (Fig. 2). The M1 proceeds over the spiral, in the antero-lateral direction and divides into two small branches, one of which innervates 3 spiracular muscles (SM) in the prothorax, while the other loops round the spiracle and innervates two dorsal muscles in the dorsolateral region of the mesothoracic hemi-segment. The M2 moves in the postero-lateral direction, just below the spiral and innervates three SM, in metathorax. In all, each TN of TG2 innervates 8 muscles (6 SM + 2 DM) in each hemi-segment and 16 muscles (12 SM + 4 DM) in the whole segment. The MN of thoracic segments does not contain sensory branches and hence appears to be purely motor in function. Further, the TN on each side receives a median nerve connective (MNC) from the dorsal nerve (DN) of the second thoracic ganglion in the ventral region of the hemi-segment (Fig.2).

The Median Nerve of AG6: It originates from the posterior end of AG6 (Fig.3). Its TN runs over the inner face of the ventral, lateral and dorsal muscles at the anterior end of the intersegmental wall and divides into three branches, which ultimately split into five motor branches (M1, M2, M3, M4, and M5) and two sensory branches (S1 and S2). The first branch of the TN runs down in the ventralateral region of the hemi-segment and divides into a motor branch M1 and a sensory branch S1. The M1 innervates 6 lateral internal muscles or musculi laterales interni (LI) present in a hemi-circle around the spiral in the lateral region, and 2 LI in the dorsolateral region, while the S1 splits into two minute branches and innervates the body wall in the lateral and dorsolateral regions of the hemi-segment. The second branch of TN splits into a motor branch M2 and a sensory branch S2 in the mid-lateral region over the spiral. The M2 splits into 4 minute terminals and innervates 4 LI, while the second sensory branch, S2 bifurcates into two small branches and innervates the body wall in the lateral and ventrolateral regions of the hemi-segment. Finally, the third branch of
the TN proceeds towards the dorsal region. While doing so, it gives a small motor branch, M\textsubscript{4} in the dorsolateral region, which innervates 2 LI above the spiracle. Finally, it ends up as M\textsubscript{5}, and extends into the dorsal region along the intersegmental wall, giving smaller nerve terminals on either side, which innervate about 16 dorsal internal muscles or \textit{musculi dorsales interni mediales} (DIM) in the sixth and seventh abdominal segments. Ultimately, the terminal end of M\textsubscript{5} extends into the middorsal region, probably making a direct contact with the dorsal tubular heart (not shown in Fig.3). In each, hemi-segment, the TN of AG\textsubscript{6} innervates about 30 muscles (14 LI + 16 DIM), thus making a total of 60 muscles in both the fifth and sixth abdominal segments on both left and right halves.

The TN in its course receives two median nerve connectives (MNCs), one each from the DN of the seventh (AG\textsubscript{7}) and VN of the sixth (AG\textsubscript{6}) abdominal ganglia. The MNC of DN of AG\textsubscript{7} arises from its dorsal aspect and connects the TN in the ventral region of the segment, while that of the VN of AG\textsubscript{6} arises from its ventral aspect as a separate branch and meets the TN in the ventrolateral region (Fig.3).

**DISCUSSION**

The origin, location and peripheral distribution of the median nerves (MNs) of\textit{ B. mori} are similar to those observed in certain other insects (Nararain, 1974 and Yadav, 2003). Though, the nerve topography sticks to a
basic pattern in the thoracic and abdominal segments, certain interregional variations are seen in between the median nerves emanating from the thoracic and abdominal ganglia. The thoracic and abdominal median nerves show similarities with regard to their origin, location, peripheral distribution, muscular innervation and connectivity with the ganglionic segmental nerves such as the median nerve connectives (MNCs). However, they differ from each other in three respects. Firstly, the TN of TG₁ has two motor branches (M₁, M₂) but does not have sensory branches, while the TN of AG₆ has five motor (M₁ to M₅) and two sensory branches (S₁, S₂). Thus, the former appears to be purely motor in function while the latter seems contain mixed fibres with both sensory and motor functions. Secondly the TN of TG₁ has one MNC that maintains connectivity with the DN of TG₂, while the TN of AG₆ has two MNCs that maintain contiguity with the VN of AG₇ on its anterior side and the DN of AG on its posterior side. Thirdly, the TN of TG₁ innervates 8 muscles (6 SM + 2 DM), while that of AG₆ innervates 30 muscles (14 LI + 16 DIM) in two adjacent hemi-segments.

The present study confirms the prevalence of close physical contact between the median nerves and spiracular muscles, which hitherto remained an assumption (Schmitt, 1965). The innervation of spiracular muscles (SM) in the prothorax or first thoracic segment (TS₁) and lateral internal muscles or musculi laterales interni (LI) in the sixth abdominal segment (AS₆) by the TN in the lateral region of the hemi-segment suggests its possible role in respiration by influencing the spiracular movements as suggested by Burrows (1982 a, b). It is likely that the contraction and relaxation of SM in the TS₁ and LI in AS₆ bring about the opening and closing of the stigmata and thereby facilitate free ventilation during respiration. It may be recalled that the SM of thorax and LI of abdomen are homologous muscles with same structure and function, but named differently in the thoracic and abdominal segments (Snodgrass, 1935; Sivaprasad and Muralimohan, 2009 a, b). The present study is in consistence with the earlier findings (Lewis et al., 1973; Burrows 1982 a, b) that the MN coordinates the ventilatory movements of spiracles. In B. mori, one to one relationship between the MN and

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Fig. 2. Origin and distribution of median nerve of the first thoracic ganglion in the fifth instar larva of Bombyx mori. The part of the musculature of the pro- and mesothoracic segments is also shown. DM: dorsal muscles; DN: dorsal nerve; M₁ and M₂: first and second motor branches; MN: median nerve; MNC: median nerve connective; SP: spiracle; SM: spiracular muscles; TG₁ and TG₂: first and second thoracic ganglia; TN: transverse nerve; TS₁ and TS₂: first and second thoracic segments (prothorax and mesothorax). VM: ventral muscles; VNC: ventral nerve cord.
SP is non-existent. For the entire cephalothoracic region that comprises the head and three thoracic segments, only one pair of spiracles (TSp.) is present in the prothorax; but three median nerves are given, one each from the three thoracic ganglia (TG\(_1\), TG\(_2\), and TG\(_3\)). On the other hand in the entire abdomen, with eight segments and eight pairs of spiracles (ASp.1 – ASp. 8), only six median nerves are given, one each from the first to sixth abdominal ganglia (AG\(_1\) to AG\(_6\)). It is likely in B. \textit{mori}, that the corresponding median nerve always need not innervate the spiracular muscles present in the same segment. In fact they receive innervations from the nerves of two different ganglia, located in two adjacent segments. For instance, the lateral internal muscles or \textit{musculi laterales interni} (LI) of first abdominal segment receive innervations from the dorsal nerve (DN) of AG\(_1\) \cite{Sivaprasad and Muralimohan, 2009 b} and MN of TG\(_3\). Likewise, the LI of each of first seven abdominal segments receive innervations from the MN of preceding ganglion and DN of the corresponding ganglion, with the sole exception of ASp.8 of eighth abdominal segment, whose muscles (LI) are innervated by only the DN of AG, as the corresponding MN is absent \cite{Sivaprasad and Muralimohan, 2009 b}. Obviously, three situations exist in B. \textit{mori} with regard to the control of spiracles.

1) If both the spiracles and median nerves are present in a particular segment, the spiracular muscles receive innervations from the DN of corresponding segment and MN of the previous segment. This arrangement is the most common one and is seen in all the seven abdominal segments.

2) In the event of the spiracle being present and MN being absent, the spiracular muscles are innervated by the DN of the corresponding segment, as seen in case of the muscles of ASp.8 \cite{Sivaprasad and Muralimohan, 2009 b}.

3) If only MN is present, and the spiracles and its associated muscles are absent in a particular segment, the former simply spreads throughout the segment, probably playing a different role altogether. This kind of arrangement is present in the mesothorax of silkworm (Fig.1). One point is clear from the present study that undoubtedly, median nerves are involved in the regulation of respiration, but control mechanism is not solely vested

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**Fig.3.** Origin and distribution of median nerve of the sixth abdominal ganglion in the fifth instar larva of Bombyx \textit{mori}. The two interganglionic connectives are stretched apart to show the location of median nerve and its bifurcation into transverse nerves. The part of the musculature of the sixth and seventh abdominal segments is also shown, together with the muscles innervated by the transverse branch of median nerve. AG\(_6\), AG\(_7\), AG\(_8\); sixth, seventh and eighth abdominal ganglia; AS\(_6\), AS\(_7\); sixth and seventh abdominal segments; DIL: musculi dorsales interni laterales; DIM: musculi dorsales interni mediales; DN: dorsal nerve; LI: musculi laterales interni; M\(_1\), M\(_2\), M\(_3\), M\(_4\), M\(_5\): first, second, third, fourth and fifth motor branches; MN: median nerve; MNC: median nerve connective; S\(_1\) and S\(_2\): first and second sensory branches; TN: transverse nerve SP: spiracle; VIL: musculi ventrales interni laterales; VIM: musculi ventrales interni mediales; VN: ventral nerve.

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with them. The dorsal and ventral nerves of the segmental ganglia also coordinate this function through appropriate contacts with TN and the muscles involved in respiration (SM / L). Further, its projections, peripheral distribution and innervation patterns suggest that the MN performs functions other than respiration as well. What is more interesting finding in the silkworm is that, a motor branch (M₅) of the MN of AG, after innervating some dorsal muscles (DIL and DIM), extends middorsally into the vicinity of the dorsal tubular heart, a feature that probably indicates its cardiac regulatory function. Further, its connectivity with DN and VN of different ganglia, and its innervation patterns in abdominal segments shows a kind of intersegmental coordination through which its circulatory function appears to be regulated. Such a coordination is apparently, achieved at three levels. The first level coordination is provided by the median nerve connectives (MNCs), such as those link the TN of TG with DN of TG, (Fig. 1) and the TN of AG, with DN of AG, and VN of AG. (Fig.2) The second level coordination is achieved by the contact between the TN and the spiracular muscles and dorsal muscles of adjacent segments. Third level coordination is facilitated strategic location of the transverse nerves in the intersegmental folds and its cross-boarder contacts (Fig. 3). Such an integrated neural network obviously, couples the spiracular ventilatory movements with locomotory movements of segments and the combined force (increased stiffness of abdominal musculature) so generated is used to exert pressure on the dorsal tubular heart to pump haemolymph through the haemocoel of silkworm – a common feature of some insects and arthropods (Iles and Pearson, 1969; Ramirez and Pearson, 1989). Though, the present investigation is not exhaustive, nevertheless, it throws light on the fact that insect heart is neurogenic and its neural control emanates from the median nerves and its connectives. This observation is in consistence with the fact that the MN and TN of insects act as neurohaemal organs, where the neurosecretory material is stored and released into the haemocoele (Taghert and Truman, 1982; Gupta, 1983; Santos et al., 2006). Probably, the neurosecretory products released from the branches of the median nerves and their fine terminal arborizations could modulate multiple functions in B. mori - a point that needs further elucidation.

Conclusion

It may be concluded that the median nerves are principal intersegmental nerves that facilitate intersegmental, interregional and inter-functional coordination in B. mori. The intersegmental coordination is achieved by their strategic location in the intersegmental folds and by maintaining contiguity with the nerves, muscles and spiracles of adjacent segments. The interregional coordination between thorax and abdomen is achieved by the presence of link-nerves such as the MNCs, extended connectives such as the TN of TG, and cross-boarder muscular innervations. The inter-functional coordination among the vital physiological processes such as the respiration, circulation and locomotion, is facilitated by its specific innervations of the muscles involved in these vital physiological processes. Such an integrative mechanism is probably necessary for growth and survival of the silkworm and to ensure effective and efficient spinning of the silk cocoon at the end of fifth instar larval development.

ACKNOWLEDGEMENTS

The author thanks the University Grants Commission (SERO), Hyderabad, for providing financial assistance in the form of a minor research project during 2006 - 2009.

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