The Ultrastructure of Neurons and Neuroglia
in the Cerebral and Pleuro-Pedal Ganglia
of Haliotis asinina Linnaeus

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ABSTRACT

The ultrastructure of neurons and neuroglia in the cerebral and pleuro-pedal ganglia of
Haliotis asinina are described. There are four types of neurons (NR 1-4) and three types of neuroglia.
(NG1-3). The NR1, which is the largest nerve cell, has a round nucleus with a thin rim of heterochromatin
attached to the nuclear envelope. The cytoplasm contains numerous rough endoplasmic reticulum,
mitochondria, but only a few small elementary granules. The nuclei of NR2 , NR3
and NR4 have increasingly
condensed heterochromatin. The cytoplasm of these cells contain relatively few organelles such as rough
endoplasmic reticulum, mitochondria and elementary granules. The NG1 is spindle-shaped with little
perinuclear cytoplasm. The NG2 and NG3 are highly ellipsoid in shape. The cytoplasm of NG1 and NG3
is very thin and contains ribosomes, a few rough endoplasmic reticulum and mitochondria. The cytoplasm
of NG2 is extremely thin and contains only ribosomes. There are no elementary granules in the neuroglia.

KEYWORDS: Haliotis asinina, neuron, neuroglia, ultrastructure.

INTRODUCTION

Bullock and Horridge1 classified the neurons in
the ganglia of gastropods on the basis of morphology
(size and nuclear - cytoplasmic ratio of the cell body
and perikaryon). In the pulmonate snails such as
Achatina fulica Bowdich, Helix pomatia Linnaeus,
Arion ater Linnaeus and Limax maximus Linnaeus,
the classification of neurons is based on cell size.2-4
They are giant neurons, ordinary neurons and globuli
cells. The giant neurons are characterized by their
large size and irregularly-shaped nucleus.4 The nuclei
are often flattened or lobated.5 The nucleoli are
usually large and annular.5 The surface of the giant
neuron is often deeply indented by processes of glial
cells.7 The ordinary neurons are divided into large,
medium and small cells.14 They are similar to the
large neurons in having large clear nuclei with one
or several nucleoli, abundant cytoplasm and thick
processes.1 The ultrastructure of ordinary neurons
has been described in many gastropod species such
as Aplysia californica Cooper,7 Lymnaea stagnalis
Lamarck8. Archachatina marginata Lamarck,9 Helix
aspersa Müller,10 A. fulica,11 Bithynia tentaculata
(Linnaeus)12 and Haliotis rufescens Swainson.13 The
cytoplasm contains a large number of small
mitochondria, innumerable cisternae of rough
endoplasmic reticulum, free ribosomes, polyribosomes,
lysosomes, numerous Golgi complexes and
elementary granules. The free ribosomes and
cisternae of the rough endoplasmic reticulum are
especially concentrated near the nucleus and in the
region of the axon hillock.1 The globuli cells contain
chromatin-rich nuclei. The cytoplasm is scant and
contains organelles similar to those found in the
ordinary neurons.11

Glial cells are distributed between the neurons
and their sheath cells and along the outer surfaces
of blood vessels.7,14,15 They are round or oval. There are two types of glial cells in L. stagnalis.8,14
The first type of neuroglia indents into the
perikaryon and into the axon of the large neurons.
Cell organelles are scarce in the cytoplasm of those
glial cells except for mitochondria. Usually some
glycogen is present. A second type of glial cells
(filamentous glial cells) is characterized by a large
number of thin filaments (5 nm) which are
comparable to tonofilaments. Moreover, these cells
contain abundant mitochondria, a rather extensive
rough endoplasmic reticulum, numerous Golgi
complexes, and lysosome-like structures.8 The
ultrastructure of glial cells of L. stagnalis is similar
to those of A. marginata14 and A. californica.7

From the previous reports on the ultrastructure
of neurons and neuroglia in the ganglia of various
species of gastropods, it is apparent that the
knowledge of ultrastructure of neurons and
neuroglia is still lacking in Haliotis asinina Linnaeus.
Therefore, the present study reports on the fine structure of neurons and neuroglia in the cerebral and pleuro-pedal ganglia of *H. asinina*.

**MATERIALS AND METHODS**

The adult abalone *H. asinina*, with a shell length of 4-5 cm, were obtained from the Coastal Aquaculture Development Center, Klong Wan, Prachuap Khiri Khan Province, Thailand. Abalones were anesthesized with 5% MgCl₂, after which their shells were removed. The cerebral and pleuro-pedal ganglia were dissected out and fixed in a mixture of 4% glutaraldehyde and 2% paraformaldehyde in 0.1M Millonig buffer (pH 7.8) at 4°C for 24 hours. Specimens were washed six times with 0.1 M Millonig buffer. They were postfixed in 1% OsO₄ in 0.1 M Millonig buffer for 2 hours, then dehydrated through a graded series of ethanol. They were embedded in Spurr's resin. The sections were stained with uranyl acetate in 70% ethanol and lead citrate and examined with a Hitachi H-300 transmission electron microscope operating at 75 KV.

**RESULTS**

From the histological observation, there are four types of neurons (NR1-4) and three types of neuroglia (NG1-3) in the cerebral and pleuro-pedal ganglia16,17.

**Type 1 neuron (NR1).** The cell body is round or oval with very large size (14x28 µm). The nucleus is round (9 µm in diameter) with a thin rim of heterochromatin attached to the nuclear envelope. Most of the remaining chromatin is finely dispersed euchromatin that is scattered uniformly throughout the nucleus (Fig 1A). The cytoplasm contains numerous mitochondria and stacks of rough endoplasmic reticulum (Fig 1A). Only few small elementary granules are present.

**Type 2 neuron (NR2).** The cell body is oval and about 4-6 µm in diameter. The nucleus is round (9 µm in diameter) with a thin rim of heterochromatin attached to the nuclear envelope. Most of the remaining chromatin is finely dispersed euchromatin that is scattered uniformly throughout the nucleus (Fig 1B). The cytoplasm is very thin and contains the usual organelles such as, rough endoplasmic reticulum, mitochondria, ribosomes, and some elementary granules (Fig 1B). These cells are rarely observed in the cerebral ganglia, while they are more numerous in the pleuro-pedal ganglia.

**Type 4 neuron (NR4).** These cells are round in shape and about 4-6 µm in diameter. The nucleus contains thin patches of heterochromatin along the nuclear envelope, while most of the nucleoplasm in the central area is clear (Fig 1D). The cytoplasm is thin and contains the usual organelles such as, rough endoplasmic reticulum, mitochondria, ribosomes, along with some elementary granules (Fig 1D). These cells are rarely observed in the cerebral ganglia, while they are more numerous in the pleuro-pedal ganglia.

**Type 1 neuroglia (NG1).** The cell body is spindle-shaped with little perinuclear cytoplasm. The nucleus is also spindle-shaped with patches of heterochromatin attached to the periphery of the nuclear membrane, and few large blocks of heterochromatin occur in the central area (Fig 2A). The cytoplasm contains a few rough endoplasmic reticulum, mitochondria and ribosomes (Fig 2A). No elementary granules were observed. These neuroglia are intermingled with neurons in all layers of the cortex.

**Type 2 neuroglia (NG2).** The cell body is spindle-shaped with little perinuclear cytoplasm. The nucleus is also spindle-shaped with patches of heterochromatin attached to the periphery of the nuclear membrane, and few large blocks of heterochromatin occur in the central area (Fig 2B). The cytoplasm is extremely thin and contains only ribosomes (Fig 2B). These neuroglia form a single sheet of continuous cell layer adjacent to the basement membrane, which is surrounded in turn by ganglionic connective tissue capsule.

**Type 3 neuroglia (NG3).** The cell body and nuclear characteristic of these neuroglia are similar to those of NG2, but they are smaller in size and the nuclear membrane is more indented (Fig 2C). The nucleus contains dense heterochromatin (Fig 2C). The cytoplasm is very thin and contains ribosomes, a few rough endoplasmic reticulum and mitochondria (Fig 2C). These neuroglia are interspersed amongst the nerve tracts in the neuropil.

**DISCUSSION**

The neurons in the cerebral and pleuro-pedal ganglia of *H. asinina* can be divided into 4 types (NR1, 2, 3, 4) based on the nuclear characteristic. NR4 is the largest neuron and exhibit the ultrastructural features similar to typical motor neurons of vertebrates such as ventral horn motor cells of the spinal cord and
Fig 1. A. A low power micrograph of type 1 neuron (NR₁) in the cerebral ganglia showing a round nucleus (Nu) with a prominent nucleolus (No). The cytoplasm contains numerous mitochondria (Mt) and stacks of rough endoplasmic reticulum (RER).

B. A medium power micrograph of type 2 neuron (NR₂) in the cerebral ganglia showing an oval nucleus (Nu) with patches of heterochromatin (He) along the nuclear envelope and in the central area.

C. A medium power micrograph of type 3 neuron (NR₃) in the cerebral ganglia showing an oval nucleus (Nu) with completely dense heterochromatin (He) and a thin rim of cytoplasm.

D. A medium power micrograph of type 4 neuron (NR₄) in the pleuro-pedal ganglia showing a round nucleus (Nu) with thin patches of heterochromatin (He) along the nuclear membrane. The cytoplasm contains a few rough endoplasmic reticulum (RER), mitochondria (Mt) and elementary granules (EG).
Fig 2. A. A medium power micrograph of type 1 neuroglia (NG,1) in the pleuro-pedal ganglia showing a spindle-shaped nucleus (Nu) with patches of heterochromatin (He) in the peripheral and central regions. The cytoplasm contains a few rough endoplasmic reticulum (RER) and mitochondria (Mt). B. A medium power micrograph of type 2 neuroglia (NG,2) in the pleuro-pedal ganglia showing a nucleus (Nu) with very dense heterochromatin (He). The cytoplasm is extremely thin and contains only ribosomes. C. A medium power micrograph of type 3 neuroglia (NG,3) in the pleuro-pedal ganglia showing a nucleus (Nu) with dense heterochromatin (He). The cytoplasm contains a few rough endoplasmic reticulum (RER) and mitochondria (Mt).
Purkinje cells in the cerebellum. Their chromatin is completely euchromatic with a prominent nucleolus, while the cytoplasm contains abundant rough endoplasmic reticulum and mitochondria, but only a few typical elementary or neurotransmitter granules.

Other types of neurons (NR_2, NR_3, NR_4) may belong to the same group. They are characterized by increasing condensation of heterochromatin. The cytoplasm is small but contains sizable numbers of organelles particularly polyribosomes and mitochondria. There are small elementary granules present in the cytoplasm of these neurons. These features are similar to association neurons in the vertebrate nervous system, such as small neurons in the molecular layer of cerebellum of mammals.

The ultrastructure of ordinary neurons had been studied extensively in the ganglia of several pulmonates such as Limax stagnalis, Helisoma tenta.ne (Phillippi), A. californica, H. pomatia, H. aspersa, A. marginata and A. fulica. The studies on the neurons of prosobranchs were reported in Helix rufescens and B. tentaculata. The ultrastructure of the ordinary neurons in H. asinina is rather simple compared with those in the pulmonates. They contain the usual cyttoplasmic organelles such as rough endoplasmic reticulum, mitochondria, ribosomes, polyribosomes, similar to the neurons of pulmonates but in smaller numbers. Their Golgi bodies and lysosomes are not observed as frequent as those of the pulmonate neurons. In addition, the ordinary neurons of pulmonates usually contain a large number of elementary granules. There are only a few elementary granules in H. asinina neurons. These granules are not stained by chromo-hematoxylin and paraldehyde-fuchsin. It is possible that the small vesicles of the neurons might incorporate neurotransmitters, which are non-peptides or glycoprotein. Similar granules, thought to contain neurotransmitters have been described in the neurons of L. stagnalis and A. marginata.

In addition to the elementary granules, special types of inclusions and secretory granules have been reported in the neurons of H. rufescens and B. tentaculata. In H. rufescens, the cerebral neurons contain large membrane-bound inclusions showing various degrees of organization such as packed membranes or filaments, clumps of strongly osmiophilic material, and homogeneous pale material. Frequently, a single inclusion shows all three features. It is possible that these inclusions are secretory in function. No such inclusions were observed in H. asinina neurons. Andrews reported the presence of lipofuscin spherules containing carotenoid pigments in B. tentaculata neurons. We did not observe these spherules in the neurons of H. asinina.

The neuroglia of the cerebral and pleuro-pedal ganglia of H. asinina contain spindle-shaped nuclei and little perinuclear cytoplasm. The NG_1 contains a few rough endoplasmic reticulum, mitochondria and ribosomes, while the NG_2 and NG_3 contain only ribosomes. These neuroglia of H. asinina are different from those described in B. tentaculata, L. stagnalis and A. marginata which have several mitochondria, rough endoplasmic reticulum, Golgi complexes and lysosome-like structures. Furthermore, they are interspersed amongst the neurons, but do not indent into the cytoplasm of the neurons like those described for the neuroglia of pulmonates.

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References


