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ZOOLOGY

Structure of the Glial Cells in the Nervous System of Parasitic and Free-living Flatworms

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Abstract—This study is devoted to ultrastructural and immunosytochemical investigation of the nervous system in parasitic and free-living platyhelminthes to learn if glial cells exist in the nervous system of flatworms. We described the ultrastructure of different types of glial cells and the peculiarities of myelinization of gigantic axons; immunoreactivity to the S100b protein is revealed. Comparative analysis of the glia structure of annelids and platods is given; structural, functional, and evolutionary aspects of myelinization of gigantic axons, which are revealed in cestodes, are discussed.

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Glial cells were revealed for the first time in brains of vertebrates. It was thought for a long period of time that they are absent in the nervous system of higher animals. Glial cells of vertebrates occupy up to 50% of the brain volume; they have wide structural diversity and are of various origins, from neuroectoderm, mesenchyma, neuromesoderm, and stem cells. Glia is topographically divided into central (astrocyte, olygodendrocytes, and their derivatives) and peripheral (Shwann's cells and satellites), which form myelin membranes of nerves. Morphological types of glial cells differ in structure and specific molecular markers, but may also transform from one type into another. It is proved that glial cells of vertebrates and invertebrates make a contribution to functioning of neurons and are able to perceive signals from them (Coles, Abbot, 1996). The electrophysiological and metabolic answer to the action of many transmitters and modulators is registered in the glial cells (Cooper, 1995). Close functional interactions between neurons and glial cells of many invertebrates are established.

It is not known yet at what stage of evolutionary development structural and functional differentiation of glial cells occurred. Ultrastructural and immunocytochemical studies confirm the existence of glia in nemerteans and annelids. Glia of nemerteans is presented by numerous small cells, which are revealed in cerebroganglion and in lateral trunks. Their cytoplasm is rich with fibrils; processes form a membrane of neurons and neuropile located between the fibrillar plate and neural elements (Golubev, 1982; Sotnikov et al., 1994). Glial cells of annelids are necessary components of the nervous system of all studied species. They form ganglion and neuropile membranes and differ in the presence of fibrils and filaments as the main components of cytoplasm of perikaryon and trunks (Hulsebosch, Bittner, 1981). Glia of annelid worms is divided into cortical, which forms the external membrane of ganglions, and medullar, which participates in isolation of neuropiles and trunks (Golubev, 1982). Structural differences between cortical and medullar cells of glia are most strongly pronounced in suctorial annelids, the medullar glia of which is composed of gigantic glial cells of neuropiles. A constant number and location and a clearly expressed zonality of the cytoplasm, which includes fascicles of intermediate filaments by their width replying glial filaments of vertebrates (Riehl, Schlue, 1998), are typical for them. The cortical membrane is made up of microglial cells, which are able to move (Golubev, 1970; 1982; Sotnikov et al., 1994). Oligochaetes possess a developed system of glia-neuron interactions; myelin is revealed in them, and gigantic nerve fibers in the trunk are isolated by a myelin-like membrane (Zoran et al., 1988). The myelin membrane of the earthworm consists of 20–200 layers and is spirally twisted in the ends in the area of the isthmus as in vertebrates (Roots, Lane, 1983; Roots et al., 1991). The difference from vertebrates is in the unequal compact structure of myelin; sometimes layers of cytoplasm are left between membranes in the form of a sandwich (Gunther, 1976).

There is still no answer to the question whether specialized glial cells in the nervous system of free-living and parasitic flatworms exist. A review of the literature indicates that turbellarian worms, cestodes, trematodes, sturgeon cestodes, temnosephallides, and monogenes have cells that form membranes of ganglia and trunks, which participate in wrapping of axons

and regions of neuropiles, whose nature is not yet clear. The most likely function of these cells is thought to be their participation in trophic and mechanical support and probably electric isolation of neurons (Rohde, 1970; Ferrero et al., 1985; Rohde, Webb, 1986; Reuter, Palmberg, 1990; Sukhdeo, Sukhdeo, 1994; Bedini, Lanfranchi, 1998; Biserova 2000a, b; 2008a, b).

The goal of the study is to reveal the presence and describe the structure of membranes of the nervous system of cestodes *Christianella minuta* and *Echinobothrium typus* and to conduct comparative analysis of the structure of the membranes of the central nervous system (CNS) of platods and other worms.

MATERIAL AND METHODS

Adult sexually mature individuals of *Christianella minuta* (Trypanorhyncha) and *Echinibothrium typus* (Diphyllidea) from the spiral valve of ray *Raja clavata* were caught in the coastal waters of the Black Sea. Free-living *Dugesia tigrina* is kept in culture on the invertebrates' zoology subdepartment of the biological department of MSU. For ultrastructural studies objects were fixed in 2% glutaric aldehyde (SERVA, Germany) on 0.2 M of a phosphate buffer adding 0.1 M of saccharose with further fixation in 2% osmium tetroxide (Moscow chemical plant) on the same buffer. After dehydration objects were put into araldite (MERK, Germany) under 37 and 60°C. For immunochemical detection of glia marker S100b, *C. minuta* and *D. tigrina* were fixed in 4% paraform on a 0.1 M phosphate buffer (PB), pH 7.4, washed, and put into 5% Triton X100 (Sigma, USA) on 0.01 M PB. Preincubation in a 10% solution of normal goad serum (NGS) on 0.01 M PB with 1% Triton X100, 30 min. Incubation in primary antibodies: Anti-s100b from rabbit 1 + 20 in 0.01 M + 1% Triton X100 + 10% NGS, 4 days, 4°C. Flush with 0.01 M PB + 1% Triton X100, 3 h. Conjugation with secondary antibodies: Alexa 546 anti-rabbit from goat, 1 + 300 in 0.01 M PBS, 22 h. Flush by buffer 0.01 M PB + 0.35 % Triton X100, 1.5 h, 4°C. Whole mounts were put into 50% glycerin on a phosphate buffer and looked at on the Leica TCS SPE confocal microscope (Germany).

RESULTS AND DISCUSSION

ULTRASTRUCTURAL IDENTIFICATION OF GLIAL CELLS IN THE NERVOUS SYSTEM OF CESTODES

The fine anatomy and ultrastructure of CNS of cestodes was studied on serial sections, and the presence of glia-like cells in cerebroganglia and major nerve trunks was established.

Echinobothrium typus (Diphyllidea). The pair cerebroganglion is situated just under the basal support lamella of the muscular bulbus of the proboscis; left and right lobes are connected by cerebral commissure. Neurons send a part of processes upwards around the bulbus and form proboscis (anterior) nerves. Another part of processes goes in the central neuropile and then in the main lateral trunks or in the cerebral commissure. The main trunks lie not very deeply in the subtegumental layer.

Ganglionary neurons and neurons from proboscis nerves are surrounded by a loose membrane as vast electron-light reservoirs limited by a membrane, which separates cerebroganglia from other cell elements of scolex parenchyma (Figs. 1a and d). These cytoplasmic extensions belong to cells that fill the space between the support plate of the proboscis bulbus and interior nerves and also surround ganglion lobes. Their processes, which contain fine-dispersed material, rare vacuoles, and mitochondria, affiliate proboscis nerves passing parallel to the neuritis but not forming specialized contacts. There are thin filaments, which fill intercells, among processes of satellite cells, which surround ganglionary neurons. Perikaryons of glia-like cells also have few organoids; the cytoplasm contains big and small vacuoles and free ribosomes (Fig. 1d). We refer to satellite cells as support glia-like cell elements. Their nuclei are encountered on the surface of the main lateral trunks (MLT) in the central region of the scolex. Cell processes penetrate not only among nerve elements but fill the space among the main muscle bundles and excretory vessels near ganglia. They probably perform an important role in metabolism along with support function. Nevertheless, there is clear specialization of satellite cells as membranes of cerebroganglion. Processes of support cells accompany all ganglionary neurons and sometimes form simple contacts. Filamentary material of intercells penetrates in invaginations of ganglionary neurons forming a kind of hemidesmosoma (Fig. 1c). In the anterior nerves, the proboscis of support cells makes up a significant part of the nerve volume separating the nerve as a whole structure. This assists in lowering of stress from pressure and deformations, which appear in the process of movements of the proboscis and muscle bulbus and work of the muscular system of hamulus, in which the form of the scolex changes significantly. Around the main nerve trunks, a multilayer membrane is formed in the form of a sandwich, in which thin light processes of glia-like cells alternate with wider interlaying fibrillar material of intercells (Fig. 1d). At the same time, the primitive condition of neuro-glial interactions in representatives of diphyllides *E. typus* should be pointed out. No differences in the ultrastructure of glial cells and character of their interactions with neurons were revealed.

Christianella minuta (trypanorchyncha). In comparison with *E. typus*, the central system of *C. minuta* is mostly concentrated and made up of elongated cerebroganglion, of which four lobes are united in the middle part at first by dorsal and ventral and then by cross and median commissures, and the median commissure is surrounded by neurons and forms the fifth cen-

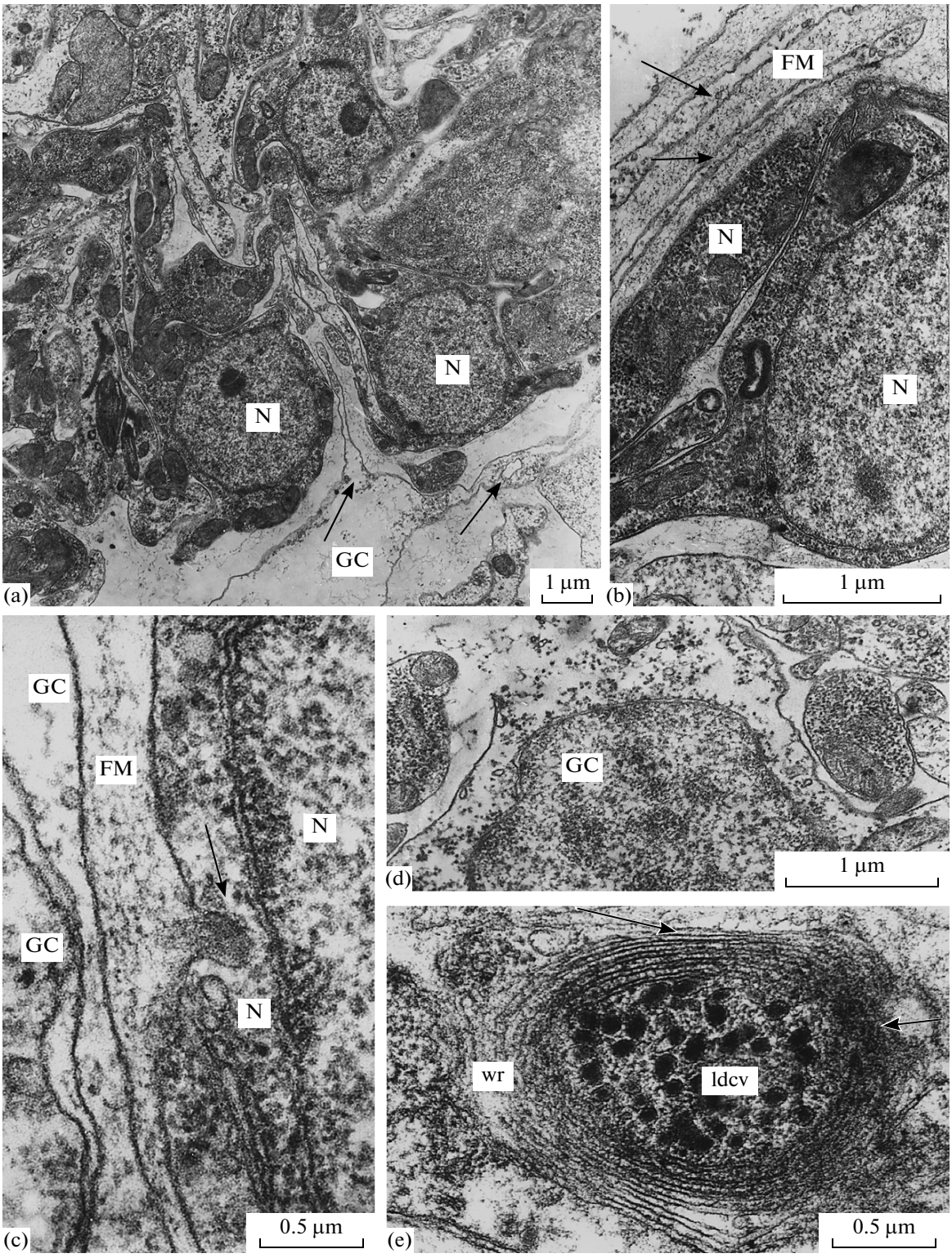

Fig. 1. Membrane structure in CNS of *Echinobothrium typus* (a–d) and *Triaenophorus nodulosus* (e). (a)—Glial membranes (GC) of cerebroganglion, arrows—cell processes limiting ganglion lobes; (b)—neuron (N) wrapping in the form of a "sandwich"; glial processes atrernate with layers of the fibrillar matrix (FM); (c)—structure of fibrillar matrix moored in neurilemma of ganglionary neuron (arrow); (d)—perikaryon of glial cell (PG); (e)—main trunk of *T. nodulosus*: myelin-like wrapping (wr) of the nerve terminal with large electron-dense vesicles (ldcv); the compact region with glued membranes (long arrow) and the region of isthmus (short arrow) are marked.

tral lobe. Bulbar nerves with gigantic axons in their content go from each lobe to muscle bulbuses of the proboscis. All parts of the central nervous system (CNS) of the scolex have thin membranes, which distinctly separate them from the surrounding parenchyma and are presented by cell processes and intercellular fibrilles.

The cerebroganglion has two types of processes: dark and light (Fig. 2). Light processes go from cells, of which perikaryons are in immediate proximity to nerve accumulation, and are in the composition of ganglia lobes and proboscis nerves. Thin prolonged bundles of electron-light cytoplasm practically do not contain organoids and form 1–2 layer membranes around neurons bodies and compact groups of axons or nerves and also penetrate deeply in neuropiles separating differently directed compartments of processes. Inside neuropiles light thin processes sometimes form a multilayer membrane from bundles tightly adjoining to each other.

Cell bodies, which form light processes, are located cortically, tightly adjoining the surface of the nerve (Fig. 2a). The nucleus is small, has an oval-lobar form, and chromatin is distributed unevenly; the cytoplasm near the perikaryon contains ribosomes, small vacuoles with transparent and granular content, rare small mitochondria, and sometimes microtubules. Processes surround the nerve in thin layer in the form of a "film."

Dark processes are located loosely, have widening, which contain ribosomes, beta-glycogen, lipidic drops, and a thick structured cytoplasm. They usually do not adjoin to nerve elements but often form stretched contacts with each other limiting the intercellular space along the nerve column, the internal space of cerebroganglion. The intercellular space of processes is filled with thin fibrilles. Determination of where dark processes belong, to one cell nucleus or another, is complicated; a part of them may be both processes of muscle cells, which do not contain miofibrilles, and processes of cells, which form walls of excretory channels.

Another type of membranes and cells, which they are formed of, is revealed in bulbar nerves. Bulbar nerves are surrounded by an electron-dense thin membrane, and each gigantic axon in the nerve is wrapped in electron-dense laminated material (Fig. 2b). Cell nuclei, which form membranes, lie on the periphery of the nerve and have an oval-triangle form with regions of concentrated and diffuse chromatin. The cytoplasm surrounds the nucleus in a thin layer, is densely structured, and contains rare mitochondria and forms very thin processes tightly adjoining to each other, which wrap axons. External membranes of glial processes quite often adjoin each other so tightly that the layer of the intercellular space could not be singled out (Fig. 2b, arrows). The final regions of processes form specialized contacts. The intercellular space, where it may be followed, contains a fine-dispersed matrix of medium electron density.

Thus, in CNS of *C. minuta*, two types of glia-like cells, which form the neuron and their processes membranes, were revealed: multilamellar cells in cerebroganglia and cells that form a myelin-like membrane of gigantic axons in bulbar nerves.

IMMUNOCYTOCHEMICAL DETECTION OF GLIA IN FLAT WORMS

Glia-like cells, which were revealed by ultrastructural methods in different groups of flat worms, do not answer the question about the nature of these cells. That is why the attempt to confirm whether cell elements belong to a certain type with the help of immunocytochemical detection of the specific glial protein S100b was made.

While studying the nervous system of *C. minuta* on a confocal microscope in the central part of the ganglion, big cells with positive immunoreactivity (IR) to AB S100b were revealed (Figs. 2c and d). Bodies and processes of S100b - IR cells surround the central neuropile, which is left dark. In the anterior proboscis nerves bodies of S100b - IR cells are stretched along the longitudinal axis of the body sending thin processes along the nerve. In addition, intensive S100b - IR was revealed below the level of bothrium in bulbar nerves, which go along the proboscis receptacle (Fig. 2e). S100b - IR elements are located in the periphery of the nerve in the form of thin bundles and stretched widening, which correspond to nuclear regions of immunoreactive cells.

Besides cestodes, preparations of turbellaria *Dugesia tigrina* were treated by antibodies to S100b. Studies indicated an intensive reaction to antibodies in rounded small cells, which were found in abundance in the worm body. However, the distribution of S100b - IR elements was relatively diffusive, that is why interpretation of the obtained results is complicated and needs further study.

DISCUSSION

PECULIARITIES OF THE THIN STRUCTURE OF GLIA IN DIFFERENT GROUPS OF FLAT WORMS

Cestodes. Cortical and medullar glia represented by several types of cells are described in CNS of *Grillotia erinaceus* (Trypanorchyncha) (Biserova, 2008). The cortical membrane of cerebroganglia is formed by fibroblastlike glial cells, which synthesize fibrilles and participate in active transport (Fig. 3). Gigantic axon and bulbar nerves have a myelin-like membrane formed by specialized glial cells, which contain prolonged membrane structures. Multilayer cortical membranes of neuropiles of the main trunks of *G. erinaceus* are formed by glial cells, the processes of which

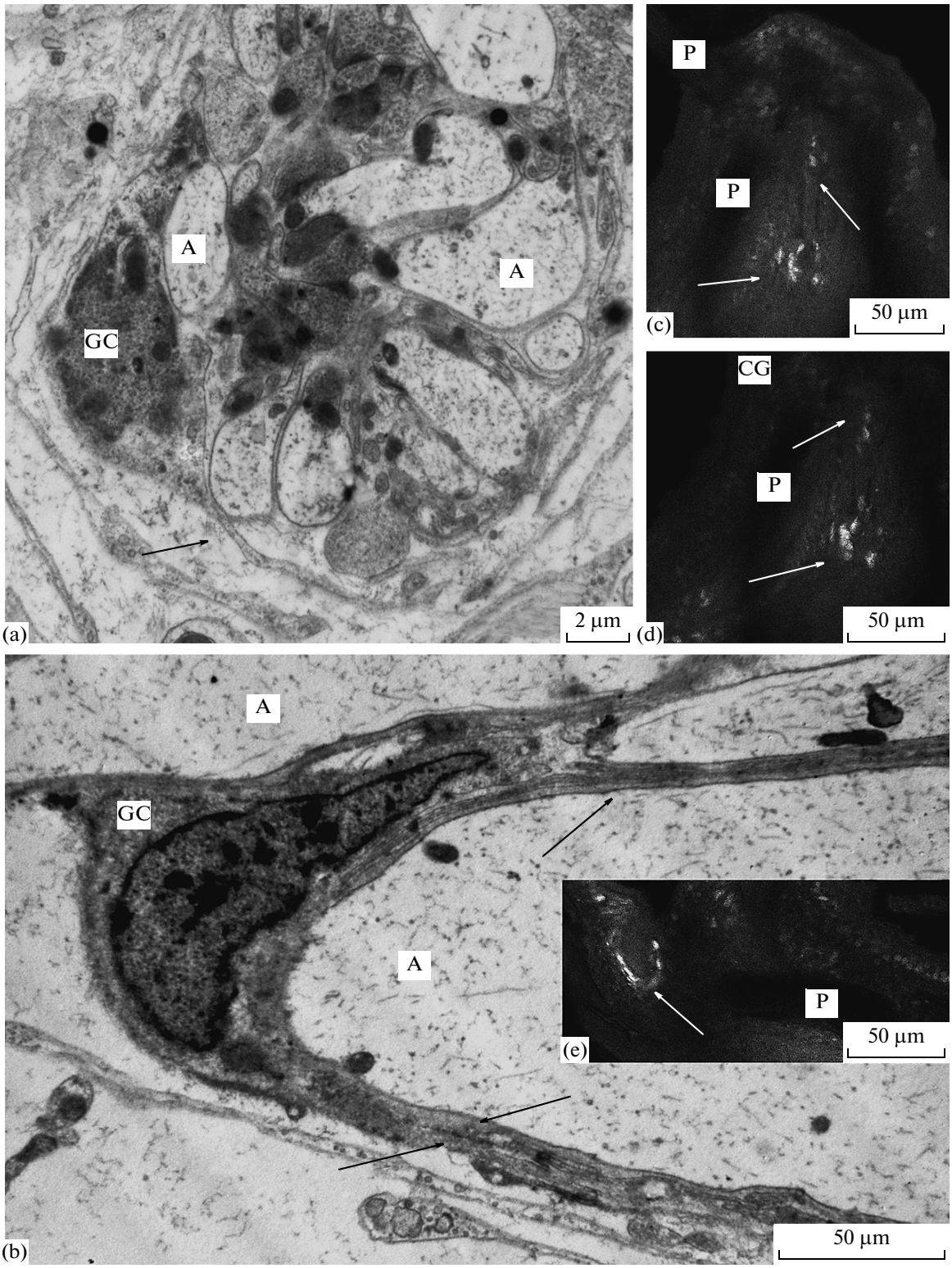

Fig. 2. Ultrastructure and immunocytochemical identification of glia in CNS of *Christianella minuta*. (a)—Multilamellar cell of 1 type (GC), processes (arrows) of which participate in wrapping of proboscis nerves; (b)—glial cell of type 2, which forms myelin-like membranes of gigantic axons; arrows indicate regions of tightly glued layers; (c, d)—s100b-IR cells (arrows) in cerebroganglion and proboscis nerves; serial optical section, 0.5 μm; (e)—s100b-IR cells (arrows) in bulbar nerve. A—axon, P—proboscis.

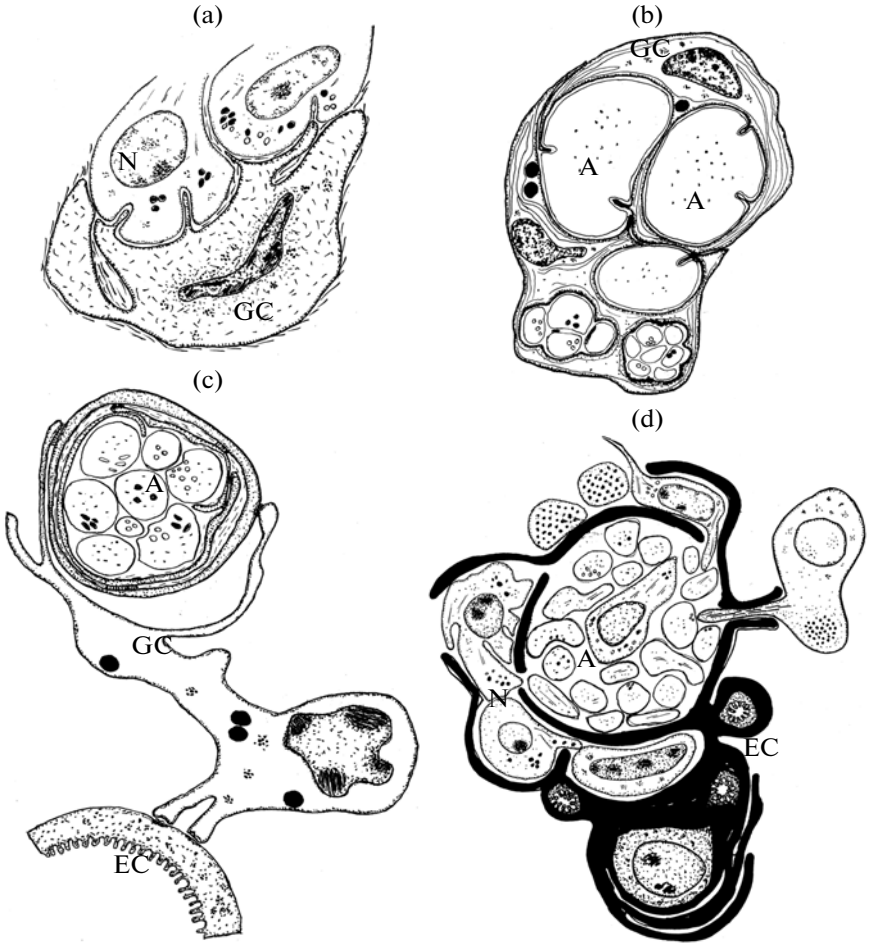

Fig. 3. Types of glial cells (GC) and glia–neuron interactions in parasitic flat worms. a—fibroblast-like cell in cerebroganglion; b—myelin-like membrane of gigantic axons; c—multilayer "sandwich" membrane of neuropile of the main trunk; c—membranes of main trunk formed by growths of epithelial cells of walls of excretory channels (EC). A—axon.

excrete collagen-like fibrilles and are connected by prolonged contacts. The neuropile membrane looks like "multilayer sandwich"; neuron bodies and glial cells are left outside the membrane. Cells of medullar glia were revealed in the central part of the trunk and participate in wrapping groups of processes and rare interneurons (Biserova, 2008b).

Cortical membranes of the main nervous trunks of plerocercoids *Ligula intestinalis* (Diphyllobothriids) are formed by small dark cells of prolonged form (Biserova, 2008a), which form with each other during prolonged specialized contacts. It is interesting that the number of glial cells in the main nerve trunks is approximately five times higher than the number of neurons. The number and location of these cells corresponds to elements immunoreactive to s100b in MLT and ganglia of *L. intestinalis* (Biserova, 2000b; 2008a). By peculiarities of the structure, these cells are close to multilammelar cells of free-living flat worms.

Cortical membranes of MLT and ganglia of *Triaenophorus nodulosus* (Bothriocephallidea) are formed by widening of the epithelium of excretory vessels (Fig. 3d) (Biserova, 1997; Biserova, Salnikova, 2002; Biserova, Korneva, 2006). At the same time in the central part of the main trunks, axons were revealed, which have a multilayer myelin-like membrane, which does not belong to cells of the excretory epithelium (Fig. 1e). Thin layers are presented by light cytoplasm without organoids. Processes wrapping axons are located in ordered concentric layers; there are zones of membrane adhesions (Fig. 1e, long arrow) and zones of "isthmus" (Fig. 1e, short arrow). Bodies of cells, which form myelin-like wrapping of axons, have not been revealed yet; but they apparently belong to medullar glia of MLT.

Specialized glial cells and developed glia-neuron interactions are described in *Amphilina foliacea* (Amphilinidea) (Biserova, 2000a, b; Biserova et al., 2000, Biserova, 2004). Cortical glial cells form multilayer membranes around cerebroganglia, the central commissure of main trunks, and caudal ganglia. Processes of glial cells enter the neuropile and isolate synaptic glomerula, surrounding the neuron body. There are differences in the ultrastructure of glia in different

regions of CNS. In cerebroganglia they are rich with mitochondria and ribosomes; in the main trunks they lack organoids, contain glycogen and lipidic drops, and support fibrilles. Glia of caudal ganglia are notable for their thick cytoplasm, which contains ribosomes, glycogen, lipids, and the presence of reservoirs with a thin fibrillar matrix (Figs. 4a and b). Glial processes form stretched contacts with each other; the intercellular space is filled with a thin-fibrillar support matrix forming a structure like a "sandwich." The processes in the "sandwich" are similar in diameter, do not contain organoids, and sometimes form tight contacts with neuritis (Figs. 4a and b). By the ultrastructural characteristics, glial cells of *A. foliacea* are closest to glial cells that form myelin-like membranes of the gigantic nerve fiber of *G. erinaceus* and *C. minuta* (Figs. 2b, 3b).

Trematodes. In cerebroganglion of *Fasciola hepatica*, two types of cells are revealed (Sukhdeo, Sukhdeo, 1994), which participate in membrane formation: (1) multilamellar cells, which form membranes wrapping ganglion and separating it from the surrounding parenchyma; (2) cells penetrating the big nerve processes in the type of trophospongia. By the morphological criteria, it is supposed that these cells may be early glia-like cells. As in cestodes, glial cells of the second type deeply penetrate in axolemma invaginations and are connected with gigantic (more than 12 μm in diameter) axons of *F. hepatica*, enter in the composition of nerve trunks and diametral commissure. It turned out in studying the ontogenesis of *F. hepatica* that gigantic axons appear only at the last stage of development in adult worms as glial cells connected with them (Sukhdeo, Sukhdeo, 1990). In the main trunks of *Multicotyle purvis* (Aspidogastrea) there are multilamellar cells, which form concentric membranes around nerve processes (Rohde, 1970, 1971). Their structure is similar to glial cells, which form membranes of cerebrogangia of *F. hepatica*.

Turbellaria. The structure and stage of development of glia in brains of free-living turbellaria is very hard to systematize due to the small number of studies and heterogeneity of the taxon itself. Such ultrastructural features as (1) cytoplasm poor with organoids, (2) tight adjoining and accompaniment of neurons and groups of processes, (3) small size and stretched form of nuclei, (4) formation of multilamellar outgrowth of cytoplasm, and (5) the presence of fibrilles and filaments in cytoplasm and intercells were revealed (Golubev, 1982; Reuter, Palmberg, 1990; Sotnikov et al., 1994; Bockerman et al., 1994; Bedini, Lanfranchi, 1998). As opposed to parasitic platods, the fibrillar component of turbellaria is poorly developed. In the main trunks of turbellaria unlike the brain, glial cells are easily differentiated by location. Light proboscises of satellite cells surround neurons and groups of axons by concentric lamellas; light cytoplasm contains glycogen, multivesicular bodies; intercellular space is filled by fine-dispersed filamental material. Multilamellar cells are described in nerve trunks of *Dugesia drotocephala*, *Armilla livanovi*, *Rimacephalus pulvinar*, *Stenostomum leucops*, Strongilostoma *simplex*, and *Procerodes littoraslis* (Golubev, 1982; Bedini, Lanfranchi, 1998; Mantyla et al., 1998).

IMMUNOCHEMICAL STUDIES OF PLATODS GLIA

The first experiments on immunocytochemical detection of glia in cestodes were conducted on *A. foliacea*. In its nervous system positive immunoreactivity to S100b was revealed (Biserova, 2000b). S100b - IR elements are registered in cerebroganglia and in the periphery of the main nerve trunk. A specific reaction to the marker of glial cells is revealed for *L. intestinalis* (Biserova, 2008a). S100b - IR elements are located in the surface of ganglion and main trunks in the form of small cells and varixes, which are often located asymmetrically on the surface of the main trunk. Cells have stretched fusiform and thin processes. In some regions of the main trunks, nuclei of S100b - IR cells are situated in pairs or in threes. The absence of a reaction in the central region of the nerve trunk was shown, which indicates the absence of big glial elements in the neuropile (Biserova, 2004; Biserova, 2008a). Along with the main trunks, this reaction is revealed in longitudinal lateral trunks, which lie in the cortical parenchyma, which are well-developed in ligula.

Thus, the results of immunocytochemical studies indicated the presence of cells that are immunoreactive to S100b in the nervous system of three species of cestodes: *C. minuta*, *L. intestinalis*, and *A. foliacea*. There is no data about immunocytochemical studies of glia of flat-worms in the literature; the studies of glia of lower worms are very limited in general. In annelids, the glial fibrillar protein GFAP is revealed in a gigantic glial cells of neuropiles of the medicinal leech *Hirudo medicinalis* (Riehl, Schlue, 1998). Positive anti-GFAP-IR was obtained in the brain of the nemertean *Linneus gesserensis* (Salnikova, Golubev, 2003). Intensive immunoreactions on glial proteins were revealed on the periphery of ganglion, whereas in the central zone, an intensive reaction on tubulin was detected, which corresponds to data of ultrastructural studies about the location of glial and nerve elements in the ganglion of nemerteans.

FORMATION OF THE BASAL MATRIX OR "NEURAL LAMELLA"

One of the stages of evolutionary formation of the brain is formation of a protective capsule. The presence of lamella of the fibromembrane, in the simplest cases neural lamella, or fibrillar lamella, which separates the brain from the surrounding tissues, is typical for brains or cerebroganglia of annelids, higher inver-

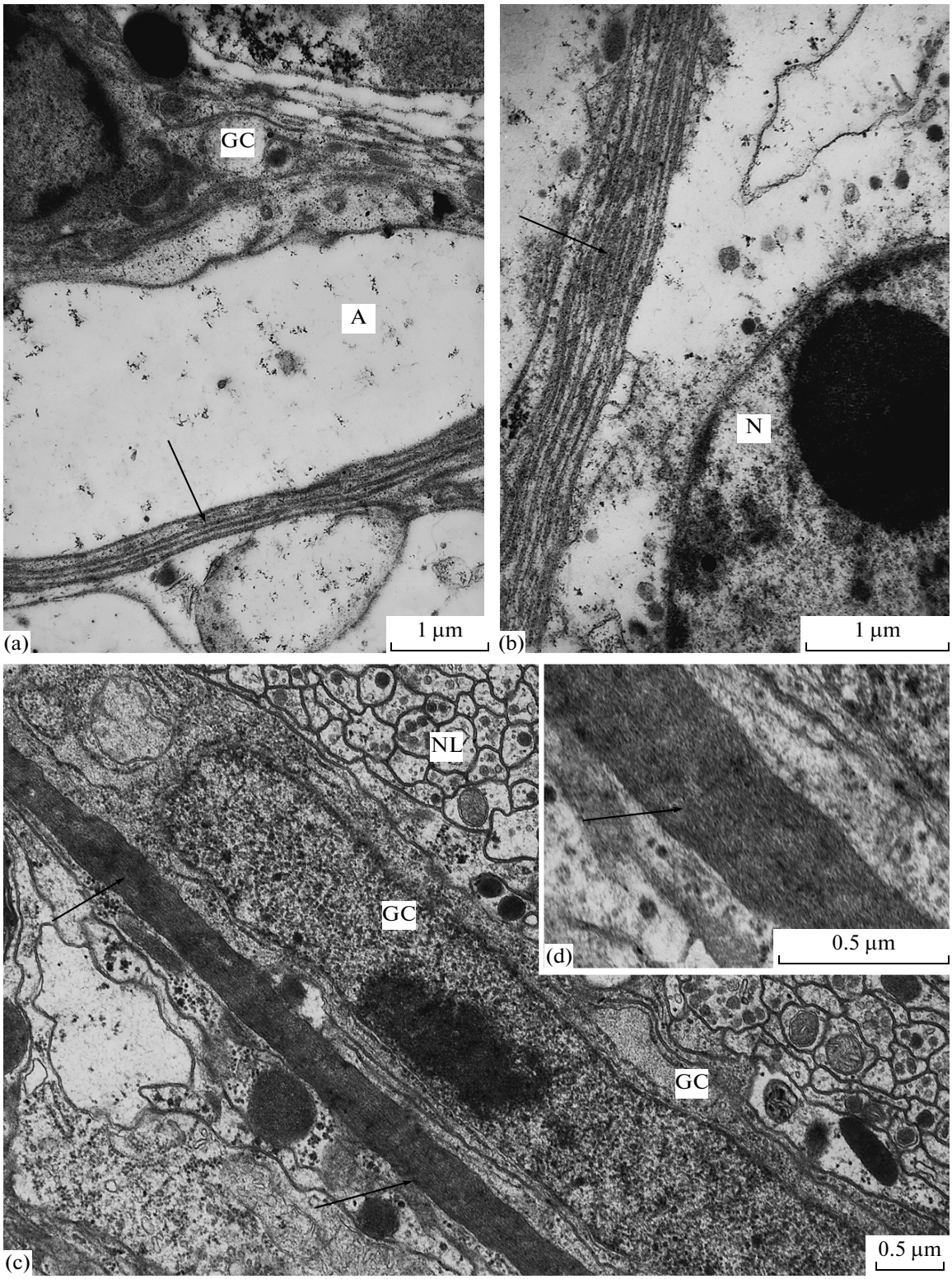

Fig. 4. Peculiarities of the structure of *Amphilina folicacea* and *Linneus gesserensis*. (a, b) Glial cell forming wrapping of gigantic serotonergic neuron in caudal ganglia of *A. foliacea*; arrows indicate myelin-like membranes of gigantic axon (a) and bodies of neuron (b); c, d—membranes (arrows) of nerve trunk and glial cells of nemertean *L. gesserensis*; e—lamellar structure of membrane (arrow).

tebrates, and vertebrates. Under the lamella, glial cells of the cortex are situated.

The fibrillar lamella was revealed in some representatives of cestodes in the process of study of ultrastructural organization of ganglia and nerve trunks. Most often the basal matrix, which limits cortical surface of ganglia or trunks, is quite loose and is not expressed as an independent layer of membrane. At the same time, trypanorhyncha and diphyllida have a significantly developed fibrillar layer, which covers lobes of the cerebroganglion (Figs. 1, 2). A clearly expressed fibrillar layer, which limits both the cerebroganglion and main nerve trunks, is present in CNS of plerocerdoid and adult *T. nodulosus* (Biserova, Salnikova, 2002), the cortical membranes of which are formed by outgrowths of epithelial cells of excretory vessels (Fig. 3d). Analysis of the ultrastructure of the nervous system of other parasitic and free-living flat worms indicated the presence of a well-developed connective tissue membrane, or capsule, which surrounds the brain, in representatives of acantacephallides *Echinorynchus gadi* and *Carynosoma strumosum* (Salnikova et al., 2007; Salnikova, Golubev, 2008) and the nemertean *L. gesserensis* (Salnikova, Golubev, 2003). Nemerteans have both an external fibrillar membrane, which unites nerve elements and simultaneously limits CNS from surrounding parenchyma, and an internal fibrillar membrane around the central neuropile. The external membrane of main nerve trunk of *L. gesserensis* has not a fibrillar nature but a lamellar nature (Fig. 4d), which is why its origin needs special study. The brain of many turbellaria has an external and internal fibrillar capsule; S. leucops has a thin external fibrillar membrane and neurons and processes of the neuropile are separated from each other by thin fibrillar lamella (Reuter, Palmberg, 1990).

Thus, we may conclude that, in the nervous system of free-living and parasitic platods, cortical and medullar glia are composed of several types of glial cells. Multilamellar cells of indefinite form with light cytoplasm are situated mainly in the main trunks and rarely in ganglia (turbellaria, cestodes, trematodes) (type 1). Fibroblast-like cells contained in cytoplasm and excreting a great amount of fibrillar components are located in ganglia (cestodes, trematodes) (type 2). Glial cells, which are connected with gigantic nerve fibers (or myelin-like) have membrane lamellas in the cytoplasm, form a myelin-like membrane, and fully wrap axons and nerves penetrating in invaginations of the axolemma, are situated in separate nerves or commissures, which contain gigantic fibers (cestodes, trematodes) (type 3). “Sandwich” cells, which are formed of multilayer wrapping of neuropiles in the form of a “sandwich"-membrane, in which cytoplasm layers alternate with layers of extracellular fibrilles and pericakyons are left outside, are revealed in the main trunks (cestodes) (type 4).

In the majority of flatworms studied, only cortical glial cells are revealed, which are situated on the surface of ganglia and trunks. In some cestodes in the main trunks, centrally located glial cells are detected. The question about the presence of medullar glia in different taxons of flatworms is poorly studied; when studied in detail these cells will probably be found. Platods also have cells in cerebroganglia, which are not present in trunks, and there are also cells of the Schwann type, which participate in wrapping of nerves and are not revealed in ganglia. Most likely, in the nervous system of the flatworm, the process of differentiation into central and peripheral glia is occurring. The position of separate types of glial cells in cerebroganglia (type 2, fibroblast-like), or in the main trunks (type 4, “sandwich"-cells), or in gigantic axons (type 3, "myelin-like”) is in our opinion connected with the functional specialization of glia and the nervous system of platods. The degree of glia development in different groups of platods is strongly connected with the degree of CNS development, peculiarities of habitation, and the behavior and size of the animal. The least specialized elements of glia of platods should be multilamellar cells, which perform a supportive and uniting function.

Comparative analysis revealed a number of similar features in the structure and function of glial cells of platods and annelids. In some parts of CNS of platods, the number of nuclei of glial cells is much greater than the number of neurons, which are absent in, for example, bulbar nerves of trypanorhyncha or regions of trunks between ganglionary widenings of amphyllinides. Glial cells of annelids belong to a type of supportive glia (Baskin, 1971a, 1b), which forms concentric membranes around ganglia and nerves, which consist of dense fibers of processes with fibrillar material, and the moor nerve trunk in the epidermis a forming net-like frame structure. As in annelids, glial cells of platods surround neurons and processes uniting them, making the internal medium and simultaneously isolating and protecting them from sharp locomotor contractions. Fibrilles of the intercellular matrix, which are excreted by glial cells, fasten in invaginations of neurons and form a thin layer in the form of fibrillar capsule. Such structural mechanisms are found along with annelids and cestodes in Acanthocephalia *C. strumosum* (Salnikova, Goubev, 2008), the proboscis ganglion of which is also subject to significant mechanical deformations in the work of the proboscis.

Multiprocess form of cells, concentric wrapping of neurons and processes, the presence of glycogen and smooth reticulum in the cytoplasm, formation of numerous specialized contacts among layers of membrane, and myelinization of gigantic axons belong to general features of the ultrastructural organization of glial cells of annelids (Gunther, 1976; Roots, Lane, 1983; Zoran et al., 1988; Roots et al., 1991; Riehl, Shlue, 1998) and platods. The ultrastructure of cells of cortical and medullar glia has significant differences both in cestodes and annelids.

One of the most interesting and important facts is detection of gigantic axons in some cestodes and trematods (Sukhdeo, Sukhdeo, 1990; Biserova, 2004, 2008; Biserova, 2000b, 2008a, b). As in annelids and crustaceans, gigantic axons of cestodes are isolated by specialized cells, which have in studied cases (*C. minuta*) s100b-positive immunoreactivity. These cells have complex interactions with gigantic axons of the type of Schwann's glia and form myelin-like membranes. Myelin is detected not in all vertebrates, for example it is absent in lampreys and myxines (Bullock et al., 1984). The opinion that myelin is absent in all invertebrates is still encountered (Kiernan, 2006). In the nervous system, two basal mechanisms of the growth of speed of conducting an electrical pulse have developed in evolution: (1) axon gigantism; (2) wrapping of axons in spiral or concentrically twisted multilayer membrane, i.e., isolating the plasmatic membrane or myelin membrane (Hartline, Colman, 2007). In flatworms we revealed both these phenomena, both concentrically twisted membranes and gigantic axons. The conducted investigations prove that in flat worms both directions of increasing the efficiency of conducting electrical pulses are developed in a parallel way. In all taxons of platods, glial cells form more or less developed multilayer wrappings of axons and bodies of neurons, which leads to less loss of the conducting signal. Gigantic nerve fibers are encountered in the nervous system of cestodes from the order Trypanorhyncha, which possess morphologically complicated and functionally differentiated adhesive apparatus and are capable of active translational motion and abrupt ejection of the long armed proboscis. Wrapping of gigantic axons of cestodes and trematodes is conducted by "myelin-like" glial cells which have anti-s100b-positive immunoreactivity in the studied cases (*C. minuta*). Thus, in the nervous system of platods, the same structures appear as in other animals in similar functional situations: realization of quick conduction of the pulse occurs by way of a decrease in the axon diameter and its multilayer isolation by glial cells. The obtained data indicate that all basal mechanisms of functioning of the nervous system and neuroglial interactions are present in platods.

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REFERENCES

Baskin, D.G., Fine Structure, Functional Organization and Supportive Role of Neuroglia in Nereis, *Tissue Cell*, 1971a, vol. 3, no. 4, pp. 579–587.

Baskin, D.G., The Fine Structure of Neuroglia in the Central Nervous System of Nereid Polychaetes, *Cell Tissue Res.*, 1971b, vol. 119, no. 3, pp. 295–308.

Bedini, C. and Lanfranchi, A., Ultrastructural Study of the Brain of a Typhloplanid Flatworm, *Acta Zool. (Stock)*, 1998, vol. 79, pp. 243–249.

Biserova, N., Do Glia Cells Exist in Parasitic Flatworms?, *Europ. J. Neurosci.*, 2000b, vol. 12, no. 11, p. 354.

Biserova, N., Dudicheva, V., Terenina, N., et al. The Nervous System of *Amphilina foliacea* (Platyhelminthes, Amphilinidea). An Immunocytochemical, Ultrastructural and Spectrofluorometrical Study, *Parasitology*, 2000, vol. 121, pp. 441–453.

Biserova, N., The Ultrastructure of Glia-Like Cells in Lateral Nerve Cords of Adult *Amphilina foliacea* (Amphilinida), *Acta Biol. Hung*, 2000a, vol. 51, pp. 439–442.

Biserova, N.M. and Korneva, Zh.V., Characteristics of Ontogenetic Development of the Nervous System of Cestodes and Amphilinids, *Zool. Bespozv.*, 2006, vol. 3, no. 2, pp. 157–184.

Biserova, N.M. and Sal'nikova, M.M., Ultrathin Structure of the Main Lateral Nerve Cords and Accompanying Elements of Triaenophorus Nodulosus *Cestoda pseudophyllidea*) *Tsitologiya*, 2002, vol. 44, no. 7, pp. 610–619.

Biserova, N.M., Do Glial Cells Exist in the Nervous System of Parasitic and Free-Living Flatworms? An Ultrastructural and Immunocytochemical Investigation, *Acta Biol. Hung.*, 2008a, vol. 60, suppl. 30, pp. 208–219.

Biserova, N.M., Nervous System of Cestodes and Amphilinids, *Extended Abstract of Doctoral (Biol.) Dissertation*, Moscow: Mosk. Gos. Univ., 2004.

Biserova, N.M., Stroenie nervnoi sistemy skoleksa Triaenorhorus nodulosus (Sestoda, Rseudorhulludea), *Parazitologiya*, 1997, vol. 31, no. 3, pp. 249–260.

Biserova, N.M., Tonkoe stroenie glial'nykh kletok v nervnoi sisteme Grillotia erinaseus, (Fine Structure of Glial Cells in the Central Nervous System of the Tapeworm Grillotia erinaceus (Cestoda: Trypanorhyncha)) *Tsitologiya*, 2008, vol. 50, no. 6, pp. 498–508.

Biserova, N.M., Ultrastructure of Glial Cells in the Nervous System of *Grillotia erinaceus*, *Cell Tissue Biol.*, 2008b, vol. 2, no. 3, pp. 253–264.

Bockerman, I., Reuter, M., and Timoshkin, O., Ultrastructural Study of the Central Nervous System of Endemic Geocentrophora (Prorhynchida, Platyhelmintes) from Lake Baikal, *Acta Zool. (Stockh.)*, 1994, vol. 75, pp. 47–55.

Bullock, T.H., Moore, J.K., and Fields, R.D., Evolution of Myelin Sheaths: Both Lamprey and Hagfish Lack Myelin, *Neurosci. Let.*, 1984, vol. 48, pp. 145–148.

Coles, J.A. and Abbott, N.J., Signalling from Neurons to Glial Cells in Invertebrates, *Trends Neurosci.*, 1996, vol. 19, no. Iss. 8, pp. 358–362.

Cooper, M.S., Intercellular Signaling in Neuronal-Glial Networks, *Biosystems*, 1995, vol. 34, issues 1-3, pp. 65–85.

Ferrero, E., Lanfranchi, A., and Bedini, C., An Ultrastructural Account of Otoplanid Turbellaria Neuroanatomy. I. The Cerebral Ganglion and the Peripheral Nerve Net, *Acta Zool. (Stockh.)*, 1985, vol. 66, pp. 63–74.

Golubev, A.I., *Elektronnaya mikroskopiya nervnoi sistemy chervei* (Electron Microscopy of the Worm Nervous System), Kazan: Izd. Kazan. Univ., 1982.

Golubev, A.I., Structure and Function of Glia of the Central Nervous System of the Leech, in *Voprosy evolyutsionnoi morfologii i biogeografii* (Problems of Evolutionary Mor-

phology and Biogeography), Kazan: Izd. Kazan. Univ., 1970, pp. 23–39.

Gunther, J., Impulse Conduction in the Myelinated Giant Fibers of the Earthworm. Structure and Function of the Dorsal Nodes in the Median Giant Fiber, *J. Comp. Neurol.*, 1976, vol. 168, pp. 505–532.

Hartline, D.K. and Colman, D.R., Rapid Conduction and the Evolution Review of Giant Axons and Myelinated Fibers, *Current Biology*, 2007, vol. 17, pp. 29–35.

Hulsebosch, C.E. and Bittner, G.D., Morphology and Number of Neurons in Two Species of Polychaetes, *J. Comp. Neurol.*, 1981, vol. 198, no. 1, pp. 65–75.

Kiernan, J., Anatomical Foundations of Neuroscience, Web Page 11/12/06 (http://publish.uwo.ca/ ~jkiernan/gliacell.htm).

Mantyla, K., Reuter, M., Halton, D., et al. The Nervous System of *Procerodes littoralis* (Maricola, Tricladida). An Ultrastructural and Immunoelectron Microscopical Study, *Acta Zool.*, 1998, vol. 79, no. 1, pp. 1–8.

Reuter, M and Palmberg, I., Synaptic and Nonsynaptic Release in *Stenostomum leucops*, in *The Early Brain*, Gustafsson, M. and Reuter, M., Eds., Abo: Acad. Press. Abo, 1990, pp. 121–136.

Riehl, B. and Schlue, W., Morphological Organization of Neuropile Glial Cells in the Central Nervous System of the Medicinal Leech (*Hirudo medicinalis*), *Tissue Cell*, 1998, vol. 30, no. 2, pp. 177–186.

Rohde, K. and Webb, R., Ultrastructure of Neuroglia in the Peripheral Nervous System of Temnocephala sp. (Turbellaria, Temnpcephalida), Zool. Anz., 1986, vol. 216, pp. 53–57.

Rohde, K., Nerve Sheath in *Multicotyle purvisi* Dawes, *Naturwissenschaften*, 1970, vol. 57, pp. 502–503.

Rohde, K., Untersuchungen an *Multicotyle purvisi* Daves, 1941 (Trematoda: Aspidogastrea). III. Licht-Und Elektronmikroskopischer Bau des Nervensystems, *Zool. Jahrb. Anat.*, 1971, vol. 88, pp. 320–363.

Roots, B.I. and Lane, N.J., Myelinating Glia of Earthworm Giant Axons: Thermally Induced Intramembranous Changes, *Tissue Cell*, 1983, vol. 15, pp. 695–709.

Roots, B.I., Cardone, B., and Pereyra, P., *Isolation and Characterization of the Myelin-Like Membranes Ensheathing Giant Axons in the Earthworm Nerve Cord Ann*, New York: Acad. Sci, 1991, vol. 633, pp. 559–561.

Sal'nikova, M.M. and Golubev, A.I., Ultrastructure of the Cerebral Ganglion of the Thorny-Headed Worm *Corynosoma strumosum*, in *Mater. IV Vseros. S"ezda Parazitol. Obshch. pri Ros. Akad. Nauk "Parazitologiya v XXI veke. Problemy, metody, resheniya"* (Proc. IV All-Russia Congress of the Parasitological Society, Russian Academy of Science "Parasitology in XXI Century: Problems, Methods, and Solutions), St. Petersburg: Lema, 2008, vol. 2, pp. 197–201.

Sal'nikova, M.M., Golubev, A.I., and Biserova, N.M., Fine Structure of the Cerebral Ganglion of the Horm-Headed Worm *Echinorynchus gadi* (Acantocephala) and Detection of GABA-ergic Neurons in It, in *Mater. IV Mezhdunar. Shk. po Teoretich. i Morsk. Parazitologii* (Proc. IV Int. School on Theoretical and Marine Parasitology), Kaliningrad: Izd. AtlantNIRO, 2007, pp. 189–191.

Salnikova, M. and Golubev, A., Immunohistochemical Study of Specific Neurofilaments and Specific Glial Fibrils in the Nemertini *Lineus gesserensis*, in *Simpler Nervous Systems: 7th East European Conf. of the ISIN. Kaliningrad, September 12–16, 2003*, 2003, p. 96.

Sotnikov, O.S., Boguta, K.K., Golubev, A.I., and Minichev, Yu.S., *Mekhanizmy strukturnoi plastichnosti neironov i filogenez nervnoi sistemy* (Mechanisms of the Structural Plasticity of Neurons and the Phylogenesis of the Nervous System), St. Petersburg: Nauka, 1994.

Sukhdeo, S.C. and Sukhdeo, M.V., Mesenchyme Cells in *Fasciola hepatica* (Platyhelminthes): Primitive Glia?, *Tissue Cell*, 1994, vol. 26, pp. 123–131.

Sukhdeo, S.C. and Sukhdeo, M.V., Ontogenetic Development of the Brain of the Platyhelminth *Fasciola hepatica*, *Tissue Cell*, 1990, vol. 22, no. 1, pp. 39–50.

Zoran, M.J., Drewes, Ch.D., Fourtner, Ch.R., and Siegel, A.J., The Lateral Giant Fibers of the Tubificid Worm, *Branchiura sowerbyi*: Structural and Functional Asymmetry in a Paired Interneuronal System, *J. Comp. Neurol.*, 1988, vol. 275, pp. 76–86.