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Functions of optic nerve glia: axoglial signalling in physiology and pathology

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Abstract

An early concept of glial function envisaged them as passive and unexcitable structural elements, much like the connective tissues of organs in the periphery. It is now known that glia have a widespread range of physiological roles and react to all forms of pathological insult. This paper reviews the major functions of oligodendrocytes and astrocytes, the main types of glia in the optic nerve, and examines novel NG2-glia, otherwise known as oligodendrocyte progenitor cells (OPCs). The major function of oligodendrocytes is to produce the myelin sheaths that insulate CNS axons, but they also have important roles in the establishment of nodes of Ranvier, the sites of action potential propagation, and axonal integrity. Astrocytes have multiple physiological and pathological functions, including potassium homeostasis and metabolism, and reactive astrogliosis in response to CNS insults. The bulk of NG2-glia are postmitotic complex cells, distinct from OPCs, and respond to any insult to the CNS by a rapid and stereotypic injury response. This may be their primary unction, but NG2-glia, or a subpopulation of NG2-expressing adult OPCs, also provide remyelinating oligodendrocytes following demyelination. Oligodendrocytes, astrocytes, and NG2-glia all contact axons at nodes of Ranvier and respond to glutamate, ATP, and potassium released during axonal electrical activity. Glutamate and ATP evoke calcium signalling in optic nerve glia and have dual roles in physiology and pathology, coupling glial functions to axonal activity during normal activity, but enhanced activation induces an injury response, as seen following injury, demyelination, and ischaemia.

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Introduction

The optic nerve is a typical CNS white matter tract and comprises the axons of retinal ganglion cells together with glia, which support them, namely oligodendrocytes, astrocytes, microglia, and newly described NG2-glia (Figure 1).[1] Oligodendrocytes lie in regular interfascicular rows of five or more cells along the long axis of the nerve, interspersed with solitary astrocytes regularly spaced between the series of oligodendrocytes, which extend their primary processes orthogonally and radially to separate axon bundles into fascicles and provide the nerve with its structure. Astrocyte processes form end-feet over the basal lamina of both the pial and vasculature to form a glia limitans, and extend minute collaterals that engage nodes of Ranvier.[2,3] In addition, small populations of microglia and NG2-glia are regularly spaced throughout the nerve. NG2-glia are usually slightly offset from the rows of oligodendrocytes and extend processes to contact nodes of Ranvier.[4] CNS glia have a widespread range of physiological and pathological roles, which are the subject of numerous reviews:[1] (1) oligodendrocyes form the myelin sheaths that facilitate rapid conduction of axons, but they are also essential for axon integrity and function in the segregation and clustering of sodium (Na^+) and potassium (K^+) channels (Ch) at nodes of Ranvier;[5] (2) astrocytes have been considered historically as passive structural elements, but they are now known to be dynamic cells with multiple functions in potassium homeostasis, metabolism, axon–glial signalling, and the physiology of nodes of Ranvier and the blood–brain barrier;[6] (3) NG2-glia were until recently

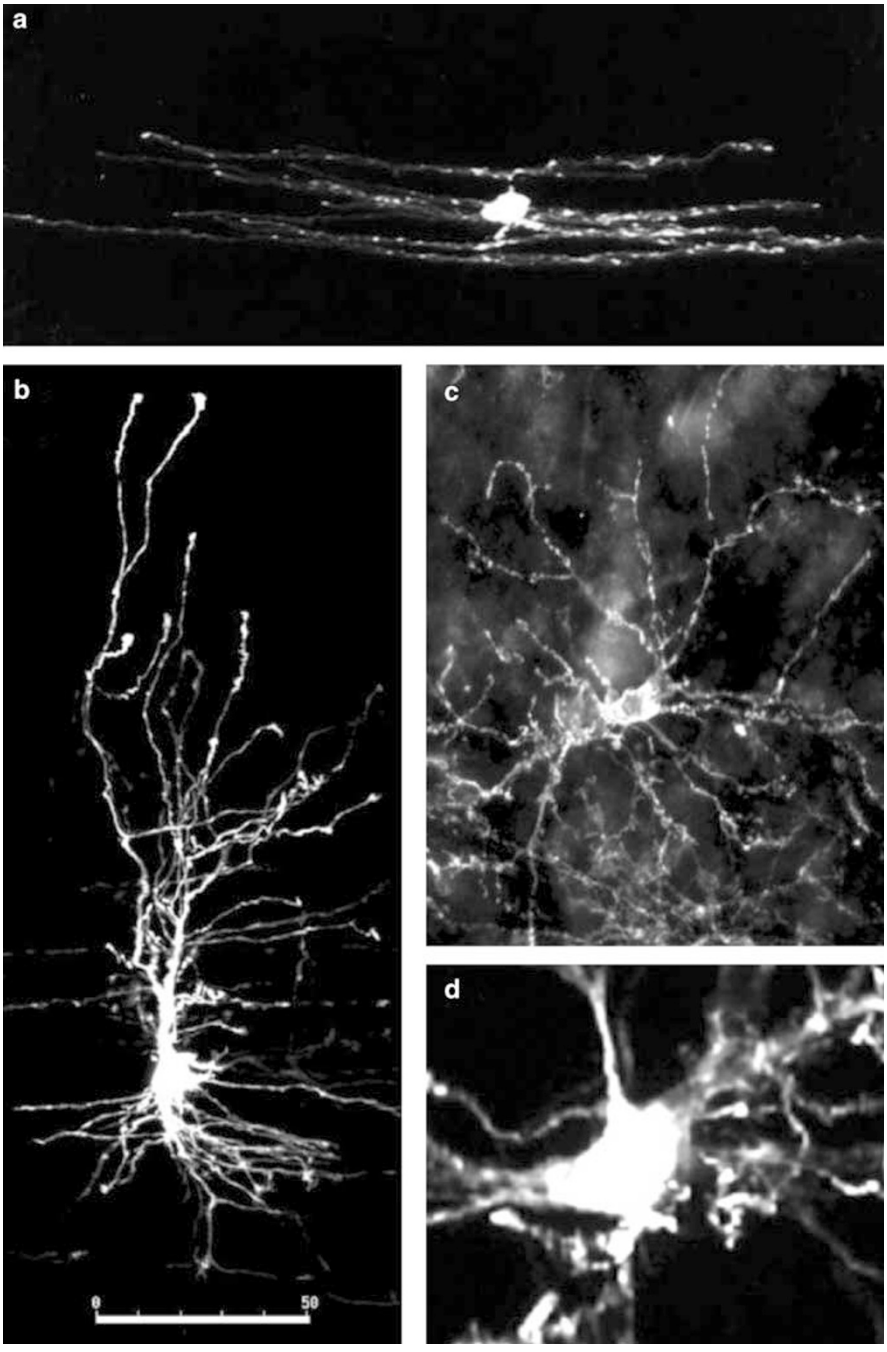

Figure 1 Confocal images of rat optic nerve glia, intracellular dye-filled with lysinated rhodamine dextra (a, b), or immunofluorescence labelled with the NG2 anibody (c, d). (a) Oligodendrocytes have 5–50 parallel processes that are the dye-filled inner and outer tongues of the internodal myelin sheath. (b) Astrocytes extend thick processes perpendicular and parallel to the axonal axis, which terminate in bulbous swellings or end feet at the pia and on blood vessels. Primary processes bear fine collaterals and spines that contact nodes of Ranvier (see Figure 4). (c, d) NG2-glia are highly complex stellate cells, with the morphological phenotype of astrocytes, but the antigenic phenotype of OPCs. Processes terminate on and extend fine spines to contact nodes of Ranvier (see Figure 7). Scale bar, 50 μm in (a–c), 20 μm in (d).

considered to be adult oligodendrocyte progenitor cells (OPCs), and an important function is to provide remyelinating oligodendrocytes following demyelination, but they are also engaged in activities not usually associated with progenitor function, such as interacting with nodes of Ranvier, axon–glial signalling, and glial scar formation following axon injury;[7] (4) microglia monitor CNS integrity via a range of ion

channels and neurotransmitter receptors and respond rapidly to changes in neuronal functions.[8] An important function of glia is their response to any forms of pathological insult, such as inflammation, injury, or ischaemia. Microglia are activated and secrete a number of cytokines and trophic factors, which can be protective or destructive, and have been reviewed elsewhere.[8] The characteristic response of astrocytes and NG2-glia is a reactive gliosis and the formation of a protective glial scar.[1,7] However, the glial scar is also highly inhibitory and NG2 is one of the most potent inhibitors of axon regeneration in the CNS.[9] Oligodendrocytes are exceptionally sensitive to any insults to the CNS, such as injury, ischaemia, or inflammation, which result in the loss of oligodendrocytes and myelin, and eventually secondary axon degeneration.[10] The present review will focus on these dynamic functions of glial cells and discuss the roles of glutamate and adenosine triphosphate (ATP)- mediated glial calcium (Ca^{2+}) signalling in optic nerve physiology and pathology.

Oligodendrocytes and axoglial interactions

The primary function of oligodendrocytes is to provide myelin sheaths for CNS axons. Intracellular dye injection demonstrates that mature myelin-producing oligodendrocytes are process-bearing cells and each terminal oligodendrocyte process ensheaths an axon to form one internodal myelin segment of variable thickness and length.[11–13] Rodent optic nerve oligodendrocytes have 5–30 parallel processes, which are the dye-filled internal and external tongue processes of the myelin sheaths, 50–200 μm long, and are connected to the cell somata by a number of fine processes 15–30 μm in length (Figures 1a and 2). Destruction of oligodendrocytes or myelin leads to disastrous consequences for optic nerve function, and underlies the optic neuritis that is often an early stage of the pathology of the human demyelinating disease, multiple sclerosis (MS).[14–17] Studies in animal models of MS have demonstrated that optic nerve oligodendrocytes are markedly affected during demyelination in experimental autoimmune encephalomyelitis (EAE),[14] viral induced demyelination,[15] and following treatment with tumour necrosis factor-α (TNF-α), a cytokine implicated in MS (Figure 2).[16] Demyelination is marked by the attenuation of and, in extreme cases, complete loss of internodal myelin sheaths in individual oligodendrocytes (Figure 2g–i). This results in focal demyelination and the slowed conduction velocities and conduction block characteristic of EAE and MS.[14,17] Remyelination and recovery of axonal function occur during remission in EAE and MS,[14] and this is a function of newly differentiated cells formed from the adult OPC pool.[18] However, in chronic MS, remyelination fails and axonal function is irreversibly compromised, despite the presence of OPCs within lesions.[19]

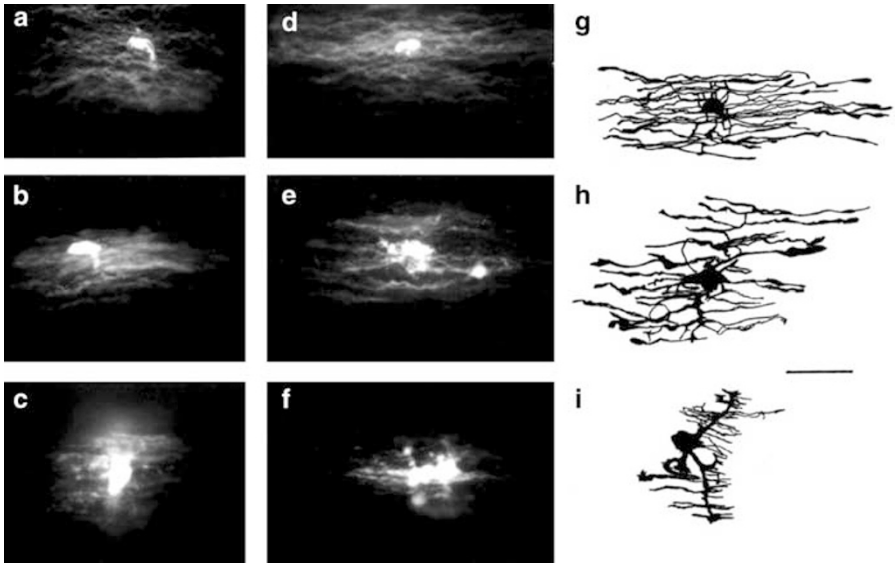

Figure 2 Morphological changes in mouse optic nerve oligodendrocytes during demyelination induced by TNF-α (a–c) and in a viral model of MS (d–i). In control nerves (a, d, g), oligodendrocytes have a uniform and symmetrical morphology when intracellularly dye-filled. Dye-filled oligodendrocytes from nerves following an intravitreal injection of TNF-α (a–c), or infection with Semliki forest virus (d–f) had abnormal features, with severely attenuated internodal myelin sheaths. (g–i) Camera lucida drawing of dye-filled oligodendrocytes illustrates the detailed anatomy of the morphological features of demyelination in single oligodendrocyte units. Compared to controls (g), internodal myelin segments begin to unravel and swell (h) and in extreme cases are completely lost (i). Scale bar, 25 μm. (From Butt *et al*[15] and Butt and Jenkins,[16] with permission).

Oligodendrocytes are essential for axon function

It is now clear that in addition to insulating axons, oligodendrocytes are important for axonal integrity and nodal function. Long-term interactions between axons and their myelin sheaths are poorly understood, but transgenic mice show signs of axonal degeneration in the absence of the myelin protein proteolipid protein (PLP), the enzyme 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), or the connexins (Cx) 32 and 47, gap-junction channel-forming proteins expressed specifically by oligodendrocytes.[20–22] Oligodendrocytes also induce Na^+Ch clustering along retinal ganglion cell axons via secreted factors and cell contact.[23–25] The clustering of Na^+Ch at developing nodes of Ranvier in the optic nerve is mediated via interactions between oligodendroglial neurofascin and axonal paranodin/Caspr-contactin complex at the myelin attachment zone.[25,26] Furthermore, oligodendrocyte disruption and demyelination result in the dispersion of axolemmal ion channels and impairment of conduction.[27,28]

Glutamate signalling and excitotoxic oligodendrocyte damage

Differentiated oligodendrocytes express ionotropic glutamate receptors (iGluR), which when activated, raise intracellular calcium ($[Ca^{2+}]_i$) (Figure 3a-c).[29] In the optic nerve, glutamate is released during axonal electrical conduction and evokes increased glial $[Ca^{2+}]_i$ in an activity-dependent manner (Figure 3d-g).[30] Glutamate is released either from axons at nodes of Ranvier or from astrocytes in response to activation by axonal action potential propagation at nodes.[31,32] The actions of glutamate in the optic nerve indicate an unresolved physiological role for Ca^{2+} signalling in oligodendrocytes. One possibility is that activation of iGluR may stimulate oligodendroglial Na^+–K^+ pumps by raising intracellular Na^+, thereby coupling K^+ uptake by oligodendrocytes with K^+ released during axonal electrical activity. The activity of glutamate is normally terminated by uptake into oligodendrocytes, astrocytes, and axons,[33,34] but in ischaemia, transporters operate in reverse and enhanced glutamate signalling is excitotoxic for oligodendrocytes.[35–37] In an experimental model, sustained activation of oligodendrocyte iGluR by injection of kainate in the optic nerve induces lesions and compromises axon conduction, which has the major features of MS and ischaemic damage.[36–38] It is

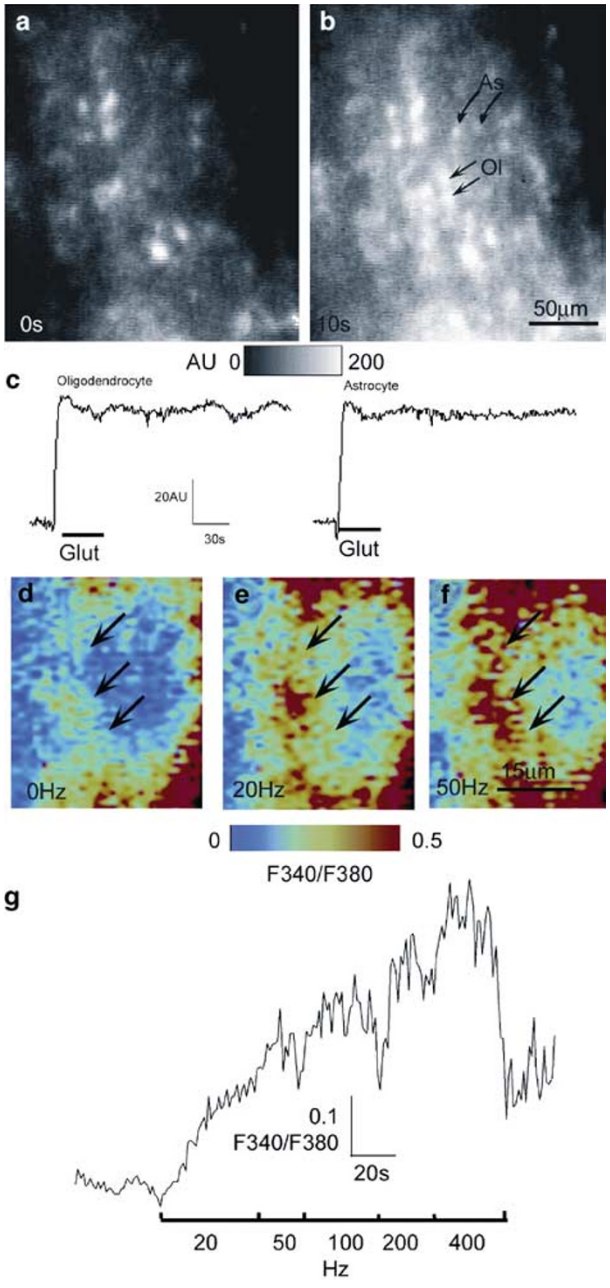

Figure 3 Glial Ca^{2+} signalling in the isolated intact rat optic nerve is evoked by glutamate (a–c) and axonal electrical activity (d–g). Optic nerves were loaded with fluo-3 (a–c) or fura-2 (d–g), and the responses of oligodendrocytes and astrocytes could be cautiously distinguished by their distributions in the nerve, either in rows of adjacent cells (some identified by arrows and Ol in b) or as large solitary cells (some identified by curved arrows and As in b). Notwithstanding this, the responses illustrated are representative of all cells analysed in the optic nerve ($n > 100$), and so it is reasonable to conclude that astrocytes and oligodendrocytes respond similarly to glutamate and axonal electrical activity. (a–c) Bath administration of 1 mM glutamate for 30 s evoked a large and rapid increase in $[Ca^{2+}]_i$, which was sustained after the removal of the agonist. (d–f) Stimulation of optic nerves via suction electrodes at increasing frequencies induced an activity-dependent increase in glial $[Ca^{2+}]_i$ between 20 and 100 Hz (arrows in d–f). The glial responses were unstable at high-frequency stimulation of 200 and 400 Hz (g).

significant, therefore, that antagonists of iGluR ameliorate the symptoms of EAE and attenuate excitotoxic injury.[39,40] The loss of oligodendrocytes and myelin results in secondary axonal damage,[10] although axons may also express iGluR, indicating that glutamate may directly compromise axons.[41]

Astrocyte signalling at nodes of Ranvier

Astrocytes contact nodes of Ranvier, the only areas of axolemma not covered by myelin in the optic nerve, and therefore the only potential site for axon–astrocyte signalling (Figure 4). A single node of Ranvier is contacted by the perinodal microprocesses of different astrocytes and also by those of NG2-glia (see below).[3,4] Astrocytes, as well as oligodendrocytes, express iGluR and their activation evokes raised intracellular Ca^{2+} (Figure 3c).[42] Astrocytes are functionally coupled via C× 43 gap junctions and following stimulation Ca^{2+} waves are propagated through the network at a rate of 7–27 μm/s.[42–44] Glutamate may be the primary axon-to-glial signaling molecule (Figure 3d-g),[30,42,44] but ATP is the primary signalling molecule between astrocytes

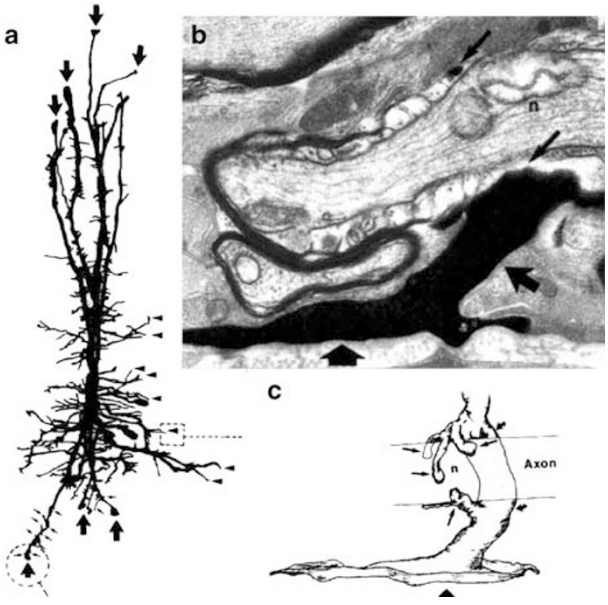

Figure 4 Astrocyte relations with nodes of Ranvier. Camera lucida drawing (a) and electron micrograph (b) of a mouse optic nerve astrocyte dye-filled with HRP. Primary processes terminated at pial end feet (large arrows in a and b), and bear microscopic collaterals and spines along their lengths that contact the axolemma at nodes of Ranvier (small arrows in a and b). The rest of the node is partially invested by the processes of other astrocytes and NG2-glia (see Figure 7), and is delimited by the paranodal loops of the myelin sheath. (c) Three-dimensional reconstruction of serial electron micrographs illustrates fine finger-like processes extending from the primary process to form multiple contacts with the node. (From Butt *et al*[3] with permission).

throughout the CNS[45–47] and in the optic nerve (Figure 5).[48–50] Astrocytes release ATP and glutamate in response to physiological stimulation and modulate the activity of adjacent neurons in culture and in the retina.[51–53] There is circumstantial evidence that axonal electrical activity in the optic nerve also triggers glutamate- and ATP-mediated glial Ca^{2+} signalling (Figure 3d-g),[30] and glutamate and adenosine, the breakdown product of ATP, have been shown respectively to compromise and protect axons in ischaemia.[10,54] Physiologically, it may be more significant that Ca^{2+} signalling triggered by glutamate released by axons and propagated by astroglial ATP can couple axonal activity and astrocyte functions, including K^+ regulation and metabolism.

Potassium regulation

A primary function of astrocytes is to regulate extracellular $[K^+]_o$,[6] which in the optic nerve is released by axons at nodes of Ranvier. The release of K^+ into the extracellular space is directly related to axonal activity,[55] and the increase in $[K^+]_o$ results in axon depolarisation and decreased conduction, as well as coupling astrocyte transport and metabolism to axonal activity (see below).[6] Intensive stimulation of the optic nerve can increase $[K^+]_o$ by 5–8 mM, but only in pathology does it exceed 12 mM, termed the K^+ 'ceiling level'.[6,55] Astrocytes remove excess $[K^+]_o$ by active uptake and cotransport, and via inwardly rectifying K^+ channels (Kirs).[6,56] The principal channel involved in K^+ regulation may be Kir4.1,[56] which is expressed by astrocytes and oligodendrocytes,[57] indicating that both glial cell types may take up K^+ released at nodes of Ranvier during axonal activity. In the optic nerve, astrocytes control the size of the activity-dependent increase in $[K^+]_o$ and speed up its return to a resting level of 3 mM, thereby directly influencing axonal activity.[6,55] Glutamate activation of astrocyte iGluR in oligodendrocytes and astrocytes (Figure 3) may also play a role in K^+ regulation at nodes of Ranvier, by stimulating Na^+ influx and activating Na–K pumps, as well as Ca^{2+} influx leading to an increase in K^+ conductance.[29] In addition, activation of glial iGluR may regulate expression of Kir4.1 (unpublished observations), although this may be more important as an early stage in reactive astrogliosis and may explain as to why K^+ regulation is impaired in gliotic tissue and ischaemic lesions.[58,59]

Axon–astrocyte signalling and metabolic coupling

Glucose is the principal energy source in the normal CNS, and astrocytes form a conduit from the blood–brain barrier to axons, through which glucose can be rapidly

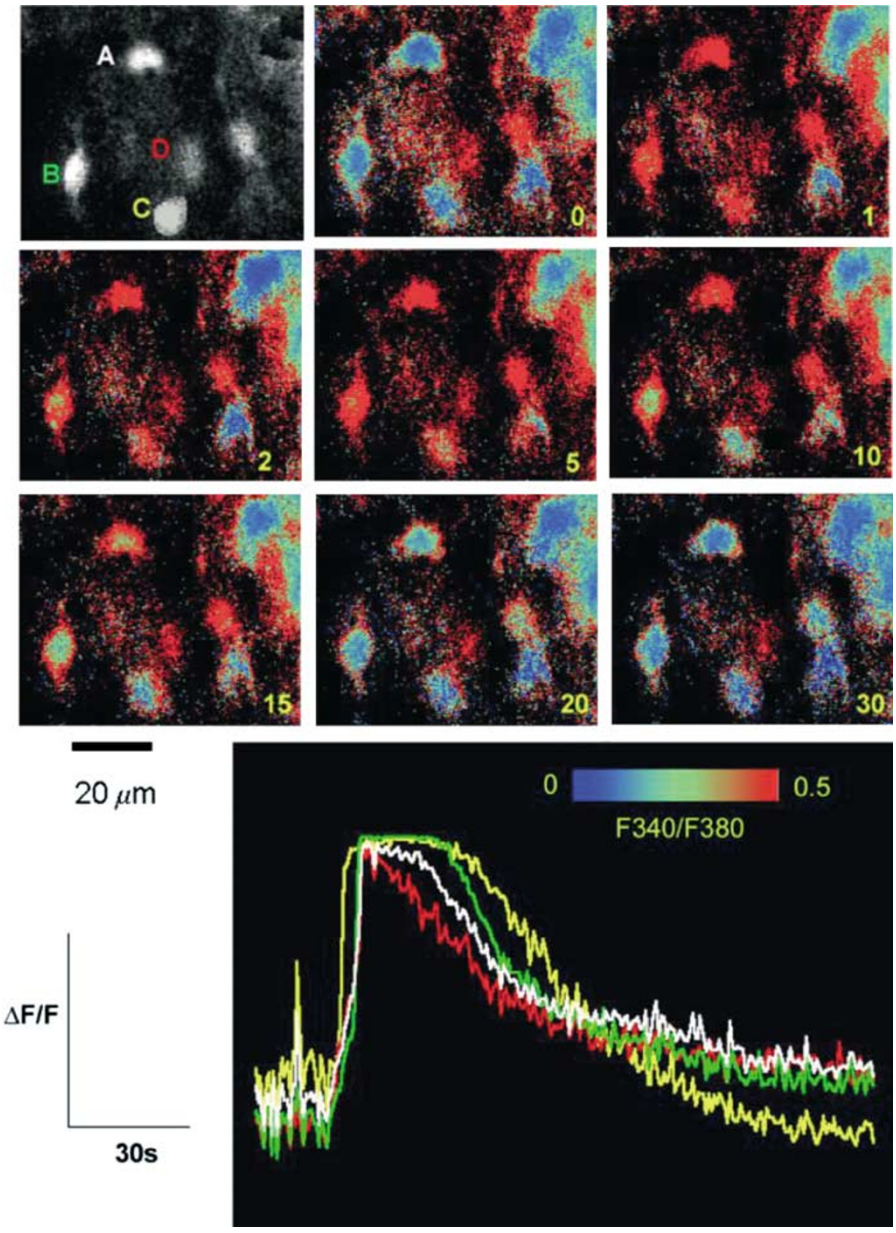

Figure 5 ATP-mediated glial Ca^{2+} signalling in the isolated intact rat optic nerve. Optic nerves were loaded with fura-2 and imaged during bath application of 1 mM ATP. Individual glial cell bodies, presumptive astrocytes (A, B), and oligodendrocytes (C, D) are observed to respond to ATP by a rapid increase in $[Ca^{2+}]_i$ which peaks within 5–10 s and decays in the continued presence of agonist. All glia analysed in the optic nerve ($n > 100$) responded similarly to ATP, indicating that both oligodendrocytes and astrocytes express functional purinoceptors, and supporting a role for ATP in optic nerve Ca^{2+} signalling.

transported.[60.] Removal of glucose, such as that which might occur when blood supply is compromised, results in an irreversible loss of optic nerve function, but astrocyte glycogen can support axonal function in the absence of glucose.[60–63] Glycogen is the single largest energy reserve in the brain and is localised entirely in astrocytes, which break it down to lactate, for transfer to axons.[60–63] Glycogen turnover is rapid and coordinated with optic nerve activity,[63] possibly via the activity-dependent increase in $[K^+]_o$ or glutamate, which are directly related to axonal activity (Figure 3g),[55] and mediate a Ca^{2+}-dependent glycogen breakdown.[64,65] Noradrenaline has also been shown to promote glycogenolysis in the optic nerve.[63]

ATP-evoked Ca^{2+} signalling in physiology and pathology

The ATP-mediated increase in glial $[Ca^{2+}]_i$ in optic nerve glia is mediated predominantly by metabotropic P2Y

purinoreceptors, with a small but significant ionotropic P2X component.[48–50]. The P2Y receptors are activated at physiological concentrations of ATP in optic nerve astrocytes and oligodendrocytes,[49] and axonal electrical activity triggers ATP-mediated Ca^{2+} signalling in optic nerve glia (AM Butt and G James, unpublished). Newman[47] calculated that the level of extracellular ATP released by retinal astrocytes following mechanical stimulation as 78 μM at the site of stimulation and 6.8 μM at 100 μm away from this site. In optic nerve glia, the latter concentration would primarily activate high-affinity $P2Y_1$ receptors, which may be representative of localised, brief, and intermittent increases in extracellular ATP during physiological signalling (Figure 6a).[49,50] However, 78 μM ATP would activate both P2Y and P2X purinoceptors and induce a large and sustained increase in glial $[Ca^{2+}]_i$ (Figure 6b).[49,50] This may reflect a pathological situation, when there is a prolonged and high increase in extracellular ATP concentration, which would activate glial P2X receptors and initiate glial cell reactivity.[66,67] Our results in the optic nerve indicate that the $P2X_7$ subtype may have a special, but not exclusive, role in glial cell pathology.[50] Furthermore, astroglial Ca^{2+} waves not only signal between astrocytes but also modulate the behaviour of oligodendrocytes and microglia, and potentially induce reactive changes distant from the site of CNS damage.[68,69] Verderio and Matteoli[69] showed that intracellular Ca^{2+} waves induced the release of ATP by astrocytes, which in turn triggered a Ca^{2+} response in microglia via $P2X_7$ receptors. There is evidence that $P2X_7$ receptors are upregulated in activated microglia following brain ischaemic injury,[70] in Müller glial cells during proliferative vitreoretinopathy,[71] and in reactive astrocytes in the enucleated optic nerve (Figure 6c).[72]

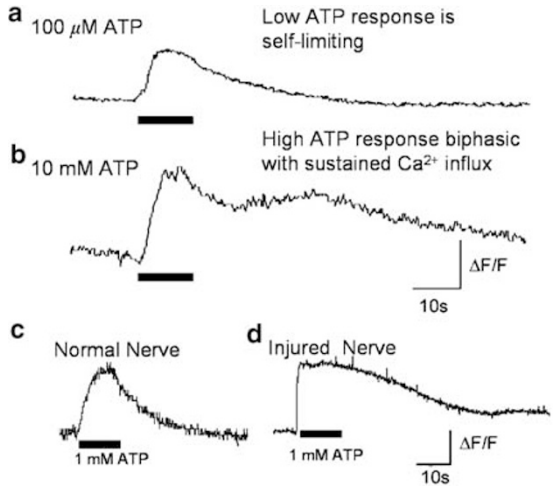

Figure 6 A role for ATP-mediated Ca^{2+} signalling in glial pathology. (a) At physiological concentrations, the ATP-evoked increase in glial $[Ca^{2+}]_i$ is transient and self-limiting. (b) At high concentrations, which can occur in pathology and following cell lysis, a 30s application of ATP evokes a large and sustained increase in glial $[Ca^{2+}]_i$, sufficient to trigger reactive astrogliosis. (c, d) Compared to the controls (c), reactive astrocytes in the enucleated rat optic nerve exhibit a sustained increase in $[Ca^{2+}]_i$ in response to 30 s application of ATP (d). The results are consistent with a role for the pore-forming $P2X_7$, receptor subtype in glial pathology.

NG2-glia (synantocytes): physiological and pathological functions of novel mature glia

A significant population of glial cells has been identified in both grey and white matter that express the NG2 chondroitin sulphate proteoglycan (CSPG). [7] NG2-glia are postmitotic, nonmotile cells with a complex stellate morphology, which extend processes to contact nodes of Ranvier in white matter (Figure 7)[4] and synapses in grey

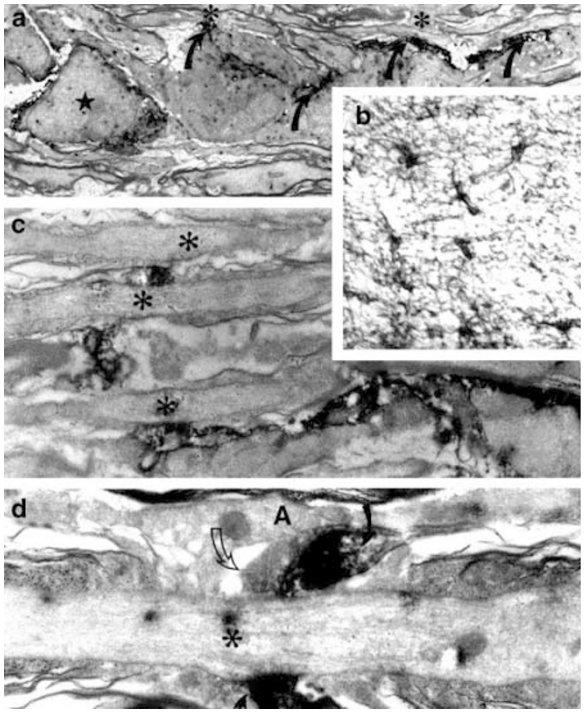

Figure 7 Perinodal NG2-glia in the rat optic nerve. Optic nerve sections were treated for immunoperoxidase labelling (b) and pre-embedding electron microscopic immunocytochemistry (a, c, d). (a) A typical NG2-glial cell (star) sits within or slightly offset from the interfascicular row of oligodendrocytes. An NG2+ process runs parallel to the glial row and axon fascicles (curved arrows) and contacts axons (asterisks) on its passage through the nerve. (b) Light microscopy of 100 μm thick section of the optic nerve showing the distribution and density of NG2-glia and the fine lattice of their parallel processes. (c) A group of neighbouring nodes (asterisks) contacted by NG2+ processes. (d) A node of Ranvier (asterisk) contacted by an NG2-glial cell process that wraps around the node to contact the axolemma on both sides (closed arrows). The same node is contacted by filipodial processes (open arrows) from astrocyte processes (a), which contain intermediate filaments and pass parallel to the axon. (From Butt *et al*[4] with permission).

matter.[73] In the adult rat optic nerve, NG2-glia comprise 7% of glial cells and are distributed uniformly throughout the optic nerve, but do not pass beyond the optic nerve head and stop at the same site as myelin. Along the length of the optic nerve, NG2-glia have a more polarised appearance, extending processes along the axonal axis (Figure 8a). At the chiasm, where the bulk of axons decussate in the rat optic nerve, NG2-glia have a radial morphology, extending fine multibranching processes along the axons of both decussating nerves (Figure 8b). NG2-glia share many morphological features with astrocytes (Figure 8c-f), but do not appear to express the conventional mature astrocyte markers, such as the intermediate filaments glial fibrillary acidic protein (GFAP) and vimentin, the calcium-binding protein S-100β or glutamine synthetase (GS), an essential enzyme in the conversion of glutamate to glutamine.[74] In addition, NG2-glia do not have the ultrastructural features of mature astrocytes and do not contain intermediate filaments, which are characteristic of both fibrous and protoplasmic astrocytes.[75]

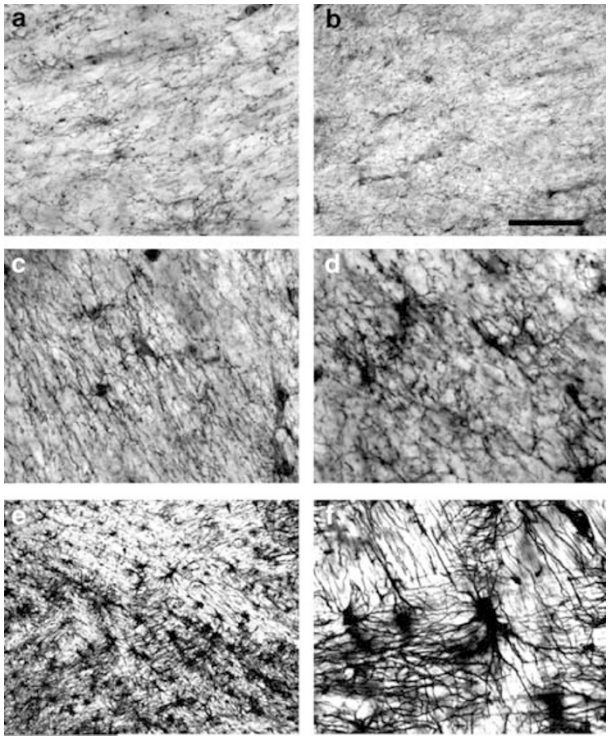

Figure 8 NG2 glia and astrocytes exhibit a stereotypic injury response to optic nerve damage. Thick 50 μm sections of optic nerve from controls (a, b) and following axon degeneration (c–f),immunoperoxidase labelled for NG2 (a–d) or GFAP (e, f). In controls, NG2 glia are small cells with a fine lattice of processes, which extend parallel to axons along the length of the optic nerve (a), and radially along decussating axons at the chiasm (b). Axon degeneration following optic nerve crush induces reactive gliosis in NG2-glia along the optic nerve length (c) and at the chiasm (d). Reactive NG2-glia are more heavily labelled with the NG2 antibody, have hypertrophic cell bodies, and thick, often matted processes (c, d). The density of NG2-glia is also increased (c, d), consistent with the reported proliferation of NG2-glia following CNS injury. The response of NG2-glia is comparable to that of astrocytes (e, f), but neither normal or reactive NG2-glia express GFAP. Reactive astrocytes are larger than NG2-glia and extend processes preferentially along the long axis of the degenerating axons, as illustrated at the chiasm (e, f). Scale bar, 100 μm in (a, b, e) and 50 μm in (c, d, f).

Relation of NG2-glia (synantocytes) to OPC/O-2A cells

Until recently, all cells expressing NG2 in the CNS have been considered adult OPC on the basis of their antigenic phenotype[19,76] and to be equivalent to oligodendrocyte-type-2 astrocyte (O-2A) cells, which *in vitro* can develop into oligodendrocytes or type-2 astrocytes, depending on the culture medium.[77,78.] However, NG2 expressing glia are distinct from OPCs or O-2A cells that give rise to oligodendrocytes during development.[79–81] For example, OPCs that are committed to differentiation into oligodendrocytes express the tetraspanin protein CD9, but only a minority (<1%) of adult NG2-glia express CD9.[75] Furthermore, NG2-glia in the optic nerve do not depend on axons,[80] in direct contrast to O-2A cells, which are lost following the optic nerve transection due to a 90% decrease in their proliferation.[82,83] Notwithstanding this, a population of NG2+ OPCs gives rise to remyelinating oligodendrocyte in MS and in animal models of EAE.[18,19] We do not currently know whether this is the function of a subpopulation of NG2+ adult OPCs.

NG2-glia (synantocytes) are highly reactive cells: a role for perinodal glutamate signalling?

NG2-glia, like astrocytes, are highly reactive cells (Figure 8c-f), and exhibit a stereotypic injury response to a wide range of insults, such as trauma, ischaemia, excitotoxicity, and demyelination.[76.] Moreover, NG2-glia express AMPA/kainate iGluR,[73,84] and so they are ideally suited to respond to glutamate released during axonal activity via their perinodal processes.[4] There is evidence that glutamate evokes raised $[Ca^{2+}]_i$ in NG2-glia or OPCs. Glutamate released from axons at nodes of Ranvier might maintain NG2-glia in a quiescent state, since glutamate inhibits the proliferation and lineage progression of NG2 expressing glia in brain slices from postnatal rats.[85] Conversely, enhanced activation of iGluR in response to high levels of glutamate, such as that which occur following injury and ischaemia, could evoke sustained intracellular Ca^{2+} (see Figure 3c) and thereby promote the proliferation and reactive gliosis characteristic of NG2-glia (Figure 8c, d). Indeed, the primary function of NG2-glia may be to monitor axonal

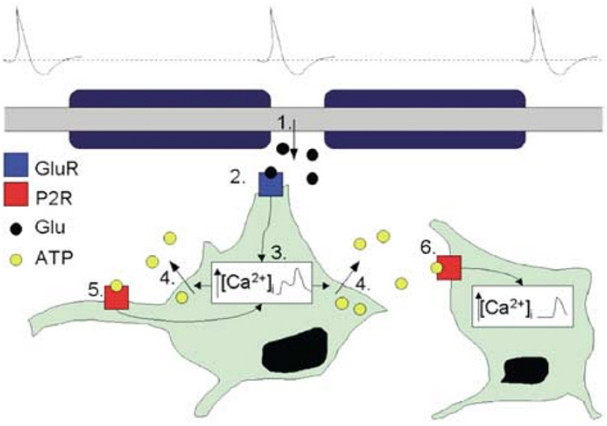

Figure 9 Glutamate- and ATP-mediated signalling at optic nerve nodes of Ranvier. (1) Glutamate is released during action potential propagation along axons, either from axons (as illustrated) or astrocytes, by unresolved mechanisms. (2) Glutamate activates AMPA/kainite iGluR on perinodal astrocytes, NG2 glia, and oligodendrocytes (astrocytes illustrated as green cells). (3) Activation of glial iGluR evokes an activity-dependent increase in $[Ca^{2+}]_i$. (4) Astrocytes release ATP (and possibly glutamate) in response to raised $[Ca^{2+}]_i$. (5) A Ca^{2+} wave is propagated through the astroglial syncytium through gap junctions and via ATP. During physiological signalling, glutamate and ATP-mediated Ca^{2+} signalling couple glial homeostatic functions with axonal activity, stimulating Na^+–K^+ pumps, increased glial K^+ conductance, glycogenolysis, and inhibition of glial proliferation.[29,65] Following insults to the optic nerve, such as ischaemia, injury, inflammation, larger concentrations of glutamate and ATP are released into the extracellular space and enhanced activation of glial receptors is toxic for oligodendrocytes and myelin, and induces reactive gliosis in astrocytes and NG2-glia. Activation of astroglial $P2X_7$ receptors by high concentrations of ATP causes manifold pathophysiological actions, including activation of cyclooxygenase-2 (COX-2). In addition, astrocytes can activate microglia at a distance from the initial insult, thereby promulgating excessive inflammatory events and the spread of damage.

activity and respond rapidly to axonal disruption, either to form a protective glial scar or regenerate oligodendrocytes.[7] To distinguish NG2-glia from true OPCs that generate oligodendrocytes during development, we have termed NG2-glia synantocytes (from the Greek *synanto*, meaning 'contact').[1,7,75] However, it is not clear at present whether there are multiple populations of NG2 expressing glia in the CNS, or a single population with multifunctional or plastic properties, which includes axoglial signalling at nodes of Ranvier, reactive gliosis following CNS insults, and their ability to dedifferentiate and generate oligodendrocytes.

Conclusions

Optic nerve glia and axons are functionally interdependent and their physiology and pathology are integrated by extracellular signals, including glutamate, K^+, and ATP (Figure 9). Glutamate released during axonal activity triggers Ca^{2+} signalling in optic nerve astrocytes, oligodendrocytes, and NG2-glia, coupling their physiological functions with axonal activity. However, overstimulation by glutamate is excitotoxic for oligodendrocytes, mediating the loss of myelin that is characteristic of MS and ischaemia, and may trigger an injury response in NG2-glia. Astrocytes communicate by Ca^{2+} waves propagated through the astroglial syncytium via gap junctions and ATP, which in turn modulate K^+ homeostasis, glycogenolysis, and axonal function. The physiological and pathological functions of ATP signalling in astrocytes may be mediated by different receptors, and $P2X_7$ receptors play a particular role in the pathogenesis of reactive astrogliosis and microglial activation. The functions of NG2-glia in axoglial signalling at nodes of Ranvier are unresolved, but they appear to be specialised to monitor and respond rapidly to changes in axonal activity, resulting in a stereotypic injury response and possibly the generation of remyelinating oligodendrocytes. It is clear from these studies that glial cells are not simple passive structural elements, but interact with axons in a dynamic manner, which is important for coupling axonal and glial functions.

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