# HISTOARCHITECTURE, SEASONAL VARIATION AND REPRODUCTIVE FUNCTION OF THE NEUROENDOCRINE ORGAN, BRAIN OF FRESHWATER PRAWN, *MACROBRACHIUM GANGETICUM*

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## ABSTRACT

**Histoarchitecture, seasonal variation and reproductive function of the neuroendocrine structure, brain of freshwater prawn *Macrobrachium gangeticum* were studied. Three types of NSCs- 'B', 'C' and 'D' were found to be concentrated in four groups in brain. These cells showed larger diameters and higher activity during breeding season. In case of females, the 'C' cells were more active during vitellogenic period. Brain extracts were found to induce gonadal maturation of both males and females.**

**Key words: *Macrobrachium gangeticum*, Brain, Neurosecreory cells, Gonads.**

## INTRODUCTION

*Macrobrachium gangeticum*, a decapod crustacean, is one of the three major freshwater prawns of India. It inhabits the middle and lower stretches of Ganga river. The biology and larval development of this species have earlier been reported from this laboratory (Singh *et* al., 1989a, 1989b, 1991a, 1991b; Roy and Singh 1997; Singh and Roy 1994 and Roy *et* al., 2006). Neuroendocrine system has an important bearing on the reproductive physiology of crustaceans. Crustacean hormones are mostly neurosecretory in origin. Neuroendocrine centres of crustaceans are composed of neurosecretory cells (NSCs) which release neurosecretory hormones (Carlisle and Knowles, 1959). Enami (1951) first time recognized the presence of the neurosecretory cells in the central nervous system of the crab, *Seasarma* sps.

The neuroendocrine system of crustaceans mainly consists of the x-organ-sinus gland complex, brain and thoracic ganglion. The NSCs of brain send their axons to the sinus gland, a neurohemal organ, located in the eye-stalk. The present communication deals with the histoarchitecture, seasonal variations and functions of the neuroendocrine structures of brain in relation to gonadal maturity.

## MATERIAL AND METHODS

### Histoarchitecture

Specimens of *Macrobrachium gangeticum* were collected from Ganga river. After acclimatization, the animals were anaesthetized with MS 222. Brain was dissected out from the specimen and fixed in aqueous Bouin's fluid for 24 hours. After the post-fixation treatment, the tissues were embedded in paraffin and sections were cut at

5-6 µm thickness. After deparaffinization, sections were stained with following stains.

1. Mallory's triple stain (Mallory, 1944)
2. Aldehyde fuchsin (AF) (Ewen, 1962)
3. Haematoxylin and eosin

The criteria used for indentification of neurosecretory cells (NSCs) in this study were that reported by Cooke and Sullivan (1982), The measurement of the cells and nucleus was made by oculometer using an oculomicrometer.

### Seasonal variation

Seasonal variation in the structure of brain was studied by its histological observation in different months as described above. Changes in neurosecretary cells of brain in relation to ovarian maturation of female prawns were also studied. For this purpose, the females were segregated according to their ovarian condition viz. pre-vitellogenic, vitellogenic-I, vitellogenic II and spent. Histological observations of brain of five prawns in each group were made. The neurosecretory profile of the previtellogenic individuals was taken as reference to compare the changes occuring in other groups.

### Effect of brain extract on ovarian maturation :

Twenty specimens of female *M. gangeticum* with immature ovaries and approximately similar size were equally divided into following four groups:

Group I - Control.

Group II - Normal animals, injected with brain extract.

Group III - Eye-stalk ablated, non injected animals.

Group IV - Eye-stalk ablated animals, injected with brain extract.

Injections of brain extract (one brain/ prawn) were prepared by homogenizing one brain in 20 microlitres distilled water and 20 microlitres of it were given on 3rd, 6th and 9th days, in abdominal musculature of prawn with the help of hypodermic microsyringe. Animals were sacrificed on the day 15 and examined.

### Effect of brain extract on testicular maturation :

For this study, 20 males with immature testes and approximately similar size were divided into following four groups.

Group I - Control.

Group II - Normal animals, injected with brain extract.

Group III - Eye-stalk ablated, non injected animals.

Group IV - Eye-stalk ablated animals, injected with brain extract.

## OBSERVATIONS

### Histoarchitecture

Brain was found to possess NSCs concentrated in four groups. The arrangement of NSCs in brain is shown diagramatically in fig 1. These groups of NSCs were named as antero-lateral groups (paired), middle group and posterior group (fig 2). The NSCs of brain were classified as 'B', 'C' and 'D' types based on size (figs 3, 4). 'A' type, the largest NSCs were found only in thoracic ganglion of this species. The three types of NSCs of brain ('B', 'C' and 'D') were measured as 24-20.1µm, 20.0-12.1µm and 12.0 - 6.0 µm respectively. All of them were round to oval. Cytoplasm of 'B' and 'C' cells was vacuolated. All these cells stained reddish with Mallory's triple and voilet with AF.

**Seasonal variation**

All the NSCs of brain ('B', 'C' and 'D') underwent some seasonal changes (Table 1). While the cellular diameters of 'B' and 'C' cells were maximum in July and August, the peak breeding period of the animal, the 'D' type of cells measured maximum in June. The diameter was found to be reduced to some extent in March, April and September also. Accordingly, the nuclear diameters of all the three type of cells were also quite reduced in March and April. The nuclear diameter of 'B' and 'C' cells, however, showed much improvement during monsoon season. However, the nuclear diameters of 'D' cells remained more or less constant throughout the year. The neurosecretory material (NSM) activity of these cells was also higher from June to August, the breeding period of the animal.

**Changes in neurosecretory cells of brain in relation to ovarian maturation**

Noticeable variation in 'B', 'C' and 'D' cells of brain of females was observed in relation to their gonadal maturation. (Table 2). Their cellular diameters were minimum in pre-vitellogenic phase but improved in vitellogenic phase. The nuclear diameters and neurosecretory materials of 'C' cells also showed increased values during vitellogenic and spent phases.

**Effects of brain extract on ovarian maturation**

The animals of group II (normal animals, injected with brain extract) showed higher values of mean GSI and oocyte diameter as compared to those of the control ones (fig 5 & 6). The Colour of the ovary got also changed from greenish white to green. Similarly, when the eye-stalk ablated animals were injected with brain extract, the mean GSI values showed significant improvement from the corresponding values of group I (control) and group III ( eye stalk ablated, non injected) animals. The oocyte diameter though significantly increased from that of group I animals, it remained a little below that of group III animals (Table 3, figs 7, 8).

**Effects of brain extract on testicular maturation**

Animals of both groups II (normal animals injected with brain extract) and IV (eye-stalk ablated animals injected with brain extract) showed significant advancement of their testicular maturity when compared with group I and group III animals respectively, as evidenced by their elongated testes as well as increased tubular diameters. These observations were further confirmed by histological examination. (Table 4, fig. 9,10,11,12).

**DISCUSSION**

The brain of crustaceans, also called supraesophageal ganglionic mass, is considered by many workers as a neuroendocrine organ (Welsh, 1991; Cooke and Sullivan, 1982; Biswas, 1991; Upadhyaya, 2000; Kumar and Pandey, 2003; Jadhav *et* al., 2001). In the present study, three types of NSCs- B, C and D, were found in brain distributed in four groups- two antero-lateral groups, a middle group and a posterior group. These findings get support from some other reports, *viz.* Parmeshwaran (1956) on *Paratelphusa hydrodromous;* Nagabhushanam and Ranga Rao (1966) on *Scylla serrata;* Nagabhushanam and Diwan (1975) on *Barytelhpusa cunicularis,* Victor and Sarojini (1985) on *Caridina rajdhari* and Jadhav *et al.,* (2001) on crab, *Uca lactea annulipes.* However, there is much variation in

Table-1: Seasonal changes in neurosecretory cells of brain of *M. gangeticum*

| Month | Cell Diameter (µm) mean ± SD | | | Nuclear Diameter (µm) | | | NSM Intensity | | |
|---|---|---|---|---|---|---|---|---|---|
| | B | C | D | B | C | D | B | C | D |
| March | 20.12±0.42 | 13.91±0.49 | 8.95±0.41 | 8.98±0.31 | 6.21±0.51 | 6.25±0.52 | + | - | + |
| April | 21.51±0.21 | 13.62±0.51 | 8.99±0.22 | 9.72±0.41 | 5.91±0.62 | 6.23±0.63 | + | - | + |
| May | 22.63±0.15 | 14.25±0.52 | 11.01±0.50 | 9.85±0.28 | 7.93±0.71 | 6.41±0.73 | + | + | ++ |
| June | 23.42±0.62 | 15.21±0.56 | 11.53±0.52 | 11.91±0.31 | 8.01±0.51 | 6.45±0.53 | ++ | ++ | ++ |
| July | 23.64±0.32 | 17.52±0.29 | 10.31±0.27 | 12.85±0.41 | 8.25±0.33 | 6.42±0.34 | ++ | ++ | + |
| August | 23.93±0.51 | 16.21±0.24 | 7.42±0.23 | 13.01±0.62 | 7.95±0.32 | 6.02±0.33 | ++ | + | - |
| Sept | 22.41±0.53 | 14.52±0.21 | 9.54±0.25 | 9.79±0.51 | 6.41±0.25 | 6.21±0.27 | + | + | + |

++ High
+ Normal
- Reduced
NSM Neurosecretory materials

Table-2: Changes in neurosecretory cells of brain of *M. gangeticum* in relation to ovarian maturation

| NSC type | Ovarian Maturation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-Vitellogenic | | | Vitellogenic-I | | | Vitellogenic-II | | | Spent | | |
| | B | C | D | B | C | D | B | C | D | B | C | D |
| Cell Diameter (µm) | 20.15±0.40 | 12.03±0.28 | 11.01±0.50 | 20.52±0.38 | 13.56±0.45 | 11.25±0.35 | 21.51±0.45 | 13.45±0.32 | 11.98±0.34 | 20.82±0.33 | 12.31±0.45 | 11.75±0.33 |
| Nuclear Diameter (µm) | 9.61±0.023 | 4.12±0.45 | 8.13±0.32 | 9.43±0.43 | 7.54±0.41 | 7.82±0.38 | 9.31±0.25 | 7.12±0.48 | 7.64±0.54 | 9.01±0.23 | 623±0.35 | 7.42±0.45 |
| NSM | + | - | + | - | ++ | - | - | ++ | + | - | + | + |

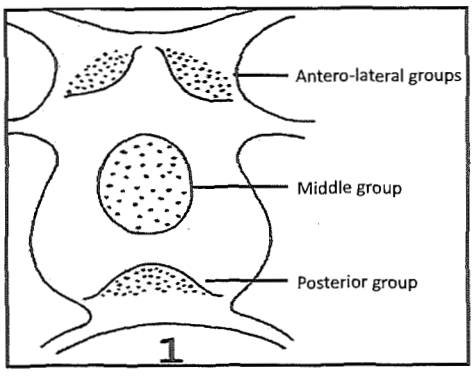
++ Moderate
+ Normal
- Reduced
NSM Neurosecretory materials
NSC Neurosecretory cells

**Table-3: Effect of brain extract on ovarian maturation of *M. gangeticum***

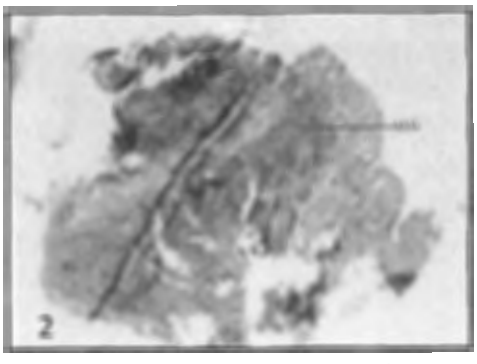
| Animals groups (size range 13-15 cm) | Description | GSI mean ± SD | Oocyte diameter mean ± SD | Colour of the ovary |
|---|---|---|---|---|
| Group-I | Control | 2.87±0.24 | 24.91±2.41 | Pale Green |
| Group-II | Normal animals injected with Brain extract | 3.12±0.41 | 29.81±3.45 | Green |
| Group-III | Eye-stalk ablated, non injected | 3.31±0.31 | 35.54±0.23 | Green |
| Group-IV | Eye-stalk ablated animals injected with brain extract | 3.91±0.35 | 34.52±1.56 | Pale Green |

**Table 4: Effect of brain extract on testicular maturation of *M. gangeticum***

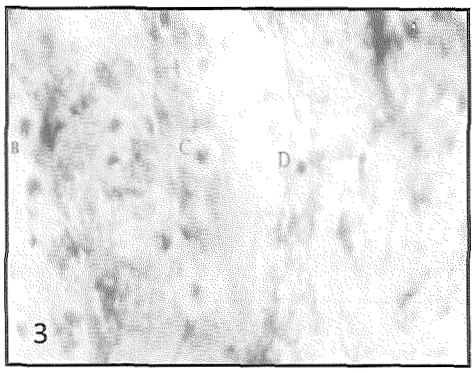
| Animal groups (size range 13-19 cm) | Description | Length of testes (cm) | Diameter of seminiferous tubule (µm) | Colour of the testes | Dominant cell type |
|---|---|---|---|---|---|
| Group-I | Control | 2.41 ± 0.54 | 30.54±3.52 | White with pink spots | spermatocytes few spermatogonia |
| Group-II | Normal animals injeccted with brain extract | 3.12±0.45 | 41.45±3.21 | White with pink sports | spermatocytes few spermatogonia |
| Group-III | Eye-stalk ablated non-injected animals | 3.21±0.59 | 44.31±4.55 | White with pink sports | spermatids, spermatozoa |
| Group-IV | Eye-stalk ablated animals injectdbrain extract | 3.52±0.61 | 48.52±4.31 | White with brownish spots | spermatozoa |



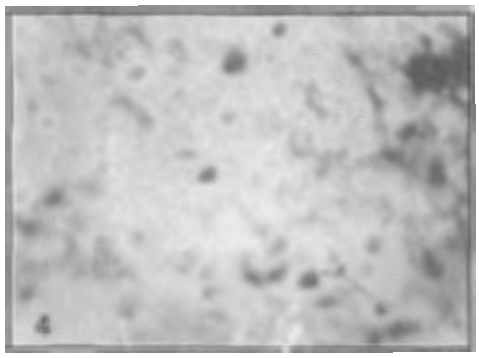

Histoarchitecture of brain.



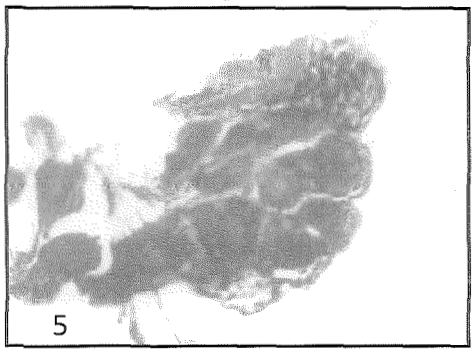

Photomicrograph of L.S. of brain showing different part (ALG- Antero-lateral group, MG - Median group and PG- posterior group) Mallory's triple x150.



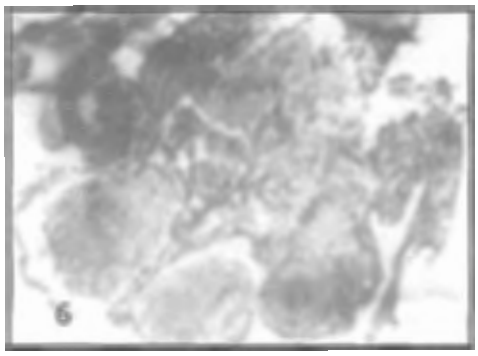

Photomicrograph of T.S. of brain showing 'B', 'C' and 'D' types of neurosecretory cells Mallory's triple x600.



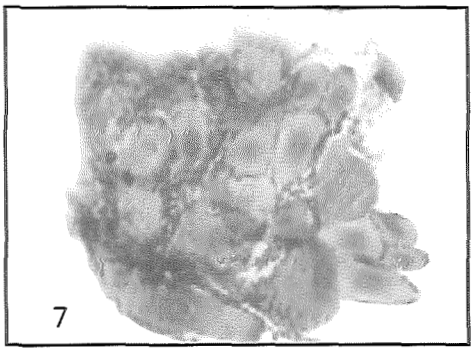

Photomicrograph of T.S. of brain showing 'B', 'C' and 'D' types of neurosecretory cells AF x600.



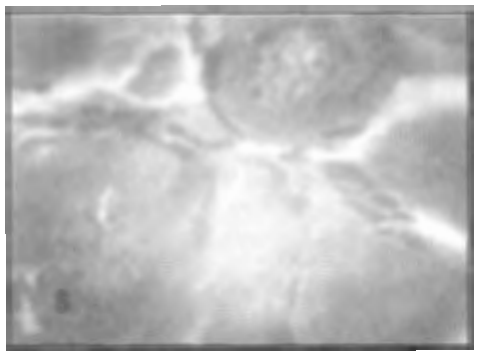

Photomicrograph of T.S. Ovary (control), Haematoxylin Eosin x150.



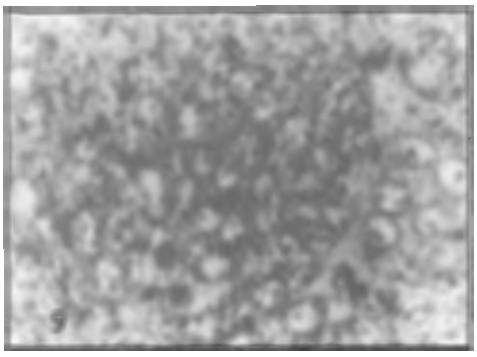

Photomicrograph of T.S. of ovary of normal animal injected with brain extract Haematoxylin -Eosin x150.



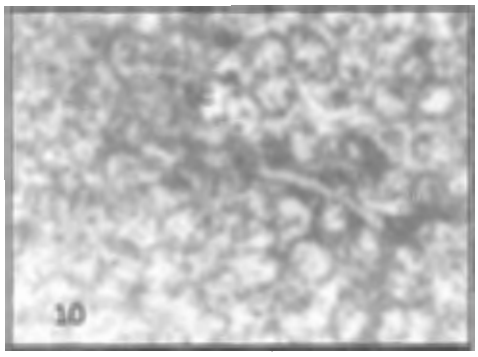

Photomicrograph of T.S. of ovary of eye stalk ablated animal Haematoxylin -Eosin x150.



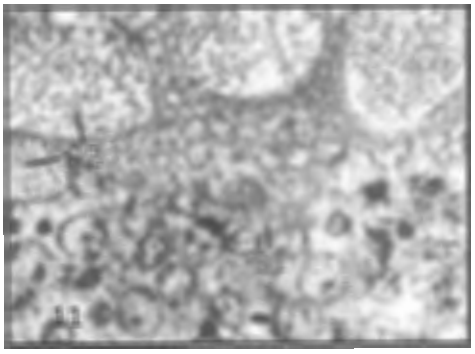

Photomicrograph of T.S. of ovary of eye-stalk ablated animal injected with brain extract Haematoxylin -Eosin x150.



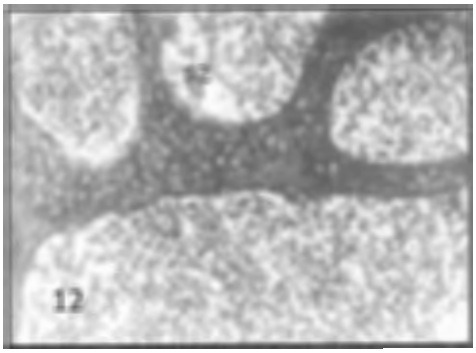

Photomicrograph of T.S. of testis (control), Haematoxylin -Eosin x1000.




Photomicrograph of T.S. of testis of normal animal injected with brain extract Haematoxylin -Eosin x1000.



Photomicrograph of T.S. of testis of eye-stalk ablated animal Haematoxylin -Eosin x600.




Photomicrograph of T.S. of testis of eye-stalk ablated animal injected with brain extract Haematoxylin -Eosin x600.
SG- Spermatogonia, SC-Spermatocytes, ST-Spermatids
SZ- Spermatozoa

the number of types of NSCs in the brain of other crustaceans reported by different workers. While Baid *et al.,* (1967) reported two types of NSCs in the brain of the crab *Potamon magnum magnum;* Joshi (1980), Gyananath (1982), Biswas (1991) and Upadhyaya (2000) have described four types of NSCs in the brain of *Palaemon stylifera, Macrobrachium lammerrei, Macrobrachium dayanum and Macrobrachium malcolmsonii,* respectively. On the other hand, Sambasiva Rao *et al.,* (1987) and Kumar and Pandey (2003) have reported upto five types of NSCs in the brain of *Metapenaeus affinis* and *Macrobrachium rosenbergii respectively.* Nagabhushanam *et al.,* (1986) have identified even eight types of NSCs in the brain of *Palaemon Stylifera.* The NSCs in the brain of *Macrobrachium gangeticum* were round to oval which is in agreement with the observation of Kumar and Pandey (2003) in *Macrobrachium rosenbergii.*

The NSCs of brain showed increased size and higher activity during breeding season (June to August) in comparison to non-breeding season. While 'D' cells measured maximum in June, 'B' and 'C' cells showed larger diameters in July and August. The cells were reduced in size in non-breeding season. In case of females in particular, the 'B', 'C' and 'D' types of cells of brain were smaller during previtellogenic phase but enlarged during vitellogenic phase. The nuclear diameter and NSM activity of 'C' cells showed a similar pattern. These observations suggest that these NSCs of brain have a positive role to play in gonadal maturation. This hypothesis gets support from various other workers. Nagabhushanam *et al.,* (1984) found type 'IV' NSCs of *Palaemon stylifera* and Biswas (1991) found type 'B' NSCs of *Macrobrachium dayanum* to be more active during the spawning phase. Upadhyaya (2000) reported 'C', 'D' and 'E' NSCs of *Macrobrachium malcolmsonii* to be larger and more active during the breeding phase. Gyananath and Sarojini (1985) indicated that type 'A' cells of brain produced ovary stimulating hormone. Sambasiva Rao *et* al.(1986)., and Sarojini *et* al., (1994) have proposed similar stimulatory role of brain NSCs in gonadal maturation. Jadhav *et* al., (2001) reported three types of NSCs ('A', 'B' and 'C') which showed increased activities during reproductive period.

Extract of brain, when injected to normal or destalked males or females, brought about precocious gonadal maturity as indicated by higher GSI values, larger oocyte diameters and greener ovaries of females and longer testes with larger seminiferous tubules and spermatozoa in males. However, the effects were more pronounced in destalked-

injected animals than those in normal ones. These observations get support from the findings of many other workers. Gyananath and Sarojini (1987) reported that brain extracts bring about testicular maturation both in normal and rather more prominently in eye-stalkless *M. lammarrei.* In *M. kistensis* also brain extract led to enhanced testicular growth in both normal and destalked males (Mirajkar, 1980 and Sarojini *et* al., 1982). The eye-stalkless animals respond more to the brain extracts, because, on one hand, they get rid of the inhibitory effects of the eye-stalks, while on the other hand, get a double dose of factors released from brain. In *M. dayanum* (Biswas, 1991) and *M. malcolmsonii* (Upadhayaya 2000) also, injections of brain extract to both normal and eye-stalk ablated animals led to enhanced gonadal maturity. All these studies grossly support the present finding in *M. gangeticum.*Thus, the present study suggests that the brain of *M. gangeticum* releases some factors which have positive effects on gonadal maturation in both males and females.

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