Review

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Sugar glues for broken neurons

Abstract: Proteoglycans (PGs) regulate diverse functions in the central nervous system (CNS) by interacting with a number of growth factors, matrix proteins, and cell surface molecules. Heparan sulfate (HS) and chondroitin sulfate (CS) are two major glycosaminoglycans present in the PGs of the CNS. The functionality of these PGs is to a large extent dictated by the fine sulfation patterns present on their glycosaminoglycan (GAG) chains. In the past 15 years, there has been a significant expansion in our knowledge on the role of HS and CS chains in various neurological processes, such as neuronal growth, regeneration, plasticity, and pathfinding. However, defining the relation between distinct sulfation patterns of the GAGs and their functionality has thus far been difficult. With the emergence of novel tools for the synthesis of defined GAG structures, and techniques for their characterization, we are now in a better position to explore the structure-function relation of GAGs in the context of their sulfation patterns. In this review, we discuss the importance of GAGs on CNS development, injury, and disorders with an emphasis on their sulfation patterns. Finally, we outline several GAG-based therapeutic strategies to exploit GAG chains for ameliorating various CNS disorders.

Keywords: chondroitin sulfate; glycosaminoglycans; heparan sulfate; nervous system disorders; proteoglycans; spinal cord injury; sulfotransferases.

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List of abbreviations

3OST, 3-O-sulfotransferase; 6OST, 6-O-sulfotransferase; 6OST1, 6-O-sulfotransferase 1; AB, amyloid-β; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE1, β-secretase-1; BDNF, brain-derived neurotrophic factor; ChABC, chondroitinase ABC; ChAC, chondroitinase AC; ChB, chondroitinase B; CNS, central nervous system; CS, chondroitin sulfate; CSPG, chondroitin sulfate proteoglycan; DRG, dorsal root ganglion; DS, dermatan sulfate; ECM, extracellular matrix; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GAG, glycosaminoglycan; Gal, galactose; GalNAc, N-acetylgalactosamine; GDNF, glial cell-derived neurotrophic factor; GlcA, glucuronic acid; GlcNAc, N-acetylglucosamine; GPI, glycosyl phosphatidyl inositol; HA, hyaluronan; HB-EGF, heparin-binding epidermal growth factor; HBGAM, heparin-binding growth-associated molecule; Hep I, II, III, heparitinase I, II, and III; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; IdoA, iduronic acid; KS, keratan sulfate; MAPK, mitogen-activated protein kinase; NCAM, neural cell adhesion molecule; NDST, N-deacetylase/N-sulfotransferase; NGF, nerve growth factor; NgR, Nogo receptor; NPCs, neuronal progenitor cells; NT, neutrophin; NFT, neurofibrillary tangles; OST, O-sulfotransferase; PD, Parkinson's disease; PG, proteoglycan; PKC, protein kinase C; PNN, perineuronal net; PTN, pleiotrophin; RPTPβ, receptor type protein-tyrosine phosphatase beta; RPTPσ, receptor type protein tyrosine phosphatase sigma; SCI, spinal cord injury; SE, status epilepticus; TGF-β, transforming growth factor beta; Trk, tyrosine receptor kinase.

Introduction

Glycosaminoglycans (GAGs) are a family of linear, sulfated polysaccharides that are associated with central nervous system (CNS) development, maintenance, and disorders. For example, the heparan sulfate (HS) chain, a member of the GAG family, regulates receptor-ligand interactions that control neurite outgrowth and pathfinding (1–3, and references therein). Similarly, the chondroitin sulfate

(CS) chain, another common GAG, is overexpressed at the scar site in spinal cord injuries (SCIs), and is a major roadblock to regeneration (4, 5). All GAGs but hyaluronan are found associated with a core protein that belongs to a special class of biomolecules called proteoglycans (PGs). HS and CS are the two most common and prominent GAG types found in the CNS. Although these chains have been reported to affect various functions in the CNS, a comprehensive understanding of their structure-function relation is still lacking. Sulfate groups present in these molecules impart negative charge at discrete positions defining not only the fine structure but also the interactions with various signaling factors. Sulfation of GAG chains is a non-template process that occurs in the Golgi apparatus and relatively little is understood about specific contributions of various 'sulfation patterns' on GAG functionality (6, 7, and references therein). However, with recent advancements in GAG synthesis and characterization, many critical functions of sugar moieties of HSPGs/CSPGs are being revealed. In this review, we focus on the role of HS and CS chains, in particular their sulfation patterns, in various CNS pathophysiological processes. Finally, we propose various GAG-based therapeutic approaches to combat different CNS disorders for which no cure exists at present.

Molecular perspective of PGs

GAGs can be distinguished into four groups depending on their sugar building blocks and the nature of their glycosidic linkages: HS/heparin, CS/dermatan sulfate (DS/CS-B), keratan sulfate (KS), and hyaluronan (HA). HS is composed of alternating glucosamine (GlcN) and glucuronic acid (GlcA) or iduronic acid (IdoA); CS/DS is composed of alternating N-acetylgalactosamine (GalNAc) and GlcA or IdoA; KS is composed of alternating galactose (Gal) and N-acetylglucosamine (GlcNAc); HA is a non-sulfated GAG, composed of alternating GlcNAc and GlcA residues (Figure 1). Furthermore, unlike other GAG chains, HA is not covalently bound to any protein and exists exclusively in the extracellular matrix (ECM) (8).

The synthesis of HS and CS polysaccharide chains involves two main steps: (i) attachment of linkage tetrasaccharide (GlcA-Gal-Gal-Xyl) to the core proteins through the serine residue, and (ii) subsequent elongation and modification of the GAG chains. GAG chains undergo diverse modifications by the action of various enzymes in a tissue-specific manner. These modifications include epimerization, N-deacetylation/sulfation, and O-sulfation by various O-sulfotransferases (OSTs), which act by sulfating residues at specific positions (Table 1). The review
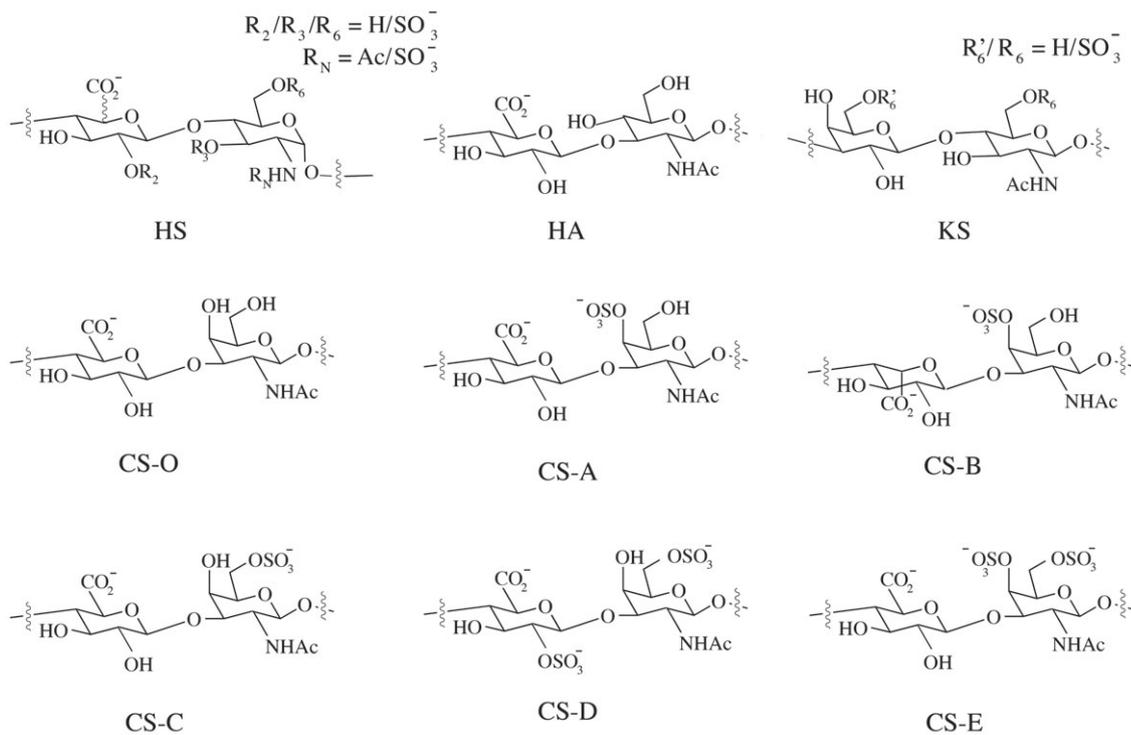

Figure 1 Schematic representation of disaccharide units found in HS, HA, KS, and CS.

Table 1 Enzymes associated with HS and CS modifications and their implications in the CNS.

Enzymes	Role in GAG modification	Known implications	References
HS C-5 epimerase	Epimerization of GlcA to IdoA of HS	*Caenorhabditis elegans* lacking the enzyme showed axonal and cellular guidance defects	(2, 198)
HS NDST (1–4)	Converts GalNAc to GalNS	Initiated sulfation of HS; required for FGF-4 signaling; disruption can impair wingless, FGF, and hedgehog signaling in mice	(199)
HS 2-OST	2-*O*-sulfation of GlcA/IdoA		(198)
HS endosulfatase	Removes 6*S*, preference for NS2*S*6*S*	Expression regulated *Wnt* signaling positively and FGF signaling negatively	(200–202)
HS 3-OST	3-*O*-sulfation	Dramatic changes in its expression were seen in developing zebrafish; expression observed in various locations during mouse development	(139–141)
HS 6-OST	6-*O*-sulfation	Modified HS chains on syndecan-1; enhanced Slit signaling; dramatic changes in its expression were seen in developing zebrafish; expression reported during mouse development	(139, 142, 198, 203, 204)
CS/DS 2-OST	2-*O*-sulfation	Knockdown led to failure of neuronal polarization; upregulated in differentiated neuronal cells	(92, 205, 206)
CS 6-OST	6-*O*-sulfation	Ratio of 4*S*/6*S* varied during development and affected neuronal plasticity; upregulated after CNS injury; upregulated in neuronal stem cells	(81, 160, 205, 207)
CS 4-OST	4-*O*-sulfation	Upregulated in neuronal stem	(205)
N-acetylgalactosamine 4-sulfate-6-OST	6-*O*-sulfation	Knockdown led to failure of neuronal polarization in mice hippocampal neurons; downregulation reduced CSPG-mediated inhibition in E18 rat cortical neurons	(92, 161)
CS/DS C5-epimerase	Epimerization of GlcA to IdoA	Upregulated in astrocytes and neurons as compared with neuronal stem cells	(205, 208)
DS 4-OST1		Mutation associated with adducted thumb-clubfoot syndrome; deficiency resulted in impaired differentiation and proliferation of neural stem cells	(209, 210)

by Sugahara and Kitagawa (6 and references therein) provides a detailed description of HS and CS biosynthesis.

Two of the most abundant HSPGs in the CNS are syndecans and glypicans. Syndecans belong to transmembrane core protein family composed of four distinct members and carry both HS and CS chains (9, 10). Unlike syndecans, glypicans are attached to the cell membrane through a glycosyl phosphatidyl inositol (GPI) linkage, are composed of six distinct members, and carry the HS chain exclusively (11 and references therein). CS chains are primarily carried by large PGs such as hyalectans or lecticans, the most abundant PGs found in the ECM of the CNS (11). The lectican family consists of four members that have been cloned thus far: versican (also called PG-M) aggrecan, neurocan, and brevican (12–15). It has been suggested that the number of GAG chains per unit length in versican, neurocan, and brevican is fairly constant. However, the number, size, and composition of the GAG chains are not only influenced by the core protein but also by the tissue from which the lectican originates. For example, the number of KS-binding domains of aggrecan varies among different species and tissues. Aggrecan DNA sequence shows the number of domains to be 13 in human, 4 in rat and mouse, and none in chicken (16). PGs other than syndecans, glypicans, and lecticans exist in the CNS; however, many of these are structurally unique. One example of such a PG is the receptor-type protein tyrosine phosphatase RPTPβ. Two of the three identified isoforms of RPTPβ have been found to carry CS chains (17). This article mainly focuses on GAGs, and detailed description of PGs can be found elsewhere (11, 18).

Influence of HS and CS in the CNS

(i) Neural development

HSPGs are expressed in actively mitotic areas of the brain. Glypican-1 transcripts have been reported in the ventricular zone, the area of neurogenesis, during CNS development

(19). Most neuronal precursor cells do not express glypican-2, -4, and -5, excluding glypican-4 (or K-glypican) expression in the ventricular zone of the cerebral cell wall (20) and glypican-5 expression in the ganglionic eminence (21). In *Drosophila*, mutations in the gene associated with glypican (*Dally* locus) results in alteration of the cell division pattern at the larval stage (22). Loss of Dally protein delays lamina precursor cells from entering into the final round of cell division. Furthermore, in humans, mutations in glypican-3 result in human X-linked Simpson-Golabi-Behmel syndrome (SGBS) (23). SGBS results in prenatal and postnatal overgrowth and has been associated with the high incidence of neuroblastoma. Syndecan-3 is the most prominent syndecan family member expressed in the adult mammalian CNS; its maximal expression in rats was found on postnatal day 7 and corresponded to glial cell differentiation, myelination, and formation of neuronal connections. The expression declined in adult neurons, where it was mainly found in axons (24).

CSPGs have also been found to influence CNS development by regulating cell division, neuronal stem cell proliferation, secondary neurosphere formation, and neurogenesis (25–27). For example, phosphacan, an RPTPβ variant, is upregulated in areas of active cell proliferation during embryonic development of rats (11, 28, 29). Neurosphere-forming cells in rat fetal telencephalons were found to express neurocan, phosphacan, and neuroglycan C. In addition, CS chains have been associated with neural stem cell proliferation through FGF-2 signaling (30).

(ii) Neuronal migration

In addition to affecting various developmental processes, PGs (mainly CS) also affect the signaling properties of the ECM that control neuronal migration. In the CNS, neuronal migration is guided by a radial glial fiber system that acts as a scaffold for migrating neurons. In early cortical neurons, CSPGs such as protein tyrosine phosphatase RPTPβ/phosphacan are localized along radial glial fibers and on migrating neurons (31). RPTPβ/phosphacan binds to several adhesion molecules, including f3/contactin, N-CAM, L1, TAG1, and tenascin (32). The most important factor that interacts with RPTPβ is pleiotrophin (PTN). PTN is a member of the HS-binding proteins that stimulates neurite outgrowth *in vitro* (33). The CS side chain and protein of RPTPβ form the binding site for pleiotrophin, and several GAGs have been reported to inhibit this binding (34). In the developing cortex, PTN is synthesized by radial glial cells and is deposited along their fibers (35). Antibodies against RPTPβ and treatment with exogenous

GAGs were reported to disturb PTN-induced migration of neurons (36). This evidence suggests that a PG-dependent ligand receptor mechanism must play a role in neuronal migration.

Studies also indicate that changes in CSPG expression are inversely correlated with migration pathways of neural crest (NC) cells. Immunohistochemical studies of chick embryos showed the restricted appearance of the CSPG versican into the CNS barrier tissues (37). In addition, surface-immobilized versican does not support the attachment of NC cells *in vitro* (38). Thus, the avoidance of versican-expressing regions by NC cells should have functional importance in their migration within the CNS. However, not all CSPGs are inhibitory. For example, highly sulfated CS motifs have been shown to enhance neuronal migration. Ishii and Maeda (39) used shRNA constructs to downregulate the production of highly sulfated CS chains, CS-D and CS-E types. The treatment of neuronal progenitor cells with such shRNA constructs in mouse embryos resulted in the accumulation of non-migrated neurons in the subventricular and intermediate zones of the cortex. This evidence further strengthens the argument that GAG type as well as their sulfation influences crucial processes in neuronal development.

(iii) Neurite outgrowth

One of the most widely studied roles of GAGs is their effect on neurite outgrowth and axonal pathfinding. Both HS and CS chains are known to be involved in neurite outgrowth. The expression pattern of HSPGs, such as glypicans and syndecans, is tightly regulated in the developing nervous system and is closely associated with neurite outgrowth (40–42). The study by Wang and Denburg (43) showed that exogenous GAGs can alter axon growth *in situ*. The involvement of HS was confirmed by using HS-degrading enzymes that led to perturbation of axonal pathfinding. Another study on the developing *Xenopus* optic pathway showed that HS binding of exogenous FGF-2, but not FGF-1, disrupts target recognition (44).

In vitro studies have indicated that HSPGs support neurite outgrowth by sequestering growth-enhancing molecules such as laminin, NCAM, heparin-binding EGF (HB-EGF), and several other midkine (MK) family members (45, 46). In addition to their growth-promoting effects, glypican-1 has been reported to serve as a receptor for Slit proteins (47). Slit proteins function as chemorepellants and inhibit axonal growth upon binding to their roundabout (Robo) receptors (48). Syndecan-3 has been identified as a possible receptor for HB-GAM (49). Inhibitors

of Src family kinases affect HB-GAM-dependent neurite outgrowth of syndecan-3-transfected cells. Therefore, Kinnunen et al. (50) suggested that syndecan-3-mediated neurite growth is associated with the cortactin-Src kinase pathway. The role of HSPGs is dependent on the developmental stage of the CNS (18). Various HS-modifying enzymes (Table 1) regulate the spatiotemporal variations in the sulfations of HS chains. The affinity of HS-signaling complexes is highly dependent on the subtle structural changes of their sulfated motifs. Hence, the diversity in HS-ligand interactions forms the basis of their observed variations in their influence on neurons. An overview of the prominent HSPG-mediated ligand-receptor interactions of the CNS is shown in Figure 2. More information in

this regard can be found in the review by Lee and Chien (3) and the references cited therein.

Similar to the influence of HSPGs, the roles of CSPGs on axonal growth are multifaceted and have been studied more extensively. CSPGs are well known to inhibit axonal growth in several regions or developmental stages of the CNS (4, 51, 52, and references therein), and in vivo evidences have shown the inability of axons to penetrate a lectican-containing glial scar (5, 53, 54). However, tissues that express higher levels of CSPGs are not always inhibitory to neural proliferation. In fact, in several studies, CS has been shown to coincide with developing axonal pathways (55–57). Neurocan and phosphacan serve as good examples in highlighting
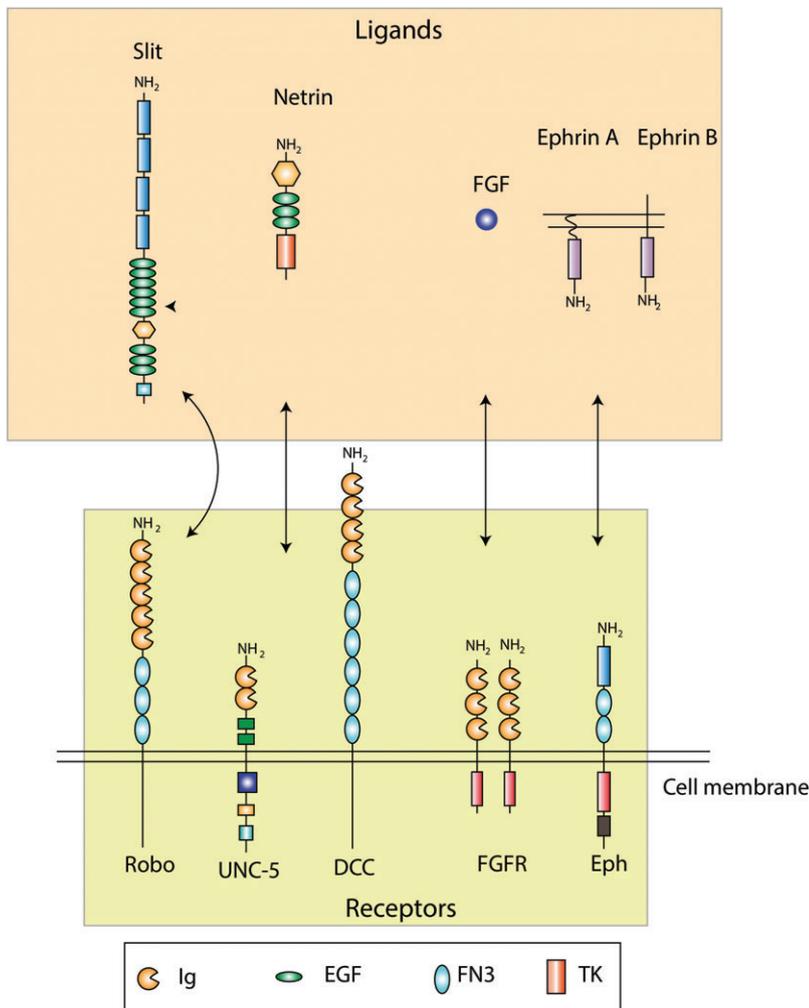

Figure 2 Receptors of HSPGs.
Prominent interactions in the CNS that are mediated by HSPGs are shown. Robo receptors bind to Slit ligands, UNC-5 and DCC (deleted in colorectal cancer) receptors bind to Netrin, FGF binds to FGFR, and ephrin receptors (Eph) bind to ephrin ligands. Box contains the definition for conserved protein domains. Ig, immunoglobulin; EGF, epidermal growth factor; FN3, fibronectin type III domain; TK, tyrosine kinase domain. [The figure has been modified from the review by Lee et al. (3).]

the contrasting roles of CSPGs. While phosphacan was reported to promote neurite outgrowth in rat cortical neurons, neurocan inhibited the growth in embryonic chick neurons (34, 58). Evidence suggests that the stimulatory/inhibitory functions of CSPGs depend on their spatiotemporal expression and interactions that are defined by their sulfation patterns.

The interactions of CS chains with various signaling molecules lead to the promotion of neurite growth. RPTPβ is expressed on migrating neurons and binds to growth factors through its CS side chains (34, 59). Several studies also claim that RPTPβ is associated with CSPG-mediated inhibition in the CNS. For example, Shen et al. (60) have shown that after dorsal column injury, sensory axons grow deeper in RPTPβ mutant mice than they do in wild-type mice.

Some mechanistic pathways are emerging from our present knowledge on the roles of CS in controlling neuronal outgrowth. For example, blood-brain barrier leakage of blood protein fibrinogen containing transforming growth factor-β (TGF-β) is thought to induce CS production in reactive astrocytes by activating the TGF-β/Smad signaling pathway (61–64). Fibrinogen, carrying the latent TGF-β complex, is then responsible for phosphorylation of Smad2 in astrocytes, leading to inhibitory scar formation

and limiting neurite outgrowth (61). CS has been reported to inhibit axonal growth by interacting with leukocyte common antigen-related phosphatase, Nogo receptors (NgR1 and NgR3), and the EGF receptors (65–67). The converging downstream effector of most CSPG-related inhibitory pathways is the activated Rho, a Ras homologue, which further activates the Rho-associated protein kinase (RhoA/ROCK) pathway. Once activated, ROCK leads to actin depolymerization through Lim kinase and cofflin stimulation. Actin filament degradation leads to immobility or collapse of growth cones present on the axons, leading to termination of axonal outgrowth (Figure 3) (68–70). Sivasankaran et al. (71) have shown that by blocking the RhoA pathway, myelin and CSPG lost their ability to activate Rho and inhibit neurite outgrowth in dorsal column neurons. However, similar growth enhancement was not observed for corticospinal tract neurons in the same animals, suggesting that the effect of CSPG signaling is specific to the neuronal cell type and region of the CNS.

(iv) CNS plasticity

HS can alter CNS plasticity by interacting with CNS ligands such as NCAM and EGF. Removal of HSPGs can lead to a
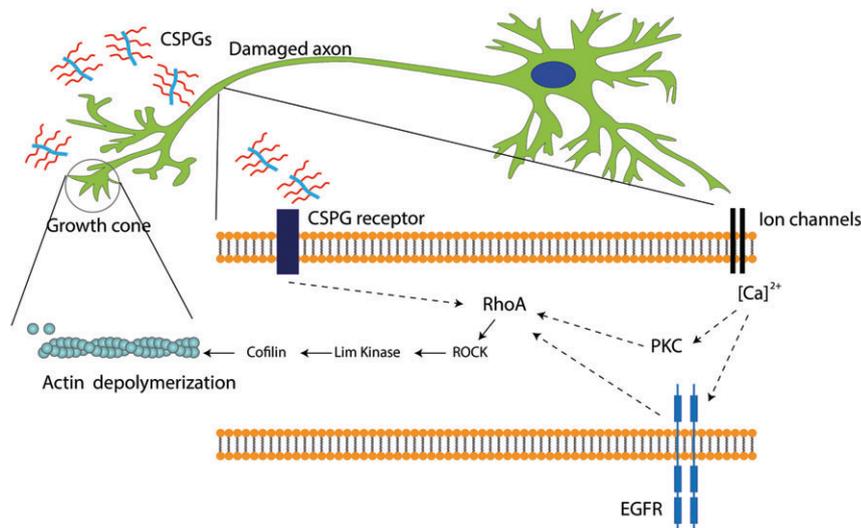

Figure 3 Intracellular signaling mechanism triggered by CSPG present in the glial scar.
CSPGs are thought to be present in axons, although their molecular identity is not well established. Recent studies have shown that CSPG can interact with leukocyte common antigen-related phosphatase, Nogo, or EGF receptors and lead to growth inhibition (65–67). RhoA activation eventually leads to actin depolymerization and the growth cone retraction. RhoA activation has also been shown to be associated with PKC pathway and epidermal growth factor receptor (EGFR) phosphorylation in a calcium-dependent manner (67, 71). However, the process of calcium influx leading to PKC activation or EGFR activation is not well defined. Dashed arrows suggest that mediators of the represented process are yet to be identified.

loss in synaptogenic activity of postsynaptically expressed PSA-NCAM (72). Conversely, loss of the HB-EGF can affect fear and spatial learning as well as alter long-term potentiation (LTP) in the hippocampus (73). Although these studies have documented the importance of GAG chains in plasticity, it remains unclear which sulfation patterns are required for influencing the plasticity. However, some evidence alludes to their importance. For example, mice that are lacking the endosulfatases Sulf-1 and Sulf-2 show shortcomings in spatial learning. Reduction of Sulf-1 alone leads to deficits in synaptic plasticity in the hippocampus (74). As evidences accumulate for the role of HS-ligand interactions in CNS plasticity, it becomes important to delineate how specific sulfation patterns can regulate CNS plasticity.

At the end of the critical period of development, loss of neuroplasticity is seen in the CNS. The critical period refers to the stage until which neuronal properties and connections can be highly modulated by experience. It has been shown that perineuronal net (PNN) formation coincides with the end of the critical period, and other evidences suggest that CSPGs have a critical role in regulating neuronal plasticity (75–78, and references therein). Aggrecan, brevican, and neurocan are major CSPGs that interact with hyaluronan and tenacins to form the PNN, which are areas of condensed ECM that surround neuronal cell bodies and dendrites (79, 80). (The role of PNN in neuroprotection has been widely studied and is discussed under the neurological disorders section.)

A widely studied model for CNS plasticity is the ocular dominance shift in the visual system, whereby the non-deprived eye becomes more represented in the visual cortex as a result of deprivation of the other eye. It has been reported that increase in the $4S/6S$ sulfation ratio of the CSPGs present in brain ECM leads to the termination of the critical period of ocular dominance in mouse visual cortex (81). Normally, the capability of plastic shift exists only until the termination of the critical period. However, an ocular dominance shift toward the non-deprived eye can also be seen in the mature CNS following treatment with chondroitinase ABC (ChABC), suggesting that CS-mediated loss of plasticity may be reversible (75). CSPGs are furthermore implicated in regulating memory formation in the CNS. The cellular correlates of memory formation are 'upward' or 'downward' shifts in the synaptic strengths of neurons in response to altered inputs, termed LTP and long-term depression, respectively (82). It was observed that ChABC treatment in the CA1 region of the mice hippocampus led to a reduction in LTP as well as LDP processes at the pyramidal cell synapse (83). Altogether, these findings suggest that, in addition to controlling their growth/migration, GAGs influence synaptic network stabilization and formation of novel synapses, thereby controlling CNS plasticity and memory formation.

GAGs in CNS injury

Even though the contribution of HS and CSPGs in the CNS is evident, in the absence of highly sensitive molecular and analytical techniques, it has been difficult to establish their structure-function relation. HSPGs interact with a diverse array of growth factors and often serve as necessary co-factors stabilizing their signaling complex (3). However, little is known about the role of HS in CNS injury. As noted earlier, as HS chains have various functional implications on CNS development, HS-mediated signaling can possibly be associated with axonal regeneration or CNS healing linked to other neurological disorders. However, the majority of research on the role of GAGs in CNS injury has mostly been focused on CSPGs.

After CNS injury such as SCI, axonal regeneration at the affected site is inhibited owing to the formation of glial scars by the reactive astrocytes (52). Glial scars in the CNS restrict the regenerative capability of injured neurons. CSPGs, which are upregulated at the injury site, are among the major components of the inhibitory scar tissues, functioning as a barrier to neuronal pathfinding (4, 84–86, and references therein). Neurocan and phosphacan are the two major CSPGs present in the scar, whereas BEHAB (brain enriched hyaluronan binding)/brevican expression has also been found to be upregulated by reactive astrocytes (87, 88). Enzymatic removal of CSPGs after injury using ChABC has resulted in partial recovery in several *in vivo* studies (89–91). Yick et al. (91) found that enzymatic digestion of CS at the lesion site led to the regeneration of Clarke's nucleus neurons into peripheral nerve grafts implanted at the site. These studies suggest that the upregulation of CS in CNS injuries is the major cause for limited axonal regeneration and therefore is the most thoroughly studied manifestation of CS in the CNS.

In the context of SCI, CS is mostly reported to have an inhibitory effect on neuronal regeneration. However, as described in the sulfation patterns section below, some CS variants were found to promote neurite outgrowth and control polarization (55–57, 92). Therefore, as further discussed in the section on the role of GAGs in sulfation patterns, one cannot draw a simplistic conclusion about the molecular role of CSPGs during CNS injury without a closer examination of their discrete sulfation patterns.

GAGs in neurological disorders

One of the most well-studied neurological disorders associated with GAGs is Alzheimer's disease (AD), in which patients show signs of cognitive deficits such as memory loss, inability to learn, and confusion (93). PGs appear to play both pathological and neuroprotective roles in AD. The disease is caused by the accumulation of neuritic plaques and neurofibrillary tangles (NFTs) in the cortex. One of the major components of the neuritic plaques is aggregated amyloid-β (AB) peptides in the brain. Highly sulfated HS has been found to be co-localized with these deposits. Less sulfated HS mediates uptake and degradation of AB in non-disease states; however, the highly sulfated HS inhibits this cellular uptake of AB (93–95, and references therein). These deposits of highly sulfated HS can be substantially degraded by Sulf-1 and Sulf-2, suggesting the presence of $6\text{-}O$ sulfates in significant amounts (96). It has also been shown that the source of this highly sulfated HS is the nearby neuronal population and that HS is important for the regulation of Alzheimer's β-secretase (BACE1) (97). Studies have reported that HS and heparin, which is similar to HS except that it is more heavily charged, bind to BACE-1 and inhibit cleavage of amyloid precursor protein (APP) (97). Other GAG chains, including CS and DS, are also found within these plaques and may contribute to plaque formation and the overall progression of the disease. Previous research also showed that HS, CS, and KS can all interact with AB peptides and enhance the shift of AB42 peptides to the β-sheet confirmation (95). GAGs have been shown to be involved in aggregation and precipitation of amyloid fibrils, leading to increase in neurotoxicity (98). Low molecular weight heparin can compete with endogenous HS for binding to AB and inhibit the formation of fibrils (95). The sulfation patterns present on the HS thus seem to be important for the formation and progression of these neuritic plaques. Non-sulfated GAGs such as hyaluronic acid have no effect on fibril formation and aggregation (95).

Sulfated GAGs have also been implicated in Alzheimer's-like changes seen in the τ protein. Incubation of this protein with heparin results in the formation of Alzheimer's-like filaments and promotes the phosphorylation of τ. This phosphorylation prevents the binding of the τ protein to stabilized microtubules and results in the rapid disassembly of microtubules assembled from τ and tubulin. The effects seen from a variety of GAGs is proportional to the extent of sulfation present on the GAG chain (99).

PGs also play a significant role in gliomas. The neural ECM is naturally resistant to cell and neurite motility.

As discussed in earlier sections, a major component of these inhibitory regions of the ECM is CS. Upregulation of BEHAB/brevican has been reported in gliomas (100, 101, and references therein). The tumor-specific BEHAB/brevican is differentially glycosylated and is cleaved by metalloproteases promoting cell motility and invasion in glioma (102, 103). Neurocan, versican, and tenascin-c are also implicated in the invasiveness of gliomas (104, 105). HSPGs are also upregulated in some gliomas and interact with factors that promote glioma growth and invasion. Specifically, several HSPGs have been found to promote the signaling and mitogenic properties of FGF-2 (106).

Sulfation of GAGs can alter the behavior of glioma cells. Studies have examined how sulfation density affects glioma proliferation and invasion; for example, reduction of sulfation using sodium chlorate treatment on glioma cells resulted in lower levels of cell proliferation (107). Intracerebral inoculation of glioma cells pretreated with sodium chlorate also lead to a decrease in size and instances of glioma, resulting in longer survival of the treated animals (108). Changes were also seen in specific sulfations on HS. Enhanced FGF-2 activity was correlated with higher sulfation levels in the HS present in gliomas with a higher amount of $2\text{-}O$ and $6\text{-}O$ sulfates (106). Sulf-2 expression was reported to regulate receptor tyrosine kinase signaling pathway through PDGFRα activation in glioblastoma cells as well as primary tumors. Knockdown of Sulf-2 in human glioblastoma cells or generation of gliomas from $Sulf2^{-/-}$ resulted in decreased growth *in vivo* in mice (109). These results indicate that endosulfatases expressing gliomas could respond to various external growth factors, and such factors can serve as potential therapeutic targets in such disorders.

Alterations in GAGs are seen in a variety of neurological disorders, including autism, epilepsy, Parkinson's disease, and schizophrenia (Table 2). A mouse model for autistic behavior, the BTBR T+tf/J mice, was recently reported to show alterations in HS associated with fractal structures of the subventricular zone (SVZ), specifically the third and lateral ventricles (110–112). This fractone-associated *N*-sulfated HS is thought to be involved in growth factor sequestering and in modulating glutamatergic synapses (110–112). Furthermore, eliminating HS in postnatal neurons in mice resulted in autistic symptoms. After conditionally inactivating *Ext 1*, an enzyme involved in HS biosynthesis in the brain after birth, there were no morphological changes; however, these mice showed stereotyped and repetitive behavior and had impaired social interactions (113). In a recent study, Pearson et al. (114) report reduction in *N*-sulfated HS in the ECM from the SVZ of brain lateral ventricles in postmortem tissue of

Table 2 Association of various PGs and GAGs with neurological disorders.

PG (GAG)	Disorder	Observed effects	References
Neurocan (CS)	Bipolar disorder and schizophrenia	SNP overlap was found in neurocan gene (*NCAN*)	(211)
Brevican (CS)	Episodic falling syndrome, related to human paroxysmal exercise-induced dyskinesia episodic ataxias	Deletion reported in brevican gene (*BCAN*)	(212)
Nauroparin (HS)	AD	Protected neurons against cholinergic lesions; increased arborization and reduced septal caspase 3 and τ immunoreactivity	(181, 182, 213, 214)
Perlecan (HS)	AD	Disordered processing was associated with amyloidosis	(215)
(HS, DS, CS)	AD	Identified in AD lesions, amyloid deposits, and neurofibrillary tangles	(216–218)
Apican (CS)	AD	APP acted as its core protein, was found in human and rat brain	(219)
(HS)	AD	*O*-GlcNAc glycosylation was upregulated	(220)
(HS/CS)	AD	Promoted formation of paired helical filaments	(221)
CSPG	HIV-1 infection	Facilitated infective entry of virus; PNN damage was observed in AIDS victims	(222, 223)
Neurocan, phosphacan, brevican (CS)	Epilepsy	Full-length neurocan was deposited after seizures, associated with axonal sprouting; phosphacan-positive PNN decreased; cleaved brevican increased in the temporal lobe and hippocampal regions of the rat brain	(224, 225)
Aggrecan, versican, phosphacan, brevican (CS)	Stroke	Plasticity increased in peri-infarct and remote regions with reduction in aggrecan, versican, and phosphacan, and accumulation of neurocan	(226, 227)
HSPGs	Gerstmann-Straussler syndrome, Creutzfeldt-Jakob disease, and scrapie	Sulfated GAGs were found to be present in amyloid plaques	(228)
HSPGs/CSPGs	Parkinson's disease	Lewy bodies were found to contain PGs	(218, 229)
Brevican, versican (CS)	Glioma	Overexpressed and promoted tumor growth, vascularization, and invasiveness	(100, 101, 230)
CSPGs	Monocular deprivation and amblyopia	ChABC led to complete recovery in rats and moderate recovery in cats	(231, 232)
HSPGs	Pick's disease	Involved in extinction of Pick bodies	(233)
HSPGs	Autism	Reduction in *N*-sulfated HS in SVZ	(114)

autistic individuals. These studies implicate that decrease in *N*-sulfated HS in specific regions of the brain could be contributing to the etiology of autism and may serve as a biomarker for the disorder.

CS chains play an etiological role in epilepsy. This is likely due to the ability of CS to stabilize synapses, reduce plasticity, and interact with GABAergic interneurons. A variety of CSPGs are thought to be involved in the onset and progression of epilepsy. Neurocan, which is primarily expressed during development, shows a renewed expression following status epilepticus (SE) in the adult hippocampus (115). Aggrecan has also been examined in relation to PNN remodeling after SE. After SE, the hippocampus showed a decrease in aggrecan expression 1 week post-SE, specifically in the dentate gyrus, followed by a decrease in aggrecan-labeled PNNs. Additionally,

the remaining PNNs show a loss of structural integrity (116).

Both HS and CS appear to play a role in Parkinson's disease (PD). Agrin, an ECM and transmembrane HSPG, has been implicated in fibril formation in AD and PD (117). NG2, a CSPG, has also been implicated in PD. In a study examining two rat models of PD, NG2-positive cells expressed calcium-binding adaptor molecule 1 and GDNF (118). It has also been seen that subcutaneous injection of a cytokine mixture containing granulocyte macrophage colony-stimulating factor and interleukin-3 ameliorated the loss of dopaminergic neurons in one of these rat PD models. One possible mechanism for this reduction of degradation is thought to be the increase in NG2-positive glia found in treated animals (118, 119). Hence, NG2-positive glia seems important for the survival of dopaminergic

neurons and presents NG2 as a possible therapeutic target for PD.

PGs have also been implicated in schizophrenia. Upon examining postmortem brains, researchers found an increase in CSPG-positive glial cells in specific brain nuclei of the schizophrenic patients. The increase was primarily seen in the deep amygdala and entorhinal cortex. A decrease in PNNs was observed in different areas of the amygdala and entorhinal cortex as well (120). However, there was no change in the presence of parvalbumin-positive GABA neurons that are most commonly associated with PNNs in the entorhinal cortex or the amygdala (121). Changes have been seen in fear learning and extinction in schizophrenia patients. This may be directly influenced by alterations in the PNN present in the amygdala, as previous studies have shown that enzymatic degradation of this structure disrupted fear learning (122, 123). This presents evidence that CS could play a key role in development of schizophrenia and its overproduction in certain areas while disruption in others may lead to improper neural functionality.

GAGs are altered in many neurological disease states; however, it remains unclear what is the exact mechanism of their involvement. Although GAGs have been implicated in several CNS pathological processes, there are ample evidences to suggest their neuroprotective role as well. Brain samples from patients with sporadic Creutzfeldt-Jakob disease revealed that PNN surrounding parvalbumin-IR neurons of the cerebral cortex disappear before these cells die. Following their death, this zone becomes occupied with protease-resistant prion protein (PrP), which then spreads into the space and interacts with other GAGs to form protease-resistant aggregates (124, 125). Likewise, cortical neurons associated with PNN were found to be resistant to neurofibrillary changes and τ pathology in AD (126, 127). *In vitro* experiments suggest that CSPGs protect PNN-associated neurons against glutamate toxicity, which also plays a role in AD (128). CS chains were also found to elicit neuroprotective effects in an *in vitro* model of calcium-dependent excitotoxicity in a sulfation-dependent manner. Treatment of rat cortical neurons with CS-E reduced cell death by N-methyl-D-aspartate (NMDA), (S)-a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, or kainate, whereas other sulfation variants of CS or HS had no such protective effect (129). Dopaminergic neurons are associated with the pathophysiology of PD and undergo significant reorganization in their aggrecan-based ECM, leading to degeneration (130). It has been suggested that polyanionic GAGs associated with PNN contribute to the reduction in local oxidative stress by scavenging redox-active ions (131). Taken together, these studies suggest a paradoxical role of PGs in neurodegenerative diseases. For example, while several evidences suggest strong interaction between GAGs and amyloid deposits and NFTs (132, 133), CSPGs containing PNNs are known to protect cells from degeneration. Therefore, it is evident that GAG structures must play different roles in various states of damaged CNS. Our understanding about the specific roles of PGs in CNS disorders is still growing, and we have summarized observations on their associations with various CNS pathologies in Table 2 and Figure 4.

The role of GAG sulfation patterns in the CNS

The elaborate molecular interactions of the HSPGs/CSPGs are tightly controlled by their sulfation patterns, which provide their ligands unique selectivity for binding (Table 3). Diverse sulfations are found in both HS and CS (134). Wang et al. (43) reported that highly sulfated HS-like polymers, in comparison with unsulfated HS, increased error in median fiber tract axonal pathfinding of pioneer axons in cockroach embryo. Furthermore, genetic studies done by deleting various sulfotransferase enzymes have shown the importance of sulfation. For example, mutation in the N-deacetylase-N-sulfotransferase gene in *Drosophila* inhibited activation of the MAPK pathway and led to defects in cell migration during embryogenesis (135). Different sulfotransferase isoforms of CS have been associated with specific regions of the brain (136). With the alternating sulfotransferase activity, the sulfation patterns of HS and CS keep changing during embryonic brain development (137, 138). Therefore, it is understood that sulfated domains of HS and CS are highly regulated by the spatiotemporal expression of various sulfotransferase and endosulfatase enzymes.

HS sulfotransferase enzymes are expressed differentially in the cerebrum during neural development. During mouse embryonic development, significant 3OST and NDST expression was observed in the ventricular zone and the cortical plate (139). NDST was also expressed in other brain areas such as the marginal zone and subplate (139). In juveniles, 3OST and 6OST expression was found to be more widespread, being found in the ventricular zone, layers V/VI, intermediate zone, and cortical plate. 3OST expression in adults was found in layers II/III and V of the cortex, and 3OST-2 and 3OST-4 were found abundantly in trigeminal ganglion neurons. 6OST showed expression in layers I and II/III. NDST expression in juveniles was
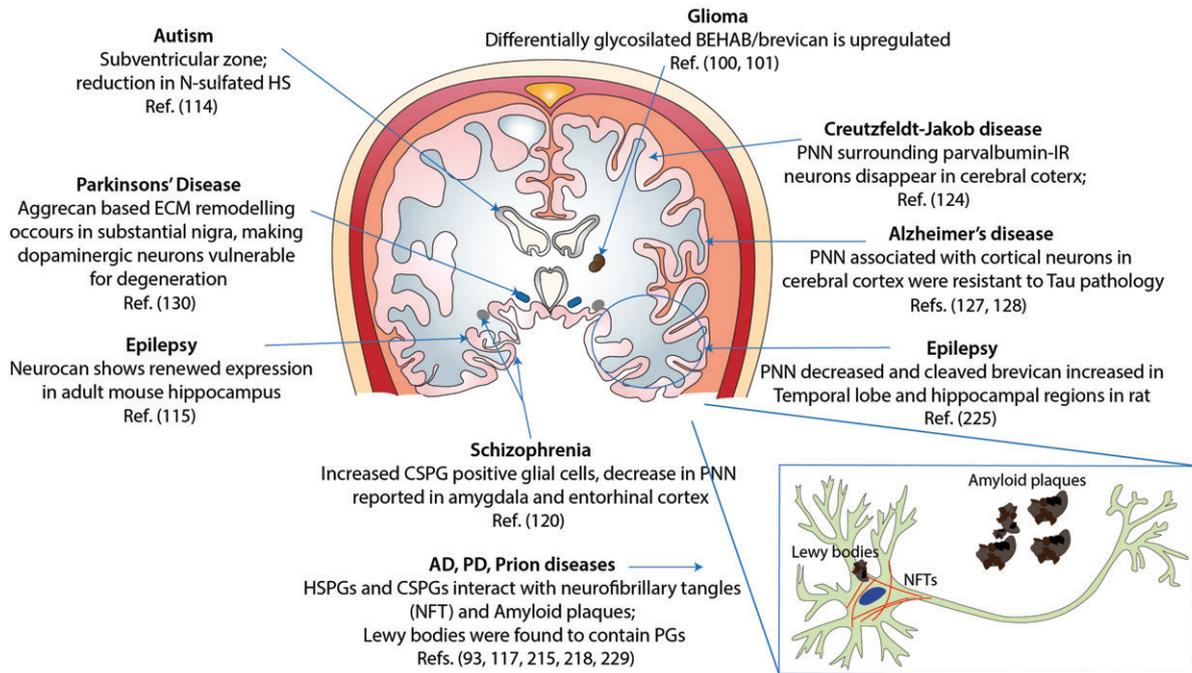
Autism
Subventricular zone;
reduction in N-sulfated HS
Ref. (114)

Glioma
Differentially glycosilated BEHAB/brevican is upregulated
Ref. (100, 101)

Creutzfeldt-Jakob disease
PNN surrounding parvalbumin-IR
neurons disappear in cerebral coterx;
Ref. (124)

Parkinsons' Disease
Aggrecan based ECM remodelling
occours in substantial nigra, making
dopaminergic neurons vulnerable
for degeneration
Ref. (130)

Alzheimer's disease
PNN associated with cortical neurons in
cerebral cortex were resistant to Tau pathology
Refs. (127, 128)

Epilepsy
Neurocan shows renewed expression
in adult mouse hippocampus
Ref. (115)

Epilepsy
PNN decreased and cleaved brevican increased in
Temporal lobe and hippocampal regions in rat
Ref. (225)

Schizophrenia
Increased CSPG positive glial cells, decrease in PNN
reported in amygdala and entorhinal cortex
Ref. (120)

Amyloid plaques

AD, PD, Prion diseases
HSPGs and CSPGs interact with neurofibrillary tangles
(NFT) and Amyloid plaques;
Lewy bodies were found to contain PGs
Refs. (93, 117, 215, 218, 229)

Lewy bodies

NFTs

Figure 4 Observed and potential associations of PGs and GAGs with neurological diseases in various regions of the human brain. This diagram represents a coronal section of the human brain and highlights evidence reported about the involvement of GAGs in various CNS disorders. Observations made in non-human brain samples may suggest that such effects persist in the human brain as well.

Table 3 Different GAG sulfation patterns and their effects related to the CNS.

GAG	Sulfation	Observed effects	References
HS	2S	Binding of FGF1 and FGF8 to several FGFRs. These interactions were associated with neural plate patterning, neurogenesis, gliogenesis, cell migration, axonal pathfinding, and neuronal regeneration	(145, 234, 235)
HS	6S	Binding of FGF1 and FGF8 to several FGFRs, associated with effects stated in the row above	(145, 235)
HS	2S/3S/6S	Associated with Slit protein binding	(236)
HS	IdoA(2S)-Glc(NS)	Associated with bFGF binding	(237)
HS	NS2S6S	Netrin 1 and semaphorin 5B binding; NS6S was preferred by Slit 2; NS2S was preferred by ephrin A1 and ephrin A2	(238)
HS	Totally desulfated/NS	Failed to bind basic FGF	(239)
CS-A	GlcA-GalNAc(4S)	Provided negative guidance cues to cerebellar granule neurons	(163)
CS-C	GlcA-GalNAc(6S)	Expressed in tissues acting as barrier to axonal advancement in chick embryo; upregulated after CNS injury; upregulation led to axonal regeneration; associated with Schwann cell motility	(159, 160, 167, 240)
CS-E	GlcA-GalNAc(4S,6S)	Stimulated outgrowth in dopaminergic, hippocampal, and DRG neurons; associated with FGF-2, FGF-10, FGF-16, FGF-18, MK, PTN, TNF-α, BDNF, HB-EGF binding; inhibited rat cortical cell binding; inhibited DRG neurite outgrowth through PTPσ pathway; assembled neurotrophin-Trk complex	(148, 150, 151, 154, 156, 162, 166, 241)
CS-D/CS-E	GlcA(2S)-GalNAc(4S)/GlcA-GalNAc(4S,6S)	Promoted growth in rat hippocampal neurons	(242, 243)
CS-B/CS-E	IdoA-GalNAc(4S)/GlcA-GalNAc(4S,6S)	Interacted with PTN; promoted neurite outgrowth in hippocampal neurons by interacting with PTN	(1, 244)
CS-A/CS-D/CS-E		Stimulated neurosphere formation through an EGF-dependent pathway	(29)
CS-B/CS-D/CS-E		Promoted FGF-2-mediated proliferation of rat embryonic neuronal stem cells	(30)

seen in layers II/III and V, whereas expression was seen in a manner similar to that of 3OST in adults (139, 140). In adults, 3OST expression can also be induced by environmental cues. Dramatic changes in the expression of various 3OST and 6OST isoforms were observed in a spatiotemporal manner in developing zebrafish (141, 142). The HS fine structure in the pineal gland showed differences when assessed in light versus dark conditions (143). The differential expression in development as well as changes in sulfotransferase expression due to environmental cues such as light implies that control of specific HS sulfation is important to normal development and neural function.

GAG functions are controlled by their sulfation pattern as well as density. The specificity of HS sulfation in determining their binding affinity for different isoforms of FGF has been investigated extensively. IdoA2S-GlcNS was found to increase the binding affinity of HS with basic FGF, and the affinity increased with increasing sulfation density (144). Sulfates seem to impart a defined structure to the HS microdomains, thus controlling their selectivity for binding to various ligands. Many such ligand receptor interactions, such as FGF-FGFR, play crucial roles in various CNS processes, such as neural plate patterning, neurogenesis, gliogenesis, axonal pathfinding, and regeneration during injury. Further details on the role of FGFs in CNS can be found in the review by Guillemot and Zimmer (145 and references cited therein).

Although several distinct roles have been proposed for HS- and CS-mediated signaling, the existence of overlap in their CNS interactions cannot be excluded completely. In fact, crystallographic analysis of RPTPσ revealed a shared binding site for HS and CS (146). In fact, Coles et al. (146) proposed that both HS and CS can bind to RPTPσ and still mediate their differential response on neurons. The computational model suggested that due to the difference in their sulfation densities, HS and CS are able to differently affect RPTPσ signaling. The discretely sulfated domains of HS may promote RPTPσ clustering, resulting in compromised phosphatase activity and hence enhanced life of neurite-promoting phosphorylated proteins. In contrast, the uniformly sulfated domain of CS results in higher phosphorylase activity mediated by RPTPσ, leading to neuronal inhibition (146). Others have also observed the contrasting roles of HS and CS mediated by RPTPσ. The role of RPTPσ as a CSPG receptor is well known (60). RPTPσ have also been reported to promote growth in chick retinal ganglion cell axons in response to basal lamina, which contains HSPG ligands (147). Although the presence of IdoA imparts very distinct structural features to HS, the presence of IdoA in certain other CS variants (CS-B and CS-D) can likely enhance their binding with

HS binding proteins. This suggests that the HS and CS structure may regulate their CNS interactions in two ways. Owing to the gross structural similarity between HS and CS, they may interact with common receptors. However, owing to the distinct, fine differences in their sulfation, the outcomes of such interactions can vary widely.

Distinct sulfated motifs of CS have been shown to control neuronal outgrowth differently. Tully et al. (148) have shown that surface-immobilized CS-E tetrasaccharide can promote outgrowth of hippocampal neurons. Highly sulfated CS structures such as CS-D and CS-E are known to bind many proteins, including FGFs, MK, PTN, EGF, brain-derived neurotrophic factor (BDNF), and chemokines (see Table 3) (149–151). The CS-E motif is enriched in a developing rat brain (136); interacts with several PGs; and acts in association with appican, syndecan-1, syndecan-4, neuroglycan C, and phosphacan (136, 152, 153). In the study by Gama et al. (151), it was shown that BDNF selectively binds to the CS-E motif with a 20-fold preference over those of CS-A and CS-C at physiologically relevant concentrations. Moreover, surface plasmon resonance experiments show that CS-E bound to MK as strongly as heparin, which was followed by other GAGs (154). Similarly, exogenously added CS-E and CS-D were seen to block pleiotrophin-RPTPβ interaction, resulting in abnormal morphogenesis of Purkinje cell dendrites (155). The observations made on binding of highly sulfated CS with HS-binding growth factors such as MK further strengthens the hypothesis that a combination of gross and fine structural features mediates the interactions of HS and CS with common receptors. Furthermore, the high sulfation density of CS-D and CS-E might structurally be more similar to HS in terms of charge distribution and the presence of 2S-containing IdoA (in CS-D). Hence, these subtle features may enhance the binding of CS motifs to HS-binding growth factors, leading to similarity in their growth-promoting outcomes.

In spite of evidences demonstrating the influence of distinct CS motifs on neurons, the underlying mechanistic pathways are not yet fully understood. Rogers et al. (156) demonstrated another aspect of CS-E signaling by modulating the neutrophin (NT)-tyrosine receptor kinase (Trk) interactions. CS-E, but neither CS-A nor CS-C, was shown to assemble the NT-Trk complex, which led to the formation of CS-E-nerve growth factor (NGF)-TrkA and CS-E-BDNF-TrkB complexes (156). NT-Trk binding mediates neurite outgrowth, differentiation, and survival through the extracellular signal-regulated kinase, phosphatidylinositol 3-kinase, and phospholipase Cγ pathways (157, 158). Multiple signaling pathways may regulate CS-mediated neuronal growth or inhibition; thus, CS-E-dependent NT-Trk binding presents another way in which CS-E plays

a role in neuronal growth and proliferation. Although NT signaling is mediated by Trk receptor dimerization, the possibility of CS-E in dimerizing Trk itself cannot be excluded completely.

In contrast to highly sulfated units, the monosulfated CS-A and CS-C were shown to have inhibitory influences on neuronal growth. The composition of three barrier tissues between the spinal cord and hind limb of the chick embryo illustrates the expression of peanut agglutinin and CS-C at the time when they are avoided by growing axons (159). In a more recent study, it was shown that CS-C synthesis is upregulated in CNS after injury (160). The study also analyzed mRNA levels of the enzyme chondroitin 6-*O*-sulfotransferase 1 (CS-6OST1) and showed its upregulation in most glial cells around cortical injuries. Although these results indicate that CS-C acts as an inhibitor for axonal regeneration, others have suggested CS-E or CS-A to be the inhibitory component of the glial scar (161–163).

The role of GAGs in enhancing amyloid aggregation revealed the following order: heparin>HS>CS=DS, suggesting that highly sulfated GAGs such as heparin were most effective in forming plaques. Furthermore, in the case of heparin, the order was found to be heparin>*N*-desulfated *N*-acetylated heparin>completely desulfated *N*-sulfated heparin>completely desulfated *N*-acetylated heparin. These results clearly show that the sulfate moiety of GAGs plays a critical role in the amyloid-β fibril formation (164, 165). Table 3 summarizes various evidences on the influence of CS and HS sulfation patterns in relation to the CNS.

Although there are a growing number of evidences that suggest distinct roles of different sulfation patterns of CS on neurons, a consensus is still lacking. For example, there is overwhelming evidence on the neurite-promoting activity of CS-E; however, Brown et al. (166) have recently shown that CS-E inhibits neurite outgrowth of dorsal root ganglion (DRG) neurons. Likewise, Karumbaiah et al. (161) reported that CS-E is upregulated in rats after SCI and is inhibitory for the growth of cortical neurons. Similarly, some studies point out the growth-promoting effects of 6-*O*-sulfation. Lin et al. (167) reported that highly 6-*O*-sulfated CS (CS-C motifs) in glial scar formed after CNS injury promoted axonal regeneration in nigrostriatal axons. Overall, contradictory evidence on the role of distinct CS motifs have been shown in the recent literature, and it seems plausible that the influence of such motifs depends on the neuronal cell type, site, and developmental stage of the CNS. Therefore, a systematic characterization of a library of CS variants is required to examine the relative influence of various sulfation patterns on neuronal growth and plasticity.

Owing to the variations in the chain length and the sulfation pattern of the CS chains used in different studies that also involved different neuronal models/sources, an understanding on the role of specific sulfation patterns is currently lacking. Although many studies utilize CS structural variants isolated from natural sources, these chains do not contain uniform sulfation patterns. Most of the commercially available CS chains are classified on the basis of the dominant sulfation motif present on them. We therefore characterized the sulfation profiles of five major CS variants found in the mammalian brain: CS-A, dermatan sulfate (CS-B), CS-C, CS-D, and CS-E, and assessed their influence on embryonic day 18 rat hippocampal neurons. Structural analysis of CS chains revealed a vast diversity in their sulfation content. Surface-immobilized CSs were used in the neurite growth assay by conjugating CS chains to the poly-L-lysine-coated surfaces. Single CS plain-field patterns revealed differential inhibitory potential of the CS variants. Interestingly, over the three time points of 24, 48, and 72 h, we observed that the neurite length and the number of neurites per cell were maximum in neurons growing over CS-D surfaces, whereas CS-C surfaces were the most inhibitory (unpublished data). These findings support previous reports on the inhibitory role of CS-C toward neuronal growth (160, 168). As already mentioned, disulfated CS chains have been reported to promote neuronal adhesion, migration, and neurogenesis. Likewise, we observed relatively better neuronal growth for both CS-D and CS-E among the CS chains tested. Interestingly, the neurite length and the number on CS-D-coated surfaces were significantly higher than in the other CS variants used (unpublished data). Most importantly, the study demonstrated that sulfation pattern variation could lead to diverse cell response to the GAGs, which might be the basis of the opposing roles of CSPGs in the CNS.

Expert opinion

It is clear that GAGs play a major role in CNS development and maintenance. The goal for the future will be to exploit these GAGs as therapeutic agents in CNS disorders. From the reported evidence, we understand that bimolecular interactions mediated by GAGs are influenced by the fine sulfation patterns present on HS and CS chains. Multiple approaches are being explored to utilize these molecules for developing solutions for the neurological injuries and disorders discussed earlier. Presently, the efforts on developing therapies for CNS injury/disorders are headed in three directions: (i) removing inhibitory CSPGs from the

injury site (85); (ii) delivering neurotrophic factors for stimulating regeneration (169); and (iii) using stem cells to support neuronal regeneration (170). However, as none of these approaches have shown complete functional restoration independently, there is a need to design combinatorial therapies that modulate the complex neuroinhibitory environment present at the scar/disease site to facilitate neuronal regeneration.

Digestion of CSPGs at the scar site after injury is one of the most widely investigated therapeutic approaches for CNS injuries. Several studies have demonstrated the therapeutic efficacy of combining the digestion of CSPGs with infusions of neurotropic factors (171–174). However, ChABC treatment results in compete degradation of CS chains and may lead to loss of growth-promoting or guiding motifs. Therefore, digestion of specific CS chains using enzymes such as ChAC or ChB should be explored to selectively remove inhibitory motifs and lead to better therapeutic outcomes than ChABC. Such an approach may also be beneficial in AD and schizophrenia, where upregulation of HS or CS leads to disease progression. Partial or complete digestion of such GAG structures with combinations of chondroitinases, heparitinases, or endosulfatases could be explored for functional effective restorations recovery in such disorders.

In spite of promising results of ChABC, delivery of the active enzyme for prolonged periods has practical limitations. ChABC has been reported to be thermally unstable, decreasing its activity significantly at body temperature (175). Therefore, Lee et al. (90) have reported the use of trehalose to thermostabilize ChABC for prolonging its activity. The stabilized enzyme could digest CS chains *in vivo* up to 2 weeks after injury. Another potential approach to circumvent the limitations of ChABC could be to modulate GAG biosynthesis using xylosides. As xylosides are small molecules, they can be efficiently targeted and delivered at the scar site to alter the production of inhibitory CS chains. Previously, click-xylosides, containing various aglycone residues, were shown to prime a variety of different GAGs in Chinese hamster ovary (CHO) cells (176). Additionally, β-D-xylosides have been used on astrocytes to enhance neuronal growth (177). Moreover, 4-deoxy-4-fluoro xylosides (fluoro-xylosides) could be exploited to inhibit GAG production at the affected region. A number of fluoro-xylosides have been found to inhibit GAG biosynthesis in CHO and endothelial cells (178, 179). Small molecular inhibitors of GAG sulfotransferases are yet another option that can be used to modulate the HS or CS sulfation pattern at the damage site. By selectively targeting one or more sulfotransferase enzymes, highly specific HS or CS motifs can be generated to stimulate

neuronal regeneration. As GAGs may enhance formation of protease-resistant plaques, inhibiting this binding may increase the turnover of these pathological aggregates by prolonged exposure to proteases. Polysulfonated GAG mimetics have been used for this purpose and were found to be protective against amyloid fibril-induced effects (180–182). Furthermore, 4-deoxy-*N*-acetyl glucosamine was shown to attenuate plaque formation and improve effects of AD (183). Therefore, several permutations and combinations of small molecular inhibitors of GAG biosynthesis could be utilized to create clinically applicable solutions for degrading or modifying pathological PGs in the CNS.

GAG-based approaches may pose some limitations as GAGs have multifunctional roles in CNS injury and pathology. First, even though PGs bind to amyloid fibrils through GAGs, an additional role of the protein core of PGs cannot be excluded. Next, although the use of ChABC or fluoro-xylosides may promote short-term neuronal recovery or plasticity by PNN degradation, it is uncertain whether such non-specific degradation or inhibition of CSPGs will be beneficial for the CNS in the long term. It has been shown that scar formation may have a beneficial role of limiting the injury and restricting the infiltration of inflammatory cells (184). Therefore, limited or transient degradation of CSPGs may be required for optimal recovery, thus making the time window of therapeutic intervention an equally important factor.

Various growth-stimulating factors such as neurotrophin-3, cyclic adenosine-mono-phosphate, and sonic hedgehog have been used to stimulate axonal regeneration at scar sites (169, 185). Many such factors target endogenous progenitor cells that can be stimulated to produce neurons and associated cell members at the injury site. In addition, spinal cord-derived neuronal stem/NPC cells can be transplanted to improve the regenerative capacity of the spinal cord, and to facilitate the functional recovery of experimental models (170, 186). To provide a stimulus for these stem cells to differentiate, they are generally seeded in polymeric scaffolds that mimic the architecture of the healthy spinal cord (170). However, instead of using synthetic polymers, GAG-based natural polymers can be developed to enhance the functionality of the scaffolds and to reproduce the molecular signals found in the developing CNS. HA is an excellent candidate for such applications because of its biocompatibility and its ability to maintain tissue organization, facilitate ion transport, and promote cell proliferation and differentiation (187). HS and CS have numerous effects on neurogenesis and neurodifferentiation, which can be exploited in conjunction with growth factor delivery. Heparin-PEG hybrid hydrogel has

been used to explore its applicability toward neuronal cell replacement strategies (188). Although the functions of KS are largely unknown, KS chains are shown to be associated with some critical aspects of the CNS (189). KS is downregulated in AD (190), is the predominant GAG in the cornea of eye (191), and is involved in glial scar formation (192). The coming years will reveal more about the biology of KS and it is likely that all GAG types will become indispensible in therapies for many neurological disorders including AD. As discussed earlier, the influence of sulfation patterns of GAG in the CNS is highly dependent on the neuron type and the state of the CNS. Therefore, different sulfation motifs or GAG combinations may lead to enhanced neuronal regeneration or recovery for different sets of disorders and injury conditions.

Besides enhancing growth and regenerating neurons, it is imperative to guide axons across the scar site to reform severed neuronal wiring. One proposed treatment involves creating a neuronal bridging device that can facilitate connections of the spinal cord regions separated by the wound site. For this approach to succeed, a growth-supporting biomaterial must act as a conduit for the directional growth of neurons. To provide guidance cues for neuronal pathfinding, the bridging scaffold can be 'sugar coated' with HS and CS chains to create a directional gradient of the growth-promoting ligands. Previously, protein micropatterning techniques have been utilized to create patterns for directing neuronal outgrowth (193, 194). By utilizing an array of differentially sulfated GAG structures, one can create unique signaling combinations
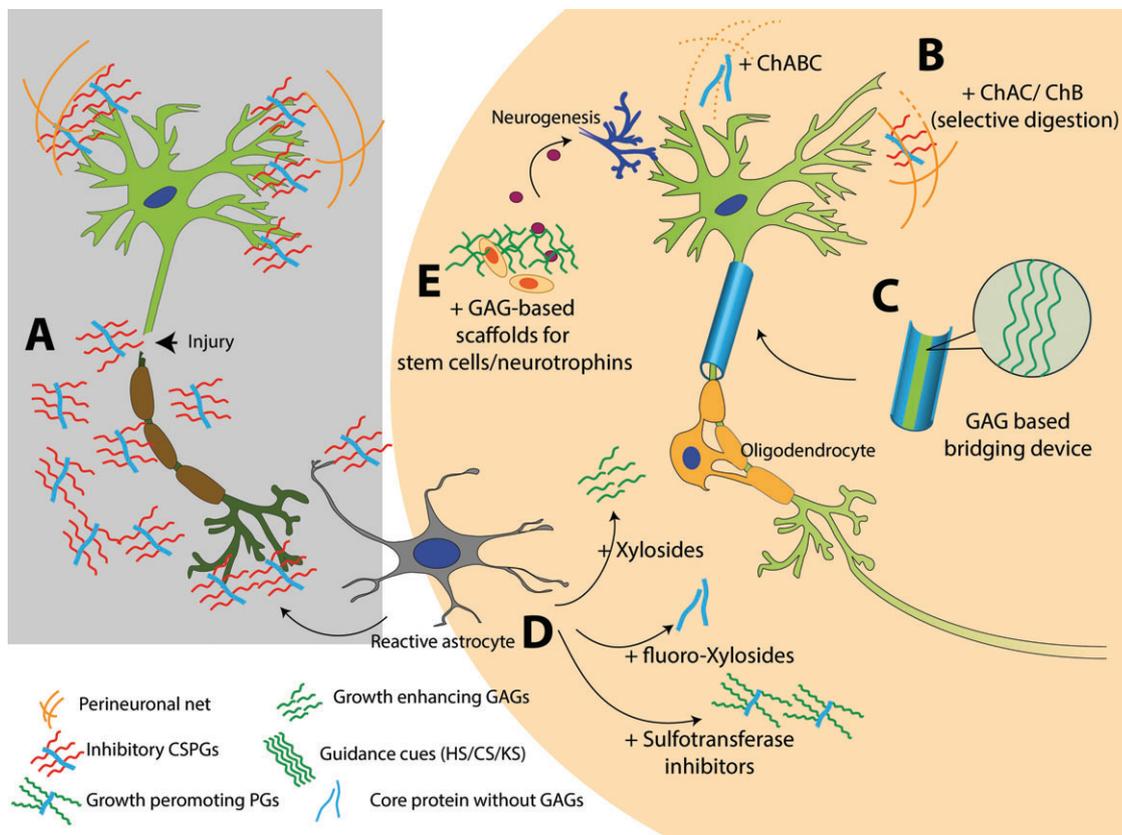

Figure 5 Use of sugar glues for broken neurons and GAG-based therapies for injured CNS.
(A) Neuronal injury results in overexpression of inhibitory CSPGs from reactive astrocytes. (B) Degrading CS chains to stimulate neuronal growth. ChABC is widely studied for this application; however, unspecific digestion of GAGs can damage growth-promoting motifs and other important structures such as PNN. Selective digestion can be done using ChAC and ChB enzymes. (C) Designing GAG-based bridging device to guide regenerating axons. Surface presented HS, CS, DS, KS, or HA domains can be used to direct axonal pathfinding. (D) Using small molecules to modulate GAG production at the scar site. Xylosides would change the composition or sulfation pattern of the GAGs secreted from reactive astrocytes. Addition of fluoro-xylosides would inhibit GAG biosynthesis and result in generation of core protein without inhibitory GAG chains. Sulfotransferase inhibitors can be used to stop production of inhibitory sulfation patterns present in CSPGs released from reactive astrocytes. (E) Bioactive scaffolds can be designed by using GAGs such as HA, HS, CS, DS, or KS. Such scaffolds can be used to deliver stem cells or neurotropic factors at the scar site. A combination of two or more such approaches can be utilized to create effective therapies for neurological disorders.

that enhance regeneration, functionality, and directed growth of neurons.

It is clear that GAGs can influence neuronal development through their structural heterogeneity; however, obtaining structurally uniform GAG chains has been a challenging task. As we present the concept of exploiting sulfation patterns to modulate the outcome of GAG-neuron interactions, obtaining uniform structures with defined sulfation motifs is a prerequisite. Significant advances have been made recently in this direction with the development of various chemoenzymatic processes to obtain defined oligosaccharide and polysaccharide structures of HS chains (195–197). Tully et al. (148) have utilized chemically synthesized CS structures in various neuronal assays. These processes can further be extended to create several uniform GAG structures of HS, CS, DS, or KS backbones for use in various CNS applications. Figure 5 summarizes various GAG-based therapies that can be utilized to develop clinical solutions for CNS disorders. It is hence clear that the biology of sulfation patterns in HS and CS chains has finally begun to take shape. The structure-function relations of GAG-mediated interactions can now be utilized to understand CNS signaling mechanisms and to design novel therapies for several neurological disorders.

Highlights

– GAGs play pivotal roles in CNS development, injury, and diseases.

– Sulfation patterns of GAG chains have a major influence on the regulation of GAG-protein interactions in the CNS.
– There are ample evidences that suggest the sulfation pattern of HS and CS chains affect the development and maintenance of CNS.
– The presence of remarkable heterogeneity in sulfation patterns of naturally occurring GAG chains is a major impediment in deciphering the role of GAG sulfation patterns in the CNS.
– Recent advances in GAG synthesis and analysis have set the stage for defining and exploiting the sulfation pattern of GAG chains to enhance CNS regeneration.
– We propose various promising therapeutic approaches to tackle CNS disorders, including the use of GAG-degrading enzymes in combination with molecular scaffolds that modulate GAG biosynthetic pathways to facilitate functional regeneration.
– The coming decade is expected to witness novel GAG-based therapeutic approaches for treating several debilitating neurological conditions.

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