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ORIGINAL ARTICLE

Fine structure of the central brain in the octopod *Eledone cirrhosa* (Lamarck, 1798) (Mollusca–Octopoda)

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Abstract

This study aims to investigate the fine structure of the different cell types in the central brain of *Eledone cirrhosa*; the organelles in the neurons and the glial cells; the glial hemolymph–brain barrier; the neuro-secretions and the relationships between glial and nerve cells. The brain is surrounded by a non-cellular neurilemma followed by a single layer of perilemmal cells. Ependymal cells, highly prismatic glial cells, astrocytes, oligodendrocytes and epithelial processes were observed. The perikarya of the neurons are filled with slightly oval nuclei with heterochromatin, a strongly tortuous ER, numerous mitochondria and Golgi apparatus with two types of vesicles. In the cellular cortex, glial cells are much less numerous than the neurons and they are located preferably at the border between perikarya and neuropil. Furthermore, they send many branching shoots between the surrounding neuron perikarya and the axons. The glial cytoplasmic matrix appears more electrodense than that of the neurons. Only few ribosomes are attached to the membranes of the ER; the vast majorities are free. In the perikarya of the glial cells, mitochondria, multi-vesicular bodies, various vacuoles and vesicles are present. The essential elements of the hemolymph–brain barrier are the endothelial cells with their tight junctions. The cytoplasm contains various vesicles and mitochondria. However, two other cell types are present, the pericytes and the astrocytes, which are of great importance for the function of the hemolymph–brain barrier. The cell–cell interactions between endothelial cells, pericytes and astrocytes are as close as no other cells.

Keywords Central brain · Neurons · Glial cells · The glial hemolymph–brain barrier · Neuro-secretions · Organelles

Introduction

The fine structure of the brain in cephalopods is not sufficiently covered in the available literature. The descriptive anatomy of the nervous system of *Octopus vulgaris* is first investigated (Young 1971). Previously, the central nervous systems of *Nautilus* and *Loligo* are described (Young 1965, 1974, 1976, 1977, 1979, 1988). Authors have tried to discuss certain aspects of the nervous system in cephalopods, but the description of the different cell types in the brain of the octopus *Eledone cirrhosa* is still missing. A series of previous studies showed what did the authors investigated in the nervous system in cephalopods. The fine structure of synaptic components of *Octopus* brain (Gray and Young 1964), the anatomy of the subpedunculate lobe in *Octopus* brain (Froesch 1974; Shomrat et al. 2008), the statocyst–oculomotor system of *Octopus vulgaris* (Budelmann and Young 1984), neuropeptidergic control of the optic gland of *Octopus vulgaris* (Di Cosmo and Di Cristo 1998), The *Octopus* genome (Albertin et al. 2015), the fine structure of the vertical lobe of *Octopus* brain (Gray 1970; Rechenbach and Wolburg 2005), electrophysiology and bio-physics of the squid giant axon (Abbott et al. 1985; Adelman and Gilbert 1990), cephalopod neurobiology (Abbott and Maddock 1995; Nave and Trapp 2008). The interest of neurobiologists has increasingly focused recently on the functional relationships among the neurons and the surrounding glial cells (Simons and Trajkovic 2006; Verkhratsky et al. 2010). Distinctly, the two elements are coupled in the brain not only in the course of electrical processes (Nakajima et al. 1965), but also in various metabolic reactions. This relationship had been demonstrated ribonucleic acid metabolism in glial cells (Abbott et al. 1981; Sun and Tsai 2011) and for various oxidative processes (Kettenmann

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and Verkhratsky 2008; Verkhratsky et al. 2010). The functional relationship among neurons and glial cells manifested itself also in pathological processes in which after an initial derailment of the glial function, the activity of the nerve cells is secondarily affected (Simons and Trajkovic 2006; Kettenmann et al. 2010). Such relationships have been studied almost exclusively in annelids and ticks (Nicholls and Kuffler 1964; Binnington and Lanem 1980). Yet little is known about the neuron-glial relationship in insects whose brain has much simpler construction. The exceptions are the light microscopic studies by (Lane and Treherne 1972) on the perineural junction complexes and the sites of uptake of microperoxidase and lanthanum in the cockroach central nervous system. This light microscopic study is partly confirmed electron microscopically on the frog *Rana esculenta* (Akert and Sandri 1976). The relationship among neurons and glial cells in insect nervous system on electron microscopic studies is partially described (Richard et al. 1984; Nixon and Young 2003).

According to Young (1971, 1988), the brain in *Octopus vulgaris* is divided into two regions: the supra- and the sub-esophageal masses which are joined laterally by basal and the dorsal magnocellular lobes. This alignment suggests that the embryonic brain is developed from two partially circum-esophageal cords which included the middle and posterior sub-esophageal masses. The cords are fused dorsally with a third cord called the supra-esophageal mass, which is confirmed by Holland et al. (2013). Besides, the supra- and sub-esophageal masses were further connected by the cerebro-brachial connective that run between the anterior regions. The latter connection involves the anterior sub-esophageal mass and the superior buccal lobes. The brain in cephalopods is assembled through a series of ganglia of molluscan origin to form lobes which are fused together into masses. These masses are connected peripherally by many nerve trunks regulating the arms, viscera and other part of the animal's body (Boycott 1961; Haszprunar and Wanninger 2012; Shigeno and Ragsdale 2015). These authors conclude a vertebrate-like neural system is present in cephalopods. The neurosecretory cells in *Octopus* are analogous to the hypothalamus (Butler 2008), and cephalopods have higher sensory centers analogous to the thalamus and the peduncle lobe is analogous of the cerebellum (Hochner and Shomrat 2014; Holland et al. 2013). The nervous control on reproduction in *Octopus vulgaris* is studied (Fernández-Rueda and García-Flórez 2007; Dunlop and King 2008). The olfactory lobe receives distant chemical stimuli and appears as an integrative center. It contains a variety of neuropeptides which are involved in controlling the onset of sexual maturation of *Octopus*, via the optic gland hormone (Young 1988; Di Cristo et al. 2009). In crustacean, insects and cephalopods, glial cells become an important part of both sensory organs and body functions where they assume multiple roles and have a much higher significance (Plan 1987; Kettenmann et al. 2010; Farris 2013; Waddell 2013).

The hemolymph–brain barrier protects the brain from pathogens, toxins and messengers which circulate in the hemolymph (Stephens and Young 1969; Abbott et al. 1981). It represents a highly selective filter, through which the nutrients required by the brain are supplied and the resulting metabolic products are removed (Crone 1986). The supply and disposal are ensured by a number of special transport processes (Budelmann and Young 1997; Gotow and Hashimoto 1984). A disruption or damage to the hemolymph–brain barrier is considered as a very serious complication. Unlike other organs in the body, the brain has extremely low nutrient or oxygen reserves (Lane et al. 1981). Also, the nerve cells are unable to meet the energy needed anaerobically, that is, without elemental oxygen (Oldendorf et al. 1977). Fluctuations in the hemolymph pH must not pass on to the brain. Fluctuations in the potassium concentration would alter the membrane potential of nerve cells. The neurotransmitters circulating in the hemolymph must not enter the brain, since they would significantly disturb the flow of information in the existing synapses there (Reese and Karnovsky 1967). The brain, as a centrally controlling organ, must also be protected from the effects of foreign substances, such as pollutants in the sea water. The extensive opacity of the hemolymph–brain barrier against pollutants, pathogens and antibodies makes it as an immunological barrier. Due to the high energy requirement of the brain, compared to other organs, disproportionately large amounts of metabolic degradation products dissipate via the hemolymph–brain barrier (Abbott et al. 1981).

This study aims to observe the fine structure of the different cell types present in the central brain of the octopus *Eledone cirrhosa*, the organelles in neurons and glial cells and the glial hemolymph–brain barrier, neuro-secretions and the structural conditions of the relationships of glial and nerve cells, thus creating a basis for future study.

Materials and Methods

The octopus *Eledone cirrhosa* (Lamarck, 1798) is a benthic, neritic cephalopod, very sedentary and lonely; it breaks its individualism in reproduction time (Jereb and Roper 2005). It inhabits rocky backgrounds with abundance of cracks and stones in marine ecosystems. Adults were collected by Pots, craft gear and decoys during summer 2017 from the Mediterranean Sea of Alexandria, Egypt. Samples are transported in aquaria to the laboratory and dissected after carbon dioxide anesthesia. A tissue blister containing the brain is removed. Pre-fixation is done in phosphate-buffered glutaraldehyde (6%, pH 7.2) at 4 ℃ for 20–24 h. Post-fixation is done in osmium tetroxide solution (1%, isotonic, pH 7.2,

Veronal acetate buffer) at 4 ℃ for 3 h. They are dehydrated in alcohol series; Embedded in Epon 812 with interposition of propylene oxide. Polymerization is done in a heat cabinet per 24 h at 35–45 ℃ for 36–60 h. The sections obtained with the "Ultrotome" (LKB) are post-contrasted with uranyl acetate and examined in "Elmiskop I" (Siemens). Ethical clearance for this study is obtained from Alexandria University ethics committee.

Results

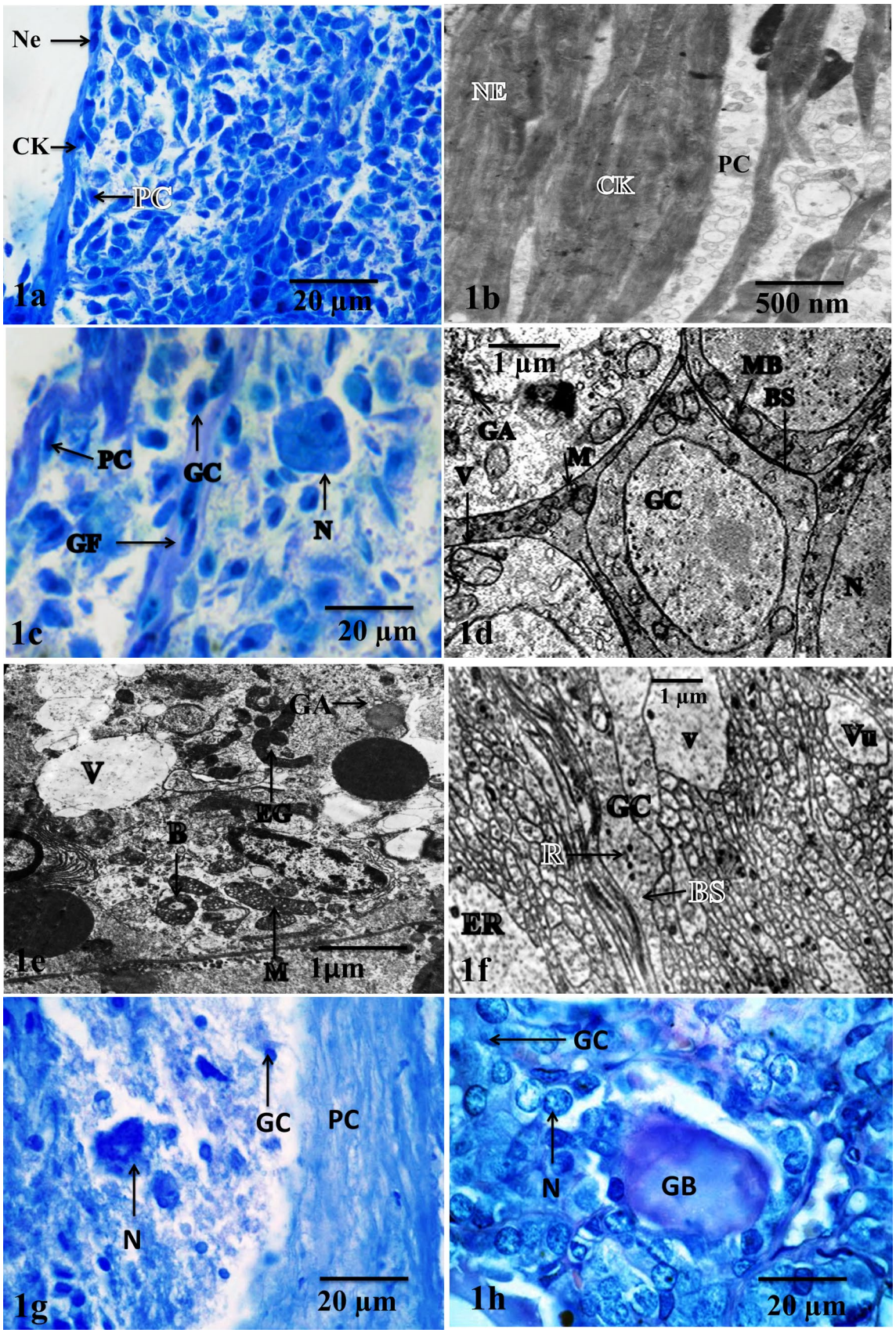
In the presented study, the brain of octoped *Eledone cirrhosa* is divided morphologically into three parts; the central brain which surrounds the esophagus and is situated inside the cartilaginous capsule. The other two parts of the nervous system, the optic lobe and the nervous system of the arms; they are situated outside the brain capsule. A pair of statocysts are embedded inside the cartilage at the lower part of the brain capsule. The first layer of the brain, the neurilemma is homogeneous connective capsule and cell-free with a thickness of 0.8–2.9 µm (Figs. 1a, b). This capsule shows striated bundles of fibrils reminiscent of collagen. Immediately following the neurilemma is a single layer of perineurium of perilemmal cells. These cells are spread flat or cubic with 1–7 µm thickness (Fig. 1c). Their nuclei are mostly oval and small. The cytoplasm is replete with large mitochondria, vesicles of various sizes, vacuoles, dense bodies and multi-vesicular bodies. The Golgi apparatus is very different in appearance from that of the neurons (Fig. 1d). The aggregates of the flat cisterns are much less extensive and are surrounded by a little number of larger bubbles. The bubbles show a little electrodense content, which often contain a central brightening (Fig. 1e). Rarely, centrioles could be recognized. Often in the cytoplasm of perilemmal cells, accumulations of very electrodense granules appear (Fig. 1f). They are often grouped together to form large, rosette-shaped complexes. The size and shape of these granules correspond to those occurred in the extra-cerebral cells may be fat or glycogen. Immediately following the perineurium is the cerebral cortex in which the perikaryon layer of the neurons is delineated by the perilemma of one or more layers of flat glial cells (Fig. 1g). The cerebral cortex comprises many cell layers and is composed of three elements, namely the neurons, the glial cells and the glial hemolymph–brain barrier cells (Fig. 1d–j). The perikarya of the neurons are irregularly polygonal and are joined together in an epithelial cell structure. The remaining gaps between the neurons are filled by glial pedestals and sparse giant fiber bundles. In any case no interstitial glial lacunae are visible. Each neural perikaryon of the cerebral cortex gives off only a single branch, which runs directly to the central masses of the giant fibers and into a dendrite tuft. The brain cerebral cells are therefore unipolar, and the perikarya are separated spatially from the underlying neural layer (Fig. 1j).

Neurons

The perikaryon has a diameter of 5–15 µm, which is largely filled with a round or slightly oval nucleus (Figs. 2a, b). The latter showed in contrast to those of glial cells where no indentations or lobes are present. The interior of the nucleus composed of a fine-granulated network, in which electrodense complexes are embedded. The complexes are heterochromatin and thus correspond to the nucleoli. The core matrix is uniform and finely granulated. The nucleus is surrounded by a typically built nuclear membrane, a perinuclear cistern that communicated with the cavities of the endoplasmic reticulum (Fig. 2c). Numerous pores with an outer diameter of app. 0.1 µm closed by a typical barrier are observed (Fig. 2d). The narrow cytoplasmic roof shows a very sparse and strongly tortuous endoplasmic reticulum, which is almost completely obscured by the numerous, partly free and partly attached ribosomes (Fig. 2e). The formation of ergostoplasmic complexes never occurs, which apparently explain the lack of a light-microscopically visible Nissl substance and the presence of diffused basophilia in the cytoplasm. Furthermore, numerous mitochondria and typical Golgi apparatus are observed, which consist of a dense assembly of mostly three flattened sacks and subsequent small bubbles (Fig. 2f). It is remarkable that centrioles could often be found in the nerve cells. In about half of the neurons struck in a thin section, different electrodense bodies (vesicles) are recognized, which have a diameter of 0.3–1.5 µm and are completely surrounded by unit membranes. According to their structure, two types could be distinguished, which are not different in size or localization. The first type (Fig. 2g) contains unit membranes vesicles embedded in a moderately electrodense and granular matrix, which are more or less regularly parallel or concentric. The spatial part of the membrane complexes with respect to the matrix change from agranulated to granulated. The second type (Fig. 2h) contains densely packed vesicles, in different swirling and tangling thick unit membranes with irregularly limited, roundish, less electron-dense spaces. Both types could occur side by side. In the control of a large number of cut neurons, there are vesicles of different sizes. It was very scarcely to find transitional forms of the two types in one vesicle, so that a close relationship can be assumed.

Glial cells

It was markedly noticed that glial cells are much less numerous than the neurons. They are distributed in the cellular cortex, but preferably located at the border between perikarya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◂**Fig. 1** **a** Semithin section showing the neurilemma of the brain of homogeneous connective capsule and cell-free. **b** TEM section the capsule showing striated bundles of fibrils reminiscent of collagen. **c** Semithin section showing a single layer of perineurium of perilemmal cells. These cells are spread flat or cubic. **d** TEM section showing the cytoplasm of a perilemmal cell was replete with large mitochondria, vesicles of various sizes, vacuoles, dense bodies and multi-vesicular bodies. The Golgi apparatus was very different in appearance from that of the neurons. **e** TEM section showing the aggregates of the flat cisterns are surrounded by a smaller number of larger bubbles. These bubbles show a little electron-dense content. **f** TEM section showing accumulations of electron-dense granules in the cytoplasm of perilemmal cells. **g** Semithin sections showing the perikaryon layer of the neurons in the cerebral cortex is delineated by the perilemma of one or more layers of flat glial cells. **h**, **i** Semithin sections showing the cerebral cortex was composed of three elements, namely the neurons, the glial cells and the glial hemolymph–brain barrier. **j** TEM section showing the neuron perikarya of the cerebral cortex give off only a single branch, which ran directly to the central masses of the giant fibers and neurons are unipolar with the perikarya separated spatially from the underlying neural layer. Neurilemma Ne; Connective Capsule CK; Striated Bundles of Fibrils SB; Perilemmal Cell PC; Mitochondrion M; Vesicle V; Multi-vesicular Body MB; Golgi Apparatus GA; Bubbles B; Electron-dense Granules EG; Perikaryon Layer PL; Neuron N; Cerebral Cortex CC; Glial Cell GC; Glial Hemolymph–brain Barrier GB; Giant Fibers GF

and neuropil. The cell bodies of glial cells appear usually similar to neurons. In contrast, the cores show the most varied forms. They could be more or less rounded or elongated and often have a strong lapping and numerous invaginations (Fig. 3a). Their forms are quite similar to the glial cores of the optic nerve of *Sepia officinalis* (Abbott et al. 1981) and are denser than the neuron nuclei. The cytoplasmic courtyard shows a widely varying width; it sent many branching shoots between the surrounding neuron perikarya and axons (Fig. 3b). The glial cytoplasmic matrix appears more electronically dense than that of the neurons, which is a welcome aid to identification. On strong enlargement, this is due to a very dense accumulation of ribosomes and the compaction of the matrix (Fig. 3c). Only a few ribosomes are attached to the membranes of the endoplasmic reticulum; the vast majority is free. Many free ribosomes are also found in the thin foothills of glial perikarya (Fig. 3d). In the perikaryon of the glial cells mitochondria, multi-vesicular bodies, various vacuoles and vesicles could be observed (Fig. 3e). Sometimes, centrioles could be noticed. Another striking difference to the neurons and perilemma cells lie in the formation of the Golgi apparatus. It consists only of a few, strongly tortuous and closely spaced canals of the smooth surfaced endoplasmic reticulum. There is small number of bubbles around (Fig. 3f). Gliosomes—each 0.5–1.2 µm diameter—and are surrounded by thick unit membranes and their interior consists of an electrodense granular matrix. In this basic substance, cubic complexes are built in, which consist of a regular array of strictly parallel dark lines. The processes of the glial cells often decayed and form a system of optically empty lacunae. For this reason, in osmium fixation, pre-fixing the tissue with glutaraldehyde was necessary.

The diameter of each glial cell varies within a wide limit; it always adapts to the local conditions and could be measured without cell membrane about 1 µm. Glial cells ramifications are somewhat exaggerated in relation to the other dimensions, since the present overview magnifications would not have permitted a resolution of the cell membranes. There are two or more glial ramifications that are sandwiched between two neuronal perikarya at different sites. Sometimes, they belong to the same cell, but other times the connection is unclear. The brain of the octopod *Eledone cirrhosa* contains multi-rows of highly prismatic glial cells which, in addition to astrocytes, oligodendrocytes, and epithelial processes, other cell type with fine structural features of ependymal cells are observed. Both glial and ependymal cells are highly granulated than different types of nerve cells.

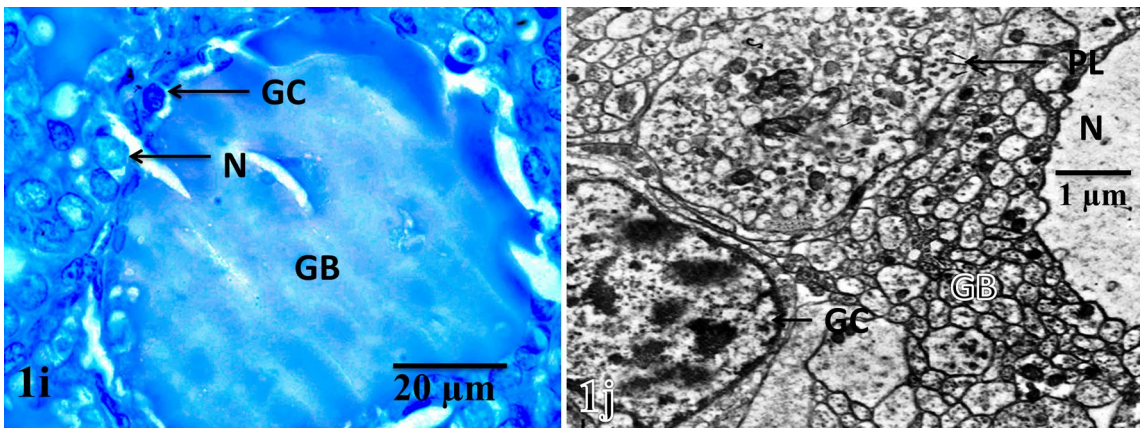
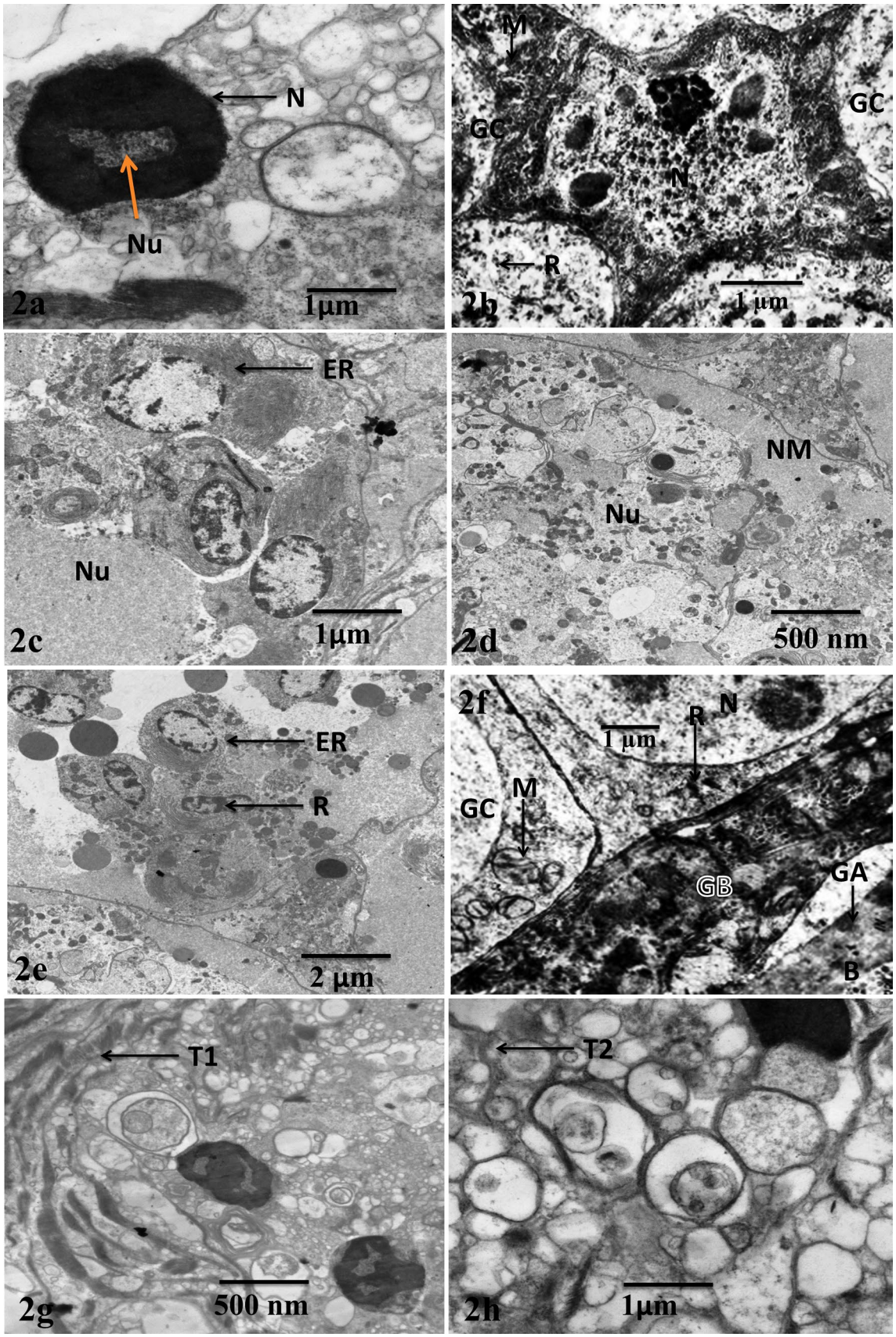


Fig. 1 (continued)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◂**Fig. 2** **a**, **b** TEM section showing the perikaryon of the neuron is largely filled with a round or slightly oval nucleus. **c** TEM section showing the nucleus is surrounded by a typically built nuclear membrane, a perinuclear cistern that communicated with the cavities of the endoplasmic reticulum. **d** TEM section showing numerous pores are closed by a typical barrier. **e** TEM section showing the narrow cytoplasmic roof showed a very sparse and strongly tortuous endoplasmic reticulum, which is almost completely obscured by the numerous, partly free and partly attached ribosomes. **f** TEM section showing numerous mitochondria and typical Golgi apparatuses are observed, which consisted of a dense assembly of mostly three flattened sacks and subsequent small bubbles. **g**, **h** TEM sections showing Type I and type II of different electron-dense bodies (vesicles) which are surrounded by unit membranes. Perikaryon P; Neuron N; Nucleus Nu; Heterochromatin H; Nuclear Membrane NM; Endoplasmic Reticulum ER; Ribosomes R; Golgi Apparatuses GA; Bubbles B; Type 1 Electron-dense Bodies T1; Type 2 Electron-dense Bodies T2; Vesicle V

From the cisterns of the endoplasmic reticulum of glial cells, bright secretions leak through and penetrate the cytoplasm. Bright secretory matter provided the raw material for dense secretory granules that are formed in the Golgi apparatus. The granules occur in different variants are apically attached. Isolated cells are so densely filled with secretory vacuoles, and the cytoplasm is reduced to narrow and dense struts. The nucleus seems as pyknotic. It was observed that the secretions vacuoles contain very little flaky material. In the glial cells which are located ventrally, secretory vacuoles coalesce into large intracellular cavities into which microvilli occasionally protrude. Finally, a kind of apocrine secretion is observed. Some glial cells have nearly homogeneous protrusions that reach to the neurons. Organelle-free cytoplasmic areas are observed in some neurons. The glial blood–brain barrier possesses different width endothelia. The basement membrane is widened in many places and enclosed small and bright districts. Often there is an elevated perivascular space; it is stuffed with disordered filaments or collagen fibrils and occasionally contains adventitial ependymal cells.

Glial hemolymph–brain barrier

The essential element of the hemolymph–brain barrier is the endothelial cells with their tight junctions. The cytoplasm of these cells is thin and only expands at the sites where one of the rare cell nuclei are embedded (Fig. 1d–j). The cytoplasm contains various vesicles and mitochondria. However, two other cell types, the pericytes and the astrocytes, are of great importance for the function and development of the hemolymph–brain barrier. The cell–cell interactions among endothelial cells, pericytes and astrocytes are as close as no other cells. They make up the hemolymph–brain barrier, for review see (Abbott et al. 1981; Wentzell et al. 1987). The endothelium had fenestrations of about 50 nm in diameter and intercellular gaps of 0.1 to 1 µm in width for the exchange of water and dissolved or suspended substances between the hemolymph and the extracellular fluid of the surrounding tissue. In contrast, there are no fenestrations and no intracellular gaps between the endothelial cells in the brain. The number of pinocytotic vesicles that allow endocytosis of solutes is very low in the endothelium of the brain. The number of mitochondria is about 5 to 10 times higher than in the other cells. This is an indication of a high energy requirement of endothelia including active transport processes and high metabolic activity, for review see (Abbott et al. 1981; Gray 1970).

Adhesion junction in the hemolymph–brain barrier was carried out by the pericytes which represents an important component of the hemolymph–brain barrier. They are characterized by their ability to contract and their phagocytotic activity. About 1/4 of the outer endothelial surface of the hemolymph–brain barrier is covered by the relatively small, oval-shaped pericytes, and every second to fourth endothelial cell is associated with a pericyte. Preferably, the pericytes are located at the junction of two endothelial cells. The degree of endothelial pericyte coverage correlated directly with the tightness of the tight junctions. A special feature is evidently observed in the pericytes located on the cerebral capillaries; they function there as macrophages. Thus, there are many lysosomes in the cytoplasm, and in vivo they showed the ability to take up soluble or materials from the surrounding, for review see (Abbott et al. 1981; Gray 1970)

Astrocytes are star-branched, compared to the pericytes significantly larger cells from the macroglia family. They are in direct interaction with the endothelia. Astrocytes induce the function of the hemolymph–brain barrier in the endothelium. They emit a number of messengers that can modulate the permeability of the endothelium in the second to minute range. However, the main task of astrocytes is assumed to supply the neurons with nutrients and to regulate extracellular ion concentrations, for review see (Büssow 1980; Gotow and Hashimoto 1984; Rechenbach and Wolburg 2005; Hamilton and Attwell 2010).

Discussion

The general organization of the brain of the octopod *Eledone cirrhosa* corresponds to the cerebral blueprint for cephalopods, which has already been extensively researched on the basis of light microscopy (Young 1971; Nixon and Young 2003). This study shows that the brain is completely separated from the surrounding tissues of the head by a cell-free membrane (neurilemma) and by the layer of perilemmal cells. The same observation is noticed in (Akert and Sandri 1976). There is no hemolymph system between the brain and

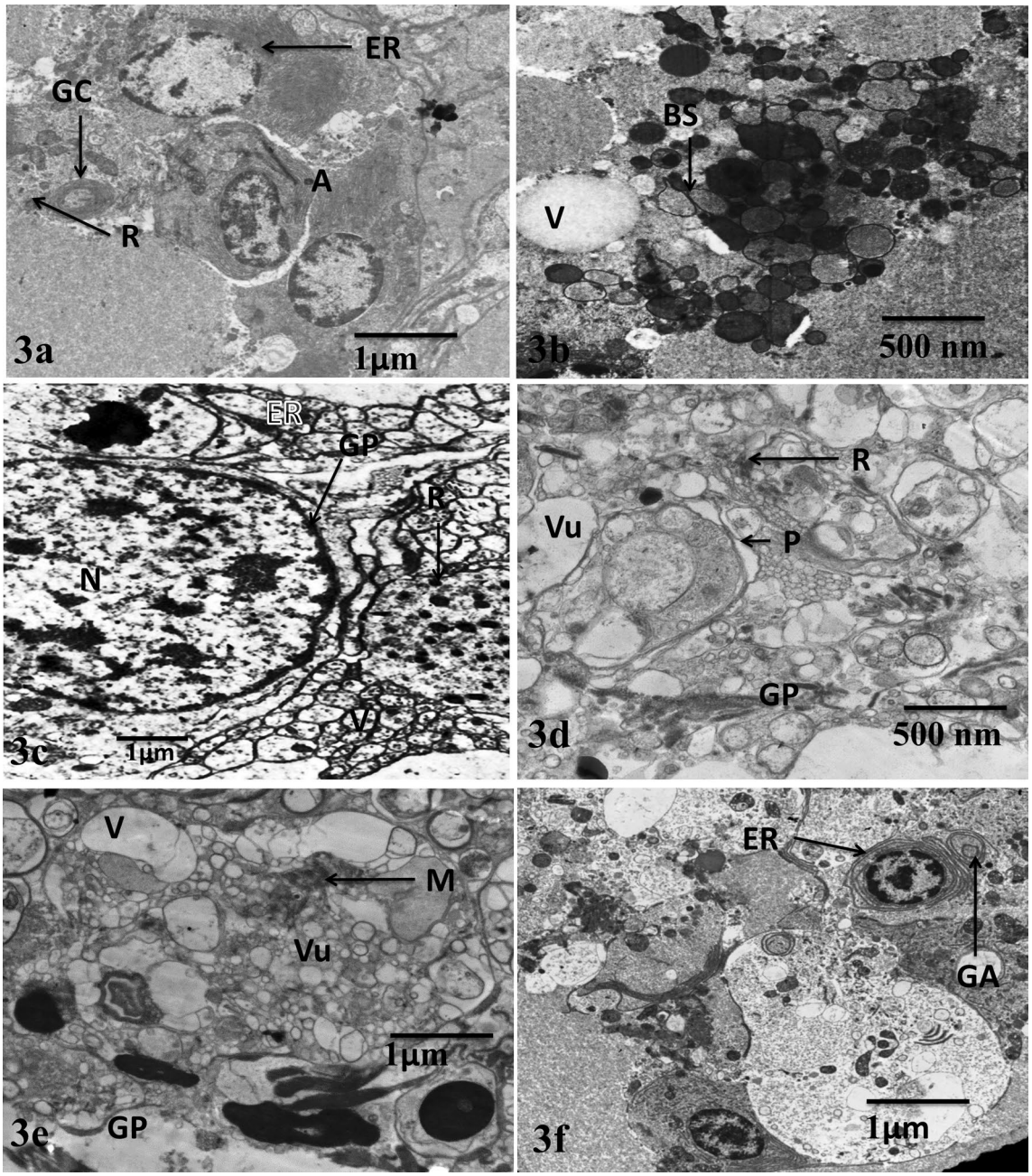

Fig. 3 **a** TEM section showing the cell bodies of glial cells appeared about the same of the neurons. The cores showed a strong lapping and numerous invaginations. **b** TEM section showing glial cells sent many branching shoots between the surrounding neuron perikarya and axons. **c** TEM section showing a glial cell with a very dense accumulation of ribosomes and the compaction of the matrix. **d** TEM section showing many free ribosomes are found in the thin foothills of glial perikarya. **e** TEM section showing in the perikaryon of the glial cells mitochondria, multi-vesicular bodies, various vacuoles and vesicles. **f** TEM section showing the formation of the Golgi apparatus. This consisted only of a few, strongly tortuous and closely spaced canals of the smooth surfaced endoplasmic reticulum. There are very few bubbles around. Glial Cell GC; Neuron N; Branching Shoots BS; Perikaryon P; Axons A; Ribosomes R; Glial Perikarya GP; Mitochondrion M; Multi-vesicular Bodies MB; Vacuoles Vu; Vesicles V; Golgi Apparatus GA; Endoplasmic Reticulum ER

the rest of the body. Only there is the glial hemolymph–brain barrier, which serves to transport the respiratory gases and nutrients and penetrates through the two layers into the brain. The perikarya of the neurons are mainly grouped close to the surface and are joined together in a dense bandage, which is referred to as the perikaryon layer of the cerebral cortex. This layer is extremely low in fibers as each cell sent only one cell process and even no direct dendritic or axonal branches from other cells. The corresponding contacts are found invariably in the next layer which contain

the synapses (Nakajima et al. 1965; Simons and Trajkovic 2006). Another characteristic of the cerebral cortex is the numerical inferiority of glial cells compared to the nerve cells. However, this inferiority is at least partially made up by the enormous surface development of the individual glial cells. Incidentally, these general architecture principles are found not only in the *Eledone cirrhosa*, but also in the brain of cephalopods (Abbott and Maddock 1995; Budelrnann 1995; Buresi et al. 2016). Neurons are extremely small; each neuron has a diameter of only 5–15 µm. This seems to be a characteristic to *Eledone cirrhosa*. A similar result is shown in puffer fish (Nakajima et al. 1965). There are two features of the internal structure: first, the small perikaryon is occupied by the nucleus so that only a sparse cytoplasmic space remained, and second, the endoplasmic reticulum is relatively poorly developed. It is crowded with ribosomes which never regularly close together. They are highly curved and widely distributed between a great number of free ribosomes and never form the basophilic plaques that can be represented as a Nissl substance (Nave and Trapp 2008; Williamson and Chrachri 2004). The result is only the diffuse basophilia described in the cytoplasm of neurons in insect (Holland et al. 2013). Even with interference microscopy, no Nissl complexes could be found in invertebrates (David et al. 1961). Thus, Nissl substance described in the neurons of insects was due to a misunderstanding (Moussa and Banhawy 1958). In the picture of this author, one can recognize that the differentiation of the endoplasmic reticulum and the arrangement of the ribosomes are largely corresponding to those of *Eledone cirrhosa*.

The inclusion bodies are frequently found in the cytoplasm and referred to as vesicles of type I and II. Such vesicles have already been observed in nerve cells of insects and gastropod nerve ganglia (Lane and Swales 1976). Thus, in this study, vesicles of type I with a diameter of 0.3–1.5 µm are obviously corresponding in structure to the electrodense inclusions of neurons of *Locusta migratoria*, which has a diameter of 0.5–1.0 µm (Doreen and Chapman 1962; Hochner and Shomrat 2012). There are two histochemically distinguishable bodies in the neurons of *Periplaneta americana*; inclusion bodies type I and II (Lane and Treherne 1972). According to these authors, lipids and glycolipids associated with proteins can be detected therein. In this study, the vesicles Type II correspond microscopically to the one from in the cells of the fat body of insects and gastropod (Lane and Swales 1976; Farris 2013). These authors summarized these vesicles as transformed mitochondria and associated them with protein storage. The glial cells differ fundamentally from the neurons in their internal structure and the surface design. The interior of the cell has the following features: electron-tight lobed nuclei, relatively greater density of cytoplasmic inclusions and dense ribosome structure, abundant glycogen granules, minimally formed Golgi apparatus and the presence of characteristic gliosomes. They are extensively studied in *Sepia officinalis* (Abbott et al. 1981), where they are very common. After (Kettenmann et al. 2010), these cells contain glycolipids and phospholipids associated with proteins. In the case of *Eledone cirrhosa*, its structure seems much more orderly than that of the *Locusta migratoria*. While the neuron is built rounded or polyhedral with a single process, the numerous and widely branched foothills are noticeable in the glial cell. Richard et al. (1984) and Haszprunar and Wanninger (2012), who spoke of a truss of glial cells, and Nave and Trapp (2008), who described the honeycomb scaffold of the glial cells, show that these processes are not pseudopod-like structures, as in vertebrate astrocytes, but cup-shaped envelopes.

Glial cells runners in the brain as intra-neuronal partitions appear to be widely distributed in invertebrates, as it has been found in the tick *Boophilus microplus* (Binnington and Lane 1980; Kandel et al. 2012), insects and gastropods (Lane and Swales 1976; Perry and Barron 2013) and cephalopods (Abbott et al. 1985; Abbott and Maddock 1995; Dunlop and King 2008). In the case of the small neuron perikarya of the brain of *Eledone cirrhosa*, penetration of the glial cells into the nerve cell bodies could not be detected. According to the results of this study, these structures occur only between neurons. In addition to mitochondria and glycogen granules, the glial cells also contain ribosomes. The functional importance of the RNA of the glial cells in relation to the metabolism of the neurons is still unclear.

Lane and Treherne (1972) demonstrated by light microscopy that *Periplaneta americana* has a concentration gradient for glycogen, which is responsible for transporting carbohydrate from the extra-cerebral fluid to the perilemmal cells and into the glial network. His findings are confirmed electron microscopically by (Abbott et al. 1981; Richard et al. 1984) and are also consistent with the present observations. Remarkably, the neurons are glycogen free, which raised the question of whether glycogen in significant quantities reached the nerve cells. Nicholls and Kuffler (1964) could not answer that question confidently; they pointed out the possibility that the glycogen with apparently intraneuronal location might be localized in the tropho-spongial processes of the glial cells. In any case, it may be assumed that the glycogen, after passing the neurallamella in a suitable form from outside, was released through the perilemmal cells to the underlying glial cells. Furthermore, the glial network takes over the transport into the depths of the brain and the distribution to the nerve cells. It is still unknown in what form the nerve cells receive the carbohydrates. The glycogen, like other nutrients, such as carbohydrates, lipids and proteins, which are not represented by electron microscopy, is likely to be partially metabolized by the glial cells (Kettenmann et al. 2010). A similar glycogen content

of the glial cells is demonstrated by (Keay et al. 2006; Sun and Tsai 2011) in the visceral ganglion of *Aplysia californica* (marine gastropod). In vertebrate astroglia, glycogen is rarely present (Hamilton and Attwell 2010). It seemed, therefore, that the glial cells tasks are related to the nutrient supply of the neurons (Gotow and Hashimoto 1984; Rechenbach and Wolburg 2005). The glial cells contain in their cytoplasm a heavy metal-containing oxygen vehicle which is analogous to hemoglobin or hemocyanin and circulates in the processes and causes the greater contrast in the electron microscope. There are heavy metal-free respiratory ferments in the broader sense in the glial cells which have a suitable, small redox potential energy is generated (de Lange and Van Minnen 1998; Keay et al. 2006; Sun and Tsai 2011). The astrocytes represent an ion reservoir for the exchanges during the depolarization of the neuron membrane. The same situation apparently also arose in the area of the supporting cells of *Octopus* retina (Büssow 1980).

To provide functions of care, disposal and homeostasis, the glial hemolymph–brain barrier has a number of structural and functional differences compared to the hemolymph system. This differentiation leads to a substantial separation of the brain from the surrounding extracellular space and is for the protection of sensitive neuronal tissue as well as for the maintenance of a constant internal environment of essential importance (Abbott et al. 1981; Lane et al. 1981; Crone 1986).

Authors interested to study the structure of the brain and the glio-vascular brain barrier of *Sepia officinalis* and *Octopus vulgaris* by light and electron microscopies (Boycott 1961; Abbott et al. 1981; Abbott et al. 1985; Budelmann and Young 1997; Rechenbach and Wolburg 2005; Simons and Trajkovic 2006; Kettenmann and Verkhratsky 2008; Di Cristo et al. 2009; Kettenmann et al. 2010). In *S. officinalis*, two types of glial cells are recognized referred to as the fibrous and protoplasmic glia. Most blood vessels are surrounded by extracellular matrix containing collagen, smooth muscle cells and fibrocytes. Inside matrices, there are glio-vascular strands that penetrate the neuropil in a complex network (Abbott et al. 1985). Neurons have thin glial sheets to their surfaces which may indent the cells to form small trophospongia. It may be correlated with the absence of dendrites (Haszprunar and Wanninger 2012). The present study investigated the fine structure of the different cell types of the central brain of *Eledone cirrhosa.* The neurilemma is a homogeneous connective capsule possessing striated bundles of collagen reminiscent fibrils. The perilemmal cells are flat or cubic with 1–7 µm in diameter with nuclei mostly oval and small. The cytoplasm has accumulations of very electron-dense granules and is replete with large mitochondria, vesicles, vacuoles and multi-vesicular bodies. The Golgi apparatus is very different in appearance from that of the neurons. These granules appear similar to those present in the extra-cerebral cells and may be fat or glycogen. The cerebral cortex comprises many cell layers and is composed of three elements, namely the neurons, the glial cells and the glial hemolymph–brain barrier cells. These descriptions of the brain tissue are never dealt with in any previous publications and considered as novel findings. The median superior frontal axons of *O. vulgaris* contain microtubules and neurofilaments, cored vesicles and synapses with trunks of the amacrine cells (Gray and Young 1964; Young 1971). The present study shows that the perikarya of the neurons are irregularly polygonal and join together by their processes. The remaining gaps between neurons are filled with glial pedestals and sparse giant fiber bundles. In any case, no interstitial glial lacunae are visible. The perikarya of the cerebral cortex give off only a single branch, which run directly to the central mass of the giant fibers. Two types of electron-dense vesicles are identified. Cores of glial cells showed numerous invaginations. In their forms, they are quite similar to the glial core of the optic nerve of *Sepia officinalis* (Abbott et al. 1981). They are denser than those of neurons. Glial cells sent many branching shoots between the surrounding neuron perikarya and axons. The glial cytoplasmic matrix appears more electronically denser than that of neurons. This is probably due to a very dense accumulation of ribosomes and the compaction of the matrix. The brain contains multi-rows of highly prismatic glial cells which, in addition to astrocytes, oligodendrocytes and epithelial processes, other cell type with fine structural features of ependymal cells were observed. Both glial and ependymal cells are highly granulated than neurons. From the cisterns of the endoplasmic reticulum of glial cells, bright secretions leaked through and penetrated the cytoplasm. Bright secretory matter provided the raw material for dense secretory granules formed in the Golgi apparatus. The granules that occur in different variants are apically attached. The secretion vacuoles contain very little flaky material. In the glial cells that locate ventrally, secretory vacuoles coalesce into large intracellular cavities into which microvilli occasionally protrude. Some glial cells have nearly homogeneous protrusions that reach to the neurons. Organelle-free cytoplasmic areas are observed in some neurons. The glial blood–brain barrier has different width endothelia. Often there is an elevated perivascular space; it is stuffed with disordered filaments or collagen fibrils and occasionally contain adventitial ependymal cells. The blood–brain interface is studied in the cuttlefish *S. officinalis* (Abbott et al. 1981). Layers lining blood vessels in the vertical lobes of the brain include a layer of endothelial cells and associated basal lamina, a layer of pericytes and a second basal lamina and perivascular glial cells. Similar results were noticed in *O. vulgaris* (Gray and Young 1964). A layer of glial cells is always interposed between blood and neural tissue, except where neurosecretory endings reached the second basal lamina. The walls of

venous channels are formed by lamellae of overlapping glial processes. In arterial vessels, the pericyte layer is thicker and more complete, with characteristic sinuous intercellular clefts. The glio-vascular channels observed in *Octopus* brain (Hochner and Shomrat 2012) are not a prominent feature of *Sepia* vertical lobe (Abbott et al. 1981). The later authors concluded that the blood–brain interface in *Sepia* is designed to restrict permeability between blood and brain. The author of this study preferred to use the terms hemolymph and glial hemolymph–brain barrier; not the terms blood, blood vessels, arteries, veins and glial blood–brain barrier which were used by previous authors in this field of study (Gray and Young 1970; Abbott et al. 1981). The present study showed that the essential elements of the hemolymph–brain barrier are the endothelial cells with their tight junctions. Similar results are recorded for *O. vulgaris* (Gray and Young 1970). The cytoplasm contains various vesicles and mitochondria. However, two other cell types are present, the pericytes and the astrocytes, which are of great importance for the function of the hemolymph–brain barrier. Similar results are found in *Octopus* brain (Hochner and Shomrat 2012). The cell–cell interactions between endothelial cells, pericytes and astrocytes are as close as no other cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abbott NWR, Maddock L (eds) (1995) Cephalopod neurobiology. Oxford University Press, Oxford

Abbott NJ, Bundgaard M, Cserr HF (1981) Fine-structural evidence for a glial blood-brain barrier to protein in the cuttlefish, *Sepia officinalis*. J Physiol Lond 316:P52–P53

Abbott N, Bundgaard M, Cserr HF (1985) Brain vascular volume, electrolytes and blood-brain interface in the cuttlefish *Sepia officinalis* (Cephalopoda). J Physiol 368:197–212

Adelman WJ, Gilbert DL (1990) Electrophysiology and biophysiscs of the squid giant axon. In: Gilbert D, Adelman H, Arnold J (eds) Squid as experimental animals. Plenum Press, New York, pp 93–132

Akert K, Sandri C (1976) The fine structure of the perineural endothelium. Cell Tiss Res 165:281–295

Albertin CB, Simakov O, Mitros T, Wang ZY, Pungor JR, Edsinger-Gonzales E et al (2015) The octopus genome and the evolution of cephalopod neural and morphological novelties. Nature 524:220–224

Binnington KC, Lane NJ (1980) Perineurial and glial cells in the tick *Boophilus microplus* (Acarina: Ixodidae); freeze-fracture and tracer studies. J Neurocytol 9:343–362

Boycott B (1961) The functional organization of the brain of the cuttlefish *Sepia officinalis*. Proc R Soc Lond B Biol Sci 153:503–534

Budelmann BU, Young JZ (1984) The statocyst-oculomotor system of *Octopus vulgaris*: extraocular eye muscles, eye muscle nerves, statocyst nerves and the oculomotor centre in the central nervous system. Phil Trans R Soc Lond B 306:1127. https://doi.org/10.1098/rstb.1984.0084

Budelmann BU, Young JZ (1997) Brain pathways of the branchial nerves of *Sepia* and *Loligo*. Philos Trans R Soc Lond B(315):345–352

Budelrnann B (1995) The cephalopod nervous system: What evolution has made of the molluscan design. In: Breidbach O, Kutsch W (eds) The nervous system of invertebrates: an evolutionary and comparative approach. Birkhauser Verlag, Basel, pp 115–138

Buresi A, Andouche A, Navet S, Bassaglia Y, Bonnaud-Ponticelli L, Baratte S (2016) Nervous system development in cephalopods: how egg yolkrichness modifies the topology of the mediolateral patterning system. Dev Biol 415:143–156

Büssow H (1980) The astrocytes in the retina and optic nerve head of mammals: a special glia for the ganglion. Cell Tiss Res 206(3):367–378

Butler A (2008) Evolution of the thalamus: a morphological and functional review. Thalamus Relat Syst 4:35–58

Crone C (1986) The blood-brain barrier as a tight epithelium: where is information lacking? Ann N Y Acad Sci 481:174–185

David GB, Brown AW, Mallion KB (1961) On the Identity of the 'Neurofibrils', 'Nissl complex', 'Golgi Apparatus', and 'Trophospongium' in the Neurones of Vertebrates. Q J Microscop Sci 102(4):481–489

de Lange RPJ, van Minnen J (1998) Localization of the neuropeptide APGWamide in gastropod molluscs by in situ hybridization and immunocytochemistry. Gen Comp Endocrinol 109(2):166–174

Di Cosmo A, Di Cristo C (1998) Neuropeptidergic control of the optic gland of Octopus vulgaris: FMRF-amide and GnRH immunoreactivity. J Comput Neurol 398(1):1–12

Di Cristo C, De Lisa E, Di Cosmo A (2009) GnRH in the brain and ovary of *Sepia officinalis*. Peptides 30(3):531–537

Doreen E Ashhurst, Chapman JA (1962) An electron-microscope study of the cytoplasmic inclusions in the neurones of *Locusta migratoria*. Q J Microscop Sci 103(2):147–153

Dunlop C, King N (2008) Cephalopods: Octopuses and cuttlefish for the home aquarium. Publications, Neptune City

Farris SM (2013) Evolution of complex higher brain centers and behaviors: behavioral correlates of mushroom body elaboration in insects. Brain Behav Evol 82:9–18

Fernández-Rueda P, García-Flórez L (2007) *Octopus vulgaris* (Mollusca:Cephalopoda) fishery management assessment in Asturias (North-West Spain). Fish Res 83:351–354

Froesch D (1974) The subpedunculate lobe of the octopus brain: Evidence for dual function. Brain Res 75(2):277–285

Gotow T, Hashimoto PH (1984) Plasma membrane organization of astrocytes in elasmobranchs with special reference to the brain barrier system. J Neurocytol 13:727–742

Gray EG (1970) The fine structure of the vertical lobe of octopus brain. Philos Trans R Soc Lond B(258):379–394

Gray EG, Young JZ (1964) Electron microscopy of synaptic structure of Octopus brain. J Cell Biol 21:87–103

Hamilton NB, Attwell D (2010) Do astrocytes really exocytose neurotransmitters? Nat Rev Neurosci 11:227–238

Haszprunar G, Wanninger A (2012) Molluscs. Curr Biol 22:510–514

Hochner B, Shomrat T (2012) An embodied view of octopus neurobiology. Curr Biol 22:887–892

Hochner B, Shomrat T (2014) The neurophysiological basis of learning and memory in an advanced invertebrate—the octopus. In: Darmaillacq A-S, Dickel L, Mather JA (eds) Cephalopods cognition. Cambridge University Press, Cambridge

Holland LZ, Carvalho JE, Escriva H, Laudet V, Schubert M, Shimeld SM et al (2013) Evolution of bilaterian central nervous systems: a single origin? Evo Dev 4:27

Jereb P, Roper CE (eds) (2005) Cephalopods of the World, an Annotated and illustrated Catalogue of Cephalopod Species Known to Date. Publicatios, Neptune City, p 1

Kandel E, Schwartz J, Jessell T, Siegelbaum S, Hudspeth AJ (2012) Principles of neural science, 5th edn. McGraw-Hill, New York

Keay J, Bridgham JT, Thornton JW (2006) The *Octopus vulgaris* estrogen receptor is a constitutive transcriptional activator: evolutionary and functional implications. Endocrinology 147(8):3861–3869

Kettenmann H, Verkhratsky A (2008) Neuroglia: the 150 years after. Trends Neurosci 31:653–659

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2010) Physiology of microglia. Physiol Rev 91:461–553

Lane NJ, Swales LS (1976) Interrelationships between Golgi, Gerl and synaptic vesicles in the nerve cells of insect and gastropod ganglia. J Cell Set 22:435–453

Lane NJ, Treherne JE (1972) Studies on perineurial junctional complexes and the sites of uptake of microperoxidase and lanthanum in the cockroach central nervous system. Tiss Cell 4:427–436

Lane NJ, Harrison JB, Bowerman RF (1981) A vertebrate-like blood-brain barrier, with intraganglionic blood channels and occluding junctions in the scorpion. Tiss Cell 13:557–576

Moussa TA, Banhawy M (1958) Studies on the Nissl substance, neurofibrillae and intracellular trabeculae of insect neurones. J R Microsc Soc 78:114–119

Nakajima Y, Pappas GD, Bennett MVL (1965) The fine structure of the supramedullary neurons of the puffer fish, with special reference to endocellular and pericellular capillaries. Am J Anat 116:471–492

Nave KA, Trapp BD (2008) Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci 31:535–561

Nicholls JG, Kuffler SW (1964) Extracellular space as a pathway for exchange between blood and neurons in the central nervous system of the leech: ionic composition of glial cells and neurons. J Neurophysiol 27:645–671

Nixon M, Young JZ (2003) The Brains and Lives of Cephalopods. Oxford University Press, Oxford

Oldendorf WH, Cornford ME, Brown WJ (1977) The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. Ann Neurol 1:409–417

Perry CJ, Barron AB (2013) Neural mechanisms of reward in insects. Annu Rev Entomol 58:543–562

Plan, T. (1987). Functional neuroanatomy of sensory-motor lobes of the brain of *Octopus vulgaris*. Ph.D. thesis, University of Regensburg

Rechenbach A, Wolburg H (2005) Astrocytes and ependymal glia. In: Kettenmann H, Ransom BR (eds) Neuroglia. Oxford University Press, New York

Reese TS, Karnovsky MJ (1967) Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 34:207–217

Richard L, Saint M, Stanley DC, Che C (1984) The glial cells of insects. Insect Ultrastruct 50:435–475

Shigeno S, Ragsdale CW (2015) The gyri of the octopus vertical lobe have distinct neurochemical identities. J Comput Neurol 523:1297–1317

Shomrat T, Zarrella I, Fiorito G, Hochner B (2008) The octopus vertical lobe modulates short-term learning rate and uses LTP to acquire long-term memory. Curr Biol 18:337–342

Simons M, Trajkovic K (2006) Neuron-glia communication in the control of oligodendrocyte function and myelin biogenesis. J Cell Sci 119:4381–4389

Stephens PR, Young JZ (1969) The glio-vascular system of, cephalopods. Philos Trans R Soc Lon B(255):1–11

Sun B, Tsai S (2011) Agonadotropin-releasing hormone-like molecule modulates the activity of diverse central neurons in a gastropod mollusk, *Aplysia californica*. Front Endocrinol 2(36):1–8

Verkhratsky A, Parpura V, Rodriguez JJ (2010) Where the thoughts dwell: the physiology of neuronal-glial "diffuse neural net. Brain Res Rev 66(1–2):133–151

Waddell S (2013) Reinforcement signalling in *Drosophila*; dopamine does it all after all. Curr Opin Neurobiol 23:324–329

Wentzell MM, Martínez-Rubio C, Miller MW, Murphy AD (1987) Functional receptors for neurotransmitters on astroglial cells. Neuroscience 22:381–394

Williamson R, Chrachri A (2004) Cephalopod neural networks. Neurosignals 13:87–98

Young JZ (1965) The central nervous system of *Nautilus*. Philos Trans R Soc Lond B(249):1–25

Young JZ (1971) The anatomy of the nervous system of *Octopus vulgaris*. Clarendon Press, Oxford

Young JZ (1974) The central nervous system of *Loligo*. I. The optic lobe. Philos Trans R Soc Lond 5(267):263–302

Young JZ (1976) The nervous system of *Loligo*. II. Suboesophageal centres. Philos Trans R Soc Lond. 5(274):101–167

Young JZ (1977) The nervous system of *Loligo*. III. Higher motor centres: the basal supraoesophageal lobes. Philos Trans R Soc Lond 5(276):351–398

Young JZ (1979) The nervous system of *Loligo*. Y. The vertical lobe complex. Philos Trans R Soc Lond 5(295):311–354

Young JZ (1988) Evolution of the cephalopod brain. In: Clarke MR, Trueman ER (eds) The Mollusca, vol 12. Paleontology and Neontology of Cephalopods. Elsevier, Amsterdam, pp 215–228