Müller Cells in the Healthy and Diseased Retina

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Contents

Abbreviations

A	adenosine
AC	amacrine cell
ADP	adenosine 5'-diphosphate
AG	astroglia
AGE	advanced glycation end product
AMP	adenosine 5'-monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANP	atrial natriuretic peptide
AP-1	activator protein-1
Apo	apolipoprotein
AQP	aquaporin
ATP	adenosine 5'-triphosphate
BC	bipolar cell
Bcl	B cell lymphoma
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor, FGF-2
BK	big conductance potassium
BL	basal lamina
BMP	bone morphogenetic protein
BV	blood vessel
BzATP	2'-/3'-O-(4-benzoylbenzoyl)-ATP
CA	carbonic anhydrase
cAMP	cyclic adenosine 5'-monophosphate
cGMP	cyclic guanosine 5'-monophosphate
CNS	central nervous system
CNTF	ciliary neurotrophic factor
CRALBP	cellular retinaldehyde-binding protein
Crx	cone rod homeobox
CTGF	connective tissue growth factor
DHA	docosahexaenoic acid
Dkk	dickkopf
DNA	desoxyribonucleic acid
DOPA	dihydroxyphenylalanine

Dp	dystrophin gene product
EAAT	excitatory amino acid transporter
EGF	epidermal growth factor
EPSC	excitatory postsynaptic current
ER	endoplasmic reticulum
ERK1/2	extracellular signal-regulated kinases 1 and 2, p44/p42 MAPKs
FGF	fibroblast growth factor
flt-1	fms-like tyrosine kinase-1, VEGF receptor-1
G	GTP-binding
GABA	γ-aminobutyric acid
GAT	GABA transporter
GC	ganglion cell
GCL	ganglion cell layer
GDNF	glial cell line-derived neurotrophic factor
GFAP	glial fibrillary acidic protein
GFR	glial cell line-derived neurotrophic factor receptor
GLAST	glutamate-aspartate transporter
GLT	glutamate transporter
GluR	AMPA receptor subunit
GlyT	glycine transporter
GSSG	glutathione disulfide
GTP	guanosine 5'-triphosphate
HB-EGF	heparin-binding epidermal growth factor-like growth factor
HDL	high-density lipoprotein
H-E	hematoxylin-eosin
HGF	hepatocyte growth factor
HVA	high threshold voltage-activated
ICAM	intercellular adhesion molecule
IGF	insulin-like growth factor
IGFBP	IGF binding protein
IL	interleukin
ILM	inner limiting membrane
INL	inner nuclear layer
IP_3	inositol 1,4,5-triphosphate
IPL	inner plexiform layer
JAK	Janus kinase
JNK	c-Jun N-terminal kinase
K_A	fast transient (A-type) potassium
KA	kainate receptor subunit
KDR/flk-1	kinase insert domain-containing receptor/fetal liver kinase-1, VEGF receptor-2
K_{DR}	delayed rectifying potassium
Kir	inwardly rectifying potassium
L	long-lasting
LDH	lactate dehydrogenase

LDL	low-density lipoprotein
LIF	leukemia inhibitory factor
LRP1	low-density lipoprotein-related protein, CD91
LVA	low threshold voltage-activated
M	Müller glia
MAPK	mitogen-activated protein kinase
MC	Müller cell
MCE	Müller cell endfoot
MCP	monocyte chemoattractant protein
MG	microglia
mGluR	metabotropic glutamate receptor
MHC	major histocompatibility
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
Na,K-ATPase	sodium and potassium-dependent ATPase, sodium pump
NAD(P)H	nicotinamide adenine dinucleotide (phosphate)
NFL	nerve fiber layer
NGF	nerve growth factor
NMDA	*N*-methyl-*D*-aspartate
NO	nitric oxide
NP	natriuretic peptide
NPY	neuropeptide Y
NR	NMDA receptor subunit
NTPDase	nucleoside triphosphate diphosphohydrolase
OAP	orthogonal arrays of membrane particles
OLM	outer limiting membrane
ONL	outer nuclear layer
OPL	outer plexiform layer
P	postnatal day
P2X	ionotropic purinergic
P2Y	metabotropic purinergic
$p75^{NTR}$	low-affinity neurotrophin receptor
PAP	peripheral astrocytic process
Pax6	paired box gene 6
PCNA	proliferating cell nuclear antigen
PDGF	platelet-derived growth factor
PDR	proliferative diabetic retinopathy
PDZ	post synaptic density protein, Drosophila disc large tumor suppressor, and zonula occludens-1 protein
PEDF	pigment epithelium-derived factor
PGP	peripheral glial process
PI3K	phosphatidylinositol-3 kinase
PK	pyruvate kinase
pO2	oxygen partial pressur
PR	photoreceptor

PRS	photoreceptor segments
PVR	proliferative vitreoretinopathy
RAGE	AGE receptor
RCS	Royal College of Surgeons
rds	slow retinal degenerative
RI	refractory index
RPE	retinal pigment epithelium
Shh	sonic hedgehog
Src	sarcoma
STAT	signal transducers and activators of transcription
SVR	surface-to-volume ratio
T	transient
TASK	TWIK-related acid-sensitive potassium
TEA	tetraethylammonium
TGF	transforming growth factor
TNF	tumor necrosis factor
Trk	tropomyosin receptor kinase, high-affinity neurotrophin receptor
TRPC	transient receptor potential canonical
UDP	uridine 5'-diphosphate
UTP	uridine 5'-triphosphate
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal peptide
Wnt	wingless-type MMTV integration site family
Y	NPY receptor

Chapter 1
Introduction

At first glance one may wonder why an entire book is devoted to Müller cells, a cell type that clearly represents a minority in our central nervous system (CNS): Out of an estimated total number of about 200 billions of cells in our CNS, the 8–10 millions of Müller cells in our two eyes constitute not more than some 0.005%. To make things even worse – Müller cells do not belong to the highly esteemed neurons but to the glia, a family of cells which for more than a century had been thought of as a sort of mere filling material between the neurons... So one may wonder even more why our research group – together with an increasing number of scientists worldwide – focuses their research on these cells now for a quarter of a century, with no end in sight.

Gentle reader! We hope to convince you that Müller cells deserve both our concerted research effort and your effort to read the book. While everybody will agree that the eye is a very special and versatile sense organ, it turned out over the recent years that Müller cells are very peculiar and multipotent glial cells. In the retina of most vertebrates and even of many mammals, Müller cells are the only type of (macro-) glial cells; thus, they are responsible for a wealth of neuron-supportive functions that, in the brain, rely upon a division of labour among astrocytes, oligodendrocytes, and ependymal cells. Even beyond such a role as "model glia" in CNS, Müller cells adapted to several exciting roles in support of vision.

Before going into detail, however, it appears appropriate to provide two introductory chapters, one about glial cells – the family of neural cells Müller cells are members of – and another one about the vertebrate retina – their habitat and workplace.

1.1 Glial Cells – the "Second Cellular Element" of Neural Tissue

1.1.1 Definition, Origin, and Functional Role(s) of Glia

The term "neuroglia" (Greek for "nerve glue") was introduced in the nineteenth century by the German pathologist Rudolf Virchow (Virchow, 1858). On his search for a connective tissue of the nervous system, he discovered cells which apparently were

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no nerve cells (i.e., neurons) and called them glia, according to what he expected to find. Nowadays, it may be speculated that this unattractive name (reflecting the poor hypothesis behind it) was one of the reasons why these cells were neglected by most neuroscientists for the next 100 years. It was only some neuroanatomists who were fascinated by their complex shape in Golgi-impregnated brain slices, and the neuropathologists who soon realized that glial cells are crucially involved in virtually all brain injuries and diseases. Then during the last 3 decades, the rapidly developing advanced methodology in neuroscience enabled the development of innovative paradigms and approaches which eventually revealed that there is virtually no event during ontogenetic development, mature functioning, and pathology of the CNS which would not involve a crucial contribution of glial cells.

Per definition, the term "glia" applies to all cells within the CNS that (i) are not neurons and (ii) do not belong to mesenchymal structures such as the blood vessels and the meninges. The glia proper can be divided into two "sub-families", macro- and microglia. The term "macroglia" summarizes a wide diversity of cell types arising from the primitive neuroepithelium together with the neurons, including ependymoglia (radial glia incl. tanycytes and Müller cells, ependymocytes, choroid plexus epithelial cells and pigment epithelial cells), several subtypes of astrocytes, and oligodendrocytes (Fig. 1.1). By contrast, microglial cells are blood-borne macrophages which, during late ontogenesis, invade the brain via the establishing blood vessels. As will be mentioned later, microglial cells intensely interact with Müller cells in the injured retina where they play an important role; however, in this chapter we will focus upon the macroglial cells. So if we want to define and/or to identify a (macro-) glial cell as such, we need to make sure that it stems from progenitor cells of the embryonic neuroepithelium but is not a neuron. The latter point appears as a trivial task if one has a typical neuron (e.g., a cerebellar Purkinje cell) and a typical glial cell (e.g., an astrocyte) in mind but if all types of neurons and glial cells are considered, a general discrimination becomes less easy with every new discovery in glial cell research (see, e.g., Kimelberg, 2004). So it has been shown that – in strong contradiction to the traditional dogma of neuroscience – glial cells do express a wealth of voltage-dependent ion channels, ligand receptors, transmembrane transporters, second messenger pathways, and even release mechanisms for signaling molecules which they share with the neurons. This is less of a surprise if one considers that CNS neurons and glial cells share the same progenitors. In insects, "*glial cells missing*" *(gcm)* has been identified as a binary genetic switch for glia versus neurons. In the presence of gcm protein, presumptive neurons become glia, while in its absence, presumptive glia become neurons (Jones et al., 1995). Although a corresponding mechanism remains to be identified in vertebrates, apparently a similar "switch" causes quantitative shifts in the protein expression profiles of neurons and glial cells, rather than "all-or-none" decisions. What remains safe to say is that glial cells, in contrast to neurons, are not direct parts of information processing chains – this is, they do not perceive specific environmental stimuli and/or transmit them to specific brain centers, for example.

So if we know what glia fail to do, what do we know about their actual functions? Mostly prompted by morphological observations such as the "strategic"

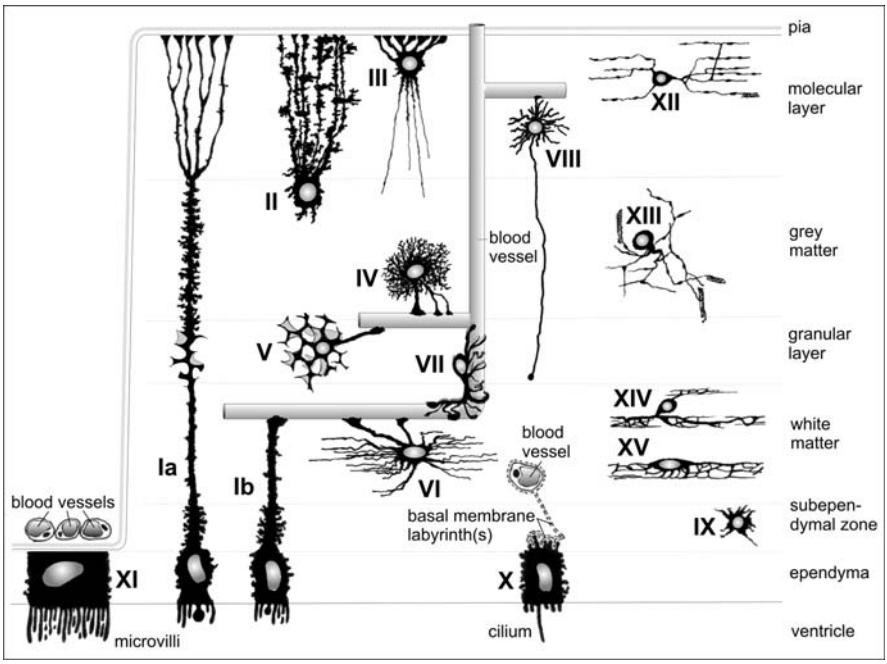

Fig. 1.1 Semischematic survey of the main (morphologically identifiable) macroglial cell types and their localization in different layers or in specialized regions of the central nervous tissue. *I*, tanycyte (*a*, pial; *b*, vascular); *II*, radial astrocyte/Bergmann glial cell; *III*, marginal astrocyte; *IV*, protoplasmic astrocyte; *V*, velate astrocyte; *VI*, fibrous astrocyte; *VII*, perivascular astrocyte; *VIII*, interlaminar astrocyte; *IX*, immature glial cell/glioblast; *X*, ependymocyte; *XI*, choroid plexus cell; *XII*, type 1 oligodendrocyte; *XIII*, type 2 oligodendrocyte; *XIV*, type 3 oligodendrocyte; *XV*, type 4 oligodendrocyte. Modified after Reichenbach and Wolburg (2005)

interposition of astrocytes between neurons and blood vessels, an impressive list of putative functions has been proposed already more than 100 years ago (Lugaro, 1907). The intense research efforts of the recent decades have generated sound evidence for most of these proposed functions (and for many more); in fact, much of this book is aimed at depicting the functions of Müller cells as a "model glia". Nonetheless, there remains a difficult step from demonstration of a functional mechanism in a cell or cell type to unequivocal proof of its importance for proper functioning of the CNS. It has been shown that if murine Müller cells are experimentally induced to die, the entire neural retina degenerates soon (Dubois-Dauphin et al., 2000) and that neuronal damage results from glial exhaustion in chronic liver failure (hepatic encephalopathy) in various mammals including man (Norenberg et al., 1992). Whereas this certainly argues for a crucial role of glial cells in the maintenance of neuronal integrity and survival, it prevents the acquisition of direct evidence by ablation of the glial cells and studying the (remaining) functions of the neurons normally associated with them. This problem is presently being addressed by the development of transgenic mouse models

where (desirably: conditioned) deletion of certain functional components such as glia-specific enzymes, ion channels, or cytoskeletal elements helps to understand the contribution of these glial molecules to neuronal functioning; some examples of this approach will be presented later.

Another approach has been possible in "lower" avertebrates. Before going into detail, it must be mentioned here that glial cells or "glia-like cells" occur not only in the CNS of vertebrates but can also be found in our peripheral nervous system (Schwann cells in the peripheral nerves, satellite cells in the dorsal root ganglia, enteric glia in the enteric nervous system, so-called supporting or sustentacular cells in the acoustic and olfactory sense organs, and others) as well as throughout the diverse nervous systems of most avertebrates. According to this more general defintion, the term "glia" applies to all cells which are morphologically and functionally associated with sensory or ganglionic neurons, or with nerves (but do not belong to the vasculature or connective tissue). Only in very primitive organisms such as polypes, individual sensory neurons and ganglion cells are scattered through the the tissue layer(s) of the outer and inner surface, not accompanied by any specialized glia-like cells (Fig. 1.2a). Obviously, these single neurons do not require glial cells

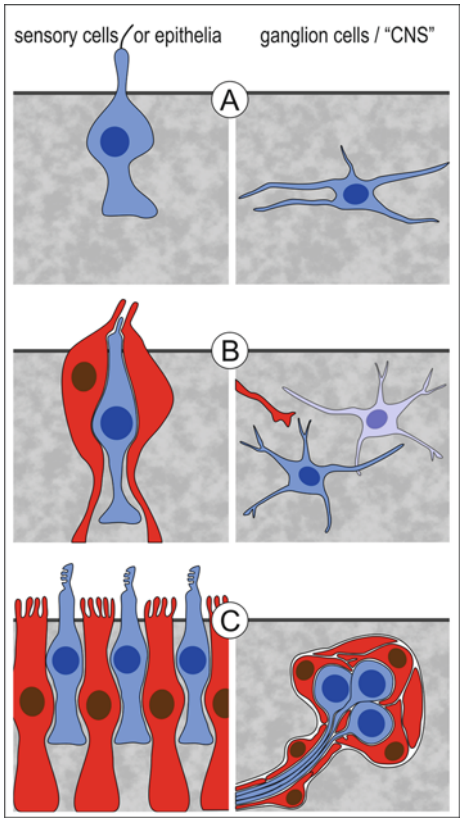

Fig. 1.2 Schematic survey of the evolutionary differentiation stages of sensory and ganglionic neurons (*blue*) and glial cells (*red*). Modified after Reichenbach and Pannicke (2008)

for their differentiation, function, or survival. Then the differentiation of larger/more complex animals resulted in the development of spezialized small sensory organs, and of small groups of loosely associated ganglion neurons (Fig. 1.2b). At this stage, the sensory neurons (but not the ganglion neurons) usually are associated with glia-like cells.

Recently, Bacaj et al. (2008) demonstrated that neurons in the major sensory organ of *Caenorhabditis elegans* survive the elimination of ensheathing glia but display functional deficits. In particular, the glia-deprived sensory neurons partially or totally lost the sensitivity for their adequate stimuli despite of an apparently normal expression of their structural and functional proteins. One of the identified glial transmembrane proteins, a KCl cotransporter, may be involved in the crucial glial contribution to osmosensivity of the ASH neuron in *C. elegans*, perhaps by providing a normosmotic microenvironment at (part of) the receptor neuron, as kind of a "standard" against which environmental changes can be measured. This had lead to the view that the homeostatic functions of "sensory-associated" glia may be required for the selection, transformation (and even transduction) of adequate stimuli by their adjacent neurons (Reichenbach and Pannicke, 2008) (in a wider sense, the absorption of excess light by pigmented glial cells close to photoreceptor neurons may also be considered as a case of homeostasis). From an evolutionary point of view, this first ancestral glia may have been separated off the neural progenitor line because this allowed to increase the signal-to-noise ratio of specific stimulus perception in non-sessile animals with a more complex behavioral repertoire.

Once established, the glial cells as "novel neural cell type with homeostatic capabilities" may have occupied the large nervous centers of more advanced animals where many neurons accumulate in ganglia or even brains (Fig. 1.2c). In this complex environment, the perisynaptic glia becomes essential for synaptic transmission (Araque et al., 1999). It should be kept in mind that signal transmission via chemical synapses can be considered as a special case of chemosensation by the postsynaptic neuron, with its adequate stimulus being the specific neurotransmitter substance released by the presynaptic terminal. Thus, an agglomerate of chemical synapses in the ganglia may raise similar homeostatic needs as chemosensation in the sensory organs. In fact, at chemical synapses a special case of division of labor can be observed between neurons and glial cells. The neuronal compartments (i.e., pre- and postsynapse) are highly specialized for rapid release and sensation of the neurotransmitter, respectively. For this purpose, they are endowed with a battery of proteins involved in the synthesis, release, and perception of the neurotransmitter. The perisynaptic glial compartments, by contrast, dominantly express transporter proteins for rapid and efficient uptake of the neurotransmitter, and specific enzymes for its conversion into a non-signaling precursor molecule. This neuron-glia interaction is called "neurotransmitter recycling" (cf. Section 2.4). Again this difference is not all-or-none, however: the neurons express some uptake carriers, and the glial cells express neurotransmitter receptors as well as molecules allowing the release of neuroactive substances (see below and Section 2.7).

Eventually, the homeostatic functions of glia appear to constitute the basis for their currently-evaluated "more exciting roles" including direct involvement in neuronal information processing (Araque et al., 1999). On the one hand, glial homeostatic functions may be modulated in their activity, or even "switched off" in order to increase the effectiveness of neuronal transmitter release by increasing the concentration and duration of presence in the synaptic cleft (Oliet et al., 2001). On the other hand, the molecular machinery required for neurotransmitter recycling may even be elaborated into active neuron-controling mechanisms such as gliotransmitter release (Araque et al., 1999) (cf. also Section 2.7).

In addition to an enhanced turnover of signaling molecules, the dense crowding of neurons in large sensory epithelia or ganglia (or brains) of complex metazoa causes other problems. Both types of tissues are typically encapsulated against their non-neural environment, usually involving a blood-brain barrier to which glial cells contribute (Wolburg et al., 2009). The insulated, highly active neurons depend on efficient nutrient delivery and clearance of waste products. This raises the need for extracellular homeostasis in an extended sense, involving supply of nutrients (glucose or lactate/pyruvate) and removal of CO_2 and water (cf. Section 2.4 and 2.5). As neuronal excitation is accompanied by Na^+ influx from and K^+ efflux into the extracellular space, and as elevated extracellular $[K^+]$ modulates the excitability of neurons, extracellular K^+ homeostasis is an important task of glial cells (see Section 2.4.2). Together, these problems may have been the driving force for the ubiquitous appearance and further multiplication (Reichenbach, 1989c) of glia in "higher" and/or bigger animals.

To summarize from this excurse what one needs to understand the functions of Müller cells, the essentials are that

- glial cells associated with sensory neurons increase the signal-to-noise ratio of perception, by assisting their adjacent neurons in the selection, processing, and even transduction of adequate stimuli; much of this involves homeostatic functions;
- glial cells associated with ganglion neurons increase the signal-to-noise ratio of signal transmission, mainly by a homeostatis of neurotransmitter molecules;
- glial cells in large sensory organs and in brain constitute a neural waste management system and play an important role in the coupled ion and water homeostasis.

Noteworthy, the retina is both a large sensory epithelium and a part of the brain (see Section 1.2) such that all of the above-mentioned functions fully apply to Müller cells. It should also be mentioned here that, after the glia-cellular system had been established in phylogenesis, it became available for a variety of other functions including guidance of neuron migration and axon pathfinding during embryogenesis, interactions with the immune systems, and many others. Most of these functions rely upon the very special morphology of glial cells which is introduced in the following chapter.

1.1.2 Basic Structural and Ultrastructural Features of Glia in Vertebrate CNS

To understand the highly complex morphology of many glial cell types (e.g., Figs. 1.1 and 1.6), one needs to keep in mind that most glial cells found in the adult vertebrate CNS had been generated – and differentiated – relatively late in ontogenesis, and that there are three types of "preferred" contact elements towards which glial cell processes grow, resulting in three basic types of glial cell processes (Reichenbach, 1989b). These are, (i) ventricle-contacting processes, (ii) blood vessel- or pia-contacting processes, and (iii) neuron-contacting processes (Fig. 1.3). Depending on how many and which of these process types a given cell establishes, the cell can be classified as, e.g., radial glial cell, astrocyte, or oligodendrocyte (Fig. 1.3) (Reichenbach, 1989b). After a "newborn" glial cell begins to differentiate,

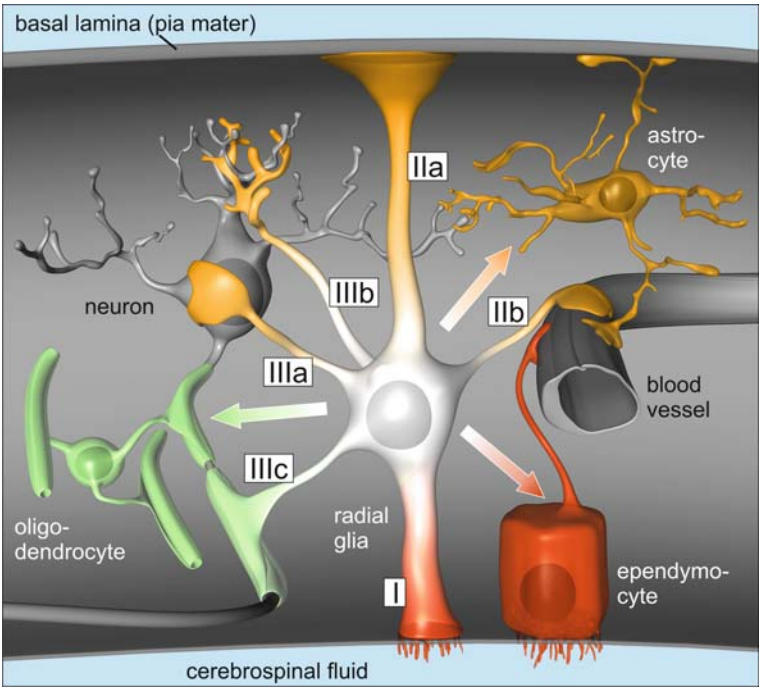

Fig. 1.3 Hypothetical macroglial cell displaying all possible types of cell processes (and microenvironmental contacts, respectively), and its real derivatives. The presence *vs*. absence of the three basic types of cell processes defines the four basic macroglial cell types, *viz* radial glial cells (all three), ependymocytes (only two), astrocytes (only two), and oligodendrocytes (only one). *I*, ventricle-contacting process; *II*, pia- (*IIa*) or blood vessel- (*IIb*) contacting processes; *III*, neuron-contacting processes (*IIIa*, to neuronal somata; *IIIb*, to "neuropile" incl. synapses; *IIIc* to axons). Modified after Reichenbach (1989b)

it must (try to) establish its pre-programmed contacts, a procedure which involves cell process growth (and even migration, in some instances). As mentioned above, most of the "target structures" (the pia mater, blood vessels, and neurons including their elaborate neurites, including axon bundles) are already formed at this stage, and the glial cells and their processes must fit into this preexisting environment. In accordance with this scenario, it has been recently shown that glial cells are softer than neurons (Lu et al., 2006). Thus, one might get some idea about the ingrowth of glial cell processes into the neuronal (and vascular) network if one imagines the buildup of a concrete construction, when the fluid concrete mass is poured into the interspaces between the steel scaffolding. Once (one to three of) the glia-typical contacts were established and the glial cell processes are differentiated, they display typical morphologies.

(A) The ventricle-contacting process generally ends with microvilli (in some cases, also cilia) extending into the fluid compartment (Figs. 1.3, 2.9 and 2.10). The apical microvilli provide a large membrane surface area at the interface between fluid and tissue, and thus facilitate the transport of molecules between the two compartments. Additionally the processes form, together with those of adjacent glial cells or neurons, adherent junctions that appear to be important for the maintenance of the biomechanical stability of the tissue and for other functions including the correct ontogenetic development of the tissue. In the retina, this network of zonulae adherentes is visible even in H/E-stained histological sections, and is called "outer limiting membrane (OLM)" (Fig. 2.10). In some specialized brain regions (the circumventricular organs), the ventricle-contacting processes are even connected by tight junctions such that a brain-cerebrospinal fluid-barrier is constituted (Wolburg et al., 2009).

(B) The pia-contacting and the perivascular glial processes abut a basal lamina by so-called endfeet (Figs. 1.1, 1.3 and 2.11). These glial endfeet form, together with the basal lamina and with the mesenchymal cells at its opposite surface (e.g, endothelial cells or pericytes, or meningeal fibroblasts), the other interface between the neural tissue and a fluid-filled compartment (note that the vertebrate CNS is epithelial and thus displays two opposite surfaces; cf. Section 1.2.1). The endfeet usually contain large amounts of smooth endoplasmic reticulum (Fig. 2.11b). Their basal lamina-abutting surface is endowed with a wealth of transport and receptor proteins as well as Ω–shaped membrane indentions indicative of active exo- and/or endocytosis. Apparently, there occurs a lively exchange of ions and even larger molecules between the endfeet and the fluid compartments behind the basal lamina. A particular characteristic feature of many glial endfeet is their dense packing with so-called orthogonal arrays of membrane particles (OAP) visible by freeze-fracture electron microscopy (Wolburg and Berg, 1987, 1988; Wolburg, 1995). A water channel, aquaporin 4, has been identified as one of the transport molecules which are accumulated in these OAPs (Yang et al., 1996; Verbavatz et al., 1997) (cf. Section 2.4.3).

(C) The neuron-contacting glial processes abut at – or rather, ensheath – neuronal cell somata and/or processes (Fig. 1.3). According to the complex shape of the ensheathed structures this type of glial processes shows the most complex structure (Fig. 1.6; cf. Grosche et al., 1999). In the *grey matter* of the CNS which contains a high density of synapses, the dominant glial cells are so-called protoplasmic astrocytes (Fig. 1.1). The many irregularly shaped processes of these cells give rise to numerous very thin, convoluted cytoplasmic tongues, also called lamellar processes (Wolff, 1968), lamellipodia and filopodia (Chao et al., 2002), or peripheral astrocytic/glial processes, PAPs (Derouiche and Frotscher, 2001) or PGPs (Reichenbach et al., 2004), respectively.

The PGPs contain only a minor portion of the glial cytoplasm volume but a majority of the cell surface area. This large membrane area in small volume compartments appears to be required to give space for a wealth of ion channels, ligand receptors, and uptake carrier proteins necessary to maintain the variety of glia-neuron interactions. In many instances, a number of such PGPs together belong to a complex subcellular structure called a "microdomain" (Grosche et al., 1999, 2002). Such a glial microdomain consists of a thin stalk and a small garbagge-like head from which latter the PGPs arise; it may contain one or a few mitochondria and is thought to be capable of a more or less autonomous interaction with the ensheathed group of a few synapses (cf. Section 1.1.3).

The *white matter* of the CNS is constituted by large numbers of axons or axon bundles. Usually if the thickness of an axon exceeds a threshold of about 0.2–0.4 μm, its internodes (i.e., the sections between two consecutive nodes of Ranvier) are myelinated by highly specialized cytoplasmic tongues arising from the processes of oligodendrocytes (Waxman and Black, 1995). Bundles of thin, non-myelinated axons are loosely ensheathed by cytoplasmic tongues arising from the processes of the so-called fibrous astrocytes (Fig. 1.1). The processes of these cells appear less complex than those of the protoplasmic astrocytes (because there are almost no synapses in the white matter) but are preferentially aligned in parallel to the axon bundles. Besides ensheathing the unmyelinated axons, these astrocytic processes extend finger-like tiny processes into the perinodal spaces of the myelinated axons (Hildebrand et al., 1993; Butt et al.,1994; Sims et al., 1985). It has been proposed that these "glial fingers" monitor neuronal activity, and trigger glial responses to it (Chao et al., 1994b).

Finally there are some CNS regions which contain densely packed small neurons, *nuclear layers*, such as the granule cell layer in cerebellum. Groups of such neuronal cell bodies are there ensheathed by the honey comb-like cytoplasmic tongues of so-called velate astrocytes (Fig. 1.1). Analyzing this short compilation of basic data it becomes evident that the shape as well as the ultrastructure of glial cell processes are largely determined by the local environment and, specifically, by the type of the contacted/ensheathed element (cerbrospinal fluid/basal lamina/synapses, axons, or somata of neurons). For a recent comprehensive review of the various types of glial cell processes and their specific structural and ultrastructural adaptations, see Reichenbach and Wolburg (2009).

Going back from individual cell processes to entire glial cells, this "adaptive character" of glia is evident, as well. The prime glia of the vertebrate CNS, the radial glia, traces back to the so-called support cells of the starfish nervous system (Fig. 1.7) and is characterized by contact to both surfaces of the epithelium (fluid environment and basal lamina) as well as to the inherent neurons. It is present everywhere in the embryonic CNS even of mammals, and constitutes the dominant glia in the adult CNS of many fish, amphibians, and even reptilians (Fig. 1.4). If further differentiation and growth of the CNS increases the thickness of the wall of the neural tube to more than a few hundred micrometers, full-length radial glial cells appear to become inefficient and/or unable to survive as such; they undergo mitotic division and the daughter cells (as well as later-generated glial cells) lose – or fail to establish, respectively – one or more of the three principal contacts (Reichenbach, 1989b). This results in the emergence of astrocytes (basal lamina and neuron contact) and oligodendrocytes (neuron contact only) (cf. Fig. 1.1).

The embryonic and fetal radial glial cells are indistinguishable from neural progenitor cells; indeed, they have been shown to generate both neurons and glial cells after mitotic division (Götz and Barde, 2005; Götz and Huttner, 2005). Moreover, they provide a scaffold for the guided migration of newborn neurons from the

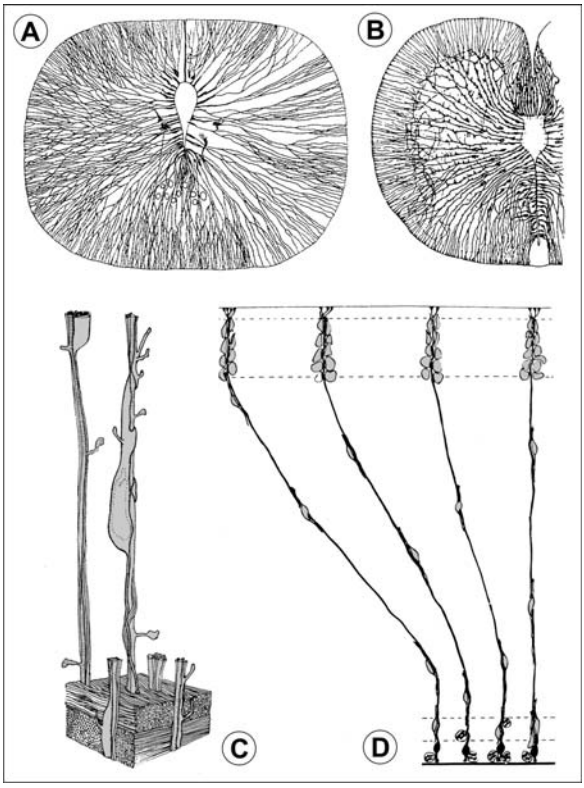

Fig. 1.4 Radial glial cells. **a**, mature radial glial cells in the spinal cord of *Petromyzon* (lamprey, agnatha; from Retzius, 1893); **b**, fetal radial glial cells in the embryonic human spinal cord (44 mm long embryo; from Ramón y Cajal, 1952); **c** and **d**, fetal radial glial cells in the monkey brain cortex (modified after Rakic, 1972, 1981). The latter two images show how the newborn neurons migrate along (bundles of) radial glial cells from their place of birth (the ventricular zone) towards their site of destination, the future cortical plate

ventricular surface of the neuroepithelium (where cell multiplication occurs) to the distant sites of their destination (Rakic, 1988) (Fig. 1.4). Likewise, the processes of fetal radial glial cells are used guidelines by the neurites of the young postmitotic neurons growing towards their targets (Silver et al., 1982; Norris and Kalil, 1991). It has been suggested that the cohort of neurons migrating along the same radial glial "climbing pole" later maintains and elaborates much of the contacts inevitably arising between the leading and trailing processes of cells migrating together, and that the cells maintain a particular relationship or even "symbiosis" with the glial cell(s) along which they migrated (Rakic, 1978; Reichenbach et al., 1993a; Reichenbach and Robinson, 1995). This may be the ontogenetic basis of functional columnar units or domains (see Sections 1.1.3 and 2.2.2) in the adult CNS, exemplified by the orientation columns in visual cortex (Mountcastle, 1957).

In relatively thin-walled brains or CNS regions (Reichenbach, 1990) the fetal radial glial cells eventually become postmitotic and differentiate into adult radial glia. On their course through the entire thickness of the CNS tissue the processes of these cells may pass through different local tissue specializations such as synapse-rich grey matter and axon-rich white matter in the frog spinal cord, for instance. Accordingly, such processes adopt a complex shape like protoplasmic astrocytes within the grey matter but assume a rather smooth shape like fibrous astrocytes when they enter the white matter (Fig. 1.1). The Müller cells, as radial glial cells of the mature CNS, display all three principal types of glial processes, as well as all three types of specialized neuron ensheathment. A specific description of their structure and ultrastructure is given in Section 2.1.

1.1.3 A hierarchy of Neuronal/Glial/Vascular Domains in the CNS

As already mentioned, a typical piece of CNS tissue consists of neurons, glial cells, and blood vessels (and extracellular spaces). It has been estimated that astrocytes make up some 30% of the brain volume (Nicholson and Sykova, 1998). There are other estimates (differing in dependence on the methods used, and on the brain areas/animal species studied) but it appears to be reasonable to assume that roughly 1/3 of the brain volume is occupied by glial cells and their processes, a little more than that by neurons and their processes, and a little less than 1/3 by extracellular clefts and blood vessels. Considering the huge size of the human brain, for example, and the very complex shapes of both neurons and glial cells, it appears to be highly improbable that the glial cells could fulfil their role as mediators between neurons and blood vessels (cf. Section 1.1.1) if the various compartments were randomly arranged. Indeed, it can be shown that the CNS is structurally and functionally compartmentalized into so-called domains at many hierarchical levels (Reichenbach and Wolburg, 2009). Per definition, such a domain is constituted by neuronal and glial elements; it (i) can be structurally distinguished from other adjacent compartments, and (ii) may function autonomously (i.e., independent on hierarchically higher structures) at least under some conditions; (iii) the range of

elements interacting within or across the limits of a hierarchical level is variable according to the present and previous activity of information processing (e.g., of the strength and/or frequency of stimulation) as well as to the metabolic conditions of the tissue.

To illustrate these rather theoretical considerations by some more vivid examples, let's climb the hierarchical levels of domains in cerebellum and brain cortex. At the lower end of the scale, small sub-regions of presynaptic terminals have been shown to contain specific subtypes of neurotransmitter receptors and uptake carriers (Dorostkar and Boehm, 2008). Although compelling evidence remains to be provided, it appears reasonable to assume that such neuronal "nanodomains" are faced to adjacent glial nanodomains which specifically interact with them. One step ahead, individual synapses or small groups of them are long-identified "smallest units" of information processing. It has been shown that these neuronal microdomains are accompanied by ensheathing glial structures called glial microdomains, with which they appear to interact specifically (Grosche et al., 1999, 2002). Then further on, an individual Purkinje neuron can be considered as a cellular neuronal domain; it interacts with its surrounding Bergmann glial cells which, thereby, constitute a (oligo-) cellular glial domain (by the way, the numerical relation between neurons and glial cells may vary at this level; one "velate" astrocyte in the cerebellum ensheathes – and probably interacts with – several granule neurons). At the next level(s) of integration, columnar arrays of hundreds or thousands of neurons may form functional units ("mesodomains") such as the direction-sensitivity columns (Mountcastle, 1957) and the ocular dominance columns (Müller and Best, 1989) in the visual cortex, and the barrel fields (Rice and Van der Loos, 1977) in the somatosensory cortex of rodents. The pendants of these neuronal mesodomains are networks of gap junction-coupled astrocytes; it can be shown that neuronal excitation within these functional units is accompanied by glial responses such as, e.g., Ca^{2+} rises (Aquado et al., 2002; Schummers et al., 2008). Finally at the upper end of the scale, so-called neuronal macrodomains involve one entire cortical area or even several of them which are activated together during cognitive tasks (Horwitz, 2004), or even a whole hemisphere or the entire cortex during arousal/sleep or in pathological instances such as spreading depression, epileptiform activity or migraine. Again, this widespread neuronal activity is accompanied by glial responses within the same tissue compartments (Schipke and Kettenmann, 2004; Amzica, 2002).

Noteworthy, as soon as the hierarchically growing domains involve more than a few neuronal and glial cells (i.e., of their size exceeds the maximum distance for easy diffusion of oxygen and other molecules), they are accompanied by a "third element", *viz* by blood vessels. The vascular bed perfectly fits to the size and shape of "its" corresponding domain (e.g., Fig. 1.5c). The state of neuronal activity within such domains is continuously "measured" by their glial inhabitants (Schummers et al., 2008) which then, according to the current metabolic needs, control the local blood flow by eliciting vasoconstriction or vasodilatation of the local arterioles (Gordon et al., 2008).

Inherent to this concept of hierarchical domains is an apparent paradox, as on the one hand even small domains can function in an autonomous manner, and on

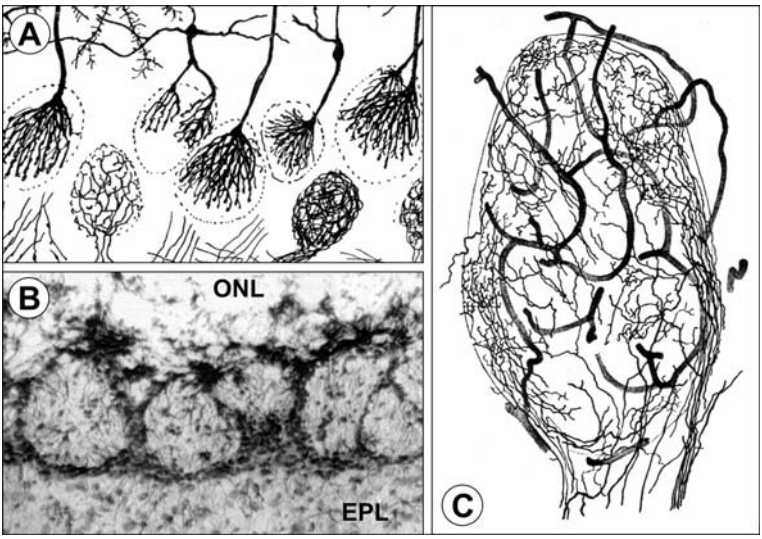

Fig. 1.5 The congruence of neuronal, glial, and vascular domains, exemplified for the olfactory bulb. **a**, the so-called glomerula, constituted by afferent and efferent neuronal processes and their synapses (newborn kitten, from Ramón y Cajal, 1952); **b**, the glial "envelope"of these glomerula, visualized by GFAP immunohistochemistry (adult frog; modified after Bailey et al., 1999); **c**, the blood supply of a glomerulum (ink injection; cat; from Kölliker (1896); the nerve fibers are also drawn)

the other hand they may work as interdependent cogwheels in the machinery of larger domains. Obviously the concept requires the presence of controlled gates between neighbouring domains of the same rank as well as towards higher-ranking domains. The relatively long, thin stalk of the microdomain shown in Fig. 1.6 may serve as an example for such a "gate". Its cytoplasmic longitudinal resistance constitutes an obstacle against the spread of Ca^{2+} rises, triggered in the head by neuronal activity in the ensheathed synapses, towards the glial stem process or adjacent microdomains. Furthermore, together with the shunt conductance of the stalk membrane, it prevents the electrotonic propagation of even large depolarizations of the head membrane (Grosche et al., 2003). Whereas these estimates explain why individual microdomains may exclusively display Ca^{2+} responses in response to low-frequency single-axon stimulation, it has also been shown that stronger/more frequent and/or extensive stimulation may cause Ca^{2+} rises in several neighbouring microdomains or even in the whole Bergmann glial cell (Grosche et al., 1999, 2003). This may be due to a spread of the activation within the neuronal compartments (simply bypassing the glial gates), as well as by an "overrun of the gates" by the accumulation of high Ca^{2+} levels during repetitive release from the stores and/or by saturation of Ca^{2+} binding proteins in the glial cytoplasm, for instance. A similar overrun of glial gates may play a causative role in pathological events such as spreading depression and epileptiform discharges (De Keyser et al., 2008).

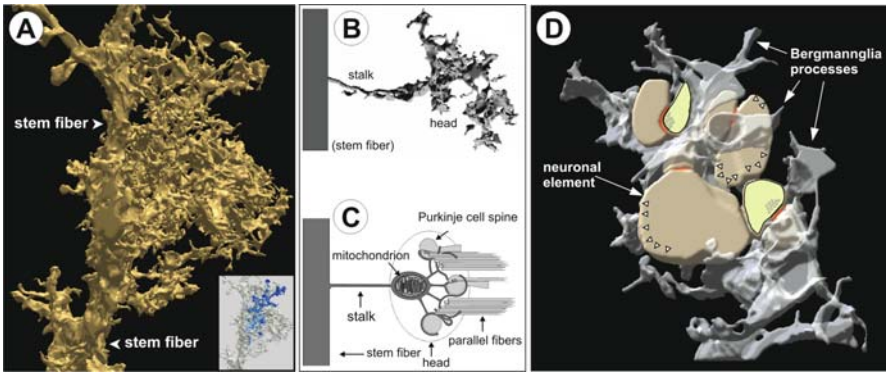

Fig. 1.6 Bergmann glial cell processes and glial microdomains. **a**, 3-D reconstruction of a part of a Bergmann glial cell process. The living cell was dye-injected in a perfused cerebellar slice; then, after fixation and dye-conversion, about 600 consecutive serial ultrathin sections were photographed in the electron microscope, and the images of the dye-labeled profiles were reconstructed by a computer program. The inset shows a substructure labeled in blue; this part was quantitatively analyzed (see **b**, **c**). **b**, Glial microdomain as part of the 3-D reconstruction shown in **a**. **c**, Schematic drawing of such a glial microdomain and its relationships to the neuronal elements. **d**, 3-D reconstruction of a group of neighboring cerebellar synapses (*yellow-green*; synaptic clefts: *orange*) together with the surrounding leaflets provided by the injected Bergmann glia (*blue-grey*). The arrowheads point to neuronal surfaces not covered by glial sheaths from the labeled cell. With permission, from Reichenbach et al. (2004)

However, there are also active mechanisms of glial gate control. Glial networks are coupled via gap junctions, the conductance of which is under control of a variety of signals including well-established intracellular second messengers (Rörig and Sutor, 1996; Rouach et al., 2000).

Thus, Ca^{2+} waves arising in one glial cell may pass to a variable number of neighbour cells, depending on the current functional state of the gap junctions between the cells (Enkvist and McCarthy, 1992; Venance et al., 1995). Moreover, there are extracellular "bypassing" glial signalling pathways; for instance, a stimulated glial cell may release ATP as a "gliotransmitter" which then activates ATP receptors on adjacent glial cells, which triggers intracellular Ca^{2+} rises in these cells and eventually causes ATP release from them, and so far (Cotrina et al., 1998; Nedergaard et al., 2003).

Furthermore, the activity of glial homeostatic mechanisms such as uptake carriers in their membrane can be modified by these signals and/or by the metabolic state of the cells; this, in turn, will modify the extracellular propagation of signal molecules released by neurons and glial cells ("volume transmission": Syková and Chvátal, 2000). Finally it should be kept in mind that large blood vessels cross the borders between different domains. In cases of stroke, for instance, the metabolism and activity of neurons and glial cells may be altered in wide areas, independent of the glial gates.

There are two conclusions from these considerations which appear to be important for an understanding of glia-neuron interactions in the retina (as a part of the

CNS: see Section 1.2), (i) the topographical relationship between neuronal and glial (plus vascular) elements reflects their intimate functional collaboration and interdependence, at many hierarchically scaled dimensions from sub- to multicellular levels, and (ii) a propagation or "ascent" of activity across the limiting "gates" of the hierarchical levels is possible via several different mechanisms which may be carried by neuronal, glial, or even vascular elements. For the role of domains in the retina, see Section 2.2.3.

1.2 The Vertebrate Retina as a Part of the CNS

Much of the above-mentioned insights into the interplay between neurons and glial cells of the brain also applies to the retina which is a part of the CNS both by embryology (it arises from an evagination of the neural tube; see Section 1.2.1) and by function (in addition to stimulus perception it performs complex signal processing; see Section 1.2.2). In addition, the retina is a sensory organ, which causes a number of specific requirements. So for instance, the retina must have access to its adequate stimulus, the light, which means that many parts of its surrounding ocular structures must be transparent and a high-quality image of the environment must be delivered to the photoreceptor cells. These and many other tasks including the generation and renewal of light-sensitive photopigments, the maintenance of the enormous energy demands of the specific transduction mechanism of the photoreceptors, as a few examples, add to the already high complexity of neuron-glia interactions in other parts of the CNS.

1.2.1 Some Phylogenetic and Ontogenetic Basics

To understand the complex and, in some sense, even counterintuitive makeup of the vertebrate retina, it is essential to keep in mind that vertebrates belong to the deuterostomian animals and that our ancestors must be searched among the relatives of recent starfish and sea urchins. If the nervous system of the starfish (Fig. 1.7) is used as a model of the origin of our CNS, two things become immediately apparent. First, this nervous system is not only embedded in the "skin" epithelium, it is by itself epithelial. It spans between the outer surface of the body, where it directly contacts the seawater as a fluid environment, to the basal lamina deliminating the epidermal cells from the mesenchymal compartments. The second important observation is that this epithelial nervous system is polar, as well as its cells are non-randomly oriented and polar. For instance, the so-called supporting cells – which can be considered as the ancestors of radial glial cells (Reichenbach and Robinson, 1995) – span the entire thickness of the epithelium from "watery" surface, into which their apical processes extend microvilli, to the inner basal lamina where their basal processes form endfoot-like structures. The similarity of these two types of cell processes to the ventricle-contacting processes and to the pia-contacting processes, respectively, of "modern" radial glial cells (Figs. 1.1 and 1.3) is apparent. The sensory cells are also polar; their sensory processes extend into the

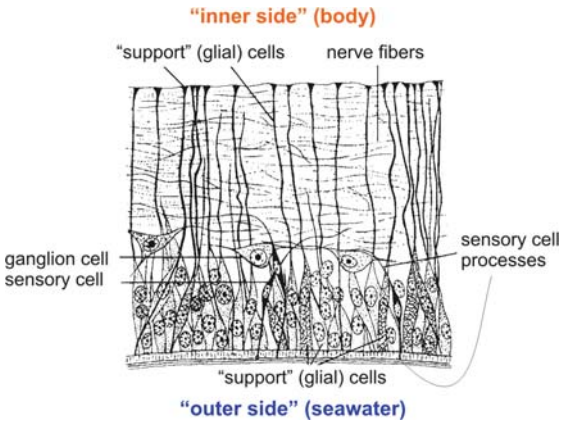

Fig. 1.7 The starfish nervous system as a (pro-)vertebrate "prototype CNS". Cross-section through the radial nerve of *Asterias rubens*. The sensory and ganglionic neurons are surrounded by so-called supporting cells which send radial processes towards the basal lamina (delimiting the ectoderma from mesoderma) which they abut with the conical endfeet of these processes. These cells may be considered as "ancestral radial glial cells". Redrawn after Figs. 1.4 and 1.12 in Meyer (1906)

maritime environment of the animal as the source of the (hitherto unknown) stimuli to be monitored, whereas their axons reach towards the ganglion cells as the sites of information processing. Notably, this polarity is obviously "correct" and easily comprehensible.

In the further course of evolution, the epithelial nervous system was maintained as such, but was enrolled into a tube and moved down under the surface of the body by the overfolding or -growing skin and subepidermal layers. Similar events occur during our embryogenesis when the – originally superficial – neural plate is enrolled and overlayed in a process called neurulation (Fig. 1.8). Inevitably, this mechanism is accompanied by an inside-out turn of the polarized epithelium: the sensory cells which had faced the environment at the surface of the body now extend their sensory

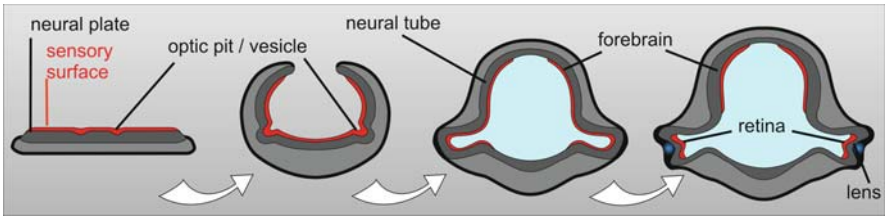

Fig. 1.8 Embryonic development of the retina by evagination of the eye anlage (optic pit/vesicle) from the neural plate/neural tube (from *left* to *right*). Later on, the *outer wall* of the optic vesicle becomes invaginated by the developing lens. This part differentiates into the neural retina whereas the *inner wall* becomes the retinal pigment epithelium; the stalk is transformed into the optic nerve (see Fig. 1.10 for more details). Initially the "sensory surface" (*red*) constitutes the outer face of the neural plate (and the embryo) but it is translocated to the inner surface by the invagination of the neural tube; original

processes into the lumen – i.e., the inner surface – of the neural tube. Perhaps, this had not been much of a problem in the most ancient small animals; still in the recent hemichordates this lumen is continuous with the surrounding seawater, and chemical and/or osmotic stimuli may be detected without crucial delay. However, later when the lumen was closed against the outside world, and filled by a substitute of the seawater – the cerebrospinal fluid – these receptors lost their original function as environmental receptors, and had to be functionally replaced by "novel" receptor types and sense organs at the surface of the animals (this was perhaps the evolutionary driving force for the emergence of the peripheral nervous system).

Unfortunately, the origin and early evolution of the vertebrate retina cannot be studied on recent relatives of our ancestors; the lancelets as "most advanced" chordates have no retina but the most primitive jawless hagfish already possess a well-developed retina almost indistinguishable from that of advanced vertebrates including mammals (Walls, 1963). However, there are sufficient facts and analogies allowing for a reasonable hypothesis. It seems as if visual information has been gathered by different types of receptor neurons in the anterior part of the neural tube ("brain") of early ancestors. In the recent lancelets (*Branchiostoma spec.*) there are groups of light-sensitive cells that may be homologous to pineal and lateral eyes of vertebrates, and that express AmphiPax-6, the single amphioxus Pax6 gene (Glardon et al., 1998). It appears to have been a distinct "innovative step" in (pre-) vertebrate evolution when a transverse stripe near the anterior end of the neural plate (in the anlage of the diencephalon) was determined for a "visual fate or potency" by a novel combination of homeobox genes, including Pax6 as a "master gene" in early eye development of vertebrates (Reichenbach and Pritz-Hohmeier, 1995) (Fig. 1.9). This area gives rise to light-sensitive neurons not only in the "main" lateral eyes

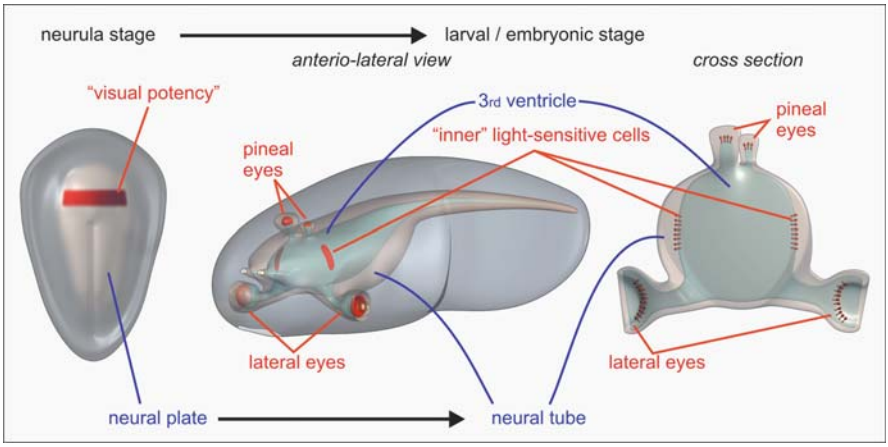

Fig. 1.9 Occurrence of light-sensitive cells in the vertebrate CNS. The neural plate contains a transversal stripe of tissue with "visual potency" (*left side*). This stripe is involved in the evagination of both the lateral eyes and the one or two pineal eyes, as well as in the formation of the lateral wall of the third ventricle; consequently, all these regions may contain light-sensitive cells (*red*). Schematic vertebrate larva; original

(corresponding to our eyes) but also in one or two dorsal eyes (pineal or parietal) and in the wall of the third ventricle (as well as to visually specialized areas of the midbrain). Still in recent fish, amphibians and reptilians all these visual sensory organs can be found (Fig. 1.9). Embryonic birds have a pineal "retina" which is transformed into a neurosecretory organ during later developmental stages; such a pineal retina fails to occur in mammals.

Do these developmental steps explain for the "odd" orientation of our retina (Fig. 1.11), with the photoreceptor cells directed away from light? Indeed, during the evagination of the optic vesicle from the diencephalic area of the neural tube the "sensory surface" – which had been turned from out- to inside during neurulation – remains at the inner, "wrong" surface (Fig. 1.8). During the subsequent secondary "counter-invagination" of the optic vesicle into the optic cup, only the distal area of the vesicle (i.e., the inner wall of the cup) develops into the sensory retina (Fig. 1.10). This part undergoes no change in orientation such that still after full differentiation of the retina the photoreceptors are directed towards the inner side, away from light entrance. Thus, the normal developmental mechanisms of our eyes inevitably lead to an inverted retina.

However, this is only half of the story. During invagination of the optic cup, the proximal area of the optic vesicle (i.e., the outer wall of the cup) is turned around such that its "sensory surface" now becomes directed towards the outer surface of

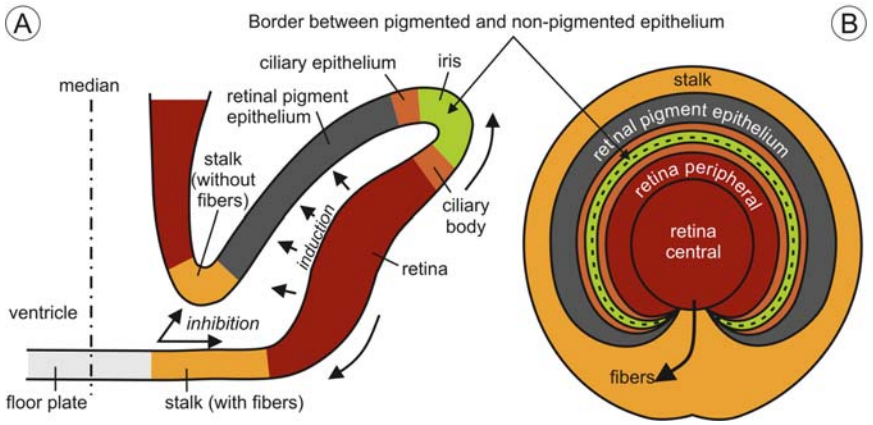

Fig. 1.10 Fate of the early eye anlagen. **a**, Frontal section through the optic cup, in the region of the optic fissure. The midline neuroepithelium may release inhibitory signals that determine the optic stalk (but prevent the formation of a retina). The same or other signals may act as chemoattractants for the first optic fibers (outgrowing from the first postmitotic ganglion cells, located close to the stalk). Another signal(s) is released by the (future) neuroretina; it determines the RPE (but prevents the differentiation of a retina in the outer wall of the optic cup), and later also the tissue at the border between neuroretina and RPE. **b**, Projection of the future tissue specifications onto the ocular field of the neural plate. Note that after evagination of the optic vesicle, the stalk region remains narrow while the distal regions undergo further growth. Modified after Petersen (1923) and Reichenbach and Pritz-Hohmeier (1995)