

## Histochemistry of the neurosecretory systems in a stomatopod crustacean *Squilla holoschista*

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MS received 18 January 1983; revised 3 August 1983

**Abstract.** The histochemistry of secretory material in neurosecretory cells (NSC) of *Squilla holoschista* has been investigated. The cells of eyestalk, thoracic ganglia and brain contain different substances with regard to reproductively active and quiescent phases. The NSC of eyestalk *C* and *D* are rich in protein, associated with sulphur containing aminoacids. The *B* type of NSC of thoracic ganglia is rich in lipoprotein, whereas the *C* type of the brain is glycolipoprotein.

**Keywords.** *Squilla holoschista*; neurosecretory cells; protein; lipoprotein; glycolipoprotein.

### 1. Introduction

Neurosecretory cells (NSC) were described in Crustacea for the first time by Enami (1951) who distinguished three types of cells in the brain of the crab *Sesarma*. By using mostly the paraldehyde fuchsin (PF) and chrome-alum haematoxylin phloxine (CHP) techniques, the cells were located in different nerve centres of Crustaceans (Gomori 1941, 1950). In Crustacea there have been only limited studies on the histochemistry of the neurosecretory cells. These studies were only supplementary to morphological studies in neurosecretion and have been done in decapod crustaceans. Gabe (1952a,b) observed the presence of PAS-positive material cells in the organs of Hanstrom and sinus glands in *Sphaeroma serratum*. Miyawaki (1956a,b) reported the presence of PAS-positive material in the NSCs in the crab *Telmessus cheiragonus*. Further, Miyawaki (1960a,b) reported the presence of cytoplasmic globules rich in RNA, cerebrosides and phospholipids in the nerve cells of crabs. Rehm (1959) described five types of proteinaceous neurosecretory material in the crab *Carcinus maenas*. A few observations on the histochemistry of the neurosecretory systems in the entamostracan groups have been published by Barnes and Gonor (1958), Lake (1970) and McGregor (1967). Recently, studies on the histochemistry of the neurosecretory materials have increased using different histochemical techniques. However, information gathered by these methods shows some divergence. Deecaraman and Subramoniam (1983) reported the types of neurosecretory cells found in the eyestalk, thoracic ganglia and brain of *Squilla holoschista* and their cyclical activity in relation to reproductive phases. The present paper deals with the nature of the neurosecretory material elaborated by the NSC of the three centres in *S. holoschista*.

## 2. Material and methods

*S. holoschista*, which breeds all through the year along the Madras Coast, were collected and maintained in the laboratory in aquarium tanks. For the histochemical analysis, eyestalks, thoracic ganglia and brain were collected from both mature and reproductively quiescent females. The quiescent females were identified by the absence of cement glands on 6, 7 and 8 thoracic segments (Deecaraman 1980; Deecaraman and Subramoniam 1980) which is taken as an index to differentiate the mature, reproductively active females from the quiescent females. The eyestalks, thoracic ganglia and brain were fixed in Bouin's, 10% neutral buffered formaldehyde, Keley's, Carnoy and Perney's fluids. Paraffin sections (6-8  $\mu\text{m}$  thickness) were stained in paraldehyde and chrome-alum haematoxylin phloxine (Gomori 1941, 1950). Besides paraffin sections, cryocut sections were also used. The histochemical tests were made following the methods outlined by Pearse (1968), and Bancroft and Stevens (1977).

## 3. Results

It has been reported (Deecaraman and Subramoniam 1983) that the eyestalk consists of 4 types of NSC namely *A*, *B*, *C* and *D* and the thoracic ganglia and brain, three types namely *A*, *B* and *C*. The activities of certain NSC of the eyestalk (*C* and *D*), thoracic ganglia (*B*) and brain (*C*) show fluctuations in their secretory activity between reproductively active and reproductively quiescent stages in the synchronous growth of ovary and cement glands, suggesting their involvement in the control of reproductive activities.

### 3.1 Histochemical staining reaction

The tissues of different endocrine centres of NSC cells are subjected to various histochemical tests of two stages (reproductively active and quiescent stages) and table 1 denotes the chemical characterization.

The cytoplasm of different NSC cells stains purple with PF, and bluish with CHP; the NSC cells show mild differences in their staining reaction with regard to reproductively active and quiescent stages. This is evident with these two stains; however, the staining reaction with histochemical dyes are more conspicuous.

The *C* and *D* type of eyestalk NSC of reproductively quiescent state stain more intensively with acidic and basic protein stains indicating the abundance of proteinaceous substances in the secretory granules (figures 1 C,D, 4 and 5). The granules are also positive to tyrosine and sulphur containing amino acids; however, the presence of tryptophan and arginine were not detected in the granules of these two cell types. The presence of RNA in the cytoplasm is clearly shown by methyl green pyronin method. The negative histochemical reaction for oil red O, Nile blue sulphate, Sudan black, Luxol fast blue and acid haematin indicate the absence of lipid substances. Steroid is also not detected. The presence of carbohydrate substance is rather doubtful. It is therefore inferred that the neurosecretory substances synthesized in *C* and *D* type of cells are primarily proteinaceous and that they are not linked to any other prosthetic groups such as carbohydrate and lipid.

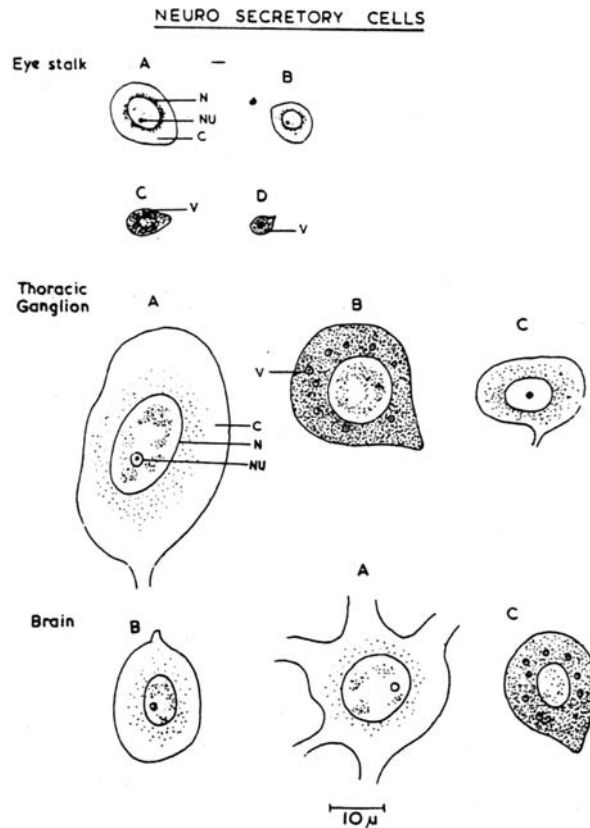
Table 1. Staining and histochemical characterization of the NSC cells of *S. holoschista*

Sl. No.	Tests and Stains	To demonstrate	Eyestalk						Thoracic ganglia						Brain			
			A	B	C	D	A	B	C	A	B	C	A	B	C			
1.	Haematoxylin (Lillie 1954)	Cytoplasm and Nucleus	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2.	Mallory's (Mallory 1938)	Cytoplasm and Nucleus	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR	BR
3.	Masson's trichrome (Papan 1948)	Cytoplasm and Nucleus	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
4.	Paraldehyde fuchsin (Gomori 1950)	Neurosecretory cells	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
5.	Chrome-alum-haematoxylin phloxine (Gomori 1941)	Neurosecretory cells	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV	BV
6.	Ninhydrin-Schiff (Pearse 1968)	Protein bound NH <sub>2</sub> groups	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.	Ninhydrin-Schiff after deamination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	Mercuric bromophenol blue (Maize <i>et al</i> 1953)	General and acidic blue	-	-	+++B	+++B	±	±	+++B	±	+++B	±	+++B	±	+++B	±	+++B	±
9.	Mercurin bromophenol blue after pepsin digestion		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.	Aqu. bromophenol blue (Johri and Smyth 1956)	Basic protein	-	-	+++B	+++B	±	±	+++B	±	+++B	±	+++B	±	+++B	±	+++B	±
11.	Aqu. bromophenol blue after deamination		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

continued







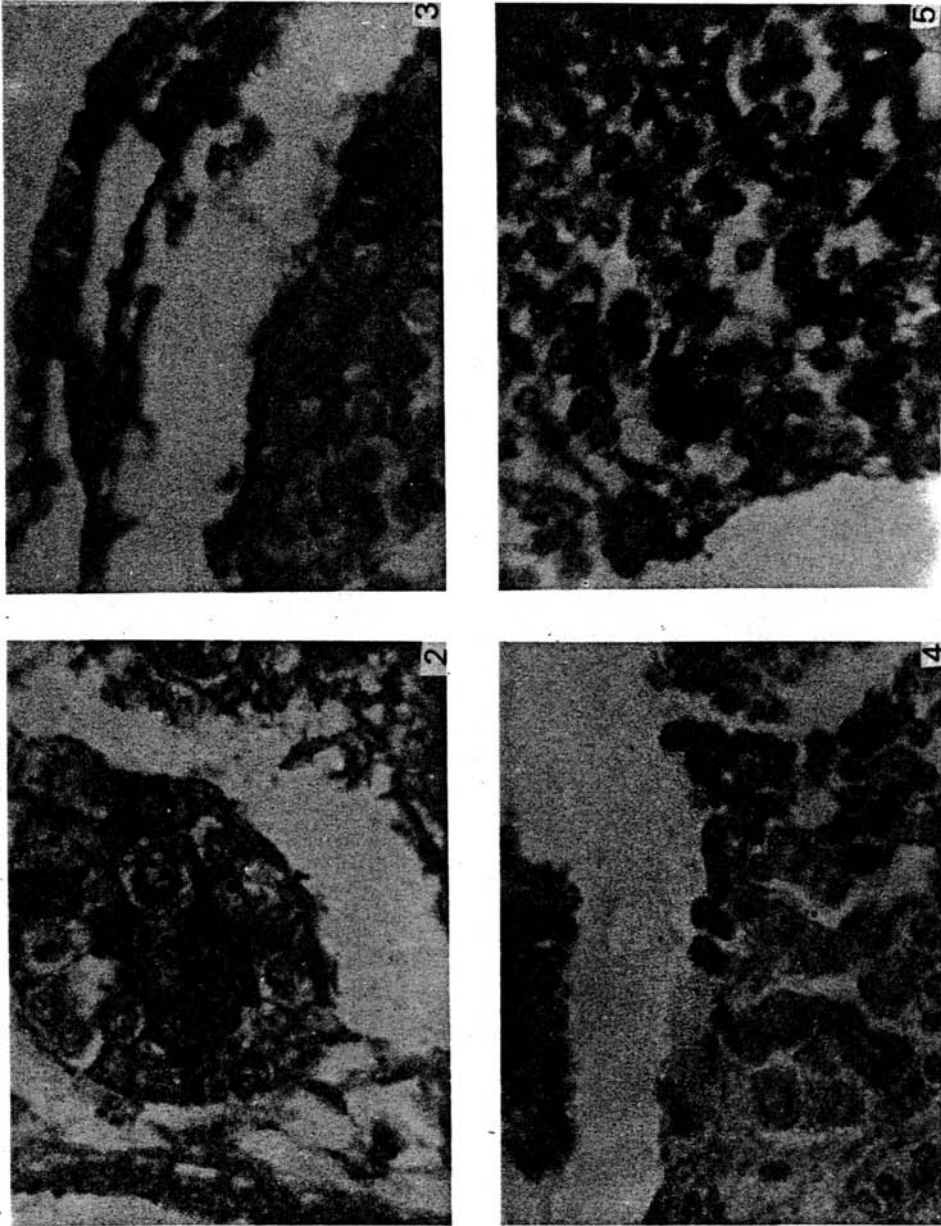
**Figure 1.** Camera lucida drawing showing the types of neurosecretory cells in eyestalk, thoracic ganglia and brain. (i) Eyestalk. Note the secretory activity of *C* and *D* type of neurosecretory cells at reproductively quiescent state. (ii) Thoracic ganglia. Note the active phase of *B* type of neurosecretory cell at reproductively active state. (iii) Brain. Note the active phase of *D* type of neurosecretory cell at reproductively active state.

The other types of NSC namely *A* and *B* of the eyestalk fail to show any substantial reaction to any of the histochemical tests in different phases of reproductive periods (figures 1A,B, 2 and 3).

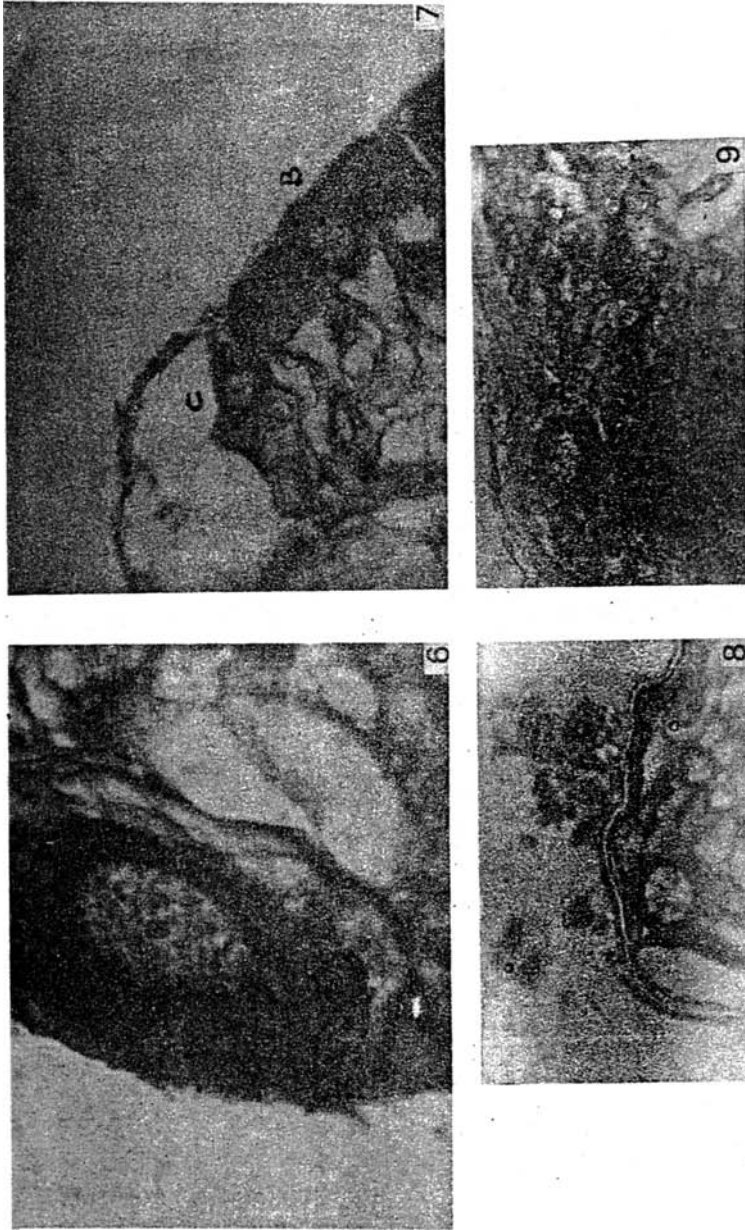
The *B* type of NSC of the thoracic ganglia also shows differences in its histochemical reaction in different phases of reproductive cycles (figures 1B and 7). This type of NSC is positive to basic and acidic proteins. Besides, the granules are positive to sulphhydryl ( $-SH$ ) and disulphide ( $-S-S$ ) reactive groups indicating the presence of sulphur containing amino acids. The other *A* and *C* NSC fail to show any appreciable histochemical reactions (figures 1A,C, 6 and 7).

The occurrence of RNA is also detected with methyl green pyronin test. The positive reaction for acid haematin, luxol fast blue and also for Sudan black may reveal the presence of phospholipid in the secretory granules of this type of NSC in the thoracic ganglia. The carbohydrate, however, is not detected. Evidently the secretory substance emanating from the NSC of thoracic ganglia is a lipoprotein.

Among the three types of NSC that are found in the brain, only the *C* type shows mild positive histochemical reaction to acidic and general proteins (figure 1C).



**Figures 2-5.** ( $\times 1125$ ). 2,3. Neurosecretory cells at reproductively active state (stained in aqueous bromophenol blue). 2. *A* type 3. *B* type. 4,5. Neurosecretory cells at reproductively quiescent state. 4. *C* type (stained in chrome-alum-haematoxylin phlorine). 5. *D* type (stained in aqueous bromophenol blue).



**Figures 6-9.** (6,8,9 X250; 7 X1125). 6,7. Neurosecretory cells of thoracic ganglia (stained in aqueous bromophenol blue). 6. A type at reproductively quiescent state. 7. B and C types. 8,9. Neurosecretory cell of brain at reproductively quiescent state (stained in Etlrich's haematoxylin). 8. A type. 9. B type.



The granules also stain for lipid as indicated by the Sudan Black dye. The PAS, toluidine blue, and Best's Carmine are also positive to these granules indicating that the secretion is a glycolipoprotein, however the concentration of the staining reaction is mild. On the other hand, the *A* and *B* NSC fail to show any significant histochemical reaction as that of *C* NSC (figures 1A,B, 8 and 9).

#### 4. Discussion

The present results show the diverse nature of secretory substances elaborated by the different NSC located in different centres namely eyestalk, thoracic ganglia and brain.

The *C* and *D* types of NSC of the eyestalk show at reproductively quiescent state more protein moiety associated with tyrosine and sulphur containing amino acids. The presence of RNA is also detected in the cytoplasm of these cells. On the other hand carbohydrate and lipid substances are absent, suggesting that these cells secrete substances which are highly proteinaceous. This finding is in agreement with the view of Kleinholtz (1966) and Adiyodi and Adiyodi (1968) that gonad inhibiting hormone in the crustacean may be in the form of a peptide or polypeptide.

The *B* type NSC of thoracic ganglia at reproductively active state also shows much secretory activity and the elaborated substance in lipoprotein associated with protein reactive groups namely S-S and -SH. On the other hand the *C* type of NSC of the brain at reproductively active state shows the presence of protein, lipid and carbohydrate substances, indicating glycolipoprotein complex.

At this point it is of interest to note in *Paratelphusa hydrodromous* (Parameswaran 1956), the presence of phospholipid in the neurosecretory cell of the thoracic ganglia. Similarly the positivity to Sudan black and also for protein-bound S-S and -SH groups have been reported in the cephalic nervous system of *Chirocephalus diaphanus* (Lake 1970) and *Rivulogammarus syriacus* (Dabbagh and Baid 1976). The presence of PAS-positive substances in the neurosecretory material have been reported in isopod (Gabe 1952a,b). Miyawaki (1958) has also made similar observation in an isopoda *Idotea japonica*. Similarly the amylase-digestible PAS positive neurosecretory granules have been recorded in one of the two types of neurosecretory cells in barnacles (Barnes and Gonor 1958).

In conclusion it may be established that the neurosecretory cells of eyestalk, thoracic ganglia and brain of *S. holoschista* contain histochemically different substances. The material of *C* and *D* cells of eyestalk is rich in protein substances. The neurosecretory material of *B* type of cells is lipoprotein and the *C* type of NSC of brain shows positivity to glycolipoprotein substances.

#### Acknowledgements

The authors thank Profs. K Ramalingam and S Augustine Chellappa for encouragement and facilities and also Dr S Jayadev Babu for critically going through the manuscript. MD gratefully acknowledges financial assistance from UGC. Thanks are also due to Mrs V Jayalakshmi Deecaraman.

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