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"Neurosecretory Cell Response to Sodium Chloride Exposure in *Perionyx excavates* Ganglia"

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Abstract

The histological examination of supra and subpharyngeal ganglia in *Perionyx excavates*, stained with Mallory's triple stain, revealed distinct neurosecretory cells, identified as "A" and "B" cells based on morphological characteristics. "A" cells, concentrated dorsally in the brain and dorsolaterally in the subpharyngeal ganglia, exhibited oval or rounded shapes with prominent nuclei and occasional vacuoles in the cytoplasm. Their cytoplasmic granules were stained deep red or violet, and secretory granules were found within axonal tracts. "B" cells, predominantly located posteriorly beneath "A" cells, displayed oblong perikarya with numerous vacuoles in the cytoplasm. Their axons were longer than those of "A" cells, often containing neurosecretory material. Following exposure to a sublethal dose of sodium chloride, significant alterations were observed in neurosecretory material intensity, cell area, and nuclear diameter in both ganglia. Control worms exhibited healthy neurosecretory cells, while experimental worms showed damaged structures with distorted neurosecretory cells. These findings highlight the vulnerability of neurosecretory cells to environmental stressors such as sodium chloride exposure.

Keywords: "A" cells, "B" cells, *Perionyx excavates*, neurosecretory cells, secretory granules, sodium chloride exposure.

Introduction

The realization of the endocrine nature of the nervous system is one of the outstanding developments in biology. The endocrine portion of the nervous system is composed of neurosecretory cells. The neurosecretory cells are the modified neurons that have developed secretory functions to such an extent that they become morphologically distinguishable from other neuronal cells. Such glandular neurons synthesize substances that may act as neurohormones. The neurohormones are retained in circulation for a longer time and evoke appropriate physiological responses at distant target organs. The concept of neurosecretion has undergone advancement with the development of histological techniques and electron microscopy Das (2022).

The nervous system of an earthworm is very simple and consists of the brain, or cerebral ganglion, subpharyngeal ganglia, ventral nerve cord, and peripheral nerve. The main trunk, i.e., the ventral nerve cord, runs below the gut in close contact with the coelom from the last segment of the body to the fourth segment from the front. The ventral nerve cord is swollen into segmental ganglia in each segment. Anteriorly, the nerve cord passes into the subpharyngeal ganglia and then bifurcates into the circumpharyngeal connectives, which pass up encircling either side of the pharynx and ultimately converge into a bilobed brain or cerebral ganglion at the dorsal surface of the pharynx in segment three (Patil, 2002).

The neurosecretory systems in annelids are of classical interest as they represent the first class of coelomates exhibiting a significant pattern of neurosecretory activity. Annelids do not show the possession of epithelial endocrine glands. So any hormonal control over development seemed to be due to neurosecretion, according to Highnam and Hill (1977). This is characterized by the synthesis of proteinaceous ‘glandular’ products the neurosecretory granules (neurohormones) in the versatile neurons. The granule itself has an active principle. Tagged with an inactive protein carrier that possesses specific staining properties and, as such, bears ultrastructural characteristics. Transport of neurosecretory elaborations is monitored through axoplasmic flow and reaches the axon terminals, where the neurohormones are generally released through exocytosis and thereby diffused into the blood stream or terminated in the surrounding tissues.

Neurosecretory cells have been reported throughout the central nervous system (CNS) of the three major classes of annelids. Furthermore, most studies on annelids have substantiated the status of the CNS as an endocrine organ which produces neurohormones as well as neurotransmitters Al-Yousuf (1992). Neurosecretory cells within the CNS of earthworms are regarded as the source of hormones controlling different vital physiological functions such as growth, osmoregulation Takeuchi (1980) reproduction Herlant-Meewis (1975) and thermal acclimation Chaudhuri *et al.* (1998). The morphology of the neurosecretory system of annelids has been well studied. Herlant-Meewis (1975), Lakhani, L. (1991, Kodarkar (1979). Mizutani *et al*. (2000) noted the fictive locomotion induced by octopamine in the earthworm. Patil (2002) studied the responses of neuroendocrine centers to some agrochemicals like ammonium sulfate, urea, suffala, and sampurna in the worm *Perionyx excavatus*. "Previous studies have shed light on the physiological responses of earthworms, particularly Perionyx excavates, to various environmental stressors. Vaidya (2023) investigated the effects of heavy metal contamination on antioxidant enzyme activity and oxidative stress in Perionyx excavates, revealing insights into the organism's response mechanisms. Furthermore, Vaidya and Jadhav (2024) explored the impact of zinc, copper, and mercury on earthworm enzymes, providing valuable information on the toxic effects of these metals on earthworm physiology. Additionally, Vaidya (2020) examined the toxic impact of mercuric chloride on glutathione-s-transferase (GST) activity in Perionyx excavates, contributing to our understanding of the biochemical responses to heavy metal exposure." The available literature shows that not much is known about the effect of soil salination on the responses of the neuroendocrine centers of *Perionyx excavates;* hence, it was selected for this study.

Materials and Methods

The earthworm *Perionyx excavates,* having approximately equal size and weight, were collected from an upland, non-irrigated field in the Ahmednagar area. The soil characteristics are the same as described in Chapter II. Earthworms were kept half immersed in glass petri plates containing 30 ml of tap water at 25 ± 0.5 oC for 24 hours to evacuate their guts, as proposed by Dash and Patra (1977). The study was carried out in plastic culture pots under laboratory conditions.

The experiment was designed to study the histological effect of a 24-hour sublethal dose of sodium chloride (1.05 g/kg soil) on worms exposed for 5 days. The earthworms were dissected by making an incision in the anterior six segments with the help of a sharp scalpel. The brain was exposed and separated into two parts, like the supra and subpharyngeal ganglia, by carefully severing circumpharyngeal connectives at the lateral sides. Since the suprapharyngeal ganglion overlies the pharynx and is connected to the ventral subpharngeal ganglia, the incision was extended up to the posterior end, and the ventral nerve cord was detached from the body wall and separated. All tissues were fixed in aqueous Bouins fluid for 24 h. After dehydration through different grades of alcohol and followed by cold and hot impregnations, the tissues were embedded in paraffin wax (M. P. 58–60 oC), and serial sections were cut at 5-7 µm in thickness, mostly in transverse and a few in longitudinal planes. The ribbons containing sections were stretched on albinized slides and dried.

For histomorphological studies, Mallory's triple strain (Mallory, 1944), chrome heamatoxyline phloxin (CHP) (Gomori, 1941), and paraaldehyde fuchsin (PAF; Meola, 1970) were used to stain the section using counterstaining with Halmi's mixture. Of the three stains employed in the study, Mallory's triple strain yielded satisfactory results, so the same stain was used in future routine work. The histomorphological features and changes in the neurosecretory cells of different ganglia were described after measuring the neurosecretory cell area (µm2), nuclear diameter (µm), and neurosecretory material staining intensity using the methods described by Kulkarni and Fingerman (1985).

In brief: D x d x Π/4 (where D=longer diameter; d = small diameter) for an oblong neurosecretory cells, and 4/3 Πr^2 for oval and round shaped cell. The neurosecretory material (NSM) staining intensity was judged visually by using the following index number:-

- Neurosecretory granules absent in the cell body
- very few neurosecretory granules
- Intermediate between 1 and 3
- large number of granules present in the cell body

For every observation 100 randomly selected cells from different sections were examined under light microscope and results are averaged.

Results

A histological survey of supra and subpharyngeal ganglia, stained with Mallory's triple stain, revealed distinct neurosecretory cells. These cells, characterized by prominent nuclei and abundant cytoplasm, exhibit unique staining patterns indicative of their neurosecretory nature (Scharrer and Scharrer, 1945). Classified as "A" and "B" cells based on morphological criteria, their responses to stimuli were examined in the earthworm Perionyx excavates. (Figs. 6.3 I and II illustrate their morphological features.)

"A" Cells:

'A' cells, located beneath the ganglion capsule and concentrated dorsally in the brain along the anterior-posterior axis, occupy dorsolateral positions near the junction of connectives in subpharyngeal ganglia. Within segmental ganglia, they are found along ventral and lateral borders. These oval or rounded cells possess prominent, centrally placed nuclei with a single conspicuous nucleolus showing affinity for acid-fuchsin stain. Cytoplasm occasionally contains vacuoles, and granules stain deep red or violet with Mallory's triple stain. Secretory granules are observed within the axonal tracts penetrating the neuropile. Although some cells are filled with neurosecretory material while others are empty, most cells display a transitional state between these extremes, indicating dynamic activity (Fig. 6.3 I and II).

Cytoplasmic granules of "A" cells stain deep red or violet with Mallory's triple stain, indicating their neurosecretory nature. Secretory granules are located within axonal tracts, suggesting the utilization of axonal pathways for neurosecretory material transport. In transverse ganglia sections, axons are typically not visible, and "A" cell axons are typically perpendicular to the neuropile (Fig. 6.4 A & B). Interestingly, while some cells are filled with neurosecretory material, others may be empty, with most cells exhibiting a transitional state, indicating dynamic activity.

"B" Cells:

"B" cells are primarily located posteriorly beneath "A" cells in the brain, with distribution throughout subpharyngeal and ventral nerve ganglia (Fig. 6.5 I & II). These cells feature oblong or pear-shaped perikarya with round nuclei typically positioned near the axon hillock. Prominent nucleoli are present, and cytoplasm contains numerous irregularly shaped vacuoles. Secretory material stains pink with Mallory's triple stain. "B" cell axons are longer than those of "A" cells, often containing neurosecretory material. Their axons may be perpendicular or show diagonal curvature before entering the neuropile (Fig. 6.4 A & B). "A" cells are more abundant in the brain, while "B" cells predominate in segmental ganglia. Neurosecretory material is dispersed intercellularly and along circumpharyngeal connective tracts, with a gradual decrease in cell numbers from anterior to posterior segmental ganglia (Fig. 6.5 I & II). Unlike in other species, Perionyx excavates axons do not form bundles or bulbs.

Table 6.1 and Figures 6.10 & 6.11 illustrate significant changes in neurosecretory material (NSM) in both supra and subpharyngeal ganglia of Perionyx excavates after exposure to a sublethal dose of sodium chloride (1.05g/kg soil) for 5 days. In the suprapharyngeal ganglia, A cell NSM intensity decreased by 66.66%, while cell area and nuclear diameter increased by 8.6% and 29.16% respectively. Conversely, B cell NSM intensity increased by 66.66%, with cell area and nuclear diameter increasing by 20% and 36.84% respectively. Control worms exhibited healthy neurosecretory cells A and B with distinct lobes, while experimental worms showed damaged structures with distorted neurosecretory cells after exposure (Fig. 6.6 and Fig. 6.7).

In the subpharyngeal ganglia, A cell neurosecretory material (NSM) intensity decreased by 66.66%, while cell area and nuclear diameter increased by 20.14% and 42% respectively. Conversely, B cell NSM intensity increased by 66.66%, with cell area and nuclear diameter increasing by 22.36% and 69% respectively. Control worms exhibited healthy neurosecretory cells A and B with distinct lobes in the subpharyngeal ganglia, while experimental worms exposed to a 24-hour sublethal dose of sodium chloride for 5 days showed damaged structures with distorted neurosecretory cells (Fig. 6.8 and Fig. 6.9).

Table No. 6.1: "Effects of Sodium Chloride Exposure on Neurosecretory Cell Activity in *Perionyx excavates*"

Treatments	Suprapharyngeal ganglia					
	A cell			B cell		
	NSM Intensity	Cell area (μm^2)	Nuclear Diameter (µm)	NSM Intensity	Cell area (μm^2)	Nuclear Diameter (µm)
Control	3*	20.7*	4.8*	3*	13.4*	3.80*
Experimental (Sodium chloride 1.05g/kg soil)	1.002* (-66.66%)	18.92* (+8.6 %)	3.5* (+29.16%)	1.0* (+66.66%)	10.72* (+20 %)	2.401* (+36.84%)
	Subpharyngeal ganglia					
	A cell			**B cell**		
Control	3*	19.56*	5.2*	3*	15.2*	4.3*
Experimental (Sodium chloride 1.05g/kg soil)	1.002* (-66.66%)	15.62* (+20.14%)	30.01* (+42 %)	1.00 (+66.66%)	11.8* (+22.4%)	1.33* (+69 %)

* - Original values NSM – Neurosecretory material + - % increase - - % decrease

Figure 6.10 "Effects of 24-Hour Sublethal Sodium Chloride Exposure on Neurosecretory Cell Types 'A' and 'B' in Suprapharyngeal Ganglia of *Perionyx excavates*"

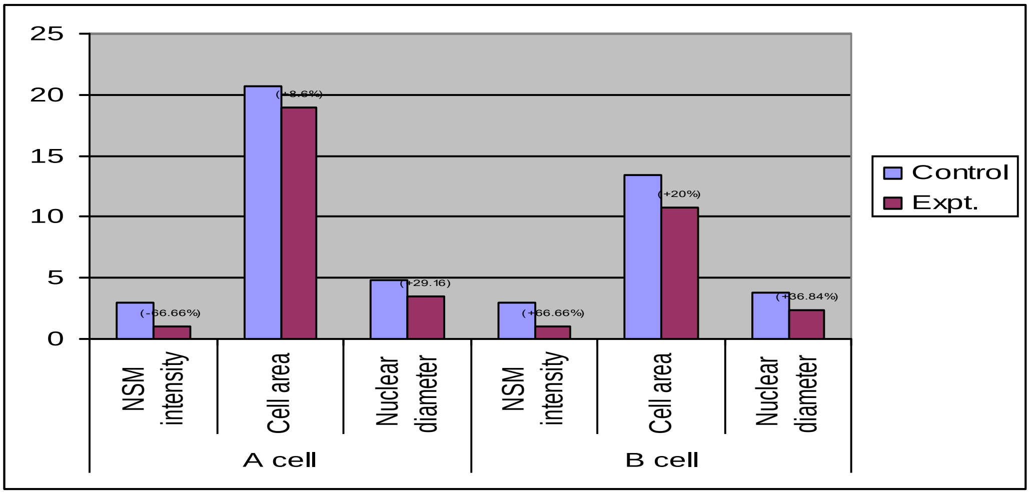
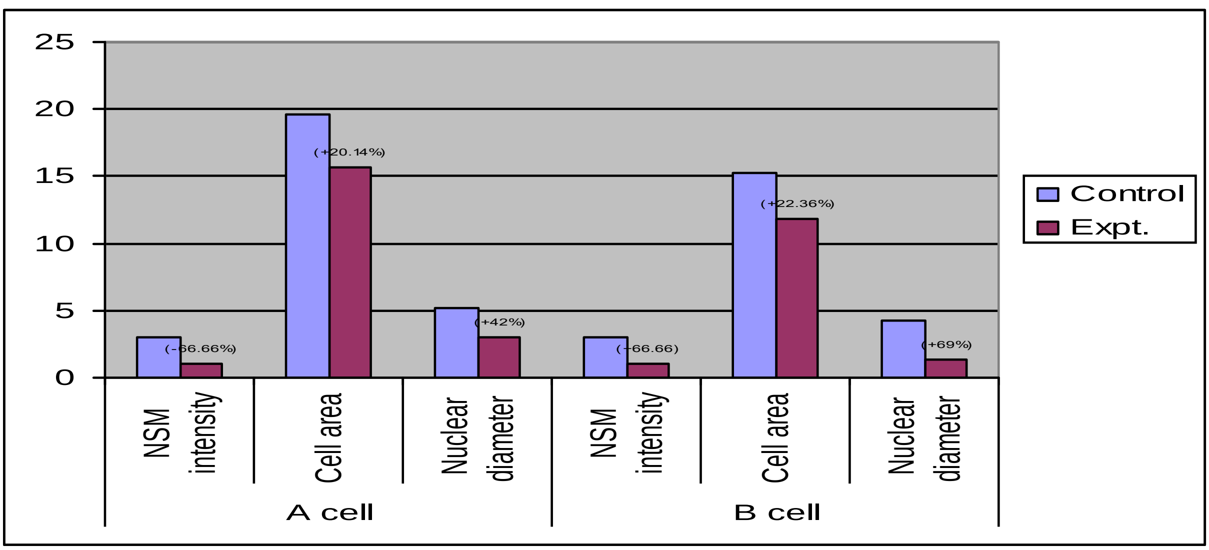


Figure 6.11 "Neurosecretory Cell Activity in Perionyx excavates After Sodium Chloride Exposure"



Discussion

The histological examination of the supra and subpharyngeal ganglia in *Perionyx excavates* has uncovered the presence of neurosecretory cells distinguished by their unique cytochemical profiles and morphological traits. These cells, categorized into "A" and "B" types, play pivotal roles in modulating various physiological processes within the nervous system. "A" cells, predominantly situated beneath the ganglion capsule along the dorsal axis, exhibit oval or rounded shapes with centrally positioned nuclei and occasional cytoplasmic vacuoles. These cells harbor deep red or violet-stained cytoplasmic granules, indicative of their neurosecretory activity. The axons of "A" cells traverse through the neuropile, implying the active transport of neurosecretory material. Notably, the activity of "A" cells appears to be dynamic, suggesting a fluctuating state of secretion within the nervous system.

Conversely, "B" cells are primarily localized in the posterior part of the brain, below "A" cells, and are dispersed throughout the subpharyngeal and segmental ganglia. These cells feature elongated or pear-shaped perikarya with round nuclei and prominent nucleoli. The cytoplasm of "B" cells contains numerous irregularly shaped vacuoles and pink-stained secretory material. Their axons, longer than those of "A" cells, also contain neurosecretory material and exhibit various curvatures before entering the neuropile. Exposure to sublethal doses of sodium chloride induced significant alterations in the neurosecretory material of both supra and subpharyngeal ganglia in *Perionyx excavates*. This included a decrease in the intensity of neurosecretory material in "A" cells and an increase in "B" cells, accompanied by changes in cell area and nuclear diameter. Histological examination revealed damaged structures and distorted neurosecretory cells in experimental worms compared to control specimens.

This study offers valuable insights into the morphological characteristics and responses of neurosecretory cells in earthworms, particularly *Perionyx excavates*, under sodium chloride exposure. However, further research is warranted to elucidate the underlying mechanisms and physiological implications of these observed changes. Recent research in the field of neuroendocrinology, such as studies by Banovacki and Matavulj (2013), Banik and Chaudhuri (2020), and Chaudhuri (2022), has highlighted the significance of neurosecretion in invertebrates and its potential implications for understanding the neuroendocrine control of physiological processes. Additionally, investigations by Das (2022) and Banik and Chaudhuri (2019) have provided insights into the cytological and histogenetic aspects of neurosecretory systems in earthworms, contributing to our understanding of their structure and function. These studies underscore the importance of further exploration into the neuroendocrine mechanisms underlying earthworm physiology.

Conclusion

The histological examination of supra and subpharyngeal ganglia in *Perionyx excavates* revealed distinct neurosecretory cells, classified as "A" and "B" cells based on morphological characteristics. "A" cells primarily located dorsally in the brain and dorsolaterally in subpharyngeal ganglia, displayed oval or rounded shapes with prominent nuclei and occasional vacuoles in the cytoplasm. In contrast, "B" cells were predominantly located posteriorly beneath "A" cells and exhibited oblong perikarya with numerous vacuoles. Following exposure to a sublethal dose of sodium chloride, significant alterations were observed in neurosecretory material intensity, cell area, and nuclear diameter in both ganglia. Control worms exhibited healthy neurosecretory cells, while experimental worms showed damaged structures with distorted neurosecretory cells after exposure. These findings underscore the susceptibility of neurosecretory cells to environmental stressors, highlighting the potential impact of sodium chloride exposure on the neurophysiology of *Perionyx excavates*.

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