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Review Article

CNS Glia: From Passive Glue to Excitable Cells

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Received: July 21, 2017; **Published:** August 07, 2017

Abstract

This review highlights new findings related to the CNS glial and their role in the neural network.

Our brain is made up of many cells, including neurons and glial cells; astrocytes, oligodendrocytes, NG2 cells, microglia and ependyma.

Neurons were thought to be the basic information processing unit of the brain, so most neurological research has focused mainly on neurons "neuron doctrine", ignoring other types of CNS cells. However, recent studies provide that glial cells are much more than just the "glue" that holds together the neurons of the CNS!

Glial cells provide support functions for the neurons and they are far more numerous than neurons.

A novel hypothesis stated that glia and neurons can speak and comprehend the same chemical language, thus the dysfunction of glial cells results in pathological neuro-glial interactions that, in turn impairs the functionality of neuronal cells.

Keywords: *CNS Glia; Optogenetics; NG2 Cells; Astrocytes; Oligodendrocytes; Microglia; Ependyma*

Abbreviations

CNS: Central Nervous System; NG2: Neuron Glia Antigen; OPCs: Oligodendrocyte Progenitor Cells; MBP: Myelin Basic Protein; MAP2: Microtubule-Associated Protein 2; GFAP: Glial Fibrillary Acidic Protein; SVZ: Subventricular Zone; BBB: Brain Blood Barrier; GS: Glutamine Synthetase; ROS: Reactive Oxygen Species; DDR: DNA Damage Response; ALS: Amyotrophic Lateral Sclerosis; GLT1: Glutamate Transporter 1; GLAST: Glutamate Aspartate Transporter; Kir4.1: The Inward Rectifying Potassium Channel; AMPARs: The A-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor. A transmembrane receptor for glutamate that mediates fast synaptic transmission in the central nervous system; Ins(1,4,5)P3 R2: Inositol-1,4,5-Trisphosphate Receptor Type 2. Are intra-membranous calcium releasing channels; ΔR/R0: The Normalized Electrical Resistance Changes; GPCR: G Protein-Coupled Receptors; AMPARs: α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptors; SBEM: Serial Block-Face SEM; ONH: Optic Nerve Head

Introduction

Advances in medicine have enabled us to replace almost all body organs except the brain. The brain is the most complicated tissue, responsible for control of vital processes, cognition, personality and is the center of motor functions. Traumatic brain injury and aging are commonly associated with varied degrees of loss of brain functions. About five percent of the older populations have neurodegenerative disorders, including mild cognitive impairment, cerebrovascular disease, Alzheimer's disease and Parkinson's disease.

So, a better understanding of the cellular and molecular processes involved in the brain is needed to maintain normal brain functionality [1].

The brain is made up of many cells, including neurons and glial cells. Neurons are cells that send and receive the electro-chemical signals. There are about 100 billion neurons in the human brain. Glial cells provide support functions for the neurons, and are far more numerous than neurons [2].

Until recently, neurons were thought to be the basic information processing unit of the brain. Most neurological research has focused mainly on neurons "neuron doctrine" and ignoring other types of CNS cells, implying that neurodegenerative disorders are diseases of neurons [3].

Interestingly, the difference in the morpho-physiological complexity of neurons between humans and other species is relatively small. While, there are many differences between human glia and those of other species. For example (Figure 1), protoplasmic astrocytes derived from human neocortex manifest a threefold larger diameter and have tenfold more primary processes than those of rodents [4,5].

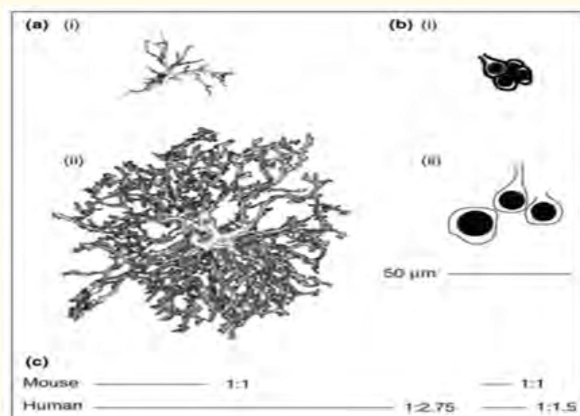


Figure 1: *Evolution of astrocytes and neurons [4]. (a) Graphical representation of glial fibrillary acidic protein (GFAP) immunostaining of mouse (i) and human (ii) cortical astrocytes. (b) Graphical representation of microtubule-associated protein 2 (MAP2) immunostaining of mouse (i) and human (ii) cortical neurons. (c) Bars illustrating the sizes of human astrocytes (left) and neurons (right) relative to the sizes of these cells in mice. Human cortical astrocytes are almost threefold larger, have approximately tenfold more GFAP-positive processes, and are more symmetrical than mouse astrocytes. The increase in complexity and size of astrocytes from mouse to man is disproportionate to the evolution of neuronal structure, possibly reflecting the increasing importance of astrocytes in the brain function of higher organisms.*

Recent studies lead to the idea that glial cells, astrocytes in particular, are much more than just the ‘‘glue" that holds together the neurons of the CNS. Glia can release ‘‘gliotransmitters" and have many of the same receptors as neurons, indicating that glia and neurons can speak and comprehend the same chemical language [6]. Also, they can act as pluripotent neural precursors for adult neurogenesis [7].

A novel hypothesis stated that dysfunction of glial cells results in pathological neuro-glial interactions that, in turn impairs the functionality of neuronal cells [1].

Therefore, this review is highlighting the CNS glial cells and focusing on recent findings on their role in the neural network.

Types of CNS glial cells

Historically, glia in the mammalian CNS has been classified as astrocytes, oligodendrocytes, microglia and ependyma (Figure 2).

Another major glial cell called "polydendrocytes" (owing to their multi-processed morphology and lineal relationship to oligodendrocytes) that is distributed throughout the developing and mature CNS. Polydendrocytes are defined as CNS parenchymal cells (non-vascular cells) that express the neuron glia antigen-2 "NG2" (also known as NG2 cells). Recent reports on the lineage and electrophysiological properties of polydendrocytes have accepted that polydendrocytes are oligodendrocyte progenitor cells (OPCs) that generate oligodendrocytes in the developing and mature CNS and serve as the primary source of remyelinating cells in demyelinated lesions [8] (Figure 3).

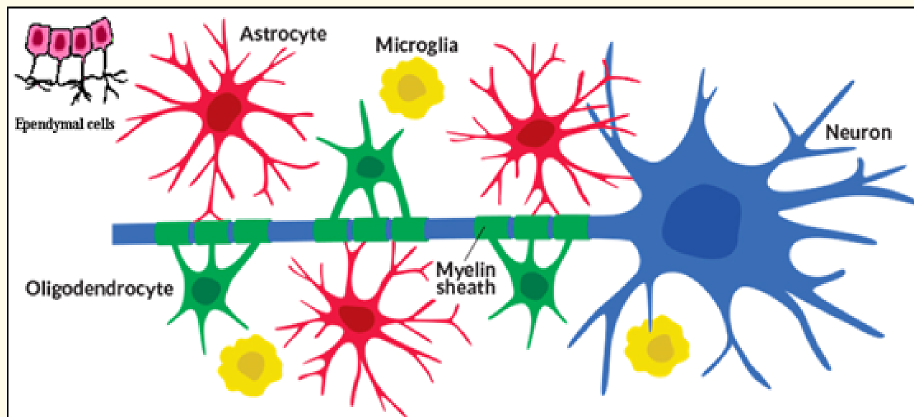
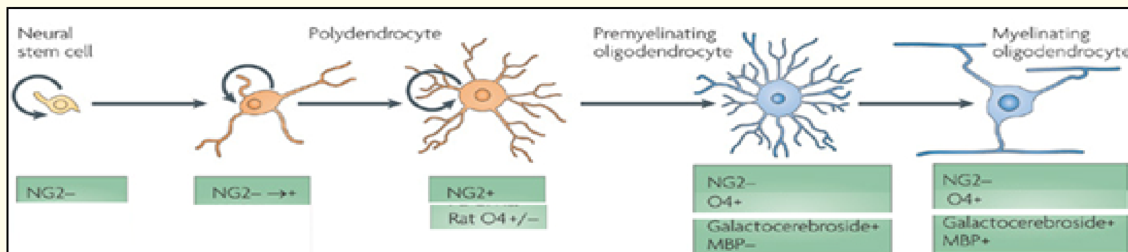


Figure 2: *This image shows the four different types of glial cells found in the central nervous system: Astrocytes (red) and oligodendrocytes (green) influence the way chemical and electrical signals travel from neuron to neuron (blue) and may shape the way information is stored. Microglial cells (yellow) help protect the brain. Ependymal cells (light pink).*

Artwork by Credit: E. Otwell.



As polydendrocytes undergo terminal differentiation into mature oligodendrocytes they lose the expression of NG2 and begin to express the immature oligodendrocyte antigen O4, followed by galactocerebroside and subsequently myelin basic protein (MBP).

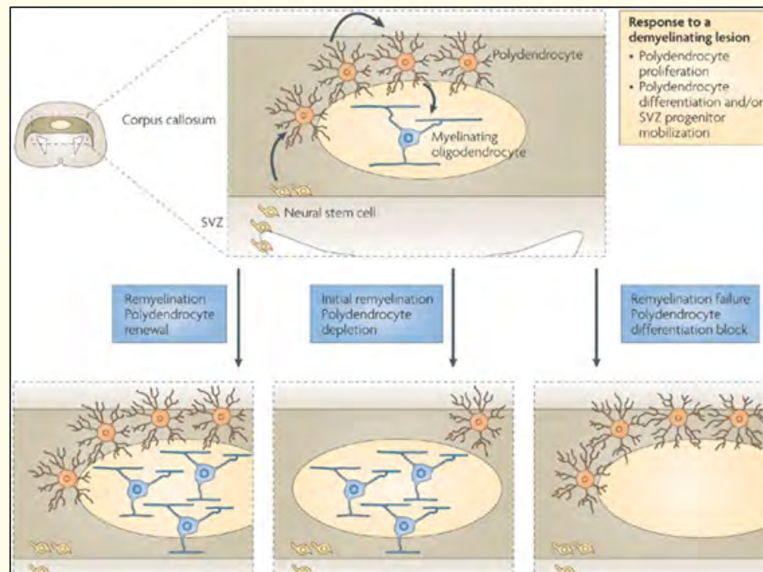


Figure 3: *Following a demyelinating lesion (yellow oval), polydendrocytes that reside in the rat corpus callosum or those that are recruited from the subventricular zone (SVZ) proliferate around the lesion and differentiate into oligodendrocytes, which myelinate demyelinated axons in the lesion (top panel). The extent of remyelination can therefore be affected by both the ability of the polydendrocytes to self-renew and their ability to differentiate into myelinating oligodendrocytes. Successful remyelination might occur when polydendrocytes proliferate and differentiate into oligodendrocytes, and in this scenario the polydendrocyte population would be replenished (left-hand bottom panel). In the absence of substantial proliferation, the polydendrocytes would not be repopulated and a second demyelinating lesion would therefore not be successfully remyelinated (central bottom panel). In the other possible scenario, remyelination would not occur because proliferated polydendrocytes fail to differentiate into oligodendrocytes, or newly differentiated oligodendrocytes fail to ensheath axons (right-hand bottom panel). [8].*

The physiological role of glial cells in brain function and homeostasis

Brain homeostasis is critically dependent on the functionality of the glial cells as summarized by Kaminsky., *et al*. [1] (Figure 4).

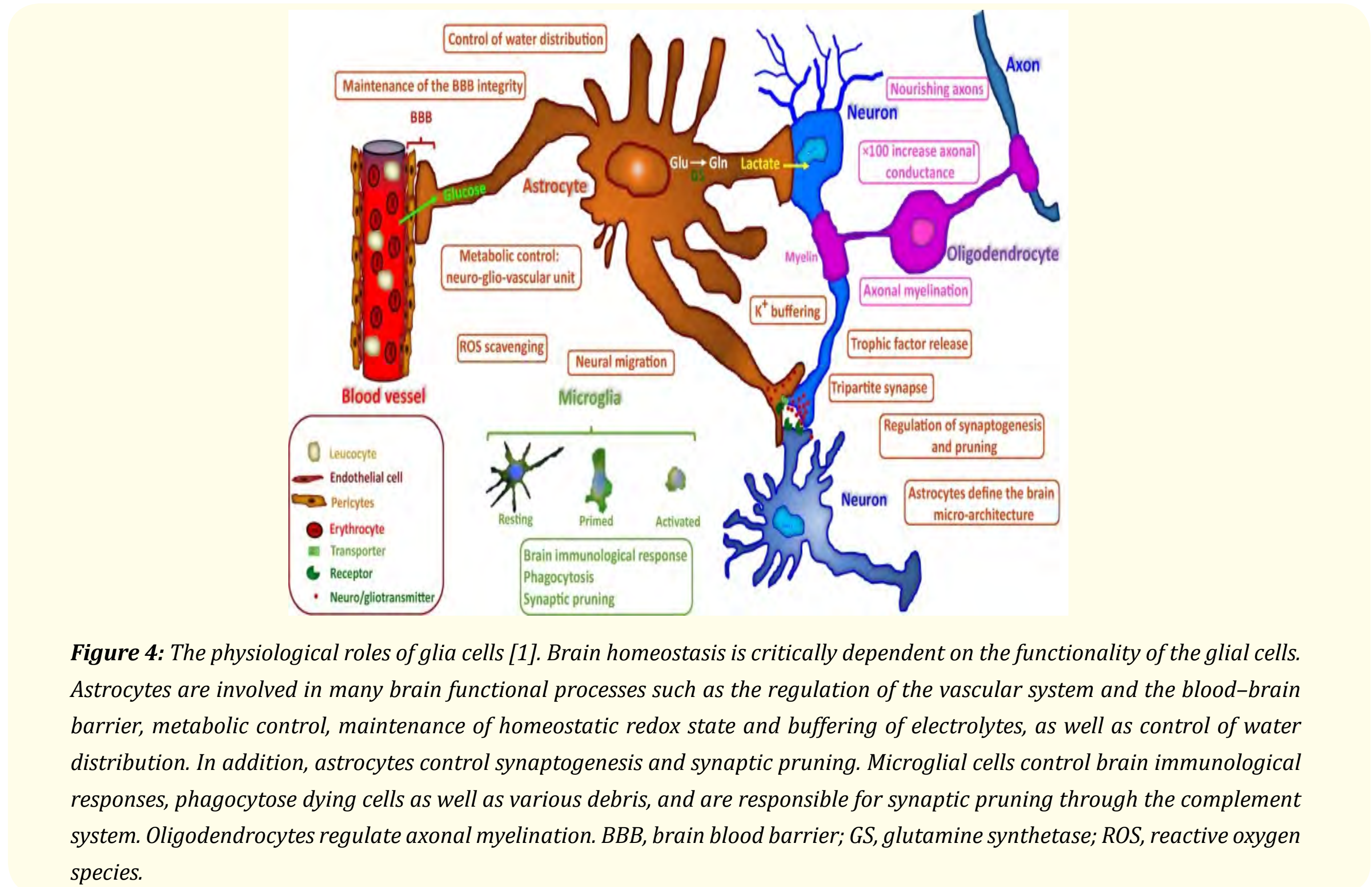


Figure 4: *The physiological roles of glia cells [1]. Brain homeostasis is critically dependent on the functionality of the glial cells. Astrocytes are involved in many brain functional processes such as the regulation of the vascular system and the blood–brain barrier, metabolic control, maintenance of homeostatic redox state and buffering of electrolytes, as well as control of water distribution. In addition, astrocytes control synaptogenesis and synaptic pruning. Microglial cells control brain immunological responses, phagocytose dying cells as well as various debris, and are responsible for synaptic pruning through the complement system. Oligodendrocytes regulate axonal myelination. BBB, brain blood barrier; GS, glutamine synthetase; ROS, reactive oxygen species.*

Optogenetic approach in glial cells

Optogenetics is an innovative biological technique which involves the use of light of specific wavelength to control cells in living tissue that have been genetically modified to express light-sensitive ion channels (Figure 5).

It is generally regarded that, neuronal, but not glial, activity is affected by membrane potential. Thus, optogenetics have been experimentally used mainly to manipulate neuronal activity *in vivo* [10]. However, recent studies showed that glial cell activity can be also optogenetically manipulated (Figure 6), which may serve as a new tool to elucidate the role of glial cells in higher brain functions [9,11].

Malfunctioning glial cells are involved in a variety of brain diseases as summarized by Kaminsky., *et al*. [1] (Figure 7).

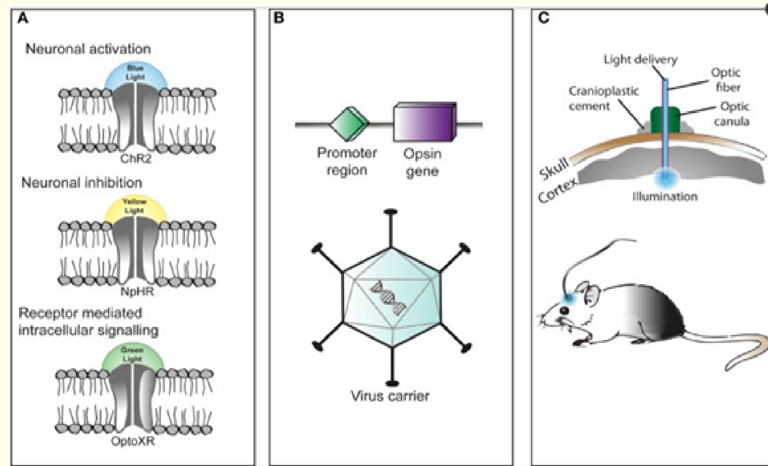


Figure 5: *Three primary components in the application of Optogenetics are as follows:*

(A) Identification or synthesis of a light-sensitive protein (Opsin). (B) The design of a system to introduce the genetic material containing the opsin into cells for protein expression such as application Adeno-Associated-Virus (genetically engineer a microbial opsin based on the gating properties (rate of excitability, refractory period, etc.) required for the experiment (microbial opsin)→ infect certain cell. Another approach is the creation of transgenic mice where the optogenetic actuator gene is introduced into mice zygotes with a given promoter. (C) Application of light emitting instruments connected to a computer. Application of light can be placed at the terminal ends or the main region where the infected cells are situated. Recent advances include the advent of wireless devices that gives the animal more freedom of mobility to reproduce in vivo results [9].

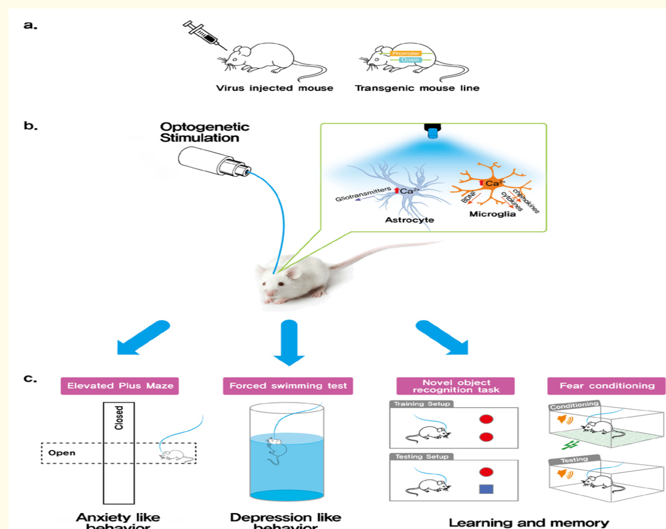


Figure 6: *A diagrammatic research scheme to dissect in vivo function of glia in higher brain function using optogenetics. This scheme shows glial cell type-specific opsin gene expression by using viral vectors or cell type-specific transgenic mice (a). Upon optogenetic glia activation/inhibition (b), the in vivo glia function can be assessed by subjecting the mice in a series of behavioral tests (c). The function of glia in anxiety can be measured by elevated plus maze that is based on the aversion of mice to open spaces. The forced swimming test is the most frequently used behavioral test for assessing depression-related behaviors. To assess the recognition and aversive memory, behavioral tests such as novel object recognition and fear conditioning task can be used [11].*

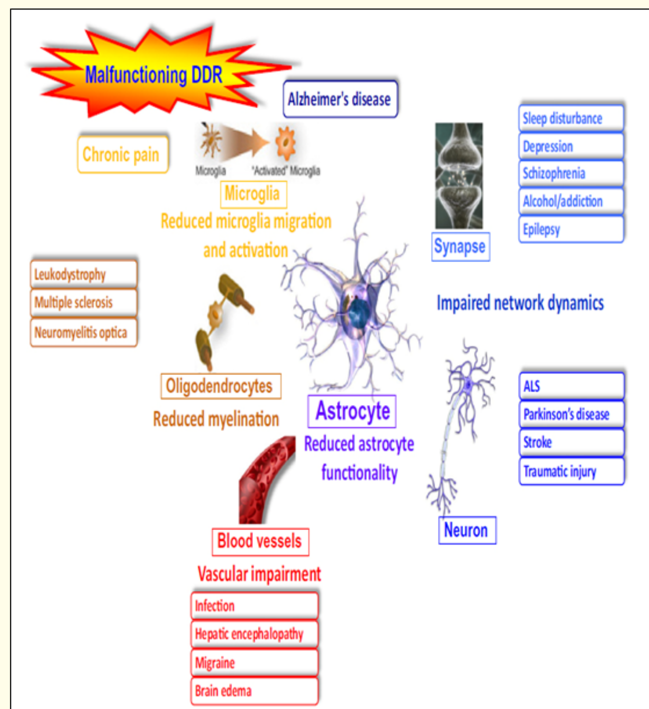


Figure 7: *The role of glial cells in brain degenerative diseases [1].*
Malfunctioning glial cells are involved in a variety of brain diseases as depicted in the Figure. DDR, DNA damage response; ALS, amyotrophic lateral sclerosis.

Astrocytes

Astrocytes are highly heterogeneous in the morphological appearance [12-14]. There are many distinct subsets of astrocytes that can be distinguished based on their morphology and biochemical characteristics (Figure 8) [15].

The main types of astroglia are:

1. Protoplasmic astrocytes located in the gray matter of the brain and spinal cord; usually have 5 - 10 primary processes with extremely elaborate branches.
2. Fibrous astrocytes localized in the white matter of the brain and spinal cord, and in the nerve fiber layer of the retina; they have long (up to 300 um) processes that run parallel to axons.
3. Radial glia, are a common feature of the developing brain. They are bipolar cells with an ovoid cell body and elongated processes. After its maturation, they disappear from most brain regions except in the retina (Mũller glia) and cerebellum (Bergmann glia).
4. Velate astrocytes are protoplasmic astrocytes found in the cerebellar molecular layer.
5. Astrocytic neural stem cells (type-B cells) are found in the sub ventricular zone (SVZ) of the lateral ventricle.
6. Pituicytes of the neurohypophysis. They are considered as astrocytes as their cytoplasm presents glial fibrillary acidic protein (GFAP).

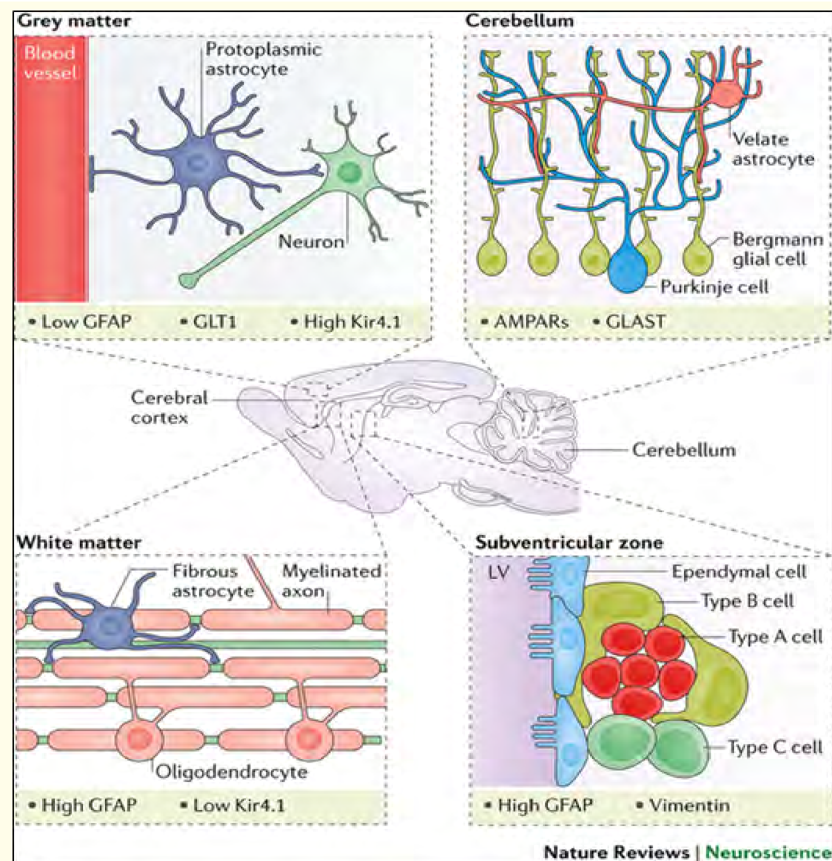


Figure 8: *The schematics illustrate the location and characteristics of several different types of astrocytes in the rodent brain. Protoplasmic astrocytes in the grey matter have a radial morphology and contact neuronal synapses and blood vessels (upper left panel). By contrast, fibrous astrocytes in the white matter have an elongated morphology and are in close contact with oligodendrocytes and myelinated axon tracts (bottom left panel). In the cerebellum, Bergmann glia extends long processes into the molecular layer and enwraps Purkinje cell dendrites (upper right panel). Velate astrocytes are protoplasmic astrocytes found in the cerebellar molecular layer. In the rodent subventricular zone, astrocyte-like type B cells line the lateral ventricles (LVs). These different types of astrocytes differentially express generic astrocyte markers. These include intermediate filament proteins (glial fibrillary acidic protein (GFAP) and vimentin, glutamate transporter 1 (GLT1) and glutamate aspartate transporter (GLAST), the inward rectifying potassium channel Kir4.1 and AMPA receptors (AMPARs). In addition to these different types, protoplasmic astrocytes can also display inter- and intra-regional molecular differences [15].*

Astrocytic cellular connectivity

Nerve cells display no direct connections between their higher-order branches. This structure is also apparent for prominent primary processes of astrocytes. But, their ultrathin protrusions can fuse and form cytosolic connections by gap junctions “connexions” – both homocellular (astrocytes to astrocyte) and heterocellular (astrocyte to oligodendrocyte and sometimes to neurons), providing a powerful communication channel that is permeable to both small ions and larger macromolecules [16].

Although EM has been the only tool that can resolve delicate cellular architecture of brain astrocytes, its critics point out that the associated tissue fixation and preparation procedures might, at least in theory, rapidly alter delicate cellular structures. Inevitably, the goal posts of research in this area have moved towards the exploration of live astrocytes *in situ*.

Rusakov [17] explored the live brain astrocytes *in situ* by nanoscopic physiology. He used two-photon excitation (2PE) microscopy and associated techniques that provide an opportunity to collect fluorescence from live cells deep in organized brain tissue. Genetically encoded, astrocyte-specific labelling of live astrocytes revealed cloudy 'fuzzy' fluorescence, suggesting local Ca^{2+} signals in the fine astrocyte processes (Figures 9Aa, Ab). Electron microscopic study of the cultured astroglia revealed reflexive gap junctions in ultrathin astroglial protrusions (Figure 9B). Rusakov [17] suggested two possible modes of arbor connectivity in astroglia: classical tree-like flow and inter-branch, lateral flow via reflexive gap junctions (Figure 9C). The term reflexive gap junction is introduced to describe gap junctions between adjacent processes from the same cell.

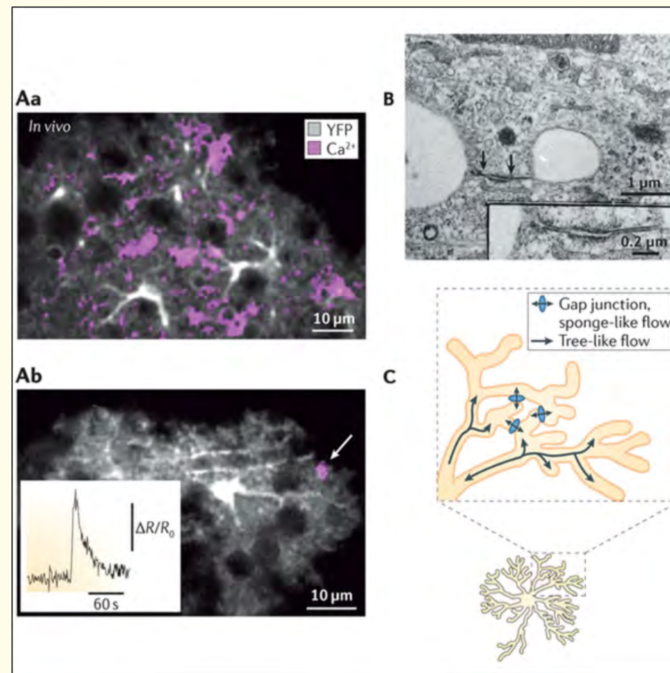


Figure 9: *Cellular connectivity and Ca^{2+} homeostasis in astroglial protrusions.*
Aa | Astroglia imaged in the somatosensory cortex in vivo. Ca^{2+} signalling domains (colored) are localized to the meshwork of fine astrocyte processes (white areas). Ab | Local Ca^{2+} signals (indicated by the arrow) documented in the fine astrocyte processes of inositol-1,4,5-trisphosphate receptor type 2 (Ins(1,4,5)P3 R2)-deficient mice (Ins(1,4,5)P3 R2 mediates the most commonly documented Ca^{2+} signalling cascade in astroglia). ΔR/R0 indicates the baseline normalized Ca^{2+}-sensitive fluorescence intensity signal. B. | Cellular connectivity and Ca^{2+} homeostasis in ultrathin astroglial protrusions. The left panel shows electron micrographs of reflexive gap junctions (that is, between processes of the same cell; indicated by arrows) in cultured astroglia (which are magnified in the inset). C. | The schematic shows two possible modes of arbor connectivity in astroglia: classical tree-like flow and inter-branch, lateral flow via reflexive gap junctions [17].

Agulhon., *et al*. [18] and Cho., *et al*. [11] stated that astrocytes *in situ* not only listen and react to ongoing neuronal activity but also could modulate this activity via the release of gliotransmitters. Ca^{2+} elevations in a small fraction of astrocytes and under certain conditions *in situ* can result in the release of gliotransmitters, including glutamate, ATP, and D-serine, that bind to pre- and/or postsynaptic neuronal receptors to modulate synaptic transmission and activity (Figure 10).

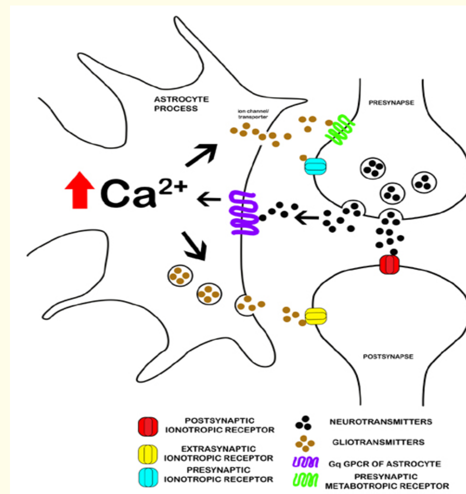


Figure 10: *Schematic Depicting the Tripartite Synapse [11].*
The tripartite synapse is composed of presynaptic and postsynaptic terminals with astrocytic processes enwrapping the synapses. The release of neurotransmitter from the presynaptic terminal acts on the postsynaptic terminal as well as with astrocytic receptors mediating intracellular Ca^{2+} elevation via Gq GPCR. Ca^{2+} elevation then triggers the release of gliotransmitters that react with the presynaptic and postsynaptic terminal receptors to modulate synaptic transmission.

It is now established that astrocytes are not solely supporting elements for neuronal activity but active and dynamic players in different aspects of neuronal function.

Mitochondrial Transfer between astrocytes and neurons

Mitochondria are organelles that perform many essential functions, including providing the energy to cells. Cells remove damaged mitochondria through a process called "mitophagy". Mitophagy is considered a subset of a process called "autophagy" which means "self-eating", in which the cell degrades its own mitochondria by enwrapping and delivering it to lysosomes. However, a new mitochondrial mechanism of neuroglial crosstalk between neurons and astrocytes has been reported.

Falchi., *et al*. [19] reported that cultured astrocytes (obtained from 8- to 10-week-old whole fetal brains from medically induced or spontaneous abortions) shed extracellular large membrane vesicles that contain mitochondria (Figures 11 and 12).

Using 3D electron microscopic volumes produced by serial block-face SEM (SBEM) (Figure 13) and high resolution TEM, Davis., *et al*. [20] showed large numbers of mitochondria were shed from neurons of the mice optic nerve head, to be degraded by the lysosomes of adjoining glial cells (Figure 14). They found sub-axolemmal accumulations of mitochondria clustered within healthy axons specifically at the sites of direct contact between the axons and astrocyte processes. Microtubules were found near the mitochondria, demonstrating that the evulsions shed from axons contain axoplasmic components other than just mitochondria. They suggested that neurons can release and transfer damaged mitochondria to astrocytes for disposal and recycling.

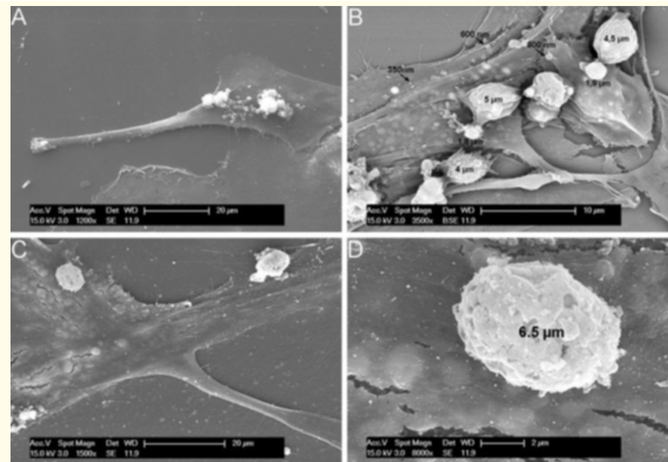


Figure 11: *SEM images. a The image shows many membrane vesicles of different sizes shedding from the cell body of an astrocyte with a long process. b Magnification of membrane vesicles shedding from overlapping astrocytes. Extracellular vesicles with different sizes are clearly visible still attached to the cell. Size of membrane vesicles larger than 1 um are shown. Arrows indicate smaller vesicles. c Image of two large-size vesicles shedding from the plasma membrane and d detail of the same vesicle observed in c [19].*

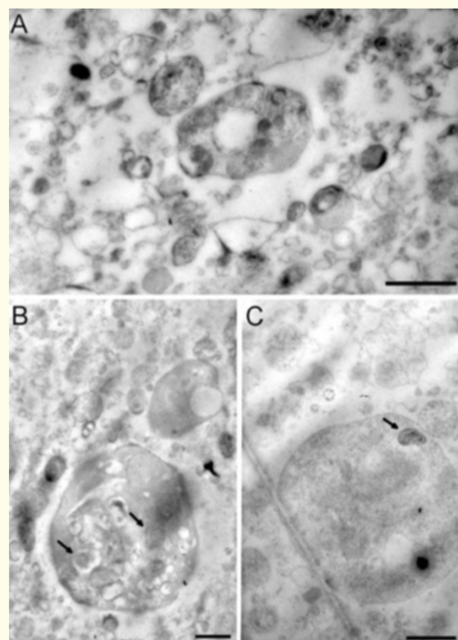


Figure 12: *TEM images of isolated vesicles. a Transmission electron micrographs of isolated vesicles obtained from cell-free supernatants of astrocyte cultures. At the ultrastructural level the membrane vesicles show evident heterogeneity in their size and morphology. b, c Membrane vesicles larger than 1 um were also observed. These large membrane vesicles display membrane content that may represent what remains of mitochondria and other membranous organelles (arrows) after the ultracentrifugation process. Bars 1 um [19].*

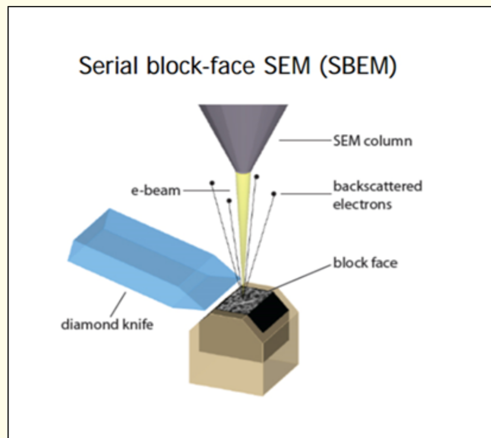


Figure 13: *3D electron microscopic technique. Serial block-face SEM (SBEM), in which in-situ ultramicrotome installed inside the electron microscope. The image of the block face will be collected, then the ultramicrotome will cut the sample to expose the next layer to be imaged, in steps as small as 15 nm.*

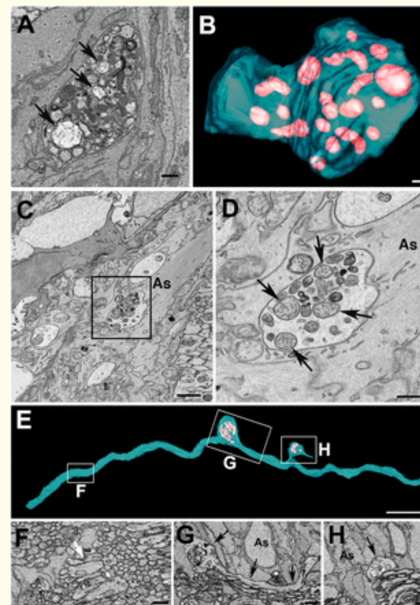


Figure 14: *EM demonstrates that mitochondria are shed through formation of large protrusions that originate from otherwise healthy axons. Evulsions of retinal ganglion cell axons within the optic nerve head (ONH) contain mitochondria. An SBEM single section (A) and serial reconstruction (B) of axonal evulsion within the ONH of a 3-mo-old C57BL/6J mouse containing morphologically distinct mitochondria (arrows in A and pink volumes in B). Transmission EM (C) and enlarged view of the boxed area (D) showing mitochondria with normal morphology (arrows) and mitochondria remnants within the same evulsion. (E) SBEM-based reconstruction of a single axon displaying two protrusions (boxes G and H). (F–H) Sections through the areas boxed in E. The white arrow in F points to close apposition between axons without intervening glia, and the black arrows in G and H point to direct contacts between the axon and astrocyte processes (As). (Scale bars: A, B, and D, 0.5 μm; C, 1 μm; E, 5 μm; F–H, 2 μm) [20].*

A metabolic-coupling between astrocytes and neurons following hypoxia was reported by Pluchino., *et al* [21]. They stated that astrocytes can transfer functional mitochondria (both via direct cell-to-cell contact and/or extracellular vesicles) to hypoxic neurons, thereby increasing aerobic respiration. Damaged/dysfunctional mitochondria can be transferred from neurons to astrocytes to support their degradation via trans-mitophagy (Figure 15).

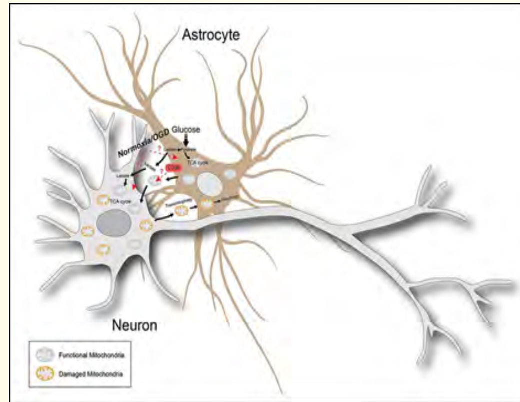


***Figure 15:** Metabolic-coupling between astrocytes and neurons following hypoxia. Astrocytes can transfer functional mitochondria (both via direct cell-to-cell contact and/or extracellular vesicles) to hypoxic neurons, thereby increasing aerobic respiration. Damaged/dysfunctional mitochondria can be transferred from neurons to astrocytes to support their degradation via trans-mitophagy [21].*

Very recently, Hayakawa., *et al*. [22] showed that astrocytes released functional extracellular mitochondria which were transferred and entered the adjacent neurons to support neuronal viability after the induction of transient focal cerebral ischemia, amplifying cell survival signals. This finding may contribute to endogenous neuroprotective and neuro recovery mechanisms ischemic stroke [22]. Electron microscopic study confirmed the presence of extracellular particles containing mitochondria in conditioned media from rat cortical astrocytes (Figure 16).

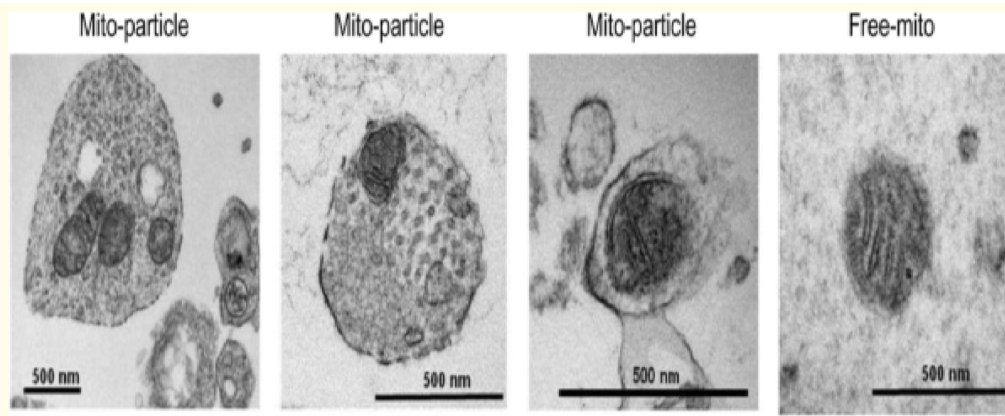


***Figure 16:** Electron micrographs showing the presence of extracellular particles containing mitochondria in conditioned media from rat cortical astrocytes [22].*

Role of astrocytes as neural stem cells in the adult brain

The subventricular zone, the largest niche of adult neural stem cells, is located along the lateral walls of the lateral ventricles in the

forebrain. Alvarez-Buylla., *et al*. [23] found a subpopulation of astrocytes in the SVZ labeled with bromodeoxyuridine (BrdU) which is a cell proliferation marker, suggesting that these glial cells corresponded to "stem cells". These multipotent astrocytes are denominated as type-B cells, which typically express the glial fibrillary acidic protein (GFAP). Later reports confirmed these observations indicating that the GFAP-expressing astrocytes are candidate stem cells in the SVZ [24,25]. Interestingly, astrocytes collected from multiple brain regions before postnatal day 10 may behave as neural stem cells *in vitro*. After that time, only the SVZ astrocytes retain this 'stemness' capacity [26]. Type-B astrocytes of the SVZ produce oligodendrocyte precursor cells that help maintain the oligodendrocyte population in the corpus callosum, fimbria fornix and striatum [14,27] (Figure 17).

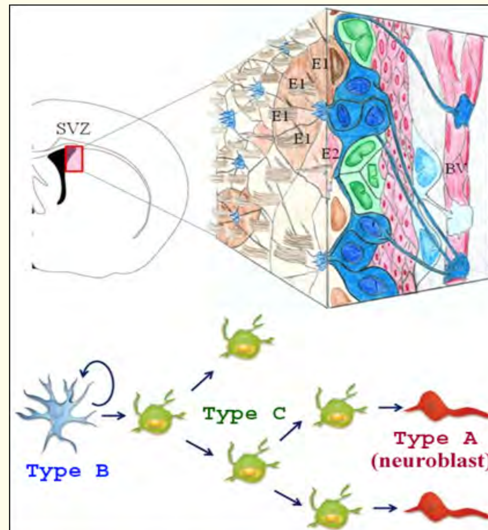


Figure 17: *The adult subventricular zone (SVZ) is lining the striatal wall of the lateral ventricles in the brain (coronal section left). A 3-D model of the SVZ is shown to the right. This region contains astrocytic neural stem cells (type-B cells depicted in blue). Type-B cells give rise to rapidly dividing intermediate progenitor cells (type-C cells depicted in green), which in turn produce migrating neuroblasts (type-A cells depicted in red). Type-B astrocytes have a long basal process contacting blood vessels (BV) and an apical ending at the ventricle surface. Note the pinwheel-like organization composed of multiciliated (Type-E1) and bi-ciliated (Type-E2) ependymal cells encircling apical surfaces of astrocytes [14].*

These findings may shed light on the genetic and molecular basis of stem-cell behavior in SVZ astrocytes, changed the perception about glia and advanced our understanding of neural stem cells, which in turn will allow designing novel therapies against neurodegenerative diseases.

Oligodendrocytes

From the initial description, oligodendrocytes have been subdivided into the interfascicular and perineuronal satellite types (Figure 18), the former is myelinating cells distributed mainly in the white matter. On the other hand, perineuronal oligodendrocytes mainly reside in the gray matter, and appose directly to neuronal cell bodies without forming any morphological specialization between them. They are assumed to be unmyelinating cells in normal brains, but are capable of producing myelin when remyelination of axons is induced after demyelination. Interestingly, the number of perineuronal oligodendrocytes is reduced prominently and significantly in the prefrontal cortex of patients with schizophrenia and mood disorders [28]. Takasaki., *et al*. [29] assumed that perineuronal oligodendrocytes differentiate in the developing cerebral cortex to meet increasing demands of principal neurons for metabolic support.

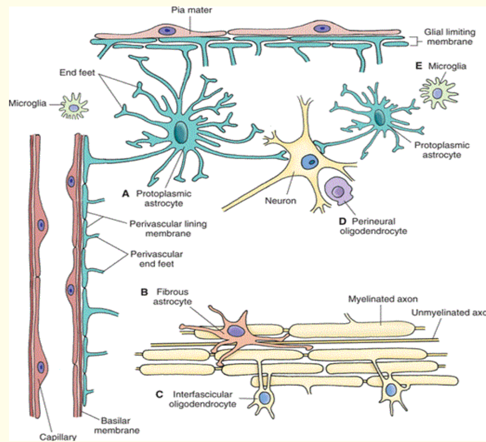
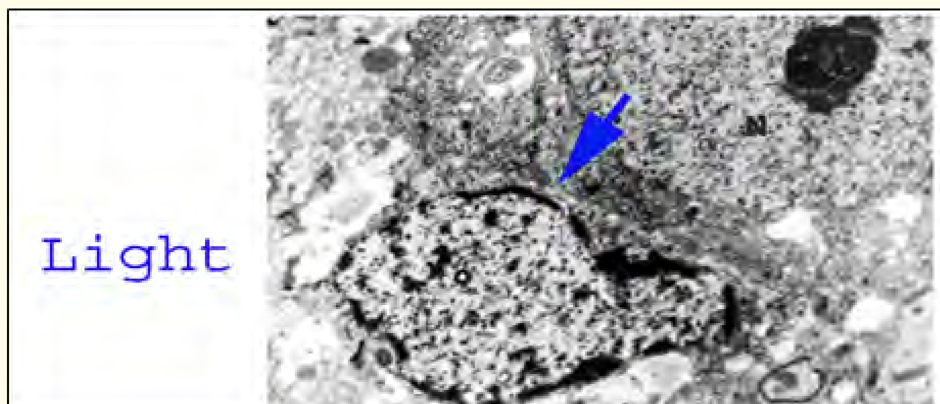


Figure 18: *Interfascicular and perineuronal oligodendrocytes. (https://healtheappointments.com/chapter 5 histology of the nervous system).*

Verkhratsky., *et al.* [30] classified oligodendrocytes, according to morphological appearance, into four types: Type I oligodendrocytes are mainly present in the gray matter and have small rounded somata and a complex process arborization, with fine branching processes that myelinate 30 or more small diameter axons with short internodes. Type II oligodendrocytes are most common in the white matter and have small somata and parallel arrays of intermediate length internodes (100 - 250 mm). Type III oligodendrocytes myelinate large diameter axons (e.g., in the medulla oblongata or the spinal cord funiculi); they are characterized by larger cell bodies and extend one or more thick primary processes that rarely branch and myelinate a small number of axons with long internodes (250 - 500 mm). Type IV oligodendrocytes myelinate a single large diameter axon with a very long internode (up to 1000 mm in length) and are localized at the entrance of CNS nerve roots.

Studies on the level of the electron microscope allowed for observation of three types of oligodendrocytes: those of light, medium and dark cytoplasm (Figure 19). The number of dark oligodendrocytes increased with age. Histochemical studies indicated the participation of oligodendrocytes in the metabolism of iron. The cause of the occurrence of oligodendrocytes with various cytoplasm densities might relate to the accumulation of iron in their cytoplasm (Figure 20) [31].



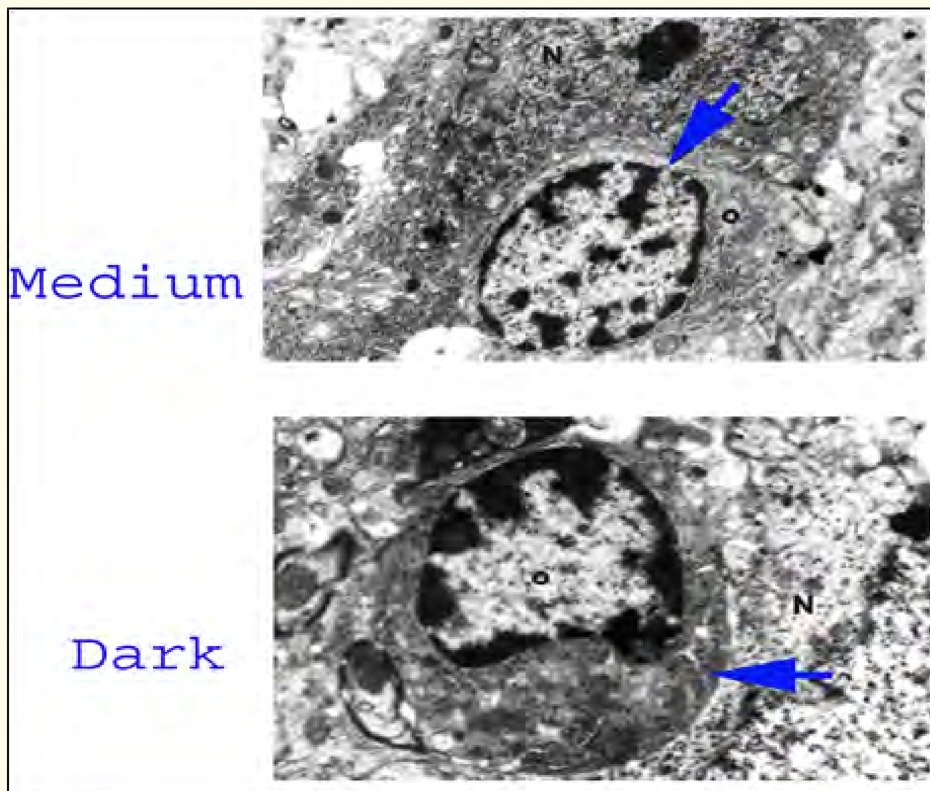


Figure 19: *Electron micrographs from the frontal cortex rat show light oligodendrocyte (of a 90-day-old), medium oligodendrocyte (of a 120-day-old), dark oligodendrocyte (of a 150-day-old) [31].*

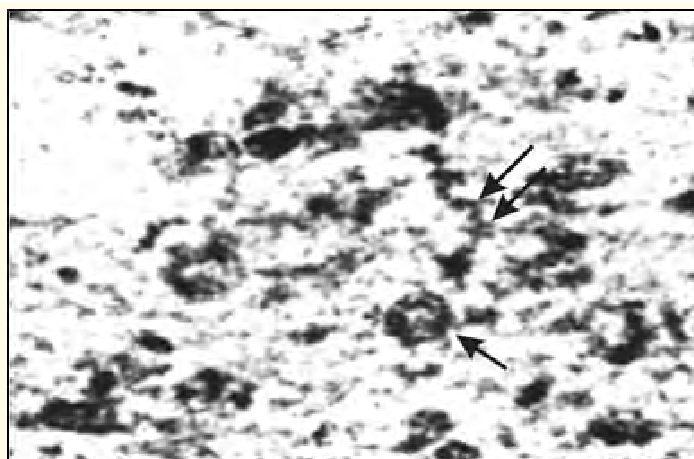
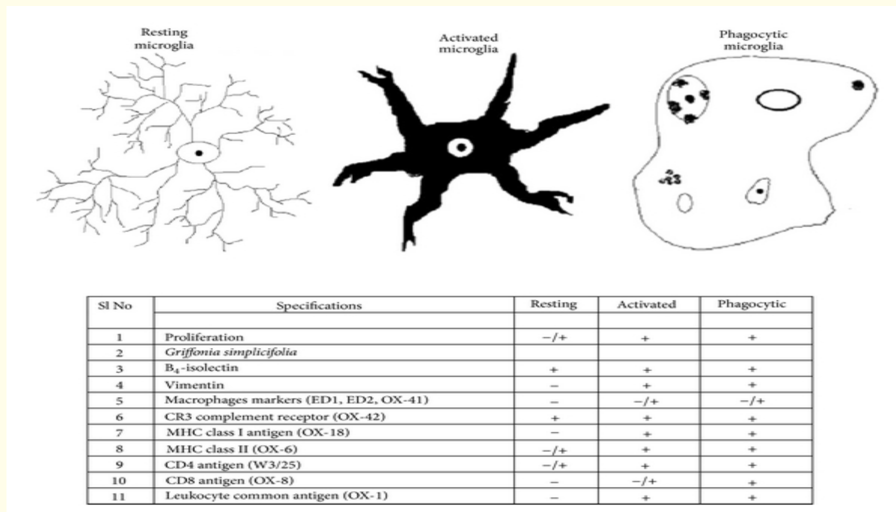


Figure 20: *The deeper layers of the frontal cortex of a 120-day-old rat. The product of the histochemical reaction to the presence of iron occurs in the cytoplasm and oligodendrocyte processes, (arrow) as well as between these cells (double arrows); x 1400 [31].*

Microglia

Microglia transits through different stages of development to attain its maturity and functionality in the CNS (Figure 21). The first stage is "activated or ameboid microglia'. It is called "macrophage of the brain" as it shares common immunological, histochemical and morphological features with macrophages outside the CNS. In due course of time and under certain circumstances, some parts of the embryonic ameboid microglia get degenerated and some develop thin processes to become "resting or ramified microglia" in an adult brain [32]. This "resting" form of microglia is characterized by a small cell body. In the normal brain, their ramified processes are constantly scanning the surrounding brain tissue and rapidly moving towards sites of acute injury or danger signals. Unlike activated or ameboid microglia, ramified microglia does not phagocytose cells but they are able to search for and identify immune threats in the CNS [33].



Sl No	Specifications	Resting	Activated	Phagocytic
1	Proliferation	−/+	+	+
2	*Griffonia simplicifolia*			
3	B_4-isolectin	+	+	+
4	Vimentin	−	+	+
5	Macrophages markers (ED1, ED2, OX-41)	−	−/+	−/+
6	CR3 complement receptor (OX-42)	+	+	+
7	MHC class I antigen (OX-18)	−	+	+
8	MHC class II (OX-6)	−/+	+	+
9	CD4 antigen (W3/25)	−/+	+	+
10	CD8 antigen (OX-8)	−	−/+	+
11	Leukocyte common antigen (OX-1)	−	+	+

Figure 21: *Different states of microglia and associated phenotypic characteristics (adopted from Neuroglia; Second Edition; Edited by Helmut Kettenmann and Bruce R. Ransom).*

In response to variety of brain injuries and inflammation, the ramified microglia is capable of dramatically changing its structural and chemical morphology into a reactive or an ameboid microglia [34]. These activated microglia become highly motile, secreting inflammatory cytokines, migrating to the lesion area, and phagocytosing cell debris or damaged neurons [35] (Figure 22).

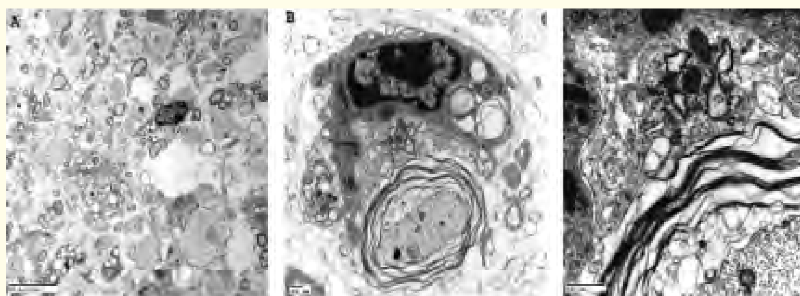


Figure 22: *Ultrastructural appearance of a perineuronal phagocytic microglial cell in the chronic demyelinating plaque of RSA59 infected mouse spinal cord. Electron micrograph (a) shows microglial cells surrounding the demyelinated axon in a demyelinating plaque region of RSA59 infected mouse spinal cord. Microglia has a heterochromatic nucleus with multiple vacuoles. High-magnification images (b) and (c) demonstrates the presence of microglia surrounding the unraveling myelin sheath in an axon. The microglia cell membrane is in intimate contact with the outer portion of the myelin sheath as the microglia is stripping away (phagocytizing) the myelin sheath and engulfs the myelin sheath. Multiple vacuoles with myelin fragments are seen within the cytoplasm of the activated microglia (phagocytotic in nature) [36].*

Iba1 (a microglia/macrophage-specific calcium-binding protein) is a better marker for morphological differentiation of resting-ramified microglia versus activated-amoeboid- phagocytotic microglia (Figure 23) [37].

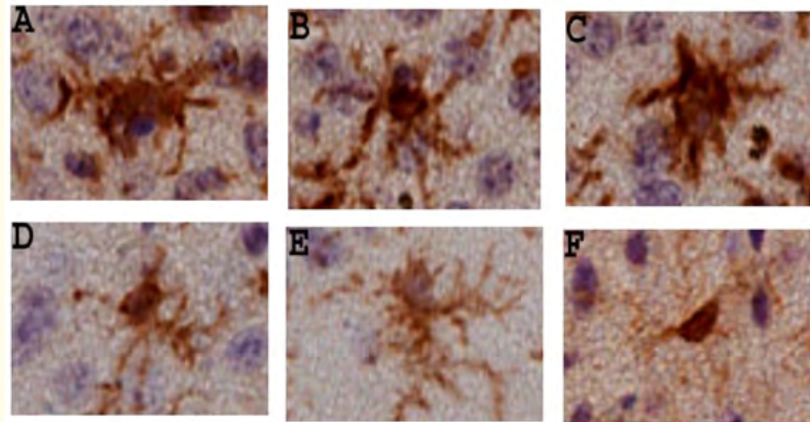


Figure 23: *Comparative morphology of activated amoeboid microglia to resting quiescent ramified microglia was taken from acute inflamed brain to resting brain tissue. Different shape of amoeboid microglia, which is embracing neuron immunostained with Iba1 and counter stained with haematoxylin(a)–(e) (1000x); (f); quiescent resting microglia in a non-pathological brain of 4-week-old mice. (Data taken from Das Sarma laboratory; unpublished data) [37].*

Bisht., *et al*. [38] recently characterized a microglial phenotype that is induced by chronic stress, aging or Alzheimer disease pathology. This 'dark' microglia appears overly active compared with the normal microglia, reaching for synaptic clefts, and extensively engulfing pre-synaptic axon terminals and postsynaptic dendritic spines. Dark microglia is characterized by electron-dense cytoplasm and nucleoplasm and display ultrastructural features of cells undergoing oxidative challenge. Bisht., *et al*. [38] hypothesized that dark microglia could be specifically implicated in the pathological remodeling of neuronal circuits, which impairs learning, memory, and other essential cognitive functions (Figure 24).

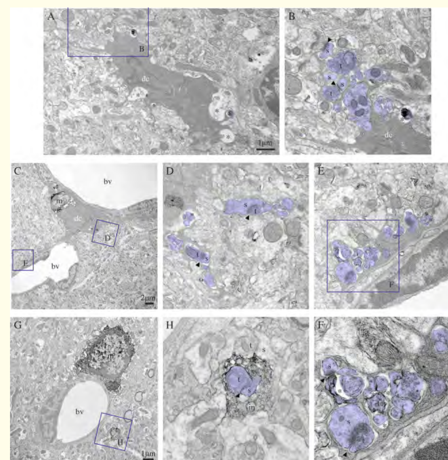


Figure 24: *Dark microglia's interactions with synapses. [38] A–F: Examples of dark microglial cells (dc) typically contacting synaptic elements (colored in purple) with their profusion of highly ramified and extremely thin processes, reaching for synaptic clefts (arrowheads), while encircling axon terminals (t) and dendritic spines (s), in the CA1 lacunosum-moleculare of stressed CX3CR1 knockout mice. In (C), the dark microglia is simultaneously contacting two blood vessels (bv) and a normal microglia (m) that is stained for IBA1. Its processes are extensively encircling various types of synaptic elements, including shrunk axon terminals surrounded by extracellular space (asterisks) in the process of being digested and an entire synapse (see the inset in F). By comparison, an example of IBA1-stained microglia (m) that is extending a single process, discontinuous from its cell body in ultrathin section, is shown in (G). Contrary to the dark microglia processes, it is bulkier and showing obtuse (instead of acute) angles. It nevertheless contains several phagocytic inclusions (in), among which a synapse between an axon terminal (t) and a dendritic spine (s), in addition to making focal contacts (instead of encircling) synaptic elements. Scale bars = 1 um for (A) and (G) and 2 um for (C).*

Ependyma

Ependymocytes line the ventricular system of the brain and the spinal cord. These cells are involved in the creation and secretion of cerebrospinal fluid (CSF) and beat their cilia to help circulate the CSF and make up the blood-CSF barrier. Their apical surfaces are also covered with microvilli, which absorb CSF, and motile cilia to circulate the CSF [39] (Figures 25 and 26).

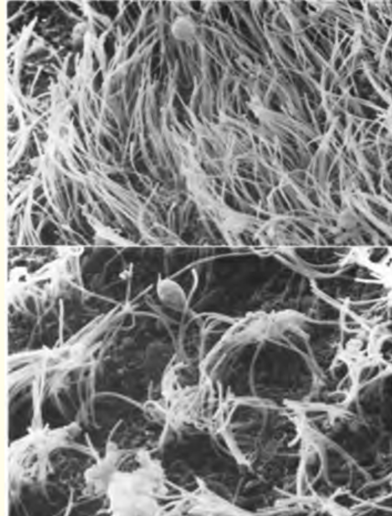


Figure 25: *Scanning electron microscope (SEM) studies of human ependyma from the lateral ventricle. Upper: Low power, X 2300. Lower." Medium power, X 3100. Note the troughs marking the ependymal cell borders and the central position of the ciliary tufts [40].*

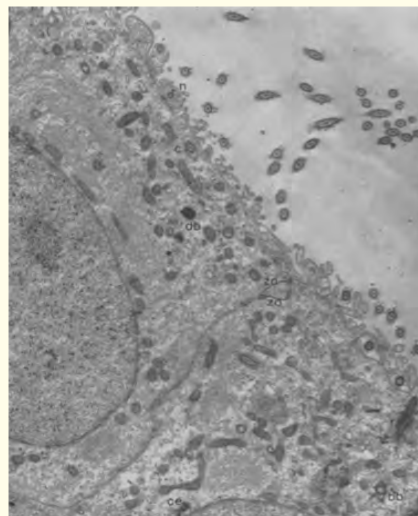


Figure 26: *Ependymal cells of the third ventricle. The free surface is thrown into numerous irregular folds which partially or completely envelop the ciliary shafts (c). The apical cytoplasm contains basal bodies (bb) and their rootlets (r), mitochondria, scattered elements of the granular endoplasmic reticulum, small vesicles, Golgi complex, and dense bodies (db). The smooth, oval nuclei are surrounded by whorls of filaments sectioned longitudinally and transversely. Two apically situated zonulae adherents (za) are joined by a zonula occludens (zo). One of the cell processes (n) lying within the ventricle may be neuron in origin. A centriole (ce) appears adjacent to the nucleus of the lower cell. X 17000 [41].*

The ependymal cells in the ventricles are loosely joined together by "desmosomes". This loose junction enables the diffusion of the CSF. While, those surrounding the choroid plexus are connected by "tight junctions", which prevent the leakage of substances from the blood into the CSF. Another type of ependymal cell, known as a "tanycyte" is found only in the lining on the floor of the third ventricle in the brain. These cells are unique from other ependymal cells in that they have "long" processes and large "end feet" (Figure 27).

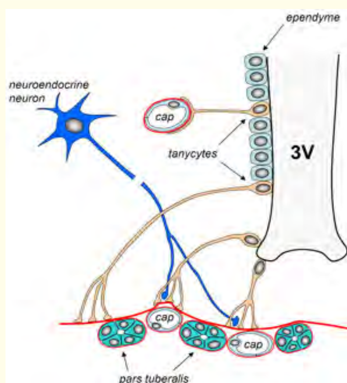


Figure 27: *Tanycytes extend processes which surround the neurosecretory terminals of the hypothalamo-pituitary system [42].*

The long processes of tanycytes allow them to carry and transport hormones in the brain [39].

Ependymal cells are also thought to act as neural stem cells. Johansson., *et al*. [39] provided evidence that ependymal cells act as reservoir cells in the forebrain, which can be activated after stroke and as *in vivo* and *in vitro* stem cells in the spinal cord. However, these cells did not self-renew and were subsequently depleted as they generated new neurons, thus failing to satisfy the requirement for stem cells. Scientists believed that ependymal cells from the lining of the lateral ventricle can be transplanted into the cochlea to reverse hearing loss [43]. More recently, tanycytes have been identified as a key relay of the photoperiodic melatonin message to the hypothalamus. Alterations in the ependyma, consisting on in its flattening or loss, have been reported in some cases of hydrocephalus [44].

Conclusion

Glial cells are much more than just the " glue" that holds together the neurons of CNS. Astrocytes modulate the neuronal activity via the release of "gliotransmitters". They transmit functional mitochondria to hypoxic neurons. Also, they can act as pluripotent neural precursors for adult neurogenesis. Oligodendrocytes participate in iron metabolism. Dysfunction of microglia could be implicated in the impairment of cognitive functions (learning and memory). Ependymal cells can be transplanted into the cochlea to reverse hearing loss. Dysfunction of ependymal cells has a role in hydrocephalus.

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