ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (119-124), Month: April - June 2018, Available at: www.researchpublish.com

Ultramicroscopic Changes in the Secretory Dynamics of the Neurosecretory Cell in the Earthworm *(Metaphire Peguana)*

[1]Trijit Nanda, [2]Malabika Bhattacharjee

[1]Assistant Professor, [2] Head and Assistant Professor,
[1,2]Department of Zoology, Vivekananda College, Thakurpukur, Kolkata, India

Abstract: **As regards to regeneration of NSC_s in the developing cerebral ganglion scientists opined that this process occurs through evolution similar to the sequence of changes with normal growth, de novo by the mitotic proliferation and differentiation of unspecialized cells (neoblast) already present in the central nervous system.Earthworm through their activities of feeding, burrowing and casting activities modify the physical, chemical and biological properties of soil, this supporting above ground vegetation. Soil physical property affected include aggregate stability and porosity, while soil biological and chemical properties modified includes nutrient cycle, formation of plant-available nutrients, organic matter dynamics, pH, microbial and faunal activities, decomposition rate etc. Consequently above ground plant production may be affected by activities of earthworms in which three main ecological groups can be recognized: epigeic, anecic, and endogeic. The SEM observations on both the sub and supraoesophgeal ganglia of the species under study are made. This has the relevance with reference to morpho-anatomical relationship of cellular complements in relation to the intraganglionic capillaries and accumulation of NSM –neurosecretory materials within ganglion in question.The brain represents the detailed surface profile with particular reference to its posterodorsal region. Indeed ring like elevation from where divergent of processes constitute the vascular complex clarifies the extensive vascularization of the cerebral ganglion. In case of suboesophageal ganglion surface conFiguration shows both depression and elevation.**

Keywords: **Neurosecretory Cells (NSC), neoblast, mitotic proliferation: epigeic, anecic, and endogeic.**

1. INTRODUCTION

The importance of central nervous system in the phenomenon of oligochaete regeneration has been elucidated by several classical investigators [1]. These observations have been substantiated by Farber [2] reiterated that neurosecretion of the VNC has a profound in the segmental regeneration of *L. terrestris*. In her detailed analysis Herlant-Meewis [3] opined that C_3 cells of each segmental ganglion exhibit spectacular cytological response to the loss of anterior segments. Synchronous release and synthesis of NSM in the ganglia immediately proximal to the level of amputation of either anterior or posterior segments in ***E. foetida*** have been recorded by Marcel [4] who also concluded that neurosecretory system promotes some aspects of regeneration. Nanda and Chaudhuri [5] investigated the extent of histomorphic changes in the neurosecretory system of the ventral ganglionic complements following anterior amputation. They have also recorded the sequential reactive response in the ganglia concerned. The secretory dynamics in the present investigation ensures synthesis from rough endoplasamic reticulum, packaging by the golgicomplex and release, axonal transport to the next neurosecretory cells.

2. MATERIALS AND METHODS

Healthy adult worms of average length 120mm were collected from neighbour hood of Kolkata and acclimated in the laboratory in the two temperature regimes- 28^0C and 35^0C respectively. (**Fig.1)**

- Anterior transaction of 5 segments for requisite number of specimens were made by sterilized paragon knife at one stroke.
- Placement of the amputed individuals within the petridish was done carefully so that the provision for the free access to the air may be possible following the closer of the lids [5].

- The operated individuals were left for 2 weeks for the normal growth of regeneration buds as well as subsequent segment proliferations.
- However controls were maintained all through.
- Regenerants of 10,21,35,49-days old were chosen for morphological studies of regeneration buds and their fate, anatomical studies of the nerve ring and sequential cytomorphic topography of neurosecretory elements in the regenerated cephalic ring
- Incidentally it may be stated that sometimes the oldest regenerants were considered on the basis of similar regenerative progress.

2.1 ULTRAMICROSCOPY:

2.1.1 SCANNING ELECTRON MICROSCOPY (SEM)]:

1. FIXATION:

a. Fixation prior to the superficial incision of the nerve ring of ***M.peguana*** was done in 2.5% gluteraldehyde with 0.2 M phosphate buffer (Ph-7.0) initially for 2 hours

b. Fixation after superficial incision

The nerve ring was subjected to superficial slicing at a frontal profile by means of sharp knife inorder to expose the internal components. The prolonged treatment (fixation) with 2.5% gluteraldehyde in 0.2M phosphate buffer for another 24 hours at 4^0C was done.

2. DEHYDRATION:

After fixation the samples were washed 3-4 times in 0.2 M phosphate buffer and dehydrated through graded alcohols as well as cold absolute acetone.

3. CRITICAL POINT DRYING (CPD):

The tissues were then dried to CPD by the isoamyl acetate and carbon –di-oxide at 31.7^0C and 71.9mm atmospheric preassure.

4. METAL COATING:

Properly dried samples were physically fixed on aluminium stubs with help silver DAG-915. The samples were the coated with gold palladium alloy (thickness $180A^0$) through spatter coater.

The samples were now ready for observations under HITACHI -530SEM

2.1.2. TRANSMISSION ELECTRO MICROSCOPY (TEM)

1. FIXATION:

The brain of the species under investigationwere dissected out and immediately fixed in 3% gluteraldehyde in o.1 M phosphate buffer (pH7.2) for three hours at 4^0C. The samples were washed in 0.1M phosphate buffer three times in 10 min interval for each.

2. POST FIXATION:

The samples were then post fixed in 1%osmium tetroxide (OSO_4) in 0.1M phosphate buffer at 4^0C for 3 hours.

3. DEHYDRATION:

The tissues were then dehydrated in the graded ethanols as follows:-

30% Ethanol	-15 minutes
50% Ethanol	-15 minutes
70% Ethanol	-15 minutes
90% Ethanol	-15 minutes
100% Ethanol	-45minutes (2 changes)

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (119-124), Month: April - June 2018, Available at: www.researchpublish.com

4. CLEARING:

The tissues were cleared by graded propylene oxide (with ethanol) as follows:-

30%Propylene oxide	20 minutes
50%Propylene oxide	20 minutes
70%Propylene oxide	20 minutes
90%Propylene oxide	30 minutes
100%Propylene oxide (2 changes)	45 minutes

5. INFILTRATION:

The dehydrated tissues were transferred to ascending grades of propylene oxide plastic mixture accrding to the following schedule:-

5.1. PREPARATION OF PLASTIC MIXTURE:

5.1.1. Solution A- the combination of the following mixture:

1.	Epon 812	-25 ml
2.	Araldite 600	-5-15 ml
3.	Dibutylpthalates	-3 ml
Mixed thoroughly by continuous stirring in a magnetic stirrer for 1 hour at room temperature.		

5.1.2. Solution –B Ddecenyl succinic anhydrite:

5.1.3. Working plastic Mixture:

1. Solution A	-9 Parts
2. Solution B	-11 Parts
• Both of these are quickly mixed by stirring with a clear glass rod. To this mixture 20-30 drops(1-15) drops per ml of DMP-30 (Dimethyl-monomethyl-phenol) were added.	
• This plastic mixture along with the propylene oxide is mixed in the following ratio:	
Propylene-plastic mixture (Ratio)	**Time For Infiltration (Hours)**
2:1	1
1:1	1
1:1	2
Pure Plastic Mixture	2

2.2. SECTIONING:

The blocks were carefully trimmed and initially 1µm thick sections were cut on a LKB ultratome 8800 using glass knives and stained with 1% toluidene blue for section of desired area from which section of ultrathin sections could be made. There after the selected areas of the tissues were trimmed into a smaller pyramid and ultrathin frontal sections (300-500Å) and individually collected on 400mesh copper grids pre cleaned within HCL and water and finally dried with alcohol.

2.3. STAINING:

The ultrathin sections mounted in the grids were stained for 30 minutes with 1% uranyl acetate and for 5-10 minutes with lead citrate in co_2 free atmosphere and finally washed clearly in triple distilled water and air dried.

2.4. PHOTOGRAPHY:

The sample were examined under **JEOL CX100 TEM** at accelerating voltage of 80KV and photographs are taken as per need.

3. RESULTS AND DISCUSSION

3.1. SCANNING ELECTRON MICROSCOPIC STUDIES (SEM) [CYTOMORPHOLOGICAL ANALYSIS]

3.1.1. SUBOESOPHAGEAL GANGLION:

The overall pattern in the distribution of cellular complements resembles with the cerebral ganglion. Indeed both large and small types of cells with undulating surfaces are visible especially at the lateral wall of the ganglion (**Fig. 2a**). Besides such types of cells are also aggregated towards the bifurcating edges of the ganglia. Orientation of the cells are especially

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (119-124), Month: April - June 2018, Available at: www.researchpublish.com

remarkable at the median axis of the ganglion (**Fig.2b**). The morphological nature of all these cells demonstrate the neurosecretory nature, although clear intracellular profile is not vivid (**Fig.2a**). Axonal processes may be understood but individual identity is not always confirmed **(Fig.2b**). Overall cellular architecture gives the impression of three dimensional profile contained by the ganglionic complements.

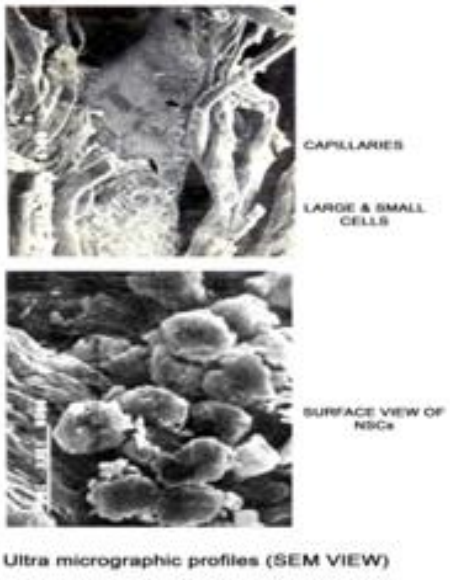
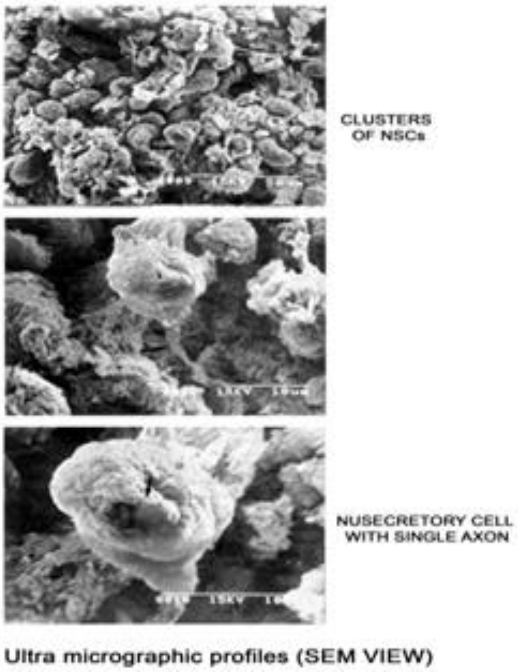


Fig. 1 **Fig. 2a, 2b [SEMX4000]**

3.1.2. SUPRAOESOPHAGEAL GANGLION:

Neurosecretory cells are in the form of clustures are well discernible (**Fig. 3a**). The cell profile represent variable appearance owing to corrugated outline of their surface. Moreover impression of the axonal processes in some cells confirms axoplasamic flow on the basis of their monoliform contour. Nuclei are distinguishable because of sharp contrast from that of cytoplasamic area (**Fig. 3b**). Detailed observations farther reveal that the brain process in both large and small cells with impression of their respective nuclei as well as the typical pattern of distribution (**Fig. 3b**).



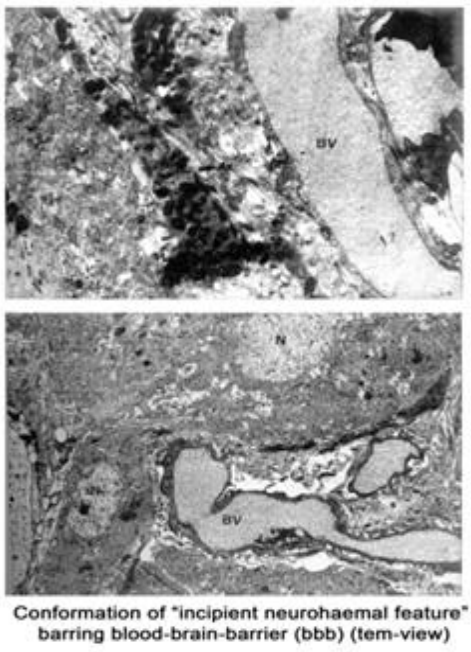


Fig.3a, 3b [SEMX4000] **Fig.4a, 4b [TEMX6000]**

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (119-124), Month: April - June 2018, Available at: www.researchpublish.com

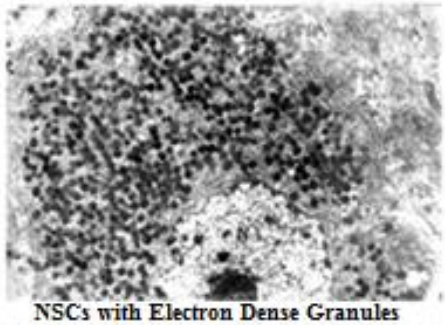
3.2. TRANSMISSION ELECTRON MICROSCOPIC STUDIES (TEM):

Both supra and suboesophageal ganglia reveal that the NS elements possess electron dense granules which are in the various state may be within the perikarya, at the axon hillock region or terminal ends of the axons. The close relationships of the axon terminals and the intraganglionic capillaries and NSM enriched are well conceived. Evidence for exocytosis are not so frequent but approximation of axon terminals and intraganglionic capillaries ready to receive electron dense particles is quiet clear. Incidentally the major part of investigations are the cells of large dimensions since the clarity of all the organelles are more spectacular.

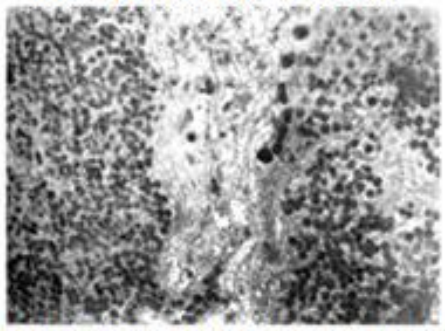
3.3. CYTOMORPHOLOGICAL STUDIES:

3.3.1. *SUPRAOESOPHAGEAL GANGLION:*

The neurosecretory cells in appearance in the electron micrograph are mostly enriched with electron dense granules having variable dimensions. Besides these the quanta of granules reveal graduation in their electron density. Fluctuation in the density of granules does not appear to be correlated with their size. Majority of these granules are detectable in the vicinity of the nucleus and remain more electron dense (**Fig. 4a**). The neurosecretory granules contain moderately dense homogeneous cores and represented rounded clustures (**Fig.4b**). Secretion rich granules are identifiable within the perikarya because they not only accumulate in the cell body but also along NS fibres. The axon terminals too in some cases may contain large amount of electron dense particles. Other organelles in varying quanta are visible along with some vacuoles. A few cells contain no granules or vacuoles but are entirely filled with ergatoplasm (**Fig.4b**).

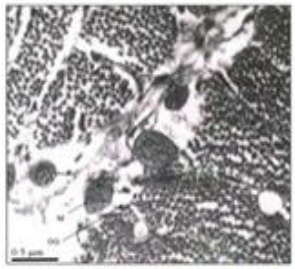


NSCs with Electron Dense Granules



Adjacent NSCs with their respective Electron Dense Granules

Fig.5a [TEMX8300]



SUPRAOESOPHAGEAL GANGLION. TEM FIGURE showing arrangement of Mitochondria and Electron Dense Granules before release of Secretion

SUPRAOESOPHAGEAL GANGLION. TEM FIGURE showing Electron Lucent and Electron Dense Granules side by side

Fig.5b [TEM X8300]

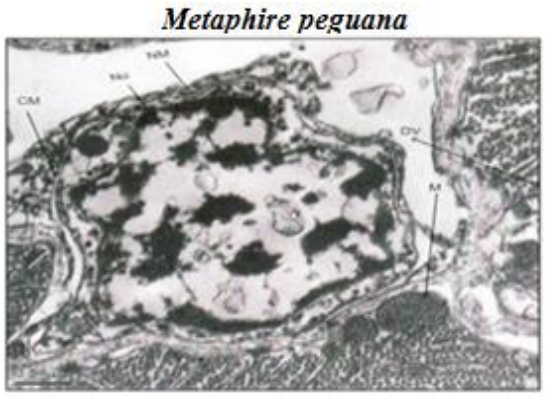
3.3.2. SUBOESOPHAGEAL GANGLION:

A close resemblance of the electron micrographic profile with the supraoesophageal ganglion has been confirmed. Generally availability of the electron dense granule in the vicinity of the nuclei is not so extensive as observed in the cerebral ganglion. In some cases neurosecretory cells contain huge amount of secretory granules of variable sizes within the perikarya but the electron density are not so high as observed in the supraoesophageal neurosecretory cells. Instances of migration of the granules along the axonal processes has also encountered. (**Fig.5a** and **Fig.5b**).

3.3.3. SUPRA OESOPHAGEAL GANGLION:

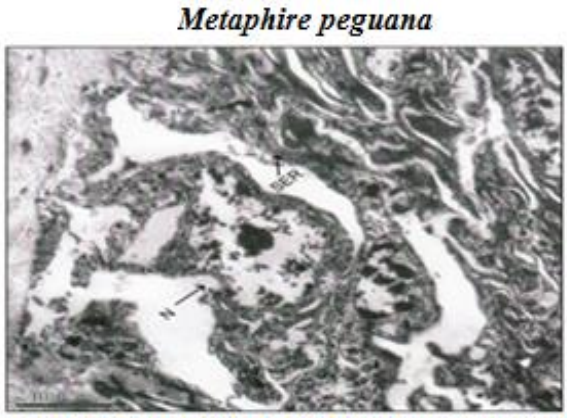
In general neurosecretory cells contain large nuclei with prominent nucleoli. Nuclear membrane may show small clefts all around. In some instances Golgicomplex may demonstrate packaging activities of the (**Fig.6a** and **Fig.6b)**

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (119-124), Month: April - June 2018, Available at: www.researchpublish.com



SUPRAOESOPHAGEAL GANGLION: TEM VIEW-
Large Nuclear Dimension with Defined Nuclear Membrane, Dense Vesicle Mitochondria and Cell organelles

Fig.6a [TEMX8000]



SUPRAOESOPHAGEAL GANGLION: TEM VIEW-
SER and part of Nucleus

Fig.6b [TEM X8000]

4. CONCLUSION

Ultrastructural studies on the neurosecretory cells of the cerebral ganglia of several species of oligochaetes indicate the possibility of hormone secretion from their cellbodies and release modulators / transmitters from their axon endings atleast from some of the neurosecretory cells [6]. Indeed clear cases of release from the perikarya into the extracellular space or directly into the blood vessels have been documented in the brain of the earthworm.Indeed clear cases of of release from the perikarya into extra cellular space is due to exocytosis from the cell bodies. Peripheral abundance of neurosecretory materials in the neural lamella following their release from the superficial cortical cells is striking. According to Tombes [7], the neural lamella serves as a acellular diffusion and possible reservoir for neurosecretory products. The existence of neurohaemal organ in the earth worm too is better fit in this general scheme than would the intracereblar location previously thought to exist. Be that as it may in the absence of well developed neurohaemal organ in *M.peguana* there exist a phenomenal amassing of neurosecretory granules in the accumulation zone and the margin of the neuropile or tissue space in the viscinity of the blood capillaries or beneath the neural lamella.

REFERENCES

[1] Morgan T.H. 1902. Experimental studies of internal factors of regeneration in earthworm Arch. Entw- Mech 14, 562-591

[2] Farber, P.A. 1965. neurosecretory activity and anterior regeneration in *Lumbricus terrestris* Anat. Rec. 151, 348.

[3] Herlant-Meewis, H. 1962. Neurosecretory phenomena during regeneration of nervous centers in *E. foetida.*Neurosecretion (Heller, H and Clark R.B.ed.) Academic Pres, New York. Pp267-274.

[4] Marcel, R. 1973 *A. cycles* secretaries de cellules de la chaine nerveuse au cours de la regeneration chez *Eisenia foetida* Sav.f . typical (Annelide, Oligochaete) Gen.Comp.Endocrinol.21,45-49

[5] Nanda, D.K. and Chaudhuri, P.S. 1983. Regeneration of the neurosecretory system of the nerv ring in earthworm *Metaphire peguna* Acat. Biol. Cracov 25, 63-67

[6] AL-Yosuf, S.A.H.1988. Distribution and ultrastructure of the neurosecretory cells in the cerebral ganglion of the earthworms.J.Mophol.,197 :1-20

[7] Tombes,A. S 1977 Type 2 Neurosecretory axons at the base of cerebral ganglion of *Perineris cultrifera* Grube (Annelida: Polychaeta) Gen.Comp. Endocrinol., 32(4): 407-410