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Organization of catecholaminergic system of *Pygospio elegans* and *Platynereis dumerilii* (Annelida)

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ABSTRACT: Catecholamines (CA) are known to play an important role in the physiology of most invertebrates. Here we provide the results on catecholaminergic systems study of two annelid species: *Pygospio elegans* (Spionidae) and *Platynereis dumerilii* (Nereididae). The nervous system was studied with the histochemical method of monoamine condensation with glyoxylic acid in combination with confocal laser scanning microscopy. Both animals possess developed catecholaminergic systems with similar general organization. However, in *P. dumerilii* considerably fewer cells were detected. In both species, CA-positive cells were found in the brain, ventral nerve cord as well as in the stomatogastric system, body wall, palps, prostomium, and regions around the chaetae. According to the cell morphology and their location in the most agile parts of the animal, we suggest CA-positive cells to be mostly proprio- or mechanoreceptors.
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KEY WORDS: Nervous system, Catecholamines, Glyoxylic acid fluorescence, Confocal microscopy, Annelida, *Pygospio elegans*, *Platynereis dumerilii*.

Организация катехоламинергической системы *Pygospio elegans* и *Platynereis dumerilii*

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РЕЗЮМЕ: Катехоламины (КА) — важные нейромедиаторы, играющие ключевую роль в физиологии беспозвоночных. В настоящей работе представлены результаты исследования катехоламинергических систем двух видов кольчатых червей: *Pygospio elegans* (Spionidae) и *Platynereis dumerilii* (Nereididae) с использованием гистохимического метода конденсации моноаминов с глиоксиловой кислотой в комбинации с конфокальной лазерной сканирующей микроскопией. Оба исследованных вида обладают развитой катехоламинергической нервной системой со сходной общей организацией. Однако, у *P. dumerilii* нервных клеток обнаружено гораздо меньше, чем у *P. elegans*. У обоих видов КА-положительные клетки были обнаружены в головном мозге, брюшной нервной цепочке, а также в стоматогастрической системе, стенке тела и в составе некоторых органов чувств. Судя по морфологии клеток и их расположению в наиболее подвижных частях тела животного, мы предполагаем, что КА-положительные клетки являются в основном проприо- или механорецепторами.
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КЛЮЧЕВЫЕ СЛОВА: нервная система, катехоламины, глиоксиловая кислота, конфокальная микроскопия, Annelida, *Pygospio elegans*, *Platynereis dumerilii*.

Introduction

Catecholamines (CA) are monoamine neurotransmitters, derivatives of 1,2-dihydroxybenzene, including dopamine, adrenalin, and noradrenaline (Vulpian, 1856; Gnegy, 2012). They are widely distributed in the animal kingdom and play important roles in animal physiology and behavior. Monoaminergic neurons in vertebrates and in most invertebrates have been shown to regulate reproduction, digestion, and feeding behavior (Croll *et al*., 1999). Nevertheless, the data on the functions and distribution of CA in some groups of invertebrates is still fragmentary. This also applies for annelids. This lack of data is partially caused by difficulties in CA detection. Catecholamines can be revealed by specific histochemical techniques such as glyoxylic acid-induced fluorescence of CA (de la Torre, Surgeon, 1976) or with the formaldehyde–glutaraldehyde fluorescence (FaGlu) (Furness *et al*., 1977). Although both methods are variable in terms of staining results, they result in fluorescent staining of catecholamine-containing cells, which could be studied using a confocal laser scanning microscope. Among lophotrochozoans molluscs are the best-studied group (Zaitseva, Shumeev, 2017; Dickinson *et al*., 2000; Voronezhskaya *et al*., 2008; Croll *et al*., 1999; Zaitseva *et al*., 2019). Since CA-ergic nerves innervate the musculature of the body wall, foot, and pharynx, they were assumed to participate in the regulation of sensory functions of digestive system and in feeding behavior. The regulation of the digestive tract activity by CA system was also suggested for flatworms (Welsh, Williams, 1970; Joffe, Kotikova, 1991), and nemerteans (Zaitseva *et al*., 2019, 2020).

Information on catecholaminergic systems of annelids is extremely scarce. Several studies were carried out on such well-known objects as *Hirudo medicinalis* (see Crisp *et al*., 2002), *Lumbricus terrestris* (see Bieger, Hornykiewicz, 1972), and *Nereis* sp. (see Dhainaut-Courtois *et al*., 1972). Several errant polychaetes such as *Nephtys* sp. (see Clark, 1966), *Ophryotrocha puerilis* (see Schlawny *et al*., 1991a, b) have been studied as well as the sedentary polychaete *Sabellastarte magnifica* (see Díaz-Miranda *et al*., 1992).

In order to acquire more comparative data on the CA-system in annelids and provide information to further understanding of the catecholamines' role in annelids we studied in detail

the CA-system in two annelids, *Platynereis dumerilii* and *Pygospio elegans*, belonging to the two main annelid clades Errantia and Sedentaria respectively. While the errant polychaete species *P. dumerilii* is a well-known model organism (Özpolat *et al*., 2021), *P. elegans* is a small tube-dwelling worm, living in the sandy intertidal zone (Starunov *et al*., 2020). Using the histochemical method of glyoxylic acid-induced fluorescent labeling combined with laser scanning confocal microscopy we described CA-containing cells and their projections in both central and peripheral parts of the nervous system. The results of the present study were compared with previous studies on other annelids to provide comparative analysis for further understanding of CA function in annelids.

Materials and methods

Pygospio elegans Claparède, 1863 was collected in the intertidal zone of the Barents Sea near the marine biological station Dalnie Zelentsi (69°07' N, 36°05' E). Worms were transported to the laboratory and kept at 18 °C in plastic containers filled with sand and artificial sea water (Red Sea Coral Pro salt) with a salinity of about 30–32‰. Under such conditions, animals showed proper activity and built sand tubes. They were fed with a mixture of powdered dried algae, mostly *Pylaiella littoralis* (Linnaeus) Kjellman, 1872, harvested from the collection site.

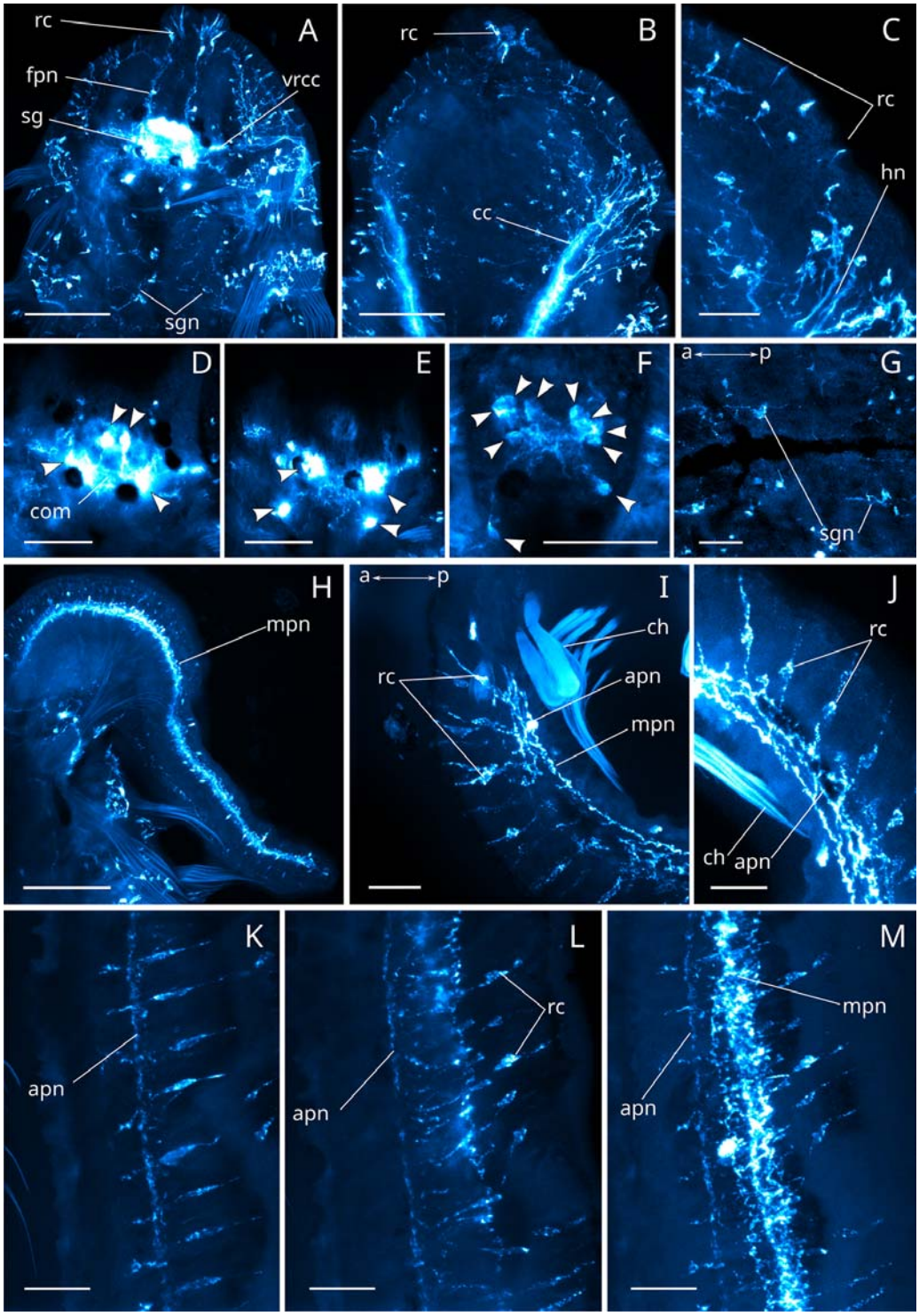
Platynereis dumerilii (Audouin et Milne-Edwards, 1834) worms were taken from laboratory culture of the embryological department of St. Petersburg State University. The culture have been contained under the following conditions: polycarbonate containers with artificial sea water (30‰, Red Sea Coral Pro salt); a permanent temperature of 18 °C; a light cycle of 18 hours of light and 6 hours of darkness; fed once a week with fine-grounded pre-frozen spinach leaves and *Artemia* sp. nauplii.

For visualization of CA-containing cells, a glyoxylic acid fluorescent technique was used. The method is based on the condensation of glyoxylic acid with monoamines including catecholamines resulting in the production of a fluorescent chemical as described by Lindvall & Björklund (1974). Animals were relaxed in 7.5% solution of $MgCl_2 \bullet 6H_2O$ and immersed in a freshly prepared buffered sodium glyoxylate-sucrose solution (500 mM sodium glyoxylate, 150 mM sucrose, 0.5 M Hepes buffer, pH 7.4) in 2 ml plastic eppendorf tubes. After 30 minutes of incubation the solution was removed, the specimens were placed on glass slides and air-dried at room temperature for 30 minutes. The slides were then heated to 60 °C for 30 minutes, embedded in liquid paraffin, and examined using Leica TCS SP5 laser scanning microscope (405 nm excitation laser, emission detection at 457–490 nm). In total more than 100 samples of each species were examined. The resulting confocal stacks of images (1024x1024 pixels, 40–120 slices per stack) were processed with Fiji (Schindelin *et al*., 2012).

Results

PYGOSPIO ELEGANS. The catecholaminergic part of the nervous system of *P. elegans* is well-developed and consists of nervous elements belonging to the brain, circumoesophageal connectives, ventral nerve cord, segmental nerves, longitudinal nerves, stomatogastric system, and nerve plexus of the body wall. Hereafter in our descriptions we follow the terminology suggested by Richter *et al*. (2010). The brain of *P. elegans* possesses numerous CA-positive cells and fibers (Fig. 1A, D–F). CA-positive fibers are distinctive in the cerebral commissures and in the circumoesophageal connectives, mostly in their ventral roots (Fig. 1B). At the level of the ventral cerebral commissure CA-positive fluorescence of the neuropil is extremely high and to some extant interferes with the signal from cell somata in this region (Fig. 1A). However, we were able to locate up to 6 pairs of symmetrically arranged CA-positive cell somata in the brain (Fig. 1D–F). The first pair of cell somata is situated in the anterior part of the brain above the commissural fluorescent spot (Fig. 1D). It is connected with the dorsal commissure by a pair of thin nerves. The cell somata 2–5 are located in close proximity to the posterior pair of eyespots (Fig. 1D–F). The sixth pair of CA-positive somata lies in the posterior part of the prostomium between the posterior eyespots and nuchal organs (Fig. 1E, F). The cells of this latter pair send their processes to the dorsal commissure of the brain.

Numerous CA-positive nerve fibers run from the cerebral ganglion to receptors placed in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body wall of the prostomium (Fig. 1A, B). The largest of them are frontal prostomium nerves that connect the supraoesophageal ganglion with 6 pairs of large fusiform receptor cells situated in the terminal part of prostomium (Fig. 1A). Other CA-positive cells scattered through the prostomium wall, mostly on its lateral sides, send their projections to the circumoesophageal commissure. They may be either separate neurites or take part in two pairs of lateral neurite bundles (Fig. 1A–C).

Numerous CA-positive receptor cells were also found along the palps of *P. elegans* (Fig. 1H–M). The most abundantly CA-innervated part is the food groove, a shallow trench running ventrally from the tip of the palp to its base, slightly biased to the medial side. On the bottom of the food groove there is the main palp nerve which comprises approximately 3–6 CA-positive fibers. In some parts of the palp the division of this nerve into two parts of nerve bundles can be seen (Fig. 1J). The larger one lies on the bottom of the food groove and a smaller one closer to the surface of the palp. There are also thin additional palp nerves that run along its ventrolateral and ventromedial sides (Fig. 1I, K–M). They are connected with the main palp nerve via numerous anastomoses. CA-positive receptor cells are situated mostly on the lateral and abfrontal parts of the palp surface and in the food groove (Fig. 1H–M). Receptor cells of the food grove form a regular pattern along its length, with a distance between CA-positive receptor cells of 20–40 micrometers (Fig. 1I,J).

CA-positive cells were also found in the stomatogastric nervous system of *P. elegans*. Here they form a loose plexus supplying the pharynx and intestine (Fig. 1A, G).

P. elegans has a heteronomous segmentation. Body segments can be divided into three groups: 12 first “thoracic” chaetigers without branchiae, then some branchiate “abdominal” chaetigers, and several “tail” segments devoid of branchiae (Starunov *et al*., 2020). Despite the differences in general morphology, the innervation of the segments in different regions follows the same pattern with only minor deviations. The CA-positive structures are observed in the ventral nerve cord, segmental nerves, parapodial nerves and body wall.

The segmental ganglion includes up to 5 pairs of CA-positive cell somata situated in the center of each segment with a slight bias to its posterior part (Fig. 2A, B). In each thoracic ganglion there is a commissure connecting right and left parts of the nerve cord (Fig. 2A). In the abdominal and tail segments the corresponding CA-positive commissure is not detected. Within the ventral connectives CA-positive elements are present in all four main nerves, with two being the most distinct: the largest nerves are situated laterally and the thin are placed medially (Fig. 2G, H).

The commissures of thoracic segments are represented by one thick nerve with four cell bodies lying anterior to it (Fig. 2A). In the segmental nerves CA-positive fibers were found in four pairs in each segment. The nerves of the

Fig. 1. Catecholamine-positive elements in prostomium and palps of *Pygospio elegans*. A, B — innervation of dorsal (A) and ventral (B) sides of prostomium; C — receptor cells in the wall of prostomium; D–F — partial Z-projections of cerebral ganglion in different specimens, arrowheads label catecholamine-positive cells; G — catecholamine-positive elements of the intestine; H–M — catecholamine-positive elements of the palps; K–M — sequential optical sections of one area of a palp.

Abbreviations: apn — additional palp nerve, cc — circumoesophageal connective, ch — chaetae, com — cerebral commissure, fpn — frontal prostomial nerves, hn — head nerves, mpn — main palp nerve, rc — receptor cells, sg — supraesophagial ganglion, sgn — stomatogastric nerve plexus, vrcc — ventral root of the circumesophageal connectives. Scale bars: A, B, H — 100 μm; C–G, J–M — 30 μm; I — 20 μm.

Рис. 1. Катехоламин-положительные элементы в простомиуме и пальпах *Pygospio elegans*. A, B — иннервация дорзальной (A) и вентральной (B) частей простомиума; C — рецепторные клетки в стенке простомиума; D–F — частичные Z-проекции оптических срезов через церебральный ганглий у различных образцов; стрелочными головками отмечены катехоламин-положительные клетки; G — катехоламин-положительные элементы в кишке; H–M — катехоламин-положительные элементы в составе пальп; K–M — последовательные оптические срезы через пальпу.

Обозначения: apn — добавочный нерв пальпы, cc — окологлоточная коннектива, ch — щетинки, com — церебральная комиссура, fpn — фронтальные нервы простомиума, hn — нервы головы, mpn — главный нерв пальпы, rc — рецепторные клетки, sg — надглоточный ганглий, sgn — стоматогастрический нервный плексус, vrcc — вентральный корешок окологлоточной коннективы. Масштаб: A, B, H — 100 мкм; C–G, J–M — 30 мкм; I — 20 мкм.

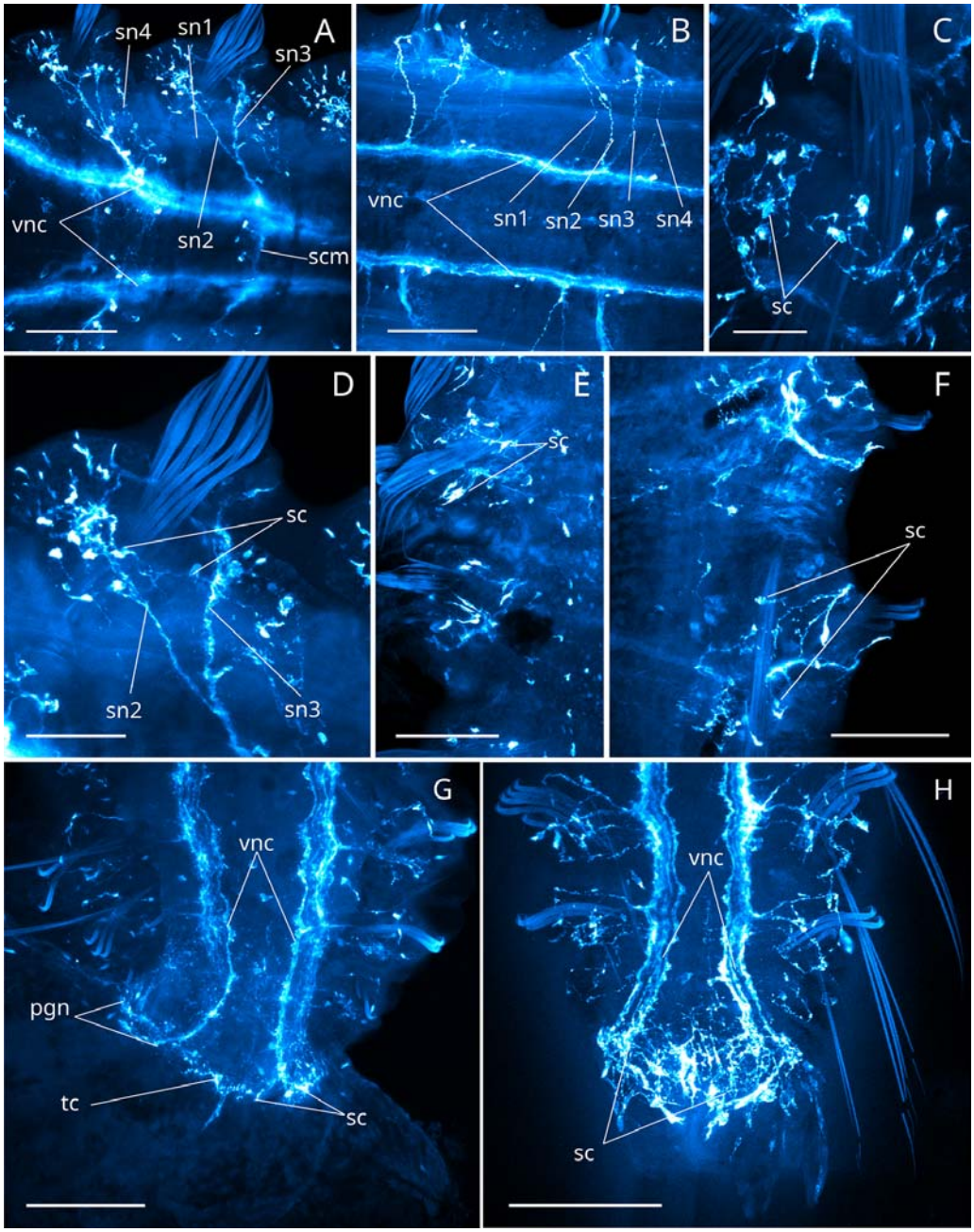


Fig. 2. Catecholamine-positive elements in body segments and pygidium of *Pygospio elegans*. A — innervation of thoracic segments; B — innervation of abdominal segments; C–E — innervation of parapodia of thoracic segments (C — lateral view, D — ventral view, E — dorsal view), arrows label cell somata of CA-positive cells in ventral ganglia; F — innervation of parapodia of abdominal segments, dorsal view; G — innervation of pygidium; H — innervation of a regenerated pygidium, 7 days post amputation.

Abbreviations: pgn — pygidial nerve, sc — sensory cell (receptor cell), scm — segmental comissure, sn1-4 — segmental nerves 1–4, tc — terminal commissure, vnc — ventral nerve cord. Scale bars: A, B, E–H — 100 μm; C — 30 μm; D — 50 μm.

first pair are thin and run from the ventral cord to the receptor cells in the body wall ahead the parapodium. The nerves of the second and third pairs run to the base of the parapodium. They exit the ganglion as a single nerve and split on the way to the parapodium. The nerves of the fourth pair extend to the CA-positive plexus placed in the body wall behind the parapodium. The second and the third segmental nerves branch off thin fibers running to the receptor cells of the ventral and lateral surfaces of the body wall (Fig. 2C, D).

Both abdominal and tail segments show no visible CA-positive commissures (Fig. 2B, G, H). The first and fourth segmental nerves do not differ from those of the thoracic segments while the second and the third exit the ganglion separately. Another difference of the abdominal region is that the fourth pair of segmental nerves innervate small highly CA-positive areas on the back of the parapodia that have a gland-likely appearance (Fig. 2B).

CA-positive elements are widely presented in the body wall mostly as receptor cells and peripheral nerves. The highest concentration of CA-positive cells is in the areas of the parapodia and on the boundaries between segments. CA-ergic innervation of the parapodia in all segments of *P. elegans* body is similar and well expressed (Fig. 2C–F). Each of the parapodial nerves gives off branches surrounding the chaetal sacs. Ventral branches are thinner than dorsal, usually possess fewer receptor cells, and may interconnect at the ventral sides of the parapodia (Fig. 2D). Dorsal branches are thicker and include numerous axons of receptor cells whose somata lie on the anterior and posterior surfaces of the parapodium (Fig. 2C, E, F). Capillary chaetae are innervated more densely than the hooded hooks (compare Fig. 2D and Fig. 2F).

CA-positive elements of the pygidium mostly belong to the paired pygidial nerves which are terminal parts of the ventral nerve cords (Fig. 2G, H). Newly regenerated pygidia show much more CA-positive elements compared to the pygidia of intact worms (Fig. 2H). The pygidial nerves pass around the anterior border of the pygidium and fuse on the dorsal side. They branch numerous thin fibers that enter the pygidial lobes. A terminal commissure is situated on the ventral side of the pygidium. A number of receptor cells are placed around the anal opening and at the bases of the pygidial lobes.

PLATYNEREIS DUMERILII. We observed numerous CA-positive elements in *P. dumerilii* in both the central and peripheral nervous systems. In the prostomium CA-positive fluorescent signal is found in the brain (Fig. 3B–D), though details of its structure are not visible. The only distinct elements are several large CA-positive cells encircling each of the four eyes (Fig. 3F). We did not find any CA-positive elements belonging to the head nerves and antennae. In only one specimen CA-positive bipolar cells were found in the palp bases (Fig. 3E). Besides, solitary thin CA-ergic fibers are observed in the peristomial cirri.

We found highly developed CA-innervation of the pharynx consisting of thin circular and thick longitudinal nerves forming a massive plexus (Fig. 3G). Two small clusters of CA-positive neurons are located at the posterior part of the plexus.

CA-positive elements of the body segments in *P. dumerilii* are mostly concentrated in the segmental ganglia (Fig. 3A). Each segmental ganglion consists of five pairs of CA-positive neurons that lie in the anterior part of the ganglion. The pairs 1–4 are situated medially, pair 5 — laterally behind the parapodia. The ventral nerve cord possesses two pairs of CA-positive nerves, the medial and lateral. In each body segment CA are observed within two pairs of segmental nerves (Fig. 3A, H). As the ventral nerve cord

Рис. 2. Катехоламин-положительные элементы в туловищных сегментах и пигидии *Pygospio elegans*. A — иннервация торакальных сегментов; B — иннервация абдоминальных сегментов; C–E — иннервация параподий торакальных сегментов (C — вид сбоку, D — вид с брюшной стороны, E — вид со спинной стороны), стрелками отмечены перикарионы катехоламин-положительных клеток в ганглиях брюшной нервной цепочки; F — иннервация параподий абдоминальных сегментов, вид со спинной стороны; G — иннервация пигидия; H — иннервация регенерировавшего пигидия, 7 дней после ампутации.

Обозначения: pgn — пигидиальный нерв, sc — сенсорная клетка (рецепторная клетка), scm — комиссура сегментарного ганглия, sn1-4 — сегментарные нервы 1-4, tc — терминальная комиссура, vnc — брюшная нервная цепочка. Масштаб: A, B, E–H — 100 мкм; C — 30 мкм; D — 50 мкм.

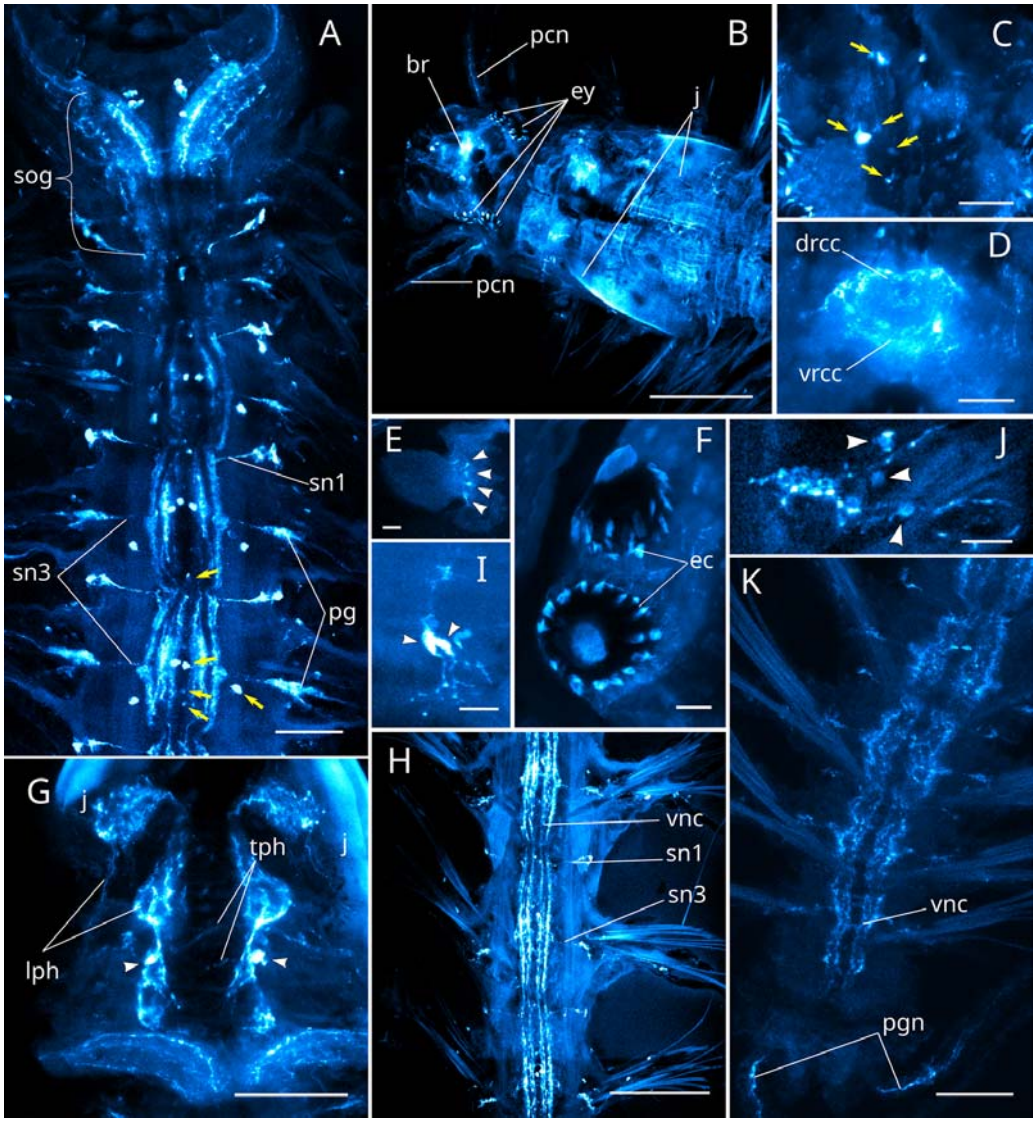


Fig. 3. Catecholamin-positive elements of *Platynereis dumerilii*. A — anterior part of the ventral nerve cord, yellow arrows label cell somata of the segmental ganglion; B — innervation of the anterior end, dorsal side; C, D — partial Z-projections of the brain region, yellow arrows label cell somata; E — receptor cells of the palp (arrowheads); F — CA-positive cells surrounding the eyespots; G — innervation of the pharynx, arrowheads label clusters of CA-positive cells; H — CA-positive elements in the ventral nerve cord and body wall; I — receptor cells (arrowheads) between segments; J — neurons in the area of the parapodial ganglion (arrowheads); K — innervation of the posterior end.

Abbreviations: br — brain, drcc — dorsal roots of the circumoesophgeal connectives, ec — CA-positive cells of the eyes, ey — eyespot, j — jaws, lph — longitudinal pharyngeal nerve, pcn — peristomial cirri nerve, pg — parapodial ganglion, pgn — nerve of the pygidial cirrus, sog — suboesophageal ganglion, sn1-3 — first and third pairs of segmental nerves, tph — transversal pharyngeal nerve, vnc — ventral nerve cord, vrcc — ventral roots of the circumoesophgeal connectives. Scale bars: A, G, K — 50 μm; B, H — 100 μm; C, D — 25 μm; E, F, J, I — 10 μm.

Рис. 3. Катехоламин-положительные элементы *Platynereis dumerilii*. A — иннервация переднего отдела брюшной нервной цепочки, желтыми стрелками указаны тела нейронов сегментарного ганглия; B — иннервация переднего конца тела, вид с спинной стороны; C, D — частичные Z-

ganglia are slightly shifted anteriorly and protrude to the previous body segment, the first pair of segmental nerves is situated at the posterior margin of the previous segment and runs to small clusters of 3–4 CA-positive cells located ventrolaterally at the segment boundary. The sensory projections of these neurons run backward to the intersegmental groove (Fig. 3I). The second pair of CA-positive nerves corresponds to the third pair of segmental nerves (the parapodial nerves) in *P. dumerilii*. At the bases of parapodia they run through the parapodial ganglion seen as a slight thickening with an increased number of varicosities, and in the parapodia wall they come in contact with multiple CA-ergic sensory bipolar cells belonging to the parapodium (Fig. 3A, H). Several neuronal somata were found in association with the parapodial ganglion in some specimens (Fig. 3J). Behind the parapodial ganglion the parapodial nerve bifurcates and its branches embrace the chaetal sacs. Some receptor cells are situated at the bases of the parapodia, near the chaetal sacs and parapodial cirri (Fig. 3H). There is no distinctive CA-ergic innervation in the pygidium of *P. dumerilii* apart from solitary thin fibers belonging to the pygidial cirri (Fig. 3K).

Discussion

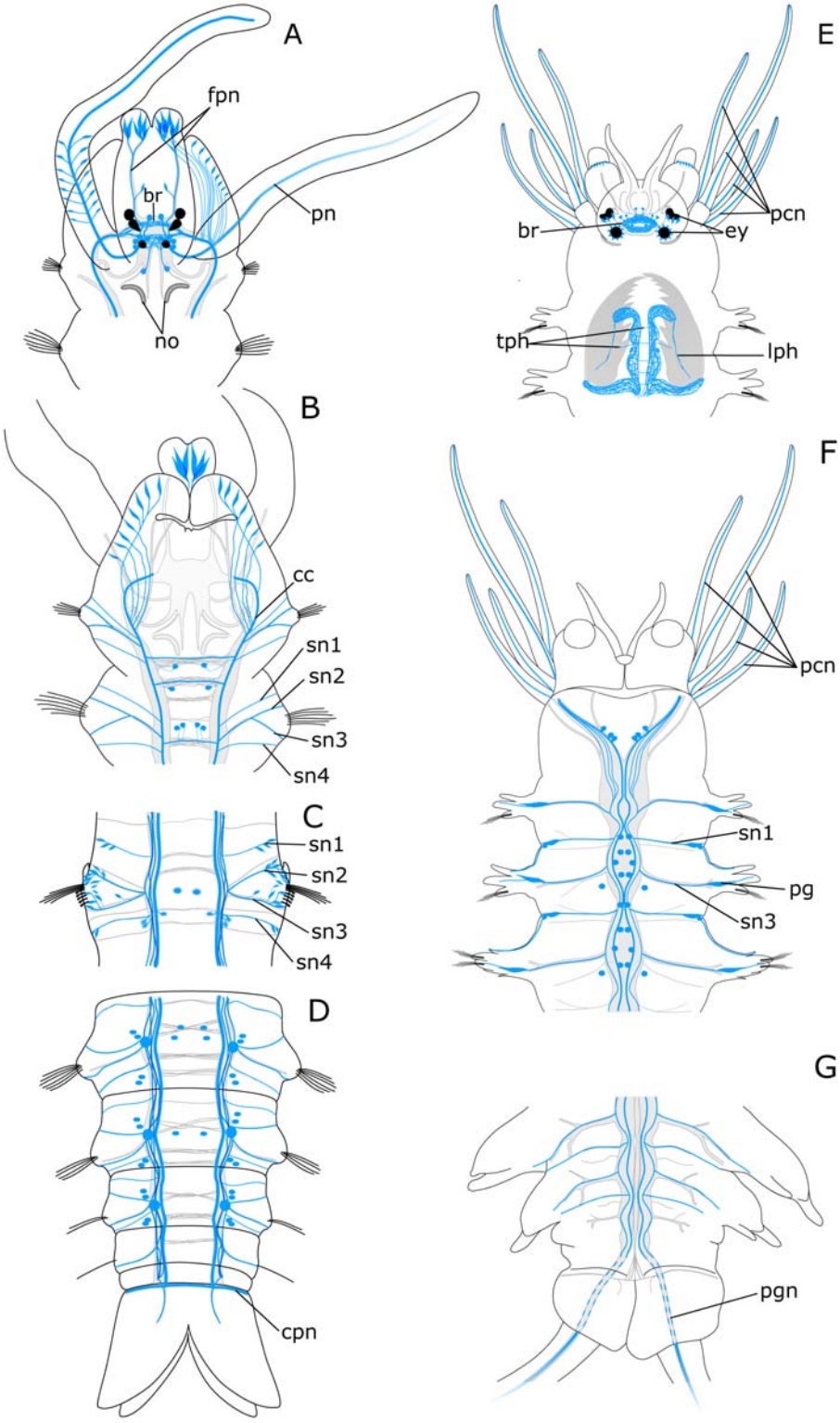
Both studied annelids possess highly developed systems of CA-ergic neurons and show a general similarity in their organization. The generalized schemes of CA-ergic systems in both species are shown in Figure 4 (A–G). The shapes and sizes of CA-positive neurons and sensory cells in both species are quite similar. Most unipolar cells belong to the central nervous system and show the typical morphology of brain cells and segmental ganglia (Heuer, Loesel, 2008; Miron, Anctil, 1988; Schlawny *et al*., 1991b). Bipolar cells are specific for the peripheral nervous system. Most of found cells have the following features: location in the body wall, solitary placement, bipolar shape (presence of one long and one short appendage, the latter getting in contact with the surface of the body). Such features of CA-positive cells have been shown to be characteristic for receptor cells in different groups of animals (Richter *et al*., 2010; Zaitseva *et al*., 2019).

We detected a greater number of CA-positive cells in *P. elegans* than in *P. dumerilii*. Currently we have no precise explanation of this striking difference. However, two explanations may be suggested. The first one is the difference in some biochemical pathways that may interfere the reaction in *Platynereis*. The studied species belong to the two main clades of annelids, Errantia and Sedentaria, which have evolved independently for a long time (Struck *et al*., 2011) leading them to the difference in numerous metabolic pathways. The second one is that *P. dumerilii* possesses less CA-positive cells in the body wall than *P. elegans* caused by the differences in the lifestyle conditions of these two annelids: the errant *P. dumerilii* is much more agile than the sedentary *P. elegans*.

In the brain of *P. elegans* we observed six pairs of CA-positive cells: one pair lies frontally, four pairs between the eyespots, and one pair is situated posteriorly. *P. dumerilii* also possesses CA-positive cells in the brain, however, on our preparations it was impossible to clearly define their exact number. A one-to-one comparison is complicated because of significant difference in brain morphology in nereidids and spionids (Orrhage, 1993; Orrhage, Muller, 2005). Another reason complicating the direct comparison is an inability to combine glyoxylate-induced fluorescence with antibody staining (e.g. against acetylated α-tubulin, revealing

проекции в области церебрального ганглия, желтыми стрелками указаны тела нейронов; E — рецепторные клетки пальпы (стрелочные головки); F — катехоламин-положительные клетки вокруг глазных пятен; G — иннервация глотки, стрелочными головками отмечены кластеры катехоламин-положительных клеток; H — катехоламин-положительные элементы в брюшной нервной цепочке и стенке тела; I — рецепторные клетки (стрелочные головки) между туловищными сегментами; J — нейроны в области параподиального ганглия (стрелочные головки); K — иннервация заднего конца тела.

Обозначения: br — мозг, drcc — спинной корешок окологлоточных коннектов, ey — глазное пятно, j — челюсти, lph — продольный нерв глотки, pcn — нерв перистомиального усика, pg — параподиальный ганглий, pgn — нерв пигидиального усика, sg — сегментарный ганглий, sn1, 3 — первая и третья пары сегментарных нервов, tph — поперечный нерв глотки, vnc — брюшная нервная цепочка, vrcc — вентральный корешок окологлоточных коннектов. Масштаб: A, G, K — 50 мкм; B, H — 100 мкм; C, D — 25 мкм; E, F, J, I — 10 мкм.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most nerve fibers). Nevertheless, in juvenile worms 7 pairs of cells were reported earlier (Starunov *et al*., 2017). Their position is comparable with *P. elegans*: four pairs surrounding the eyespots, two pairs on both sides of the brain neuropil, and one pair in front of it. We did not find CA-positive cells so closely related with the eyespots in *P. elegans*. The cells of 2–5 pairs in *P. elegans* brain are closely related with the eyespots and may be related with photoreceptor functions, however this suggestion needs further verification.

The posterior pair of brain cell somata described in *P. elegans* may be related to nuchal organs by their position behind the brain (Schlötzer-Schrehardt, 1987). However, they do not receive any other CA-positive fibers from body wall receptors or other organs. In *P. dumerilii* we did not find any cell related with nuchal organs. This finding suggests that CA-positive cells in studied annelids do not participate in chemoreception function (do not act as primary chemoreceptors) in nuchal organs. The posterior cells of *P. elegans* may be a secondary gain and perform some integrative functions due to highly developed nuchal organs in Spionidae (Jelsing, 2003). The most comprehensive study of the catecholaminergic system in annelids was performed by Schlawny with coworkers (Schlawny *et al*., 1991b) on eunicid annelid *Ophryotrocha puerilis*. The authors detected 53 fluorescent cells in the brain region, which by our rough estimations, is far more than in *P. elegans* or *P. dumerilii.* According to the provided schemes and images of whole-mount preparations, the cell somata also show some tendency to clustering. Though direct comparison with our results seems premature, two important points can be distinguished. First, the authors did not mention any cells or processes connected with the nuchal organs, that corroborates our hypothesis that CA do not participate in chemoreception. Second, in *O. puerilis* paired cell clusters that send projections to the eyespots were found. The eyes of *P. dumerilii* are also highly innervated with CA: each of the four photoreceptor organs is surrounded by cells that are presumably involved in signal transmission from the eyes to the brain. According to the ultrastructural data we suggest these cells to be either photoreceptor or supportive cells (Fischer, Brökelmann, 1966). Nevertheless, it is impossible to undoubtedly define these cells with the use of glyoxylic acid fluorescent technique only. We did not find such CA-positive cells around the eyespots of *P. elegans*, however, some cells in the brain are in direct contact with the posterior pair of the eyes. It is difficult to ascertain that these cells are related with photoreception, further studies would shed light on this question.

High level of CA-innervation of *P. elegans* palps and concentration of the neurons around the food groove reflects the role of these structures in the feeding process. The long mobile

Fig. 4. Generalized scheme of catecholaminergic system organization of *Pygospio elegans* (A–D) and *Platynereis dumerilii* (E–G). Main nerve tracts of the central nervous system are outlined in grey. A — innervation of the dorsal side of the prostomium of *P. elegans*; B — innervation of the ventral side of the prostomium and thoracic segments of *P. elegans*; C — innervation of abdominal segment of *P. elegans*; D — innervation of the posterior end of *P. elegans*; E — innervation of the dorsal side of the prostomium of *P. dumerilii*; F — innervation of the ventral side of the peristomium and body segments of *P. dumerilii*; G — innervation of the posterior end of *P. dumerilii*.

Abbreviations: br — brain, cc — circumoesophageal connectives, cpn — circular pygidial nerves, ey — eyespots, fpn — frontal prostomial nerves, lph — longitudinal pharyngeal nerve, no — nuchal organs, pcn — nerves of the preistomial cirri, pg — parapodial ganglion, pgn — nerves of pygidial cirri, pn — palp nerves, sn1-4 — segmentary nerves, tph — transversal pharyngeal nerve.

Рис. 4. Обобщенная схема организации катехоламинергической системы *Pygospio elegans* (A–D) и *Platynereis dumerilii* (E–G). Серым обозначены общие контуры нервных волокон центральной нервной системы. A — иннервация дорсальной стороны простомиума *P. elegans*; B — иннервация вентральной стороны простомиума и торакальных сегментов *P. elegans*; C — иннервация абдоминального сегмента *P. elegans*; D — иннервация заднего конца тела *P. elegans*; E — иннервация дорсальной стороны простомиума *P. dumerilii*; F — иннервация вентральной стороны перистомиума и туловищных сегментов *P. dumerilii*; G — иннервация заднего конца тела *P. dumerilii.*

Обозначения: br — мозг, cc — окологлоточная коннектива, cpn — кольцевой нерв пигидия, ey — глазные пятна, fpn — фронтальные нервы простомиума, lph — продольный глоточный нерв, no — нухальные органы, pcn — нервы перистомиальных усиков, pg — параподиальный ганглий, pgn — нервы пигидиальных усиков, pn — нервы пальп, sn1-4 — сегментарные нервы, tph — поперечный глоточный нерв.

palps of Spionidae are used both for suspension and deposit-feeding which involves reactions of chemoreception and, especially, mechanoreception (Lindsay *et al*., 2008). The additional palp nerve also supplies the receptors of the abfrontal surface. Similar cells at the abfrontal palp surface were shown by FMRFamide antibody labeling in different spionids by Forest & Lindsay (2008). These authors suggest chemosensory functions for such cells. The CA-positive cells of the palps and prostomium body wall are similar in morphology and orientation to CA-containing cells, found in nemerteans and mollusks (Zaitseva *et al*., 2019, 2020) which were identified as mechanoreceptive. Thus, we can suggest a model where in the palps of *P. elegans* FMRFamide-positive cells are chemosensory and CA-positive cells are mechanosensory (or mixed mechano- and chemosensory).

P. dumerilii possesses palps that are shorter and less mobile in comparison with *P. elegans*. They are not used for suspension-feeding but play an important role in deposit-feeding as an organ of both chemoreception and mechanoreception. Surprisingly, no CA-positive nerve fibers or receptor cells have been detected in palps of *P. dumerilii*. Only one specimen showed some cells at the palp base arranged similarly to those found in palps of *Ophryotrocha puerilis* (Schlawny *et al*., 1991b). At the same time, the peristomial cirri possess distinct CA-positive nerve fibers in all specimens studied. Such dramatic difference in palp and cirri innervation may suggest that CA in *P. dumerilii* do not perform such important function in reception in comparison to *P. elegans*.

CA-innervation of the pharynx in *P. dumerilii* is far more complicated than in *P. elegans*, which correlates well with the overall differences in pharynx organization (Tzetlin, Purschke, 2005). Nevertheless, in *O. puerilis*, which also has a complicated pharynx with jaw apparatus, the stomatogastric system shows general similarity with *P. dumerilii* (Schlawny *et al*., 1991b). The stomatogastric nerves give rise to pharyngeal and oesophageal longitudinal nerves. The oesophageal nerves are connected by a commissure as well as several separated neurites. There are also CA-positive cells in the oesophageal ganglia on either side of the commissure. Thus, the stomatogastric system complication follows the overall foregut complication. Spionids possesses foregut with dorsolateral ciliary folds and relatively simply organized pharyngeal organ (Purschke, Tzetlin, 1996) that corroborates with our data. *P. elegans* stomatogastric nervous system is organized in a plexus. *O. puerilis* has a complicated jaw apparatus that is supplied by longitudinal nerves with commissures and paired oesophageal ganglia (Schlawny *et al*., 1991b). The most complicated is *P. dumerilii* axial pharynx innervation forming a complicated system of longitudinal and transversal nerves. CA-positive elements may be involved in mechanoreception or motor innervation of the pharyngeal region.

Despite the great difference in gross-morphology, the CA-positive components of the ventral nerve cord show a high degree of similarities between the two studied species. Segmental ganglia of *P. elegans* include up to 5 pairs of CA-positive somata, placed in the first third of the segment. In *P. dumerilii* the segmental ganglia also comprise five pairs of CA-ergic somata, located mostly anteriorly. The arrangement of CA-positive fibers in the ventral connectives is also similar in both species. No distinctive CA-positive commissures can be seen neither in *P. elegans* abdominal segments nor in *P. dumerilii* (all segments). This is partially explained by the common ground plan of the annelid nervous system (Orrhage, Müller, 2005; Müller, 2006). A similar pattern was described in *Ophryotrocha puerilis* (Schlawny *et al*., 1991a, b) with four main CA-positive longitudinal nerves and few pairs of neurons (two pairs of unipolar cells per ganglion in untreated animals and up to 9 pairs after dopamine or noradrenaline treatment). The CA-positive commissure of *O. puerilis* is similar to those found in *P. elegans* thoracic segments. The small number of CA-positive cells in ventral nerve cord ganglia was also described in other annelid species (Clark, 1966; Dhainaut-Courtois, 1972; Myhrberg, 1967; Lent, 1982). Further comparative physiological studies are needed to answer this question.

The general body wall organization is comparable in both species. Within the segments of *P. elegans* CA are found in four pairs of segmental nerves. The first pair innervates the area ahead of the parapodium, the second and the third innervate the parapodium, while the region where the fourth pair runs has an appearance of a small cluster of cells and may corre-

Table. The main characteristics of the catecholaminergic system in annelids
Таблица. Основные характеристики катехоламинергической системы разных аннелид

Character	***Pygospio elegans***	***Platynereis dumerilii***	***Nephtys* (after Clarck, 1966)**	***Ophryotrocha puerilis* (after Schlawny *et al*., 1991b)**
Brain	CA-positive fibers in both commissures, 6 pairs of neurons	CA-positive fibers in both commissures, paired neurons scattered in the brain	CA-positive fibers in neuropile, paired neurons scattered in the brain	Strong CA-positive signal in neuropil. Numerous CA-positive cells in the brain.
Segmental ganglia	5 pairs of neurons, 2 pairs of longitudinal nerves	5 pairs of neurons, 2 pairs of longitudinal nerves	3 pairs of neurons, 2 pairs of longitudinal nerves	2 pairs of neurons (9 after DA treatment), 2 pairs of longitudinal nerves
Stomatogastric nervous system	Nerve plexus	Complicated meshwork of longitudinal and transversal nerves with paired ganglia	No data	Two pairs of longitudinal nerves with anterior commissure and paired ganglia
Palp and antennae innervation	CA-positive fibers in main and additional palp nerves. Numerous bipolar cells along the palps	Bipolar cells in the palpophors	Few CA-positive fibers in the antennal nerves, no CA-positive cell somata in the antennae	Groups of bipolar cells in the palpophors and bases of antennae
Prostomium innervation	Prominent prostomial nerves. Numerous bipolar cells scattered through the prostomium and mouth region	No discernible signal detected	Faint prostomial nerves with a few cells associated with them	Bipolar cells sending their axons directly to the brain neuropil
Segmental nerves	4 pairs of CA-positive nerves	2 pairs of CA-positive nerves	3 pairs of CA-positive nerves	3 pairs of CA-positive nerves
Body wall and parapodia innervation	Numerous bipolar cells in the body wall with concentrations around chaetal sacs	Single bipolar cells in the body wall and parapodia. Prominent parapodial ganglia with several neurons	CA-positive fibers and bipolar cells in the epidermis. Single neurons in parapodial ganglia and in parapodia wall	CA-positive subepidermal plexus. Parapodial ganglia present. Bipolar cells in the parapodial wall. CA-positive innervation of the aciculae
Pygidium	Paired nerves encircling the pygidium and branching fibers towards the pygidial lobes. Terminal commissure present	Paired longitudinal nerves running to the tip of the pygidial cirri	No data	Paired longitudinal nerves with a terminal commissure. Bipolar cells in the pygidial cirri

spond to the cells of parapodial glands that were described for Spionidae (Meißner *et al*., 2012). The second and third pairs of segmental nerves demonstrate different levels of fusion in thoracic and abdominal regions of the body of *P. elegans*, which may be interpreted as two separate pairs of nerves that tend to merge, or, in the contrary, originally a solitary pair of nerves that splits in two to different degree. In *P. dumerilii* CA-positive elements are found only in two of the four main segmental nerves (Hamaker, 1898). According to their position, these two nerves are the first and the third pairs respectively. The CA-positive fibers innervate the parapodia and the segmental borders suggesting their mechanosensory functions. Similarly, in *O. puerilis* and *Nephtys* (Clark, 1966; Schlawny *et al*., 1991b) most CA-positive fibers contribute the parapodial nerves, and only few neurites were found in some other segmental nerves.

Parapodia of both species possess a number of CA-positive sensory cells, however, they differ significantly in number. In *P. elegans* numerous receptor cells encircle the base of the parapodium. In *P. dumerilii* only solitary cell somata that were interpreted as receptors are seen. Nevertheless, in other errant annelids, *O. puerilis* and *Nephtys*, more CA-positive cells were found (Clark, 1966; Schlawny *et al*., 1991b).

CA-innervation of the pygidia in the two species studied is also very different. While in *P. dumerilii* only some fibers were detected inside the pygidial cirri, in *P. elegans* there are numerous receptor cells around the anus and in the base of a pygidium. During regeneration of *P. elegans* the first CA-positive cells appear on the third day post amputation in parts of the peripheral nervous system (Starunov *et al*., 2020). After a week post amputation, newly formed segments possess fully developed CA-ergic systems, and the number of CA-positive cells is higher in comparison with intact parts (Fig. 2G–H). This fact needs further investigation and may be a result of a changing cell chemism during regeneration, or due to the fact that in intact animals the level of CA could be below the detection limit.

Thus, our data support the general idea that peripheral CA-positive cells perform mainly sensory functions. We suggest for them proprio- or mechanoreceptor function due to their presence in the highly agile parts of the body and on the areas of folds. Nevertheless, it is still not clear whether the CA perform motor function. Some evidence for the motor functions are available from *Sabellastarte magnifica* and *Lumbricus terrestris* (Myhrberg, 1967; Díaz-Miranda *et al*., 1992). Clark also suggested that CA perform both receptor and motor functions in Nephtys (Clark, 1966). With modern neurophysiology data, we may prove that the presence of several neurotransmitters performing quite similar functions in one organism is a common situation (Cifuentes, Morales, 2021).

In conclusion, we suggest that although all of the studied species share common features in the organization of their CA-systems, the differences between errant and sedentary species are even more striking than between different species of Errantia (Table). They include presence or absence of receptor cells in the prostomium and pygidium, number and shapes of cells in the parapodia and the level of involvement of CA in the innervation of sensory organs. Nevertheless, no clear picture is still forming since only a limited number of species has been studied so far. It is likely that such variability of nervous systems in Annelida is a result of long-term adaptation to different life conditions. Recent studies have shown an impressive level of the annelid nervous system plasticity and a high level of convergence in annelid nervous system evolution (Helm *et al*., 2018). Further broad taxon studying is urgently needed for the comprehensive comparisons of CA functions among different annelid species.

Compliance with ethical standards.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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