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GENERAL BIOLOGY

Distribution of Serotonin and FMRF-Amide in the Nervous System of Different Zooidal Types of Cheilostome Bryozoa: A Case Study of *Arctonula arctica*

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Abstract—In the White Sea bryozoans *Arctonula arctica,* the structure of the nervous system and distribution of 5-hydroxytryptamine (5-HT) and FMRF-amide were studied for the first time using immunohistochemical methods and confocal scanning microscopy. The neurotransmitters studied have been actively involved into the integrative processes, gut functioning, and regulation of motion activity. In avicularia, 5-HT and FMRF-amide receptors are capable of performing the same functions, except for participation in the gut functioning, because they have no digestive system.

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The phylum Bryozoa is an abundant group of colonial, mostly attached invertebrates that are active filter-feeders living in both marine and fresh waters [1, 2]. Phylogenetic relationships within the phylum and with other phyla of the animal kingdom are still intensely debated. In accordance with different modern concepts on Animalia phylogeny, bryozoans are assigned to the group of Lophotrochozoa along with Phoronida and Brachiopoda, or these three groups are considered unrelated [3–5].

Although morphology of both fossil and modern Bryozoa has been studied since the XIX century, our knowledge of the nervous system (NS) is still unsufficient in various groups of these animals. A feeding zooid (autozooid) is the basic module in bryozoan colonies. The autozooid NS has been mostly studied on the freshwater bryozoans using utine histological methods, methylene blue staining, electron and confocal laser microscopy, and immunohistochemistry [6–10]. Reports on the marine Bryozoa studied by immunohistochemical methods and confocal microscopy are not numerous [11, 12].

A distinct feature of marine bryozoan colonies is that, apart from autozooids, they contain several types of polymorphic zooids (heterozooids) performing various functions. Avicularia that also belong to heterozooids, are, modified zooids presumably carrying a defense function. The movements of their mandible help sweeping off larvae and small predators from the colony. The problem of polymorphic zooids, particularly avicularia, has been attracting researchers' interest since their discovery by Ellis in 1753 [13]. However, the number of reports on avicularum structure is scanty [14, 15]. Modern methods, such as confocal microscopy and immunohistochemistry (serotonin detection), have been used only for the NS of a single species of the genus *Bugulina* to study the avicularium NS [15].

This is the first comparative study of the nervous system in autozooids and avicularia of marine bryozoans using immunohistochemical and histochemical methods and the confocal laser microscopy. NS elements were detected by means of antibodies against serotonin (5-HT), FMRF-amide and α-tubulin. Fluorescence-labeled phalloidin staining was used to study musculature. The colonies of the bryozoan *Arctonula arctica* (M. Sars) were collected in the vicinity of the White Sea Biological Station of the Saint Petersburg State University Chupa Inlet were the subjects of our study.

Arctonula arctica is characterized by a structured location of autozooids and heterozooids in a colony: each autozooid is connected with two avicularia to form a second-order module. Autozooid consists of two parts: a polypide and a cystid. The polypide includes a retractable crown of tentacles (lophophore), which sieves food particles out of water; it also includes the gut and associated muscle groups. The

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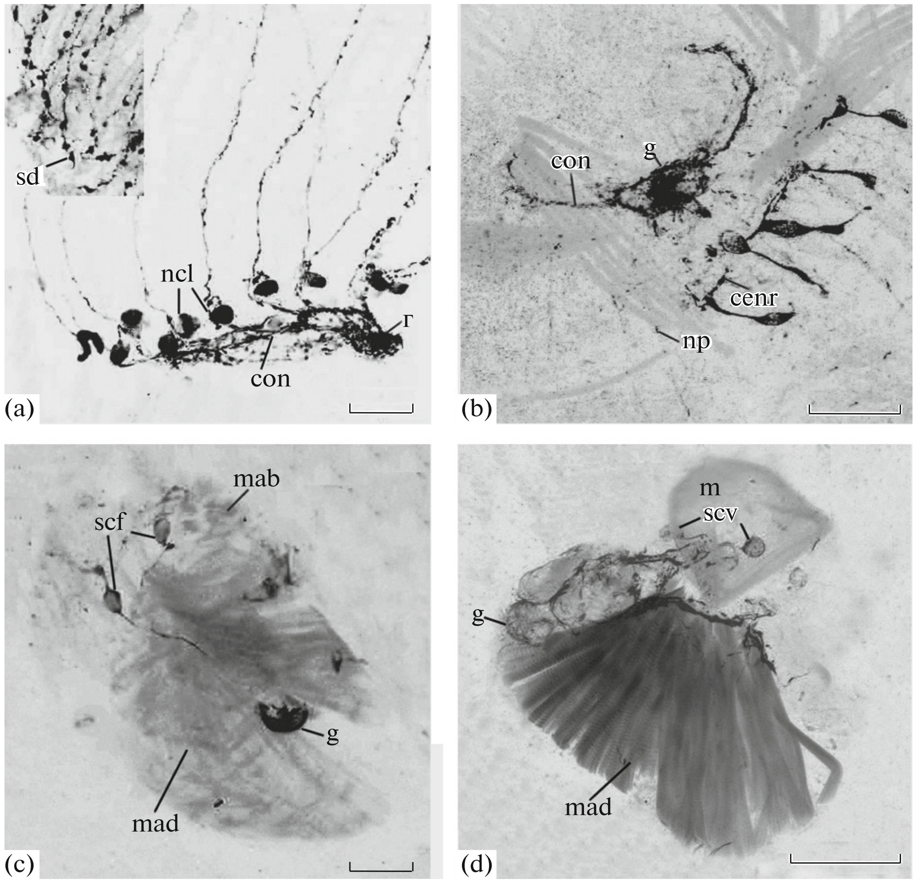

Fig. 1. (a, c) Serotonin- and (b, d) FMRF-amide-immunopositive elements in the nervous system of (a, b) an autozooid and (c, d) an avicularium of *Arctonula arctica*. Muscles detected using phalloidin staining. Scale: (a, c) 30 and (b, d) 40 μm. Abbreviations: g, ganglion; m, mandible, mab and mad, muscles abductors and adductors, respectively; scv and scf, sensory cells of vestibulum and frontal membrane, respectively; sd and ncl, sensory and nerve cells of lophophore, respectively; con, circumoral nerve ring; cenr, cardioesophageal nerve ring; np, nerve processes innervating lophophore.

cystid is a calcified part of the body wall with epithelium and peritoneal lining, where the polypid can be retracted. The NS of *A. arctica* consists of a cerebral ganglion at the lophophore base, which is connected with the circumoral nerve ring, sensory cells and the nerves innervating the lophophore, body wall, parietal muscles, and gut. The lophophore tentacle nerves extend from the circumoral nerve ring. The gut is innervated by visceral nerves of cerebral ganglion. The nerves extending from the ganglion also innervate the main muscle groups: diaphragmal muscles, retractors, opercular muscles lowering operculum on the orifice through which the polypide is retracted into the cystid, and parietal muscles participating in the ascus wall inflexion and the polypide protrusion out of the cystid. In *A. arctica*, the sensory cells were found in the tentacle epithelium, in the frontal wall and vestibular area (above the diaphragmal sphincter). Their central processes reach the cerebral ganglion within the corresponding nerves.

The 5-HT-immunopositive pericarya were found in *A. arctica* autozooids at the base of most of the tentacles. Their peripheral processes innervate tentacles, while the central ones passing via the circumoral nerve ring reach the cerebral ganglion neuropil, where they form a bulk of terminal branching (Fig. 1a). In addition, the primary intraepithelial sensory cells located in the tentacles and cardioesophageal nerve ring of the gut (at the boundary between the esophagus and gastric cardia) can also be assigned to 5-HT-containing elements (Fig. 1a). Central processes of 5-HT-immuno-positive sensory cells of tentacles reach the cerebral ganglion within the tentacle nerves; processes of the sensory cells of the gut-within the visceral nerves. The same 5-HT-cell distribution was found in autozooids of the marine Bryozoa *Cribrilina annulata* and *Bicelariella ciliata* [11]. In the freshwater Bryozoa, 5-HT-immunopositive cells are involved in the lophophore innervation; the intraepithelial sensory cells have also been shown in the tentacles [6–9]. The FMRF-amide-immunopositive pericarya were detected in the cerebral ganglion of autozooids (Fig. 1b). These cells are involved in the tentacle innervation, and their peripheral processes extend from the cerebral ganglion via the circumoral nerve ring to the tips of the tentacles. Lophophore innervation by the FMRF-amide-containing cells was also characteristic of the freshwater Bryozoa [6–9]. As in the case with serotonin, sensory FMRF-amide-containing cells were detected in the cardioeasophageal nerve ring of the gut. Their central processes extend to neuropil of the cerebral ganglion via the medial visceral nerve to the neuropile of the cerebral ganglion.

An avicularium of *A. arctica* is a zooid significantly reduced in size; it is incapable of independent feeding or reproduction. Its length is typically less than 120 μm, which is six times smaller than that of an autozooid, averaging about 780 μm. Nevertheless, all the main parts of avicularia are retained, being homologous to the corresponding autozooid structures. The upper part of the avicularium polypide consists of a single short tentacle in a special sheath; it is homologous to the lophohore of autozooid. In addition, the avicularium has a modified operculum (mandible), and contains cerebral ganglion, the main muscles (abductors and adductors) that correspond to parietal and opercular autozooid muscles, the diaphragmal muscles, and polypide retractors. The cerebral ganglion of avicularium is located immediately under the rudimentary tentacle. The nerves extending from the ganglion reach the mandible-lowering adductors muscles and abductors muscles responsible for inflexion of the frontal membrane, as well as diaphragmal muscles and retractors.

Sparse 5-HT-immunopositive neurons, as well as nerve fibers, were found in the avicularium cerebral ganglion (Fig. 1c). Two bipolar sensory cells on the opposite sides of the cystid under the frontal membrane can also be assigned to 5-HT-elements. They are presumably act as mechanoreceptors (Fig. 1c). The sensory processes of these cells reach the lower surface of the frontal membrane, while the central ones reach the ganglion. A few sensory FMRF-amide-containing cells were detected in avicularia in the area of the vestibulum under the mandible, under the frontal membrane, and in the rudimentary tentacle. Their central processes reach the ganglion. The FMRF-amide-containing neurons that innervate the abductor and adductor muscles, diaphragmal muscle, and retractors were also found in the cerebral ganglion (Fig. 1d).

Our data suggest that 5-HT and FMRF-amide are actively involved in regulation of the sensory function, digestive processes, motion activity, and integrative processes in both freshwater and marine Bryozoa. As in many other invertebrate groups, 5-HT probably participates in the ciliary activity of lophophore cilia in Bryozoa. In spite of a significant reduction of the nervous system in avicularia, 5-HT and FMRF-amide have proved to fulfill the same physiological functions except for the digestive activity, because there is no gut in these heterozooids.

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