Insect Sci. Applic. Vol. 15, No. 3, pp. 351–360, 1994
Printed in Kenya.

0191-9040/94 $3.00 + 0.00

THE REGULATORY MECHANISM OF THE MALE ACCESSORY REPRODUCTIVE GLAND (ARG) OF *SERINETHA AUGUR* (FABR) (HETEROPTERA: COREIDAE) —A COTTON PEST

P. BASKER[2], L. S. RANGANATHAN[1]* and P. PADMANABHAN[1]
[1]Dept. of Zoology, Annamalai University, Annamalai nagar-608 002, Tamil Nadu, India;
[2]Centre for Applied Genetics, Dept. of Zoology, Bangalore University, Bangalore-560056, India

(*Received* 7 *January* 1993; *accepted* 30 *September* 1993)

Abstract—The present study deals with the interrelationship of the neuroendocrine complex (NEC) and male accessory reproductive gland (ARG) of a cotton bug, *Serinetha augur*. The NEC consists of brain (cerebral ganglia), corpus cardiacam (CC) and corpus allatum (CA). Based on the staining reaction of aldehyde fuchsin (AF) and chrom alum haematoxylin-phloxin (CAHP), four types of neurosecretory cells have been identified in the brain. The neuroendocrine control over the ARG in *S. augur* was investigated through ARG extirpation (and gonadectomy)-induced hypertrophy of CA. The changing pattern of proteins in the brain during the pre- and post-mating periods (as judged by electrophoretic investigations) further supports the interrelationship of the NEC and ARG.

Key Words: ARG, corpus cardiacum, corpus allatum, extirpation, hypertrophy

Résumé—L'étude actuelle s'agit de la entreparenté du complexe néuroendocrine (CNE) et de la glande reproductrice accessoire mâle (GRA) d'une punaise cotonière, *Serinetha augur*. Le CNE consiste en cerveau (cerebral ganglia), corpus cardiacam (CC) et corpus allatum (CA). Fondé sur la réaction tachante d'aldehyde fuchsin (AF) et de chrom alum haematoxyline et phloxine (CAHP), quatre sortes de cellules néurosécrétoires ont été identifieés dans le cerveau. Le contrôle néuroendocrine sur la GRA dans *S. augur* était recherché à travers l'extirpation de la GRA et la gondectomie hypertrophie du CA. Le dessin changeant des proteins dans le cerveau pendant la période d'accouplement avant et aprés (par des recherches électrophoretiques) soutient l'entreparente du CNE et la GRA.

Mots Clés: GRA, corpus cardiacum, corpus allatum, extirpation, hypertrophie

INTRODUCTION

The neuroendocrine complex of adult insects consists of neurosecretory cells (NSC) of the brain, corpora cardiaca (CC) and corpora allata (CA). The pioneering work on the NSC of brain of honeybees was by Weyer, (1935). Since then, several investigations have been carried out on the histomorphology of the NSC, CC and CA in other insects (Thompson, 1965; Mason, 1973; Panov and Melnikova, 1974; Highnam and Hill, 1977; Kannan and Prabhu, 1985). The neurosecretory and the reterocerebral endocrine systems of hemipteran insects have been described by many authors (Johanson, 1958; Srivastava, 1970; Mason, 1973; Panov and Melnikova, 1974; Samal and Ramalingam, 1981).

The accessory reproductive gland (ARG) has many vital roles pertaining to sperm transfer activities including sperm activation, motility and maturation (Odhiambo, 1969; Leopold, 1976; Happ, 1984). Hence it is thought that a study of the changes of the histomorphology of the neuroendocrine system and ARG in relation to sperm transfer activities will throw more light on the role of these factors in spermatogenic cycle of the hemipteran, *S. augur*, which is a pest of cotton, *Gossipium hirsutum*.

*Corresponding author.

MATERIALS AND METHODS

Adult *S. augur* were collected in and around the Annamalai University campus, and reared in wooden cages of 30 x 33 x 45 cm at a temperature of 29 ± 1°C and 60% R.H. About 25 adult males and females were kept together in one cage and were fed with bunches of fresh tendrils of *Cardiospermum halicacabum* (Sapindacea) and bolls of cotton.

Histology of ARG and CA

The male bugs were dissected in insect Ringer solution following the method of Ephrussi and Beadle (1936). The ARG and the brain complex including CA were removed and fixed in different fixatives such as Bouin's and Zenker's fluid. The tissues were then dehydrated, cleared in xylene and embedded in paraffin wax (58–60°C). Serial sections of 6μ thickness of the ARG and CA were stained with haematoxylin and eosin. With the aid of an ocular micrometer, the length, width and nuclear diameter of at least 25 glandular epithelial cells in the middle of the free ends of follicles were measured. Nuclear volume and glandular volume were determined by the procedure of Siew (1965) and Tembhare and Thakare (1976).

Gonadectomy and ARG extirpation

Three-day old adult males were selected for gonadectomy (removal of testis) and ARG extirpation experiments. Before the operation, insects were anaesthetised with ether. Two longitudinal incisions were made along the mid ventral line (third to fifth sternal plates). Testes were located by piercing the intersegmental membrane at the junction of seventh and eighth sterna and extirpated. With the help of fine forceps, the ARG was snapped at the base and pulled out. The wound was sealed with molten paraffin wax after sprinkling the area with antibiotic powder. Per cent mortality in gonadectomy is about 8 and per cent ARG extirpation is 21. Fifty insects were used for each operation. Sham operations consisted of all procedures of real operation except that the testes and ARG were not removed.

Electrophoresis

Electrophoretic separation of protein from the brain complex was carried out following the method of Davis (1964). Brain complex (50 mg) of pre- and post-mating cycles was stored in 1 ml of Tris (0.02 M) —HCl buffer (0.1N) at pH 6.8. It was homogenised with 0.5 ml of cold saline and centrifuged at 15,000 g (RPM) at 4°C for 20 min. Samples of 0.04 ml mixed with an equal volume of 40% sucrose were added onto the top of each jel tube. Bromphenol blue (0.5%) was used as marker. Electrophoresis of water-soluble proteins was carried out in Tris - glycine buffer (pH 8.8) at a constant current 3mA/ gel tube. Gels were stained with 1% Coomassie brilliant blue to identify protein bands. The stained gels were scanned in a Perkin Elmer Schimadsu CS 910 Dual wavelength TLC/Gel scanner at 620nm.

RESULTS

Morphology

Sections referred to in Figs 1–10 except 4 Bouin fixed Heidenhein haematoxylin and counterstained with eosin.

Brain. The brain of *S. augur* is divisible into three regions, namely proto- deuto- and tritocerebrum (Figs 1a and b). Protocerebrum lies at the anterior region, deutocerebrum is in the latero mid region and tritocerebrum is in the latero posterior region of the brain. The neurosecretory cells in the brain complex have been arbitrarily classified into four types (A, B, C and D; Fig. 2) and are connected by pars-intercerebralis. Neurosecretory cells in this region are designated as median neurosecretory cells.

A type cells are the largest neurosecretory cells present in the tritocerebrum with an average diameter of 13–18 μm. These cells can be identified by their weak staining reaction with chrom alum haematoxylin-phloxine (CAHP) and aldehyde fuchsin (AF). This reaction may be due to lower concentration of neurosecretory materials (Table 1).

B type cells are pear-shaped with an average diameter of 10–12 μm. The cytoplasm was stained dark purple with AF and blue black with CAHP indicating a higher concentration of neurosecretory material. The B cells were found mostly in the protocerebral region during the pre and post mating periods.

C type cells appear relatively smaller than B type cells, measuring about 5–10 μm in diameter. The cytoplasm showed lesser amounts of neurosecretion as evidenced by moderate staining and reaction with AF and CAHP. These cells were found mostly in the deutocerebrum.

D type cells are the smallest neurosecretory cells with an average diameter of 6–8 μm found in the protocerebrum. They stain green with AF and red with CAHP. The cytoplasm of these cells showed a weak staining reaction with AF and CAHP, suggesting a minimum quantity of neurosecretory materials in them.

Corpus allatum (CA). This is a single median gland attached to the corpus cardiacum posteriorly (Fig. 3). The size of the gland increases with increase in the volume of the gonad.

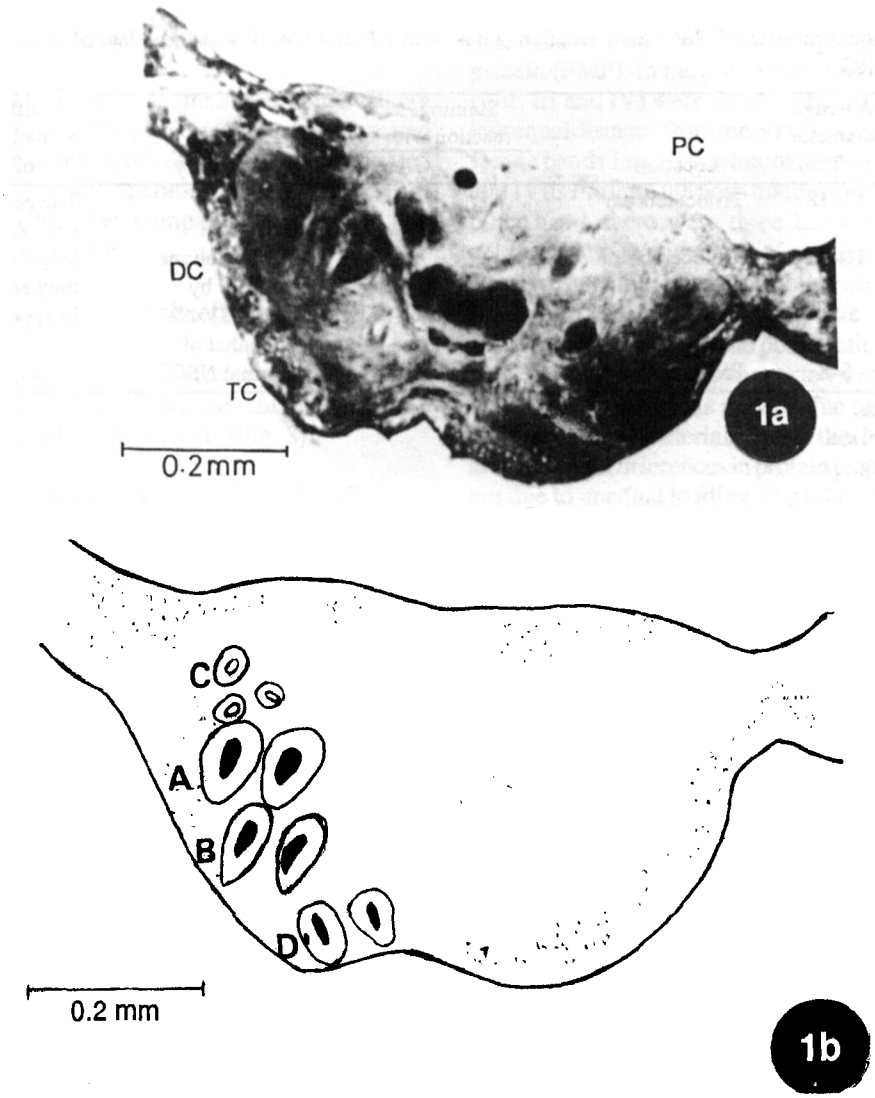


Fig. 1a. Frontal section of the brain showing the three lobes of the brain—Protocerebrum, deutocerebrum and tritocerebrum. Dc—Deutocerebrum; Pc—Protocerebrum; Tc—Tritocerebrum
Fig. 1b. Showing the distribution of neurosecretory cells in the brain of *Serinetha augur*

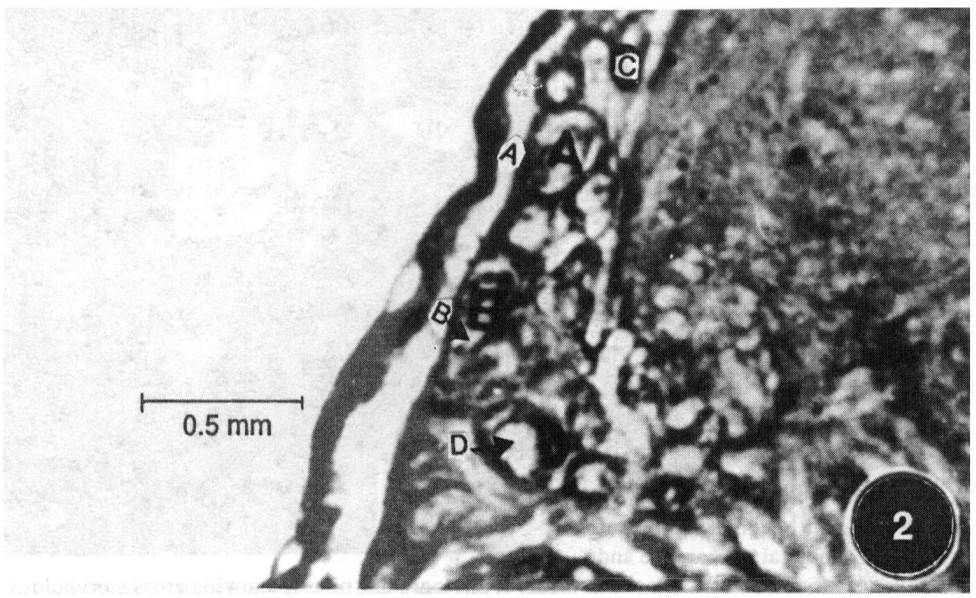


Fig. 2. Section of the brain showing different types of neurosecretory cells (A, B, C and D)

Table 1. Morphometric data of NSC, their reaction status with AF and CAHP and the effect of gonadectomy and extirpation of ARG

S. No.	Cell type	Average diameter in (μm)	Location	Staining reaction with AF	Staining reaction with CAHP	Effect of gonadectomy	Effect of extirpation of ARG
1.	A	13–18	Tritocerebrum	+	+	Observed reduction of the volume of NSC by about 31.4% than that of normal NSC	The neurosecretory cells A and B are larger in size and they are vacuolated in appearance
2.	B	10–12	Protocerebrum	+++	+++		
3.	C	5–10	Deutocerebrum	++	++		
4.	D	6–8	Protocerebrum	+	+		

\+ — Feeble.
++ — Moderate.
+++ — Strong.

Accessory reproductive gland. The accessory reproductive gland (ARG) is situated around the common ejaculatory duct which lies at the postero median end of the abdominal cavity below the junction of the two vasa deferentia (Fig. 4). The gland consists of a single layer of columnar epithelium (Fig. 5). At the posterior region, towards the inner side of the gland, on either side of the common ejaculatory duct, a group of squamous epithelial cells are present (Fig. 6). The mode of secretion appears to be holocrine in the squamous epithelial cells (Fig. 5) and apocrine in the peripheral columnar epithelial cells (Fig. 5).

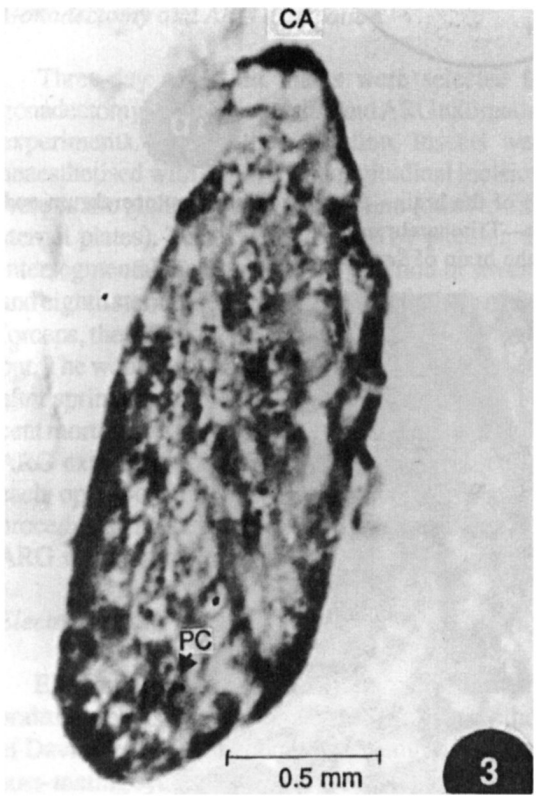


Fig. 3. Frontal section showing the histomorphology of the corpus allatum. Note the syncytial appearance and closely packed cells. CA—corpus allatum; Pc—Packed cells

Histology

The corpus allatum of the adult male bug of *S. augur* is a small ovoid structure. Since the constituent cells are closely packed, it is difficult to make out the cellular boundaries. The nuclei are placed eccentrically, with eosinophilic cytoplasm. The chromatin granules are distributed homogeneously and stain intensely with haematoxylin. The volume of the CA was 144,237 μm³ in the pre-mating period. However, it increased to 186,624 μm³ during the post-mating period (an increase of about 25%).

Changes in the neurosecretory cells of brain during post-mating period

The neurosecretory materials in the neurosecretory cells (of A and B types) were reduced after mating and as such the cells appeared to be vacuolated.

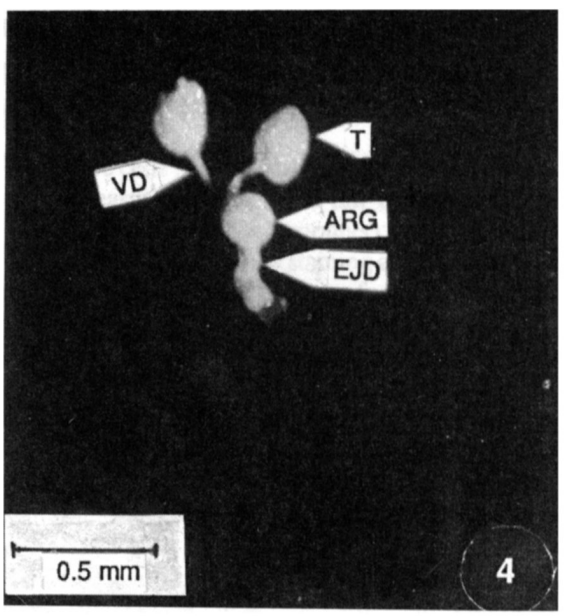


Fig. 4. Photographic picture showing gross morphology of the male reproductive system of *Serinetha augur*

Changes in CA after gonadectomy

The gonadectomy of the male adult bugs did not affect their reproductive activity. The gonadectomised insects mated on the eighth day just as did unoperated males. However, this operation resulted in increase of the CA by 9% when compared to that found in a pre-mating insect (Fig. 7).

Effect of gonadectomy on the NSC of brain

Gonadectomy resulted in reduction of the volume of neurosecretory cells. The mean volume of 234.15 μm^3 was reduced to 158.65 μm^3 (Fig. 8).

Effect of extirpation of ARG on CA and NSC

In the absence of ARG, the male insects attempted to mate on the eighth day. However, the mating behaviour such as caressing and chasing were absent in the experimental insects. The CA became hypertrophied and the neurosecretory cells A and B were larger and vacuolated (Figs 9 and 10).

Effect of sham operation

Sham operations of gonadectomy and ARG extirpation did not cause any noticeable change in the behaviour or activities of the CA, NSC or ARG.

Electrophoretic studies on the brain complex

The electropherograms of brain complex in pre- and post-mating insects showed quantitative changes of protein (Figs 11 and 12). There were four bands of protein in the brain complex of pre mated insects (I, II, III and IV). Those bands found near the origin were designated as slow-moving protein (SMP) and those found towards the marker front, as fast moving protein (FMP). In the pre-mating insects, four bands (I, II, III and IV) were found to be placed at more or less equal distance from the origin towards the marker. Hence bands I and II were considered as SMP and III and IV as FMP. In the post-mating individuals, on the other hand, there were three bands (I, II and IIA) coinciding with the SMP of the pre-mating ones. These results indicate a possible shift in the protein fractions, i.e., a reduction in both the SMP and FMP when the animals are in the post-mating condition. It is, therefore, tentatively suggested that the reduction in the protein fractions may be due to the release of neurosecretory materials from the brain complex after mating. Differences in protein pattern is certainly not due to unequal loading as 0.04 ml of sample was used uniformly in each run of electrophoresis.

DISCUSSION

Median neurosecretory cell types

The neurosecretory cells in the brain of insects are identified on the basis of their staining affinities. In the present study on the brain of *S. augur*, four types of median neurosecretory cells (MNC) namely A, B, C and D were recognised on the basis of their affinity to AF and CAHP. This finding agrees with observations where many types of neurosecretory cells have been reported (Rajendiran and Ramalingam, 1977; Sridharan, 1984). In *Oncopeltus fasciatus* and *Schistocerca gregaria* four types of median neurosecretory cells have been reported in the brain (Johansson, 1958; Highnam and Hill, 1977). On the other hand three types of neurosecretory cells have been reported in the brain of *Poecilocerus pictus* (Raziuddin et al., 1978) and *Chrysocoris purpureus* (Sridharan, 1984), two types in *Iphita*

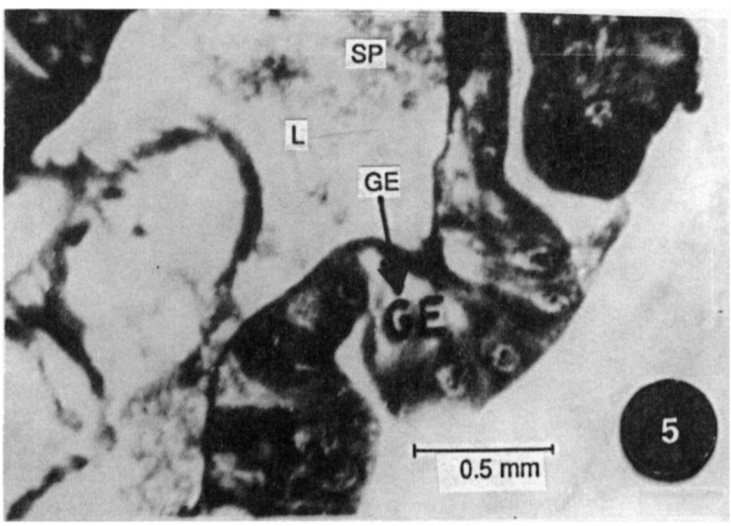


Fig. 5. Section of the glandular epithelium of the peripheral region of the ARG. GE—Glandular epithelium; L—Lumen; SP—Secretory product

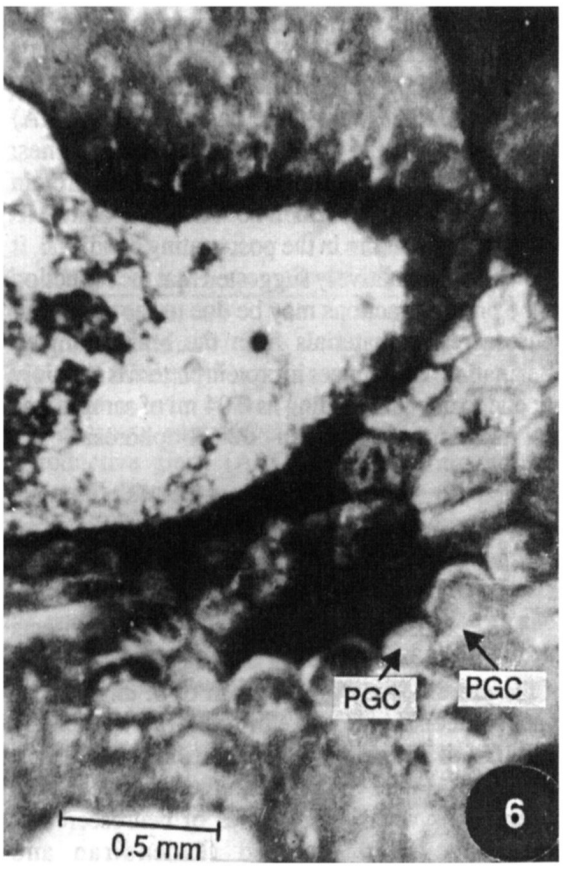


Fig. 6. Section of the polygonal cells at the postero lateral region of the ARG. PGC—Polygonal cells

limbata (Nayar, 1958), *Dysticus marginalis* (Abraham, 1966), *Musca nebulosa* (Deoras and Bhaskaran, 1966), *Gryllotalpa africana* (Rajendiran and Ramalingam, 1977) and only one type in *Belostoma indica* (Dogra, 1969), *Melonoplus sanguinipes* (Dogra and Ewen, 1970) and *Pyrilla perpusilla* (Krishnanandam and Ramamurthy, 1971).

MNC and secretory activity of male ARG

Studies on the role of neurosecretory system in regulating egg maturation and oviposition in insects are numerous (de Wilde and de Boer, 1969; Elliott and Gillott, 1976; Ranganathan, 1982). However, studies involving the relationship between the reproductive processes and the neuroendocrine activities in male insects are very few (Dupont-Raabe, 1952; Mason, 1973; Barker and Davey, 1981, 1983). Different views have been put forward regarding the nature of neurosecretory cells in insects. For instance, it is reported that in *Phasmids*, neurosecretory cells are active when they are filled with the neurosecretory material (Kadhirvel, 1985). However, in *Iphita limbata*, an inverse relationship has been established between the activity of neurosecretion and reproductive systems (Nayar, 1958). On the other hand, Kadhirvel, (1985) has reported that the neurosecretory cells are less active when they have more neurosecretory material. In the present study on *S. augur*, it has been observed that only A and B type of neurosecretory cells are loaded with neurosecretory material on the fourth day of

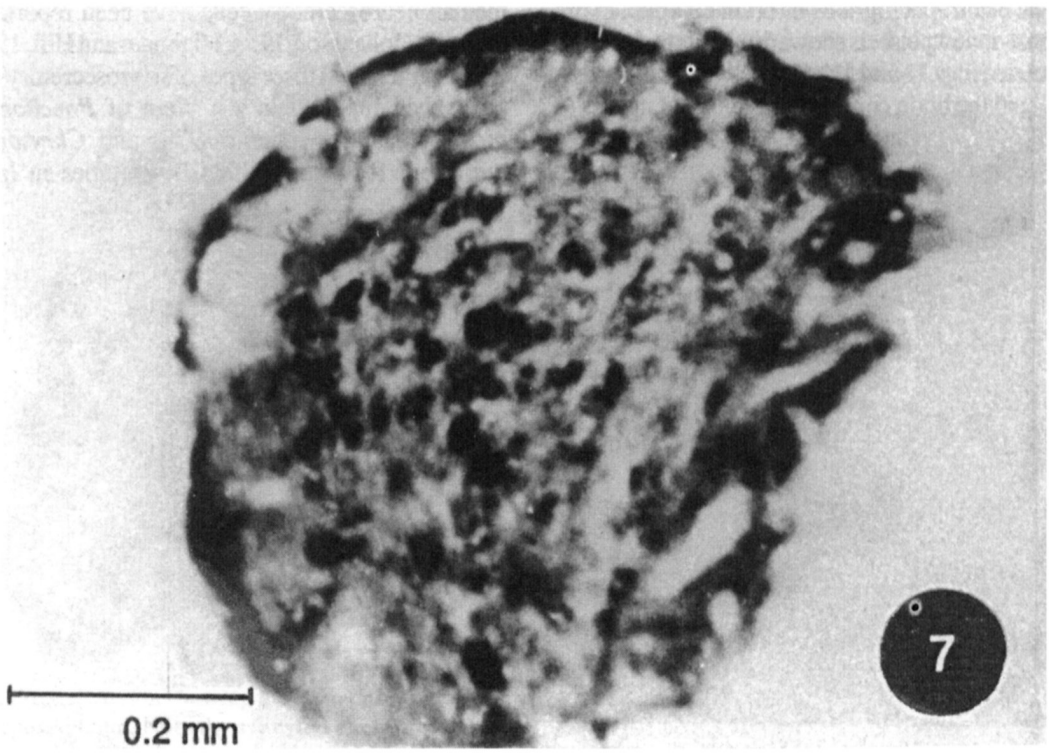


Fig. 7. Section showing hypertrophied nature of corpus allatum (CA) after gonadectomy

adult life (before mating). After mating, the neurosecretory substances of A and B cells get reduced, indicating their secretory activity. Simultaneously, the secretory activity of the ARG also seem to increase. Such a phenomenon may be due to the regulatory role of these types of neurosecretory cells in the secretory activity of the ARG. Further, it is observed that in *S. augur*, A and B cells become active after the extirpation of ARG. Similar observation has been made in *Odontopus varicornis* (Kadhirvel, 1985). It has been established that in *Rhodnius prolixus*, the neuroendocrine system controls the activity of ARG (Barker and Davey, 1983). The existence of a MNC-CA-ARG axis has

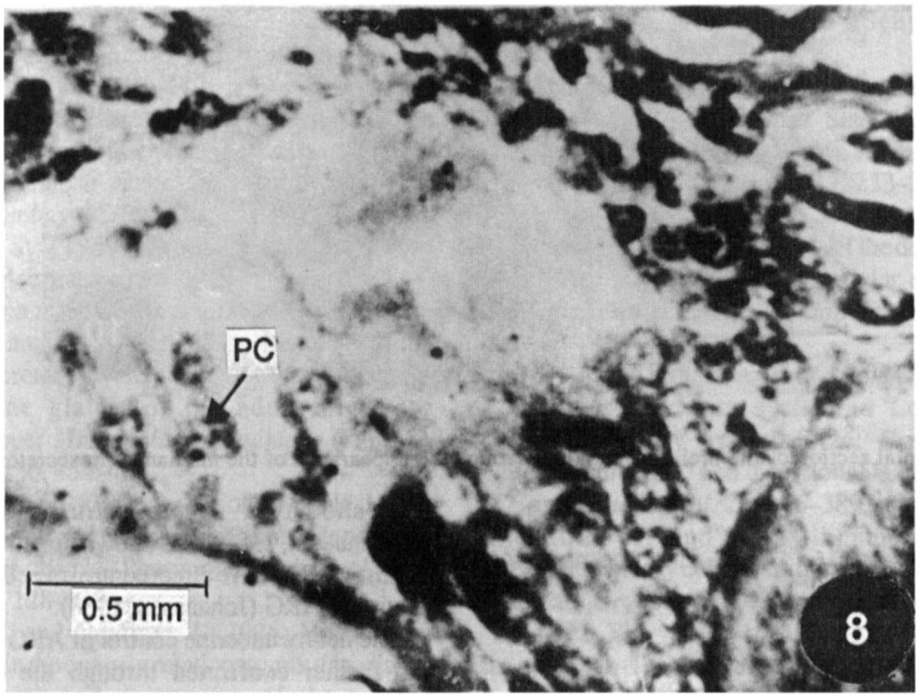


Fig. 8. Frontal section of the brain showing the neurosecretory cells after gonadectomy. Note the tightly packed cells in all the regions of brain complex and dense appearance. PC—Packed cells

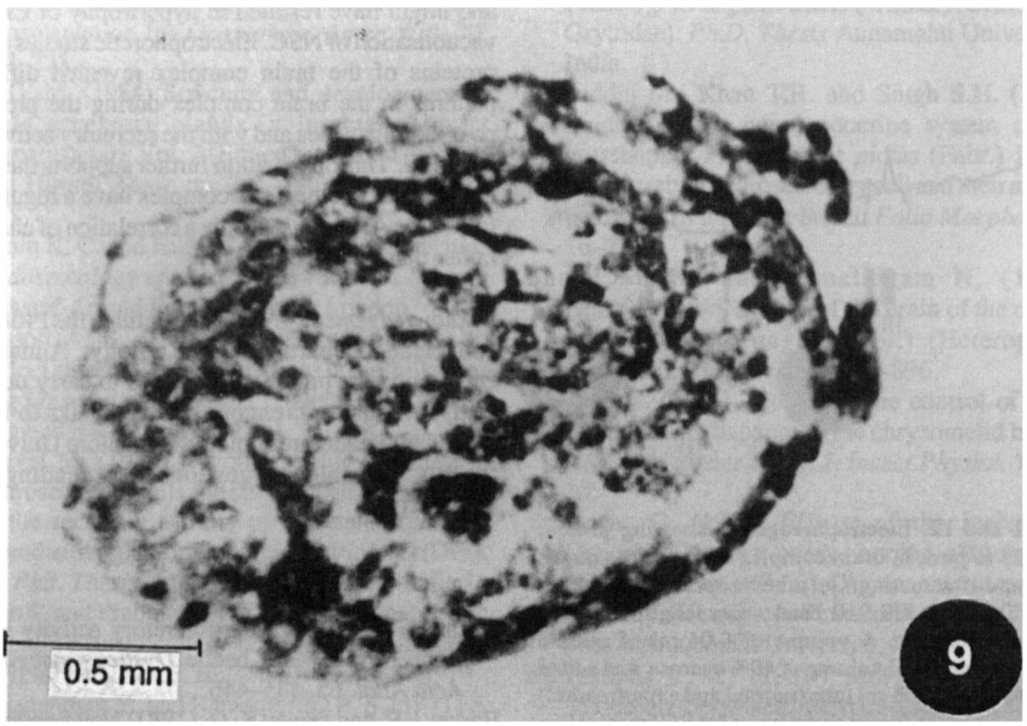


Fig. 9. Section showing the hypertrophied corpus allatum (CA) after extirpation of ARG

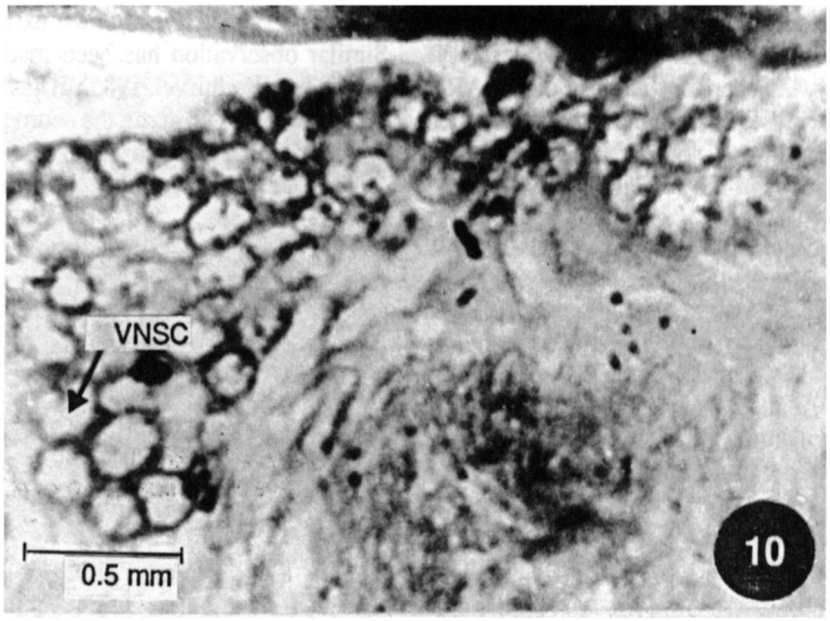
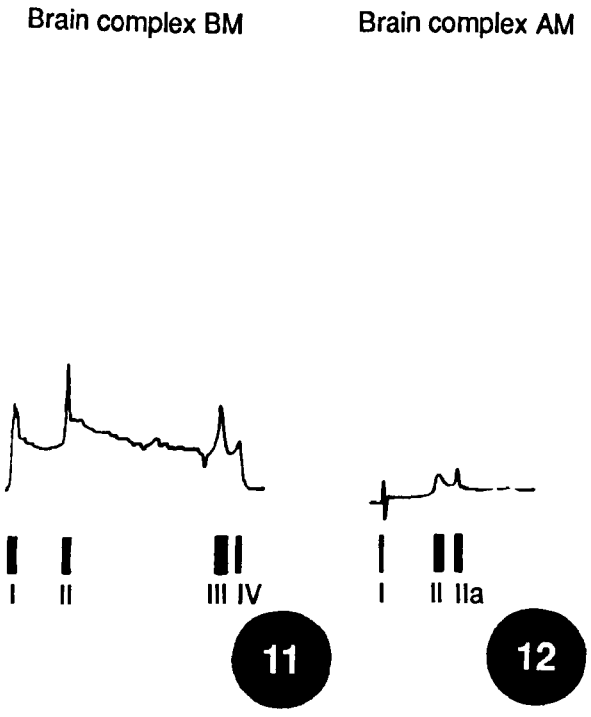


Fig. 10. Frontal section of the brain showing the vacuolated appearance of the median neurosecretory cells



Figs 11 and 12. Electropherograms showing protein fractions as peak in brain complex of *Serinetha augur* before and after mating. Gel tubes are scanned in Perkin Elmer Shimadsu CS 910 Dual wave length Gel/TLC scanner at 620 nm. A volume of 0.04 ml of sample mixed with an equal volume of 40% sucrose was added on to the top of each gel tube (normal and experimental) and 50 mg tissues (brain complex and ARG) were taken for this study. BM—Before mating; AM—After mating

been shown in *Plebiogryllus guttiventris* (Ranganathan, 1982). It is known that in *Oncopeltus fasciatus*, MNC have direct control over the secretory activity of ARG (Johansson, 1958).

The neuroendocrine control of ARG in *S. augur* was further confirmed through the extirpation experiment of ARG and its effect on the neurosecretory cells (NSC) and CA. In the absence of ARG, the feed back to CA/NSC could have been lost and might have resulted in hypertrophy of CA and vacuolisation of NSC. Electrophoretic studies on the proteins of the brain complex revealed different patterns in the brain complex during the pre- and post-mating cycles and with the secretory activity of the ARG. This observation further supports the view that the NSC in the brain complex have a regulatory role in the ARG. This is only a correlation of changes rather than a casual relation.

Acknowledgement—We are grateful to the Professor and Head, Department of Zoology, Annamalai University, for providing facilities to carry out this study. We also express our sincere thanks to Dr. S. Krishnan, Reader in Zoology, Bangalore University for his valuable suggestions and reading this manuscript.

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