Fine structure and axonal organization in the buccal ganglia nerves of *Aplysia* (Mollusca, Gastropoda)

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**Summary.** The morphology of the *Aplysia* buccal nerves and connectives has been studied by electron microscopic analysis. In these nerves the fine structure of the elements (connective sheath, glia, axons and their vesicular and cytoplasmatic content) is similar to that of other molluscan nerves. Some features seem to be comparable to other invertebrate groups such as Crustacea and Annelida. The axons have been divided into four classes on the basis of their calibre, and each type has been counted in all the nerves. The number of axons relating to identified buccal neurons is discussed. Finally, some speculations about relationships between buccal ganglia and peripheral regions connected by buccal nerves are proposed.

**A. Introduction**

The nervous system of gastropod molluscs has been widely utilized for neural circuit studies. Substrates of several types of behaviour have been identified in many species, their neural elements studied and analysed, especially from a functional rather than structural point of view (Kandel 1979; Getting 1985).

In *Aplysia*, morphological and ultrastructural studies have been carried out mainly on abdominal ganglia, focusing on the neuron-glia relationships, the identification of neurotransmitters, the synaptic ultrastructure, the count of ganglion cell number and axons in the connectives and peripheral nerves relating to their functional complexity (Bailey et al. 1981; Coggeshall 1967; Price and McAdoo 1979; Tremblay et al. 1979).

The ultrastructural study of the *Aplysia* buccal ganglia, on the other hand, has received less attention, although much research has been carried out recently on the functional analysis of buccal elements underlying feeding behaviour, a complex stereotyped behaviour of particular interest due to the ease with which it can be evoked and observed (Gardner 1971, 1977; Fiore and Meunier 1979; Fiore and Geppetti 1981; Sussweinn and Byrne 1988).

At present the only morphological and ultrastructural results available in literature concern the synaptic and cellular morphology of identified buccal elements of the motor circuit, and the cellular distribution of neuropeptides (FMRF-amide, CCK, gastrin, SCPa and SCPb) localized in buccal neurons through optic and electronic immunocytochemical techniques (Kreiner et al. 1986, 1987; Lloyd et al. 1987; Ono 1986; Reed et al. 1988; Sossin et al. 1987). As a whole, these studies share the knowledge of the structural correlates of central mechanisms, functionally studied, which are involved in the integration of the feeding motor circuit. At the same time, they supply partial results on the morphological analysis of the neural elements that connect the buccal ganglia with peripheral buccal and intestinal regions.

The aim of the present work is to cover some gaps by supplying ultrastructural data on the organization of buccal nerves and connectives, with particular attention to the axon shape, calibre and number. The relationships between axons and glial elements have also been considered.

**B. Materials and methods**

Specimens of *Aplysia depilans* (Bohatsch, 1761), which were captured in sea zones near Leghorn (Italy), were kept in aquaria filled with natural sea water, filtered and aerated at 15°C and fed with *Ulva*.

The buccal ganglia with emerging nerves and connectives were surgically removed from the animal. After dissection the nerves were cut from the ganglia and separately fixed for 4-6 h in a solution of 1% glutaraldehyde with 2% paraformaldehyde, buffered in phosphate 0.13 M (pH 7.4) with the addition of CaCl₂ 0.01 M and sucrose 0.05 M. Nerves were postfixed for 2 h in 2% OsO₄ in the same phosphate buffer, dehydrated in graded ethanol and embedded in an epon-araldite mixture. The ultrathin sections, cut with an LKB ultratome, were stained with both uranyl acetate and lead citrate and observed under a Siemens Elmiskop 1 A elec-
The semithin sections stained with toluidine blue were observed under a Zeiss light microscope (LM) and photographs taken with a Nikon camera were made in order to calculate the diameters and the total area of each nerve.

**Statistical analysis.** The number of axons in each nerve was determined in five specimens. Two procedures were employed to estimate the total number of fibers in transverse sections. For axons with a diameter greater than 10 µm LM photographs from integral semithin sections were observed and that total number of axons was counted (standard error of mean [sem] n=5). Axons with a diameter less than 10 µm were counted on thin sections. The total nerve profile was divided into six segments. For each segment two electron micrographs were randomly selected. For each micrograph the total number of axons was counted. This number was multiplied by the number of micrographs, derived from the nerve total area calculation. The mean value of the total number of axons was obtained calculating the sem where the number of samples was 12.

### C. Results

#### General anatomical features

Five nerves – first, second, third buccal (1b, 2b, 3b), oesophageal (on) and radular (rn) nerves – start from the buccal ganglia. They innervate the pharynx, the salivary glands and the oesophagus. Furthermore, a cerebro-buccal connective (cbc) connects the buccal ganglia to the cerebral ganglia (Fig. 1). In these nerves there are axonal projections of identified buccal neurons (A, B, C, D and s cells) which constitute the neural ground of the feeding behaviour consummatory pattern, producing the generation and the execution of the feeding motor output (Bedini et al. 1983; Fiore and Meunier 1979; Fiore and Geppetti 1981; Gardner 1971). Another neuron group, the L cells, project their axons in the cerebro-buccal nerve and are involved in the feeding motor control and integration (Fiore and Geppetti 1985).

For the sake of convenience, since emerging from buccal ganglia we have improperly extended the definition of nerve to cbc, even though it is a neural pathway of morpho-functional connection between the buccal ganglia and the cerebral ganglia.

#### Light microscope structure

The buccal nerves are variable in length and calibre (Table 1) but they present the same basic morphological features. Each nerve is enveloped by a fibrous sheath about 20–60 µm thick consisting of connective cells interposed between collagenous fibre bundles. In some nerves there are also muscle cells intermingled in the fibrous component of the sheath. A perineural lamina, 2 µm thick, lines the fibrous sheath. Internally the nerves are made up of axons and glial cells (Fig. 2). The cytoplasmic projections of the glial cells surround one or more axons which are massed in bundles relative to the calibre. In this way they determine a radiate configuration which characterizes the topography of each nerve.

On the basis of the axonal area configuration, which has been determined by glia distribution, the following can be distinguished (Fig. 3):

A) Nerves consisting of many semicircular-shaped bundles of small axons with the top turn on the nerve centre (1b).

B) Nerves subdivided into two separated regions: the first mainly occupied by large-sized axons, the second by small- and medium-sized axons (2b, on).

C) Nerves in which the separation of axonal areas is missing, a uniform distribution being present (3b, cbc).

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Calibre</th>
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<tbody>
<tr>
<td>1b</td>
<td>0.16 ± 0.011</td>
</tr>
<tr>
<td>2b</td>
<td>0.23 ± 0.027</td>
</tr>
<tr>
<td>3b</td>
<td>0.16 ± 0.010</td>
</tr>
<tr>
<td>on</td>
<td>0.21 ± 0.036</td>
</tr>
<tr>
<td>rn</td>
<td>0.26 ± 0.032</td>
</tr>
<tr>
<td>cbc</td>
<td>0.18 ± 0.0048</td>
</tr>
</tbody>
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**Table 1. Calibres of buccal nerves calculated on ×200 LM sections of a whole nerve (sem n = 5)**

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**Fig. 1. Schematic reconstruction of the buccal ganglia with their emerging nerves and connectives: first buccal nerve (1b), second buccal nerve (2b), third buccal nerve (3b), oesophageal nerve (ON), radular nerve (rn), cerebro-buccal connective (CBC). Buccal neurons are indicated with the classification of Fiore and Meunier 1979, Fiore and Geppetti 1985**