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# Graphene Glial-interfaces: challenges and perspectives

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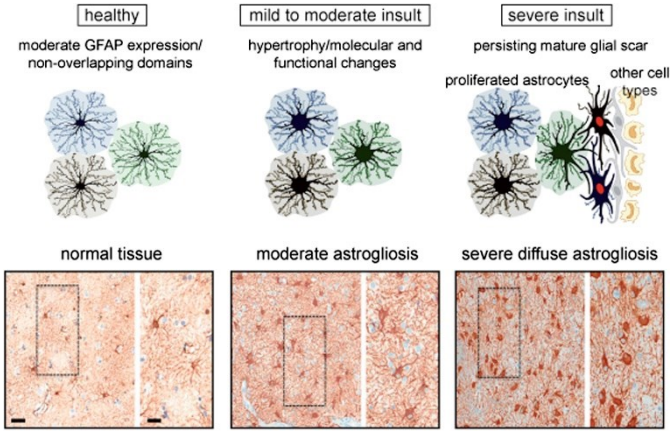


**Fig. 1** Astrogliosis. Forms of reactive astrogliosis in the human brain, depending on the severity of the lesion. Reproduced with permission from ref. [1] (licensed under the Creative Commons CC BY license, Copyright 1969, Elsevier)

## Reactive gliosis and glial pathologies

Analysis of most-mortem human brain tissues and animal models of CNS diseases have revealed that astroglial dysfunctions and microglial-mediated inflammation contribute to the pathogenesis of several neurological and psychiatric disorders.[1,2,3] In all types of brain pathologies, regardless of aetiology, astrocytes trigger a complex response mechanism called reactive astrogliosis. In reactive gliosis, astrocytes activate a complex pattern of alterations in their morphological, bioelectric and physiological properties, in response to injuries; according to the severity of the disease (Fig. 1).[2,4]

Astrogliosis manifests through hypertrophy, proliferation and alterations in protein expression, such us glial fibrillary acidic protein (GFAP) overexpression. This reaction aims at isolating the lesion site, rebuilding the blood-brain barrier and remodelling the neuronal circuits in the areas surrounding the injury, until the formation of a glial scar and the release of growth factors to facilitate synaptogenesis.[5,6] Specifically, the reaction of astrocytes located in the vicinity of the primary lesion is very different from that of astroglial cells distant from the lesion. Astrocytes located immediately around the damaged area undergo hypertrophy and proliferation, to until completely replace the existing tissue architecture with a permanent glial scar around the necrotic area. In areas further away from the injury site or in milder traumatic forms, reactive changes are much more moderate and do not distort the architecture of the nervous tissue or astrocyte organization into individual distinct domains, but rather release growth factors and facilitate synaptogenesis and neuronal network remodelling. The primary signals, which trigger both forms of astrogliosis, derive from damaged cells in the lesion core, and are represented by neurotransmitters (mainly glutamate and ATP), cytokines, adhesion molecules, growth factors and blood factors. The combination of these detrimental signals determines the type of reactive astrogliosis in the area surrounding the lesion, in different neuropatholgies (Fig.2).[1]

Insults to the brain always result in prominent changes of glial-cell properties including morphology, metabolism and proliferation. The triggering signals that produce these long-term changes are not yet well understood. Since it is apparent that glial $Ca^{2+}$signalling is an important pathway of glia-glia and neuron-glia crosstalk, it is important to understand its role during pathological conditions. *In vitro* and *in vivo* ischaemic models have revealed that brief exposures of astrocytes to

hypoxic or hypoglycemic conditions trigger [$Ca^{2+}$] elevation arising from activation of both voltage-gated $Ca^{2+}$ channels and $Ca^{2+}$ release from internal stores. The ischaemia-induced elevation in $[Ca^{2+}]_i$ could be induced either by a propagating wave of elevated $[K^+]_o$, which accompanies injury-induced spreading depression, or by neuromediators massively released upon brain damage. Increases of [$Ca^{2+}$] in astrocytes induced by brain insults might alter the cell functionality and could possibly induce the release of growth factors or result in astrocytic volume changes.[1]

Microglia are viewed as the cellular sensory element for brain pathology. Damage of the CNS triggers a complex cascade that results in a step by step activation of microglial cells and astrocyte mediated by signal cascades where $Ca^{2+}$ signalling might play a central role.[7]

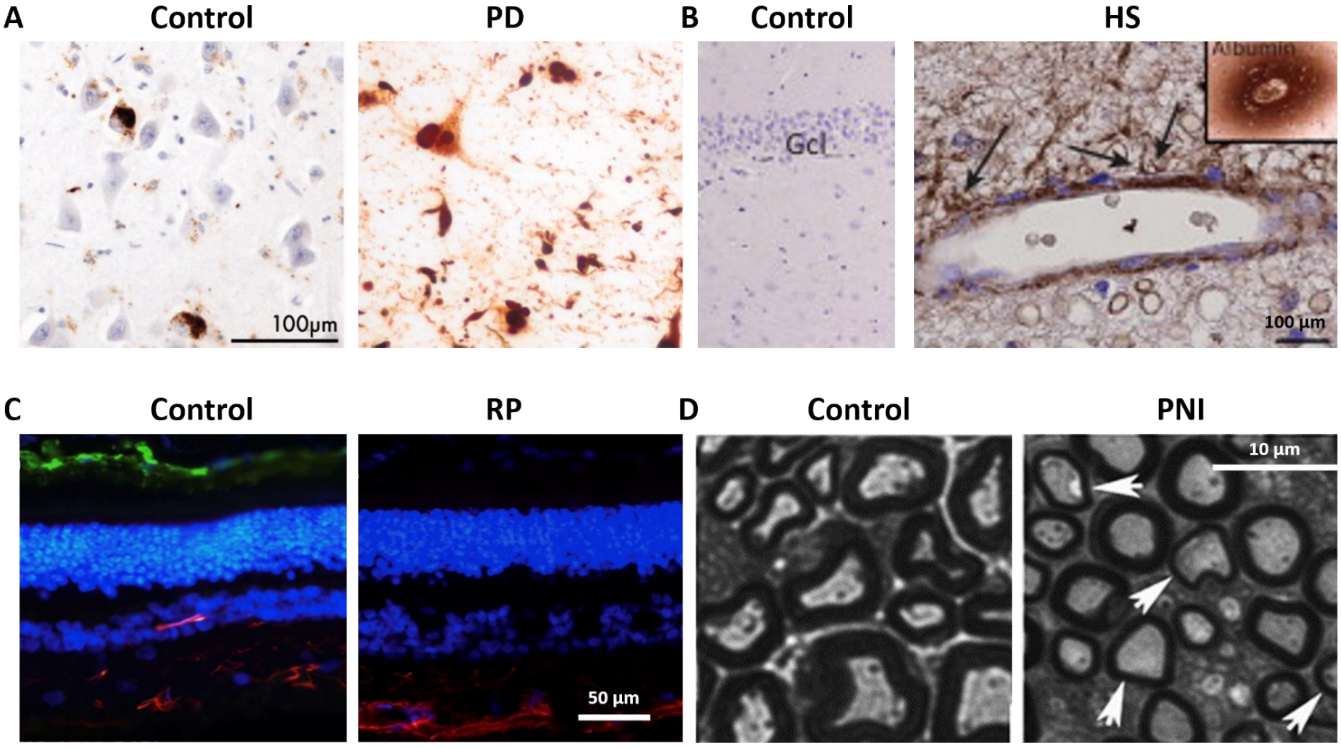


**Fig. 2** Gliotic cells in pathologies. A) Parkinson Disease (PD). Astrocytes in healthy brain tissue and astrocytes accumulating α-synuclein in the cytoplasm from PD patients. Adapted with permission from ref. [16] (Copyright 2010 Movement Disorder Society, John Wiley and Sons) B) Epilepsy. Inflammatory changes in hippocampal tissue from an autopsy control subject and in a surgically resected specimen from a patient with temporal lobe epilepsy (TLE) and associated hippocampal sclerosis (HS). Adapted with permission from ref.[13] (Copyright 2012, Elsevier) C) Retinitis pigmentosa (RP). Comparison of the glia in controls and adult dog model of RP, RPE65-/-. Adapted with permission from ref. [17] (Copyright 2015, The Authors, Elsevier) D) Peripheral nerve injury (PNI). Comparison of uninjured and demyelinated Schwann cells in mouse models of chronic nerve compression. Adapted with permission from ref. [18] (Copyright 2011, Wiley Periodicals).

**Alzheimer's disease (AD)** is the most common cause of senile dementia.[8] AD is characterized by the rapid impairment of episodic memory, followed by a serious decline in cognitive functions. Histological features of AD are represented by the formation of deposits of beta-amyloid protein (Aβ) in blood vessels, which leads to the accumulation of amyloid plaques in grey matter and the formation of abnormal intraneuronal tangles of tau protein.[9] The involvement of astrocytes in the AD pathogenesis was firstly hypothesized on 1910, supported by the data that the expression of GFAP and astrocytic hypertrophy was observed in the vicinity of amyloid plaques, confirming the correlation between astrocytes changes and progression of AD. At AD early stage, neurons begin to overproduce Aβ protein, which causes degeneration of dendrites. The release of neuronal substances triggers the activation of astrocytes, which can extend their processes towards Aβ deposits and detect them. They surround damaged neuron to eliminate neuronal debris and absorb and degrade amyloid plaques. During the progression of AD, astrocytes display alterations affecting cytoskeleton, gap junctions, K+ buffering and homeostasis of neurotransmitters (GABA and glutamate).[1,9] Calcium signalling as well as AQP4 expression are correlated with dementia and AD.[1,5,6] A more recent and intriguing hypothesis states that so-called glymphatic system alteration hamper the proper clearance of extracellular milieu from waste and provoke amyloid-β b amyloid accumulation that is causal for AD. The glymphatic system is a brain clearance pathway. Influx of cerebrospinal fluid (CSF) depends upon the expression and perivascular localization of the astroglial water channel AQP4.[10] Technologies enabling to modulate glymphatic system might thus be promising biomedical device-based approach for the therapy of AD. On this regard, it should be noted that recent evidence demonstrates that calcium signalling and water

transport can be triggered by electric field application through organic transistor architecture in cultured astrocytes *in vitro*. [11]

**Epilepsy** is one of the most common neurological diseases and it is characterized by repeated epileptic seizures, associated with motor behavioural and consciousness disorders. An astrocytic basis for epilepsy has been proposed [13,14] (Fig. 2 B), as reactive astrogliosis characterizes all forms of epilepsy, but it is particularly evident in hippocampal sclerosis, associated with epilepsy of the medial temporal lobe (MTLE). MTLE is characterized by the loss of hippocampal pyramidal neurons (CA1 and CA3) and it is widely investigated in the astroglial field. Changes in the activity of astroglial channels and receptors suggest the possible involvement of astrocytes in hyper-excitation and spread of epileptic events.[1,12,13] Observations on brain tissue samples from epileptic patients have shown alterations in the expression, localization and functionality of astroglial proteins such as Kir 4.1, AQP4, glutamate and GABA transporters. A potential impairment of AQP4 function has been proposed in TLE patient hippocampus. In particular, over expression of AQP4 and reduced levels of dystrophin (a protein involved in anchoring AQP4 to the membrane in perivascular endfeet) were observed in TLE hippocampus tissues. However, AQP4 density along the perivascular membrane domain of astrocytes was reduced by half in MTLE CA1 region, while no difference was found in AQP4 density on astrocyte membranes facing neuropil. These changes were secondary to altered perivascular dystrophin expression in sclerotic areas, which caused the loss of perivascular AQP4 and resulted in an impaired water flux through astrocytes. Given that in presence of high neuronal activity $K^+$ and water are taken up by the astrocyte membrane facing the neuropil, transported through astrocytic syncytium and siphoned into blood through perivascular endfeet membrane. This altered flow of water affected extracellular $K^+$ buffering and contributed to epileptogenicity. Enhanced gap junctional communication between neurons is considered a major factor underlying the neuronal synchrony driving seizure activity. However, in most studies of cerebral tissue from animal seizure models and from human patients with epilepsy, there is up-regulation of glial, gap junctional proteins Cx43 and 32 mRNA and protein, but not of neuronal ones.[15]

All these evidences indicate that astroglial dysfunctions are associated with epileptogenesis processes and can be considered as alternative targets for the development of new antiepileptic drugs.[1]

**Glioma** represent the main form of primary brain tumours that originate from glial cells. In particular, astrocytomas, gliomas deriving from astrocytes, are the most common subtype of brain gliomas.[19] Several studies have identified astrocytes functional changes in astrocytomas, mainly related to alterations in extracellular glutamate levels and membrane channel (such as voltage-dependent channels and gap junctions). From investigations of human tissue resections, it has been found that GFAP overexpression correlates with the presence of glioma, hence GFAP is used as an immunohistochemical marker to verify the astrocytic origin of glial tumour. Alterations in the expression of other structural proteins of the intermediate filaments, such as keratin and vimentin, are linked to the degree of astrocytoma malignancy.[1]

**Peripheral nerve injury (PNI)** is a global clinical disease that seriously impacts people health and there is no available strategy for successful treatment (Fig.2 D). Peripheral nerves convey signals between the spinal cord and the rest of the body, by motor, sensory, and autonomic neurons. Efferent neurons (motor and autonomic) receive signals from neurons of the central nervous system, primarily using the neurotransmitter acetylcholine among others. Afferent (sensory) neurons receive their signals from specialized cell types. These signals are sent to the CNS to provide sensory information to the brain and possibly interneurons in the spinal cord when a reflex response is necessary. Peripheral nerve injuries range in severity from mild compression causing decrease in conduction nerve velocity to severe crush and full transection of axons and connective tissue resulting in the complete discontinuity of the nerve. Schwann cells are critically involved in determining the peripheral nerve injury outcome, wherein a degraded, thinner myelin sheath is observed, as evidenced by an increased g ratio (a ratio of the axon diameter to the axon plus myelin sheath diameter) and a decreased internodal length (the distance between adjacent nodes of Ranvier). [20] This is accompanied by Schwann cell proliferation, dedifferentiation, and an increase in metabolic activity necessary for re-myelination. Schwann cells play a substantial role in promoting axonal regeneration, as they are the main source of neurotrophic factors, which alter the gene expression profile of the neuron to promote regeneration. Nerve growth factors (NGF) is under expressed within healthy nerves, but it is upregulated in Schwann cells during injury to promotes cell growth and proliferation. Many neurotrophic factors have been discovered, with functions ranging from improving cell survival through mechanisms of apoptosis prevention, to promoting regenerating factors in the neurons and in Schwann cells. Schwann cells produce neurite-promoting factors, which are incorporated in the extracellular matrix such as fibronectin and laminin.

The axonal changes at the distal end of the severed nerve eventually lead to the breakdown of the nerve stump to make way for a newly regenerating axon. The hallmark of this phase is the granular disintegration of the cytoskeleton. This occurs after a sudden inflow of extracellular ions, primarily $Ca^+$ and $Na^+$, leads to a cascade of events resembling apoptosis, which serves to recruit macrophages using signals elaborated from Schwann cells. It has been shown that the distal nerve stump is capable of transmitting an action potential some hours after transection. More recently, mRNA of brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) were found to be up-regulated while neurotrophin-3 and ciliary neurotrophic factor (CNTF) were down-regulated in the distal stumps of transected tibial nerves. Chronically denervated distal nerve stumps can maintain these changes 6 months after injury if regeneration has not occurred.[20,21]

**Parkinson's disease (PD)** The hallmark of this very important disease is the presence of Lewy bodies in the Nervous System. The full mechanism of PD is still debated; recent studies have shown that α-synuclein, a protein commonly found in the brain, plays an important role in the formation of Lewy bodies. This has generated a new disease concept, that of α-synucleinopathies.

Oxidative and inflammatory processes affecting the dopaminergic neurons of the substantia nigra may play a crucial role in the etiology of Parkinson disease (PD). Since glia are the main generators of these processes, the possibility that PD may be caused by glial dysfunction needs to be considered. Reactive astrocytes and microglia are abundant in the substantia nigra of PD patients (Figure 2 A). In particular, microglia may be the main triggering components since they produce large numbers of superoxide anions and neurotoxins. [3] Their toxicity towards dopaminergic neurons has been demonstrated in tissue culture and various animal models of PD. More recently, it has been reported that α-synuclein-immunoreactive inclusions also occur in both astrocytes and oligodendrocytes.[22] New evidence indicates that Bergmann glia in the cerebellar cortex can also be affected by filamentous accumulation of α-synuclein in α-synucleinopathies. Immunohistochemical observations from PD, diffuse Lewy body disease (DLBD) or multiple system atrophy (MSA) patients have revealed the presence of α -Synuclein-positive doughnut-shaped structures in the cerebellar molecular layer. Immunofluorescence and immunoelectron microscopy studies have shown that these granulo-filamentous α-synucleinpositive structures were located in the GFAP-positive radial processes of Bergmann glia. [23]. BG have never received much attention with regard to neuropathology. Proliferation of Bergmann glia has been described as a common response following Purkinje cell degeneration. However, the presence of abnormal filamentous accumulation of tau in tauopathies [24] as well as of α synuclein in α -synucleinopathies suggest the involvement of Bergmann glia in these pathologies. Thus, Bergmann glia are emerging as therapeutic targets of α -synuclein pathology in order to develop novel therapeutic approaches directed to PD and other α –synucleinopathies.

**Retinal degenerations,** a leading cause of blindness worldwide, are characterized by the degeneration of photoreceptors that impair the ability of the retina to detect light (Figure 2 C). Despite enormous efforts in the clinical treatment of many eye diseases, no established method to prevent or cure photoreceptor degeneration has been as yet identified.

Age-related macular degeneration (AMD) and Retinitis pigmentosa (RP) are the two most prevalent forms of retinal degenerative diseases.[25,26] AMD begins by primarily affecting cone photoreceptors in macula, leading to blurred central vision. AMD affects 30-50 million people in the world. RP initiates with progressive degeneration of rod photoreceptors in peripheral retina, manifesting early symptoms as loss of peripheral vision and night vision. Degeneration of rods is followed by deterioration of cones, resulting in the visual decline from "tunnel vision" to blindness. RP is estimated to affect 1.5 million people in the world. Morphometric analyses have shown that over 50% of the ganglion cells survive wet AMD and the ganglion cell density in dry AMD does not differ significantly from that in normal eyes. Morphologic studies in severe human RP patients reveal moderate preservation of inner retinal neurons: 70-80% of the bipolar cells and 25-40% of the ganglion cells. These diseases affect the photoreceptors but preserve the inner retinal layers. Tradition therapeutic approaches attempt to restore vision with retinal prostheses to reactivate the spared inner retinal neurons by electrical stimulation, bypassing the damaged photoreceptor layer.[27,28] However pathophysiological roles of glial cells must be fully understood to adequately engineer technological solutions devoted to prevent or treat the dysfunctions of retinal cells. AMD is a neurodegenerative disorder in which pathological alterations are related to age. In AMD, old microglia suffer age related dysfunctions in their homeostatic properties and dynamic, morphology and motility capacities. Additionally, they are less responsive to external stimuli like ATP and their translocation to the outer retina may be an essential event that triggers chronic AMD. In RP, not only microglia but Müller glia are more activated in the early stages of the disease. The expression of low-affinity p75 receptor in Müller cells resulted increased in a dog RP model, as well as vimentin and GFAP overexpression was observed, although more prominently in younger animals than in adults. [29]

Müller cells can sense the damage in the outer retina and transmit the information to the inner retina. This phenomenon was investigated in dog models carrying a mutation in the RPE65 gene, which encodes the 65 kDa RPE-specific isomerase involved in visual pigment regeneration and photoreceptor viability. [29] When the retinal degeneration originates in the RPE, Müller cells, as well as neighbouring astrocytes and retinal ganglion cells, sense the changes in the damaged outer retina and respond by signalling to the inner intact retinal layers. [17] Due to its ability to sense and react to dysfunctions, Müller cells represent a strategic target for sensing and modulation of glial signals in the retinal disorders and visual pathologies.

**Glaucoma** is characterized by the damage of retinal ganglion cells and their axons in the optic nerve that can lead progressively to blindness.[31] Among risk factors influencing glaucoma, the elevation of intraocular pressure (IOP) is particularly important. The function of astrocyte and Müller cell activation is unknown as yet, although one week after the IOP elevation more astrocyte gliosis and its subsequent decreasing were observed. A possible explanation may be that the astrocytes detect the increase in pressure directly, or through microglial or Müller cells, and they react by the release of neurotrophic signals to support ganglion cells survival.

In glaucoma, reactive astrocytes may potentially overexpress toxins, while Müller cells overexpress glutamine synthetase, Structural changes caused by glaucoma are described by vimentin cytoskeleton disorganization. AQP9 expression is reduced in RGCs of glaucomatous eyes, while glaucoma induces AQP7 expression in the Müller cell endfeet. Alternatively, the

 

expression of both AQP7 and AQP9 is reduced in the ciliary body of glaucomatous eyes. Therefore, AQP expression seems to play some relevant role in glaucoma. [32]

Macular edema is an ocular inflammation resulting from a progressive fluid accumulation in the macula. A major cause of macular edema is the trauma of ocular tissue, associated to mechanical stress of retinal neurons, nerve fibers and capillaries that lead to the disruption of the blood-retinal barrier and swelling of retinal cells. Müller cell swelling may contribute to the progression of edema, since they are responsible for fluid absorption by AQP channels.[33]

Retinal cell swelling can be also a consequence of retinal detachment occurring after surgical insertion of an implant device. Hence Müller cells role in edema onset represents an important aspect that need to be evaluated in long-term implantation of retinal prosthesis. [34,35]

A recent literature reports that Müller TRPV4 channel, which function as thermosensor, osmosensor and mechanosensor, can be activated by mechanical stimuli caused by retinal detachment-induced swelling in Müller cells and that TRPV4 activation by Müller glia swelling was potentiated by body temperature. These events trigger photoreceptors degeneration, with an adverse impact on retinal viability. Together, these results indicate the functional importance of TRPV4 in the molecular pathway underlying edema, and suggest the new possibility to selectively modulate glial channel properties in order to improve performance of implant device for recovering visual functions. [36]

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