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Chapter 13

Neurosecretory Responses of Native, Endogeic Earthworm *Metaphire posthuma* (Vaillant, 1868) to Sub-acute Hyperthermic and Hypothermic Stress

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Abstract

Temperature is regarded as an important factor that influences the growth and reproduction of earthworms. During summer, earthworms experience hyperthermic stress, while in winter, they undergo hypothermic stress. Topsoil, endogeic, native earthworm *Metaphire posthuma* [1], when subjected to sub-acute hyperthermic stress (35°C) as well as hypothermic stress (4°C), both types of neurosecretory cells (NSCs; A and B cells) show significant neurosecretory changes. Under sub-acute hyperthermic stress, exudation of profuse mucus and coelomic fluid from the earthworm's body was noticed, leading to decreased body weight. The sub-acute hyperthermic stress also exerted the presence of cytoplasmic vacuolation as well as depletion of neurosecretory materials (NSM) in both types of NSCs, followed by axonal transport of NSM to the "zone of accumulation". Under hypothermic stress, exudation of a small amount of mucus and ejection of scanty coelomic fluid were noticed. The hypothermic stress exerted an increase in the number of deeply stained Type A cells in the cerebral ganglia, besides having partial depletion of nuclear volumes and retarded axonal transport. Accumulation of NSM in the NSCs was also noticed, while the zone of accumulation showed scanty distribution of NSM. The results indicate that the impact of thermal fluctuations is mediated through supra-oesophageal ganglionic NSCs (both type A and type B cells) of *Metaphire posthuma* [1] as evidenced by histo-morphic variations and changes in secretory dynamics, furnishing a working hypothesis for their 'adaptive value' to thermal gradient.

Keywords: Thermal stress, *Metaphire posthuma*, Neurosecretory cells, Cerebral ganglion, Earthworm.

1. Introduction

Earthworms, the crucial components of the soil ecosystem, are included under the order Oligochaeta of the phylum Annelida [2]. They play a vital role in food webs and are considered soil bioengineers and rejuvenators [3]. According to ecological categories earthworms are of types: epigeic, endogeic and anecic species. Due to oxidation of soil organic carbon, most of the Indian earthworms are considered to be endogeic. Top soil endogeic earthworms are subjected to various kinds of physical stresses including the thermal stress. Temperature is regarded as one of the most important factors controlling growth and reproduction in animals [4]. Earthworms are subjected to a hot summer condition from March to May in the Indian subcontinent, when the high surface temperature is much more limiting to earthworms than low temperature [5] which they face during the winter from January to February. During winter the surface soils become cold and the earthworms move deep inside the soil [4]. When the earthworms are subjected to such kind of stress, they respond through cytological changes in the CNS – NSCs [4].

Earthworms are lower group of coelomate animals that lack endocrine glands and neurohaemel organ. The CNS due to its rich vascularisation and presence of enormous number of neurohormone producing NSCs acts as endocrine gland. Their CNS comprises of paired cerebral ganglia, paired subesophageal ganglia and ventral nerve cord. In earthworms, NSCs are of two types, peptide secreting type A and amine secreting type B cells. The deeply stained type A cells outnumber the lightly to moderately stained type B cells in the earthworm brain. In contrast, the type B cell density dominates in the subesophageal and ventral nerve cord ganglia. Beneath the cortical tier of NSCs

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(type A & type B) lie the medulla or neuropile made up of axons coming out of NSCs and ordinary neurone. [6]. As they do not possess any epithelial endocrine gland, any kind of physiological activity such as thermal acclimation is under the control of neurosecretion [7, 8].

Since there are no reports in the literature on the neurosecretory response of the cerebral ganglia of the *Metaphire posthuma* [1] when subjected to thermal stress, we aimed to study the cytomorphological changes in the cerebral neurosecretory system in thermal stressed topsoil, endogeic, native earthworm *Metaphire posthuma* [1].

2. Methodology

Adults of *Metaphire posthuma*, were collected from soil and brought to the laboratory. In the laboratory, these worms were acclimatized (temperature 28°C and RH 80%) and cultured in an earthen pot having moist soil. Sprinkling of water into the soil was done periodically to maintain the natural condition of the soil bed. After a week-long acclimation in an earthen pot under laboratory conditions, worms were taken out for the experiment and were divided into 3 groups, each group comprising 5 individuals. The worms of Group I were narcotised in 10% alcohol and were carefully subjected to head amputation in the laboratory for further histological processing, which acted as the control. The worms of group II were placed in the incubator maintaining 35°C for 45 minutes and then at room temperature for 15 minutes. The worms of Group III were put inside the refrigerator with an average temperature of 4°C. After 45 minutes of exposure in the refrigerator at 4°C, followed by 15 minutes at room temperature. Then the worms of Group II and III were carefully sacrificed in the laboratory by carefully beheading till 6^{th} – 8^{th} segment. The mean weight of each group's earthworm was measured before and after conducting the experiment. The amputated heads were then immersed in the Bouin's fluid in small glass vials for 18 hours for proper fixation of the tissue [9]. Tissues were dehydrated in gradations of alcohol, cleared in xylene and embedded in paraffin wax (58–60°C). Serial transverse sections (7 μm thick), stretched on glass slides were stained with Gomori's Chrome Alum Haematoxylin Phloxin (CAHP) [10] and simplified Paraldehyde Fuchsin (PAF) [11]. The stained slides containing Cerebral ganglion were observed and studied under compound microscope and micro photographs were taken with the help of digital camera.

3. Results and Discussion

3.1. Behavioural and physiological changes

Earthworms kept under sub-acute thermal stress (35°) initially showed restless movement, exudation of mucus and coelomic fluid from the body. These changes resulted in lowering of mean individual body weight (gm).

Aggregation of worms together was observed when subjected to 4°C in the refrigerator. Movement was reduced after 7 minutes and completely stopped after 15 minutes. Exudation of little amount of mucus and ejection of scanty coelomic fluid were noticed.

Figure 1: Showing the earthworms of Group II before sub-acute hyperthermic stress (35°C)

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Figure 2: Showing the earthworms of Group II after sub-acute hyperthermic stress (35°C)

Figure 3: Showing the earthworms of Group III before hypothermic (4°C)

Figure 4: Showing the earthworms of Group III after hypothermic (4°C)

3.2. Changes in bodyweight

Loss in body weight was seen in earthworms of Group II and Group III. The loss of body weight in both cases was observed due to the exosmosis of water from the body into the surrounding environment.

Table 1: Showing decrease in the bodyweight (%) of experimental earthworms

	The average weight of each worm (gm)		Changes in body weight (gm)	Decrease in body weight (%)
	Initial weight (n=5)	Final weight (n=5)		
Group II	1.58	0.92	0.66	41.7
Group III	1.14	0.86	0.28	24.5

3.3. Histomorphic Changes in Neurosecretory system

Control

Cerebral ganglia of *Metaphire posthuma* kept under normal laboratory temperature 27°-29°C showed occurrence of 2 types of neurosecretory cells- deeply stained type A cell and light to moderately stained B cell. Type A cells occur as a compact tier of cells beneath the perineurium. Type A cells are generally pear shaped or oval shaped cells with axon terminating towards neuropile. Type B cells which are larger than the A cells, lie in between tier of deep stained cells at the centre of neuropile. Neuropile is fibrous in nature and is made up of axon coming out of NSCs and some ordinary neuron. Rich vascularization is noticed in the cortical tier of cell at central neuropile.

Zone of accumulation lies in between cortical tier of cells (both type A and type B) and central neuropile. Neurosecretory axons coming out mostly from the NSCs transport neurosecretory materials at the margin of neuropile. The NSCs (Type A and Type B) are in various stages of secretion. In type A cells colloidal secretion and in the moderately stained cells fine coarse secretory materials is noticed. Besides this, type B cells show numerous cytoplasmic vacuoles.

Table 2: Showing the properties of type A and type B neurosecretory cells that are observed in the cerebral ganglion of *Metaphire posthuma*

Cell types		Cell shape	Arrangement and location	Cytoplasmic characteristics	Staining affinity of NSCs		Axonal transport	Chemical nature of secretion	Physiological characteristics
					CAHP	AF			
A	A1	Ovoid with more than one axon. Generally, one axon directed towards the perineurium.	One or two layered. Just beneath the perineurium, dorsal and postero-lateral in position. Distributed throughout the CNS.	Colloidal with ill-defined nucleus. No cytoplasmic vacuole. No secretory cycle.	Strong affinity, dark blue.	Strong affinity, dark purple.	Well demarcated.	Peptide in nature.	Growth and reproductive cycle, thermal acclimation, osmotic balance.
	A2	Typical pear shaped, unipolar or bipolar. Axon generally terminates towards neuropile. Larger than A1 cells.	Multilayered (3-6). Below A1 cells. Outnumber all types of NSCs. Distributed throughout the CNS.	Colloidal with well-defined nucleus and occasional cytoplasmic vacuole. Distinct secretory cycle.					
B		Variable in shape. Apolar or unipolar. Axons may or may not be present.	Dorso-lateral as well as ventral in location. Number much less than A type cells. Located in between cortical A cells and central fibrous neuropile. Found throughout the cerebral ganglia.	Fine granular cytoplasm with abundance of cytoplasmic vacuoles. Distinct secretory cycle.	Light to moderate affinity, Light blue.	Light to moderate affinity, Light purple.	May or may not be present, perikaryal discharge often noticed.	Amine in nature.	Regeneration, thermal acclimation, osmotic balance, metabolism.

3.4. Experimental

Group II: (After 45 minutes of sub-acute hyperthermic stress)

Sub-acute hyperthermia in *Metaphire posthuma* led following changes in cerebral neurosecretion-

1. Massive to marginal depletion of neurosecretory materials in both type A and type B cells.
2. Axonal transport of NSM to the ”zone of accumulation” was noticed in both type of cells.
3. Both the types of cells showed cytoplasmic vacuolation.
4. Axon oriented voluminous nuclei was noticed.
5. Accumulation NSM in the extra cellular space and also blood capillaries were noticed.

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Exposure to high temperature triggers metabolic activity in the worm, which in turn stimulates NSCs to promulgate an accelerated rate of NSM release than the rate of Synthesis. This concentration may be followed by intensification of cytoplasmic vacuolation, leading to depletion and enlargement of the nuclear volume. Cytoplasm vacuolation coupled with depletion of Neurosecretory cells and axonal transport of NSM indicate utilization of neurosecretion to prevent loss of water and mucous from the body for their survival. Indeed, exposure to high temperature result in water loss through desiccation factor. So, if the elaboration of antidiuretic factor [12] by these hormonogenic cells is considered, the phenomenon of quick NSM release goes down since worms are threatened with severe desiccation in the course of sublethal hypothermic stress. Although the rate of type A NSC is confirmed during thermal acclimatization, the role of type B cell is not been confirmed but involvement of cells in neurosecretory mediation in the event of metabolic compensation is obvious [12]. During summer-heat the surface soils become dry and the poikilothermal top soil earthworms face hyperthermia stress as they cannot move away quickly due to less mobility. The physiological features of earthworms due to hyperthermia stress on earthworm simulates those of dehydration, the neurosecretory changes are generally similar in the two cases. So, during extreme summer heat, NSCs (chiefly type A cells) show the signs of depletion.

Group III: (After 45 minutes of hypothermic stress)

Hypothermia in *Metaphire posthuma* led following changes in cerebral neurosecretion-

1. Accumulation of neurosecretory materials (NSM) in the neurosecretory cells (NSCs) followed by partial to major depletion in NSCs irrespective of their type.
2. Distinct appearance of A-cells at periphery of cortex and beneath the neuropile.
3. Axonal transport of NSM at the margin of neuropile is visualized.
4. Nuclear volume increased as studied visually.
5. Zone of accumulation shows scanty distribution of NSM.

During the winter, earthworms move deep inside the soil and become inactive with metabolism running at a low gear. So NSCs in the 'winter brain' of earthworm become loaded with neurosecretion in contrast to their 'summer brain'.

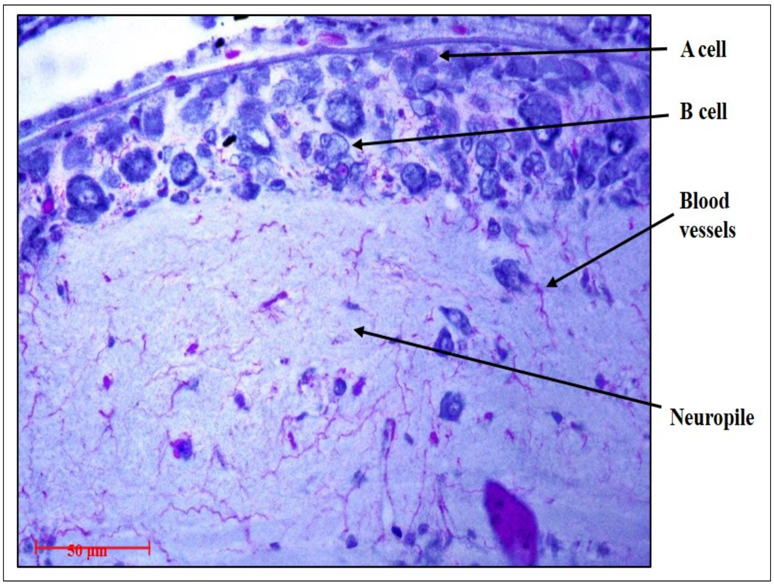

Figure 5: Frontal section Showing A cell, B cell, blood vessels and neuropile in cerebral ganglia of *Metaphire posthuma* in the control earthworms (CAHP, X400)

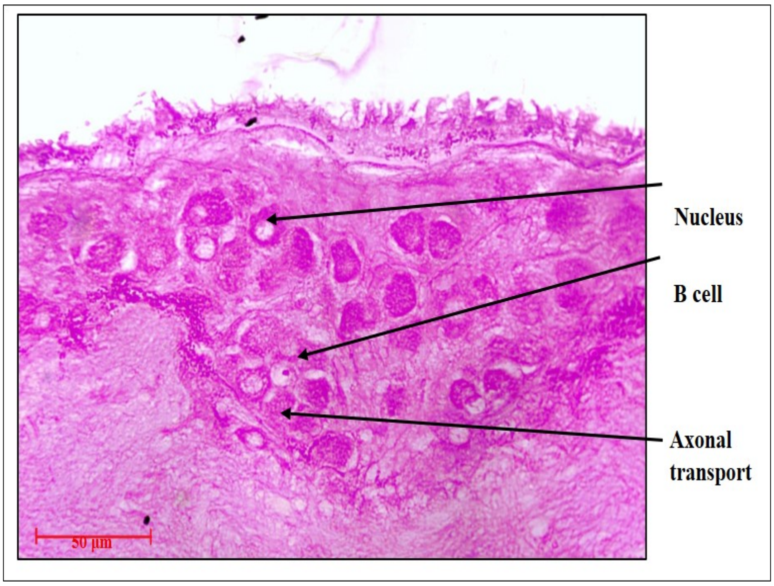

Figure 6: Frontal section showing B cell, nucleus and axonal transport in cerebral ganglia of *Metaphire posthuma* in the control earthworms (PAF, X400)

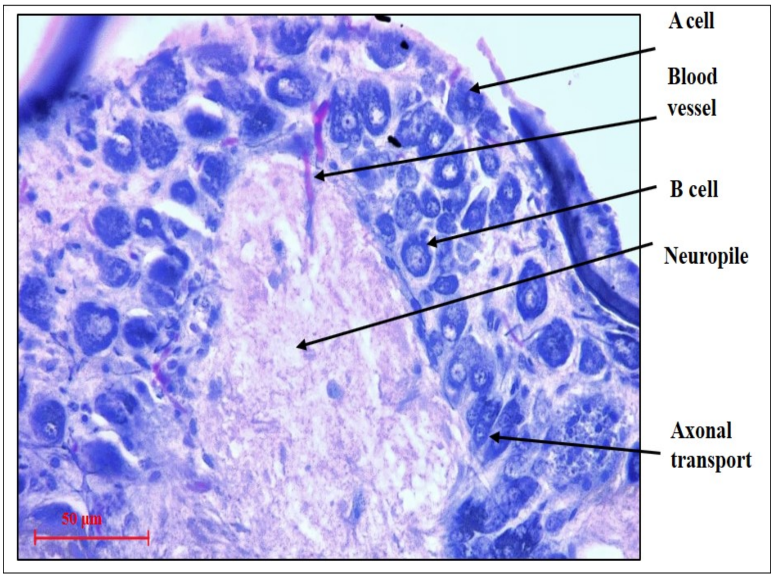

Figure 7: Frontal section showing A cells, B cells, blood vessels, neuropile and axonal transport in cerebral ganglia of *Metaphire posthuma* after 45 minutes of sub-acute hyperthermic stress (35° C) (CAHP, X400)

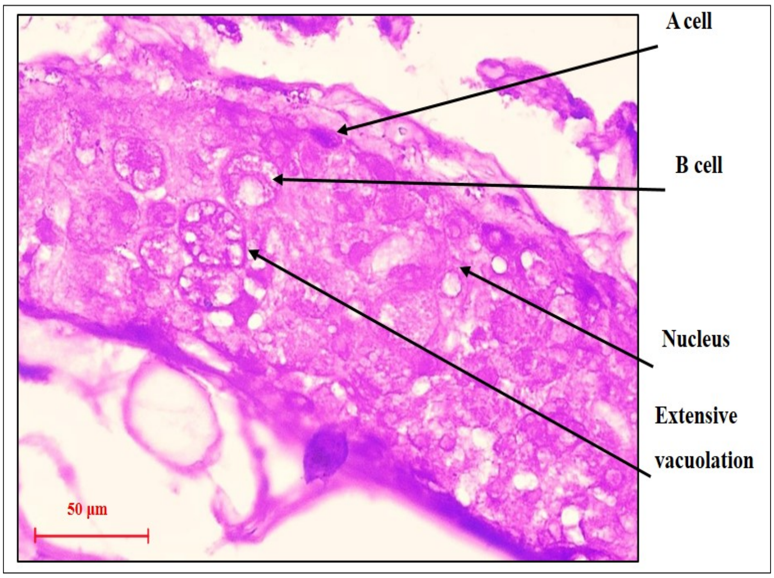

Figure 8: Frontal section showing A cell, B cell, nucleus and extensive vacuolation in cerebral ganglia of *Metaphire posthuma* after 45 minutes of sub-acute hyperthermic stress (35° C) (PAF, X400)

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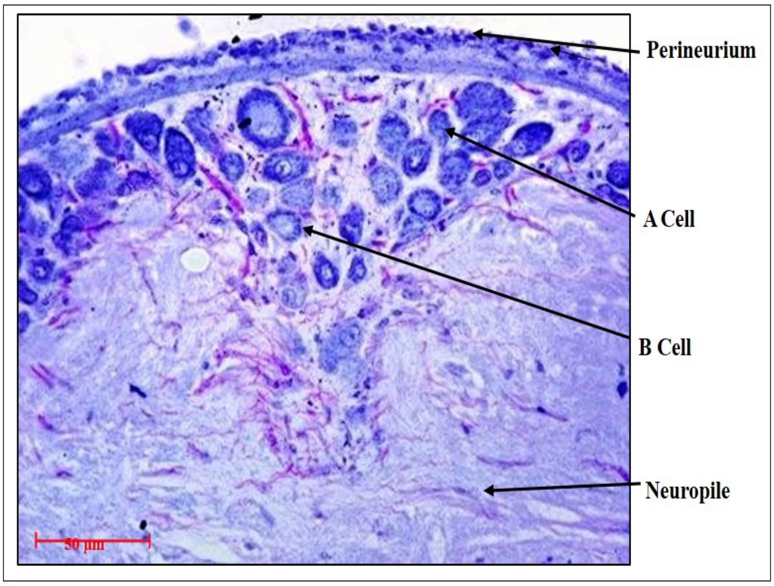

Figure 9: Frontal section showing A cell, B cell, neuropile and perineurium in cerebral ganglia of *Metaphire posthuma* after 45 minutes of hypothermic stress (4° C) (CAHP, X400)

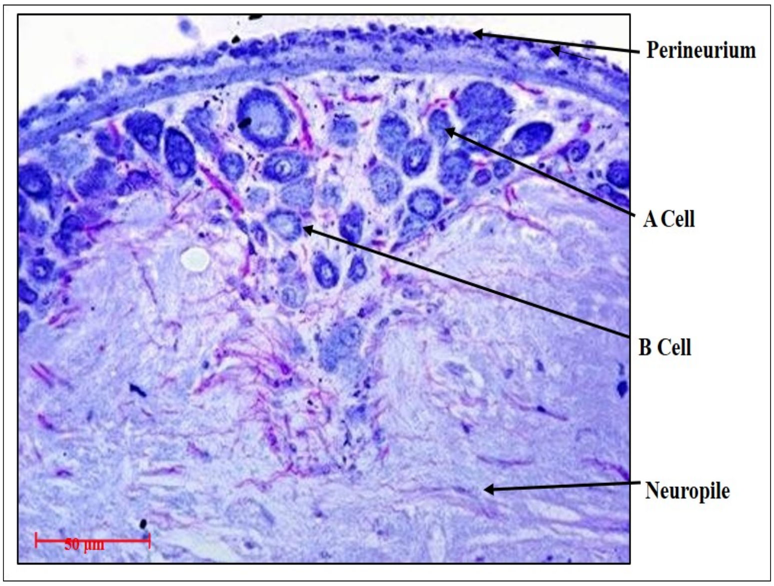

Figure 10: Frontal section showing A cell, B cell and nucleus in cerebral ganglia of *Metaphire posthuma* after 45 minutes of hypothermic stress (4° C) (PAF, X400))

4. Conclusion

The effects of thermal stress on earthworm neurosecretory system clearly indicates that neurohormones secreted from the central nervous system neurosecretory cells (type A and type B) of earthworm have a possible role to combat different seasonal or occasional stresses in nature. Cytological changes in the NSCs such as depletion of NSM is noticed during hyperthermia and accumulation of NSM during hypothermia furnish a working hypothesis for their 'adaptive value' to thermal gradient. This may be considered as a case of homeostasis which is imperative to cope with temperature extremes experienced by this tropical earthworm, *Metaphire posthuma* [1], during its life time.

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