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Early development of nervous system in *Litopenaeus vannamei* (Boone, 1931) (Crustacea: Decapoda)

Larval nervous system, genesis, differentiation

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Abstract: The nervous system plays pivotal role in co-ordination of body activities and coping with the task of controlling the behavioral activities during larval development.

In present study, we give a basic description in the early stage morphology of nervous system in *L. vannamei* by classical histological techniques. Results revealed that neuropil first emerged in N_1 stage and the front part of nervous system started getting structural shape in N_3 where the ganglions were coalesced in front part of head.

The complete structure of central nervous system including proto-, deuto- and tritocerebrum was observed in N_6 stage. The structural expansion of nervous system in successive stages was continued and protocerebrum differentiated into anterior and posterior median protocerebrum at Z_3 stage. The optic ganglions, sub-esophageal ganglions and pleon ganglions were distinct in zoea stage. Furthermore, we discuss the relativity between the development of olfactory lobes and the voluntary movement of larva.

Keywords: *Litopenaeus vannamei*, development, nervous system, histology.

1. INTRODUCTION

The nervous system of vertebrates and invertebrates varies in structural complexity. In annelids, molluscs and arthorpods the nervous system is more centralized into one or several ganglia in order to co-ordinate and store information received from the environment (Castro and Huber, 2007). The basic investigations on genesis of nervous system are necessary in order to reveal its functional establishment, the hormonal regulation and physiological activities. Furthermore, the morphological assessment of NS provides the valuable information for phylogenetic relationships (Purschke *et al.*, 2000) because meager changes occur during its development.

Until now number of studies had conducted on development of arthropod nervous system including investigations on decapod crustacean, based on immunocytochemical and molecular studies, the wealth of knowledge on neurogenesis of crustaceans is continuously growing. Some of the popular articles on the subject are (Elofsson, 1969; Nakamura and Seki, 1990; Harzsch and Dawirs, 1993, 1996; Harzsch *et al..*, 1999; Harzsch, 2001., 2003; Xue, 2005; Vilpoux *et al.*, 2006; Zhang *et al.*, 2007; Schmidt, 2007; Semmler, *et al.*, 2008, Ungerer and Scholtz., 2008; Song *et al.*, 2009; Fischer and Scholtz, 2010).

The morphology of decapods nervous system is well known and the shrimp nervous system is composed of dorsal brain and ventral nerve cord, the dorsal brain can further be described into proto- deuto- and tritocerebrum (Young, 1959). Besides studies on adult nervous system, scanty information is available on the structural morphology and development of the larval nervous system in penaeidae shrimps. The investigations on the larval nervous system of penaeidae species are only restricted to the studies of (Elofsson, 1969; Nakamura and Seki, 1990 and Zhang *et al.*, 2007). The literature suggested that appearance of nervous system related structures in larval stages are not specific.

The present study designed to investigate the morphology and development of early nervous system in *L. vannamei*, a prime aquaculture species, using classical histological methods. The study provides academic evidences on probing into the functional establishment of nerve cell.

2. MATERIALS AND METHODS

L. vannamei embryos (blastula, gastrula, limb bud embryo, embryonic nauplius), larvae (nauplius (N_1-N_6), zoea (Z_1-Z_3), mysis (M_1-M_3) and post larva (P_1-P_3) were obtained from a shrimp hatchery in Zhanjiang, China during 2009.

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For histological observations, specimens of each stage were first fixed in Bouin's solution for 24 h. Smaller samples (N_1-N_6, Z_1-Z_3), were first washed thrice with 70% ethanol, and treated with 50% ethanol and distal water for 20 min respectively for re-hydration, and then dehydrated in ascending series of ethanol 30 min for each grade after embedded in agar, furthermore cleared in xylene two times 15 min each. Other samples (M_1-M_3, P_1-P_3) were dehydrated directly in ascending series of ethanol 30 min each after washed three times with 70% ethanol, and the methods for clarity and embedment are the same as described above. The serial sections in transverse, longitudinal and horizontal for each specimen were obtained at 6-7 µm thickness using a rotary microtome. Slides were then stained by haematoxylin and eosin (H&E) dye, examined and captured images by a Nikon E80i microscope.

3. RESULTS

Genesis and differentiation of brain

The neuropil surrounded by an incomplete single layer of nerve cells, initiated distinctly in N_1 stage **(Fig. 1A)** and expand the range along the margins in N_2 stage **(Fig.1B)**. Furthermore the protocerebrum was visible in N_3 stage **(Fig. 1C)** and the structural appearance of brain kept progress in successive stages and a complete structure of brain including proto-, deuto- and tritocerebrum occurred in N_5 and N_6 **(Fig. 1D, E)**. During zoea, the brain becomes larger and more distinguishable, and focused mainly its differentiation in increasing the number of nerve cells and expanding the range of neuropil **(Fig. 2 A~C)**.Until Z_3, the protocerebrum differentiated into two parts, named anterior median protocerebrum (AMPN) and posterior median protocerebrum (PMPN) **(Fig. 2D)**. Moreover, the brain of *L. vannamei* showed its expansion in mysis and post larval (M_1-M_3 and P_1-P_3).

Genesis and differentiation of olfactory lobes and optic ganglions

The olfactory lobe was demarcated at Z_1 stage and located at the outside of the tritocerebrum **(Fig. 2B)**. As an important structure for chemoreceptor, this organ expanded gradually during the larval development to participate in food capture and environment **(Fig. 2E, F)**.

The appearance of central pit of eyes emerge in N_2 stage and the steady increase of cell number was noted which are responsible to form compound eye in forthcoming stages. The ganglions of compound eye appeared in zoea stages and differentiated into Lamina ganglions, medulla externa, medulla interna and medulla terminalis **(Fig. 2I)**, and the volume considerably increased in myses and post larval stages. The increase of different part in optic ganglions was unequal; the most enlarged optic ganglions was medulla terminalis, medulla interana, and then medulla externa, lamina ganglions respectively. Until P_3 stage it has been observed that the increase in volume of medulla terminalis and medulla externa is comparatively more rapid than rest of two optic ganglions **(Fig. 2J)**. The optic ganglions linked with protocerebrum by protocerebral tract **(Fig. 2C),** and kept on expanding in mysis and post larval stages.

Genesis of ganglions

The sub-esoghageal ganglions including pleon ganglions are well demarcated in N_6 stage **(Fig. 1F)** and their expansion continued in myses and post larval stages **(Fig. 2G, H)**. Each pleon ganglions situated at the anterior margin of the sternum of the corresponding segment. The pleon ganglions are connected with protocerebrum with circum-esophageal connectives.

4. DISCUSSION

This study focused on basic neuroanatomical organization of nervous system in larval and post larval stages of *L. vannamei*, a prime aquaculture species. In *Penaeus,* the nervous system is more specialize and comprises of dorsal brain and ventral longitudinal nerve cord connected by a pair of circum-esophageal commissures (Tirmizi and Khan, 1970). Like other penaeidae, the brain of *L. vannamei* lies on the dorsal side at base of the rostrum and has been divided into three basic divisions, proto-, deuto- and tritocerebrum in N_5 and N_6.

The brain neuropil originated at N_1 stage in *L. vannamei* and the result accorded with the *F. chinesis* (Zhang *et al.*, 2007), but disagreement with *P. japonicus* where the slight differentiation of nervous tissue was observed in N_2 stage (Nakamura and Seki, 1990). Furthermore, the brain in *L. vannamei* was divided into proto-, deuto and tritocerebrum at N_5 and N_6 stage, however, in *F. chinensis*, the brain differentiation is in Z_1 (Zhang *et al.*, 2007). Xue (2005) reported that brain was distinguished from the proto-, deuto-, and tritocerebrum at embryonic nauplius stage II in *Portunus trituberculatus*. Ours results did not show any disagreement in basic structure of CNS with that of the family penaeidae, *P. japonicus* (Nakamura and Seki, 1990), *Hynas araneus*, *Carcinus maenas* (Harzsch and Dawirs, 1993, 1996) and *F. chinensis* (Zhang *et al.*, 2007), although the differences in emergence time of structures existed. The findings of Sandman *et al.*, (1992) in *Cherax destructor*, *Schylla serrata* and *Panulirus argus* also showed the similar structural organization of nervous system.

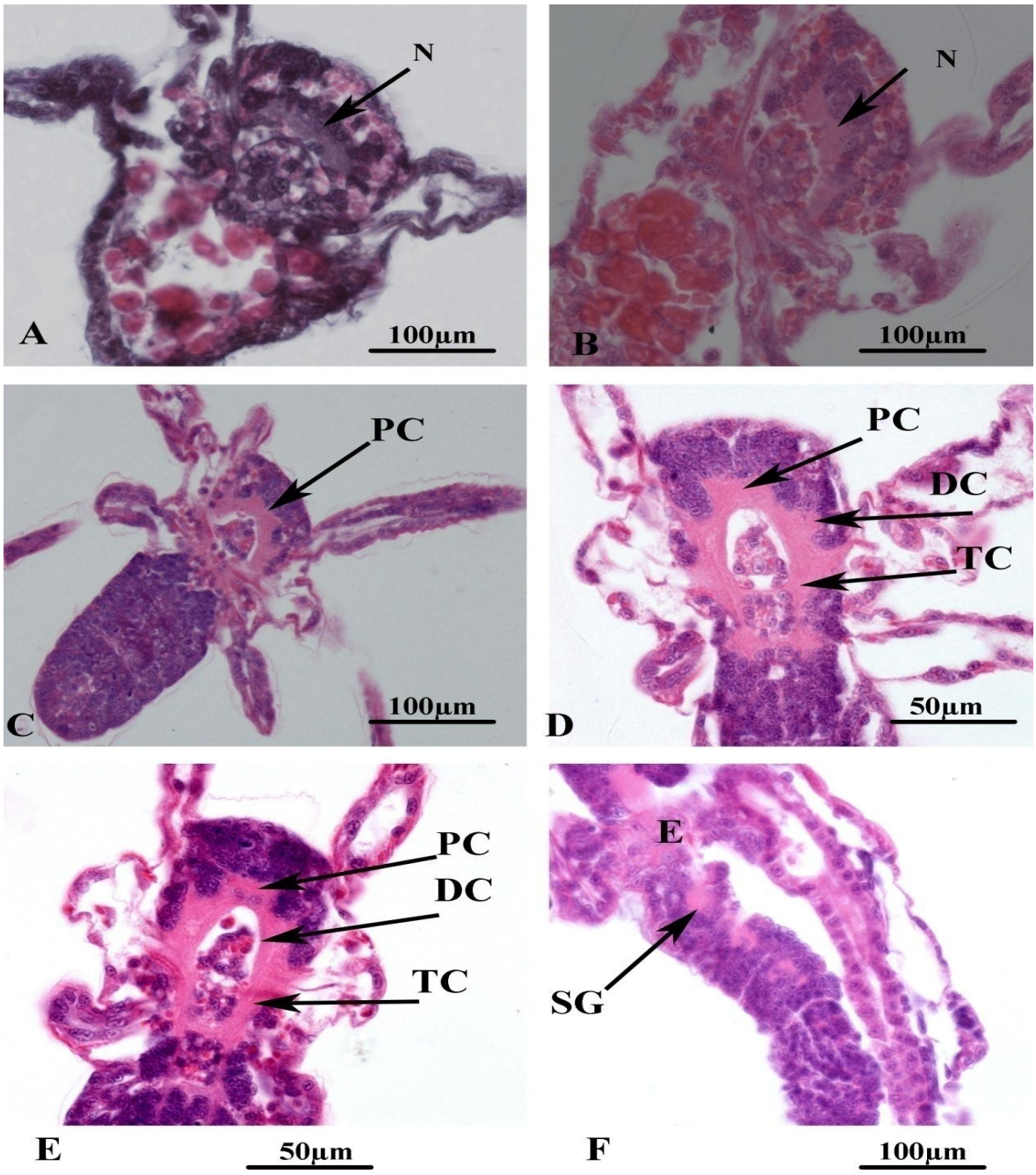


Fig.1. Genesis of nervous system in *L. vannamei*.: A and B, Horizontal section of N_1 and N_2 showing neuropil; C, Horizontal section of N_3, showing further development of nerve organization; D and E, Horizontal sections of N_5 and N_6, showing the structural shape of proto- deuto- and tritocerebrum. F, Longitudinal section of N_6, showing sub-esophageal ganglions.
DC, Deutocerebrum; E, Esophagus; N, Neuropil; PC, Protocerebrum; SG, Sub-esophageal ganglion; TC, Tritocerebrum.

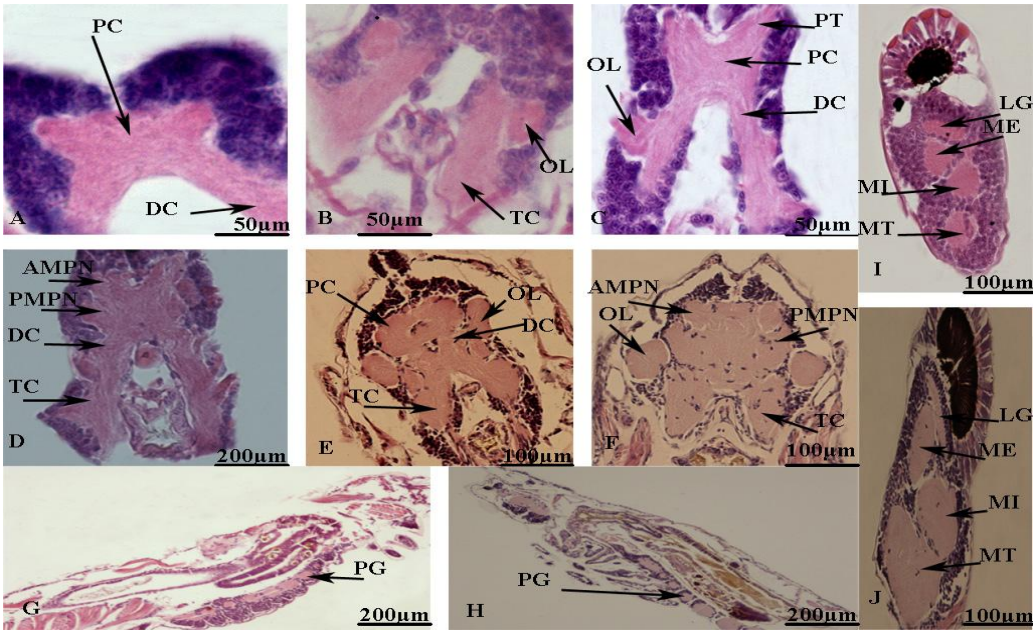


Fig. 2. Differentiation of nervous system in *L. vannamei.*: A and B, Z_1. Showing the proto-, deuto-, tritocerebrum and olfactory lobe; C, Z_2. Showing proto-, deutocerebrum and protocerebral tract; D, Z_3. Showing protocerebral tract, AMPN and PMPN, deuto- and tritocerebrum respectively; E, M_3. Showing the greater expansion of nervous system; F, Horizontal sections of P_3. Showing distinct differentiation and greater expansion of complete nervous system; G and H, Showing the pleon ganglions in M_3 and P_3; I & J, Horizontal section of eye stalk of Z_3 and P_3 respectively.
AMPN, Anterior median protocerebrum; DC, Deutocerebrum; E, Esophagus; ME, Medula externa; MI, Medula interna; LG, Lamina ganglion; OL, Olfactory lobes; PC, Protocerebrum; PG, Pleon ganglion; PMPN, Posterior median protocerebrum; PT, Protocerebral tract; TC, Tritocerebrum; TM, Medula terminalis.

In *L. vannamei* the central pit of eyes emerged in N_2 stage and the appearance of optic ganglions were observed in zoea stages and could be differentiated into Lamina ganglions, medulla externa, medulla interna and medulla terminalis. The organization of optic ganglions is remarkably constant with other groups of decapods such as Cray fish, crab, spiny lobster and *H. araneus*, respectively (Sandeman et al., 1992; Harzsch and Dawirs, 1996). Although in the certain crustaceans the optic ganglions could not be differentiated such as cyprid (Harrison and Sandeman, 1999), the brachyurans have large protocerebral tract which suggested reliance on the visual system (Sandeman *et al.*, 1992). The protocerebral tract of *L. vannamei* is also large enough which connects the optic ganglions with protocerebrum.

The ventral nerve cord in larval stages of *C. maenas* is composed of the sub-esophageal ganaglion (SEG), five thoracic ganglia and six abdominal ganglia (Harzsch and Dawirs, 1993). The sub-esophageal ganglions (SEG) in *L. vannamei* was first observed at N_6 stage while the thoracic ganglions and pleon ganglions respectively were found in Z_1 and Z_2 stage in *F. chinensis* (Zhang *et al.*, 2007) however, the sub-esophageal ganglions was noted at N_4 stage of *P. japonicus* (Nakamura and Seki, 1990). The results suggested that a time difference of nervous system development existed surely among the species in crustaceans.

The nervous system in *L. vannamei* at N_6 stage contains the full neural elements which are necessary for mediating complex interactions with the environment. The voluntary movement of larva is co-related with the development of nervous system. Nakamura and Seki (1990) reported that N_1 of *P. japonicus* does not respond voluntarily and our observations accord with them. In early stages, the nervous system is in its preliminary developmental level meanwhile the movement of appendages is spontaneous activity. The same phenomenon is also reported by Nakamura and Seki (1990). In *L. vannamei*, the complete willing movement and balancing begin from N_6 and zoea because olfactory lobes are well demarcated in zoea which play role in behavioral activities. More knowledge of the neuroanatomy is required to follow up the complete behavioral function of nervous system in penaeid shrimps. Moreover, the advance techniques for further study of embryonic and larval development of nervous system in shrimps will contribute the depth and important direction to the subject.

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