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NEUROSECRETORY CELLS IN CERTAIN EARTHWORMS SPECIES (OLIGOCHAETE)

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ABSTRACT

Typical Neurosecretory cells of the cerebral ganglion were studied in three oligochaete families: *A. porrectodea caliginosa, Dendrodrilus rubidus :* (Lumbricidae); *Amynchas corticis :* (Megascolidae); *Dichogaster bolaui :* (Octochaetidae). In the first species five types of neurosecretory cells (A_1 - A_5) were studied in details by light and electron microscope. Each type has differentiated shape, size and location within the cerebral ganglion. Ultrastructurally, each type has highly distinctive elementary granules. Compartive survey was made for the other species using light microscopy only.

INTRODUCTION

Neurosecretory cells (NsCs) occur in a large number in the cerebral ganglia of all annelids, Scharrer and Scharrer (1945) stated that "Almost one half of the cerebral ganglion is glandular".

Various opinions have been expressed about the types of NsCs, present in the cerebral ganglion (Harms, 1968; Herlant–Meewis, 1955, 1956a,b; Aros *et al.*, 1965; Aros *et al.*, 1977,1980; Demorias *et al.*, 1979; Kinoshita and Kawashima, 1986). Al–Yousuf (1984) established a full description of the NsCs in the earthworms *Lumbricus terrestris, Eisenia foetida, Octolasion cyaneum, Dendrobeona subrubicunda* and *Allolopophora longa.* Type A_1 – A_5 cells are abundant in these species and occur only in the cerebral ganglion. Other NsCs types also occurs in the cerebral ganglion and widely distributed through the nervous system (Al–Yousuf, 1984). Similar results were obtained in this study which confirmed the main pattern of NsCs classification and distribution in local earthworm species.

MATERIALS AND METHODS

The oligaochaetes *A. caliginosa, D. rubidus, A. corticis* and *D. bolaui* were collected from Roudat Al–Faras, State of Qatar.

For light microscope, the earthworms were submerged in 0.85% saline solution. The cerebral and nerve ganglia were dissected, immediately fixed in Bouin's fixative for 24 hours at room temperature, washed in several changes of 70% ethanol, dehydrated in an ascending series of ethanol, cleared in xylene of methylbenzoate, and embedded in in paraffin wax (MP 50°C). Serial sections of 4–6 μm thick, were stained with paraldehydefuchsin (PAF) (Gabe, 1966).

For electron microscope, freshy dissected cerebral and nerve ganglia of *A. caliginosa* were fixed at 4°C in 1% OsO_4 in veronal acetate buffer pH 7.2 or pH 7.4 for 1 hour, washed in buffer for 15 minutes, dehydrated in a ascending series of ethanol, and processed for epoxy embedding. 1 μm semithin sections were stained with 1% toluidin blue (TB) and used for the light microscope for general survey. Adjacent, ultrathin sections were double–stained with uranyl acetate and lead citrate examined in Joel transmission electron microscope operating at 120 KV (In King Abdulaziz University, Faculty of Science, Jeddah, Saudi Arabia).

The criteria use to distinguish NsC types were the topography, shape, average size of the cells and the staining affinity of the secretory contents. Ultrastructurally, size and appearance of the elementary granules in each type of NsCs were considered as the most important criteria.

RESULTS

Basic work in distinguishing NsCs was done on *A. caliginosa* (Fig. 1) then a comparative survey was made for the other three species; *D. rubidus* (Fig. 2); *A. corticis,* (Fig. 3) and *D. bolaui* (Fig. 4). Some NsCs are very widely distributed throughout the whole nervous system, (Fig. 5). In contrast, NsCs which described here, are only located within the cerebral ganglion.

In the four species, five types ($A_1 - A_5$) of NsCs are observed in the dorso–posterior regions of the cerebral ganglion. Their distribution is easily observed in wax or semi–thin sections stained with TB or PAF. Different cell types have affinities for TB and PAF stains (Figs. 1–4). Table (1) contains, comparison of NsC another two types NsCs, namely C and SEF types

(Al–Yousuf, 1984) were observed by electron microscope with the cerebral ganglion of *A. caliginosa*. However, they were hardly observed in other species using light microscope only.

No major differences in NsCs characteristics were observed among the four species. However, cell size varies in these species proportional to the cerebral ganglion size (Table 2).

At the ultrastructure level in *A.caliginosa,* type A_1 cells form most of the peripheral cells lying beneath the brain capsule. Their axons make contact with the brain capsule or penetrate it to the perineurium. Their distal processes extend to the neuropile region. The NsC granules are round to oval in shape and are 1300 – 2500 A^oin diameter. Their contents range from being moderately to highly electron opaque and are surrounded by a thin clear irregular halo. No correlation between density and granule size could be observed (Fig. 6).

Type A_2 cells form the second layer of cells beneath the peripheral one. Most of their axons enter the neuropile while a few axons make contact with the brain capsule or penetrate it to the perineurium. Their NsC granules are 1400 –2400 A^o in diameter, oval in shape and have moderate electron opaque–contents with no surrounding halo. They can easily be distinguish by their homogenous density (Fig. 7).

Type A_3 cells are clustered in the midline of the ganglion dorsal region and are mingled with the first layer (Fig. 1), most of their axons extend towards the perineurium and a few of them run toward the neuropile. They have NsC granules, 900 – 1600 A^o in diameter and similar in shape and dinsity as those of type A_2 cells (Fig. 8).

Type A_4 cells are mingled with type A_1 cells in the peripheral layer and their axons resemble those of type A_1 (Fig.1). Their NsC granules measured 200 – 1800 A^o and vary in shape from elliptical to oval. They differ greatly in appearance owing to the variable electron density of their contents. No correlation is observed between the size and density of these NsC granules (Fig. 9).

Type A_5 cells are scattered among type A_2 cells and their axons follow a course similar to those of A_4 cells. Type A_5 granules are similar to those of type A_4 cells except that the granules of the former type are larger in size (1100–2600 A^o in diameter) and their contents are less electron opaque than those of the latter (Fig. 10).

DISCUSSION

In the present study, five NsC types are observed in the cerebral ganglia of the four local earthworm species. These results are similar to that (Al−Yousuf, 1984) which described similar types of NsC in British earthworm fauna.

These cell types are identified on the basis of closely correlated observation of semi−thin resin sections stained with TB and the adjacent ultra−thin section. OsO_4 / veronal acetate buffer gives each type of the NsCs a characteristic degree of TB staining affinity. This makes the link between light microscope and electron microscope comparatively easy (Al−Yousuf, 1984).

Such clear differentiation of cell type is not always possible in other species, and not even possible in the mammal hypothalamo−hypophysial system, although immunocytochemistry reveals that two distinct cell types secret oxytocin and vasopressin, respectively (Martin and Voigt, 1981).

In other endocrine systems, a multiplicity of cell types sinus gland of Crustacea (review by Bern and Hagadorn, 1965 is correlated with a diversity of distinctive granule types, e.g.: Nephtys cerebral ganglia (Zahid, 1974) and pars intercerebralis of insects, (Maddrell and Nordmann, 1979).

Many of the features which distinguish different granules types may well be considered as fixation artifacts, but this does not underestimate their diagnostic value. For example, the almost lucent appearance of the contents of many granules in types A_4 and A_5 is a reminiscent of "mature" granules in the vertebrate pituitary, awhich have been called "granule ghosts".

This effect is caused by fixation damage since when a fixative of pH 5.0 is used all granules appear densecored (Morris and Cannata, 1972). Similarly in this study, more "granule ghosts" are observed with OsO_4/veronal acetate fixative of pH7.4 than following faxative at pH 7.2.

Many authors try to classify NsCs in the earthworms on the basis of their affinity to PAF stain (Zahid, 1977; Herlant−Meawis, 1962). Such classification may lead to an understimation of the complesity of the cerebral NsC system. Gallisian and Girardie (1972) attemted to combine observations made by light microscope with those by electron microscope. However, glutaraldehyde was used as a primary fixative which may produce severe problems, such as shinkage of tissue and high density of the ground cytoplasm; furthermore, no attempt was made to correlate their findings with those of other authors.

An early attempt was made by (Bern and Hagadorn, 1965) to establish the equivalence of NsCs described by various authors in earthworms at the light microscope level. Later investigations in the laboratories of both Herlant - Meewis, (1974) and Aros, (1977) have been extended the observations to the ultrastructural level. Table (3) is an attempt to correlate the results of several previous studies with that of the present study. The equivalence of cell types is based similarities of general features and ultrastructural appearance in the published micrographs. In view of the present finding together with those of previous studies, it is quite possible that types $A_1 - A_5$ (which are identical to the classical NsCs in their staining affinity and ultrastructural) are sources of certain hormones. Changes in the secretion is an indiction of an endocrine role of these cells (Herlant–Meewis, 1955; Aros *et al.*, 1965; Al–Yousuf, 1987a).

Other lines of evidence for their endocrine character include their exclusive localization within the brain. In the polychoete Nephtys all the exclusively cerebal cells (and only they) contribute to the neurohaemal complex (Zahid, 1974).

Cytochemical studies have implicated the posterior region of the cerebral ganglion as the source of hormones (Al–Yousuf, 1987a; Saussey, 1970; Gallissian, 1968; Gallissian and Girardie, 1972). Recently, attention has been given to establish the nature and role of peptidergic secretions within the brain of the earthworm. A limited number of peptides has been demonstrated immunocytochemical; enkephalin and B–endorphin have been shown to be present in the cerebral ganglion (Alumets *et al.*, 1979) whereas endorphin and CRF is located in the nerve chain ganglia (Remy and Dubios, 1979; Remy *et al.*, 1982). Recently, thyroglobulin like material was found in both the cerebral ganglion and the ventral nerve cord of *E. foetida* (Marcheggian *et al.*, 1985). Immunocytochemically, substance P were found in various types of NsCs while ACTH and antiopsin are located mainly in type A of NsC (Aros *et al.*, 1980) furthermore type A cells were found to react negatively with Arginine Vapsopressin and Oxytocin Antisera, whereas type B cells reacted positively (Kinoshita and Kawashima, 1986). The role of hormones in various physiological control processes can be revealed by further knowledge of the peptides released by NsCs.

Table 1

Charactieristic Features of NsC Types in *A. caliginosa*

Cell-type	Cell-shape	Cell-size	Staining Affinity of NsC	
			TB	PAF
A_1	Variable in shape Multipolar	Small, 13 μm X 21 μm	Very strong affinity dark blue	Dark puple
A_2	Typical pear shape Bipolar	Medium, 15 μm X 26 μm	Moderate affinity Blue	Moderate affinity Purple
A_3	Fusiform shape Monopolar	Small, Strong affinity 10 μm X 18 μm	Strong affinity Dark blue	Purple
A_4	Viriable in shape Bipolar	Small 14 μm X 20 μm	Weak affinity Faint Blue	Very weak affinity Faint Purple
A_5	Variable in shape, Bipolar	Moderate Affinity 21 μm X 32 μm	Moderate affinity Blue	Purple

Table 2

Dimensions of the different NsC types in various Earthworm species.

Species	A_1 Cells	A_2 Cells	A_3 Cells	A_4 Cells	A_5 Cells
A. caliginosa	13 X 21 μm	15 X 26 μm	10 X 18 μm	14 X 20 μm	21 X 32 μm
A. corticis	12 X 18 μm	13 X 21 μm	9 X 15 μm	13 X 18 μm	19 X 29 μm
D. bolaui	13 X 20 μm	14 X 23 μm	11 X 17 μm	14 X 20 μm	21 X 31 μm
D. rubidus	14 X 22 μm	16 X 25 μm	12 X 19 μm	15 X 21 μm	23 X 33 μm

Table 3

Possible equivalence of NsC types in earthworms.

Present Investigation	Aros et al. (1965)	Aros et al. (1977)	Herlant-Meewis (1956a,b)	Herlant-Meewis (1974)	De-Morais et al. (1979)	Gallissian & Giradie (1972)	Berjon & Meunier (1968)	Oosaki (1966)	Kinoshita & Kawashima (1986)
A_1	A_1	A_1	a	A_1	1	2	—	1	b Cells
A_2	A_2 (Majority)	A_2 (Majority)	Part of A	A_2	3	1	A	2	a Cells
A_3	$A_3 + A_2$ (Majority)	$A_3 + A_2$ (Majority)	—	—	—	—	—	3	c Cells
A_4	—	Part of A_3	—	—	—	3	—	—	
A_5	Possible Part of B	Possible Part of B	Grands et-Moyens neurones	—	—	—	—	—	

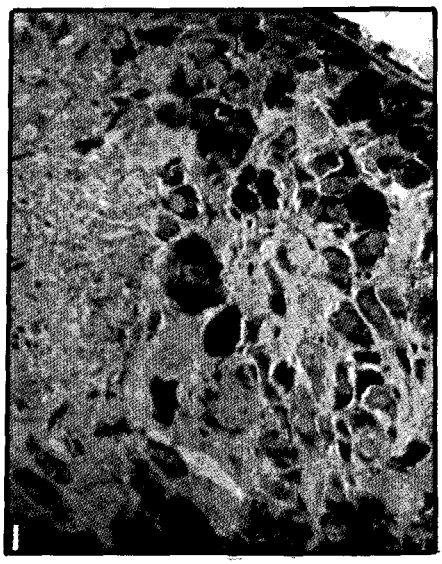

Figure 1 : ***A. caliginosa,*** **sagittal epoxy section of the brain stained with TB. Note the variation of staining affinity for each cell type.**

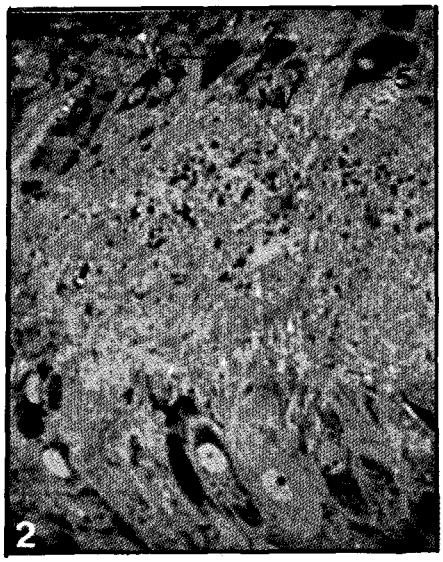

Figure 2 : ***D. rabidus,*** **ransvers epoxy section of the brain stained with TB. Note the cluster of type A_1 of NsC have the most positive affinity for the stain. Note variation of staining affinity for other cell types.**

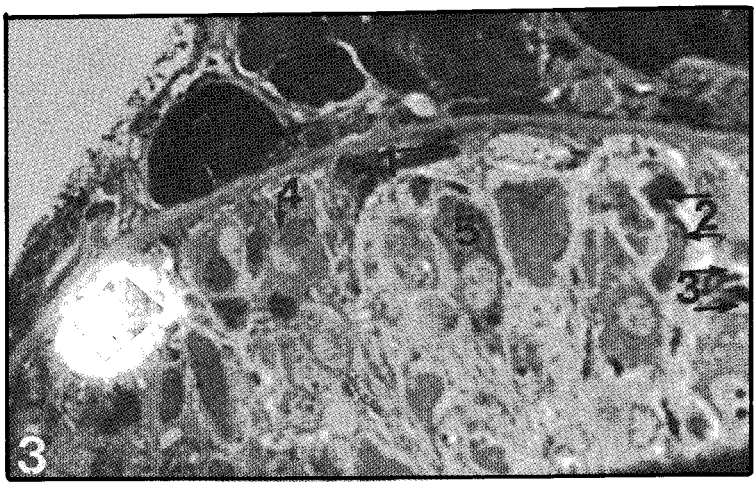

Figure 3 : *A. corticis,* transverse wax section of the brain stained with PAF. Type A_1 of NsC have the most positive affinity for the stain. Note variation of staining affinity for other cell types.

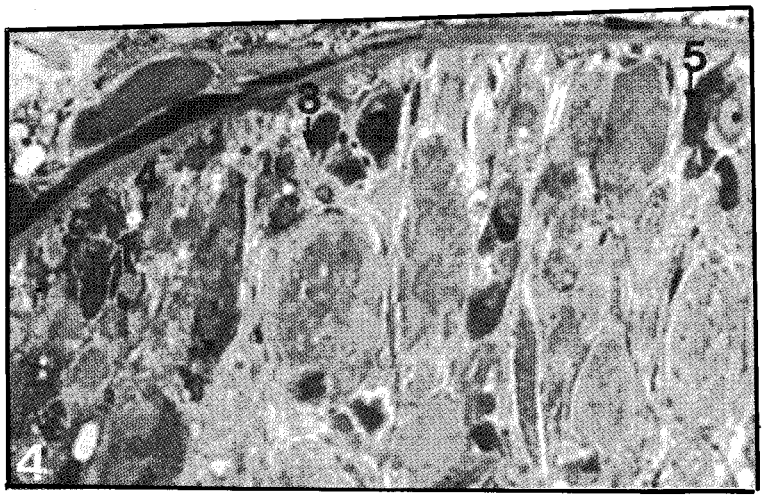

Figure 4 : *D. bolaui,* sagittal wax section of the brain stained with PAF. Note the presence of five types of NsC whicn vary in size and staining affinity.

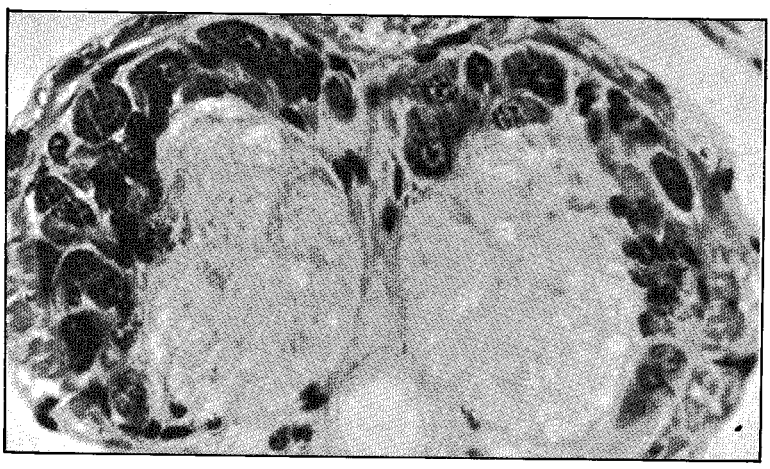

Figure 5 : *A. caliginosa,* transverse wax section of the nerve cord stained with Hematoxylin and Eosin. Note absence of types $A_1 - A_5$ NsC. Many other NsC are exist.

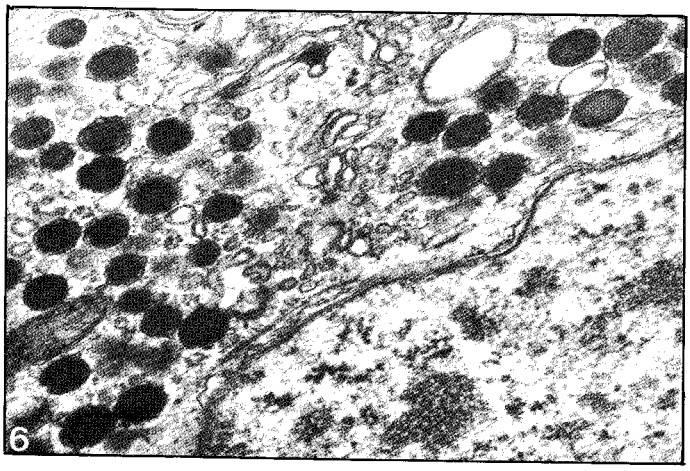

Figure 6 : *A. caliginosa,* type A_1 of NsC. Note the fine appearance of the ganules and their thin irregular halo.

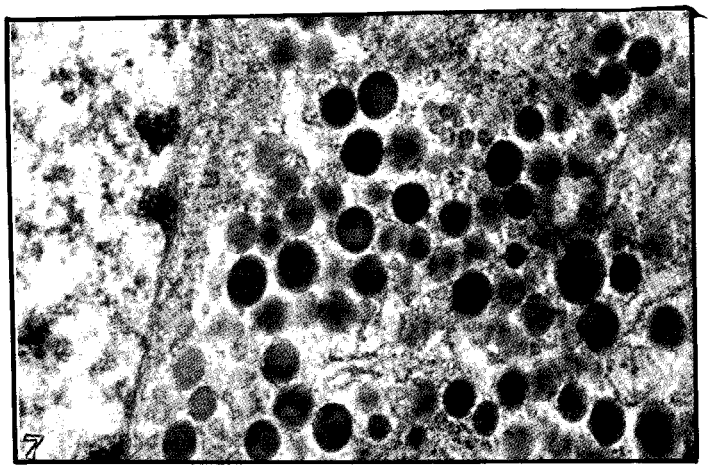

Figure 7 : *A. caliginosa,* type A_2 of NsC. Note oval shape and moderate homogenous electron density of the granules.

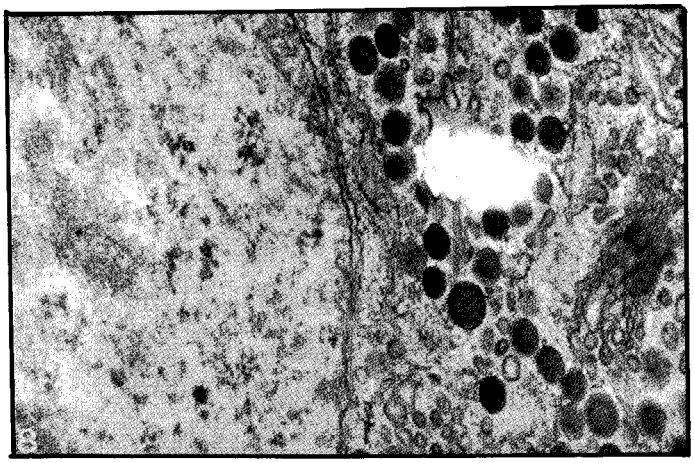

Figure 8 : *A. caliginosa,* type A_3 of NsC having small size granules. Note similarity in shape and density to these of type A_2 cells.

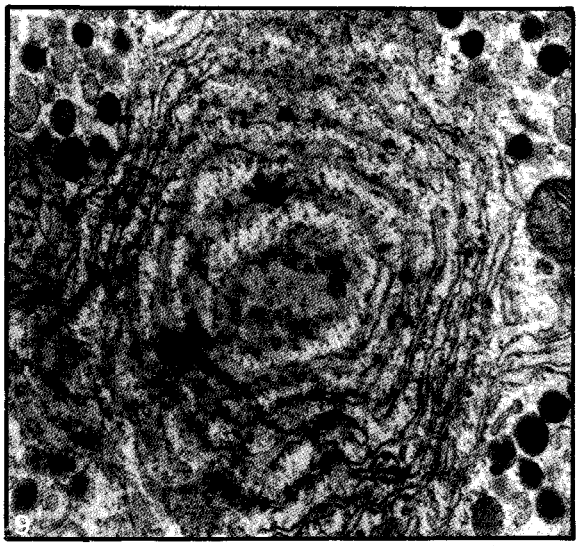

Figure 9 : *A. caliginosa,* type A_4 of NsC having medium size granules which vary in shape and density.

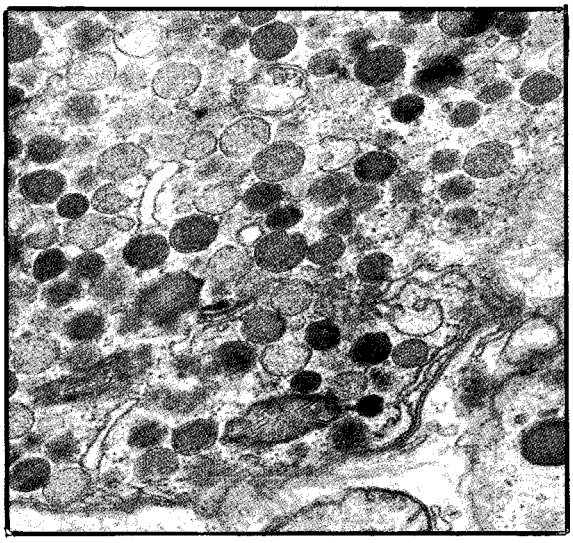

Figure 10 : *A. Caliginos,* type A_5 of NsC having large density of these type A_1 cells.

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الخلايا العصبية الافرازية في أنواع محددة من الديدان الأرضية

شـــعاع اليوســـف

تم دراسة الخلايا العصبية الإفرازية المؤلف ستالح اليوسف المنتشرة في العقد المخية لأربعة أنواع من ديدان الأرض التابعة لطائفة قليلات الأشواك وهي كالتالي :

ابريكتودا كاليجينوزا دندرودريلس روبيدس (عائلة لمبريسيدا)
أمانثاس كورتبيـــــس (عائلة مجاسكوليدا)
ديكوجاستر بـــــولاوى (عائلة أوكتوشايندا)

في الجنس الأول صنفت أهم الخلايا العصبية الإفرازية بواسطة المجهر الضوئي والمجهر الإلكتروني إلى خمسة أنواع بناء على شكل وحجم وموقع هذه الخلايا داخل العقد المخية . كما أوضحت الدراسة وجود حبيبات إفرازية لها شكل وحجم محدد لكل نوع من أنواع الخلايا العصبية الإفرازية . وقد قورنت النتائج في الأجناس الثلاثة الباقية والتي أثبتت تواجد هذه الأنواع الخمسة من الخلايا العصبية الإفرازية في جميع العقد المخية المدروسة .