

# SENSO-NEUROENDOCRINE CELLS WITHIN THE CEREBRAL GANGLION OF THE EARTHWORM *APORRECTODEA CALIGINOSA*

By

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## الخلايا الحسية العصبية الإفرازية في العقد المخية لديدان الأرض ابريكتودا كاليجينوسا

شعاع هاشم اليوسف

باستخدام المجهر الإلكتروني النفاذ تم اكتشاف نوع من الخلايا العصبية المهذبة والتي يُحتمل أن يكون لها دور حسي إفرازي في داخل وخارج العقدة المخية في دودة الأرض ابريكتودا كاليجينوسا .

وعند تتبع نهايات هذه الخلايا اتضح أنها تحتوي على تجمعات من الحويصلات الإفرازية والتي تتواجد بالقرب من غلاف العقدة العصبية أو بالقرب من الألياف العضلية خارج العقدة المخية .

ترسل هذه الخلايا أليافاً أخرى لتنتهي في وسط العقدة المخية حيث توجد العديد من التشابكات العصبية والتي تتميز بظاهرة الاستخلاء الخارجي كيميائية لخروج محتوي الحبيبات الإفرازية . الدراسة السيتوكيميائية أوضحت وجود مركبات الكاتيكولامين ضمن هذه الحبيبات الإفرازية .

Key Words: Cerebral ganglion - Earthworm Senso-neuroendocrine cells

### ABSTRACT

Ciliated neurones of probable endocrine function are present inside and outside the cerebral ganglion of the earthworm *Aporrectodea caliginosa*. Cell fibers contain clusters of synaptoid vesicles in regions adjacent to the brain capsule or muscles. The cell bodies also send axons to terminate within the neuropile, where synapses and exocytosis of their secretory granules occur. Cytochemical studies indicate the presence of catecholamines within these granules.

### INTRODUCTION

The cerebral ganglion of earthworms is regarded as the source of "vascular" hormones controlling many biological activities (Herlant-Meewis, 1977; De Morias *et al*, 1979; Mill, 1982; Al-Yousuf, 1984). The brain contains distinct categories of typical neurosecretory (NSY) cells whose fibres terminate within the neuropile and within the precapsular epithelium (Aros *et al*, 1977; Al-Yousuf, 1984, 1987, 1988a). Secretory end feet (SEF) system which contain secretory vesicles and abundant of mitochondria are examples of neuronal secretory cells. These cells differ cytologically in some respects from typical NSY cells (Golding and Whittle, 1977; Al-Yousuf, 1988a). The same authors have emphasised the existence of senso-neuroendocrine cells which might be a common phenomenon in annelids.

In oligochaetes, most sensory cell bodies lie in the epidermal layer and their axons enter the central nervous system via the

first and second segmental nerve (Gunther and Walther, 1971). Their distal ends are presumably associated with various ciliated sensory cells (Mill and Knapp, 1970). The three pairs of lateral segmental nerves contain axons of both sensory and motor neurons. The former of which number over 10,000 in each half segment; these axons correspond in number to the epidermal sensory cells (Knapp, 1970; Mill, 1978). In addition, there is a small number of sensory cells whose cell bodies lie within the central nervous system. Their axons terminate in the epidermis and in some cases leave the central nervous system in more than one nerve (Knapp and Mill, 1968; Mill, 1982). This study deals with the ultrastructure of these sensory cells.

Aros *et al*, (1971a, b) suggested that sensory cells contain densecored vesicles, 120-170 nm in diameter, with acetylcholinesterase activity. On the other hand Myrhberg, (1971) reported the presence of primary monoamine, stored in granules of 30-50 nm in diameter. This study further investigates on these

granules by cytochemical study.

Within the muscles of body wall two types of neuromuscular junctions have been described in earthworms. In the first type the axons contain spherical vesicles (30-70 nm) in diameter and there is a large synaptic cleft containing basement membrane material. In contrast, the second type axons contains large dense-cored vesicles, 90-150 nm in diameter; the small synaptic cleft is devoid of basement membrane material (Mill and Knapp, 1970; Rosenbluth, 1972). Similar junctions were observed within the muscles surrounding the brain (Al-Yousuf, 1984). Further information on the types of neuromuscular junctions is presented in this study.

In annelids, there is a wide range of photoreceptor cells varying from single cells to common eye types. In earthworms, numerous unicellular photoreceptors have been found on the prostomium and distributed in the epidermis. A number of these photoreceptors occur in the nerves of the head and caudal regions, as well as in the cerebral ganglion (Mill, 1978, 1982). The ultrastructure of the unicellular photoreceptors within the brain of earthworms is described in further detail in this report.

#### MATERIALS AND METHODS

Specimens of *A. caliginosa* (Al-Yousuf, 1987) were collected from Qatar farms. Six freshly dissected cerebral ganglia were treated in each of follows solutions at 4°C.

1. 0.8% glutaraldehyde + 1% OsO<sub>4</sub> in 0.05 M phosphate buffered at pH 7.2, for 0.5 hour, rinsed in 0.05 M phosphate buffer at pH 7.2 for 15 mins and post-fixed in 1% OsO<sub>4</sub> for one hour.

2. Amine cytochemistry method as described by (Tranzer and Richards, 1976). Fixation in 1% glutaraldehyde + 0.1 M cacodylate buffered at pH 7.2. This followed by a rinse in 0.2 M sodium chromate-potassium dichromate buffered at pH 6.5 for three hours and post-fixed in 1% OsO<sub>4</sub> for one hour.
3. Control specimens were treated as amine cytochemistry method without corresponding substrates (Chromate-dichromate). Fixation was followed as mentioned above in step (2).

The methods used for the dehydration, infiltration, cutting and staining of the tissues are given elsewhere (Al-Yousuf, 1988a). Serial ultra-thin sections were examined using a Jeol 100KV electron microscope at the Science College of King Abdul-Aziz University, Jeddah, Saudi Arabia.

#### RESULTS

Among the various types of NSY cells which occur within the brain, one type of ciliated cell was detected both outside and inside of each ganglion examined. These cells were located dorsoposteriorly and stained lightly with toluidine blue. The cell bodies are fusiform or tear-shaped, measuring about 14 × 25 μm (Fig. 1). They are distributed singly or in pairs and vary from 2 to 4 cells on each side. The perikarya of these cells are adjacent to blood vessels or the brain capsule at both sides of the ganglion. The ciliated cells are invested by epithelium but lacking associated supporting cells (Fig. 1).

From their fine structure, these cells appear to be sensory, with a single cilium or bundles of cilia. Most of the extra-cerebral sensory cells are unciliated (Figs. 1, 2). However, some bear

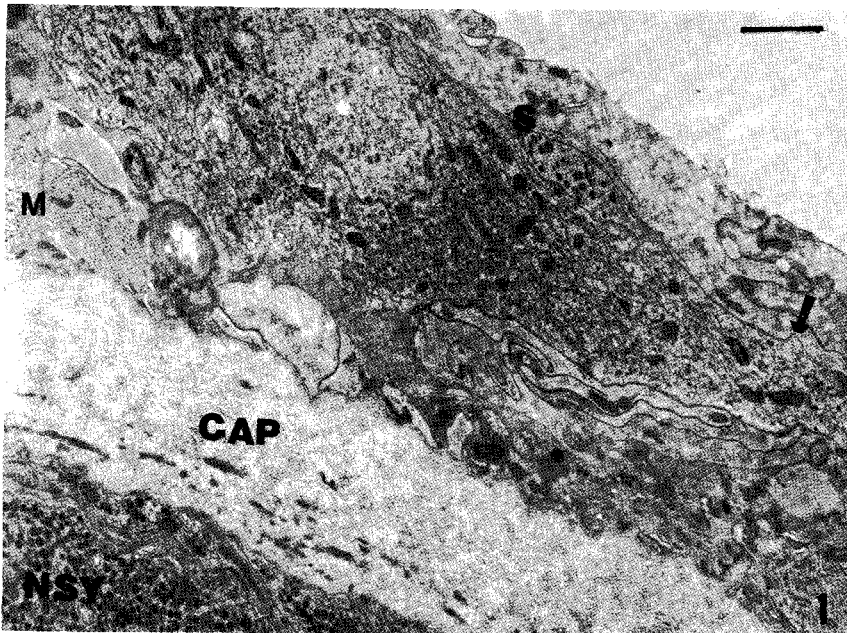


Fig. 1: Sensory cell (S) lying outside the cerebral ganglion. Note neurosecretory cell within the brain (NSY). Arrow indicates a single cilium. Muscle (M), brain capsule (CAP). Scale bar = 1 μm / × 11,000



Fig. 2: Perikaryon of an extracerebral sensory cells with single cilium (C). Note variation of density within the granules. Rough endoplasmic reticulum (R), mitochondria (M), Golgi body (G). Scale bar = 1 μm / × 33,000

large numbers of cilia varying from 25 to 42 each cell (Fig. 3). Intracerebral sensory cells are mainly multiciliated (Fig. 4). In each case the axonema possesses a full complement of microtubules i.e., a central pair and an outer ring of nine double

microtubules: (9+2). Prominent dynein arms of faint radial filaments connect peripheral and central microtubular complexes. An association with a basal body or a ciliary root has not been detected (Figs. 2-4).

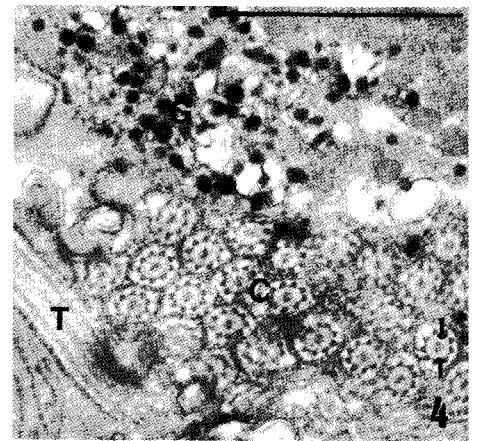
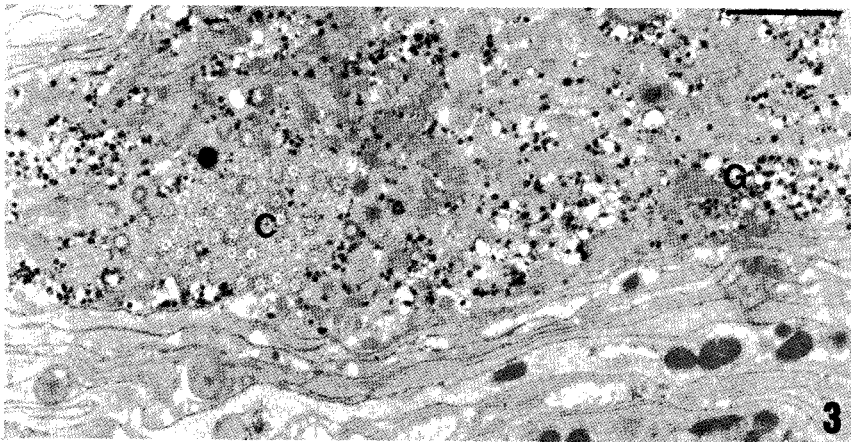


Fig. 3: An extracerebral sensory cells with many cilia (C). The granules (G) have highly electron-dense contents with amine cytochemistry. Scale bar =  $1\mu\text{m} / \times 15,000$

Fig. 4: An intracerebral sensory cell containing a large number of cilia (C) which enveloped by the unit membrane. Note prominent dynein arms and faint radial filaments interconnecting peripheral and central microtubular complexes (Arrows). Note also longitudinal section of microtubules (T). The granules (G) have a dense positive reaction with amine cytochemistry. Scale bar =  $1\mu\text{m} / \times 33,000$

The cilium also contains aggregates of electron-dense material and is surrounded by a circle of various numbers of large, evenly spaced microvilli, which are arranged in rows and extend into the cell body for about  $0.5\mu\text{m}$ . Each microvillus is  $0.1-0.2\mu\text{m}$  in diameter and  $0.6-0.8\mu\text{m}$  in length. These microvilli are avoid, with the narrow and oriented towards the cilium, and are usually filled with tonofilaments (Figs. 3, 4).

Sensory cells contain granules ranging between  $50-120\text{nm}$  in diameter and having irregular cores of variable electron density (Fig. 2). These granules give a positive cytochemical reaction for amines which reveal a highly electron-dense core (Figs. 3, 4). The granules are widely distributed in perikarya, axons, dendrites and terminals.

Characteristically, the rough endoplasmic reticulum is poorly developed and consists mainly of short threads which tend to be more common peripherally. In contrast, an abundance of smooth endoplasmic reticulum was found throughout the perikarya. Free ribosomes and a number of dense glycogen particles are dispersed throughout the cytoplasm along with lysosomes, mitochondria, neurofilaments, neurotubules and multivesicular bodies. A few Golgi bodies are found in the perinuclear region of the cytoplasm (Fig. 2).

As it is evident from serial sections sensory cells are bipolar or multipolar. Fibers from the intracerebral sensory cells either terminate within the neuropile or penetrate the brain capsule. They also and extend anteriorly where synaptoid endings may abut stroma of collagenous fibers or muscle (Figs. 5, 6). Fibers of the extracerebral sensory cells are widely distributed through the pericapsular epithelium and may form synapses at this side of the brain (Fig. 7). The same cells send axons through the brain capsule (Fig. 8) to terminate within the neuropile, where synapses and endocytotic figures were observed (Figs. 9, 10).

Visceral muscle are widely distributed outside the cerebral ganglion. The fibers of sensory cells occur either associated with the main part of the muscle fibers or with muscle tails, which are devoid of contractile material. Clear neuromuscular synapses were observed, in which the sensory fibers contain mostly small vesicles,  $30-50\text{nm}$  in diameter, and small numbers of granules. The synaptic cleft ( $18-25\text{nm}$  wide) contains no basement membrane material. The post-synaptic membrane has a little electrodense material on its inner surface (Fig. 6). Another two types of granules are involved with neuromuscular junctions but have no connection with sensory fibers. These are similar to those described by (Al-Yousuf, 1984) and also by (Mill and Knapp, 1970) and by (Rosenbluth, 1972).

Pigment cells occur within the pericapsular epithelium and give rise to processes which may be mixed with sensory fibers.

Adjacent to the sensory cells, single or paired photoreceptor cells are located within the cerebral ganglion. These cells contain an organelle composed of a transport hyaline region associated with a dense region. The inner hyaline region appeared as a lens supported by a network of anastomosing neurofibrils (Fig. 11). These cells also contain a rhabdomere-like array of microvilli, which arise as evaginations of the outer plasma membrane of the cell (Fig. 11). There is direct contact between members of pairs of these cells where interdigitation of their microvilli occur. Each microvillus is  $0.1-0.2\mu\text{m}$  in diameter and  $0.8\mu\text{m}$  in length. Several cilia may fill the cavity of the microvilli (intervillous space) but seem to be independent of them. These cilia show the  $9 + 2$  pattern of tubules associated with a basal body along with ciliary roots (Fig. 12).

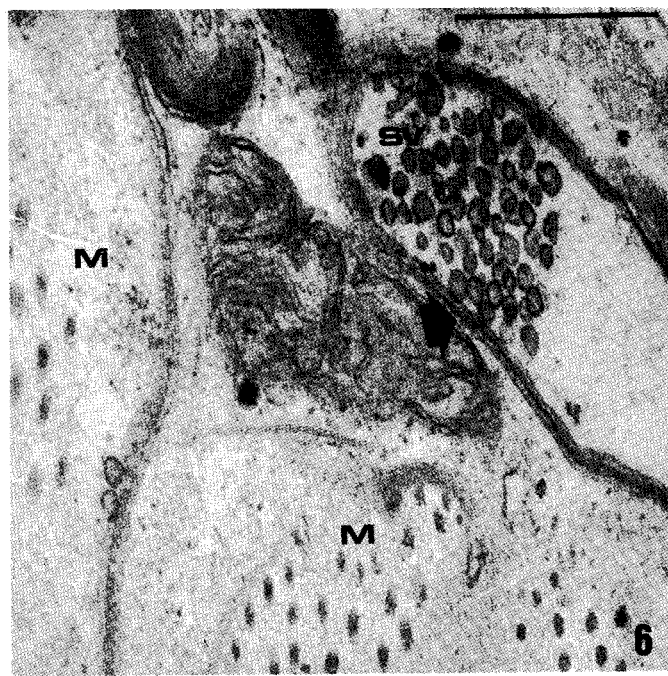
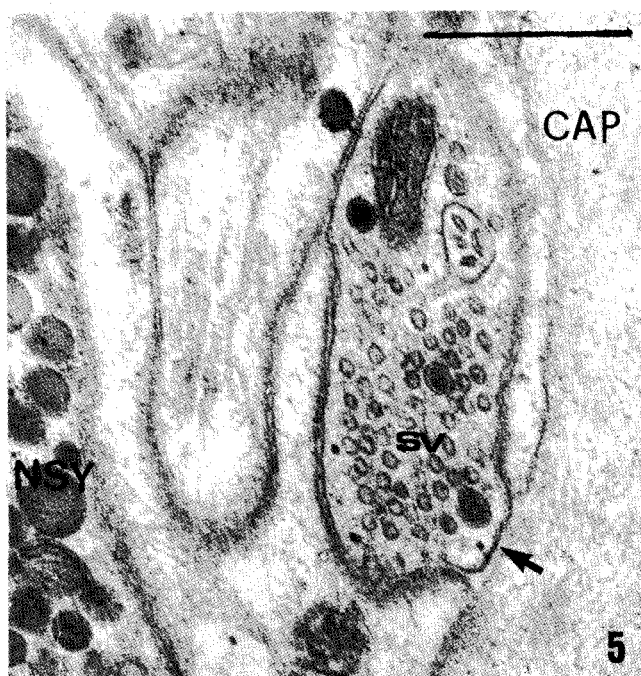


Fig. 5: Fiber of an intracerebral sensory cells followed by serial sections and found to be terminated against the brain capsule (CAP) (Arrow). Note clear synaptoid vesicles (SV) and part of neurosecretory cells (NSY) within the brain. Scale bar =  $0.5 \mu\text{m} / \times 55,000$

Fig. 6: Possible fiber of an extracerebral sensory cell containing a large number of synaptic vesicles (SV) forming a neuromuscular junction outside the brain. Arrow indicates the postsynaptic membrane, which has a little electron-dense material on its inner surface. Scale bar =  $0.5 \mu\text{m} / \times 55,000$

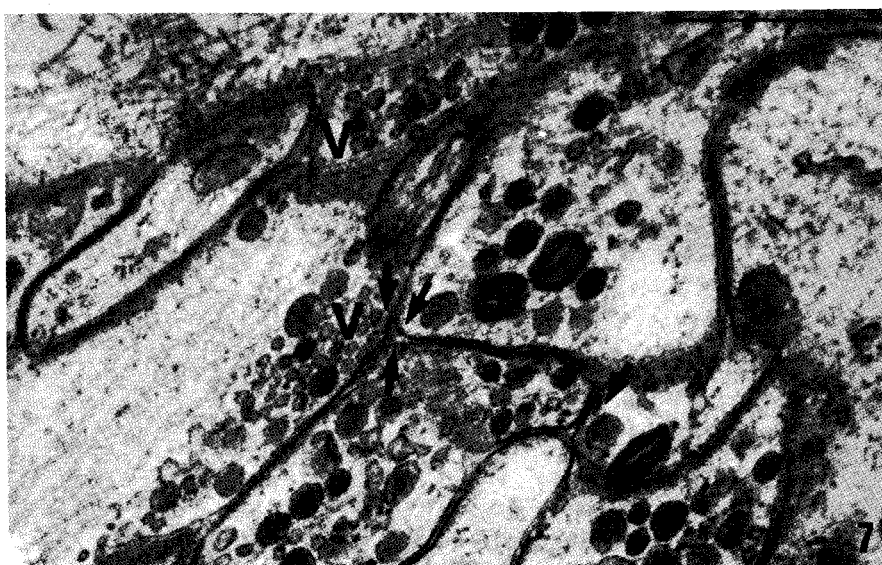


Fig. 7: Number of possible sensory fibers within the pericapsular epithelium. Note clear vesicles (V) which may indicate synapses between these fibres (Arrows). Scale bar =  $0.5 \mu\text{m} / \times 55,000$



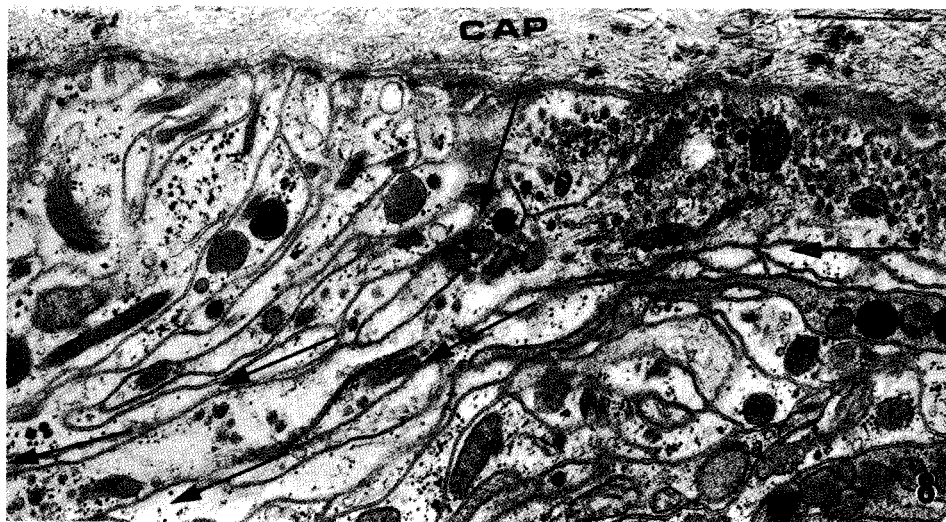


Fig. 8: Fibers from an extracerebral sensory cells followed by serial sections and found to be passing through the brain capsule (CAP) and extending towards the neuropile (Arrows). Scale bar = 1  $\mu\text{m}$  /  $\times 18,000$

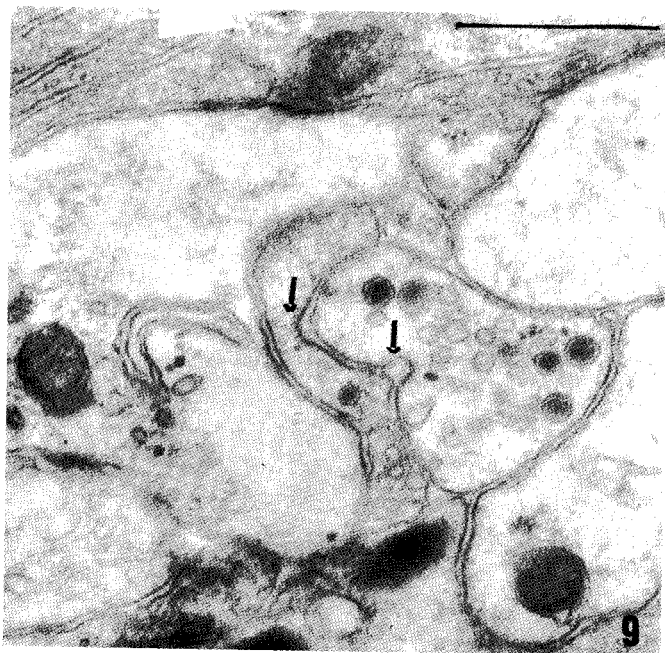


Fig. 9: Two possible sensory fibers within the neuropile. Note well defined endocytotic coated profiles (Arrows). Scale bar = 0.5  $\mu\text{m}$  /  $\times 55,000$

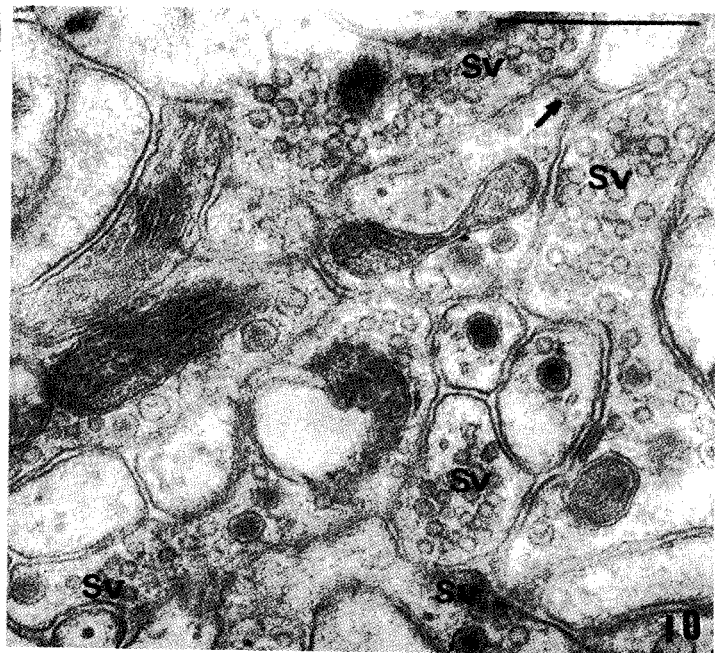


Fig. 10: Possible sensory fibers within the neuropile contain clusters of synaptic vesicles (SV). Arrow indicates possible exocytotic material. Scale bar = 0.5  $\mu\text{m}$  / = 55,000

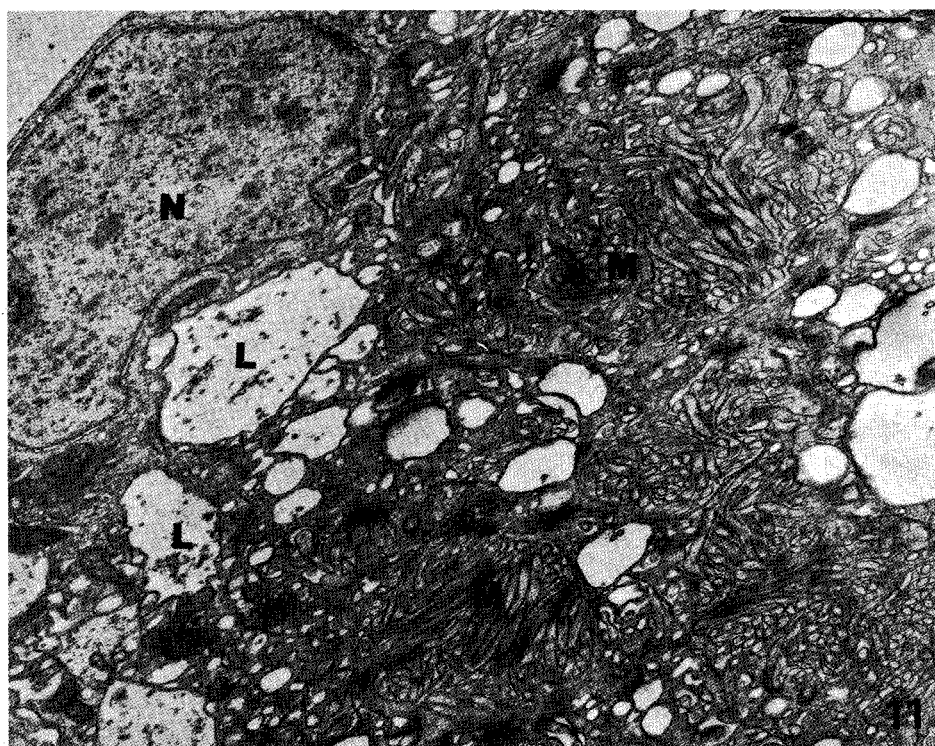


Fig. 11: Photoreceptor cell. Note number of lens (L) and the large number of microvilli (M). Nucleus (N). Scale bar = 0.5  $\mu\text{m}$  /  $\times$  33,000



Fig. 12: Photoreceptor cell contains several cilia (C) which fill the cavity of the microvillus (M). Note the basal body (B) and the ciliary root (R). Scale bar = 0.25  $\mu\text{m}$  /  $\times$  80,000

#### DISCUSSION

This is the first detailed study of the fine structure of intracerebral sensory cells within the cerebral ganglion of the earthworm. However, extracerebral sensory cells and several types of NSY cells have been classified within the cerebral ganglia of many earthworm species (De Morias *et al*, 1979;

Al-Yousuf, 1984, 1987, 1988a). The strongest evidence for the endocrine character of some NSY is their exclusive localization within the brain (Golding and Whittle, 1977; Al-Yousuf, 1988a). This applied to the sensory cells in this study which may have a sensoendocrine function. This phenomenon is also established in other invertebrates (Zahid and Golding, 1974; Golding and Whittle, 1977).

Sensory cells may have a well-substantiated potential transmitter function, since synaptic release within the neuropile was observed. Furthermore materials released by granule exocytosis may be regarded as neuromodulators which have longer lasting, and more diffuse, influences on neural activities than those produce by synaptic transmitters. The same materials may act as a hormone when released directly into the blood system or when they diffuse from the perikarya into the intercellular space (Al-Yousuf, 1988a). There is now much evidence for the diverse functional roles of neuropeptides as neurohormones, neurotransmitters and neuromodulators. Furthermore, the co-existence of several peptides and conventional neurotransmitters in a single cell has been very well established (Scharer, 1985; Hokfelt, 1990).

Generally, earthworm ciliated cells are structurally similar to the corresponding sensory cells in polychaetes (Golding and Whittle, 1977). As shown in this study, sensory cells contain biogenic amines which may have function with neuropeptides through synapses within the neuropile. These cells may give information or act as receptors for neuropeptides and so help in their functional interpretation. More studies are needed to define role of amines in physiological activity, and their roles in controlling biological processes.

Sensory fibers form a rich synaptic zone within the pericapsular epithelium as well as in the neuropile. In these zones the sensory cells communicate with other nerve cells. The integrative activities of the cerebral ganglion are presumably affected by the nerve processes in these synaptic zones. Sensory cells may involve in the mediation of feedback influences impinging upon the NSY system. Furthermore, sensory cells act as regulators of visceral movement. This regulation appears to involve double innervation, since one of the neuromuscular junctions contains small dense core vesicles and the other contains peptidergic granules (Mill and Knapp, 1970; Rosenbluth, 1972; Al-Yousuf, 1984). Visceral muscles may act in a synchronous rhythm with body lengthening and shortening (Rosenbluth, 1972). Furthermore, amines isolated by histochemical techniques, including dopamine, noradrenaline and 5-hydroxytryptamine, seem to be involved in functioning of the NSY cells in oligochaetes (Herlant-Meewis, 1977).

More recently, the immunocytochemical results suggest the existence of peptides resembling met and leu-enkephalin in sensory cells within earthworm-epidermis. The processes of these cells become collected to form sensory nerves before entering the ventral ganglionic chain. Further study suggest that opioid peptides act as sensory transmitters in earthworm (Gresser and Larsson, 1986).

The cilia of sensory cells may have a mechanical function and could contribute effectively to the circulations of the coelomic fluid (Aros *et al*, 1971a, b; Zimmermann, 1971). Zahid and Golding, (1974) suggested that sensory cells within the brain may have an osmoreceptor function. Association of sensory fibers with pigment cells supports the possibility of a photoreceptor function for the latter.

The photoreceptor organs exhibit a considerable diversity of form in annelida. Little is known concerning the functional significance of this diversity (Zahid and Golding, 1974). As shown in this study, the cilia of the photoreceptor cells extend into the central vesicle, where they intermingle with the microvilli (see also Rohlich *et al*, 1970). These cells may be

involved in the control of the NSY cells associated with them.

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