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Role of Neurosecretory Cells in the Breeding, of Freshwater Bivalve: *Lamellidens Corrianus* (Lea)

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Abstract: In the freshwater bivalve like *Lamellidens corrianus* neurosecretory cells have been detected in the cerebral, pedal and visceral ganglia. In this animal, which may releases gametes two or three times during the course of the reproductive period, cerebral neurosecretory material is observed to disappear in some or all of the cells several days prior to each discharge of the gametes. The material is replenished between successive spawning, and the cells become depleted completely by the end of the reproductive period. Emission of the gametes may depend on the lifting of an internal inhibition correlated with the disappearance of neurosecretory material from the cerebral ganglion. In the present study, removal of cerebral ganglia accelerated the growth of gametes and injection of their extracts gradually increases the growth. This was more pronounced in cerebralectomized animals than extract injected ones. Removal of cerebral ganglia than injection of cerebral ganglionic extract to *Lamellidens corrianus* shows stress on visceral and pedal ganglia which affect the spawning. Experiments were carried on 2nd and 12th day. Variation in neurosecretory cells were observed and compared as normal with experimental and results has been discussed in the light of neurosecretory shifts.

Keywords: Breeding, Freshwater bivalve, *Lamellidens corrianus*, Neurosecretory cells.

INTRODUCTION

The freshwater bivalve molluscs are suspensory feeders on the primary stage of food chain, hence they notably influences the organization and functioning of ecosystems. Amongst invertebrates, molluscs show great variability in their nervous system ranging from primitive arrangement in chitons to the complex mass of fused ganglia forming the 'brain' of cephalopods. Studies on the reproductive cycles of clams from Indian coastal water have been carried out on *Katylesia opima*,[1] Neurosecretory cells seems to exert a control upon mitosis of germ cells, meiosis, vitellogenesis (cerebral ganglia) or spawning (visceral ganglia)[2]. Most of the effector organs used for pharmacological or physiological experiments, the way in which nervous system operates may differ considerably between animals, depending on the number of neurons involved and the individual shape, size and spatial arrangement of the component about fifty percent of the nervous system is composed of non-excitable satellite glial cells which are packed between and around the neurons.

They are supposed to transport neurosecretory substance[3] and[4]. Evidence for the occurrence of a wide variety of neurotransmitters in different tissues of *Lamellibranchs* including the nerve ganglia has been discussed from the functional point of view[5]. Some of the researchers[6] stated that the significance of NSCs as connecting link between nervous and endocrine systems and neurosecretory neurons "participate either directly or indirectly in endocrine control and form all or part of endocrine organ". Hormones are consequently well suited to exert their effects over extended period of time, and the endocrine system controlling-term process within the body such as the coordinated growth of organs or the maintains of appropriate metabolite concentration in the blood and tissues.

The significance of the differential staining affinities of neurons within the nervous system of both vertebrates and invertebrates was first appreciated by the scholar[7] and also their staining differences were used as the basics for first description of NSCs and to give original definition of neurosecretion[7]. The dangers of attributing neurosecretory function to a nervous on histological grounds have been pointed out on numerous occasions[8, 9]. Neurosecretory cells have been detected in the cerebal, pedal and visceral ganglia of the orders Protobranchiata, Filibranchiata, pseudolamellibranchiata, and eulamellibranchiata[3, 10-12] and their physiology has been studied to some extent[13]. Very little work on involvement of neurosecretion in reproduction and energy metabolism is reported in case of freshwater species[14].

MATERIALS AND METHODS

To extend the knowledge in this field, the present work has been undertaken on the freshwater species using the bivalve, *Lamellidens corrianus* (Lea). This species is abundantly distributed along the banks of river in Jayakwadi backwaters (Nathsagar) at Paithan near Aurangabad this species occurs throughout the year abundantly and hence it is used in the present study. The freshwater bivalve molluscs, *Lamellidens corrianus* (Lea) were collected from Jayakwadi backwaters (Nathsagar) at Paithan, 45 km. away from Aurangabad. After brought to the laboratory, the shells of the bivalves were brushed and washed with fresh reservoir water so as to remove the fouling algal biomass and mud. The animals of 80-85 mm shell length were selected for experiment and they were acclimatized for 24h. At laboratory condition in fresh aerated reservoir water (with renewal of water at the interval of 12-13h.) during the period and no food was given to the bivalves during laboratory acclimatization and subsequent experimentation. After 24 h., reservoir water was once again renewed and aeration was given. After 1hour the animals extended their organs (foot, mantle, siphons) to maximum and soon surgical operations and injection of the ganglionic-extract were done. Soon after the operation and injection of ganglionic extracts to normal control, extirpated 30 animals of cerebralectomy, 30

animals of extract injected, bivalves were transferred to separate aquaria. Each aquarium contained 15 liter well aerated reservoir water, and experiment was run for 12 days.

The experiments were conducted for 12 days on freshly collected animals. 5 animals from each group during the experimental period were fixed in Bouins Hollande on 12th day for histological study of gonad and ganglia. The section of cerebral, visceral and pedal ganglia were stained with Mallary triple stain[15], all the sections were observed under the research binocular microscope and wherever necessary, measurements were made before microphotography. Study of neurosecretory cycle and reproduction carried out[16].

RESULTS

The histological details of neurosecretory cells in different ganglias of *Lamellidens corrianus* during different seasons were given in table (1-3) and Figures (1-3). In cerebral ganglia Type A cell showed cell length (12.8571 ± 0.8997 µm) in control, (13.7142 ± 0.4879 µm) in injection of cerebral ganglionic extract to intact control 12th day. The cell width of Type A cell were (6.7142 ± 0.4879 µm) in control, (7.4285 ± 0.5345 µm) in extract injected group. The nuclear diameter in control was (5.1428 ± 0.3779 µm); in extract, injection was (5.7142 ± 0.4879 µm). Axon length in Type A cell in control was (2.4285 ± 0.5345 µm), in ganglionic extract injected was (2.5714 ± 0.5345 µm). Type B showed the diameter (9.4285 ± 0.5345 µm) in control, (9.8571 ± 0.6900 µm) in injection of cerebral ganglionic extracts to intact control. Nuclear diameter in Type B cell were (5.4285 ± 0.5345 µm) in control, (5.7142 ± 0.4879 µm) in extract injected group. In visceral ganglia on 12th day, Type A cell showed cell length (16.8571 ± 1.2149 µm) in control, (15.8571 ± 1.3451 µm) in cerebral ganglia ablation and (17.8571 ± 1.0690 µm) in injection of their ganglionic extract. The cell width of Type A cell were (7.8571 ± 1.0690 µm) in control, (7.1428 ± 0.6900 µm) in ablated and (8.2857 ± 1.2535 µm) in extract injected group.

The nuclear diameter in control was (3.2857 ± 0.4879 µm), in ablation (4.5438 ± 0.8164 µm) and in extract injected group was (3.1428 ± 1.0690 µm). Axon length was (4.2179 ± 0.5773 µm) in control, (4.2855 ± 0.5773 µm) in ablated and (2.1428 ± 0.6900 µm) in extract injected group. Type B cell showed the diameter (9.8571 ± 1.0690 µm) in control, (8.7258 ± 0.8164 µm) in cerebral ganglia ablated and (6.5714 ± 0.7867 µm) in cerebral ganglionic extract injected group. Nuclear diameter of Type B cell in control was (5.1428 ± 0.6900 µm), in ganglia ablated was (5.7142 ± 0.9511 µm), and in extract injected was (4.5714 ± 0.5345 µm).

In pedal ganglia on 12th day, Type A cell showed the cell length (14.5000 ± 1.0488 µm) in control, (13.5714 ± 0.9759 µm) in cerebral ganglia ablated and (13.8571 ± 0.8997 µm) in cerebral ganglionic extract injected group. The cell width of Type A cell were (5.8333 ± 0.7527 µm) in control, (5.2857 ± 0.4879 µm) in ablated and (5.7142 ± 1.3801 µm) in extract injected group.

Nuclear diameter of A Type cell were (5.6666 ± 0.8164 µm) in control, (4.1428 ± 0.6900 µm) in ablated and (4.5714 ± 0.5345 µm) in extract injected group. Type A cell showed axon length (1.5000 ± 0.5477 µm) in control, (1.8571 ± 0.6900 µm) in cerebral ganglia ablation and (1.7142 ± 0.7559 µm) in injection of their ganglionic extracts groups. Type B cell showed the cell diameter (8.3333 ± 0.8164 µm) in control, (7.7142 ± 1.1126 µm) in cerebral ganglia ablation and (8.1428 ± 1.0696 µm) in injection of their ganglionic extracts. The nuclear diameter in control was (4.8333 ± 0.7527 µm), in ablation (5.5714 ± 0.7867 µm) and in extract injected group was (5.8571 ± 0.6900 µm), on 12th day.

Table 1: Changes in the neurosecretory cells of cerebral ganglia on 12th day due to ablation of cerebral ganglia and injection of extracts in *Lamellidens corrianus* (All the values are in µm)

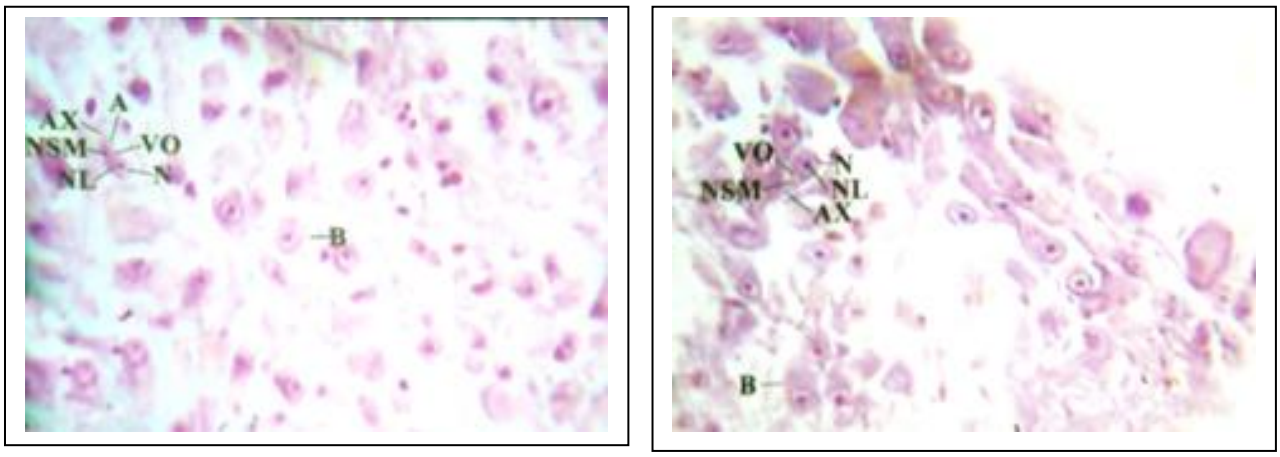
	Type A				Type B	
Group	Cell length	Cell width	Nucleus diameter	Axon length	Cell diameter	Nucleus diameter
Control normal	12.8571 ±0.8997	6.7142 ±0.4879	5.1428 ±0.3779	2.4285 ±0.5345	9.4285 ±0.5345	5.4285 ±0.5345
Injection of ganglionic extracts to intact control	13.7142 ±0.4879	7.4285 ±0.5345	5.7142 ±0.4879	2.5714 ±0.5345	9.8571 ±0.6900	5.7142 ±0.4879

Table 2: Changes in the neurosecretory cells of visceral ganglia on 12th day due to ablation of cerebral ganglia and injection of extracts in *Lamellidens corrianus* (All the values are in µm)

	Type A				Type B	
Group	Cell length	Cell width	Nucleus diameter	Axon length	Cell diameter	Nucleus diameter
Control normal	16.8571 ±1.2149	7.8571 ±1.0690	3.2857 ±0.4879	4.2189 ±0.5773	9.8571 ±1.0690	5.1428 ±0.6900
Ablation of cerebral ganglia	15.8571 ±1.3451	7.1428 ±0.6900	4.5438 ±0.8164	4.2855 ±0.5773	8.7258 ±0.8164	5.7142 ±0.9511
Injection of ganglionic extracts to intact control	17.8571 ±1.0690	8.2857 ±1.2535	3.1428 ±1.0690	2.1428 ±0.6900	6.5714 ±0.7867	4.5714 ±0.5345

Table 3: Changes in the neurosecretory cells of pedal ganglia on 12th day due to ablation of cerebral ganglia and injection of extracts in *Lamellidens corrianus* (All the values are in µm)

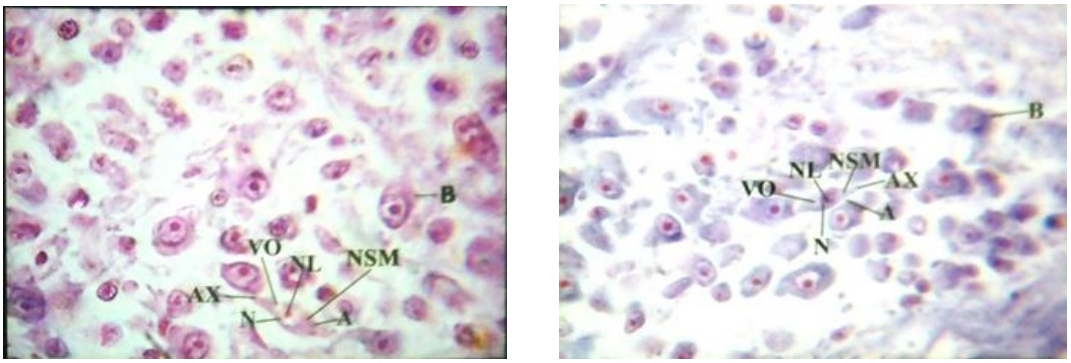
	Type A				Type B	
Group	Cell length	Cell width	Nucleus diameter	Axon length	Cell diameter	Nucleus diameter
Control normal	14.5000 ±1.0488	5.8333 ±0.7527	5.6666 ±0.8164	1.5000 ±0.5477	8.3333 ±0.8164	4.8333 ±0.7527
Ablation of cerebral ganglia	13.5714 ±0.9759	5.2857 ±0.4879	4.1428 ±0.6900	1.8571 ±0.6900	7.7142 ±1.1126	5.5714 ±0.7867
injection of ganglionic extracts to intact control	13.8571 ±0.8997	5.7142 ±1.3801	4.5714 ±0.5345	1.7142 ±0.7559	8.1428 ±1.0696	5.8571 ±0.6900



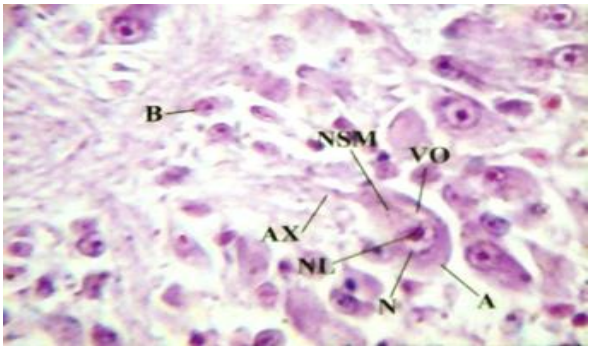
(I) Control (II) Injection of cerebral ganglionic extract

A = Type A cell; B = Type B cell; N = Nucleus; NL =Nucleolus; NSM = Neurosecretory material; AX =Axon; VO =Vacuole

Fig. 1: Histological changes in the neurosecretory cells of cerebral ganalia on 12th day due to ablation of cerebral ganglia and injection of their extracts in *Lamellidens corrianus*.



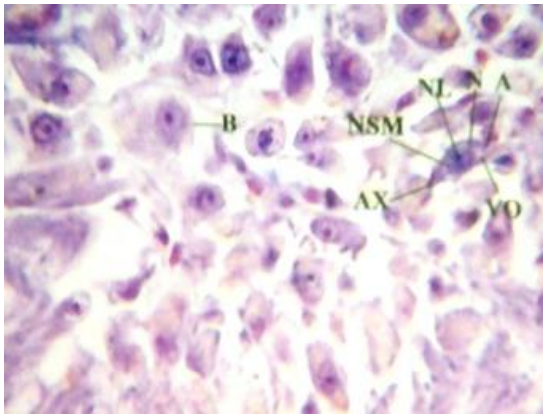
(I) Control (II) Ablation of cerebral ganglia



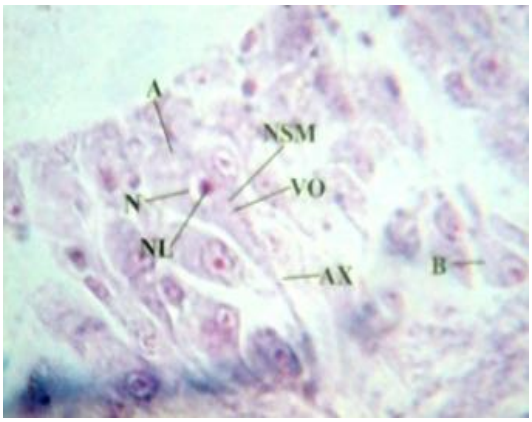
(III) Injection of cerebral ganglionic extract

A = Type A cell; B = Type B cell; N = Nucleus; NL =Nucleolus; NSM = Neurosecretory material; AX =Axon; VO =Vacuole

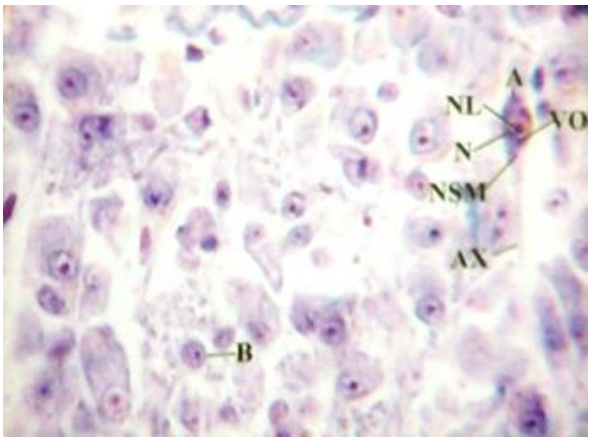
Fig. 2: Histological changes in the neurosecretory cells of visceral ganglia on 12th day due to ablation of cerebral ganglia and injection of their extracts in *Lamellidens corrianus*.



(I) Control



(II) Ablation of cerebral ganglia



(III) Injection of cerebral ganglionic extract

A = Type A cell ; B = Type B cell; N = Nucleus; NL =Nucleolus; NSM = Neurosecretory material; AX =Axon; VO =Vacuole

Fig. 3: Histological changes in the neurosecretory cells of pedal ganglia on 12th day due to ablation of cerebral ganglia and injection of their extracts in *Lamellidens corrianus*.

In the present study, histological examinations of cerebral ganglia revealed accumulation of neurosecretory material in Type A cells, also vacuoles were conspicuous. The nuclei and nucleoli were more prominent, Type B cells revealed less accumulation of neurosecretory material. In visceral ganglia Type A cells showed more accumulation of neurosecretory material. Nuclei and nucleoli stained conspicuously. Type B cells showed less neurosecretory material, the nuclei and nucleoli stained less conspicuously.

In pedal ganglia the secretary material in Type A and Type B cells rarely accumulated. Removal of cerebral ganglia caused pronounced changes in the tinctorial properties of both the type of cells from the visceral and pedal ganglia of cerebral ganglia ablation and injection of their extract to ablated bivalves. Removal of cerebral ganglion caused decreased in the neurosecretory material from both the type of cells in another cerebral ganglion during experiments. In visceral ganglia, ablated group showed decrease in the neurosecretory material in both the type of cells but in extract, injected group the material accumulated in Type A cells.

DISCUSSION

The freshwater bivalve molluscs are suspensory feeders on the primary stage of food chain, hence they notably influences the organization and functioning of ecosystems[17]. A relation between reproduction and the neurosecretory cells in the cerebral and visceral ganglia of *Mytilus* and of *Chtamys* has been suggested[18], in these animals, which may releases gametes two or three times during the course of the reproductive period, cerebral neurosecretory material is observed to disappear in some or all of the cells several days prior to each discharge of the gametes. The material is replenished between successive spawning, and the cells become depleted completely by the end of the reproductive period. Emission of the gametes may depend on the lifting of an internal inhibition correlated with the disappearance of neurosecretory material from the cerebral ganglion.

From a functional point of view, earlier researcher[3] suggest that a relationship might exist between the neurosecretory cells in the cerebropleural and visceral ganglia and reproduction in two marine mussels, *Mytilus edulis* and *Chamys varia.* During the period of gamete maturation, it was found that cerebral neurosecretory material accumulates within the perikaryon and is discharged just prior to the extrusion of gametes. This pattern of cell synthesis and release is repeated before each subsequent gamete evacuation. Surgical removal of the cerebral ganglia has little effect on maturation of the gonocytes, but accelerates their discharge. It had been concluded that the cerebroplural ganglia in some way inhibited spawning until a few days before the beginning of the reproductive period, at which time this inhibition was removed coincident with the discharge of neurosecretory substances[3]. The transport of this material may be via axons directly to a target site, thus insuring stimulation of the correct structure at the appropriate time. The mussel then becomes sensitive to the correct environmental stimuli with completion of the spawning process.

However, hormones may also be involved. Researcher[19] have studied, in *M. edulis*, the effect of various nervous centers on the storage cells (CG (cerebral ganglia) and adipogranular cells) and the gonad located in the Mantle[18] and [19]. In a hormonal organ culture medium, pieces of mantle tissue are difficult to maintain. Adding of cerebral ganglia induces the start of lysis of the CG (cerebral ganglia) and adipogranular cells, which in normal animals accompanies gametogenesis. The sex cells seem to continue their development[20]. Addition of visceral ganglia to cultures of mantle tissues, however, prevents lysis and induces an increase in reserves comparable to that observed in field specimens collected after the reproductive season. Gametogenesis stops under these conditions. It has been conclude that the visceral ganglia release a neurohormone, which stimulates the storage activity of the CG (cerebral ganglia) and the adipogranular cells, whereas the cerebral ganglia produce a neurohormone with a mobilizing action on the reserves, which is needed for the survival and development of the gametes[19]. Thus, in bivalves, the storage activity in the mantle depends on the concentration of metabolites, such as glucose, in the haemolymph (i.e. on the feeding condition) and on the action of neurohormones. Neurosecretory hormone or hormones involvedin regulation of physiological process like oxygen consumption and play a vital role in it[21].

Different categories of NSCs have been distinguished based on their size and morphology. In *Mytilus edulis* and *Chlamys varia* some NScs are pear-shaped, unipolar and upto 25 µm, while others are small and multipolar[18]. Pear-shaped (Type I) and oval-shaped (Type II) NSCs were distinguished in *Crassostrea virginica* and *Meretrix casta*[16, 22] and *Katelysia opima*[23]. Different categories of NSCs have also been reported in the freshwater mussel, *Unio tumidus*[12]. In the present study, all the ganglia of *Lamellidens corrianus* showed two types of NSCs which are in accordance with those observed in *Teredo*[11], in *Mytilus* and *Chlamys*[13], in *Crassostrea virginica*[16] for Type A cells and in *Crassostrea virginica*[16] and in *Katelysia opima*[23] for Type B cells.

The appearance and position of neurosecretory products within the perikarya vary with the stage of the secretion[3, 11, 13, 24, 25]. In some cells, neurosecretory granules are few, while in others they are abundant and remain discrete. In still other cells, neurosecretory products are present in lumps or pools. The discharge of neurosecretory products is characterized by cytoplasm and the presence of small quantities of secretary products between the vacuole and axon hillock[26]. Signs of axon transport are not very distinct in marine bivalves. Neurosecretory products have been observed in the axon hillock and proximal parts of the inter-ganglionic paths of the axons, but they disappear in the neuropile and are not seen in the communicative branches, commissures or nerves leaving the ganglia.

Investigation by worker[18] demonstrated distinct annual neurosecretory cycle in the pear-shaped NSCs of the cerebral ganglia in temperate species, *Mytilus edulis and Mytilus galloprovincialis*. The annual neurosecretory cycle and gametogenic cycle in these mussels appear to be closely correlated. Secretary material is accumulated in the cerebral ganglia during gametogenesis and evacuated from the cells, when the gametes become fully mature. The small multipolar neurons and the NSCs of the visceral ganglion in these muscles showed continuous activity throughout the year. These observations were confirmed in oyster *Crassostrea virginica*[6, 27].

CONCLUSION

In the present study a close relationship between the gonad maturation and neurosecretion in Type A cells from cerebral ganglia can be made, during the period of early gametogenesis the neurosecretory material, begin to accumulate. In the present study, removal of cerebral ganglia might have exerted a stress effect on visceral ganglia and thereby on nerves of the gills causing accelerated release of swarms with more mucus. After a lapse of 7 days *Lamellidens corrianus* after cerebral ganglia removal did not show any change in the degree of shell valves gaping and protrusion of pallial edges, siphons and feet when compared to controls.

The experiments carried out on *Lemellidens corrianus* by removing cerebral ganglia and giving cerebral ganglionic extracts to normal intact, revealed pronounced changes in both the type of NSCs from the existing ganglia. In visceral ganglion, the cell length of Type A cells decreased in cerebral ganglia ablated group and increased in cerebral ganglionic extract injected group. During experiments nuclear diameter decreased in ganglia ablated and extract injected group.

Thus, due to cerebral ganglia removal, visceral and pedal ganglia exerted stress effect in functioning of the hormonal regulation and this stress varied from one ganglion to another. The removal of cerebral ganglia accelerated the growth of gametes and injection of their extracts gradually increases the growth. This was more pronounced in cerebralectomized animals that extract injected ones. These aspects include the regulation by neurohormones/neurohumors of normal physiological functioning and homeostasis. It is tentatively suggested that the cerebral and visceral ganglia in bivalves molluscs, including *Lamellidens corrianus*, elaborate some principles including neurohormones, neurotransmitters and other unknown factors which trigger the metabolic demands of animal during different reproductive phases.

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