

# 3

## Neuroglia: Definition, Classification, Evolution, Numbers, Development

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### 3.1 Definition of neuroglia as homeostatic cells of the nervous system

*'THE NEUROGLIA is the delicate connective tissue which supports and binds together the nervous elements of the central nervous system. One part of it, which lines the central canal of the cord and ventricles of the brain, is formed from columnar cells, and is called ependyma, while the rest consists of small cells with numerous processes which sometimes branch and sometimes do not.'*

Encyclopaedia Britannica, 1910, 11th Ed., v. 19, p. 401

*'As the Greek name implies, glia are commonly known as the glue of the nervous system; however, this is not fully accurate. Neuroscience currently identifies four main functions of glial cells: to surround neurons and hold them in place, to supply nutrients and oxygen to neurons, to insulate one neuron from another, and to destroy pathogens and remove dead neurons. For over a century, it was believed that they did not play any role in neurotransmission. That idea is now discredited; they do modulate neurotransmission, although the mechanisms are not yet well understood.'*

Wikipedia, June 15<sup>th</sup> 2012 (<http://en.wikipedia.org/wiki/Neuroglia>)

To the continual surprise and confusion of everybody working in neuroglial research, the proper definition of 'neuroglia' has not hitherto been agreed upon. Many existing definitions highlight the supportive role of these cells, and some rests on their process branching and delicate morphology, but the most common definition assigned to neuroglia is "cells residing in the brain that are not electrically excitable neurones or vascular cells".

For example, Ted Bullock and Adrian Horridge defined neuroglia as 'Any non-nervous cell of the brain, cords . . . ganglia . . . and . . . peripheral nerves, except for cells comprising blood vessels, trachea, muscle fibers, glands, and epithelia . . .' (Bullock & Horridge, 1965). As a result, 'neuroglia' has become a generalised term that covers cells with different origins (ectodermal for macroglia and mesodermal for microglia), morphology, physiological properties and functional specialisation. Indeed, in the CNS, neuroglia include the cells of the choroid plexus, the oligodendrocytes, the ependymal cells, the radial glia of the retina, the immunocompetent microglia/innate macrophages and the hugely diverse astrocytes; whereas, in the PNS, they include the diverse kinds of Schwann cells, satellite glia, olfactory ensheathing cells and the highly numerous enteric glia. All belong to the family neuroglia.

There is, however, one unifying fundamental property common for all these cell types and this is their ultimate function – homeostasis of the nervous system. Indeed, as we shall see below, the evolution of the nervous system led to a specialisation of neurones, which become perfect elements for signalling and information processing. This came at the price of losing essential housekeeping

functions, as neurones are generally incapable of regulating their own immediate environment and are vulnerable to many kinds of environmental insults. These main housekeeping functions went to the neuroglia, which have themselves specialised into many types of cells to perform specific aspects of nervous system homeostasis.

This homeostatic function of neuroglia is executed at many levels, and includes:

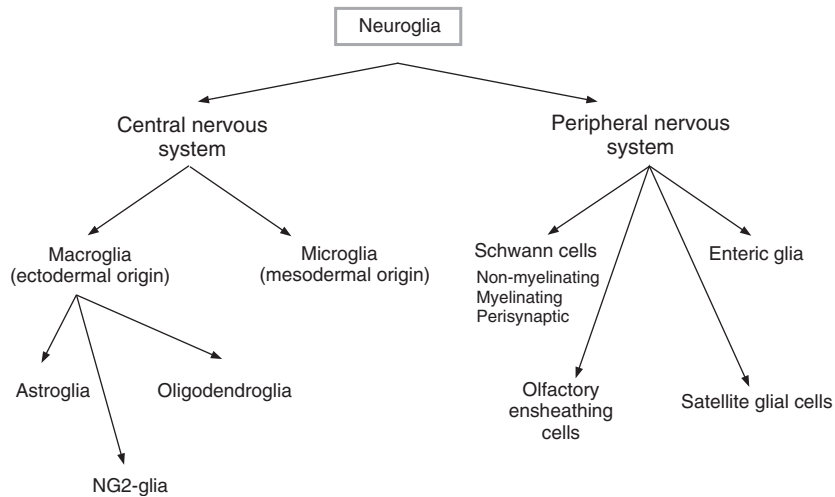
- whole body and organ homeostasis (e.g. astrocytes control the emergence and maintenance of the CNS, peripheral glia are essential for communication between the CNS and the body, and enteric glia are essential for every aspect of gastrointestinal function);
- cellular homeostasis (e.g. astroglia and NG2-glia are both stem elements);
- morphological homeostasis (glia define the migratory pathways for neural cells during development, shape the nervous system cyto-architecture and control synaptogenesis/synaptic pruning, whereas myelinating glia maintain the structural integrity of nerves);
- molecular homeostasis (which is represented by neuroglial regulation of ion, neurotransmitter and neurohormone concentrations in the extracellular spaces around neurones);
- metabolic homeostasis (e.g. neuroglial cells store energy substrates in a form of glycogen and supply neurones with lactate);
- long-range signalling homeostasis (by myelination provided by oligodendroglia and Schwann cells);
- defensive homeostasis (represented by astrogliosis and activation of microglia in the CNS, Wallerian degeneration in CNS and PNS, and immune reactions of enteric glia; all these reactions provide fundamental defence for neural tissue).

Moreover, some neuroglial cells act as chemosensitive elements of the brain that perceive systemic fluctuations in  $\text{CO}_2$ , pH and  $\text{Na}^+$  and thus regulate behavioural and systemic homeostatic physiological responses.

Therefore, the neuroglia can be broadly defined as *homeostatic cells of the nervous system*, represented by highly heterogeneous cellular populations of different origin, structure and function.

## 3.2 Classification

Generally (Figure 3.1), the neuroglia in the mammalian nervous system are sub-classified into peripheral nervous system (PNS) glia and central nervous system (CNS) glia. The PNS glial cells (see Chapter 8) include:



**Figure 3.1** Classification of neuroglia.

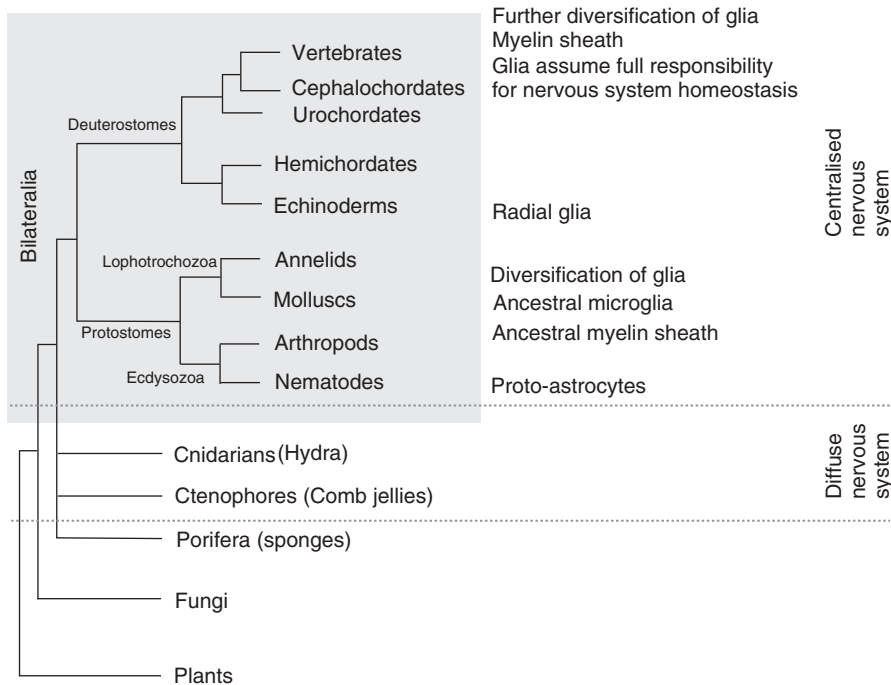
1. *myelinating Schwann cells* that myelinate peripheral axons;
2. *non-myelinating Schwann cells* that surround multiple non-myelinating axons; and
3. *perisynaptic Schwann cells*, which enwrap peripheral synapses (for example neuro-muscular junctions).

The PNS glial cells which surround neurones in peripheral ganglia are known as *satellite glial cells*, and those in the olfactory system are known as *olfactory ensheathing cells*. Finally, the PNS includes *enteric glia*, which reside in the enteric nervous system.

CNS glia are generally subdivided into *astrocytes*, *oligodendrocytes*, *NG2-glia* and *microglia*. Astrocytes, which are the main homeostatic cells of the grey matter are, in turn, subdivided into many different types, which will be discussed in detail in Chapter 4. Oligodendrocytes are the myelinating cells in the CNS (see Chapter 5), and NG2-glia act as oligodendroglial precursors (Chapter 6). Finally, microglia represent the innate brain immunity and defence (Chapter 7).

### 3.3 Evolution of neuroglia

The ‘Tree of Life’ (visit the *Tree of Life* project at <http://tolweb.org/tree/phylogeny.html>) is in constant change, as the taxonomy is being continuously revised (the literature on this topic is immense and the reader is advised to look for details in several papers published during last decade, e.g. Cavalier-Smith, 1998; Cavalier-Smith, 2009; Ding *et al.*, 2008; Dunn *et al.*, 2008; Keeling *et al.*, 2005; Parfrey *et al.*, 2006; Yoon *et al.*, 2008). Whatever taxonomic chart we may use (either dividing all



**Figure 3.2** Evolution of nervous systems and of neuroglia.

living forms into Domains of Bacteria, Archea and Eucarua, or that using Empires of Prokaryota and Eukaryota), the nervous system of which neuroglia are a part are a sole property of the Kingdom of Animalia. The cladogram of the latter is relatively well defined (Figure 3.2) and broadly comprises radially symmetrical Cnidaria and Ctenophora (which previously were regarded as members of a common family of Coelenterata, but are now considered as separate phyla) and Bilateralia that encompass the vast majority of phyla. The bilateralia are represented by Protostomia (further subdivided into Ecdysozoa and Lophotrochozoa; some taxonomists also recognise the Platyzoa as separate superphyla) and Deuterostomia, which include Echinodermata, Hemichordata and Chordata (to which vertebrates belong).

The early evolution of the nervous system can only be speculated upon, because fossils do not provide much material for analysis, and it is likely that many early life forms have not survived to our time. Nonetheless, certain generalisations can be drawn, and overall we are in possession of a rather logical system of views on the milestones of nervous system phylogeny. In this matter, of course, we shall restrict our narrative only to the very general outline of the evolutionary routes of the nervous system; for a more detailed account, readers are referred to numerous comprehensive reviews (e.g. Arendt *et al.*, 2008; Ghysen, 2003; Holland, 2003; Stollewerk & Simpson, 2005).

The very first nervous system appeared in Ctenophora (comb jellies) and Cnidarians (hydras and jellyfishes), in the form of a relatively homogeneously distributed network of neurones connected with their processes. These networks are generally known as a *diffuse nervous system*. The neurones in these networks have evolved from epithelial cells which made up the two tissue layers of all these species (the epidermis and the ectoderm); as a rule, the neurones are much denser in the epidermis.

Diffuse nervous systems are made from multipolar and unipolar neurones, which are organised in several semi-independent networks. The nerve cells in diffuse nervous systems are connected with proper chemical synapses, although we cannot exclude the existence of electrical (i.e. gap junctional) contacts between them. The appearance and evolution of the synapse is another extremely interesting topic (see, for example, Ryan & Grant, 2009).

Importantly, the main molecules needed for synapse formation had already evolved in single cell organisms. Indeed, the receptors for neurotransmitters appeared previously in bacteria (pentameric receptors and glutamate receptors) and in protozoa (purinoceptors, which are present in amoeba). Similarly, ion channels and ion pumps and many molecules of the post-synaptic density appeared very early in evolution in prokaryotes and early unicellular eukaryotes (yeast and amoeba; see Case *et al.*, 2007; Ryan & Grant, 2009). The epithelial cells that give rise to ancestral neurones are also endowed with exocytotic machinery underlying vesicular release of proto-neurotransmitters.

The next step in evolution of the nervous system is associated with the appearance of neuronal masses known as ganglia. This signalled the appearance of the *centralised nervous system*. In some Cnidarian polyps, the nerve networks already showed some concentration around the oral opening, and this was likely the beginning of the centralisation process. How this process of centralisation proceeded remains generally unknown, but several hypotheses are currently in existence.

Notably, centralisation coincided with the appearance of bilateral symmetry and emergence of Bilateralia. Initially, for example in Nematoda, the centralised nervous system was made of several ganglia localised around the oral orifice. The centralisation continued in phylogeny, and in more advanced protostomes (for example in insects and crustacea), the central nervous system is present in the form of a polyganglionic brain. Further developments led to the appearance of a layered nervous system, which evolved in Echinoderma and Hemichordata and become fully organised in Chordata.

The evolutionary origins of glial cells are obscure. There are some indications that glial have appeared in phylogeny on several occasions, and parallel evolution is likely. There is no evidence for the existence of glial cells in diffuse nervous systems and no cells associated with neurones or their processes have been detected in the comb jellies. Similarly, no glial cells were found in Cnidaria polyps, with the exception of scyphomedusae, in which some glia-like cells were apparently

reported – although neither their function nor, indeed, their glial identity, has been analysed in detail (Hartline, 2011).

Most probably, the neuroglia appeared with the emergence of a centralised nervous system, when neurones acquired specialisation and subsequently began to amass into sensory organs and ganglia. The very first glial cells are associated with sensory organs and have the same epithelial origin as neurones. These glia-like cells are described in Acoelomorpha, the primitive flat-worms, which are generally considered to be the earliest (or one of the first) Bilateralia. More advanced and much more characterised are glial cells in nematodes (in particular in *Caenorhabditis elegans*), whose properties we shall discuss below.

In Platyzoa, which are also considered to be primitive Bilateralia, glial cells ~~have a mosaic-like appearance:~~ glial cells are absent in Rotifera (wheel animals) and in many platyhelminthes (for example in tubellarian flatworms, Catenulida or Macrostomida). At the same time, glial (or accessory) cells have been found in polyclad flat-worms and in some (but not in all) triclad planaria. Neuroglia are generally present throughout Ecdysozoa and Lophotrochozoa, being well developed in molluscs, in Annelids, and even more so in Arthropods (insects and crustaceans).

In Deuterostomes, at the very base of Chordata, a new type of neuroglia emerge, the radial glial cells, which is most likely directly associated with the appearance of a layered nervous system. In many early Chordata, the radial glial cells dominate and are present throughout life, while parenchymal glia (i.e. astrocytes) are either completely absent, or remain in the minority. An increase in brain thickness triggered further development of parenchymal glia, which became increasingly heterogeneous and assumed a full homeostatic responsibility in the CNS of mammals, while the radial glia instead became mostly confined to the prenatal period and largely disappeared from the adult brain parenchyma.

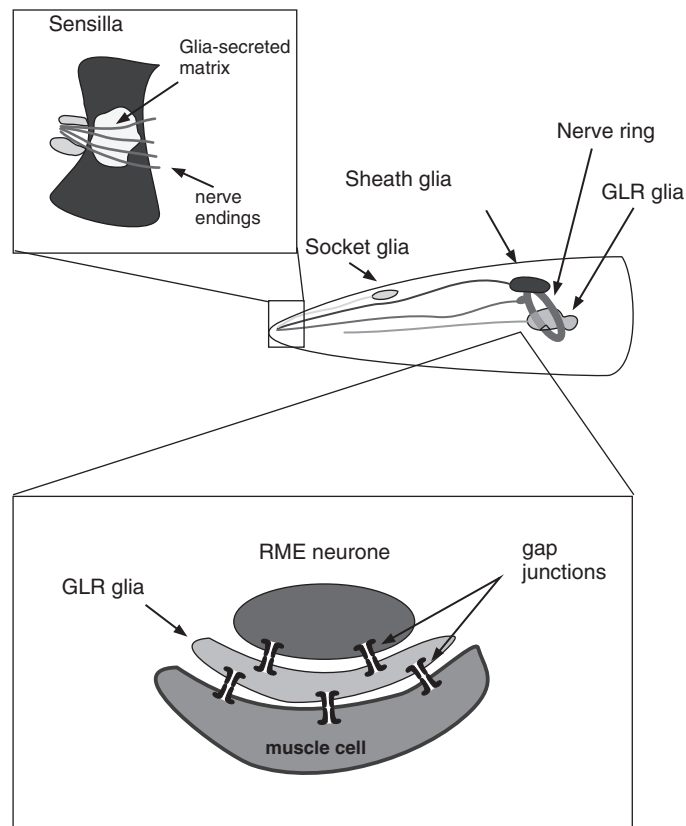
All in all, the full story of glial evolution is complex and not fully understood; there are several comprehensive reviews (Hartline, 2011; Heiman & Shaham, 2007; Oikonomou & Shaham, 2011; Radojicic & Pentreath, 1979; Reichenbach & Pannicke, 2008), to which readers are referred for further details. Below, we shall provide an account of the evolution of the main types of neuroglia.

### 3.3.1 Evolution of astrocytes

**(i) Nematoda: neuroglia in *Caenorhabditis elegans*** The nervous system of *C. elegans* is very well characterised morphologically and functionally. It contains 302 neurones and some 56 supportive/glial cells, which can be considered as proto-astrocytes; 50 of these glia are of epithelial origin, and six specialised glial cells are of mesodermal origin. The nervous system of *C. elegans* has major signs of centralisation. The sensory neurones distributed in the periphery send their processes to the nerve ring located in the frontal part of the body. The nerve ring also contains cephalic and motor neurones, which send efferent signals through the ventral and dorsal nerve cords. The sensory neurones in *C. elegans* have remarkable

specialisation and diverse modality, being sensitive to soluble and volatile chemicals (analogues of taste and olfaction), temperature, mechanical stimulation, osmotic pressure, oxygen and even pheromones.

Most of the glial cells of the worm, 46 out of 50, are associated with the endings of these sensory neurones. These dendritic endings, together with glia, form sensory organs known as *sensilla*. Incidentally, males have specific sensilla (23 in total, composed of endings from 46 neurones) concentrated in the tail and imperative for mating; their particular function is physical sensation of the vulva of a mating partner (Heiman & Shaham, 2007). The number of neurites in sensilla varies between 1 and 12, but each sensilla has a pair of glial cells known as the sheath and socket cells (Figure 3.3). Sensilla in the male tail, as a rule, contain only a single glial cell classified as the structural cell. The remaining four glial cells, known as



**Figure 3.3** Glial cells in *Caenorhabditis elegans*.

The 'brain' of *C. elegans* is represented by a nerve ring. Most of the glial cells are part of sensory organs known as sensilla. Each sensilla has two glial cells: the sheath cell and socket cell. In the anterior part, there are four CEP (cephalic) glial cells that ensheath the nerve ring. The nerve ring also has six GLR glial cells which establish gap junctional contacts between motor neurones (RME) and muscle cells.



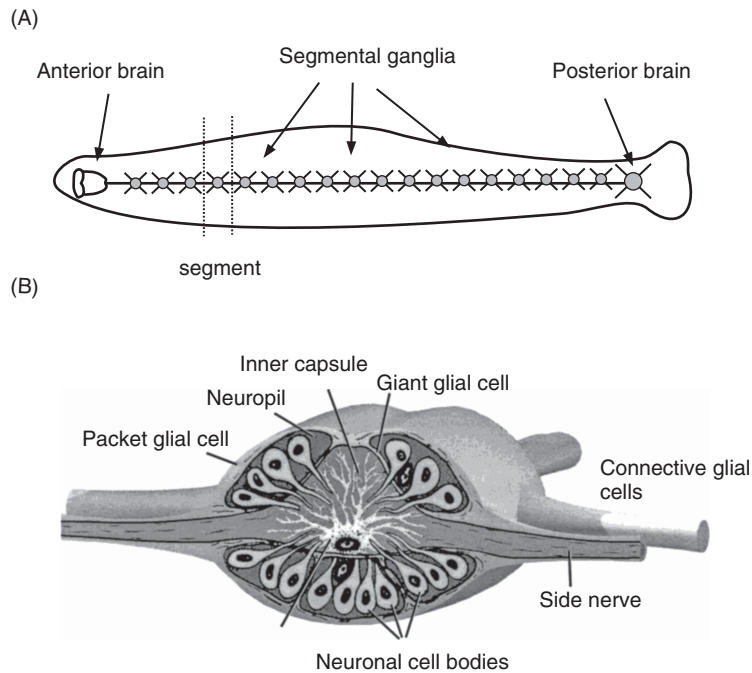
CEP sheath cells (because of their association with cephalic neurones), ensheath the nerve ring and send processes contacting synapses in the neuropil. Finally, the six mesodermally derived glia, known as GLR cells, are located around the nerve ring, where they make gap junctions with neurones and muscle cells.

The functional role of glial cells in *C. elegans* is not entirely clear, but they include: proper functioning of sensilla, possibly through increasing sensory efficacy; enwrapping and encapsulating synapses, possibly controlling ion homeostasis in perisynaptic regions; neuronal development and morphogenesis; and active neuronal-glial interactions. In addition, glial cells in *C. elegans* are also able to phagocytose dying cells during embryogenesis. At the same time, glial cells are not obligatory for *C. elegans* survival. Genetic or physical ablation of glia, although affecting sensory efficacy and neuronal morphogenesis, does not prevent the worm's nervous system from functioning and does not substantially affect animal survival (Bacaj *et al.*, 2008). Glial cells in *C. elegans* are not involved in neuronal metabolic support, either. Physiologically, glia of *C. elegans* show many neuronal features; for example, they generate  $\text{Ca}^{2+}$  signals through activation of voltage-operated channels and do not have developed intracellular  $\text{Ca}^{2+}$  stores (Stout & Parpura, 2011).

**(ii) Annelida: astroglia in leech** The medicinal leech *Hirudo medicinalis* was one of the very first animal models for studying neuroglia, as Stephen Kuffler and Richard Orkand used them for their pioneering electrophysiological experiments in the mid-1960s (Kuffler & Potter, 1964; Orkand *et al.*, 1966). The medicinal leech belongs to the Annelids and has a well defined centralised and segmented nervous system. The leech nervous system is composed of 34 ganglia. Six fused ganglia form the anterior brain, seven fused ganglia form the posterior brain and in between lies a chain of 21 ganglia, with each segment of the worm body being innervated with a single ganglion (Figure 3.4). In the frontal part of the nerve chain, four ganglia (two supra-oesophageal and two sub-oesophageal) are fused to form two neuronal masses that can be regarded as the animal's quasi-brain. These two frontal masses are linked together and form a peri-oesophageal ring. At the rear end of the leech body, another seven ganglia are fused into a caudal ganglion.

Leech neuroglia show a degree of specialisation, represented by several main types. Every single ganglion contains about 400 neurones (with the exception of the 5th and 6th ganglia innervating the reproductive system, which have  $\approx 700$  nerve cells) and ten glial cells. The glial cells comprise two giant glial cells, two connective glial cells which ensheath axons, and six packet cells which cover neuronal cell bodies. The giant glial cells, located in the ganglion central neuropil, are quite unique in their size and physiology. The somata of these glial cells have a diameter of  $\approx 100 \mu\text{m}$  and their processes extend through the whole of the neuropil, being  $\approx 300 \mu\text{m}$  in length.

The giant glial cells have an extensive complement of receptors, ion channels and transporters (Deitmer *et al.*, 1999; Lohr & Deitmer, 2006). The receptor palette includes ionotropic and metabotropic glutamate receptors, nicotinic acetylcholine



**Figure 3.4** Neuroglia in the medicinal leech, *Hirudo medicinalis*.

- A. General structure of the nervous system.
- B. Structure of the segmental ganglia, which contains three types of glial cells: the giant glial cell, packet glial cells and connective glial cells. (B - modified from Deitmer et al., 1999 with permission)

receptors, ionotropic and metabotropic serotonin receptors, metabotropic purinoceptors linked to ER  $\text{Ca}^{2+}$  release, and metabotropic receptors linked to myomodulin. The main type of ion channels are represented by potassium channels. In addition, giant glial cells express voltage-operated  $\text{Ca}^{2+}$  channels and chloride channels. Multiple transporters are involved in regulation of extracellular glutamate, choline and pH.

The giant glial cells have complex  $\text{Ca}^{2+}$  signalling, with a high degree of compartmentalisation; the  $\text{Ca}^{2+}$  signals are mainly generated by membrane channels and  $\text{Ca}^{2+}$  permeable ionotropic receptors, whereas the contribution of intracellular  $\text{Ca}^{2+}$  stores to  $\text{Ca}^{2+}$  signalling is relatively small. Neuronal activity in the neuropil activates glial receptors and triggers local  $\text{Ca}^{2+}$  fluxes which, in turn, produce highly localised  $\text{Ca}^{2+}$  signals.

Most likely, the main physiological role of giant glial cells is the regulation of ions (mostly  $\text{K}^+$  and  $\text{H}^+$ ) and neurotransmitter homeostasis in the neuropil. In Annelids (similar to many other invertebrates, such as Arthropods and Molluscs), nerve cell somata are often invaginated by glial cells processes. This structure is

known in histology as the ‘trophospongium’ (Holmgren, 1901), and it is likely to be involved in trophic support of neurones.

The connective glial cells ensheath axons and are possibly involved in mechanical and metabolic support of the latter. The packet cells enwrap neuronal cell bodies and segregate neurones in architectural microdomains known as packets; in addition, packet cells are probably involved in regulation of the perineuronal microenvironment. Therefore, neuroglia in Annelids contribute to functional compartmentalisation of the neuronal groups, a function which is present in all subsequent evolutionarily more advanced life forms. All three types of glia in the leech are coupled through gap junctions (formed by innexins, two types of which are specifically expressed in glial cells) and form a panglial syncytium embracing the whole of the nervous system. This syncytium may provide for long-range molecular diffusion through the nerve cord.

**(iii) Arthropods: astrocytes in *Drosophila* and other insects** The Arthropods and, in particular, insects, developed highly diversified neuroglia. In *Drosophila*, the nervous system has been investigated in detail, and we have reasonably deep knowledge on neuroglial morphology and function. The *Drosophila* brain is constructed from three pairs of ganglia fused into one frontally located mass. It is divided into the paired visual protocerebrum (receiving visual information), paired deutocerebrum (receiving sensory input from antennae) and tritocerebrum (which is believed to integrate information from other parts of CNS). Each part of the CNS has several relatively independent neuropil regions.

There are several classifications of *Drosophila* neuroglia, which divide these cells according to their anatomical location and morphology. Here<sup>2</sup> we adopt the classification presented by Edwards & Meinertzhagen (2010) (see also Hartenstein, 2011; Parker & Auld, 2006 for further details). The total number of neuroglial cells in *Drosophila* CNS (which comprise  $\approx 90,000$  cells) does not exceed ten per cent.

- The first type of CNS glia is the *surface glia*, which makes the haemolymph-brain barrier and is subdivided into *perineural glia* (relatively small cells lying on the ganglionic surface) and *subperineural* or *basal glia* (represented by large sheet-like cells connected with a septate junction that forms the actual barrier).
- The second type is the *cortex glia* that contact neuronal cell somata in the CNS; each glial cell establishes contacts with many neurones.
- The third type is *neuropil glia*, which are located in the neuropil and cover axons and synapses. The neuropil glia are further subdivided into *ensheathing* or *fibrous cells* that enwrap axons, and *astrocyte-like glia*, which form a perisynaptic glial cover.
- Finally, there are *tract glial cells*, which cover axonal tracts connecting different neuropils.

There is further diversity of neuroglia in different regions of the *Drosophila* nervous system. For example, in the optic lamina (the optic neuropil), as many as six different glial cell types are distinguished (*fenestrated glia*, *pseudocartridge glia*, *distal and proximal satellite glia*, *epithelial glia* and *marginal glia*). Similarly, several classes of neuroglial cells have been described in the deep optic lobe. These include *giant optic chiasm glia*, *small outer optic chiasm glia*, *medulla satellite glia* and *medulla neuropil glia*. Several other small sub-populations of glia have been identified in the eye disk, optic stalk, and in the antennae. Finally, the peripheral nervous system of *Drosophila* contains *wrapping glia* that cover peripheral axons. A similar degree of complexity is also found in other insects, such as, for example, in the housefly *Musca domestica*, or in tobacco hornworm *Manduca sexta*.

When comparing Arthropods with Annelids, we see a rather significant development of glial diversity; these heterogeneous glia assume many new functions compared to lower phyla. An important new function of glia in Arthropods is the formation of the blood-brain barrier (or, to be precise, the haemolymph-brain barrier, because Arthropods do not have a closed circulatory system, nor do they have proper blood) that effectively separates the CNS from the rest of the body and segregates molecules allowed to enter the nervous system. In addition, glia separate the intra-brain tracheoles that supply CNS with air/oxygen.

As has already been mentioned, the haemolymph-brain barrier is sealed by septate junctions that play a role similar to tight junctions between endothelial cells of the blood-brain barrier in higher vertebrates. The haemolymph-brain barrier is formed solely by neuroglia and it is critical in controlling transport of ions (especially  $K^+$ , whose concentration rises high during feeding) and various nutrients. There are some indications that nutrients can cross the glial barrier by regulated endo/exocytosis. The glial cells also define the architecture of the insect CNS, by separating functionally distinct neuronal ensembles.

In insects, glial cells regulate ion balance in the intra-CNS fluids through the activity of  $Na^+$  and  $K^+$  ion pumps. In addition, glial cells redistribute ions from regions of high concentration through intercellular diffusion via gap junctions (formed by innexins) that connect glial cells into syncytia. Insect CNS glial cells are critical for homeostasis (clearance and recycling) of the principal neurotransmitter histamine in the retina, which is achieved through a shuttle operating between photoreceptors and surrounding glial cells.

In the CNS, glial cells also control glutamate homeostasis; *Drosophila* glia specifically express two types of excitatory amino acid transporters – dEAAT1 and dEAAT2 – which belong to the extended family of EAAT transporters, also present in vertebrates. Furthermore, insect neuroglia also express glutamine synthetase and thus may be involved in glutamate-glutamine shuttling with neurones. Incidentally, glutamate is a main neurotransmitter controlling sexual behaviour, and especially courtship, in *Drosophila*, and specific glial disruption of the glutamate transporter alters courtship behaviour though disrupting proper apprehension of male-associated pheromones, resulting in homosexual courtship attempts. *Drosophila* glia also

express an enzyme, dopa decarboxylase, which is needed for synthesis of 5-HT (serotonin) and may act as a supply of the latter. *Drosophila* glia are intimately involved in controlling circadian rhythms.

Trophic support of neurones is another fundamental function of insect glia. For example, in the honeybee retina, the glial cells (known as pigment cells) convert glucose/glycogen into alanin, which is then released and taken up by neurones and acts as an energy substrate. Neuroglial cells in insects are involved in various aspects of CNS development, for example by presenting localisation clues for migrating neurones. The neuroglia in insects are obligatory for neuronal survival, and neurones degenerate in several *Drosophila* mutants with altered glial functions. Finally, glial cells in insects are involved in brain defence and are already endowed with astrogliosis capabilities; in addition, insect neuroglia are capable of phagocytosis.

#### **(iv) Neuroglia in early Deuterostomia (Hemichordata and Echinodermata)**

The Hemichordata (Acorn worms) and Echinodermata (e.g. sea urchin, starfishes, sea cucumber) are currently considered to be sister phyla of Chordata; it is still unclear whether they represent a parallel evolutionary trait or are related to Chordata. The nervous system of Echinoderms consists of a circumoral nerve ring and radial nerve cords (five in most of the species).

When comparing neuroglia of Echinoderms to the Arthropods or Molluscs, the most surprising feature is an almost complete disappearance of different glial forms and an emergence of a new type of glia. These new glia are characterised by an elongated shape, long processes that span the whole thickness of the neural parenchyma, perpendicular orientation to the surface of the neuroepithelium and high level of expression of intermediate filaments in the cytoplasm. All in all, these cells are very similar to radial glia of higher vertebrates (Mashanov *et al.*, 2009).

The function of these radial glial cells is not really known, although they are actively involved in regeneration of the nervous system, being the precursors of newborn neurones and assisting migration of these neurones through the CNS tissue. These features very much resemble the main functions of radial glia in the vertebrate brain, and we may expound that the Deuterostomia developed a new organisation of the nervous system, relying on radial glia, that determines the layered organisation of the CNS. In addition, in some Echinoderms (e.g. sea cucumber), a few glia-like cells located in CNS parenchyma have been discovered, although neither their function, nor indeed their glial identity, are yet known. We know next to nothing about neuroglia in Hemichordata. The ganglia of *Cephalodiscus gracilis*, for example, were reported to contain no glia at all (Rehkamper *et al.*, 1987).

**(v) Neuroglia in low vertebrates** In the early vertebrates, the same tendency of prevalence of radial glia in some species can be observed. In elasmobranchii (chondrichthian fish such as sharks and rays), for example, two types of brains are distinguished – the ‘laminar’ type I and ‘elaborated’ type II. The type I brains are

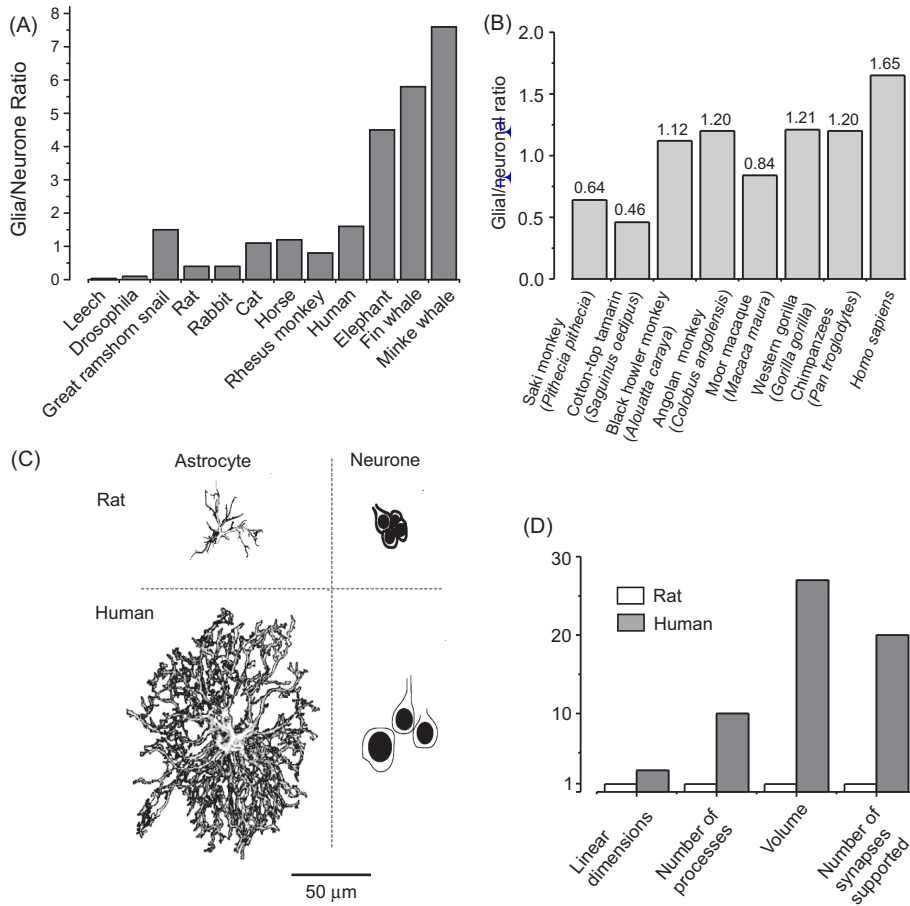
relatively thin with large ventricles; in this type of the brain neurones are mostly confined to the periventricular zone. The 'elaborated' brains are larger and thicker and neurones migrate away from the periventricular zone and form nuclei. Neuroglia in the 'laminar' brains are represented mainly by radial glia (also known as ependymoglia or tanycytes), whereas the parenchyma of 'elaborated' brains contains numerous astrocytes/astrocyte-like cells (Ari & Kalman, 2008).

The increase in parenchymal astrocytes in elaborated brains can be explained by an increase in surface-volume ratio of radial glial cells, with an increase in the thickness of the brain. This constrains homeostatic capabilities of the radial glia and, hence, prompts increase in the number/complexity of parenchymal astrocytes (Reichenbach *et al.*, 1987). Alternatively, the emergence of parenchymal astrocytes is explained in terms of increased complexity of vascularisation, requiring perivascular glial support that cannot be provided by radial glia (Wicht *et al.*, 1994). It is worth noting that astrocytes/neuroglia in sharks form the blood-brain barrier, and some of the capillaries are completely surrounded by astroglial process, being thus endocellular vessels (Abbott, 2005; Long *et al.*, 1968).

A similar preponderance of radial glia is observed in bony fish and in particular in teleosts (e.g. zebrafish). In zebrafish, radial glial cells extend through the entire width of the brain, from the ependymal coating of the ventricles to the pial surface of the brain. These radial glial cells express GFAP, have glutamine synthetase (indicating their possible role in glutamate homeostasis and metabolism) and express aquaporin-4 (indicating their role in water homeostasis – see Grupp *et al.*, 2010). The absence of parenchymal glia (astrocytes) is particularly important for the reactions of fish brains to injury. Insults to the teleost fish brain do not trigger astrogliosis; stab wounds, for example, are closed rapidly (in several days) without formation of the scar. Instead of an astrogliotic response, the zebra fish increase neurogenesis, which most likely provides new cells to fill the wound (Baumgart *et al.*, 2010). Importantly, in teleosts, the blood-brain barrier is shifted to ependymal cells. This arrangement remains in all higher vertebrates, although tight junction proteins are found in glia in the optic nerve of some species.

**(vi) Glial advance in higher vertebrates** Neuroglia attained maximal development in mammals. Moreover, an increased complexity of the brain, together with an increased intellectual power, was accompanied by remarkable increases in the numbers and complexity of glia (Oberheim *et al.*, 2006; Reichenbach, 1989). This coincided with similarly remarkable increases in glial diversity and in glial functions. The evolutionary increase in astroglial complexity is particularly obvious in the brains of primates, and especially in the brain of humans (Oberheim *et al.*, 2009).

Human astrocytes are much larger and far more complex than those in the rodent brain (Figure 3.5). In the human brain, the average diameter of belonging to a human protoplasmic astrocyte is  $\approx 2.5$  times larger than that formed by an equivalent rat astrocyte (142  $\mu\text{m}$  vs. 56  $\mu\text{m}$ ). The volume of the human



**Figure 3.5** Phylogenetical advance of neuroglia.

- A. Glia-to-neurone ratio in the nervous system of invertebrates and in the cortex of vertebrates. Glia-to-neurone ratio is generally increased in phylogeny; this ratio more or less linearly follows an increase in the size of the brain.
- B. The glia/neurone ratio in the cortex of higher primates; this ratio is highest in humans (Data taken from Sherwood *et al.*, 2006).
- C. Graphic representation of neurones and astroglia in mouse and in human cortex. Evolution has resulted in remarkable changes in astrocytic dimensions and complexity.
- D. Relative increase in glial dimensions and complexity during evolution. Linear dimensions of human astrocytes, when compared with mice, are  $\approx 2.75$  times larger, and their volume is 27 times larger; human astrocytes have  $\approx 10$  times more processes and every astrocyte in human cortex enwraps  $\approx 20$  times more synapses.

C, D adapted, with permission, from Oberheim *et al.*, 2006.

protoplasmic astrocyte domain is  $\approx 16.5$  times larger than that of the corresponding domain in a rat brain. Likewise, fibrous astrocytes populating the white matter are  $\approx 2.2$  times larger in humans compared to rodents. Human protoplasmic astrocytes have  $\approx 10$  times more primary processes, and correspondingly much more complex processes arborisation than rodent astroglia (Oberheim *et al.*, 2006). As a result, human protoplasmic astrocytes contact and integrate  $\approx 2$  million synapses residing in their territorial domains, whereas rodent astrocytes cover  $\approx 20,000$ – $120,000$  synaptic contacts (Bushong *et al.*, 2002; Oberheim *et al.*, 2009).

The brains of primates contain specific astroglial cells which are absent in other vertebrates (Oberheim *et al.*, 2009; and see Chapter 4). Most notable of these are the interlaminar astrocytes (Colombo & Reisin, 2004; Colombo *et al.*, 2004; Colombo *et al.*, 1995), which reside in layer I of the cortex; this layer is densely populated by synapses but almost completely devoid of neuronal cell bodies. These interlaminar astrocytes have a small cell body ( $\approx 10 \mu\text{m}$ ), several short and one or two very long processes. The latter penetrate through the cortex and end in layers III and IV; these processes can be up to  $1 \mu\text{m}$  long. The endings of the long processes create a rather unusual terminal structure, known as the ‘terminal mass’ or ‘end bulb’, which is composed of multilaminar structures containing mitochondria.

Incidentally, the processes of interlaminar astrocytes and size of ‘terminal masses’ were particularly large in the brain of Albert Einstein (Colombo *et al.*, 2006), although whether these features were responsible for his genius is not really proven. The function of these interlaminar astrocytes remains completely unknown, although it has been speculated that they are the astroglial counterpart of neuronal columns, which are the functional units of the cortex, and that they may be responsible for a long-distance signalling and integration within cortical columns. Interestingly, interlaminar astrocytes are altered in Down syndrome and Alzheimer’s disease.

Human brains also contain polarized astrocytes, which are uni- or bipolar cells that dwell in layers V and VI of the cortex, quite near to the white matter; they have one or two very long (up to  $1 \mu\text{m}$ ) processes that terminate in the neuropil. The processes of these cells are thin ( $2$ – $3 \mu\text{m}$  in diameter) and straight, and they also have numerous varicosities. Once more, the function of polarized astrocytes remains enigmatic, although they might be involved in para-neuronal long-distance signalling.

The evolution of neurones produced fewer changes in their appearance – that is, the density of synaptic contacts in rodents and primates is very similar (in the rodent brain, the mean density of synaptic contacts is  $\approx 1397$  millions/ $\mu\text{m}^3$ , which is not much different from humans, where synaptic density in the cortex is  $\approx 1100$  millions/ $\mu\text{m}^3$ ). Similarly, the number of synapses per neurone does not differ significantly between primates and rodents. The shape and dimensions of neurones also have not changed dramatically over the phylogenetic ladder. Human neurones are certainly larger, yet their linear dimensions are only about 1.5 times greater than in rodents. Thus, at least morphologically, evolution resulted in far greater changes in glia than in neurones, which most likely has important, although yet undetermined, significance.



### 3.3.2 Evolution of myelination

The problem of nerve impulse propagation was always a challenge to evolutionary development of multicellular organisms. Increase in animal size obviously requires faster nerve conductance, which in the simplest way, could be achieved through an increase in axon diameter (Hartline & Colman, 2007). Indeed, increase in axon diameter reduces resistance of the axon proportionally to the square of diameter, and the conductance velocity is directly proportional to the square root of the axon diameter (Hodgkin, 1954). This strategy was employed by many invertebrates, including Annelids, certain crustacea and Molluscs. In *Loligo* squid, for example, large axons (0.5  $\mu\text{m}$  in diameter) propagate action potentials with a velocity of up to 30 m/s.

Increase in axon diameter, however, implies at least two fundamental limitations which are incompatible with complex nervous systems. First, the conduction through large axons is energetically costly, because substantial  $\text{Na}^+/\text{K}^+$  pumping is needed to maintain the ion gradients. Second, large axons apply severe space constraints; for example, if the human optic nerve were composed from large axons similar to those of the squid, the diameter of the nerve would have to exceed 0.75 m (see Chapter 5).

An alternative strategy was the development of the myelin sheath, in which axons are coated with multiple layers of lipid membranes. These membranes are interrupted by gaps known as nodes of Ranvier (see Chapters 5 and 7 for details), in which the axolemma is rich in voltage-operated ion channels that generate the action potential. These lipid-rich membranes insulate parts of the axons between the nodes, thus increasing axonal transverse resistance and reducing transverse capacitance. This allows saltatory propagation of action potentials, which substantially increases the conduction velocity in relatively small diameter axons (with maximal conduction velocity in vertebrates reaching 100–120 m/s). It has been generally accepted that myelination first occurred in vertebrates, which represented a fundamental evolutionary step that allowed development of compact nervous systems with many commissural axons, connecting neurones from different parts of the CNS and allowing rapid propagation through intra-CNS tracts and peripheral nerves (Zalc, 2006).

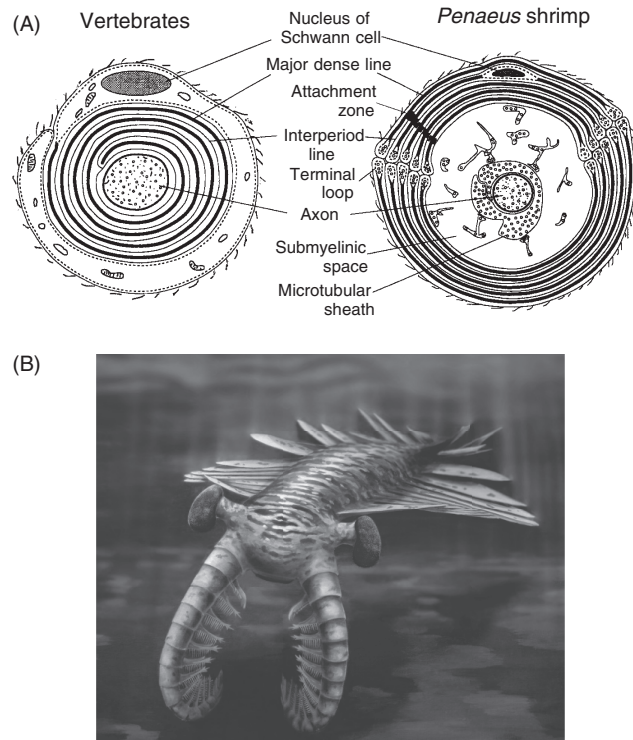
Myelin proper is, indeed, found only in relatively developed vertebrates. Compacted myelin sheaths are absent in lower vertebrates, such as hagfish and lampreys, and began to develop in sharks and bony fish. It has been suggested that myelination emerged for the first time in placoderms (extinct early jawed armoured fish that lived in the early Silurian period,  $\approx 420$  million years ago), which are phylogenetically placed at the base of Chondrichthyan and bony fishes. This suggestion is based on the fossil record which compared the foramina for oculomotor nerves in jawless primitive Osteostraci fishes (that are believed to lack myelination) and Placoderms. The diameter of nerve foramina between these two fishes is the same (about 0.1  $\mu\text{m}$ ), whereas the length of nerve in Placoderms was 10 times larger, which

logically incurred the need for myelin to preserve the same duration of action potential-mediated signal transduction (Zalc *et al.*, 2008).

Further reasoning suggests the connection between appearance of the jaw in early Gnatostomata (the jawed vertebrates, which embrace all higher vertebrates living today, including mammals) and myelination. By acquiring myelinated nerves, these fishes arguably acquired better ability to hunt their prey, while keeping the axonal diameter the same or even smaller compared to their jawless predecessors (Zalc *et al.*, 2008). This is all speculation, but what we know is that Agnathans do not have myelin, whereas even the most primitive Elasmobranchii and Holocephalans (i.e. sharks, rattfishes and chimera fish) have well developed myelin sheaths with nodes of Ranvier, this general structure persisting in all higher vertebrates.

The ensheathing of axons (which does not really involve compacted myelin) had, however, appeared much earlier in evolution (see reviews of Bullock, 2004; Hartline & Colman, 2007; Roots, 2008). Several invertebrate species (most notably some Annelids and Crustaceans) have well defined periaxonal coverage, and these covered axons conduct action potentials with high velocity. For example, in the earthworm *Lumbricus terrestris*, the central axons of 50–100  $\mu\text{m}$  in diameter are ensheathed with more than 60–200 layers of cell membranes produced by many cells, nuclei of which are scattered along the axon. These are glial cells which send processes to wrap the axon. The whole structure does not have clearly identifiable nodes, yet the conductance velocity of 20–45 m/s is greater than that in the much thicker giant axons of the *Loligo* squid. Similar axonal coverage has been found in another group of marine Annelids, Phoronids, in which axons are wrapped with many (9–20) layers of membranes. In the aquatic sludge worm, *Branchiura sowerbyi*, the axon is enwrapped by about 50 membrane layers.

The most striking example of invertebrate axonal ensheathment is found in Crustaceans – in particular, in prawns, shrimps and crabs. In the prawns of the genus *Penaeus* (e.g. Japanese tiger shrimp or Chinese white shrimp), the axonal-glial structure is quite peculiar. First, the diameter of the axon is much smaller compared to the overall fibre diameter. The axon is surrounded by glial membranes and by a large, so-called, submyelinic space, lying between the axonal membrane and the first layer of glial membranes (Figure 3.6). During excitation, the ion currents are trapped in this space as if the normal axon is surrounded by a giant axon (the submyelinic space acts, in essence, as a low-resistance pathway), which gives an unprecedented functional result as the prawn's fibres (120  $\mu\text{m}$  in diameter) conduct action potentials with the speed of up to 210  $\mu\text{s}$  (Kusano, 1966; Xu & Terakawa, 1993, 1999). The submyelinic spaces are tightly sealed at nodes, known as 'fenestration nodes', thus allowing for saltatory conduction. The nodal diameter and internodal distance are proportional to the axon diameter, and in prawns vary between 5–50  $\mu\text{m}$  and 3–12  $\mu\text{m}$ , respectively. The thickness of the glial membranous sheath is  $\approx 10 \mu\text{m}$ , and it is comprised of 10–60 layers, with 8–9 nm distance between them. Similar to vertebrates, voltage-operated sodium channels in prawns are concentrated at the nodes, where their density can reach in the order of thousands of channels/ $\mu\text{m}^2$ .



**Figure 3.6** Myelin-like sheath in shrimps.

- A. Structure of the myelin sheath in vertebrates and myelin-like sheath in *Penaeus* shrimp. The myelin sheath of vertebrates is tightly wrapped around the axon and forms a continuous spiral of membrane, with the nucleus of the Schwann cell on the outer edge of the sheath. The myelin-like sheath of the shrimp is separated from the axon by the submyelinic space, which acts as an outer axon; the nuclei of the Schwann cells are located within the sheath.
- B. The predecessor of modern shrimps was the metre-long swimming invertebrate *Anomalocaris*, the top predator in the Cambrian ocean more than 500 million years ago. These giant shrimps had exceptionally complex external eyes, composed of many tens of thousands of lenses. Arguably, these predators require fast propagating and compact axons, which could indicate that they were the first to develop myelin-like sheath.

Reproduced, with permission, from Xu, K. and Terakawa, S. Fenestration nodes and the wide submyelinic space form the basis for the unusually fast impulse conduction of shrimp myelinated axons. *J Exp Biol*, 202 (Pt 15), 1979–1989 (1999) © the Company of Biologists. The Journal of Experimental Biology: [jeb.biologists.org](http://jeb.biologists.org). Reproduced with permission from Nature (cover picture for v. 480).

There is a fundamental difference between vertebrates and prawn axonal coverage. In vertebrates, the single Schwann cell or process of oligodendrocyte spirals around the axon, forming multiple membranous lamellae; in prawns, a single myelinating

glial cell sends multiple processes, each of which encircles the axon once. Another difference is location of the nuclei of the myelinating cell. In vertebrates, this is always located at the outer edge of myelin sheath, whereas in prawns, the nuclei are randomly located between membrane laminae (Xu & Terakawa, 1999).

What was the evolutionary pressure leading to the appearance in prawns of axonal systems with exceedingly high conduction velocities? This may have an ancient phylogenetic root. In the Cambrian period (500–540 millions years ago), the giant prawns, the *Anomalocaridids*, were the largest and most ferocious predators of the sea (Van Roy & Briggs, 2011). Their length exceeded one metre and they had particularly acute vision. The compound eye of the *Anomalocaridid* was exceptionally big (according to fossil measurements, the visual surface was 22  $\mu\text{m}$  long and 12  $\mu\text{m}$  wide) and it was composed of tens of thousands of hexagonal ommatidial lenses,  $\approx 70\text{--}110 \mu\text{m}$  in diameter (Paterson *et al.*, 2011). Obviously, the rapid nervous conductance and the reduction in size provided by the appearance of a glial sheath, in conjunction with submyelinic space, would have been of paramount importance for the evolution of such a complex visual system, with the need to rapidly collect information from these tens of thousands of lenses and to support the successful predatory behaviour of such a large animal.

Notably, mammals also retain a population of oligodendrocyte progenitor cells (OPCs) in the adult, that are capable of regenerating oligodendrocytes throughout life (see Chapters 5 and 6). The abundance of these NG2-glia in the adult mammalian CNS has begged the question of the evolutionary benefit of retaining such a substantive surplus of cells. The brain of adult non-mammalian vertebrates exhibits a high proliferative and neurogenic activity, which is the function of radial glia in the telencephalic ventricular zones. In adult mammals, parenchymal NG2-glia are the main proliferating cells. Although there is evidence for NG2-glia or adult OPCs in fish and frogs, they do not appear to respond to insults by increased proliferation. It is possible that the remyelinating capacity of NG2-glia (adult OPCs) is a mammalian evolutionary development and reflects the greater complexity and cellular specialization in the mammalian brain.

In conclusion, myelination emerged early in evolution. Most likely, it developed from the neuroglia that contacted axons with purely structural and metabolic purposes. Once it had occurred, myelination gave obvious evolutionary advantages, the most important being an increase in compactness of the nervous system and reduction in energy expenditure for restoring ion balances. Parallel evolution is evident, and different phyla developed their own arrangements. The most peculiar of these are the prawn nerve fibres which, at the same time, are characterised by the fastest conduction velocities.

### 3.3.3 Evolution of microglia

The evolutionary origins of microglia remain largely unexplored. However, it is conceivable to assume that they appear in response to formation of the ancient

nervous system barriers following the emergence of compact neuronal masses. The appearance of these body/nervous system barriers obviously restricted immune/defence cells from entering the neural masses, thus leaving nervous tissue unprotected against possible insults. The evolutionary response was achieved by migration of immune cells into neuronal ganglia, where they changed their phenotype and become innate immune/defence cells of the nervous tissue. The evidence for phylogenetically early microglial cells is available for Annelids (leech), Molluscs (bivalva and snails) and some Arthropods (insects) (see Kettenmann *et al.*, 2011 for detailed review).

The leech nervous system has a surprisingly high density of microglial cells. In other cases, microglia are small with a spindle-like shape. Following injury, leech microglia migrate to the site of lesion, change their morphology and acquire phagocytic properties. Activated microglia in the leech can be stained by weak silver carbonate, a classical probe for vertebrate microglia. The leech microglial cells are also implicated in production of antimicrobial peptides in response to infectious attack (Schikorski *et al.*, 2008).

Microglial cells are present in the ganglia of Molluscs. For example, microglia in the mussel *Mytilus edulis*, a marine bivalve, can migrate in response to various signals, including NO, opioid peptides, cannabinoids and cytokines. Similarly, migrating microglia have been found in the snail *Planorbarius corneus* and in the insect *Leucophaea maderae*. In another snail, *Planorbis corneus*, the microglial cells (morphologically distinguished by phagocytic inclusions) are concentrated in the ganglia neuropil and subcapsular cortex, and the number of these phagocytic cells increases substantially after mechanical lesion (Pentreath *et al.*, 1985).

### 3.4 Numbers: how many glial cells are in the brain?

How many glial cells are there in the nervous system and, in particular, how many glial cells are in the human brain? Numerous papers and monographs (including the first edition of our book *Glial Neurobiology*) stated that, in the human brain, glial cells outnumber neurones by a factor of ten. However, this statement seems to be incorrect. In principle, we still do not know the exact number of neural cells in the human brain, but the experimental data obtained from stereological and nuclear counts indicate that, overall, the numbers of neurones and non-neuronal cells in the human brain is roughly equal.

In general, there is an assumption that the evolution of the nervous system resulted in an increase in the number of glia. Indeed in *C. elegans*,  $\approx 50$  neuroglia co-exist with  $\approx 300$  neurones; in the leech, each ganglia contains  $\approx 400$  neurones and only 10–12 neuroglial cells; in *Drosophila*, only about 9000 neuroglia populate the CNS, containing ten times more neurones. There are exceptions, however; for example, the buccal ganglia of the great ramshorn snail *Planorbis corneus* contains 298 neurones and 391 glial cells, giving the glia to neurone ratio  $\approx 1.5$  (Pentreath

*et al.*, 1985), with glial cells occupying about 43 per cent of the ganglia volume and neurones about 33 per cent.

In vertebrates, it is generally agreed that glia to neurone ratios in the cortex increase with an increase of the size of the brain. According to different estimates, the glia to neurone ratio in the cortex is about 0.3–0.4 in rodents,  $\approx 1.1$  in the cat;  $\approx 1.2$  in the horse, 0.5–1.0 in the Rhesus monkey, somewhere between 1.5–1.7 in humans, and as high as 4–6 in elephants and in the fin whale *Balaenoptera physalus* (Figure 3.5; for details see Christensen *et al.*, 2007; Dombrowski *et al.*, 2001; Friede, 1954; Hawkins & Olszewski, 1957; Lidow & Song, 2001; Oberheim *et al.*, 2006; Reichenbach, 1989; Tower, 1954).

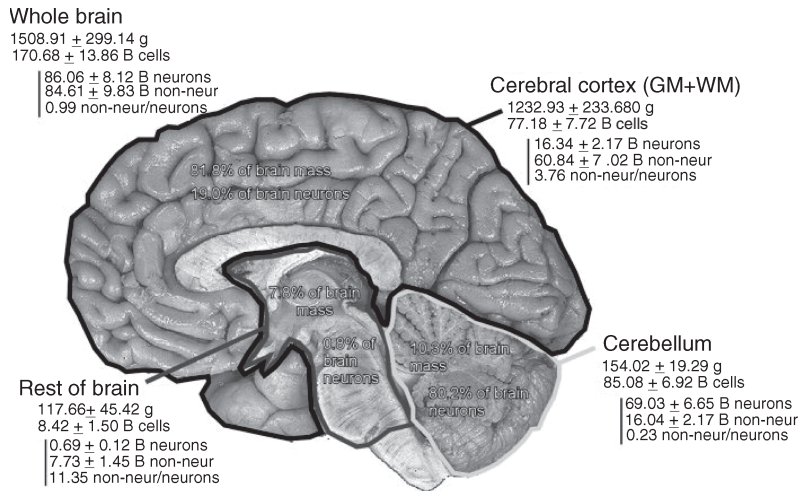
Rather surprising counts have been obtained for cortices of Göttingen miniature pigs, which (in adulthood) contain 324 million neurones and 714 million neuroglia, with a glia to neurone ratio of 2.2 (Jelsing *et al.*, 2006). The largest number of glia have been found in the neocortex of the common Minke whale (*Balaenoptera acutorostrata*), which contains  $\sim 12.8$  billion neurones and 98 billion glia, giving therefore a glia to neurone ratio of  $\sim 7.6$  (Eriksen & Pakkenberg, 2007).

The total cellular count of the cells in the human brain, however, remains quite enigmatic, because of many methodological difficulties. Stereological counts of neurones in the human cortex, for example, have yielded rather different results, with overall numbers of neurones varying between 7–10 and 28–39 billion (Lent *et al.*, 2012; Pakkenberg, 1966). The number of cells in the cerebellum has been estimated to be the largest in the brain, with counts ranging between 70–109 billion (Andersen *et al.*, 1992; Lange, 1975).

A direct approach to this problem was taken in recent years by Brazilian neuroanatomists, who used the so-called ‘isotopic fractionation’ for counting the total number of neuronal and non-neuronal cells in mammalian brains (Azevedo *et al.*, 2009; Herculano-Houzel & Lent, 2005). In this technique, the brains are homogenized and the total number of nuclei is counted; subsequently, the neuronal nuclei are stained with antibodies against neurone-specific neuronal Nuclei protein (NeuN) and counted. The remaining non-stained nuclei apparently reflect the total number of non-neuronal cells (which naturally include glia as well as other non-neuronal cells, such as vascular cells).

Using this technique, the total number of cells in the rat brain was estimated at 330 million, of which 200 million are neurones, thus giving a non-neuronal (glial) cells to neurone ratio of  $\approx 0.65$ . When the same technique was applied to the human brain, the total number of neurones and non-neuronal cells appeared to be almost equal: there were on average 86 billion neurones and about 84 billion non-neuronal cells in the brains of adult (50–70 years old) human males (Figure 3.7; Azevedo *et al.*, 2009).

Of course, the total numbers do not reflect the diversity of the nervous system, different regions of which have a very different cytoarchitecture. The nuclear counts confirmed this diversity by showing very different numbers for the glia to neurone ratio for different parts of the brain. The lowest ratio ( $\approx 0.22$ ) was found for the



**Figure 3.7** Cell numbers (neurones vs. non-neuronal cells) in different regions of the human brain. Values represent mean  $\pm$  standard deviation. Cell numbers were determined by isotopic fractionation, which determines total number of nuclei, and counts of NeuN positive nuclei give the number of neurones.

Reproduced, with permission, from Azevedo, F. A. C. et al. (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *Journal of Comparative Neurology* pp.532-541 © John Wiley & Sons Ltd.

cerebellum, with 70 billion neuronal nuclei and only 16 billion non-neuronal ones. In the cerebral cortex (i.e. in both grey and white matters), the ratio was  $\approx 3.76$ , with 60 billion non-neuronal cells and 16 billion neurones, whereas in basal ganglia the non-neuronal cells to neurones ratio was  $\approx 11.3$  with 0.69 billion neurones and 7.73 billion non-neuronal cells (Azevedo *et al.*, 2009; Lent *et al.*, 2012).

This ‘isotopic fractionation’ technique can not be considered flawless, of course. We do not know how many nuclei are lost in the process, how accurate is the staining, or how good is the spatial resolution. The numbers also have to be treated with caution; they were obtained from analysing only four different brains, from relatively old humans. Finally, this technique does not discriminate between subtypes of non-neuronal cells, and neither does it provide information about numbers of astrocytes, oligodendrocytes, NG2-glia and microglia.

Stereological counts from 31 human post-mortem tissues provided the following numbers of neurones and glia for neocortex (Pelvig *et al.*, 2008). The total number of neurones was 21.4 billion in females and 26.3 billion in males; the total number of glial cells was 27.9 billion in females and 38.9 billion in males. This gives an overall glia to neurone ratio of  $\approx 1.3$ . In this work, the authors also tried to calculate the relative numbers of glial cell types, and they found that astrocytes accounted for  $\approx 20$  per cent, oligodendrocytes for 75 per cent and microglia for 5 per cent of the total glial cell population. The identifying criteria, however, were rather doubtful, since no specific staining was employed. For example, oligodendrocytes were

defined as cells localised in close proximity to neurones or blood vessels, with a small rounded or oval nucleus with dense chromatin structure and a perinuclear halo; NG2-glia were not considered at all. In the absence of specific staining, the counts for glial subtypes should be taken with caution. In the earlier morphological studies, based on 2D counting, the distribution of glial cell types was found to be: astrocytes 40 per cent, oligodendrocytes 50 per cent and microglia 5–10 per cent (Blinkow & Glezer, 1968).

Detailed analysis of the glia to neurone ratio was performed on several primates from very primitive monkeys, such as tamarins and Saki monkey, through gorillas and chimpanzees, to humans (Sherwood *et al.*, 2006). In this study, the cell numbers were counted in special areas of cortex associated with complex tasks such as memory (area 9L in prefrontal cortex), speech-related Broka area (area 44) and anterior paracingulate cortex associated with theory of mind (area 32), as well as in primary motor cortex (area 4). It turned out (see also Figure 3.6) that human cortices have a higher glia to neurone ratio compared to all other primates, which was paralleled by a very substantial increase in human brain size (the heaviest primate brain, i.e. gorilla, weighs on average 509 gm, whereas the average human brain weighs  $\approx 1,373$  g).

An increase in glia to neurone ratio in mammalian evolution most likely reflects an increase in neuronal energy expenditure and, hence, a need for more support provided by glia. Indeed, it has been calculated that human neurones need about 3.3 times more energy to fire a single spike and 2.6 times more energy to maintain the resting membrane potential, when compared to rodents (Lennie, 2003). Another pressure is certainly provided by an increased activity of synaptic transmission and, hence, higher demand for homeostatic clearance/maintenance of balance of neurotransmitters and ions.

## 3.5 Embryogenesis and development of neuroglia in mammals

### 3.5.1 Macroglial cells

All neural cells (i.e. neurones and macroglia) derive from the neuroepithelium, which forms the neural tube. These cells are pluripotent, in a sense that their progeny may differentiate into neurones or macroglial cells with equal probability, and therefore these *neuroepithelial cells* may be defined as true '*neural progenitors*'. These neural progenitors give rise to neuronal or glial precursor cells ('neuroblasts' and 'glioblasts', respectively), which in turn differentiate into neurones or macroglial cells. For many years, it was believed that the neuroblasts and glioblasts appear very early in development, and that they form two distinct and non-interchangeable pools, committed, respectively, to producing strictly neuronal or glial lineages. It was also taken more or less for granted that the pool of precursor cells is fully depleted around birth, and that neurogenesis is totally absent in the mature brain.



In recent decades, however, this paradigm has been challenged as it appears that neuronal and glial lineages are much more closely related than was previously thought, and that the mature brain still has numerous stem cells which may provide for neuronal replacement. Moreover, it turns out that neural stem cells have many properties of astroglia. All these matters will be discussed in more detail in Chapter 4.

The modern scheme of neural cell development is as follows. At the origin of all neural cell lineages lie neural progenitors in the form of neuroepithelial cells. Morphologically, neural progenitors appear as elongated cells extending between the two surfaces (ventricular and pial) of the neuronal tube. Very early in development, the neural progenitors give rise to *radial glial cells*, which are the first cells that can be distinguished from neuroepithelial cells. The somata of radial glial cells are located in the ventricular zone and their processes extend to the pia. These radial glial cells are the central element in subsequent neurogenesis, because they act as the main neural progenitors during development, giving rise to neurones, astrocytes and some oligodendrocytes. The majority of oligodendrocytes, however, originate from glial precursors that are generated in specific sites in the brain and spinal cord (see below).

Astrocytes are generated both from radial glia and, later, in development from specific glial precursors that also give rise to oligodendrocytes; the proportion of the final population of astrocytes derived from radial glia and glial precursors depends on the region of the CNS. Radial glia not only produce neurones, they also form a scaffold along which newborn neurones migrate from the ventricular zone to their final destinations (see Chapter 4). Moreover, descendants of radial glia persist in specific neurogenic regions of the adult brain and retain the function of stem cells.

Oligodendrocytes develop from committed glial precursors through several intermediate stages (Goldman, 2007), which have been thoroughly characterized in culture systems, by using several specific antibodies (see Chapter 5). The developmental origins of astrocytes is less clear than that of neurones and oligodendrocytes. Some astrocytes appear to arise from astrocyte progenitors that migrate to their different sites in the brain, while others are derived from radial glia. In the perinatal cortex astrocytes retain proliferative capabilities and most new astroglial cells arise from symmetric division of differentiated astrocytes (Ge *et al.*, 2012). Some astrocytes derive embryonically from glial precursors with the phenotype of oligodendrocyte progenitor cells (OPCs), which fate-mapping studies show generate protoplasmic astrocytes as well as oligodendrocytes in the forebrain. In the forebrain, glial precursors from the subventricular zone migrate into both white matter and cortex, to become astrocytes, oligodendrocytes and NG2-glia (as well as some interneurones).

In the cerebellum, some Bergmann glia and other astrocytes arise from radial glia (and some share a common lineage with Purkinje neurones) and, later in development, glial progenitors migrate from an area dorsal to the IV<sup>th</sup> ventricle to give rise to all types of cerebellar astrocytes, myelinating oligodendrocytes and NG2-glia (as well as interneurones). In the embryonic retina, common precursors give rise to both

neurones and Müller glia. Glial precursors that migrate into the retina via the optic nerve give rise to astrocytes, but oligodendrocytes and NG2-glia are absent from the retina of most mammals. Astrocytes and oligodendrocytes in the spinal cord appear to arise from different precursors in separate areas of the ventricular zone. The ventral neuroepithelium of the embryonic cord is divided into a number of domains, which contain precursors that first generate neurones (motor neurones and interneurones) and then oligodendrocytes. Astrocytes most likely arise from radial glia.

### 3.5.2 Astroglial cells are brain stem cells

Neurogenesis in the mammalian brain occurs throughout the life span in specific neurogenic regions. New neurones that continuously appear in the adult brain are added to neural circuits, and these may even be responsible for the considerable plasticity of the latter. The appearance of new neurones does not happen in all brain regions of mammals; it is mainly restricted to hippocampus and olfactory bulb (although, in many non-mammalian vertebrates, neurogenesis occurs in almost every brain region).

In both hippocampus (in its subgranular zone) and in the subventricular zone (the latter produces neurones for the olfactory bulb), the stem cells have been identified as astrocytes. It remains unclear whether astroglial cells in other brain regions may also retain these stem cell capabilities (See Chapter 4 for more detailed discussion).

### 3.5.3 Peripheral glia and Schwann cell lineage

Peripheral glia arise from neural crest cells (see Chapter 8). The Schwann cell lineage starts from *Schwann cell precursors*, which are the progeny of neural crest cells. Neural crest cells also give rise to peripheral sensory and autonomic neurones and their associated satellite glia, as well as the neurones and glia of the enteric nervous system. By around the time of birth, Schwann cell precursors have developed into *immature Schwann cells*, and the latter differentiate into myelinating or non-myelinating Schwann cells. An important juncture in the progression of the Schwann cell lineage occurs when some of the immature cells establish contacts with large-diameter axons and commence the process of myelination (see Chapter 8). Immature Schwann cells that happen to associate with small diameter axons remain non-myelinated. An important difference between non-myelinating and myelinating Schwann cells is that the former maintain contacts with several thin axons, whereas myelinating Schwann cells always envelop a single axon of large diameter.

Schwann cell precursors and immature Schwann cells are capable of frequent division, and proliferation stops only when cells arrive at their terminal differentiation stage. However, mature Schwann cells (both myelinating and non-myelinating) can swiftly de-differentiate and return into the proliferating stage, similar to immature cells. This de-differentiation process underlies the Wallerian degeneration that

follows injury of peripheral nerves (see Chapter 9). After completion of nerve regeneration, Schwann cells once more re-differentiate.

### 3.5.4 Microglial cell lineage

Microglial cells derive from the myelomonocytic lineage, which in turn develops from hemangioblastic mesoderm (see Chapter 7). The progenitors of microglial cells, the primitive myeloid progenitors originating from the extraembryonic yolk sac enter the neural tube at early embryonic stages (e.g. at embryonic day 8 in rodents). These **foetal macrophages** are tiny rounded cells which, in the course of development, transform into embryonic microglia that have a small cell body and several short processes. The second wave of myeloid cell migration into the brain occurs in the early postnatal period, mainly in the corpus callosum where the dense groups of **amoeboid microglial cells** are defined as **fountains of microglia**. The amoeboid microglial cells proliferate very rapidly and migrate into the cortex, where they settle and turn into *ramified resting microglia*.

Microglia play an essential phagocytic role during development, removing the debris that arises from the large degree of neural apoptosis during development. Microglia can be 'killers' as well as 'cleaners' in the developing CNS. For example, in the embryonic retina, immature neurones express low affinity p75 receptors for nerve growth factor (NGF), which are down-regulated as neurones mature, but excess neurones do not lose their receptors and die by apoptosis in response to NGF released by microglia. Microglia are also responsible for immune tolerance to CNS antigens, by migrating into the embryonic CNS and providing a memory of 'self' before the blood-brain barrier is formed, after which the CNS becomes immune privileged and largely isolated from the systemic immune system. Amoeboid microglia in the developing CNS express many antigenic markers in common with systemic macrophages, but these are down-regulated as they differentiate into resting microglia. Any insult to the CNS results in the activation of microglia, which regain an amoeboid morphology, macrophage antigens and a phagocytic function.

Microglial cells retain their mitotic capabilities and they continue to divide (albeit at a very slow rate) in the adult.

Following insults to the adult CNS, macrophages may again enter the brain and are often indistinguishable from resident activated microglia. In these cases, most studies do not distinguish between microglia and macrophages (generally identified antigenically), and the terms are used interchangeably.

## 3.6 Concluding remarks

Glial cells appeared and evolved several times in phylogeny. The very first neuroglia were associated with sensory organs, where they assisted neuronal function. Increase in the complexity of the nervous system and its centralisation and cephalisation increased the demand for homeostatic support that was provided

by more complex neuroglia. In invertebrates, glial cells diversified and assumed many homeostatic functions related to control of ion and neurotransmitter homeostasis, metabolic support, and regulation of neuronal development.

Glial cells also formed the ancestral blood-brain barrier, thereby isolating the nervous system from the rest of the body. This isolation stipulated the further development of glial cell defensive functions, which led to an appearance of the astroglial response and emergence of phagocytotic microglia. In parallel, increases in animal size and complexity of interneuronal connections stimulated the development of the myelin sheath, which first appeared in invertebrates in several primitive forms, then was further developed in vertebrates. The evolution of myelination formed the basis for increased complexity of the nervous system that relies on interneuronal connections.

In early ancestors of vertebrates, and in early Chordata, a new type of glia – the radial glia – appeared; this was connected with the appearance of a multilayered brain. In the early forms, the radial glia dominated. An increase in brain thickness triggered another wave of evolution of astroglia that developed into the main homeostatic cell of the brain. Increased brain size coincided with an increase in the total glia to neurone ratio to provide increased support to mammalian neurones. Finally, in the brains of primates, and especially in the brains of humans, the astrocytes become exceedingly complex, and new types of astroglial cells, involved in interlayer communication/integration, have evolved.

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