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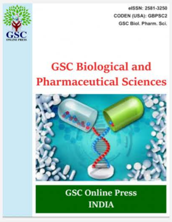

(REVIEW ARTICLE)

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Stress effects and neurosecretion in Earthworms: A Review

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Abstract

Earthworms, the poikilothermal first group of invertebrate, are subjected to stressful events, occasional (illumination), frequent (amputation due to predation) and seasonal (dehydration during summer), hydration (during monsoon), thermal (heat stress during summer, cold stress during winter) etc. These lowly evolved creatures often overcome the events of stress through eco-physiological adaptive changes in the central nervous system (CNS) neurosecretory system. In absence any endocrine gland and neurohaemal organ, their CNS is enriched with vascularisation and well defined neurosecretory cells (NSCs) (**type A** and **type B**). The **type A** NSCs - the source of peptide neurohormone and release their secretion through axonal transport, response mainly against osmotic stress (dehydration, hydration), light (when exposed to the sunlight), hypo and hyperthermia etc. The type B NSCs are source of amine neurohormone and release neurohormone mainly by perikarya and occasionally by axon. The type B cells show remarkable cytoplasmic changes in relation to metabolic compensation following food deprivation in earthworms. Both the type A and type B NSCs show adaptive response such axonal transport, cytoplasmic vacuoles followed normal secretory cycle (i.e. synthesis, storage, release) during amputation and subsequent regeneration in earthworm.

Keywords: Stress Effects; Earthworm; Dehydration- Hydration; Thermal Stress; Amputation; Neurosecretion.

1. Introduction

Stress is the natural reaction in the animal body facing unwanted changes or challenges. Stress may be physical, mechanical or chemical etc.

Earthworms, popularly called farmer's plough, live in and feed on soils. According to ecological categories earthworms are of three types, such as, epigeic, endogeic and anecic species. The epigeic species are deeply pigmented, phytophagous, non-burrower, found in organic wastes (preferably cattle dung) above the soil surface. As they are surface feeder and subjected to predator pressure and amputation of body parts during ploughing, nature has gifted them the power of high regeneration ability, as well as fecundity (*Perionyx excavatus*, *Eisenia fetida*, *Eudrilus eugeniae*). The endogeic earthworms in contrast are lightly or moderately pigmented, geophagous and live in both the top, as well as, the sub-soils depth. The tops soil (0-15cm) endogeic species (*Octochaetona beatrix*, *Pontoscolex corethrurus*) have good and sub-soil (15-30cm) species (*Polypheretima elongata*) possesses less fecundity. Most of the Indian earthworms are endogeic because of oxidation of soil organic carbon. The topsoil endogeic earthworm species possess regeneration ability, but the sub-soil species have poor or no regeneration power. The anecic species are dorsally pigmented, phyto-geophagous (*Lampito mauritii*), vertical burrower with moderate or less regeneration ability and medium fecundity.

The epigeic and top soil endogeic earthworms are subjected to the physical stresses such as hydration, dehydration, thermal, salinity, amputation and chemical / phytochemical stresses like, change in pH, exposure to different plant extracts (for worm collection) etc. When the earthworms are subjected to any kind of stress, they respond through

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cytological changes in the CNS – NSCs. 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 nerohormone producing NSCs acts as endocrine gland. Their CNS comprises of paired cerebral ganglia ("brain"), paired subesophageal ganglia and ventral nerve cord (VNC).

In earthworms, NSCs are of two types, peptide secreting type A and amine secreting type B cells (Fig 1a-1b).The deeply stained typeA 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 (type A&type B) lie the medulla or neuropile made up of axons coming out of NSCs and ordinary neurone. The subesophagel ganglia, in addition to the type A and type B possess "islets" of Hubl cells (U cells) located at the base of circum-esophageal connective (Fig 1c). The type A, type B and U cells react positively with basic stains like Chrome alum haematoxylin phloxin (CAHP) and Paraldehyde fuchsin (PAF/AF) stains.

Following exposure to different stresses, field collected and laboratory acclimated earthworms are narcotised in 5-10% the cerebraland ventral nerve cord ganglia are dissected out, fixed in aqueous Bouin's fluid and processed for tissue embedding and staining. The neurosecretory materials are acidophilic in nature and these are turned basophilic by permanganate oxidation. Methodology for tissue processing and staining has been discussed by Chaudhuri [1, 2].

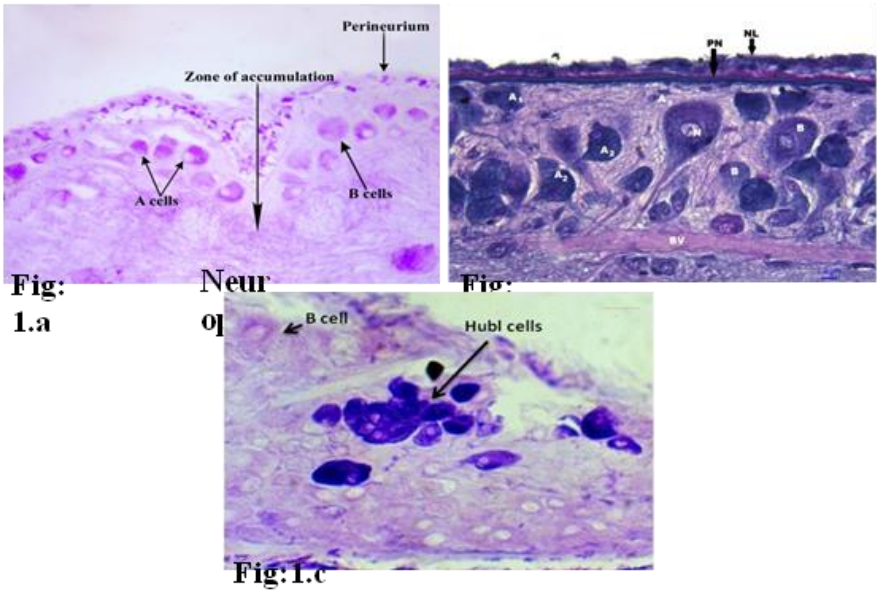

Figure 1a Control section (AF stain) and Figure 1b (CAHP stain) showing distribution of neurosecretory cells (type A & type B) in the cortical part of cerebral ganglia of earthworm. Note Zone of Accumulation in the margin of neuropile and peripherally located perineurium (PN) and neural lamella (NL). Figure 1c showing "islets" of Hubl cells (U cells) at the base of circum-esophageal connectives

2. Effects of physical and chemical stresses on neurosecretory system

2.1. Dehydration stress

Earthworms are often exposed to seasonal events like flooding of water during rainy season and face the problem of water stress due to acute desiccation during summer draught condition. During summer condition the earthworms move deeper inside the soil, while during monsoon following heavy rain they come out from their burrows. Kamemoto [3] studied the role of brain in the water permeability of the integument by removal of brain and reimplantation or injection of brain homogenates in hydrated *Lumbricus terrestris* and *Eisenia fetida*. It has been suggested that both peptidergic type A NSCs, and aminergic type B NSCs of the brain are involved to inhibit water loss in *L. terrestris* during dehydration [4]. In *L. terrestris* the neurosecretory principles from brain influence the osmotic and ionic regulation by changing the functional activities of integument or nephridia [5, 14]. A relationship exists between water conservation and ventral nerve cord neurosecretion in the tropical earthworm, *Metaphire peguana* during dehydration [6]. In *Eutyphoeus gammiei* following dehydration for 2 hrs to 6 hrs drastic decline in the number of type A cells, extensive vacuolation coupled with acute to marginal depletion in type A and type-B NSCs was reported [7]. Extensive depletion of neurosecretory material (NSM) from both type A and type B cells, discharge of NSM in the blood vessels, presence of voluminous nuclei and increase in nucleo-cytoplasmic ratios in both type A and type B cells were reported in *E. gammiei* [7] and *Eudrilus eugeniae* [8] following dehydration stress under laboratory conditions (Fig 2).

2.2. Hydration Stress

Hydration of worms (1 to 4 hrs.) following 2 to 6 hrs of dehydration leads to accumulation of NSM in both the type A and type B NSCs of the cerebral ganglia (Fig 3). These characteristics indicate functional attributes of cerebral type A and type B NSCs during possible "super-elevation" of osmotic concentration of the body fluid in earthworms. Depletion of NSM from the cerebral NSCs of dehydrated earthworms was probably due to elaboration of "anti-diuretic factor" for inhibition of water loss [7]. Following transfer of the dehydrated (6 hrs) earthworms to water (hydration) the desiccation stress was mitigated and again there was an increase in the number of type A NSCs and re -accumulation of NSM in both the type A and type B cells in the cerebral ganglia.

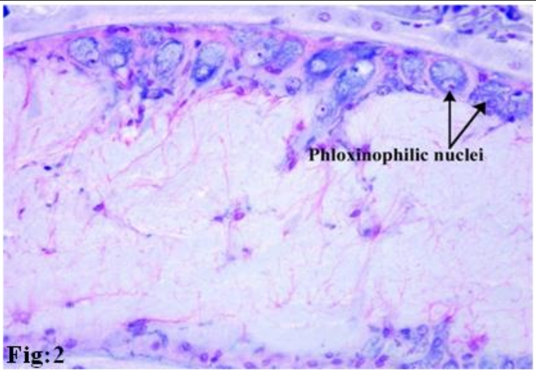

Figure 2 Experimental section showing CAHP positive NSCs in the cerebral ganglia with abundance of cytoplasmic vacuoles, extensive depletion due to discharge of NSM through axonal transport in 2hrs dehydrated earthworm *Eudrilus eugeniae* [8]

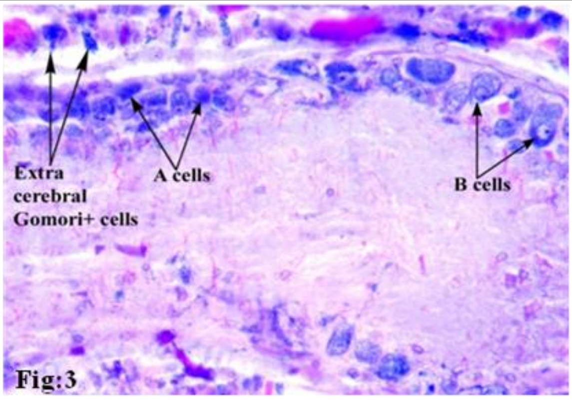

Figure 3 CAHP positive cells in the cerebral ganglia with accumulation of NSM in 2hrs hydrated earthworm, Eudrilus eugeniae following 2 hrs dehydration [8]

2.3. Thermal Stress

Temperature is the most important factor controlling growth and reproduction in animals. Temperature and moisture are usually inversely related. The high surface temperature and dry soils are much more limiting to earthworms than low temperature and water logged soils [9]. During winter the surface soils become cold and the earthworms move deep inside the soil.

2.3.1. Hyper- thermal Stress (38°C - 40°C)

Earthworms are poikilothermal animal. Under experimental hyperthermic condition (38°C - 40°C) earthworms become restless for 10-12 minutes. Exudation of profuse mucus from the worm's body surface occurs. Earthworms become inactive possibly due to the impact of direct heat to cause desiccation water loss. Hyperthermia, indeed, bring about high metabolic rate in earthworms. Majority of type A cells show marginal to acute depletion, appearance of cytoplasmic vacuoles and transport of NSM to the accumulation zone at the margin of neuropile and also the extracellular space. Nucleo-cytoplasmic ratios of the NSCs also increase. The cytomorphic changes in the NSCs simulate the conditions similar to those of dehydration stress.

2.3.2. Hypo-thermal Stress (4°C)

Hypothermal stress simulates winter condition. Earthworms subjected to hypothermia (4°C) undergo aggregation to forum a clump. Chill-coma exposure leads to massive accumulation of NSM in the type A NSCs with reduction in nuclear volume. The type B cells characterised by numerous cytoplasmic vacuoles show signs of NSM depletion. Although the involvement of type A cells based on its remarkable cytoplasmic changes is obvious, the type B cells probably play role in metabolic compensation [10].

2.4. Salt Stress

Most species of earthworms cannot tolerate salt water [9]. The coastal soil species are threatened with salinazation because of seawater encroachment and irrigation. Recently Zhang *et al.* [11] reported that *Eisenia fetida* can survive in coastal saline soils of Tianj in North China. The earthworms like *Lampito mauritii*, *Eutyphoeus incommodus* and *Metaphire* (=*Pheretima*) *posthuma* are salt tolerant species to some extent [12]. Chaudhuri *et al.*[13] used salt water (400g sodium chloride in 10 lt. water) for extraction of earthworms (*Entyphoeus gammiei*) from the soils. Fresh kitchen wastes, poultry and pig manures contain significant amount of inorganic salts that may kill earthworms. So prior to vermicomposting of kitchen wastes, salts should be removed through washing [15, 16]. That salinity exerts remarkable effect on neurosecretory activity in the brain of *E. gammiei* was reported by Chaudhuri and Chaudhuri [19]. Following submergence of the of the worms in 1% salt water for 2 hrs, the cerebral NSCs (type A) showed partial to total depletion of NSM with no change in nuclear diameter. This indicates that there is an increase in the release of NSM from the type A cells leading to depletion. Marked depletion of NSM from PAVB - positive NSCs (= A cells) in the brain and subesophageal ganglia of *Pheretima posthuma* [17], and *Perionyx excavatus* [18] following salt stress have earlier been reported. In contrast, the type B cells in the brain of *E. gammiei* had partial accumulation of PAF stained granules both in perikarya and the axons with increase in nuclear volume.

Indeed, salt-loading mimics the physiological effects of dehydration. It is reasonable to assume that in order to economise desiccation water loss, the cerebral type A cells in *E. gammiei* probably synthesize and release anti-diuretic factor as majority of these cells show partial or total depletion under salt stress. Dramatic increase in the number of type A cells coupled with their massive accumulation of NSM and decrease in nuclear volume (thereby indicating inhibition of secretion) following distilled water submergence of worms is consistent with such view [19]. The factor (hormone) for osmoregulation possibly intervenes by altering the functional activities of integument or nephridia [4, 14]. The role of type B NSCs under salt stress is not clearly understood. Probably NSM released by type B cells is involved in metabolic compensation [19].

Indeed lot of controversies are still in vogue about the nature of the principle or neurohormone that regulates water metabolism in invertebrates. This principle is considered as 'diuretic' in several insects [20, 21], *Lymnaea stagnalis* [22], (*Pheretima communissima* and *Eisenia fetida* [23] and 'anti-diuretic', in *Blabera fusca* [24] and tropical earthworms *Perionyx excavatus* [18] Interestingly, several insects have shown to have a dual control with 'anti-diuretic', as well as, 'diuretic' hormone [25]. To maintain hydrostatic and salt level balance, CNS of earthworms probably produces both diuretic and anti-diuretic principles that may or may not be confined to specific cell types in the brain of earthworm [19].

2.5. Phytochemical Stress

In North East India including Tripura, Bangladesh, West Bengal, Chennai etc. the aqueous extract of herb, *Polygonum hydropiper* (Family Polygonaceae) is extensively used as a spray against the earthworms for control of some surface activities (cast production) in dwelling places, play ground and for collecting them for angling. In fact, large number of earthworms comes out of their burrows following spraying of aqueous solution of *P. hydropiper* extract (500g crushed material in 10 lt. of water [27]. Most of the collected worms following several times of washing in water survive well. The norsesquiterpene acid named polypiperic acid and acylglucosyl sterol are the potent earthworm repellent principles isolated from the aerial part of *P. hydropiber* [27]. The extract of the herb *P. hydropiper* is a powerful stimulant, diuretic, skin irritant and anti-helminthic [28].

The earthworms (*E. gammiei*) treated with aqueous extract of *P. hydropiper* shows interesting behavioural and physiological changes associated with drastic cytomorphological alterations in the CNS-NSCs [29]. Such changes are probably due to the presence of active factors (acyl glucosyl sterol and nor sesquiterpene in the plant extract that cause excitation of the CNS through skin receptors. Cytopathological changes such as shrinkage of neurosecretory perikarya, cracky texture of cytoplasm, intense depletion of NSM, occurrence of cytoplasmic vacuoles, pycnotic nuclei in the cerebral NSCs are some of the interesting features following treatment with aqueous solution of different concentration (1:2, 1:4) of *P.hydropiper* extract. Less concentrated solution (1:6) of the plant extract had comparatively less cytomorphological alterations but increase in nucleo-cytoplasmic indices of NSCs indicating high level of cell activity than those affected by more concentrated solution (1:2, 1:4 ratio) of plant extract. It is reasonable to assume that pharmacological effects of the active compounds such as acylglucosyl sterol and polypiperic acid in the plant extract create disturbance in the neurosecretory system leading to uncontrolled release of neurosecretion as indicated by extensive cytoplasmic vacuolation and depletion in the neurosecretory cells. Such plant extract induced neurosecretory changes are much similar to the poisoning effects of insecticide treatments on the cerebral NSCs of *Periplaneta americana* [30].

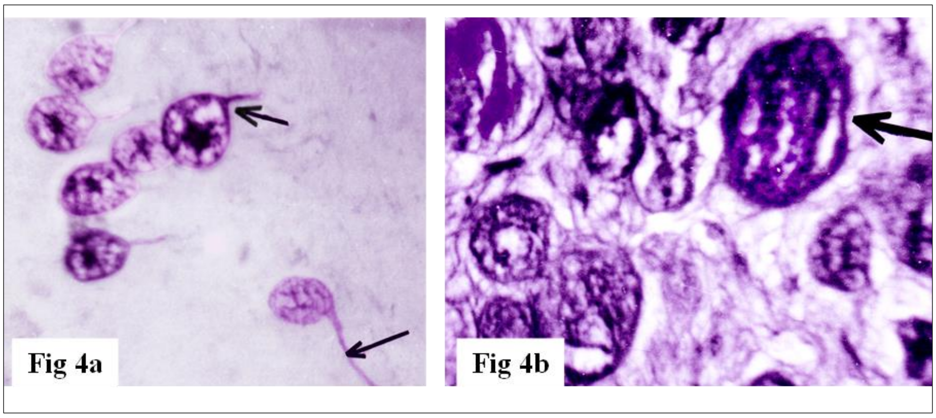

Figure 4a & 4b Frontal section showing AF stained NSCs with hypertrophy of perikarya, extensive cytoplasmic vaculations, axonal transport & cracky texture of cytoplasm in the type B cells of the cerebral ganglia following application of crude aqueous extract of Polygonum hydropiper in Eutyphoeus gammiei [29]

2.6. Starvation Stress

Earthworms use wide variety of organic materials along with soils for food. Nutritional deprivation in earthworm renders their metabolism to a low gear and is detrimental to their biomass and reproduction [9, 33]. Presence of Gomori positive secretion in the 'vegetative ganglionic nerve fibers' innervating the pharynx and oesophagus of earthworms, *Dendrobaena atheca* was earlier reported [31]. Gorgees and Baid [31] attributed possible role of the relevant ganglia in the process of digestion. Nutritional deprivation in *Metaphire peguana* [32] and *E. gammiei* [33] results into remarkable cytomorphological changes especially in the type B cells, such as enlargement in the nuclear volume of cells, reticulation in cytoplasm due to massive accumulation of cytoplasmic vacuoles encircled by NSM, remarkable depletion, NSM accumulation beneath the cell membrane and also in the axon. The NSM accumulation of amine secreting type B cells may be related to perikaryal release of neurosecretion. It is possible that neurosecretory changes during starvation stress are metabolic disturbances due to starvation.

2.7. Amputation

Earthworms in general have unique power of regeneration [35]. But the deep soil (sub-soil) dwelling earthworms generally do not regenerate because they rarely come out of their burrows and are not exposed to predator attack. Following amputation the sub-soil earthworm species show healing up of wounds. The importance of the CNS in the phenomenon of Oligochaete regeneration has been discussed by several classical investigators [36, 37, 38]. That the cerebral ganglion ('brain') was indispensable for anterior regeneration in *Lumbricus terrestris* was experimentally established [39]. Later Herlant-Meewis [40] refuted the solitary role of cerebral ganglia and advocated the involvement of VNC ganglia in both anterior and posterior regeneration in *Eisenia fetida.* Marcel [41] recorded synchronous synthesis and release of NSM in VNC ganglia immediately proximal to the level of amputation of either anterior or posterior segments in *E. fetida* and concluded that neurosecretory system promotes some aspects of regeneration. In *Metaphire peguana* following 24 hrs of cephalic amputation, Nanda and Chaudhuri[42] recorded intense accumulation of neurosecretion in majority of the anterior most ventral ganglionic NSCs (both type A and type B)followed by 'shrunken condition' of some neurosecretory perikarya, axonal transport of NSM towards neuropile (Fig 5a-5b), occurrence of cytoplasmic vaculations, voluminous nuclei after 48 hrs. and intense depletion in NSCs, accumulation of NSM at the margin of neuropile, as well as, in the neighbourhood of intra-ganglionic capillaries after 72 hrs of amputation. The shrunken conditions of some neurosecretory perikarya of anterior most ventral ganglia may be due to generalised stress action due to cephalic amputation.Temporary cessation of neurosecretory transport after 24 hrs of amputation is probably related to the event of blastema formation and restoration of lost body parts. It is advocated that adverse amputation stress in the form of injury possibly release cellular products that act as an adjunct to stimulate the NSCs of VNC for the production of 'regeneration promoting hormone' [25]. Deep burrowing earthworm *Entyphoeus gammiei* (Octochaetidae) having intact brain do not regenerate following amputation of 15 posterior most body segments [43]. Debrained earthworms, in contrast, show poor regeneration ability with respect to the replenishment of only two segments. Depletion followed by marginal accumulation of NSM in the cerebral NSCs of 24 and 48 hrs amputed earthworms and massive engorgement coupled with decrease in cell and nuclear volume after 72 hrs were reported. Chaudhuri *et al.*[43] suggested an important inhibitory role in caudal regeneration. In contrast, cerebral ganglia produce and release 'regeneration promoting hormone' in the tropical earthworm, *Perionyx excavatus* [44] and *Metaphire houlleti* [45]. Neuroendocrine manipulation through ablation of the 'brain' in the tail amputed earthworms, *Eudrilus eugeniae* promotes the process of posterior regeneration compared to those having intact brain [34]. In the latter (i.e. with the intact brain), regeneration of posterior body segments was delayed. This indicates that posterior regeneration in *E. eugeniae* is under the joint influence of regeneration inhibiting factor secreted by brain and regeneration promoting factors released from the ventral nerve cord [34]. The same authors observed cytomorphological alterations in the cerebral NSCs which were most conspicuous following 24 hrs of amputation during which acute depletion in the type A cells with cytoplasmic vacuolation and voluminous nuclei were be recorded (Fig 5c). Distinct secretory cycle with synthesis transport and release was recorded in the type A and type B NSCs of cerebral and ventral nerve cord ganglia following 48 hrs to 96 hrs of amputation in *E. eugeniae*. Recently Bhattacharjee and Chaudhuri[46] reported distinct cellular changes in the cerebral NSCs, such as release of neurohormone followed by uniform secretory cycle in cerebral type A and type B cells of *Lampito mauritii* (Fig 5d).

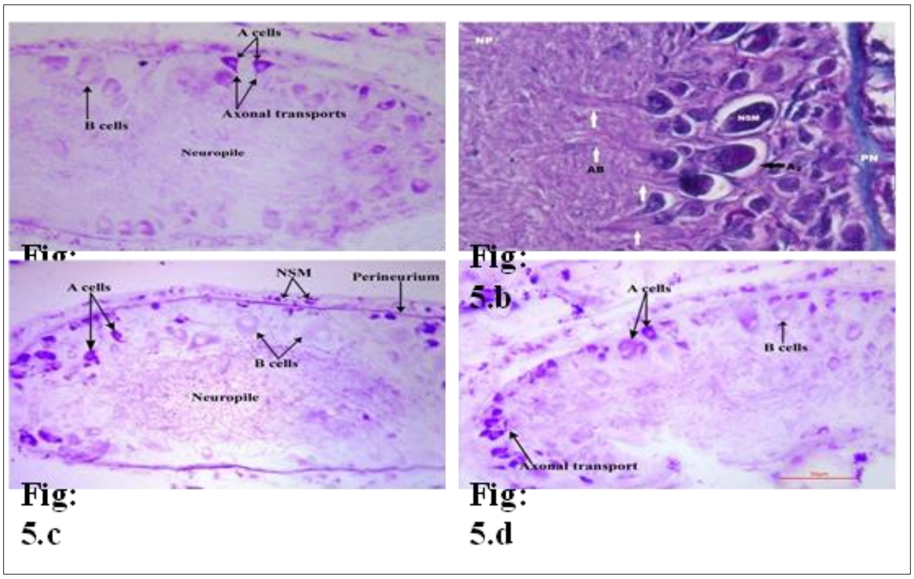

Figure 5a Frontal section (AF stain) showing axonal transport of NSM from type A NSCs towards zone of accumulation and cytoplasmic vacuolation in the type B NSCs in the cerebral ganglion of earthworm following 24 hrs of amputation; **Figure 5b** Frontal section (CAHP stain) showing axonal transport through axon bundles emerging from NSCs deep into the neuropile; note shrunken condition of neurosecretory perikarya following 24 hrs of amputation stress; **Figure 5c** Section showing accumulation of AF positive NSM in type A cells, cytoplasmic vacuolation in type B cells of cerebral ganglia; note zone of accumulation charged with neurosecretion following 48 hrs of amputation stress; **Figure 5d** Frontal section showing distinct neurosecretory cycle with synthesis, storage & release of NSM from NSCs following 72 hrs of amputation indicating revival of normal neurosecretory status [46].

2.8. Light Reaction

Earthworms are usually found to show mass migration over-ground in morning time probably in search of better food resource and other suitable ecological factors. Examples of such kind of migration include *Metaphire houlleti, Amynthas alexandri, Eutyphoeus sp., Perionyx excavatus etc.*At that time earthworms become exposed to the visible light (infra red, ultra violet radiation). As earthworms are photo negative, exposure to illumination acts as light stimulus that acts on their photoreceptor cells which are concentrated in the basal part of epidermis on the prostomium, first segment and on the last segment [9]. Effects of light stress on cerebral NSCs are similar to dehydration [47]. Interestingly the rate of depletion of NSM is directly correlated with the light intensity and duration of illumination each day [47].The stainable materials [47] reappear within the brain of *Lumbricus rubellus* when the worms are returned to darkness. Recovery of the neurosecretory system in *Allolobophora chlorotica* exposed to light without infra red occurs after 2-3 days even when illumination is maintained.

3. Discussion

In nature earthworms are subjected to different types of physical and chemical stresses during their annual cycle of activity. As they are lowly evolved coelomate invertebrate, no endocrine gland and neurohaemal organs are found in their body. The earthworms meet the challenges against different stresses such as, osmotic stress, thermal stress, light stress, amputation etc. By virtue of having enormous number of neurohormone secreting NSCs in the highly vascularised CNS, earthworms meet the challenges of stress (if not deleterious) by remarkable cellular changes in the neurosecretory system. During extreme summer heat when the earthworms are threatened with water loss from the body due to desiccation, their CNS – NSCs release neurohormone (AF, CAHP positive secretion) to conserve body water. So the NSCs show the signs of depletion such as axonal transport with appearance of cytoplasmic vacuoles, lack of AF positive secretion inside the cell body. During rainy seasons the earthworms become over hydrated due to influx of water inside the body. So release of neurohormone is inhibited by accumulation of neurosecretion in the perikarya. The peptide secreting **type A** cells are involved in the process of water balance of the body acting at the level of nephridia or integument.

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. 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'.

Salt stress draws out water from the body and mimics the condition of dehydration stress. So the earthworms following exposure to salinity, release NSM from the NSCs leading to cellular depletion in the typeA cells which is essentially in relation to water conservation. Thus it is noticeable that release of neurosecretion is recorded when the earthworms become stressed with desiccation (summer), hyperthermia (summer), salinity (exposure to coastal soils) and light stress in their habitat. Synthesis and storage of neurosecretion in the neurosecretory cell body is recorded when the earthworms are exposed to hydration (flood, heavy rains) and hypothermia (winter). Indeed lot of controversies persist regarding the nature of the factor or principle that regulates water balance in the body of earthworm. Controversies exist regarding the nature of the principle whether it is 'anti-diuretic' or 'diuretic'. It is proposed that in order to maintain hydrostatic and salt level balance the earthworms probably produce both 'diuretic' and 'anti-diuretic factors' which may or may not be confined to specific cell types in the brain of earthworms[19].

Nutritional deprivation in earthworms brings about metabolism to a low gear .The cause of cytological changes in the amine secreting type B NSCs such as large amounts of NSM encircled cytoplasmic vacuoles appearing as reticular cytoplasm, depletion of NSM in the brain and ventral ganglia of earthworms are far from clear. However these cellular changes may be related to the physiology of digestion or due to metabolic misbalance in earthworms.

Polygonum hydropiper (locally called 'Biskatali') is used as aqueous extract to collect earthworms for angling. It acts as a skin irritant to retrieve earthworms from their burrows. Acyl glucosyl sterol and polypiperic acid in the aerial part of the plant are earthworm repellent factors in the *P.hydropiper* extract [27]. The CNS neurosecretory changes like cell break down, intense depletion due to uncontrolled release of NSM, occurrence of cytoplasmic vacuoles, pyknotic nuclei in the NSCs of earthworms treated with the plant extract are similar to the insecticidal effects on the cerebral NSCs of *P. americana* [30].

Earthworms are well known for their high regeneration potential following amputation during ploughing or loss of body parts by predators [35]. Following amputation, initially neurosecretory discharge followed by synthesis, storage and release of NSM from type A and type B cells (secretory cycle) throughout the CNS during subsequent regeneration clearly indicate involvement of neurosecretion in the phenomenon of regeneration in Oligochaeta. A balance between ‘regeneration promoting hormone’ secreted by the ventral nerve cord ganglia and ‘regeneration inhibiting hormone’ from the cerebral ganglia probably play a key role in earthworm regeneration.

4. Conclusion

The review on the effects of stresses (physical/chemical) on earthworm neurosecretory system clearly indicates that neurohormone secreted from the central nervous system neurosecretory cells (type A and type B) of earthworm have possible role to combat different seasonal or occasional stresses in nature. Similar cytological changes in the type A NSC such as depletion of NSM is noticed during dehydration, hyperthermia, and exposure to salinity, light stress etc. Phytochemical (*P.hydropiper*) stress causes cell break down, uncontrolled release of neurosecretion in earthworm which are similar to insecticidal effect. Neurosecretory release through axons followed by distinct secretory cycle is noticed following amputation and subsequent regeneration.

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