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CONCURRENT EFFECTS OF LIGHT AND EYESTALK EXTRACT ON BRAIN, THORACIC GANGLION AND ON THE CHANGES IN THE GONADAL INDICES OF FEMALE CRAB, *PARATELPHUSA HYDRODROMOUS* (HERBST)

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SUMMARY

Three types of neurosecretory cells, (A, B & C) were identified in the brain and four types of neurosecretory cells (A, B, C & D) in the thoracic ganglion of *P.hydrodromous*. Continuous exposure to light for 15 days triggered the secretory activity of the neurosecretory cells and increased the gonadal indices. While saline treatment potentiated the light mediated effect, injection of the eyestalk extract inhibited the effect of light. Results of this preliminary study suggest that eyestalk hormones regulate the effect of light on ovarian development in *P. hydrodromous*.

Key words : Eyestalk extract; Gonadal index; Light; Neurosecretory cells; Neurosecretory material; *Paratelphusa hydrodromous*.

INTRODUCTION

The reproductive activity of crustaceans depends both on environmental factors and hormones (1). The presence of the ovary inhibiting hormone in the eyestalk of crustaceans including the fiddler crab, *Uca pugilator* (2 - 6) is well-known. The surgical removal of the eyestalk in crustaceans promotes ovarian maturation (7 - 8). In general, removal of both eyestalks in decapod crustaceans induced precocious yolk deposition and vitellogenesis in the ovary. In contrast, injection of eyestalk extracts or partially purified ovary inhibiting hormone or the implantation of the eyestalk neuroendocrine organs result in the inhibition of ovarian maturation (9).

Many investigators have reported that the environmental factors such as salinity, water, temperature, light and availability of food materials act as the regulators of both growth and

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reproduction in crustaceans (10 – 12). It has been established that in stalk-eyed decapod crustaceans, the neurosecretory cells present in the eyestalk, brain and thoracic ganglion mediate the effects of environmental factors (12). The effect of the chief environmental factor, the light, which influences the neurosecretion in stalk-eyed decapod crustaceans is mediated through the neurosecretory cells present in the eyestalks, brain and thoracic ganglion (12–14).

Hanumante (15) has suggested that the constant exposure of *Perionyx excavatus* to light and darkness impeded the secretion of neurosecretory cells of brain and subpharyngeal ganglia. Similarly, interception in the neurosecretion, significant increase in the nuclear diameter with a concomitant decrease in the cell area and neurosecretory materials due to constant exposure of *Poecilobdella viridis* to light has been reported by Nagabhushanam and Kulkarni (14). These authors also reported that the light acts as a stimulant on the secretory mechanisms of neurosecretory cells.

Nadarajalingam and Subramoniam (12) reported that the increased light intensity enhances the secretory activity of eyestalk neurosecretory cells in Ocypodid crabs, *Ocypoda platytarsis* and *O.macrocera* and that of brain and thoracic ganglion in *O. macrocera*. Ragunathan *et al.* (16) reported an increase in the gonadal indices of the fresh water crab, *Paratelphusa hydrodromous* treated with saline and further continuous exposure to light for 15 days. Recently, we have reported that the gonadal indices significantly decrease in the eyestalk ablated *P.hydrodromous* treated with eyestalk extract (17). In this paper, we report the changes in the NSC of brain and thoracic ganglion of a female *Paratelphusa hydrodromous* (Herbst) due to combined action of light and eyestalk extract.

MATERIALS AND METHODS

The intermoult, stage I, female *Paratelphusa hydrodromous* used in the present study were collected from the lake in Sathuvachari, which is 6 kms away from Vellore town of Tamil Nadu. They were acclimatized in the laboratory for a week in the prevailing room temperature. The eyestalk extract was prepared by ablating eyestalks from reproductively active female crabs. The two eyestalks from one animal was triturated in 0.15 ml of saline.

Forty crabs were selected and were divided into four groups (A,B,C,D) of ten each and placed in separate round plastic tubs. The water in tubs was changed daily and the crabs were fed with boiled beef meat *ad libitum*.

Crabs belong to Group a were killed on the first day of the experiment. Group B did not receive any treatment and was considered as initial control, group C received physiological saline and group D received saline - aqueous eyestalk extract. Injections were given on 1st, 5th and 10th day of the experiment. The dose of the saline and eyestalk was 50µl / crab.

Crabs belong to groups B, C and D were placed in square wooden photoperiodic chamber (12) with a side length of one meter. The chamber was covered with a thick black cloth to avoid

external light. The chamber was fitted with a fluorescent bulb of 1450 lux and necessary arrangements were made for sufficient aeration inside the chamber. The experiment lasted for 15 days. At the end of 15 days, the crabs were weighed and killed. The brain and thoracic ganglia of all the groups were dissected out and fixed in Bouin's solution for overnight. The tissues were clearly water washed and then dehydrated in graded alcohol series and embedded in paraffin wax of melting point 55°- 58° C. Sections were cut at 6-8 µm, stained with Ehrlich's haematoxylin and counterstained with Eosin (18). The neurosecretory cells lodged in the brain and the thoracic ganglion were studied.

The ovary and spermatheca were blotted, dry weighed and the ovarian and spermathecal indices were calculated (19) using the following formula.

$$\text{Index} = \frac{\text{Wet weight of the tissue}}{\text{Wet weight of the crab}}$$

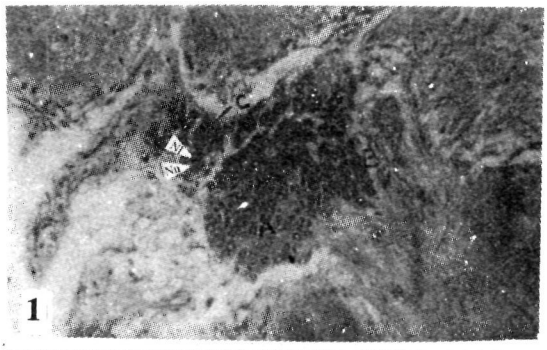
The data were statistically analysed.

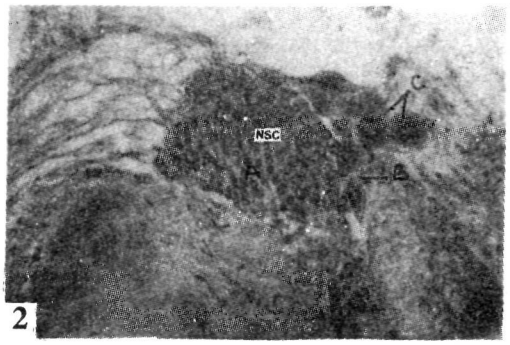
RESULTS AND DISCUSSION

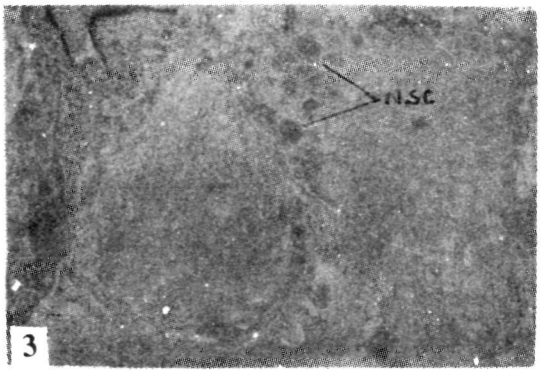
It was evident that there are three different but distinct types of neurosecretory cells (NSCs) (A,B&C) in the brain and four types of NSCs (A,B,C&D) in the thoracic ganglia. The type A NSCs found in the brain varies in shape and they are oval or spherical or elliptical or round in structure. They are very small and measured about 6.25 µm in size and the secretory granules are almost absent (Fig.1).

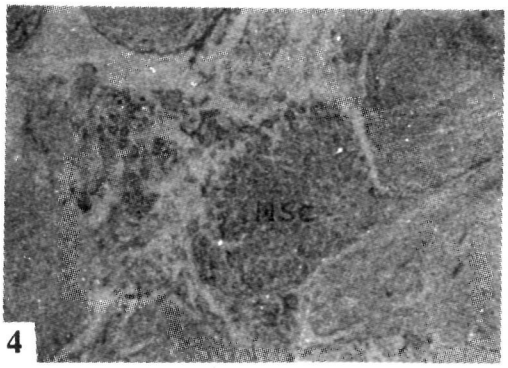
The type B NSCs are spherical in shape. They are 12.5 µm in size and are bigger than the type A NSCs (Fig. 2). The type C are larger in size (25 µm), the nucleus and nucleolus are quite distinctly seen. The staining reaction is intense with Haematoxylin and Eosin due to more neurosecretory material (NSM) in the cytoplasm. (Fig. 2).

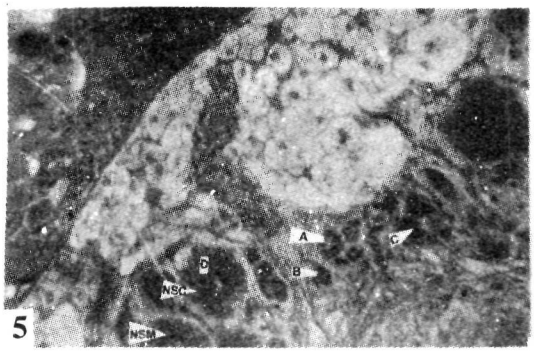
The exposure of these three groups (B, C and D) of crabs to continuous light for 15 days and further treatment with saline (group C) and eyestalk extract injection (group D) revealed that the NSCs of the thoracic ganglia of the group B crabs were comparatively smaller than the NSCs of the group C crabs. The brain of group B (light – untreated) crabs showed a moderate staining reaction with haematoxylin – eosin and also showed a moderate amount of NSM (Fig.1). The NSCs of the brain of group C animals stained intensely due to more NSM and the cells were slightly enlarged. The NSCs in the group B crabs include type A (12.5 µm), type B (18.75 µm) and type C (31.25 µm). In group C crabs type A NSCs measured 18.75 µm, type B NSCs measured 25 µm and type C NSCs measured 37.5 µm (Fig.2). The NSCs of the brain of the group D crabs were similar to that of the group B crabs (Fig.3).

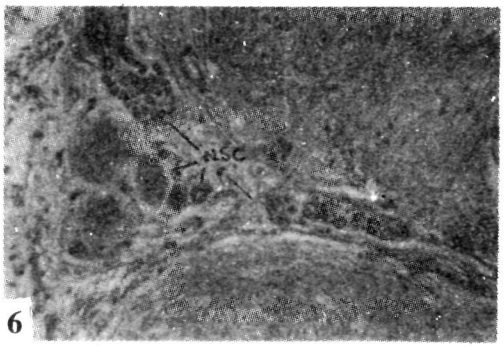












Figs 1-3 : Photomicrographs showing the neurosecretory cells in the C.S. of brain of 1) light treated 2) light plus saline treated and 3) light plus eyestalk extract treated crabs. Stained in Haematoxylin - Eosin X1500

Figs 4-6 : Photomicrographs showing the neurosecretory cells in the C.S. of thoracic ganglion of 4) light treated 5) light plus saline treated and 6) light plus eyestalk extract treated crabs. Stained in Haematoxylin - Eosin X1500

The types of neurosecretory cells (NSG) in the brain and thoracic ganglia and their response to light and ovarian stalk were studied in the fresh water crab *Paratelphusia hydrodromus* at light microscope level.

NI - Nucleolus; NSC - Neurosecretory cell; NSM - Neurosecretory material, Nu - Nucleus. A - Type A NSC; B - Type C NSC; C - Type C NSC; D - Type D NSC

The NSCs of the thoracic ganglia were comparatively larger than the NSCs of the brain and four different types (A, B, C & D) of NSCs were identified in the thoracic ganglia. Type A NSCs varied from oval to spherical or elliptical in shape (Fig.5). The nucleus was indistinct and the cytoplasm was scanty, stained moderately and were 6.25 µm in size. Type B NSCs were round in shape, and larger than the type A NSCs with a size of 12.5 µm. Cytoplasm contained moderate amount of NSM (Figs. 5 & 6). Type C NSCs were larger than the type B NSCs (18.75 µm) with a distinct nucleus and dense NSM (Figs. 5 & 6). Type D NSCs were the largest in size (25 µm) and hence called "giant NSCs (Figs. 5 & 6) and had distinct nucleus and nucleolus, surrounded with rich NSMs.

Exposure of these three groups (B, C and D) of crabs to continuous light for 15 days and further treatment with saline (group C) or eyestalk extract (group D) revealed moderate staining reaction in the NSCs of the thoracic ganglia of the group B crabs and the different types of NSC had increased size (i.e.) the sizes of Type A was 25 µm, type B was 31.25 µm, type C was 37.25 µm and type D was 43.5 µm but the NSCs were comparatively smaller than the group C crabs (type A measured 37.5 µm, type B measured 50 µm, type C measured 62.5 µm and type D measured 75 µm) (Fig.4). The NSCs of the thoracic ganglia of the group C crabs showed intense staining reaction and the NSCs were larger than the other two groups viz., B and D (Fig. 5). The NSCs of the thoracic ganglia of the group D crabs were more or less similar to that of the group B crabs (Fig. 6).

Deecaraman and Subramoniam (14 – 15) surveyed the NSCs of eyestalk, brain and thoracic ganglia of *Squilla holoschista* and their action on ovary and cement gland. They have suggested the involvement of certain types of NSCs found in the eyestalk, brain and thoracic ganglia and their subsequent elaboration on gonads and also characterized the hormones histochemically as protein, lipoprotein and glycolipoprotein complexes.

The injection of eyestalk extract and further exposure of the crabs to continuous light for 15 days produced less staining intensity of the NSCs of brain and thoracic ganglia when compared with their light plus saline treated control groups. The stimulatory effect of light on the gonadal development has been reported by many earlier workers (12, 23 - 24). The influence of light and saline as observed in the present investigation suggests the synthesis of more NSMs in the NSCs of both brain and thoracic ganglia. Contray to this, the less staining intensity and NSMs observed in the NSCs of group D crabs might be due to the inhibition of the neurosecretion in the NSCs by the gonad inhibiting hormone present in the eyestalk extract which is injected to the crab, even after the treatment of crabs to continuous light for 15 days.

The effect of light and its action on the NSCs of neuroendocrine tissues are well supported by earlier workers (12, 14). Nagabhushanam and Kulkarni (14) reported a concomitant decrease in the cell area and neurosecretory materials due to constant exposure of *Poecilobdella viridis* to light. They also reported that the light, as an external factor has triggering effects on secretory activity of the NSCs of brain and subpharyngeal ganglion. Nadarajalingam and Subramoniam (12) reported that the light intensity has resulted in high secretory activity in the

NSCs of eyestalks of *Ocypoda platytarsis* and *O. macrocera* and of brain and thoracic ganglion of *O.macrocera*.

According to Kulasekharan (22) the various types of NSCs in the brain (A, B & C) and thoracic ganglion (A, B, C & D) of a fresh water crab *Spiralothelphusa hydrodroma* are the stages of transformation of cells from the absence of secretory substances to cells of larger sizes with rich secretory substances. He has further stated that the type A NSC in thoracic ganglia and brain as the early cell stage based on their smaller size and the absence of secretory materials in the cytoplasm. This slowly increases in size as well as in its cytoplasmic contents and passes through type B and finally becomes "C" in the brain and "D" in the thoracic ganglia. After they reach the large or mature stage the secretory materials of the NSCs are released and act simultaneously on the target organs.

The ovarian index was higher in group C crabs compared to that of groups A, B and D. The increase in the ovarian index of the group C crabs was quite significant ($P < 0.001$) compared to groups A and B. The ovarian index of the group D crabs was significantly decreased ($P < 0.001$) compared to group C. The spermathecal index of the group B was higher than group A crabs ($P < 0.001$) and that of group C was higher ($P < 0.001$) than group B crabs. A significant ($P<0.001$) decrease in the spermathecal index of the group D was also noticed when compared with the group C (Table 1)

The gonadal index is an important tool in reproductive biology which clearly forecasts the development of the gonads (25). Data obtained in the present investigation show that there is a significant increase in the ovarian and spermathecal indices when crabs were exposed to light and the indices further raised in crabs exposed to light were treated with saline. A decrease in the ovarian and spermathecal indices observed in those crabs exposed to light and treated with eyestalk extract suggest that the changes in the ovarian index is proportionally related to the changes in spermathecal index.

Thus, the present study reveals increased ovarian and spermathecal indices in crabs exposed to continuous light and saline treatment potentiates the effect of light and eyestalk has an inhibitory effect on ovarian and spermathecal development.

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