J. Crop Prot. 2016, 5 (2): 179-187
doi: 10.18869/modares.jcp.5.2.179

Research Article

Configuration of nerve cord and characterization of brain neurosecretory cells in adult firefly, *Luciola gorhami* (Coleoptera: Lampyridae)

Hojjatollah Mohammadi[1*], Vilayanoor Venkataraman Ramamurthy[2] and Balla Subrahmanyam[2]

1. Department of Plant Protection, Faculty of Agriculture, Yasouj University, Yasouj, Iran.
2. Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi 110012, India.

Abstract: Nerve cord configuration and brain neurosecretory cell (NSC) characteristics were studied in adult firefly, *Luciola gorhami*, applying two methods, *in situ* and section staining. Nerve cord was of primitive type and consisted of brain, subesophageal ganglion, three thoracic and seven abdominal ganglia which were connected to each other serially through a pair of longitudinal connectives. Thoracic ganglia were separated and had the same size. All abdominal ganglia had the same size except the last one which was twice larger than the others. Abdominal ganglia were not fused with thoracic ganglia. Using *in situ* staining, 26 neurosecretory cells (NSCs) stained as median neurosecretory cells (MNSCs) and lateral neurosecretory cells (LNSCs). MNSCs consisted of 20 cells in three groups in pars intercerebralis. MNSCs had a U shaped arrangement in such a way that 4 round and large cells were located in front and two parallel groups (8 pryiform to round cells in each group) located in back. LNSCs were comprised of 6 large cells in two groups (one group on each lateral lobe of protocerebrum). MNSCs pathways were not clear but LNSCs pathways were clear and ipsilateral. Using section staining, large number of NSCs in pars intercerebralis stained gray and purple in color. Gray cells were large, more in number and appeared in many sections. Purple cells were large and grouped in the middle of gray cells. Both types of cells were on the surface area of brain and had large nucleus. Their axons were bundled together and extended backwards to the rear of brain.

Keywords: *In situ* staining, Nerve cord, Neurosecretory cells (NSCs), Section staining

Introduction

The basic organizational unit of insect nervous system is ganglion which differentiates and appears as segmentation occurs in embryo. Initially each segment has one ganglion. During the later stages of embryonic development, moulting and metamorphosis, the ganglia shift anteriorly or posteriorly and fuse with neighboring ganglia in diverse patterns of fusion depending upon the respective insect (Menees, 1961; Schmidt *et al*., 1997; Technau *et al*., 2006). Insect nerve cord and its fusion pattern has been reviewed by many authors (Matsuda, 1976; Kristensen and Nielsen, 1981; Kerry and Mill, 1987; Niven *et al*., 2008). It has been also studied at different levels of family, subfamily, tribe, genus and species to find out phylogenetic significance. It was found that coleopterans are

Handling Editor: Saeid Moharramipour

* **Corresponding author**, e-mail: H.mohammadi@yu.ac.ir
Received: 15 September 2015, Accepted: 15 December 2015
Published online: 12 January 2016

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

the most diverse of all the orders of insects in terms of ganglion fusion, varying from fusion of all thoracic and abdominal ganglia into one to three distinct thoracic and eight abdominal ganglia (Crowson, 1960; Mann and Crowson, 1983; Calder, 1989; Heath and Evans, 1990). Based on Wheeler *et al*. (2001), fusion of third thoracic ganglion with first abdominal ganglion occurred early in the evolution of insects and may have been crucial for insect flight. Yeates *et al*. (2002) showed that fusion of second and third thoracic ganglia are more prevalent than fusion of first and second thoracic ganglia.

Neurosecretory cells (NSCs), as a part of nervous system, have been defined as special neurons which have cytological characteristics of endocrine cells (William and Kloot, 1960; Johanson, 1963; Ma and Reolofs, 1995; Klowden, 2013). They are neurons in structure and probably in function while they serve as source of hormones. Structurally there is no sharp distinction between NSCs and nerve cells. Nevertheless, NSCs have bigger soma and are differentiated by staining properties from common nerve cells (Nation, 2008; Chapman, 2012). NSCs stand as a link between nervous and endocrine systems and they have been reported from different parts of nervous system but protocerebrum contains most of NSCs. The best known NSCs are located in pars intercerebralis and lateralis which are called median NSCs (MNSCs) and lateral NSCs (LNSCs), respectively. They have been investigated in many laboratory insects (Morris and Steel, 1975; Lavensaeu *et al.,* 1985; Ma and Reolofs, 1995; Rulifson *et al.,* 2002; Siga, 2003). Axonal endings of NSCs mostly terminate in neurohemal places such as corpora cardiaca, aorta wall, small sites around nerves and sympathetic or central ganglia to release their contents (Johanson, 1962 and1963; Siga, 2003).

Study of nervous system has many advantages since it facilitates unraveling the evolutionary route of insects which could be used in taxonomic studies and it is the best system to be targeted for insect control (Raymond-Delpech *et al.,* 2005; Harzsch, 2006). On the other hand, NSCs, as a part of nervous system play a major role in insect life by producing neurosecretory materials (NSM). All neurosecretory materials, except few biogenic amines (e.g. Tyramine and Octopamine), are peptides or small proteins. NSM can act as neurotransmitter, neuromodulator or neurohormone and influence almost all insect physiological processes including growth, development, moulting and metamorphosis (Predel and Eckert, 2000; Rulifson *et al.,* 2002; Roeder, 2005; Farooqui, 2007). Characterization of NSCs and their products brings a plethora of opportunities to combat insect pests in the nick of time through targeting NSCs by pesticides or make use of their neuropeptides to disturb insect physiological activities. Right now based on the available knowledge, exploiting potential of neuropeptides as an alternative to control pests in future is very promising arena. Five families of neuropeptide including adipokinetic hormone (AKH), trypsin modulating oostatic factor (TMOF), diuretic hormone (DH), pheromone biosynthesis activating neuropepetide (PBAN) and allatotropin (AT) are of special importance for pest control (Altstein *et al*., 2000; Nauen *et al*., 2001; Gäde and Goldsworthy, 2003; Altstein, 2004). Therefore, in present study, it was attempted to examine nerve cord and brain NSCs of *Luciola gorhami* as a member of coleopteran order through *in situ* as well as sectioning.

Materials and Methods

Dissection and fixation

Live adult insects were collected and dissected dorsally in insect saline under stereoscopic binocular microscope (Leica ES2, Germany). Extra parts and tissues such as dorsal vessel, digestive system, tracheal trunks and fat bodies were removed to reach the nerve cord. Intact nerve cords were taken out and immediately submerged into aqueous Bouin's solution for fixation. After 24 hours Bouin's solution was replaced by 70 percent ethyl alcohol. Alcohol was renewed daily (at least 7 days) until there was no sign of yellow picric acid. Attention was paid to this stage as light yellow tint could vitiate staining. These brains were preserved in 70 percent ethyl alcohol until used (Humason, 1979; Presnell and Schreibman, 1997).

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

Dehydration and clearing

Brains were dehydrated by passing through five increasing graded series into absolute ethyl alcohol (50, 60, 70, 80, 95 and 100 percent). Brains were kept 10 to 15 minutes in every change, each of 95 percent and absolute alcohols were applied twice. The dehydrated brains were cleared by passing through two changes of pure xylene. Each change took time 10 to 15 minutes. From this stage onwards samples were ready for *in situ* staining but they were subjected to the following procedures for section staining (Humason, 1979; Presnell and Schreibman, 1997).

Infiltration and embedding

Completely processed brains were infiltrated with paraffin (Hellige INC., USA). Infiltrated brains were embedded with paraffin inside small container or aluminum foil boxes. At this time brains were arranged properly and paraffin allowed to be solidified. Blocks were prepared and trimmed into rectangles (Humason, 1979; Presnell and Schreibman, 1997).

Slide preparation and staining

Sections were prepared with 5 to 10 µm thickness using a rocking microtome (Brunel microscopes Ltd. England) and fixed on the slides. Three stains aldehyde fuchsin, chrome-alum hematoxylin and phloxin (Merck Millipore, Germany) were prepared**.** Aldehyde fuchsin was used in a single staining for both *in situ* as well as section staining. Chrome-alum hematoxylin and phloxin were used in a double staining method only for section staining. Slides were viewed under research compound microscope (Leica DM750, Germany) to reveal NSCs and their characters (Humason, 1979; Presnell and Schreibman, 1997).

Results

Nerve cord of *L. gorhami* consisted of brain, subesophageal ganglion, three thoracic ganglia and seven abdominal ganglia. Brain was located at the level of pharynx in the frontal region of head capsule. It had two large optic lobes occupying most of cephalic region and covered with numerous ommatidia (Fig. 1 A). Subesophageal, thoracic and abdominal ganglia were located ventrally and connected to each other serially through a pair of connectives. Subesophageal ganglion was close and connected to brain through circumesophageal connectives. Three distinct and same-sized thoracic ganglia were present and mesothoracic ganglion was relatively close to methathoracic ganglion. There were seven abdominal ganglia. First abdominal ganglion shifted towards front and was near (not fused) to methathoracic ganglion. The sixth abdominal ganglion was close to last abdominal ganglion. All abdominal ganglia had approximately the same size except the last abdominal ganglion which was twice larger than the others (Fig. 1 A).

Using *in situ* staining, two types of cells were observed based on the stainability; (1) less stained cells, they were mostly observed in pars intercerebralis and their number was not clear since they could not be counted. (2) Well stained cells, at least 26 well stained NSCs were identified which could be classified as median and lateral neurosecretory cells, positionally. MNSCs consisted of 20 cells in three groups in pars intercerebralis. MNSCs had a U shaped arrangement in such a way that 4 round and large cells were located in front and two parallel groups (8 pryiform to round cells in each group) were located in back. LNSCs comprised two groups (one group on each lateral lobe of protocerebrum), and each group consisted of three large and well stained cells. Neurosecretory pathways of MNSC groups were not clear but pathways of LNSC groups were stained and were clear. LNSCs had ipsilateral pathways (Fig 1. B and C).

Using section staining, a large number of cells were stained and appeared in pars intercerebralis. Cells stained into two, gray and purple, groups. Gray cells were large, more in number and appeared in many sections. Purple cells were large and it seemed that purple cells had been grouped in the middle of gray cells. Both types of cells were on the surface area of brain compared to other nerve cells and they had large nucleus. Their axons were coming together, bundled and moving to brain backwards (Fig. 1. D).

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

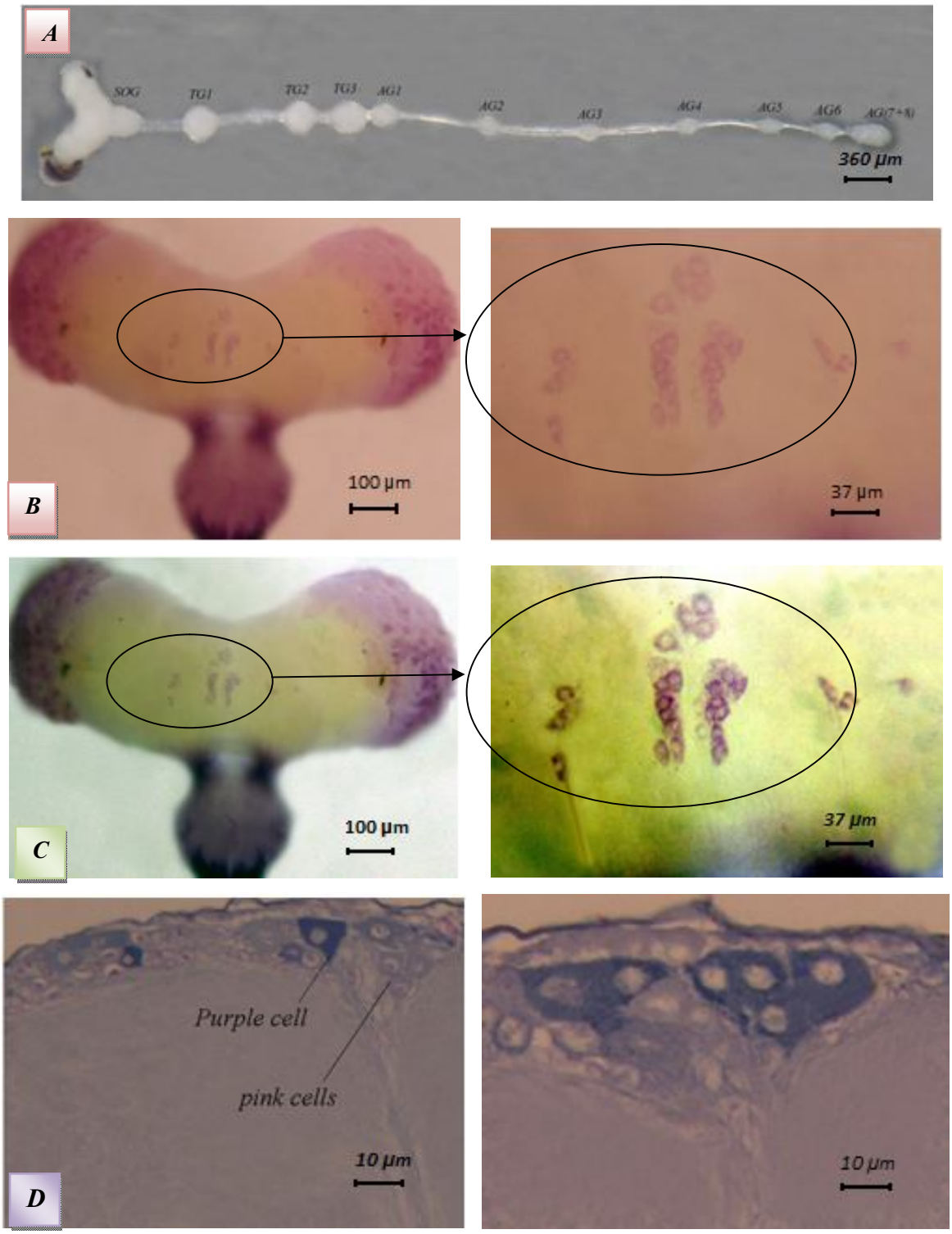

Figure 1 A) Nerve cord of *Luciola gorhami*, SOG, subesopheageal ganglion, TG, thoracic ganglion, AG, abdominal ganglion. B and C) In situ staining of MNSCs and LNSCs with original and changed contrast. Medial and two lateral groups are visible. Ipsilateral pathways of lateral groups are also visible. D) Section staining of NSCs. Two cell types (purple and gray) are observed. Pink cells are grouped in the middle of gray cells.

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

Discussion

In the earliest insect to evolve, there was probably a pair of ganglia in each segment. In the course of evolution, ganglia of the same segment fused along the midline in all extant insects. Primitive insects like apterygotes usually possess three thoracic and eight abdominal ganglia while nerve cords of pterygote are so diverse and vary from one to eleven ganglions (Wheeler *et al*., 2001; Nation, 2008; Niven *et al*., 2008). In present study, nerve cord of *L. gorhami* as a member of a higher order of insects (Coleoptera), had a primitive type and the ganglia were mostly unfused. According to Niven *et al*. (2008), although insect nerve cords have been studied by many authors over the last century, most authors have not attempted to determine the exact underlying causal factors of nerve cord structure. They hypothesized that several factors such as position of muscles especially for locomotive organs, position of sense organs, body size or shape and energy cost may affect the position and fusion pattern of ganglia along the nerve cord. We can only hypothesize about contributing factors. However, Wheeler *et al*. (2001) studied the fusion pattern of the third thoracic and first abdominal ganglia and integrated their findings with a recent insect phylogeny. It was concluded that this event occurred early in the evolution of insects and it may have been crucial for evolution of insect flight. In another study, Grimaldi and Engel (2005) proposed that one contributing factor to the complete fusion of the abdominal ganglion in Paraneoptera may be the absence of cerci in these orders which reduces sensory input to the terminal ganglion and allows it to shift anteriorly. In present study, the first abdominal ganglion was not fused with third thoracic ganglion contrary to what would be expected, since *L. gorhami* is a flying species and this fusion must have happened before evolution of flight. However, there are evidences that fusion can be reversed and ganglia be separated from one another. In larvae of *Syrphus ribesii*, the ganglia are fully fused, but during metamorphosis, the nerve cord is extended and separates into its composite ganglia (Niven *et al*., 2008). Hence, it is very likely that separation of first abdominal ganglion from metathoracic ganglion in this species or family has occurred after evolution of flight. In present study, it was seen that all abdominal ganglia had approximately the same size except the last abdominal ganglion, which was twice larger than the others. This implies that seventh and eighth ganglions might have fused into one and formed the last abdominal ganglion. A primitive nerve cord in *L. gorhami* should not be so strange because earlier works reported that members of coleopteran order are very diverse in terms of ganglion fusion, ranging from fusion of all thoracic and abdominal ganglia into one to three distinct thoracic and eight abdominal ganglia (Mann and Crowson, 1983; Calder, 1989; Heath and Evans, 1990).

Different techniques such as staining and immunocytochemistry have been devised to visualize and characterize NSCs inside intact insect brain (*in situ*) or sectioned insect brain. In fact, large size and superficial position of NSCs and the minute dimensions of insect brain have provided the possibility of *in situ* demonstrating of NSCs (Dogra and Tandan, 1964; Raabe, 1983; Yamanaka *et al*., 2005). In this study, staining method of Dogra and Tandan (1964) including *in situ* and sectioning was followed as framework to demonstrate NSCs of *L. gorhami*, but whenever results were not satisfactory, changes were made in some steps such as oxidization, bleaching, staining or differentiation to standardize the procedure. Of these, bleaching step was so crucial and required more care. At the end, removing of neurolemma was so crucial and was done after five hours or overnight clearing in cedar wood oil. However, in present study using *in situ* staining with aldehyde fuchsin, 26 NSCs in five groups were displayed which can be considered as a good result and suitable procedure for further studies. In addition, by applying section staining with chrome-alum hematoxylin and phloxin, two types of cells (purple and gray) were differentiated. Although *In situ* staining was faster and less time consuming, but only

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

the position of surficial NSCs were displayed and the other properties of NSCs could not be characterized. Ma and Roelofs (1995) applied both *in situ* and section staining to study NSCs in European corn borer moth, *Ostrinia nubilalis*. Based on their results, of the three histologically revealed groups, only one group could be revealed by *in situ* staining.

There are different dyes such as aldehyde fuchsin, chrome-alum hematoxylin and phloxin to stain and differentiate NSCs. Ittycheriah and Mark (1971) appreciated that double staining with chrome-alum hematoxylin and phloxin was the only technique which stained insect NSCs uniformly. On the contrary, Panov (1980) claimed that chrome-alum hematoxylin and phloxin was not sensitive and did not give satisfactory results. In present study, double staining with chrome-alum hematoxylin and phloxin was good for selectivity and sensitivity, provided mode of application could be standardized accordingly. Anwar and Ismail (1979) applied chrome-alum hematoxylin and phloxin for histological study of brain NSCs in adult *Gryllus bimaculatus* and recognized five types of cells (black-blue, grayish pink, purple, slight pinkish and mauve-red). Nath *et al.* (1998) visualized 17 cerebral NSCs in *Oxya hyla hyla* through staining with aldehyde fuchsin and classified them in three groups based on shape, size and stainability. Aydemir and Ergen (2001) characterized three types of NSCs in the thoracic and abdominal ganglia of *Melanogryllus desertus* through staining with aldehyde fuchsin. Tiwari and Singh (2001) classified cerebral NSCs of *Chrotogonus trachypterus* based on stainability into three groups. Type A cells were few, very stain positive. Types B and C cells were less conspicuous and did not stain darkly.

Brain NSCs could be classified based on their position, stainability, shape and size. Based on their position, they are divided in MNSCs, LNSCs, and ONSCs (optical neurosecretory cells). Of these, MNSCs are the main group and considered as mammalian hypothalamus analog because they produce neuropeptides such as prothoracicotropic and prothoracicostatic hormones (Klowden, 2003; De Loof *et al*., 2012). Based on stainability of NSCs, the most widely accepted classification is that by Raabe (1983) who divided NSCs into two major types. (1) Type A, contain neurosecretions which become acidic during oxidation and stained with basic dyes such as aldehyde fuchsin and chrome-alum hematoxylin. (2) Type B, mostly stained with acidic dyes such as phloxin after oxidization and they have a low affinity for basic dyes such as aldehyde fuchsin and chrome-alum hematoxylin. In present study, two groups of cells (MNSCs and LNSCs) were revealed positionally and two types of cells (purple and gray) differentiated based on stainability. It is obvious that purple cells are a part of Raabe's A type, while gray cells are a part of Raabe's B type. Number and size of NSCs vary in orders of insects. Usually, they may be large and few in number as in *Apis mellifera* or small and many in number as in locust (Chapman, 2012). The investigated *L. gorhami* seems to have an intermediate situation in size and number of NSCs.

References

Altstein, M. 2004. Novel insect control agents based on neuropeptide antagonists. The PK/PBAN family as a case study. Journal of Molecular Neuroscience, 22: 147-157.

Altstein, M., Ben-Aziz, O., Schefler, I., Zeltser, I. and Gilon, C. 2000. Advances in the application of neuropeptides in insect control. Crop Protection, 19: 547-555.

Anwar, I. and Ismail, S. 1979. Neurosecretory centers in the brain of adult *Gryllus bimaculatus* (Orthoptera: Grylidae). International Journal of Insect Morphology and Embryology, 8: 265-275.

Aydemir, E. M. and Ergen, G. 2001. Histological investigations on the neurosecretory cells in thoracic and abdominal ganglia of *Melanogryllus desertus* (Orthoptera; Gryllidae). Turkish Journal of Zoology, 25: 83-86.

Calder, A. A. 1989. The alimentary canal and nervous system of Curculionidae (Coleoptera):

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

gross morphology and systematic significance. Journal of Natural History, 23: 1205-1265.

Chapman, R. F. 2012. The Insects: Structure and Function. Fifth edition. Cambridge University Press. UK.

Crowson, R. A. 1960. The phylogyny of Coleoptera. Annual Review of Entomology, 5: 111-134.

De Loof, A., Lindemans, M., Liu, F., De Groef, B. and Schoofs, L. 2012. Endocrine archeology: Do insects retain ancestrally inherited counterparts of the vertebrate releasing hormones GnRH, GHRH, TRH, and CRF? General and Comparative Endocrinology, 177: 18-27.

Dogra, G. S. and Tandan, B. K. 1964. Adaptation of certain histological techniques for *in situ* demonstration of the neuroendocrine system of insects and other animals. Quarterly Journal of Microscopical Science, 105: 455-466.

Farooqui, T. 2007. Octopamne-mediated neuromodulation of insect senses. Neurochemical Research, 32: 1511-1529.

Gäde, G. and Goldsworthy, G. J. 2003. Insect peptide hormones: a selective review of their physiology and potential application for pest control. Pest Management Science, 59: 1063-1075.

Grimaldi, D. and Engel, M. S. 2005. Evolution of the Insects. Cambridge University press, Cambridge, UK.

Harzsch, S. 2006. Neurophylogeny: Architecture of the nervous system and a fresh view on arthropod phyologeny. Integrative and Comparative Biology, 46: 162-194.

Heath, R. V. and Evans, M. E. G. 1990. The relation between the ventral nerve cord, body size and phylogeny in ground beetles (Coleoptera: Carabidae). Zoological Journal of the Linnean Society, 98: 259-293.

Humason, G. L. 1979. Animal Tissue Techniques. W. H. Freeman and Company. San Fransisco.

Ittycheriah, P. I. and Marks, E. P. 1971. Performic acid-resorcin fuchsin: a technique for the *in situ* demonstration of neurosecretory material in insects. Annals of the Entomological Society of America, 64: 762-765.

Johanson, B. 1962. Neurosecretion and the transport of secretory material from the corpora cardiaca in aphid. Nature, 196: 1338-1339.

Johanson, B. 1963. A histological study of neurosecretion in aphids. Journal of Insect Physiology, 9: 727-739.

Kerry C. J. and Mill, P. J. 1987. An anatomical study of the abdominal musculature, nervous and respiratory systems of the praying mantid, *Hierodula membranacea*. Proceeding of the Royal Society of London, B 229: 415-438

Klowden, M. J. 2003. Contributions of insect research toward our understanding of neurosecretion. Archives of Insect Biochemistry and Physiology, 53 (3): 101-114.

Klowden, M. J. 2013. Physiological Systems in Insects. Third edition. Academic Press.

Kristensen, N. P. and Nielsen, E. S. 1981. Abdominal nerve cord configuration in adult non-ditrysian Lepidoptera. International Journal of Insect Morphology and Embryology, 10: 89-91

Lavenseau, L., Gadenne, C. and Trabelsi, M. 1985. Histological and ultrastructural study of the protocerebral neurosecretory cells of the European corn borer, *Ostrinia nubilalis*. International Journal of Insect Morphology and Embryology, 14: 51-62.

Ma, P. W. K. and Roelofs, W. L. 1995. Anatomy of the neurosecretory cells in the cerebral and subesophageal ganglia of the female European corn borer moth, *Ostrinia nubilalis*. International Journal of Insect Morphology and Embryology, 24: 343-359.

Mann, J. S., and Crowson, R. A. 1983. Phylogenetic significance of the ventral nerve cord in the Chrysomeloidea (coleptera: Phytophaga). Systematic Entomology, 8: 103-119.

Matsuda, R. 1976. Morphology and evolution of the insect abdomen. Pergamon Oxford, UK.

Menees, J. H. 1961. Changes in the morphology of the ventral nerve cord during the life history of *Amphimallon majalis* Razoumowski (Coleoptera: Scarabaeidae). Annals of the Entomological Society of America, 54 (5): 660-663.

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

Morris, G. P. and Steel, C. G. H. 1975. Ultrastructure of neurosecretory cells in the pars intercerebralis of *Rhodnius prolixus* (Hemiptera). Tissue and Cell, 7: 73-90.

Nath, S., Chel, G. and Ghosh, D. 1998. Some observations on the neuroendocrine system of female *Oxya hyla hyla*. Indian Journal of Entomology, 60: 233-237.

Nation, J. L. 2008. Insect Physiology and Biochemistry. Second edition. CRC Press, Boca Raton.

Nauen, R., Sorge, D., Sterner, A. and Borovsky, D. 2001. TMOF-like factor controls the biosynthesis of serine protease in the larval gut of *Heliothis virescens*. Archives of Insect Biochemistry and Physiology, 47: 169-180.

Niven, J. E., Graham, C. M. and Burrows, M. 2008. Diversity and evolution of insect nervous cord. Annual Review of Entomology, 53: 253-271.

Panov, A. A. 1980. Demonstration of neurosecretory cells in insect central nervous system, In: Strausfield, N. J. and Miller, T. A. (Eds.), Neuroanotomical Techniques. Springer-Verlag, New York, pp. 26-51.

Predel, R. and Eckert, M. 2000. Neurosecretion: Peptidergic systems in insects. Naturwissenschaften 87: 343-350.

Presnell, J. K. and Schreibman N. P. 1997. Humason's Animal Tissue Techniques. Johns Hopkins University Press. Baltimore, USA.

Raabe, M. 1983. The neurosecretory-neurohemal system of insects; anatomical, structural and physiological data, In: Berridge, M. J., Treherne J. E. and Wigglesworth, V. B. (Eds.), Advances in Insect Physiology. Academic Press, London and New York, 17: 205-303.

Raymond-Delpech, V., Matsuda, K., Sattelle, B. M., Rauh, J. J. and Sattelle D. B. 2005. Ion channels: molecular targets of neuroactive insecticides. Invertebrate Neuroscience, 5: 119-133.

Roeder, T. 2005. Tyramine and Octopamine: ruling behavior and metabolism. Annual Review of Entomology, 50: 447-477.

Rulifson, E. J., Kim, S. K. and Nusse, R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science, 296: 1118-1120.

Schmidt, H., Rickert, C., Bossing, T., Vef, O., Urban, J. and Technau, G. M. 1997. The embryonic central nervous system lineages of *Drosophila melanogaster*. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. Developmental Biology, 189: 186-204.

Siga, S. 2003. Anatomy and functions of brain neurosecretory cells in Diptera. Microscopy Research and Technique, 62: 114-131.

Technau, G. M., Berger, C. and Urbach, R. 2006. Generation of cell diversity and segmental pattern in the embryonic central nervous system of *Drosophila*. Developmental Dynamics, 235: 861-869

Tiwari, S. K. and Singh, N. P. 2001. Neurosecretory cells in adult female of surface grass hopper, *Chrotogonus trachypterus*. Journal of Entomological Research, 25: 151-154.

Wheeler, W. C., Whiting, M., Wheeler, Q. D. and Carpenter, J. M. 2001. The phylogeny of extant hexapod orders. Cladistics, 17: 113-169.

William, G. and Kloot, V. D. 1960. Neurosecretion in insects. Annual Review of Entomology, 5: 35-52.

Yamanaka, N., Hua, Y. J., Mizuguchi, A., Watanabe K., Niwa, R., Tanaka, Y. and Kataoka, H. 2005. Identification of a novel prothoracicotropic hormone and its receptor in silkworm *Bombyx mori*. Journal of Biological Chemistry, 280: 14684-14690.

Yeates, D. K., Merritt, D. J. and Baker, C. H. 2002. The adult ventral nerve cord as a phylogenic character in Brachyceran Diptera. Organism Diversity and Evolution, 2: 89-96.

[DOR: 20.1001.1.22519041.2016.5.2.14.5]

بررسی ساختار زنجیر عصبی و ویژگی‌های یاخته‌های عصبی-ترشحی مغز در حشرات کامل کرم شب‌تاب، *Luciola gorhami* (Coleoptera: Lampyridae)

حجت‌اله محمدی[1*]، ویلیانور ونکاتارامان رامامورتی[2] و بالا سوبرامانیام[2]

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران.

۲- بخش حشره‌شناسی، انستیتو تحقیقاتی کشاورزی هندوستان، دهلی‌نو ۱۱۰۰۱۲، هندوستان.

* پست الکترونیکی نویسنده مسئول مکاتبه: H.mohammadi@yu.ac.ir

دریافت: ۲۴ شهریور ۱۳۹۴؛ پذیرش: ۲۴ آذر ۱۳۹۴

چکیده: ساختار زنجیر عصبی و ویژگی‌های یاخته‌های عصبی-ترشحی مغز با دو روش رنگ‌آمیزی در جای خود و رنگ‌آمیزی برش‌های میکروسکوپی در حشرات کامل کرم شب‌تاب، *Luciola gorhami*، بررسی گردید. زنجیر عصبی ساختار ابتدایی دارد و شامل مغز، گره زیرمری، سه گره قفسه‌سینه‌ای و هفت گره شکمی می‌باشد که توسط یک جفت طناب عصبی به‌هم متصل هستند. گره‌های قفسه‌سینه‌ای از هم جدا می‌باشند و اندازه یکسانی دارند. گره‌های شکمی دارای اندازه یکسانی هستند، به‌جز آخرین گره که اندازه آن دو برابر سایرین می‌باشد. گره‌های قفسه‌سینه‌ای و شکمی در هم ادغام نشده‌اند. با روش رنگ‌آمیزی در جای خود، ۲۶ یاخته‌ی عصبی-ترشحی آشکار گردیدند. بیست یاخته‌ی میانی در سه گروه و در ناحیه‌ی میانی مغز اول قرار داشتند. این یاخته‌ها آرایش **U** مانند داشتند به‌طوری که یک گروه متشکل از چهار یاخته‌ی کروی و بزرگ در جلو، و دو گروه موازی (هر کدام متشکل از هشت یاخته‌ی گلابی تا کروی) در عقب قرار داشتند. شش یاخته‌ی جانبی در دو گروه (سه یاخته در هر طرف مغز اول) قرار داشتند. مسیر عصبی یاخته‌های میانی معلوم نبود اما مسیر عصبی یاخته‌های جانبی معلوم و به‌صورت غیرمتقاطع بود. با رنگ‌آمیزی برش‌های میکروسکوپی، تعداد زیادی از یاخته‌های عصبی-ترشحی قسمت میانی مغز اول به رنگ‌های ارغوانی و خاکستری درآمدند. یاخته‌های خاکستری دارای تعداد زیاد و اندازه بزرگ بودند و در بیش‌تر برش‌ها مشاهده گردیدند. یاخته‌های ارغوانی نیز بزرگ و در لابلای یاخته‌های خاکستری به‌صورت مجتمع قرار گرفته بودند. هر دو نوع یاخته دارای هسته‌های بزرگ بودند و در ناحیه‌ی سطحی مغز قرار داشتند. آکسون‌های آن‌ها گردهم آمده و به‌صورت دسته‌ای به طرف عقب مغز امتداد داشتند.

واژگان کلیدی: رنگ‌آمیزی در جای خود، زنجیر عصبی، یاخته‌ی عصبی-ترشحی، رنگ‌آمیزی بافت

[DOR: 20.1001.1.22519041.2016.5.2.14.5]