Regular Paper

# CYTOLOGICAL STUDY OF NEUROSECRETION IN RELATION TO REPRODUCTION IN THE MALE SCORPION, HETEROMETRUS FULVIPES (KOCH)

Sobha K[1]., Veeraiah K.[2] and Gopala Rao N.[2]

[1]Department of Biotechnology, Bapatla Engineering College Bapatla, Andhra Pradesh India Pin: 522 101 [2]Department of Zoology, Acharya Nagarjuna University Nagarjunanagar, Andhra Pradesh India. Pin: 522 510.

## ABSTRACT

The role of neurosecretion in the annual reproductive cycle of male scorpion was studied by taking monthly samples around 15th of every month and the role in courtship-mating was studied from the samples taken during courtship, immediately after mating, at 6 hours, 12 hours, 24 hours after mating and 4th and 7th days of post-mating period. From the observations, it is inferred that the neurosecretory cells of groups 2, B and 3 to 9 take part in triggering the testicular maturation. Sets I, III and group C cells possibly play a role in the gradual maturation process of the testes while set II cells indicate their involvement in the mature stage. The increase in the synthetic activity of protocerebral neurosecretory cells (NS cells) manifested by the histological appearance of phloxinophilic material inside protocerebral vacuolar spaces in the spent and slow-maturing stages is considered to suggest the involvement of these protocerebral NS cells in testicular maturation. The release of phloxinophilic secretary globules from group 3 cells and phloxinophilic accumulations near groups 5 and 6 throughout the annual cycle was inferred to signify their possible involvement in all the phases of testicular activity and/or activities supporting extra-testicular events contributing to the overall reproductive processes. The results indicated the possible involvement of set I, groups 4 and 6 in the courtship of the male scorpion. Sets II, III, group 2 of brain and all NS cell groups of sub-oesophageal ganglion except groups 4 and 6 were suggested to be possibly involved in both courtship and mating.

**Key words**: Neurosecretion, Protocerebrum, Sub-oesophageal ganglion, Reproduction, Phloxi nophilic secretion, courtship-mating, annual cycle.

## INTRODUCTION

Neuroendocrine control of female reproduction, among arthropods, is investigated to a considerable extent in crustaceans and insects. The neuroendocrine control of reproduction in male arthropods is meager and is reported in very few studies [10, 11, 17]. Among arachnids, particularly scorpions, there is limited study on the neuroendocrine control of male reproduction. A study on the seasonality of male reproduction in the scorpion, Heterometrus ful vipes revealed the occurrence of a definite breeding season in the months of July and August [24] and basing on the testicular activity, four stages were identified in the annual reproductive cycle viz., slow-maturing, fast-maturing, mature and spent stages [9]. As the occurrence of a definite breeding season and intricate courtship-mating process involve an interplay of metabolic, biochemical, physiological and more particularly endocrine factors, an attempt is made to investigate the role of neurosecretory cells (NSCs) in the annual testicular cycle and courtship-mating.

## MATERIAL AND METHODS

The Cephalothoracic nerve mass (CTNM) of the scorpion was dissected and removed to Bouin's fluid prepared with scorpion ringer [18] for 24 hours. The material was then processed and embedded by the paraffin double embedding technique [8]. Serial sections cut at 5m and 8m thickness in the frontal, sagittal and trans-

Correspondence to: Department of Biotechnology
Bapatla Engineering College
Bapatla, Andhra Pradesh
India Pin: 522 101 Email: sobha_kota@yahoo.co.in
Phone 0863-2355661(R)

verse planes were stained selectively with Gomori's chromium-haematoxylin-phloxin (CHP) [6], Paraldehyde fuchsin (PF) [2] and Mallory's heidenhain triple stain (Rapid one step method) [3]. Of the three staining procedures, CHP yielded the best results and hence was used throughout the study of neurosecretion in relation to male reproduction.

The role of neurosecretion in the annual reproductive cycle was studied by taking samples around 15th of every month. Care was taken to sacrifice the animals only between 8AM and 12 Noon in order to avoid any variation that might occur due to the strong circadian rhythms in the scorpion, Heterometrus fulvipes [16-25]. For the study on courtship-mating, samples were taken at different stages viz., during courtship, immediately after mating, 6 hrs, 12hrs, 24 hrs after mating and 4th and 7th day of post-mating phase.

Cytometric observations from randomly chosen sections of the CTNM at 450 magnification and study of histological aspects such as intensity of cytoplasmic staining, vacuolation in the cytoplasm, storage, transportation and accumulation of the secretory substance etc. were made. Using average cellular and nuclear diameters, Student's 'T' test was conducted at 5% level of significance taking the immediately preceding condition as reference to find out if any significant changes occur in any group of cells at different stages of experimentation in relation to male reproduction.

## RESULTS

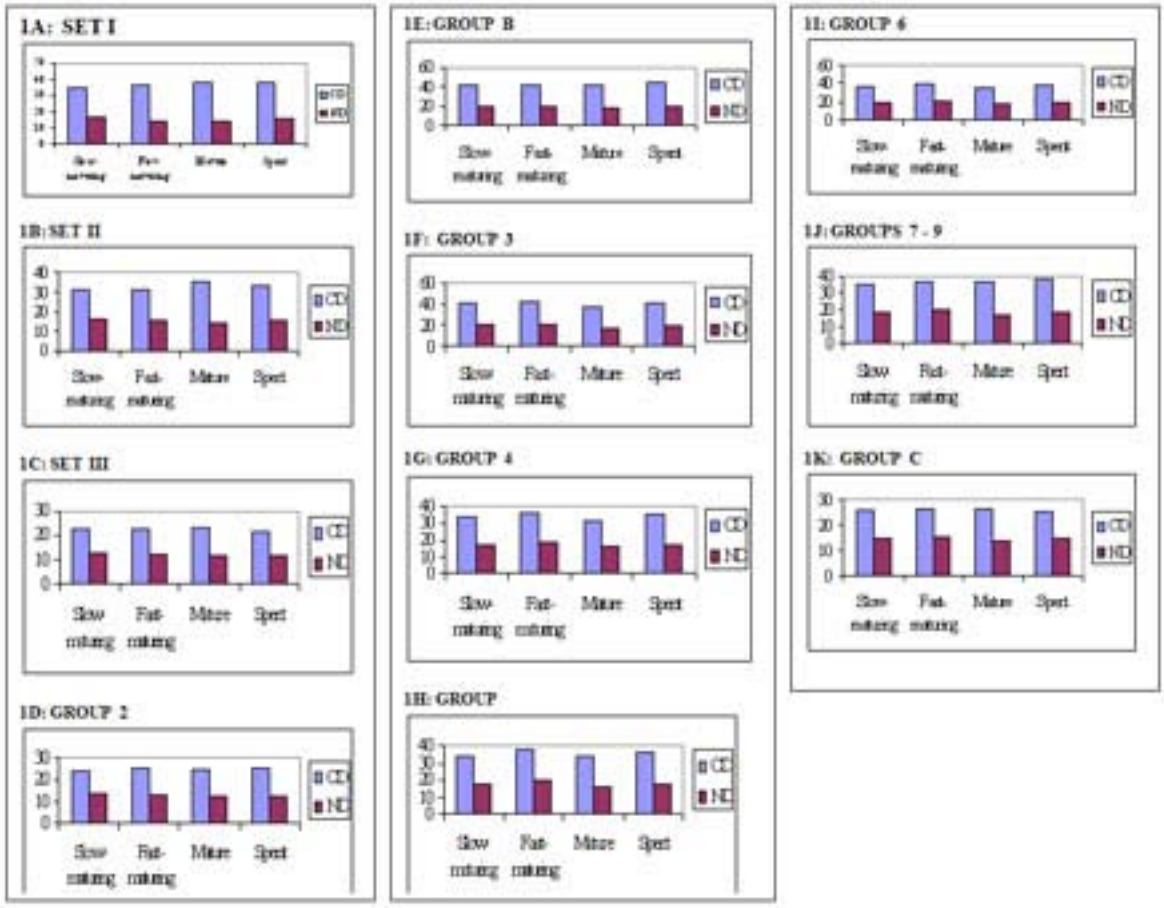
As a pre-requisite, mapping the distribution of NS cells in the CTNM was done and the different groups were named as reported by Habibullah [7]. The neurosecretory (NS) cells occurring in supra-oesophageal ganglion are divided into group1 and group 2. Group 1, further divided into 3 sets designated as sets I, II and III, is located in the median dorsal part of the protocerebrum. The number of cells in each set increased from I to III while their size decreased. Thus the cells of Set I are largest in size and smallest in number of cells occurring in the protocerebrum. Group 2 cells occur in the region of the frontal ganglion in 3 clusters, one median and two lateral. The NS cells in the sub-oesophageal ganglion (SEG) are classified into group B, groups 3 to 9 and group C. Group B cells are the largest neurosecretory cells found in the scorpion, Heterometrus fulvipes and are located at the base of the pedipalpal nerves. The NS cells occurring in the first 5 neuromeres of the SEG are located at considerable distance from one another as these neuromeres are provided with appendages while those occurring in the last four neuromeres belonging to the mesosoma become concentrated. The NS cells in each neuromere are arranged in 2 clusters, one lateral and the other median. Group C is a small cluster of cells with slightly different histological characteristics but located nearer to the cluster of cells present in the hind region of SEG. With Mallory's triple stain, these cells stain slightly purple and the nucleolus stains pink with acid fuchsin.

### Neurosecretory cells in annual reproductive cycle of the male scorpion

Cytometric observations of different groups of NS cells during annual reproductive cycle of the male scorpion are presented in Figures 1A to 1K.

**Figure 1A to 1K: Cytometric observations of different groups of NS cells during the annual cycle of reproduction in the male scorpion, *H. fulvipes***

g: Slow-maturing; p: Fast-maturing; q: Mature; r: Spent
Number in ( ) is 'n' value CD: Cellular diameter (µm), ND: Nuclear diameter (µm)




Slow-maturing stage (December to February)

Sets II and III of protocerebral NS cells and NS cell groups of SEG showed feeble to moderate staining with somatic granulation in some cells. Peripheral cytoplasmic vacuolation was found in many cells while storage of phloxinophilic secretory globules in the peripheral cytoplasmic vacuoles was noticed in group 3. Phloxinophilic accumulations near groups 5 and 6 were noticed throughout the annual reproductive cycle. Storage of phloxinophilic and in some, CH stained secretory material inside large protocerebral vacuolar spaces was noticed in this stage.

Fast-maturing stage (March to May)

All groups of NS cells in the CTNM showed moderate to intense staining with blue somatic granulation and peripheral cytoplasmic vacuolation. Storage of phloxinophilic globules in the peripheral cytoplasmic vacuoles of group 3 cells was observed.

Mature stage (June to August)

Protocerebral NS cells, particularly set II were found to show significant histological activity in the mature stage while all other groups of NS cells continued to show moderate staining. Storage of phloxinophilic secretory globules inside the peripheral cytoplasmic vacuoles of group 3 cells was observed (Fig. 4).

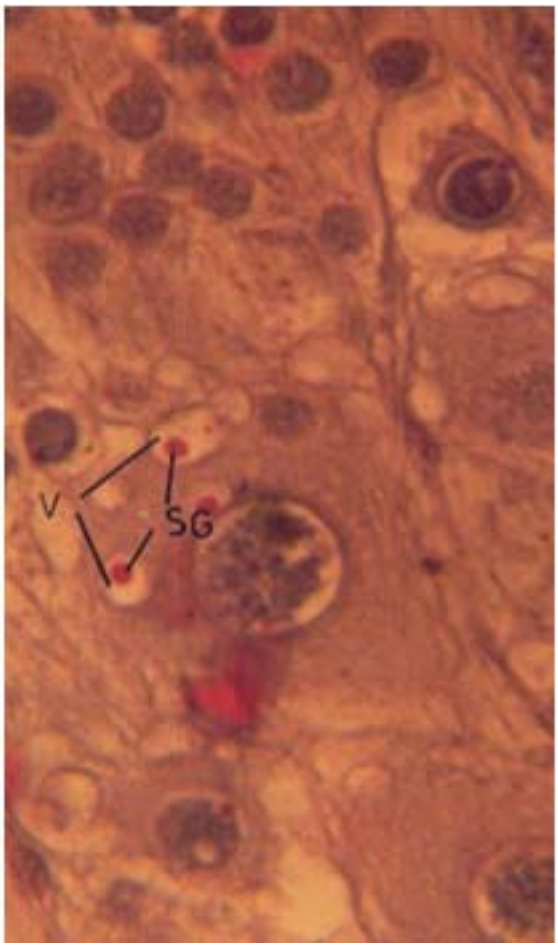



Fig. 4: Storage of phloxinophilic secretory globules inside peripheral cytoplasmic vacuoles of group 3 cells in June [Stain: CHP] X450

PA: Phloxinophilic accumulation; V: Vacuole; SG: Secretory globule Arrow indicates transportation

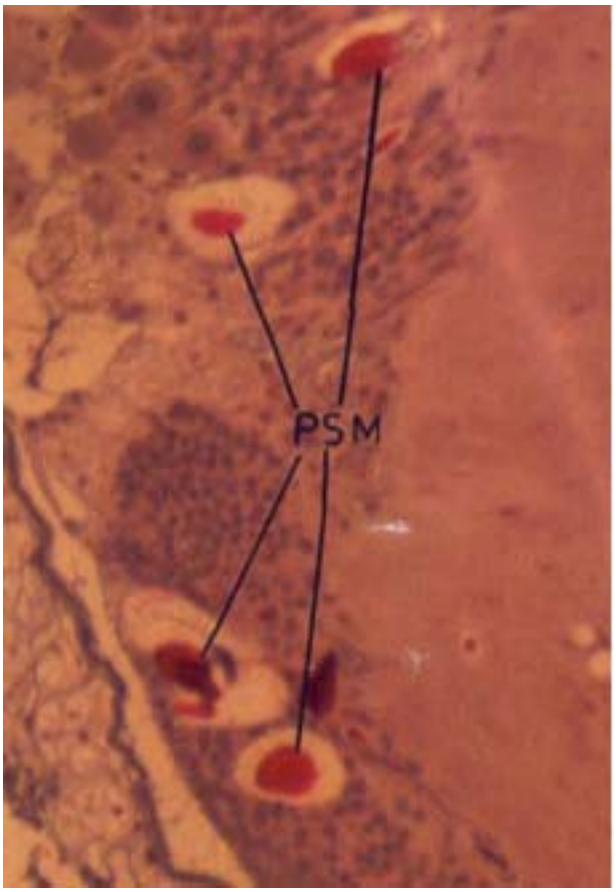



Fig. 5: Storage of phloxinophilic secretory material inside protocerebral vacuolar spaces in September [Stain: CHP] X 100
PSM: Phloxinophilic secretory material

Spent stage (September to November)

Moderately intense cytoplasmic granulation in all groups of NS cells except group 2 and group 4, storage of phloxinophilic globules in large vacuolar spaces of protocerebrum (Fig. 5)and amidst group B cells were noticed.

Transportation of phloxinophilic secretory globules from group 3 cells towards the dorso-meidan region of SEG (Fig. 3) and phloxinophilic accumulations near groups 5 and 6 were observed throughout the annual cycle (Fig. 1 and 2).

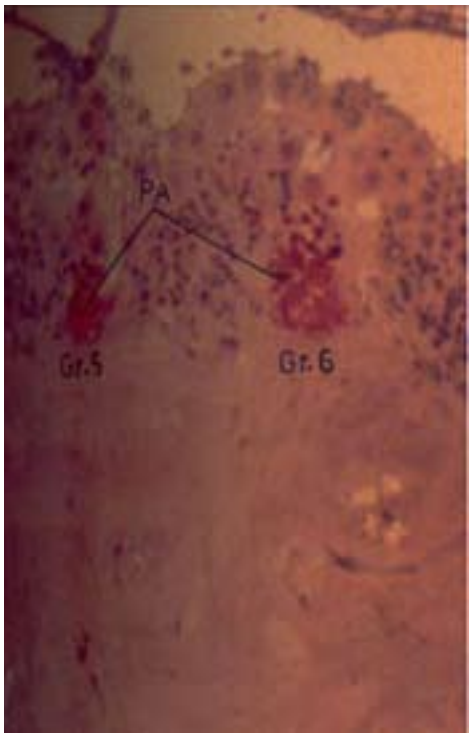



Fig. 1: Phloxinophilic accumulations of secretory product near groups 5 and 6 in July [Stain: CHP] X100

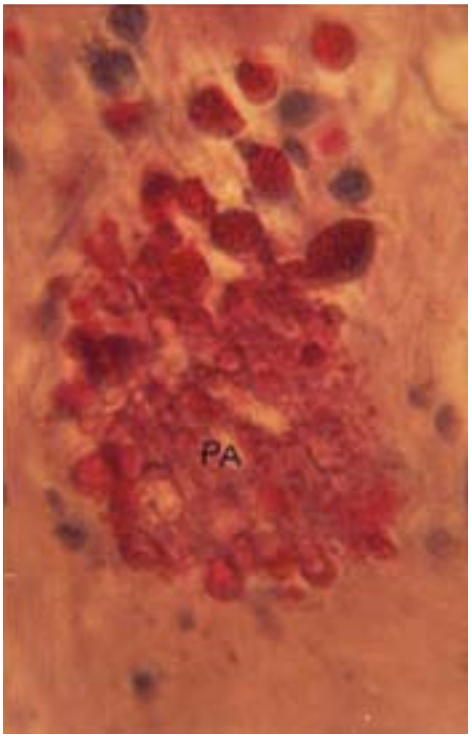



Fig. 2: Phloxinophilic accumulation near group 6 in July ( Same as in Pm. 18) [Stain: CHP] X450

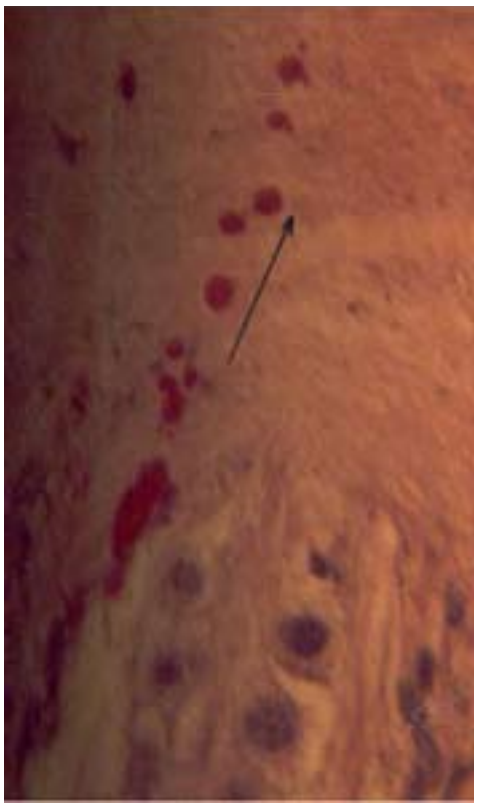


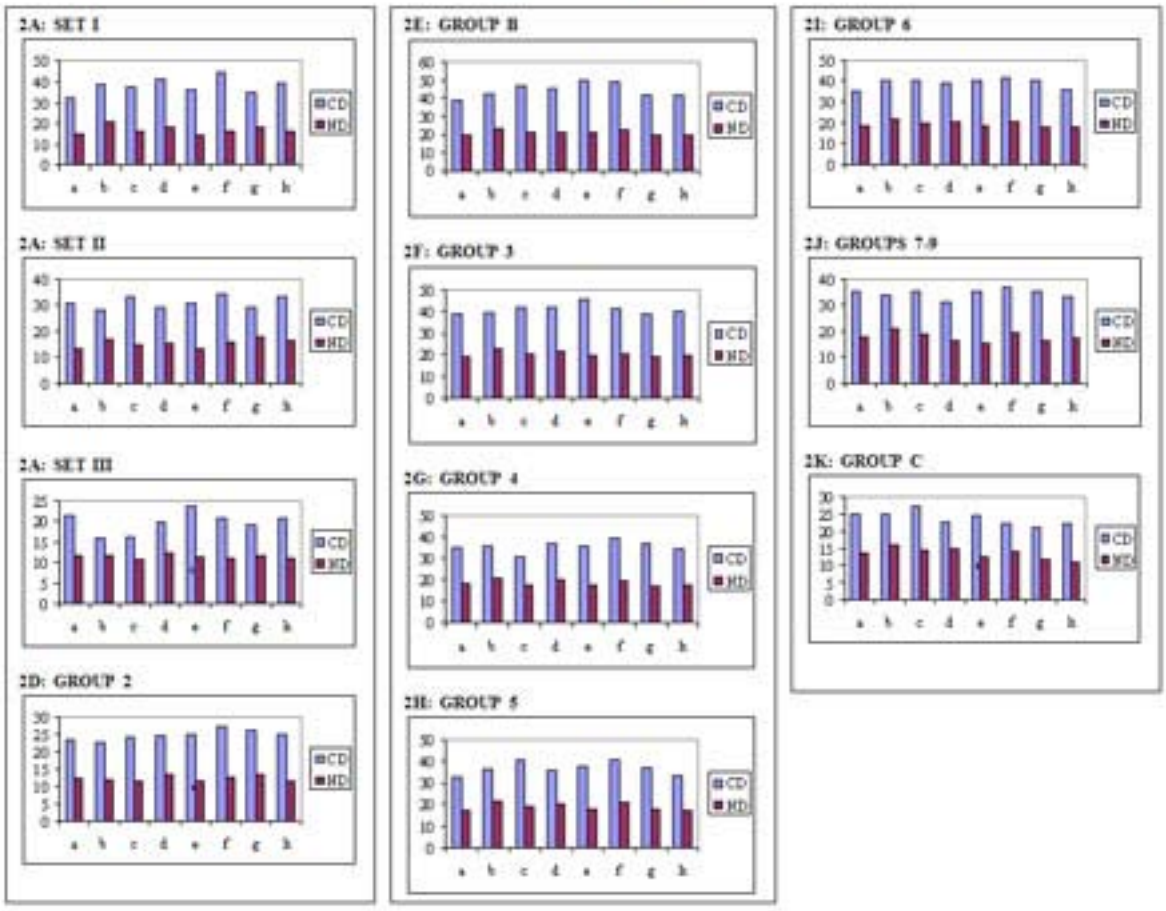
Fig. 3: Transportation of phloxinophilic secretory globules from group '3' cells towards dorso-median region of sub-oesophageal ganglion in July [Stain: CHP] X 450

Neurosecretory cells in Courtship and Mating

Cytometric observations of different groups of NS cells during courtship, mating and post-mating periods of study are presented in Figures 2A to 2K.

**Figure 2A to 2K: Cytometric observations of different groups of NS cells during the annual cycle of reproduction in the male scorpion, *H. fulvipes***

a: Control; b: During courtship; c: Immediately after mating (AM); d: 6 hours AM; e: 12 hours AM; f: 24 hours AM; g: 4th day AM; h: 7th day AM. Number in ( ) is 'n' value CD: Cellular diameter (μm); ND: Nuclear diameter (μm)




Courtship

At this stage, cytoplasm of NS cells of group 1 (Fig. 8) of protocerebrum and groups 4 and 6 of SEG showed moderately intense staining with a little somatic granulation. Peripheral cytoplasm of most cells were vacuolated.

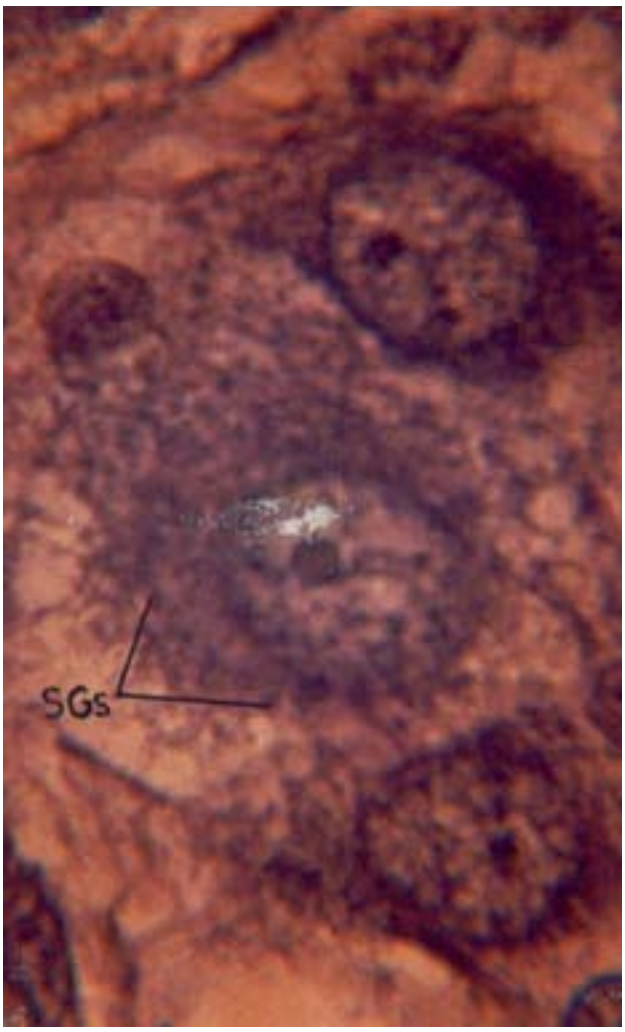



Fig. 8: Set III cells of protocerebrum showing intense somatic granulation during courtship [Stain: CHP] X1000
SGs: Somatic granules

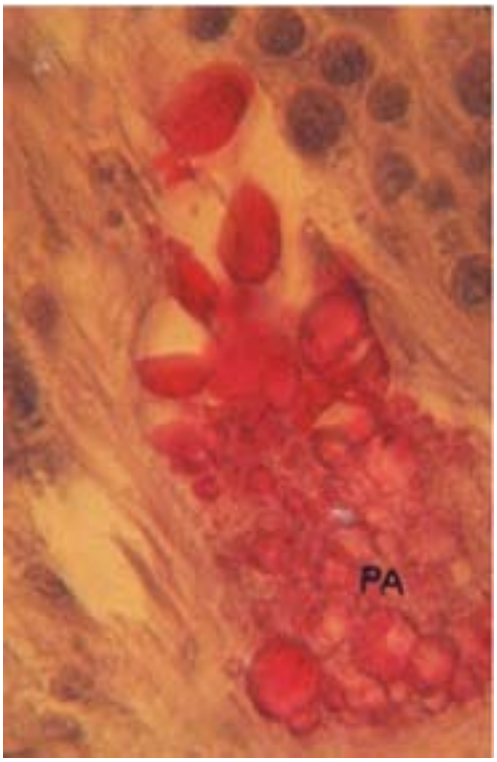



Fig. 6: Phloxinophilic accumulation of secretory product near group 6 cells immediately after mating [Stain: CHP] X450
PA: Phloxinophilic accumulation

After Mating

All groups of NS cells of SEG, except groups 4 and 6 , showed intense cytoplasmic staining with somatic granulation and peripheral cytoplasmic vacuolation in some cells. Protocerebral NS cells showed moderate or intense cytoplasmic staining with granulation during different stages of post-mating period (Fig. 9). Transportation of phloxinophilic globules from group 3 cells (Fig. 7) and phloxinophilic accumulations near groups 5 and 6 (Fig. 6) were observed throughout the post-mating period.

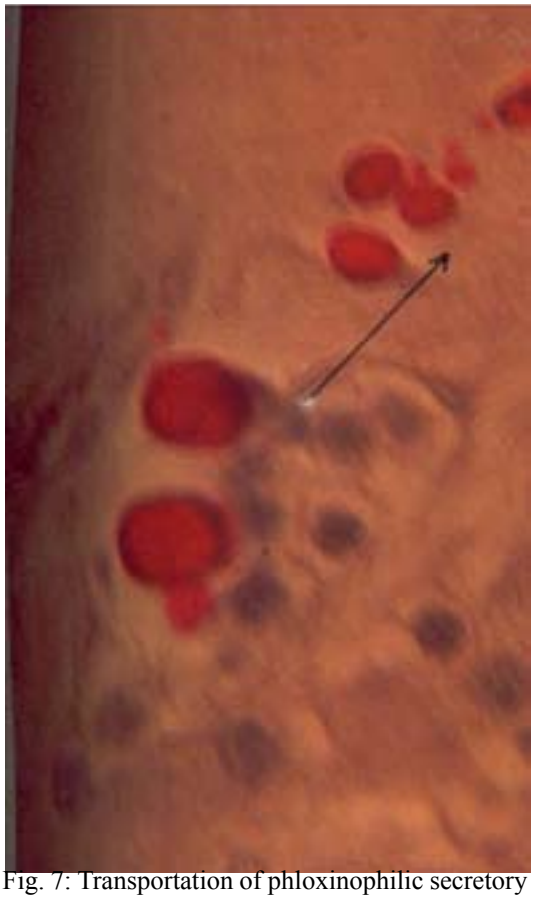


Fig. 7: Transportation of phloxinophilic secretory globules from group 3 cells towards the dorso-median region of sub-oesophageal ganglion on 4th day after mating [Stain: CHP]X450
Arrow indicates transportation

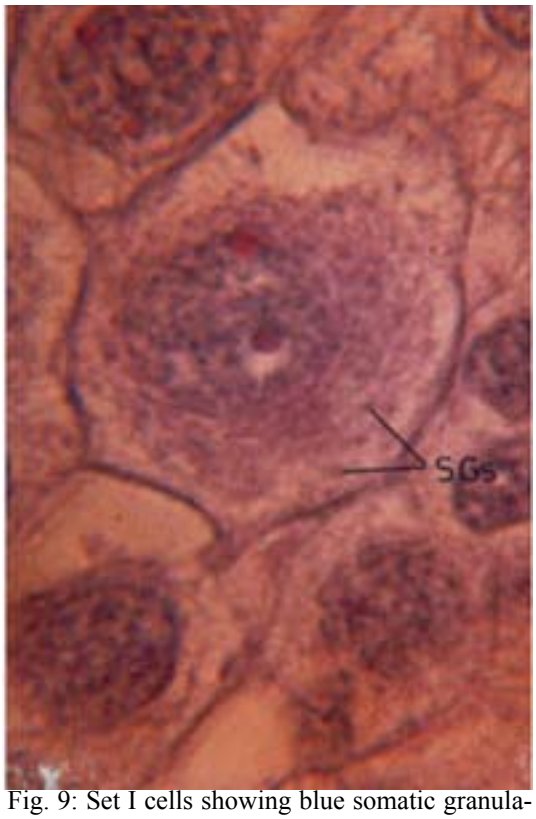



Fig. 9: Set I cells showing blue somatic granulation on 4th day after mating [Stain: CHP] X1000
SGs: Somatic granules

## DISCUSSION

Gabe [5] reported the occurrence of NS cells in two symmetrical, bilaterally placed groups on the dorsal side of protocerebrum and metamerically arranged NS cells in the SEG of all the 18 species of 5 orders of arachnids studied. Habibullah [7] described the occurrence of bilaterally placed group 1, group A and group 2 NS cells in the supra-oesophageal ganglion and groups B, 3 to 9 and C in different neuromeres of the SEG in the scorpion, Heterometrus swammerdami. In the European scorpion, Euscorpius carpathicus, three groups of NS cells in the protocerebrum, one group in the tritocerebrum and two groups near the outlet of the pedipalpal nerves were described [13].

The distribution of NS cells in H. fulvipes is in concurrence with the above mentioned investigations except for the non-occurrence of group A cells in the brain, which are found in H. swammerdami. In H. fulvipes, group B cells occurring at the base of the pedipalpal nerves are the largest of all the NS cells just as in H. swammerdami. The storage of secretory globules in the peripheral cytoplasmic vacuoles, perhaps, signifies the absence of a neurohaemal organ in the scorpion.

According to Lea and Thomsen [14], the larger nuclei and nucleoli are the main attributes of the cells synthesizing neurosecretory material where as the cells with smaller nuclei are considered to be inactive or in a state of minimal synthesis. It has also been reported that the cells with larger nuclei are more active than the cells with smaller ones [4, 20, 23]. The results of the present study are interpreted basing on the above mentioned observations.

NS cells of the supra-oesophageal ganglion (brain) (groups 1 and 2) and group B cells of the sub-oesophageal ganglion (SEG) with their highest nuclear volumes revealed their significant synthetic activity in slow-maturing stage suggesting their active involvement in the early maturation of the testes.

Significant synthetic activity of groups 3 to 9 and C as evident from their highest nuclear volumes, increasing secretory activity of sets I and III as observed from their cellular volumes, and highest secretory activity of groups 3, 5 and 6 manifested by their highest cell volumes, release and accumulations of phloxinophilic material in the fast-maturing stage prompted the suggestion that they play a contributory role in spermiogenesis. Similar studies on the role of NS cells in the reproduction of Haffterius rufoclanatus revealed the involvement of neurosecretory material from the posterior cells of the pars intercerebralis in spermiogenesis [15]. Highest secretory activity of protocerebral NS cells as signified by their highest cell volume and somatic granulation could be considered to be associated with the maximum maturation activity of the testes which includes the release of fully formed sperms into the lumen, emptying the cysts. Group C cells can also be considered to play a role in the mature stage of the testes through their highest synthetic activity. Other groups of NS cells with their lowest nuclear and cellular volumes indicative of minimal synthesis and secretion respectively, do not appear to take part in the mature stage of the testicular activity.

It is suggestive from the results that group 2, group B, group 4 and groups 7 to 9 help in bringing about the degenerative changes in the spent stage of the testes so as to restart another cycle of maturation from December onwards.

The role of NS cells in the reproductive acitivity may be either direct i.e. influencing the reproductive structures directly or indirect i.e. through their influence on mobilisation and utilization of nutrients essential to meet the energy expenditure during the testicular activity at different stages of annual cycle. Studies on the biochemical changes in different tissues of the male scorpion during different months by Janardhan Rao [9] revealed that

1. The proteins and TNPS (total ninhydrin positive substances) of testes and associated reproductive organs (ARO) increased continually from December to August followed by a decline upto December. From February onwards, hepatopancreas served as the extragonadal source of proteins to meet the enhanced demands of testicular activity and

2. the glycogen of the testes decreased from December to August and then showed an increase upto December. The glucose also followed the same trend except for an elevation during July and August. In ARO, glucose and glycogen declined from December upto June. Higher levels maintained during July and August declined in Septemeber to increase again upto December.

The results of the present study suggest the possible involvement of sets I, III and group C in the above mentioned changes in the proteins and TNPS in reproductive and associated reproductive organs. Secretory patterns of group B cells justify their possible role in the increased glycogenolysis from December to August.

Thus the changes in the synthetic and secretory activity of different groups of NSCs described during different months correspond not only with the changes in

the testicular activity but also with the changes in the biochemical constituents of different tissues. It could, therefore, be suggested that the neurosecretions exert their influence either directly or indirectly through the biochemical, metabolic or physiological activities on the reproductive activity of the male scorpion, H. fulvipes.

The four stages of testicular cycle viz., slow-maturing, fast-maturing, mature and spent periods of H. fulvipes [9] are comparable to the four successive stages identified in the reproductive cycle of the male Palaemon serrifer viz., the growing period, the mature, the ripe and spent and the degenerating and resting periods [12] respectively. Histological studies also revealed four groups of NS cells designated as A, A1, B and E in the brain and thoracic ganglia of P. serrifer of which the 'A' cells show secretory activity for the mature, ripe and spent periods and 'E' cells for the growing, the mature, the ripe and spent periods [12]. Similar studies on neurosecretion in relation to the testicular cycle of the crab, Potamon koolooense revealed the occurrence of 4 types of NS cells designated as A, B, C, and D in the thoracic ganglia and marked annual cyclic changes of synthesis and release from 'A' cells in association with the testicular cycle. Early stages of spermiogenesis were shown to depend on the neurosecretion of thoracic ganglion especially of 'A' cells. This gonadotropic influence of thoracic ganglia was found to be mediated through androgenic glands [10]. Type 'A' NS cells of brain were found to be involved in the meiosis, spermiogenesis and spermiation stages of testicular cycle in P. koolooense [11]. In Scylla serrata, 'Y' organ factor was presumed to be responsible for testicular maturation [17]. But in H. fulvipes, the present investigation revealed that all groups of NS cells take part at one or the other stage of the testicular cycle.

Set I, group 4 and group 6 cells showed an increase in synthetic and secretory activity during courtship and a decrease immediately after mating suggesting their possible role in courtship of the male scorpion. Increase in the synthetic activity of sets II, III, group 2 and groups 7 to 9 during courtship with a consequent increase in the secretory activity in the mating stage followed by either decrease (set II) or further increase (set III and group 2) at 6 hours after mating signify their probable role in courtship and mating. Increased synthetic and secretory activity of groups B, 3, 5 and C during courtship and immediately after mating, perhaps indicate their continued activity in courtship and mating phase of the male reproductive cycle. As the cells did not fall in a specific set pattern of increase or decrease in their activity at different stages of post-mating period, and also as the post-mating changes in the testicular activity were not clearly established, it was not possible to relate the neurosecretory activity with the post-mating events of the male scorpion. Transportation of phloxinophilic secretory globules towards the dorso-median region of SEG from group 3 cells from courtship stage and phloxinophilic accumulations near groups 5 and 6 from immediately after mating through different stages of experimentation prompt the suggestion that they play a role in courtship, mating and post-mating testicular and related physiological events.

Acknowledgements: We gratefully express our indebtedness to Prof. TGK Reddy and Prof. V. L. Das for their invaluable guidance and encouragement.

## REFERENCES

1. Babu KS, 1973. Histology of the neurosecretory system and neurohaemal organs of the spider, Argiope aurantia (Lucas). J. Morph. **141**: 77-98.
2. Cameron ML, Steele JE. 1959. Simplified aldehyde fuchsin staining of neurosecretory cells. Stain Technology. **34**: 265-266.
3. Cason JE. 1950. A rapid one-step Mallory-Heidenhain stain for connective tissue. Stain Technology. **25**: 225-226.
4. Ewen AB. 1962a. Histophysiology of the neurosecretory system and retrocerebral endocrine glands of the Alfalfa Plant bug, Adelphocoris lineolatus (Goeze) (Hemiptera: Miridae). J. Morph. 111(3): 255-269.
5. Gabe M. 1955. donneès histologiques sur la neurosecretion chez les Arachnides. Arch. Anat. Micr. **44**: 351-383.
6. Gomori G. 1941b. Observations with differential stains on human islets of Langerhans. American Journal of Pathology. **17**: 395-406.
7. Habibullah M. 1961. Secretory structures associated with the neurosecretory system of the immature scorpion, Heterometrus swammerdami. Quart. J. Micr. Sci. 102(4): 475.
8. Humason GL. 1971. Animal tissue technique. Third Edition. W.E. Freeman & Co. Sanfrancisco and London.
9. Janardhan Rao L. 1989. Studies on the reproductive seasonality of the male scorpion, Heterometrus fulvipes (Koch). [ Ph.D. Thesis]. Available from Ach-

arya Nagarjuna University, Andhra Pradesh, India.
10. Joshi PC, Khanna SS. 1984. Neurosecretory system of the thoracic ganglion and its relation to the testicular maturation of the crab, Potamon koolooense (Rathbun). Z. Mikrosk. Anat. Forsch. Leipiz. **98**: 429-442.
11. Joshi PC, Khanna SS. 1985. Studies on the relationship between the neurosecretion of the brain and the testicular cycle in the crab, Potamon koolooense (Rathbun) (Crustacea, Decapoda). Zool. Anz. **214**: 320-332.
12. Kim Myung-Hee, Taek-Yuille. 1992. Histological study of neurosecretory cells in the brain and the thoracic ganglion with gonadal development of Palaemon serrifer . Bull. Korean Fish Soc. 24(5): 327-339.
13. Kvartirnikov MP. 1980. Neurosecretory and endocrinological conditions in the European scorpion, Euscorpius carpathicus. Acta. Zool. Bulg O **14**: 83-87.
14. Lea AO, Thomsen E. 1962. Cycles in the synthetic activity of the medial neurosecretory cells of Calliphora erythrocephala and their regulation. Mem. Soc. Endocr. **12**: 345-347.
15. Lopez Guerrero YRMA. 1987. Analysis of the structure of the testicle and of the neurosecretory cells in the pars intercerebralis of Halffterius rufoclanatus (Jekel) (Coleoptera). Mus. Reg. Sci. Nat. Bull. (Torino) 5(2): 443-446.
16. Masthanaiah S, Doraiswami Reddy V, Chengal Raju D, Swami KS. 1978. Circadian rhythmic activity of lipase in the scorpion, Heterometrus fulvipes (Koch). Curr. Sci. 47(4): 130-131.
17. Nagabhushanam R, Farooqui UM. 1984. Changes in the activity of Y-organ cells during spermatogenesis in a marine crab, Scylla serrata (Decapoda, Crustacea). J. Adv. Zool. 5(1): 48-51.
18. Padmanabhanaidu B. 1967. Perfusion fluid for the scorpion, Heterometrus fulvipes. Nature (London) 213 (5074): 410.
19. Raju C, Bashamohideen MD, Narasimham C. 1973. Circadian rhythm in blood glucose and liver glycogen levels of scorpion, Heterometrus fulvipes . Experimentia, **29**: 964-965.
20. Ramalingam N. 1971. Studies on ovarian development and neuroendocrine system in Chrysomyia megacephala (Fabr.) [Ph.D. Thesis] Available from Annamalai University. India.
21. Reddy T Gopalakrishna. 1966. Studies on the behaviour of a selected arachnid with special reference to diurnal activity rhythms. [Ph.D. Thesis]. Available from S.V. University. Tirupathi. India.
22. Reddy T. Gopalakrishna. 1970. An enquiry into the causes driving the scorpion, Heterometrus fulvipes into its natural habitat. J. Annamalai Univ. Sci. **28**: 147-154.
23. Saini RS. 1966. Neuroendocrine control of Oocyte development in the beetle Aulacophora foveicollis Luc. J. Insect Physiol. **12**: 1003-1008.
24. Subburam V, Reddy TGK. 1978a. Breeding season and gestation period of the scorpion, Heterometrus fulvipes. J. Bombay Nort. Hist. Soc. 75(2): 513-514.
25. Venkateswara Rao P, Govindappa S. 1967. Dehydrogenase activity and its diurnal variation in different muscles of the scorpion, Heterometrus fulvipes . Proc. Ind. Acad. Sci. LXV: 243-249.