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THE CEREBRAL NEUROSECRETORY SYSTEM IN THE BRACKISHWATER TIGER PRAWN *PENAEUS MONODON* (FABRICIUS)*

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ABSTRACT

Histomorphology of the cerebral ganglion of a penaeid prawn *P. monodon* has been studied by means of classic neurosecretory staining techniques. Three kinds of neurosecretory cells namely A, B and C are distinguished on the basis of their shape, size and tinctorial characteristics of the cytoplasmic inclusions. Heterogeneous distribution of neurosecretory cells in five major groups like B_1, B_2, B_3, B_4 and B_5 has been discussed in relation to the phylogenetic status of *P. monodon*. Further occurrence of deeply stained tiny cell cluster at the mid-ventral plane of the ganglion is also reported. Intracerebral axonal tracts leading to optic lobe suggests dispatch of neurosecretory material/cellular products through axonal processes and from the body of secretory neurons as well.

INTRODUCTION

SINCE the work of Enami (1951) a comprehensive account on the cytomorphic characteristics of orientation of cerebral neurosecretory cells (NSC_s) have been furnished in *Gecarcinus* and *Cambarus* by Bliss and Welsh (1952) and Bliss *et al.* (1954) respectively. Later many other investigators (Miyawaki, 1955; Parameswaran, 1955, 1956; Durand, 1956; Matsumoto, 1958; Nagabhushanam and Rao, 1966) attempted to locate the grouping of NSC_s and their subsequent participation for transport of neurosecretory material (NSM) in the central nervous systems of *Telmessus*, *Paratelphusa*, *Oronectes* and *Scylla*, etc. Fragmentary reports, however, are on record with respect to the structural identity of brain NSC_s in natantia. Involvement of these cells for the release of NSM through axons has casually been traced upon in *Caridina laevis* (Pillai, 1961), *C. weberi* (Nagabhushanam and Vasantha, 1972), *Penaeus kerathurus* and *P. japonicus* (Ramadan and Matta, 1976) apart from their locations and ill-defined groupings.

The objective of the present investigation is to reveal the microanatomical profile of the structure, nature, distribution and groupings of the brain NSC_s in tiger prawn *Penaeus monodon* (Fabricius). This is necessary to evaluate the functional status of the neurosecretory perikarya in the course of varied physiological conditions.

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Material and Methods

The specimen (63-152 mm) used for this study were female tiger prawn *P. monodon*. They were collected from the brackishwater culture ponds at Kakdwip, 90 km south of Calcutta. Both Carnoy's (Absolute alcohol 60 ml + Chloroform 30 ml + Glacial acetic acid 10 ml) and aqueous Bouin's (Picric acid saturated solution in distilled water 75 ml + 40% Formaldehyde 25 ml + Glacial acetic acid 5 ml) fixed brains were used for cytomorphic probe. Tissues were dehydrated in alcohol, cleared in xylene and embedded in tissuemat (M.P. 56-58°C). Serial frontal sections (7-10μm) were stained in Gomori's Chrome alum haematoxylin phloxin — CAH — (Bargmann, 1949) (1% haematoxylin 50 ml + 3% chrome alum 50 ml + 5% $K_2Cr_2O_7$ 2 ml + 5% H_2SO_4: 1 ml); Heidenhain's azan (Gurr, 1956); aldehyde fuchsin — AF (Cameron and Steele, 1959) (0.50 gms of dry crystals dissolved in 100 cc of 70% alcohol) and resorcin fuchsin — RF (McGuire and Opel, 1969) (1 gm resorcin fuchsin dry dye dissolved in 90 cc. of 70% alcohol + 2 cc (12N) HCl). Always maximum diameters of both cells and nuclei were taken into account by oculometer for determination of their dimensions. Olympus (light) microscope was used for the present study.

Results

Macroscopic studies

It is confined to the cephalic region of the brain and is well protected by calcified carapace. It is situated deep beneath the carapace in the mid-line behind the eyes and above the epistoma.

Virtual exposition of anatomically three distinct lobes — the proto, deuto and triotocerebrum of the brain is not well discernible. The dorsal most lobe of the brain or protocerebrum always remains conspicuous in contrast with other two lobes — a condition which necessitates to designate it as 'cerebral mass'.

Microanatomical studies

Cell groups: In general, five NSC groups like B_1 through B_5 are encountered. B_1 remains extended from the ventral to dorsal and at the supradorsal region it is represented by bilateral anterodorsal patch which bears resemblance with hexapod parasintercerebralis region (Pl. I A). B_2 group is restricted anterolaterally and essentially comprises of two sets of small cluster to each half of the brain (Pl. I B). The third NSC group B_3 is rather extended at the lateral margin and encountered at the mid-dorsal plane of the brain (Pl. I C). The small but conspicuous B_4 group is located at the postero-lateral profile and demonstrates possession of conspicuous NSC types (Pl. I D). The last or the fifth NSC group B_5 is restricted to the postero-medial part of the ganglion and has much resemblance with B_1 group (Pl. I E). Of the five NSC groups, B_1 and B_5 remain unpaired while the rest are paired. Indeed, majority of the NSC groups are encountered at the medial than either dorsal or ventral plane of the ganglion in question. Spatial arrangement of NSC groupings is dependent upon the confluence of the neuropilar mass which is rather distinct at the dorsal than the ventral profile.

Neurosecretory cell types

A type: Largest of all the cell types with an average diameter of 60 μm and are either round or pear-shaped with or without axonal processes. Nuclei remain oval to round with an average diameter of 21 μm. The cytoplasm is more or less homogeneously distributed with secretory inclusions. Sometimes aggregation of NSM in the form of clusters

are noticeable in the vicinity of the axon hillock. Vacuoles within the cytoplasm are not seldom and may contain secretory granules. Evidence for both axonal and peripheral discharge of NSM are often encountered (Pl. I D, F).

B type : These are medium types of cell ranging from 12 to 24 μm with an average diameter of 18 μm. They may be oval, polygonal or pear-shaped in appearance and have axonal processes. The vesicular nuclei with average diameter of 9 μm are centric and possess ill-defined nucleoli. The fine cytoplasmic inclusions are scarcely distributed within the perikarya. Occurrence of peripheral vacuoles lacking secretory inclusions and coalescence of these vacuoles are often detected. Occasional transport of NSM, through axonal processes, is visible (PL. I D, F).

C type : Size ranges of these types of cells vary from 12 to 16 μm and possess average diameter of 14 μm. They are either oval, round or polygonal in shape and may have with or without axonal processes. Round nuclei with conspicuous intranuclear material are mostly centric and have average diameter of 10 μm. Discrete secretory inclusions are often distributed within the cytoplasm where presence of vacuoles is not scarce (PL. IF).

Tiny type : In association with the aforesaid types of unique identifiable neurons, existence of a few cluster of tiny deep stained cells is also noticed and their distribution is chiefly restricted at the midventral plane of the brain. Critical observations reveal that average size of this category of cells is 5 μm and are round in shape. The nuclei are, however, disproportionately large and possess average diameter around 3 μm. Sometimes possession of very short axon like processes devoid of NSM are detected in these cells (PL. I B).

Distribution of neurosecretory cell types : As regards groupwise distribution of NSC types both A - and B - cells are encountered in all the NSC groups while C - cells are restricted in B_1, B_3 and B_5. The tiny cell groups, however, are confluent with the B_2 - than any other cell groups.

Neurosecretory products of the ganglion : Majority of the NSC types do expel their elaborations through axonal transport. Evidence for the transport of NSM through the axonal tracts originating especially from B_1 and B_2 groups has been explicitly clear when intracerebral course of the axonal bundle. on their way to optic lobe is followed upons Besides these, migration of neurosecretory product in cells especially belonging to B_4 group is accomplished in a manner which can be considered as peripheral discharge. In consequence, intercellular deposition of NSM becomes conspicuous (Pl. I D).

Discussion

Location of NSC_s in the supraoesophageal ganglion of *P. monodon* reveals exposition of several cell groups. Probe on the occurrence of cerebral cell groups in crustaceans has been made to elucidate their interrelationship with respect to transport and/or release of secretory material (Matsumoto, 1958). Existence of five major groups of NSC_s in the decapodan brain is attributed as a usual feature in sub-orders like reptantia and natantia. Besides these, special emphasis has been paid to probe into details concerning indispensability and characterization of a particular group so as to categorise the latter into sub-groups (Bliss *et* al., 1954 ; Matsumoto, 1958). In the present study, the orientation of NSC groups in the brain exhibits close resemblance with that of *C. laevis* (Pillai, 1961) and *C. weberi* (Nagabhushanam and Vasantha, 1972) with reference to their morphological features and uniformity in appearance. Whether concentration of NSC_s in the form of groups in aquatic mandibulates especially natantia (*P. monodon*) has any implication with regard to their taxa or

due to diversity of behavioural and phenomenal potential within the individual of a particular group in connection with the involvement of large number of NSC is still obscured (Rowell, 1976).

Classification of NSC$_s$ in the species under study is principally based on their shapes size (in descending order) and morphological characteristics of the secretory product which, in fact, advocated by most of the crustacean neuroanatomists. Accordingly, three principal types of NSC have been distinguished in the body of the cerebral ganglion and is in conformity with the observations of other investigators (Enami, 1951 ; Bliss *et al.*, 1954 ; Pillai, 1961 ; Nagabhushanam and Rao, 1966). Indeed, such classification of NSC$_s$ is in no way stressing upon standard principle as the previous investigators followed different methods of characterisation and thus reveals 'lack of uniformity'. Furthermore, most of the earlier workers attempted to classify the neurosecretory perikarya that are located in the eyestalk and at length applied the same principle throughout the nerve centres including cerebral ganglion and ventral nerve chain (Durand, 1956) where axons from the 'perikarya of origin' terminate to neurohaemal organs of the protocephalic neurosecretory system. Although major emphasis has been given to the distribution of cell types in decapodan brain (Parameswaran, 1956 ; Ramadan and Matta, 1976) not much concrete data concerning the involvement of cell types for the formation of groups have been recorded yet (Bliss *et al.*, 1954 ; Pillai, 1961 ; Nagabhushanam and Vasantha, 1972 ; Ramadan and Matta, 1976). It was Matsumoto (1958) who first made an attempt to describe comparatively the structural entity of all the NSC groups that are available in the brain of five species of crabs. In their studies on both crab and prawn, Nagabhushanam and Rao (1966) and Nagabhushanam and Vasantha (1972) described four to five groups of NSC and in the former they have mentioned the groupwise distribution of cell types while in the later this is lacking. In our study on *Penaeus*, the existence of the NSC group is in partial agreement with crab and crayfish (Bliss *et al.*, 1954), five species of crabs (Matsumoto, 1958), a crab (Nagabhushanam and Rao, 1966) and a prawn (Nagabhushanam and Vasantha, 1972). In so far the total number of groups, only five are encountered in *P. monodon* in contrast with maximum seven out of which two can be considered as special for containing cells like D and E types as reported by Matsumoto (1958). Although Nagabhushanam and Vasantha (1972) described five groups of brain NSC$_s$ in prawn *C. weberi*, no clarification with regard to their composition has been attempted. In *Penaeus*, however, clear indication for the distribution of cell types in each group is understood. Trend for the distribution pattern of the major cell types demonstrates close parallelism with that of Matsumoto (1958). But a difference in their (A and B types) dimension as well as cytomorphological features exists. In fact, A type with its smaller dimension in *P. monodon* is found to be distributed in all the groups instead of B_1, B_2 and B_4 of five species of crab (Matsumoto, 1958), while more or less identical pattern in the distribution of B type is observed in all the NSC groups in both the cases. Thus all that matters is the cytomorphological characteristics and not with pattern of orientation. Homogeneous confinement of a particular cell type to a group(s) as envisaged by Matsumoto (1958) or Nagabhushanam and Rao (1966) is not tenable in *P. monodon* where heterogeneous composition is the rule.

In view of the available information (Pillai, 1961 ; Nagabhushanam and Vasantha, 1972 ; Ramadan and Matta, 1976) concerning the intracerebral neurosecretory pathways in decapod crustacea, it is increasingly clear

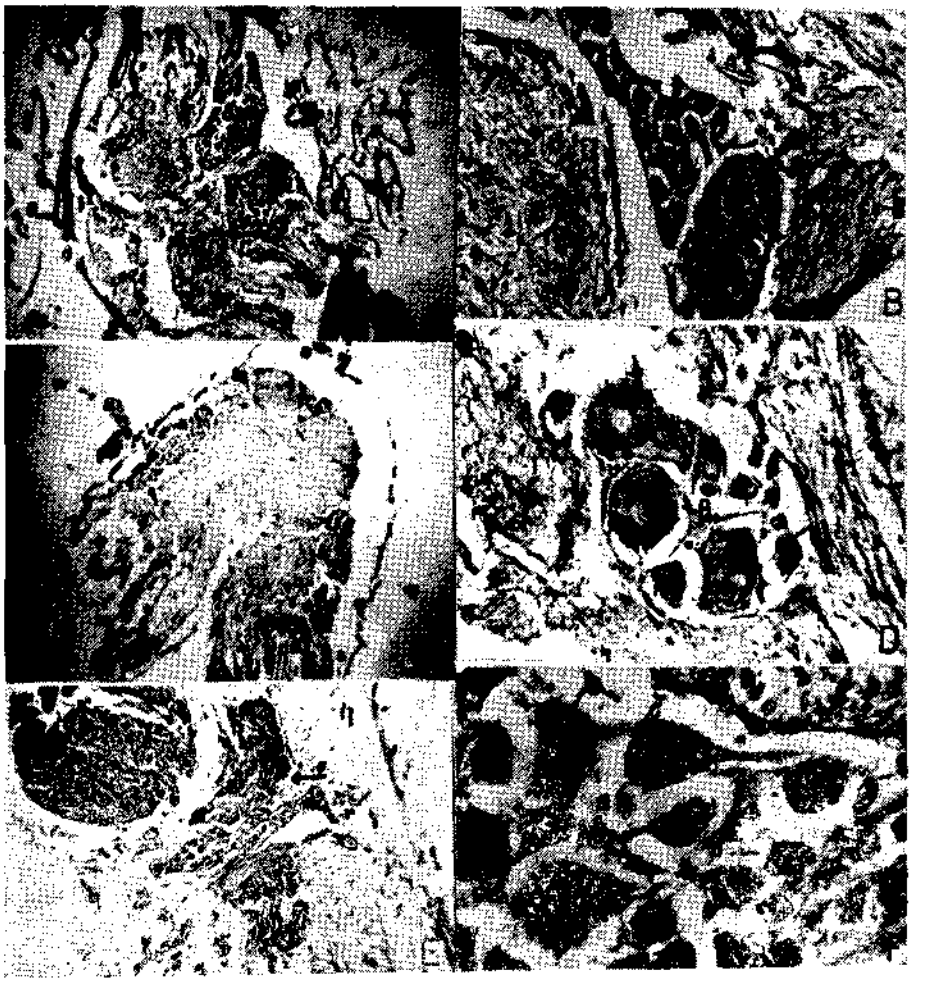

PLATE I A. The cerebral ganglion of *P. monodon* showing bilateral disposition of B_1 neurosecretory cell group (AF-reaction, × 150), B. B_2 group deep at the base of the optic nerve peduncle. Note the cluster of intensely stained tiny cells (arrow) and their relationship with B_3 group (AF-reaction, × 375), C. distribution of neurosecretory cells in B_3 group girdling at the lateral margin of the brain (Azan-reaction, × 150), D. exhibiting small group (B_4) enriched with CAH-positive A and B cell types at the posterolateral part of the brain. Note peripheral discharge (arrow) from some of the neurosecretory cell bodies (× 375), E. postero-median B_5 group situated at the ventral profile of the brain. Note its discontinuity with the B_1 group following neuropilar invasion (arrow) (AF-reaction, × 150) and F. a typical group (B_1) containing AF-positive A, B and C types of cell. Note microanatomical distinction and secretory cycle in these unique identifiable neurons (× 1500).

that axonal migration from the perikarya of origin exists for eventual discharge of the active principle (hormone?) into the circulation. Alternatively, the possibility that the pericaryons may liberate active principle directly into the tissue fluid — a fact that has been considered more particularly in connection with the NSCs of the thoracic ganglia in decapod Crustacea (Parameswaran, 1956; Matsumoto, 1958; Miyawaki, 1960) is not ruled out. Our findings, on the cerebral neurosecretory pathways in *Penaeus*, clearly indicate axonal migration via optic lobe from restricted NSC groups (B_1 and B_2) in contrast with other NSC groups where, of course, direct discharge cannot be discounted. In fine, morphophysiological characteristics, nature and localisation of NSCs in groups coupled with their involvement for the discharge of neurosecretory elaboration in the supraoesophageal ganglion of *P. monodon* represents a heterogeneous picture so as to substantiate the concept of species specification despite the existence of eco-variable factors.

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