**9,934 WORDS, 10 FIGURES, 351 REFERENCES**

# Glycans and Glycosaminoglycans in neurobiology: key regulators of neuronal cell function and fate.

Anthony J Hayes [1], ¶James Melrose[2, 3, 4]

[1]Bioimaging Research Hub,
Cardiff School of Biosciences,
Cardiff University,
Cardiff CF10 3AX, Wales, UK.

[2] Graduate School of Biomedical Engineering,
University of New South Wales,
Sydney 2052, NSW, Australia.

[3] Raymond Purves Laboratory,
Institute of Bone and Joint Research,
Kolling Institute, Northern Sydney Local Health District,
Royal North Shore Hospital,
St. Leonards 2065, NSW, Australia.

[4] Sydney Medical School, Northern,
University of Sydney at
Royal North Shore Hospital,
St. Leonards 2065, NSW, Australia.
.

¶Address correspondence to :-

James Melrose,
Raymond Purves Bone and Joint Research Laboratories,
Institute of Bone and Joint Research,
Level 10, Kolling Institute of Medical Research, B6,
The Royal North Shore Hospital,
St. Leonards, NSW 2065, Australia.
Ph +61 2 9926-4806,
Fax +61 2 9926-5266
Email: james.melrose@sydney.edu.au

**Key words:** glycocode; glycan; bikunin; appican; phosphacan; fucose; glycosaminoglycan, lecticans, PNS/CNS.

**Short running head:** Glycans and Neural Function

**Abstract**

The aim of this study was to examine the roles of L-fucose and the glycosaminoglycans (GAGs) keratan sulphate (KS) and chondroitin sulphate/dermatan sulphate (CS/DS) with selected functional molecules in neural tissues. Cell surface glycans and GAGs have evolved over millions of years to become cellular mediators which regulate fundamental aspects of cellular survival. The glycocalyx, which surrounds all cells, actuates responses to growth factors, cytokines and morphogens at the cellular boundary silencing or activating downstream signalling pathways and gene expression. In this review we have focussed on interactions mediated by L-fucose, KS and CS/DS in the central and peripheral nervous systems. Fucose makes critical contributions in the area of molecular recognition and information transfer in the blood group substances, cytotoxic immunoglobulins, cell-fate mediated Notch-1 interactions, regulation of selectin mediated neutrophil extravasation in innate immunity and CD-34 mediated new blood vessel development and the targeting of neuroprogenitor cells to damaged neural tissue. Fucosylated glycoproteins regulate delivery of synaptic neurotransmitters and neural function. Neural KS-proteoglycans were examined in terms of cellular regulation and their interactive properties with neuroregulatory molecules. The paradoxical properties of CS/DS isomers decorating matrix and transmembrane proteoglycans and the positive and negative regulatory cues they provide to neurons is also discussed.

**Abbreviations**

| | |
|---|---|
| AD | Alzheimer's disease |
| ADAM | a disintegrin and metalloproteinase domain |
| ADAM-TS | a disintegrin and metalloproteinase domain with thrombospondin motifs |
| ADCC | antibody dependent cellular cytotoxicity |
| AGE | advanced glycation end product |
| Akt | protein-kinase B |
| ALS | amyotrophic lateral sclerosis |
| APP | amyloid precursor protein |
| ATP | adenosine triphosphate |
| BDNF | brain derived neurotrophic factor |
| β3GlcNAcT | β1,3-N-acetylglucosaminyltransferase |
| CS | chondroitin sulphate |
| CSPG | chondroitin sulphate proteoglycan |
| CSL | an acronym for CBF-1/RBPJ (recombining binding protein suppressor of hairless) |
| CNS | central nervous system |
| DCC | a receptor named *Deleted in Colorectal Cancer* |
| DRG | dorsal root ganglion |
| DS | dermatan sulphate |
| DS | dermatan sulphate proteoglycan |
| ECM | extracellular matrix |
| EGF | epidermal growth factor |
| EGFR | epidermal growth factor receptor |
| ER | endoplasmic reticulum |
| ERK | extracellular signal-regulated kinase |
| FGF | fibroblast growth factor |
| FGFR | fibroblast growth factor receptor |
| FcγRIIIA | activating Fc receptor specific for IgG Fc region expressed by HNK cells and macrophages |
| FUT | fucosyl transferase |
| GAG | glycosaminoglycan |
| GlcNAc6ST | *n*-acetylglucosamine-6-O-sulfotransferase |
| GSK | glycogen synthase kinase |
| GTP | guanosine triphosphate |
| HMBG-1 | high-mobility group box-1 protein |
| HNK | human natural killer |
| HS | heparan sulphate |
| HA | hyaluronan |
| IGD | interglobular domain |
| IGFBP2 | insulin-like growth factor binding protein-2 |
| IgG | immunoglobulin G |
| KS | keratan sulphate |
| KSPG | keratan sulphate proteoglycan |
| KSGal6ST | keratan sulfate galactose 6-O-sulfotransferase |
| LAD II | leukocyte adhesion deficiency II |
| LAR | leukocyte common antigen related |
| LC-MS | liquid chromatography-mass spectroscopy |
| LRR | leucine rich repeat |
| MAb | monoclonal antibody |
| MAPK | mitogen-activated protein kinase |
| NCAM | neural cell adhesion molecule |
| NG2 | neural/glial antigen 2 |
| NMR | nuclear magnetic resonance |
| 2D MRS | two dimensional magnetic resonance spectroscopy |
| NG2 | neural/glial antigen-2 (CSPG-4) |

| | |
|---|---|
| NGF | neural growth factor |
| PKA | cAMP dependent protein kinase |
| POFUT | GDP-fucose protein O-fucosyltransferase 1 |
| POFUT | protein - fucosyl transferase |
| PG | proteoglycan |
| PNS | peripheral nervous system |
| PSGL-1 | selectin-P ligand (CD162) |
| PTPσ | protein tyrosine phosphatase σ |
| RAGE | receptor for advanced glycation end products |
| RPTP-σ | receptor-like protein tyrosine phosphatase-σ |
| SCI | spinal cord injury |
| SHH | sonic hedge hog |
| sLeX | sialyl Lewis-X antigen |
| Trk B | tyrosine receptor kinase B |
| TGF-β | transforming growth factor-β |
| TNF-α | tumour necrosis factor-α |
| TSRs | thrombospondin repeats |
| SYN | synapsin |
| RPTP-ζ | receptor protein tyrosine phosphatase-zeta |
| Wnt | this is a condensation of terms describing the *Winged* and *Int* transcription factor morphogens |

## 1. Introduction

### *1.1 Aim*

This study reviews the roles of selected glycans and glycosaminoglycans (GAGs) which decorate neural glycoproteins and proteoglycans (PGs) and examines how they contribute to neuronal function and repair processes. Due to the complexity of the large number of neural effector molecules and their broad interplay with receptors, ion channels, synaptic and axonal structures in health and disease it has not been possible for this review to provide a comprehensive coverage of all of these aspects. Rather, key interactive molecules have been focussed on and novel aspects of the functional roles of glycans such as L-fucose and GAGs such as keratan sulphate (KS) and chondroitin/dermatan sulphate (CS/DS). The role of heparan sulphate (HS) in neuronal development and function and also pathogenesis (e.g in neurodegenerative conditions such as Alzheimer's disease (AD) is a significant area of glycobiology under intense scientific scrutiny and, as such, is outside the scope of the current review. For this, the reader is referred to a number of recent studies [1-6].

### *1.2 Analysis of glycan and glycosaminoglycan complexity*

While the structural complexity of glycan structures is a daunting subject to investigate [7-10] powerful analytics have been developed to assist in these investigations. These new methodologies include ion-mobility mass spectrometry [11, 12], application of synchtrotron radiation for glycan structural analysis [13], application of high throughput automated N-glycopeptide glycoproteomic identification systems and orbitrap mass spectrometry [14-16], integrated systems glycobiology methodology incorporating glycogenomics, glycoproteomics and glycomics [17], fully automated chip-electrospray mass spectrometric analysis for the determination of CS/DS fine structure[18]. GAG microarrays for the analysis of GAG-protein interactions [19-21] have also been applied to profiling the sulphation patterns of GAGs to determine growth factor interactive sequences [22, 23] and have also identified CS-E tetrasaccharides motifs which act as TNFα antagonists [24]. Development of clickECM cell-derived azide functionalised extracellular matrices (ECMs) [25], photoactivatable and chemoenzymatic glycan labelling tools [26-28], non-invasive two dimensional nuclear magnetic resonance spectroscopy [29], glycoengineering of monoclonal antibodies (MAbs) with improved carbohydrate-protein interactive properties and immune cell targeting capability has improved their efficacy in anti-cancer therapeutics [30]. Multimodal glycosylated conductive polymer biointerfaces suitable for the evaluation of carbohydrate-protein interactions [31] and nanoscale biomatrices for studies on glycocalyx interactions [32] have been developed. Such approaches have been applied to the translation of the 'Sugar Code' into immune and vascular signaling programs with potential therapeutic application [33], such an approach may also provide a better comprehension of the complexities of altered glycodynamics in brain conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, epilepsy and neural conditions characterised by altered cognitive learning [34].

Analysis of the structural complexity of glycans has been considerably aided with the development of software packages which simplify unambiguous representation of glycans and their structural forms. These include GlycanBuilder [35], KCam[36], GlycResoft, a software package for automated recognition of glycans from liquid chromatography-mass spectrometry (LC-MS) data[37], KEGG Carbohydrate matcher (http://www.genome.jp/ligand/kcam/), SWEET-DB, annotated carbohydrate data collections[38], DrawRINGS, 2D Glycan structure Drawing Tool (http://rings.t.soka.ac.jp/java/DrawRings.html), LINUCS: linear notation for unique description of carbohydrate sequences[39], GLYDE (http://glycomics.ccrc.uga.edu/GLYDE-CT/) [40], EUROCarbDB tools to normalise and convert glycan structures: Glycan builder (http://www.eurocarbdb.org/applications/structure-tools) and analysis of MS spectra : GlycoWorkbench (http://www.eurocarbdb.org/applications/structure-ms-tools). PROCARB is a database of known and modelled carbohydrate binding protein structures with sequence based prediction tools[41]. Establishment of the Consortium for Functional Glycomics (CFG, http://functionalglycomics.org/static/consortium/consortium.html) in 2001 has aided glycan research through the extensive, highly informative reference material readily available on their web-site. Informatics tools are also available for the analysis of GAG structure[42] and conformation [43] and for the determination of interactive GAG sequences [44-49]. Glycomics databases such as EuroCarbDB (http://www.ebi.ac.uk/eurocarb/home.action) and The Functional Glycomics Gateway (http://www.functionalglycomics.org/), Databases of Conformations and NMR Structures of Glycan

Determinants [50] and software for the structural determination of GAGs by mass spectrometry [51], and for automated comparison of low molecular weight heparins from LC/MS data [52] have also been developed [51]. Nuclear magnetic resonance (NMR) spectroscopy has also been applied to the structural analysis of sulphated fucose-CS polymers [53]. Furthermore, novel high sensitivity, low toxicity alkynyl-fucose substrates have been developed for the visualisation of fucose incorporation into glycopolymers, these alkynyl-fucose substrates are incorporated into N-glycans by a wide range of fucosyl transferases[54] enabling their visualisation in cells using biotin-steptavidin Alexa-488 histochemistry and they may be extracted, separated by SDS PAGE and identified by Western blotting [53]. The complexity of glycans surpasses by several magnitudes that of the other major life biomolecules, proteins, lipids and nucleic acids [9, 10, 21, 55, 56] and their analysis has lagged behind due to this complexity however with the improvement in glycan analysis now possible with the methodology outlined above this gap is steadily closing.

Glycan biodiversity occurred over at least 500 million years of vertebrate and invertebrate evolution and an even longer evolutionary period in bacteria leading to their evolution as mediators of cellular interaction. Glycans occur in the glycocalyx of all cells and they are the first point of contact between that cell and other cells, with that cell and the extracellular matrix or with any invading organism. Thus there were heightened evolutionary pressures on these front-line glycans to develop recognition and effector roles, with this major positive selection stimulus glycans diversified into their present day level of complexity. The glyco-code could therefore be considered a biodiverse IT database which nature has developed over a very significant evolutionary period [57]. Thus many structural permutations were explored and those glycan structures that have persisted to the present day are ones which offer interactive capability with effector molecules in essential physiological processes providing improved survival traits. Deciphering this glyco-code using the sophisticated glycobiological methodology now available is an important research objective and may uncover invaluable insights as to how glycans regulate cells and be of application in repair biology.

## 2. The complexity of neural tissues

*2.1 Cell types in the central and peripheral nervous system.*

Neurons and glial cells have a common neuro-epithelial origin in the embryonic nervous system and thus share many structural and molecular characteristics [58, 59]. Neurons and glial cells display unique properties which distinguish these cell types from others. Approximately 10% of all cells in the tissues of the central and peripheral nervous systems (CNS/PNS) are neurons. Accessory cell types also include astrocytes, radial glia, oligodendrocytes, ependymal cells, microglia and microvascular endothelial cells while neural/glial antigen 2 (NG2) positive glia are also considered to be a distinct cell type. Microglia are fundamentally distinct from other brain cell types, being derived from primitive peripheral myeloid progenitors during embryogenesis. Microglia are the resident phagocytic cells of the brain, taking part in immune-mediated defense processes which clear damaged cell debris while other glial cells have roles in the nutrition of the neuron and maintenance of axonal structures [58-61].

The CNS/PNS has an extensive blood supply which services its considerable metabolic demands. Like most cells in the human body, glucose, is also the primary energy source for neurons. The brain is the most energy-demanding organ in the human body and while it may only constitute ~2% of the total mass of the human body it uses 20% of the bodies total energy production [62]. Glucose metabolism is the physiological fuel for brain function and is also required for the generation of ATP and the precursor compounds required in the synthesis of neurotransmitters needed for cell signalling. Brain functions such as thinking, memory, and cognitive learning are intricately interlinked to efficient utilisation of glucose in energy production [63]. However, too much glucose as occurs in type I and II diabetes can also be detrimental to brain function. Type 2 diabetes accelerates brain aging and accelerates functional decline in dementia resulting in significant age dependent cognitive changes in brain function.

While glycans are of particular importance in the provision of the metabolic demands of the CNS/PNS, they also have significant recognition roles in neuronal regulation. Neurons are terminal post-mitotic cells with the ability to communicate precisely and rapidly with other cells in the neural system through long cellular extensions (dendrites) that extend to distant sites in the body. Two features equip neurons with this interactive capability: (i) Neurons have receptive dendrites in the cell body and

a transmitting axon at the other end, this arrangement is the structural basis for unidirectional neuronal signaling, (ii) Neurons are electrically and chemically excitable cell types. The neuron cell plasma membrane contains specialized ion channels and receptor proteins that facilitate the regulated flow of specific inorganic ions in and out of the neuron, thereby redistributing charge and creating intracellular electrical micro-currents that alter the voltage across membranes. Such charge changes can produce a wave of depolarization in the form of action potentials along the axon and this is the usual way a signal and neurotransmitter molecules are transmitted from one neuron to another [64]. A waxy myelinated sheath surrounding the axon ensures that high conduction velocities are maintained in neurons to optimise their excitatory transmitter properties (Fig 1). Neuro-transmitters are synthesised in the Golgi/endoplasmic reticulum (ER) of the neuronal cell body (soma) and transported by a microtubular system towards the pre-synaptic membrane where they are stored in synaptic vesicles for later co-ordinated delivery into the synaptic gap for transportation to a communicating neuron. Neurons do not use their microtubular assemblies for cell division like other cells, but they use these as internal scaffolding elements for the elongation of axons and dendritic processes. Microtubules act as compression-bearing struts that contribute to the shape of the neuron and also act as directional conduits for the transport of neurotransmitters and organelles from the cell body to the synaptic terminals (Fig 2). Synaptic vesicle membranes contain the fucosylated glycoprotein synaptophysin, which forms pore-like assemblies that provide portals for the entry of Ca2+ ions in and out of these structures. Synapsin is another major fucosylated vesicle associated glycoprotein which interacts with the cytoskeleton tethering synaptic vesicles and co-ordinating their transport to the synaptic gap for eventual synchronised neurotransmitter transmission across the synaptic gap to communicating nerves in the neural network.

While glial cells are a less excitable cell type than neurons, their membranes nevertheless also contain transporter proteins that facilitate the uptake of ions as well as proteins that remove neurotransmitter molecules from the extracellular space. Thus glial cells act as accessory support cell types to regulate neuronal function and also have roles in the nutrition of neurons and assembly of the myelin sheath. In addition, they undertake running repair processes to ensure the maintenance of neuronal structural integrity (Fig 2). Sophisticated regulatory systems are in place to facilitate neuron-glial cell communication [65-69]. Phosphorylation, ubiquitination, and glycosylation of proteins facilitate weak interactions with multivalent adaptor proteins resulting in the formation of membrane-associated and soluble complexes that mediate information transfer between cells. These systems are dynamic and complex and display remarkable specificity to control signaling pathways and effective communication between neurons and glial cells.

It is estimated that there are over 100 distinct types of neurons in humans. These display molecular and cytological bio-diversity displaying different cell body shapes and arrangements of dendritic processes in variable depths of the cerebral cortex. All neurons inherit the same complement of genetic information during development, however each neuron expresses a restricted set of genes in-situ and they produce a restricted range of enzymes, structural, membrane and secretory proteins specifically designed to service their precise environmental needs. While neurons have lost the ability to replicate they, nevertheless, are capable of re-growth after injury provided the resident inhibitory cues are circumvented and they receive appropriate stimulatory cues to promote neuritogenesis. Glycan modified proteoglycans and glycoproteins have important roles to play in this area providing both stimulatory and inhibitory cues which regulate neural repair and regrowth.

Astrocytes communicate extensively with neurons, define the margins of functional areas of the brain including gliotic scars and also stabilise its internal environment. The extracellular components the astrocytes lay down (e.g. abakan) form a barrier interfacing with the blood brain barrier to exclude components from entry into brain tissues or the glial scar [70]. Astrocytes provide nutrients to neurons and maintain the integrity of neuronal components replacing old and damaged tissue. Astrocytes modify neuronal signals by secreting glio-transmitters and generating waves of Ca2+ action potentials with regulatory properties. Astrocytes also regulate blood flow through extensions which encircle blood vessels and mediate communication with the lining endothelial cells (Fig 2j). Oligodendrocytes assemble the myelin sheath around neurons. Astrocytes also attach to this encircling structure on the neuron which represents a direct line of communication between these two cell types. These astrocyte interconnections dilate and contract blood vessels and influence neuronal signaling in

a dynamic manner to regulate blood flow and neuronal action [71]. Thus the astrocyte is an important coordinative regulator of synaptic function and is believed to have important roles in cognitive learning and memory processes. A single neuron may contain as many as 100,000 synapses and the neuron relies on astrocytes to help control synaptic function through elaborate bidirectional communication between the astrocyte and the neuron. Astrocytes are an underappreciated cell type in neuronal tissues. Astrocytes, like neurons also produce neurotransmitters, generate their own calcium based action potentials and have receptors and ion channels which facilitate constant astrocyte-neuronal communication [72] .

CD34 is an important fucosylated endothelial cell surface molecule containing glycan interactive structures which affect the homing of progenitor cells in microvessels [73, 74]. CD34+ bone marrow haemopoietic stem cells are recruited to sites of brain trauma and differentiate into microglia which participate in neuronal repair processes. ALS, a complex multifactorial progressive degenerative disease with numerous intrinsic and extrinsic factors underlying its etiopathogenesis also displays degenerative vascular pathology underpinned by endothelial cell degeneration [75]. As discussed more fully later in this review, L-Fucose is a component of many *O*-linked and *N*-linked glycan modifications in a number of glycoproteins with important functional roles in many physiological and pathophysiological neural processes[76]. *O*-Fucosylation occurs at consensus sequences on two small cysteine-rich domains in Epidermal growth factor-like (EGF) repeats and Thrombospondin Type 1 Repeats (TSRs) in glycoproteins such as Notch-1, CD-34 and thrombospondin-1 [77]. Mouse Notch-1 contains three *O*-fucosylation sites in EGF repeats 1-5 and thrombospondin-1 has three fucosylation sites in thrombospondin repeats 1-3 [78]. 6-Alkynyl fucose (6AF) is an L-fucose analogue (Fig 4j) which has been developed to facilitate labelling and tracking of these L-fucose motifs in physiological processes [79]. Over 100 proteins are predicted to be *O*-fucosylated on the basis of identified consensus EGF repeat sequences [80]. The Notch receptor family have more predicted *O*-fucosylation sites than any other protein in the recorded databases [81] (Fig 5). Many groups have shown that *O*-fucosylation is essential for Notch's functional properties [80, 82-84]. *O*-fucose also has functional roles in agrin which enables this proteoglycan to cluster acetylcholine receptors in the NMJ [85]. The precise function of *O*-fucose in the vast majority of these proteins however is unknown. Thrombospondins produced by astrocytes have roles in the formation of synapses.

αL-fucose is a terminal or core monosaccharide on *N*-and *O*-linked glycan chains on many glycoproteins (Fig 4d, Fig 5a-g). It also occurs as a capping structure along with sialic acid on the KS-I and KS-II chains of PGs (Fig 4a-c) and in terminal sLeX motifs in glycoproteins (Fig 4f, Fig 5b, Fig 6f-h). KS is heavily substituted with fucose and sialic acid in ALS. The prominent terminal locations of L-fucose points to its role as a molecular recognition site for interacting proteins. Fucose occurs as a terminal sugar linked to a penultimate galactose residue in glycoconjugates or to core GalNAc residues in N-glycans (Fig 5b,f). Fucose can also be directly attached to serine or threonine residues by fucosyl transferases in *O*-linked glycans and can act as an acceptor molecule for the attachment of further saccharides to form small oligosaccharide side chains (Fig 4e).

## 3. Functional roles of the glycosaminoglycan components of brain extracellular and cell associated proteoglycans in neuroregulation

### *3.1 Neural proteoglycans*

ECM proteoglycans (PGs) play important directive roles in the growth of axons and in the navigation, plasticity and regenerative properties of neurons. PGs have paradoxical roles in neuronal growth and repair processes where they can both promote neuronal growth but in other settings can inhibit neural repair [86]. The sulphation positions and charge density of the GAG side chains of PGs can be sources of important signals to the neurons which either inhibit or promote neuronal repair [86]. Thus the CS-A and CS-C chains of lectican PGs such as aggrecan, versican, neurocan and brevican are sources of inhibitory signals and a barrier to neural outgrowth in perineural net formations (Fig 3) which surround areas of axonal damage in glial scar formations [87-90]. CS isomers of higher charge density such as the CS-D and CS-E motifs of phosphacan, bikunin and appican can actually promote neuronal repair processes. Thus, collectively, these CS isomers guide axonal growth and repair with remarkable specificity [91-94]. Another GAG present in some neural PGs is keratan sulphate (KS) and interesting interactive properties are now emerging for this GAG.

*3.2 An Emerging Role for KS in the regulation of neuritogenesis*

The sulphation status of GAGs is an important functional determinant conveying important molecular recognition and information transfer properties that control cellular behavior [57, 95-98]. GAG sulphation motifs on PGs interact with cytokines, growth factors, chemokines, morphogenetic proteins, and extracellular matrix components modulating signaling pathways which control diverse aspects of cellular behaviour such as proliferation, differentiation, migration and matrix synthesis. After the cornea, neural tissue is the next richest source of KS, however it is a relatively neglected GAG and relatively little is known of its functional properties [99]. When dordal root ganglion (DRG) neurons are cultured on a substratum of CS-PGs, neurite outgrowth is inhibited, correlating with the reduced neural repair evident in glial scar tissue where levels of CS-PGs are elevated [87, 100, 101]. Treatment of DRG neuron cultures with chondroitinase ABC or keratanase results in a recovery of neurite outgrowth and these enzymatic treatments also promote neural repair processes in models of axonal damage [102-104]. KS and CS can both be sources of inhibitory signals in neuritogenesis. Three molecular forms of KS have been identified. KS-I and KS-II are substituted with L-fucose which has recognition roles in *N*- and *O*-linked glycans [99], KS-III is also found in the brain [105]. *O*-fucosylation of the KS chains attached to aggrecan vary along its core protein (Fig 4). The KS-II chains in the KS-rich region contain capping fucose and sialic acid residues but this varies in tissues. These capping structures occur in aggrecan isolated from intervertebral disc and articular cartilage but not in aggrecan isolated from non-weight bearing cartilaginous tissues such as the trachea or nasal cartilage. KS chains interspersed within the CS-2 region of aggrecan are more heavily fucosylated than the KS chains in the KS rich region or the small KS chains found in the G1 and G2 or interglobular domains. These CS-2 KS chains are detected by MAb 3D12H7 [106]. It is not known to what extent brain aggrecan displays such KS modifications, KS chains are however heavily substituted with L-fucose and sialic acid in amyotropic lateral sclerosis (ALS) [99]. The functional significance of these L-fucose and sialic acid substitution patterns on KS has not been determined but it is conceivable that they may modify or sterically impede the interactive properties of KS with neuromodulatory molecules.

Specific KS-PGs (e.g. phosphacan) in the CNS/PNS contain highly charged KS chains and display anti-adhesive properties inhibiting the attachment of neural cells to tenascin-C and laminin and this promotes neuronal outgrowth and axonal repair processes [107, 108]. Other brain KS-PGs (e.g. abakan, PG1000, SV2, claustrin) also contain 5-D-4 positive KS chains which confer interactive properties in neurotransmission, and synaptogenesis [109]. Localization of low and high sulphation phosphacan KS motifs in the Zebra song finch brain are correlated with neural development and cognitive song-learning [110]. Low sulphation KS is diffusely distributed throughout the brain while highly sulphated KS is specifically expressed in the song nuclei centres. GlcNAc-6-O-sulphotranferase (GlcNAc6ST), the enzyme responsible for the biosynthesis of highly sulphated KS is also exclusively associated with the song nuclei. Highly sulphated phosphacan localized to the perisynaptic spaces and dendrites but not the presynapse of the mouse visual cortex has roles in synaptic plasticity [111]. GlcNAc6ST knockout mice express one half of the level of KS of wild type mice. Highly sulphated KS-phosphacan generates T-type Ca2+ channel mediated long-term potentiation of non-deprived eye responses after mononuclear deprivation. E□□□□□□□□□□□□□□□□β3GlcNAcT-7 and GlcNAc6ST-1, TGF-β and FGF-2 in adult mice is elevated in gliotic scars [112]. Fibroblast growth factor 2 (FGF-2) elevates TGF-β1 production by astrocytes and KS expression in gliotic scars which inhibit neural repair. GlcNAc6ST knockout mice display reduced KS expression and enhanced neural regeneration after brain injury [101]. KS-PGs focally upregulated in spinal cord injuries are laid down by reactive microglia, macrophages and oligodendrocyte precursor cells but not by astrocytes [113]. Astrocytes do however produce the KS-PG abakan following injury which defines functional areas and the margins of gliotic scars in the cerebral cortex [114]. Abakan is also associated with malignant astrocytic tumours [115] and glioblastoma [116]. Furthermore, highly sulphated KS levels however are severely reduced in AD with levels reduced to less than 50% of control tissue levels [117].

KS interactions with cell stimulatory molecules regulate tissue homeostasis. KS chains bind insulin-like growth factor binding protein-2 (IGFBP2) [118], Sonic Hedgehog (SHH), FGF1 and FGF2 [119]. KS is a component of neural matrix and cell membrane PGs . KS-I interactions involving highly sulphated KS detected using MAb 5-D-4 have been demonstrated in a microarray of 8268 proteins and

458 custom array of 85 extracellular nerve growth factor protein epitopes [120]. Two hundred and
459 seventeen of the 8268 microarray proteins interacted with KS including 75 kinases, several membrane
460 and secreted proteins, cytoskeletal proteins and a number of nerve function proteins. Surface plasmon
461 resonance confirmed these interactions and allowed the determination of binding their constants. Of
462 the 85 selected ECM nerve-related epitopes, KS bound 40 of these. This included Slit, two Robo's, nine
463 ephrin receptors, eight ephrins, eight semaphorins, and two nerve growth factor receptors. The Slit-
464 Robo cell-signaling pathway is central to axonal guidance, angiogenesis and neurogenesis during spinal
465 development. The slit receptors contain variable numbers of LRR motifs and 7-9 EGF repeat domains
466 which have protein interactive properties. KS interactions in the Robo-Slit cell signaling pathway
467 produces downstream activation of Rho GTPases, actin depolymerisation and cytoskeletal re-
468 organisation. Direct cell-cell interactions between Ephrins and Ephrin protein-tyrosine kinase receptors
469 also regulate a range of important intracellular signaling pathways during development, that control
470 cell migration and are involved in axonal growth cone guidance. The semaphorins, which, exist as both
471 secreted and membrane bound forms, are also involved in axonal growth cone guidance and provide
472 short-range inhibitory signals through interactions with plexin and neuropilin receptors which regulate
473 Rho family GTPases (Fig 8f, g). Such interactions are critical to neural development and neural repair.
474 As seen in Figure 4, substitution of KS-I and II with L-fucose may modulate their interactive properties
475 with the aforementioned receptors. L-Fucose has demonstrated roles in molecular recognition and
476 receptor-ligand interactions involving Notch, selectin-P ligand (PSGL-1) and CD-34 [121-126].
477
478 KS coexists alongside CS chains in brain aggrecan [89, 127] and phosphacan [103, 107, 108,
479 128, 129]. Neurite outgrowth of DRG neurons is inhibited when they are plated on to CS-PGs, and this
480 inhibitory effect is removed by either chondroitinase ABC or keratanase treatment [102, 104].
481 Keratanase treatment promotes functional recovery of spinal cord injury [103]. Developmental
482 changes in KS sulphation patterns are associated with alterations in plasticity and cognitive learning
483 and functional recovery of neural tissues. GlcNAc6ST-1 knock out mice display no gross developmental
484 phenotype, but show changes in the induction of glial scar formation [101], and better axonal growth
485 after both cortical stab wounds and spinal cord injuries [130]. These studies emphasize the importance
486 of highly charged KS chains identified by the KS antibody 5-D-4 in nerve repair processes. The 5-D-4
487 MAb recognizes KS structures containing 6-sulphated Gal and GlcNAc residues. *GlcNAc6ST1* and
488 *KSGal6ST* both contribute to the generation of the 5-D-4 epitope and are essential for 6-sulphation of
489 Gal within KS in the developing and adult brain and induced after injury [131] and in early postnatal
490 brain development. 5-D-4 reactivity is abolished in the KSGal6ST knockout mouse brain. The early
491 phases of ALS are accelerated in GlcNAc6ST-1(-/-) mice where CNS KS is also ablated [132]. KS
492 produced by M2 microglia suppress the early phases of ALS, microglia produce KS heavily modified
493 with fucose and sialic acid. GlcNAc6ST1(-/-) mice display a complete absence of microglial KS but
494 increased phagocytosis of amyloid-β protein and reduced levels of cerebral amyloid deposition [133].
495 Inhibition of KS biosynthesis by targeting GlcNAc6ST1 thus represents a therapeutic target in AD.
496 Functional roles for KS have been suggested in spinal cord development in GlcNAc6ST1 knockout mice
497 where KS binds to Shh and acts as a morphogen regulating murine embryonic spinal development
498 [134]. KS interactions in late phase *Shh* signaling acts as a morphogenetic switch regulating the
499 generation of oligodendrocyte progenitor cells from motor neurons [134]. The KS-PG, phosphacan also
500 acts as a developmental molecular switch which regulates neuronal development. KS chains inhibit
501 neuronal attachment but promote outgrowth activity, an effect reversible by keratanase treatment
502 [135].
503
504 Other lines of evidence demonstrate key roles for KS in development and repair/remodeling in other
505 tissues. For example, KS may be chondroprotective in inflammatory arthritis models [136]. Murine
506 aggrecan has a truncated core protein devoid of a KS rich region thus KS levels are low in murine knee
507 joints. Intraperitoneal administration of KS ameliorated IL-1 induced GAG release and protected
508 cartilage from arthritic changes in GlcNAc6ST1 (-/-) mice. Furthermore, GlcNAc6ST1 activity is
509 significantly reduced in macular corneal dystrophy resulting in the occurrence of low- or non-sulfated
510 KS and corneal opacity [137].
511
512 *3.3 CS/DS and their cell and matrix regulatory roles in neural tissues*
513 CS is the most abundant GAG in the human body and is *O*-sulphated at the 2, 4 and C6 positions [55].
514 GlcA may also be epimerised to α L-IdoA in the related GAG, DS, leading to structural diversity in CS/DS

with over one thousand different pentasaccharide combinations possible [55]. The large number of structural permutations possible with CS/DS facilitates interactions with a diverse repertoire of cytokines, chemokines, morphogens and growth factors with regulatory properties in tissue development and ECM remodelling [55, 138-142]. CS also occurs as a number of isoforms including the high charge density CS-D and CS-E and lesser charged CS-A, CS-B and CS-C [98]. CS-D and CS-E are enriched in the brain transmembrane PGs phosphacan, syndecan-1, syndecan-4, NG2 proteoglycan/CSPG4, neuroglycan-C/CSPG7, and ECM PGs appican (β-APP) and bikunin [143-145]. CS-A, B, C are abundant in the brain hyalectan proteoglycan family consisting of brevican, neurocan, versican and aggrecan. The CS-D and CS-E motifs embedded within the CS-A side chains of β-APP, bikunin and phosphacan convey neuroregulatory properties [108, 145]. While CS-D and CS-E can promote neural repair the same cannot be said of the CS-A, B, C side-chains of neural net PGs layed down in the gliotic scar. Perineural nets [146] have been immunolocalised in rat brain tissues using the MAb 1-B-5 to a non-sulphated aggrecan stub epitope generated by chondroitinase ABC. 1-B-5 reactivity is displayed in extensive extracellular distributions encompassing a large group of neurons (Fig 3 a, b) as well as pericellularly surrounding single or small numbers of neurons (Fig 3c, d) [147]. Formation of glial scars, seals the injury but also creates a barrier to axonal regrowth. The scar centre is highly inflammatory and populated by NG2+ glia, astrocytes seal the border of the scar but in so doing entrap axons attempting to regrow within the scar, thus activated astrocytes and ECM components laid down in the scar contribute to regenerative failure[148]. The NG2 positive glia are a progenitor cell type for oligodendrocytes which participate in neural remodelling and repair processes whereas astrocytes define the boundary of the gliotic scar and do not participate in its repair. PGs in neural tissues thus have paradoxical modes of action, CS-PGs, of the lectican family hinder axonal regrowth while the transmembrane CS-PG (NG2/CSPG4) and phosphacan, upon shedding from the cell by ADAM 10 (a disintegrin and metalloproteinase containing protein 10), promote axonal re-growth and production of synaptic adhesion molecules, promoting synaptic signaling, plasticity and functional recovery. The positive contribution of CSPG4 to neural repair processes is confirmed from knockout studies of NG2/CSPG4 mice which display aggravated tissue loss, inflammation and neurologic deficits after traumatic brain injury. Progranulin, a functional ligand of Notch and Eph2a acts in concert with NG2/CSPG4 to overcome neuronal inflammation and structural recovery of damaged neuronal tissue. Progranulin is upregulated after spinal contusion in mice [149]. Progranulin is produced by neurons and glia and has roles in inflammation and wound repair [150, 151]. Progranulin is proteolytically processed into peptide fragments (granulins) during tissue remodelling and these display different biological activity to the native molecule. Progranulin has trophic properties while the granulins act as inflammatory mediators and contribute to neuroinflammation, dementia and development of AD [151-153]. Neuronal expression of α9β1 integrin, trkB, and protein tyrosine phosphatase σ (PTPσ) , which are receptors for tenascin-C, brain derived neurotrophic factor (BDNF) and CSPGs respectively, have also been shown to significantly enhance regeneration of injured axons[154-157]. Thus with the correct expression of these cell surface receptors, growing axons can respond to appropriate guidance cues in their extracellular micro-environments by regulating their intracellular signaling pathways to modify growth cone behaviour and promote intrinsic repair [154, 156, 157]. Neuronal regeneration has been induced by transgenic integrin expression [158], lentiviral trk-B induced Erk activation [159] or by modulation of PTPσ expression [157]. PTPσ and the related leukocyte common antigen-related (LAR) and Nogo receptors 1 and 3 (NgR), bind the inhibitory glycosylated side chains of CSPGs and regulate synaptic structure and neuroplasticity [160, 161].

As already noted, progranulin expressed in mature neurons and microglia, has protective roles in neurogenerative disorders [162-164] and plays a central role in the regulation of neural inflammation, enhancing neuronal survival and stimulating neurite outgrowth activity. Progranulin achieves this through modulation of glycogen synthase kinase (GSK)-3β. Inhibition of GSK-3β has received interest as a therapeutic target in the treatment of traumatic brain injury and is neuroprotective, promoting functional recovery after intracerebral hemorrhagic stroke [165]. GSK-3β inhibitors rescue cognitive impairment in AD, Fragile X syndrome, Down syndrome, Parkinson's disease and spinocerebellar ataxia type 1 [166]. Levels of phosphorylated tau protein are elevated following traumatic brain injury and may contribute to pathological structural changes in the CNS [167]. Misfolded amyloid-β-peptides and hyperphosphorylated tau protein accumulation is a hallmark of AD [168]. Caspase-3 regulates tau phosphorylation in AD, is mediated by the GSK-3β pathway and

involves cleavage of protein-kinase B (Akt) by Caspase-3 [168]. Progranulin thus has significant roles in the promotion of neural repair processes following traumatic brain injury and it acts in concert with CSPG4 to promote these. The interaction of progranulin with neural PGs and neural receptors in specific regions of traumatic brain injury is mediated by GAGs attached to PGs in the traumatised area oversulphated CS isomers play a significant role in such binding interactions. This is consistent with progranulins interactive properties with the HS-PG, perlecan [169]. Oversulphated DS also displays neuritogenic activity in hippocampal neurons [170]. Novel CS/DS-GAGs identified in shark fin cartilage can bind neurotrophic factors and these also display neurite outgrowth promoting activity. CS-octasaccharides have been isolated from shark cartilage containing CS-D hexasaccharide sequences with neurite outgrowth promoting activity [171]. Novel oversulphated CS-E tetrasaccharides have also been isolated from squid cartilage [172] with neuroregulatory activity [173]. CS-E containing CS tetrasaccharides have been synthesized and demonstrated to have potent FGF-2 binding properties but their neurite outgrowth stimulatory profiles have not been determined [174] despite an earlier study which demonstrated this activity in a CS-tetrasaccharide [175]. Neurite outgrowths by hippocampal neurons are stimulated by CS-E tetrasaccharide, desulphated CS-E tetrasaccharide is inactive as is a CS-E disaccharide (Fig 7). CS-A and CS-C inhibit neural outgrowth activity thus collectively CS isomers can both promote and inhibit neural repair.

*3.4 Contributions from other GAG types in neuroregulation and neural repair processes*

As already noted, CSPGs in glial scars prevent neurite outgrowth in-vitro and nerve regeneration in-vivo[176]. Astrocytes stimulated with IL-1β do not upregulate any of their CSPG genes suggesting that these are not the only reactive glial scar proteoglycans. Rat cortical astrocytes produce more HS than CS in culture and these highly charged GAGs are more effective at stimulating nerve growth factor (NGF) signaling in PC12 cells. Furthermore, the heparin binding domain of laminin also promotes neurite outgrowth along with NGF [177] thus HS proteoglycans also contribute to neuritogenic events. Furthermore, domain V of perlecan delays the onset of glial scarring in rat models by down-regulating neurocan and phosphacan expression and upregulating NGF activity[178]. The balance between CS and HSPG levels can therefore either inhibit or stimulate neurite outgrowth and nerve regeneration. The laminin-like LG3 fragment of perlecan is not associated with glial scarring, mice deficient in NG2/CSPG4 have reduced glial scarring and are more permissive to axonal regrowth[148]. These animals have a similar phenotype to progranulin deficient mice[148]. Progranulin is neuroprotective [179] and binds to the C-terminal LG1 and LG2 repeats of perlecan domain V [180]. The C-terminal region of perlecan also binds CSPG4 [181] and has neuroprotective and pro-angiogenic properties in a rat ischemic model thus also contributes to neural repair processes [182]. Thus while CS GAGs are a major focus in this review any potential synergism or antagonistic effects with other GAG types also need to be considered in a holistic approach to better understand neural repair processes.

*3.5 SHH ,HS, CS, and KS interactions model tissue patterning and neural development.*

Hedgehog (HH) proteins are highly conserved morphogenetic signaling molecules with fundamental roles to play in vertebrate and invertebrate embryonic development [183-186]. The HH signaling pathway plays key roles during embryonic development and remains active in adults. The GAG chains of cell surface PGs shape HH gradients and signal transduction [119, 134, 187, 188]. Three HHs have been identified in mammals, Sonic, Indian, and Desert hedgehog, these are typically expressed in the nervous system, cartilage and testis respectively. SHH is synthesized as a 45-kDa precursor protein which undergoes autocatalytic cleavage to a 20-kDa N-terminal fragment (residues 24–197 in the human gene sequence) responsible for all known hedgehog biological activity. This is membrane-associated through a palmitic acid attachment at its N-terminus [189] and cholesterol at its C -terminus [190-192]. Patched (Ptc), a 12 span transmembrane protein SHH receptor acts as a negative regulator of SHH signaling. SHH is interactive with glypican and CS GAG isomers and these are responsible for the production of SHH gradients which are a driving force during tissue morphogenesis. Surface plasmon resonance studies have demonstrated that corneal KS has interactive properties with SHH [119]. KS regulates the switch from motor neuron to oligodendrocyte generation during development of the spinal cord [134]. Glypican and CS participate in SHH mediated cell signaling [187] regulating tissue patterning and development of the neural system. SHH cell signaling is important in foetal and postnatal brain development and regulates the proliferation of early cerebral cortex progenitor and oligodendroglial lineage cells, expansion of their numbers is critical in the development of the neocortex [183, 185, 193, 194]. SHH guides axonal development during neurogenesis, cellular

responses in early brain injury and following demyelination [195]. SHH may represent a therapeutic target to focus on in neurological disorders [196]. Co-ordinated SHH and Wnt mediated cell signaling regulates cranial nerve development [197]. SHH has roles in the differentiation of oligodendrocytes [198] and in glial neural cell communication during brain development which provides neuroprotection [186] and neuroplasticity . Neurons diversify astrocytes in the adult brain through SHH signaling [199]. SHH is a regulator of extracellular glutamate levels in epilepsy and modulates the release of gliotransmitters from cultured cerebellar astrocytes [200, 201].

*3.6 CS interactions modulate neural cell behaviour.*

CS is a prominent CNS GAG and occurs in a number of isomeric forms with differing degrees of sulphation and interactive properties [19, 20, 22, 202-204]. CS microarrays have proved useful in the assessment of CS-protein interactions [19, 20, 22] and has detected neurostimulatory and inhibitory CS species as well as a tumour necrosis factor $\alpha$ (TNF$\alpha$) antagonist [24, 175]. Interactions of neurons with CS/DS promotes cellular survival [205]. The CS glycan chains of PGs interact with a diverse collection of proteins in the CNS to promote neural growth, proliferation, differentiation and long term survival. Some CS isoforms provide chemorepulsive nerve guidance cues which regulate axonal development and repair processes following traumatic injury. CSPGs inhibit the growth cone by interaction of CS chains with laminin, collagen and cell surface integrins. Receptor type protein tyrosine phosphatase-$\sigma$ (RPTP-$\sigma$) also acts as a neural CS receptor [161] while RPTP-$\zeta$ interacts with the NCAM resulting in an inhibition of neural cell adhesion and growth (Fig 8c, d). The ecto-domain of RPTP-$\zeta$ is enzymatically released from the cell surface by ADAMS 10 generating the soluble phosphacan which can promote neural outgrowth and repair processes. Highly charged CS isomer side chains such as CS-E on proteoglycans bind FGFs and present these to FGF receptors (FGFRs) to promote cell signaling, neural growth and differentiation (Fig 8e). Interaction of the attractive guidance protein Semaphorin 5A with CS converts this to a repulsive guidance protein (Fig 8h). Semaphorin 3A is a cell membrane bound and secreted short range repulsive inhibitor guidance protein which interacts with CS-E in lectican perineural net formations to inhibit nerve regrowth. This effect is mediated by interaction with neuropilin-1 and neuroplexin neural receptors (Fig 8f). Plexin acts as a signal transduction molecule along with transmembrane neuropilin co-receptors in the neuropilin-plexin receptor complex (Fig 8f).

Eph receptors and ephrins display broad spatial and temporal expression patterns throughout the nervous system [206, 207]. During early development, these interactions contribute to neurogenesis (reviewed in [208]) and differentiation [208, 209]. Eph-ephrin signaling influences the functions of Rho GTPase proteins, which in turn regulate the actin cytoskeleton influencing neuronal migration during development. Eph-ephrin signalling can generate both attractive and repulsive interactions and can positively support neurogenesis, axonal guidance and neural repair [208, 209]. EphA2 receptor tyrosine kinase is a functional cell surface receptor for the secreted glycoprotein progranulin. Fourteen Ephrin receptors have so far been identified. Ephrin-Eph receptor cell signaling regulates cellular morphology and proliferation influencing the adhesive properties of cells during cellular migration in embryonic development, vasculogenesis and angiogenesis and has roles to play in axonal guidance, and synaptogenesis. Progranulin also promotes angiogenesis through the Ephrin receptors and upregulation of vascular endothelial growth factor (VEGF) production to modulate neuroinflammation [151, 210]. Phosphorylation of EphA2 by progranulin leads to tyrosine phosphorylation of other tyrosine kinases such as EphA4, EphB2, and EGFR through extensive cross talk (Fig 9b) among receptor tyrosine kinases [211]. Progranulin promotes the activation of the mitogen-activated protein kinase (MAPK) and Akt signaling pathways. Progranulin is secreted as a dimer containing up to 14 granulin modules per dimer which are available for protein–protein interaction. This may enable the dimer to bridge several receptors on a cell and serve as a multi-receptor signaling complex explaining the cross-talk when progranulin binds to EphA2 (Fig 9b).

The guidance of axonal development is a complex highly integrated process dependent on a myriad of inhibitory and stimulatory effector ECM proteins. Perineural net formations with hyaluronic acid (HA), tenascin-R and lectican PGs in gliotic scars are prominent stabilizing and protective structures which minimize further damage to neural tissues and protect neural cell populations in the scar from oxidative stress. Myelin-associated glycoprotein, Nogo, and the semaphorins all provide inhibitory cues over axonal development. CS and KS interact in a sulphation-dependent manner with a number of axonal guidance proteins, including slit2, netrin1, ephrinA1, ephrinA5, and semaphorin 5B

[22]. Netrin-1 modulates axonal growth direction and speed and directs F-actin reorganization, essential for mammalian neural development. The best characterized netrin-1 receptor, *Deleted in Colorectal Cancer* (DCC), is localized to growth cones, but is also observed in neuronal cell bodies [212]. Netrin-1 attracts and repels distinct motor axon populations, according to the spatio-temporal expression of Netrin receptors [213] in neural tissues. The guidance cues provided by Netrin-1 are influenced by its interactive properties with ECM PGs, a theme recapitulated by most of the axonal guidance promoter proteins. These represent complex interplays between multiple components which regulate spatio-temporal neural growth [100, 214]. Netrin-1 can also synergize with ephrin receptors to regulate axonal formation [213]. A greater understanding of these axonal guidance cues would be insightful in therapeutic strategies aimed at producing guided nerve regeneration [215-219].

CSPG4 promotes neural repair processes through upregulation of epidermal growth factor receptor (EGFR) expression [220, 221] and interaction with progranulin [148, 222, 223]. Progranulin is upregulated after spinal contusion [149]. CSPG4 is highly expressed by macrophages, microglial cells, tumour, perivascular and oligodendrocytes involved in cell adhesion and migration [224-228]. CSPG4 is upregulated in glioblastoma, astrocytoma and a number of other human tumours [221, 229, 230]. Activated microglial cells form synapses with neurons to participate in neural repair [224] and re-organisation of the gliotic scar and improve neural outgrowth [148, 231, 232]. Following traumatic injury to the brain, the cells in the impacted area upregulate aggrecan, versican, brevican, neurocan in HA-macroaggregate perineural net structures stabilised by link protein and tenascin-R providing protection from oxidative stress and further mechanical injury. Astrocytes seal the margins of these gliotic scars by upregulating the brain matrix proteoglycan abakan. These perineural nets inhibit nerve outgrowth. Chondroitinase ABC selectively depolymerises the CS side chains of the lectican PGs improving neural recovery in the gliotic scar [233] and improves spinal cord repair [102, 234-237]. Chondroitinase C also significantly improves repair of peripheral nervous tissue but appears to have a more specific mode of action [238].

ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs 4) is localised in regions of the spinal cord undergoing spontaneous repair and specifically targeting of the lectican PGs in the scar tissue [239, 240]. KSPGs are similarly up-regulated in glial scars and inhibit axonal repair [101, 113, 114, 135, 241, 242]. Mutant mice deficient in the enzyme GlcNAc6ST-1 show improved functional recovery following spinal cord injury [130]. Therapeutic use of keratanase also improves axonal repair [103]. The KSPG phosphacan is also upregulated in scar tissues where it promotes mossy fiber outgrowth and nerve regeneration [107]. Chondroitin-6-sulphate upregulated in scar tissue is reported to have nerve regenerative potential.

*3.7 RAGE in the brain*

Receptor for advanced glycation endproducts (RAGE) is a receptor which binds advanced glycation end products (AGEs) and CS in brain tissues (Fig 10). RAGE acts as a receptor for oversulphated CS isomers such as CS-E [243, 244]. AGEs modulate amyloidogenic precursor protein (APP) processing and Tau protein phosphorylation regulating AD development [245]. AGEs in glioblastoma have a modulatory role over tumour development [246]. RAGE mediates amyloid β accumulation in a mouse model of AD by regulation of β- and γ-secretase activity [247]. Targeted inhibition of RAGE reduces amyloid-β influx across the blood-brain barrier and improves cognitive deficits in mice [248]. High-mobility group box-1 protein (HMGB-1) and β-amyloid oligomers promote neuronal differentiation of adult hippocampal neural progenitors via RAGE and the NF□B pathway [249] sustaining neurogenesis counteracting the hostile AD brain microenvironment [6]. This promotes survival of vulnerable brain cell populations [249, 250]. AGEs impair NLRP3 inflammasome-mediated innate immune responses in macrophages and modulates neuroinflammation through the NF□B pathway [251].

## 4. Roles for L-Fucose in Neuro□□□□□□□□□□□□Processes□

*4.1 O- and N-linked fucosylated proteins in neural tissues*

While neuronal mitochondria utilize glucose as an obligate primary energy resource in the tricarboxylic acid glycolytic pathway to generate energy neurons are also responsive to sugars other than glucose as cell regulatory agents. Positive selection pressure over at least 500 million years of vertebrate evolution has resulted in sugars which have evolved molecular recognition and information

transfer properties equipping them as cellular mediators serving as critical determinants of protein folding, trafficking, and stability . Glycans are abundant in the brain and are involved in various neural functions including learning and memory, brain development, and spinal cord injury [102, 252-254]. The precise molecular mechanisms whereby glycans influence these processes is not well understood but it is clear that synaptic transfer of information between neurons occurs through glycoprotein mediated interactions. L-fucose exists as a terminal residue on *N*- or *O*-linked glycoproteins attached to the C-3 and C-6 position of N-acetylglucosamine or the C-2 position of galactose (Fig 4). The fucose α1-2 galactose (Fuc α1-2Gal) linkage has been implicated in cognitive processes such as learning and memory. Non-invasive two dimensional magnetic resonance spectroscopy (2D MRS) has identified six Fuc α1-2 Gal sugars in brain tissue. 2D MRS offers an unprecedented insight into the molecular mechanisms by which fucosylated sugars contribute to neuronal processes and how they alter during development, ageing and disease [29]. Fucose is an unusual sugar in that it exists as a 6-deoxy αL-galactopyranose configuration and is a prominent functional component of neural tissues such as synaptic membranes [29]. Addition of 2-deoxy D-galactose, an L-fucose analog to hippocampal neuronal cultures potently inhibits neural outgrowth activity whereas 3-deoxy D-galactose is inactive, moreover addition of D-galactose to 2-deoxy-D-galactose treated cultures results in the functional recovery of normal neuron growth characteristics. Fuc α 1-2 Gal is a non-reducing terminal component of many glycans [29] and is implicated in neurite outgrowth, synaptogenesis, neuronal development, learning, and memory [27, 28, 255, 256]. Treatment of animals with 2-deoxy-D-galactose, disrupts the formation of Fuc α 1-2Gal linkages, and causes reversible amnesia [257] interfering with the maintenance of long-term potentiation in an electrophysiological model of learning and memory [258]. Furthermore loss of 1, 6-fucosyl transferase activity also decreases hippocampal long term potentiation [259].

αL-fucose is a terminal or core monosaccharide on *N*-and *O*-linked glycan chains on many glycoproteins (Fig 4d, Fig 5a-g). It also occurs as a capping structure along with sialic acid on the KS-I and KS-II chains of PGs (Fig 4a-c) and in terminal sLeX motifs in glycoproteins (Fig 4f, Fig 5b, Fig 6f-h). KS is heavily substituted with fucose and sialic acid in ALS. The prominent terminal locations of L-fucose points to its role as a molecular recognition site for interacting proteins. Fucose occurs as a terminal sugar linked to a penultimate galactose residue in glycoconjugates or to core GalNAc residues in N-glycans (Fig 5b,f). Fucose can also be directly attached to serine or threonine residues by fucosyl transferases in *O*-linked glycans and can act as an acceptor molecule for the attachment of further saccharides to form small oligosaccharide side chains (Fig 4e).

*4.1.1 L-Fucose as a functional component of blood group substances and Immunoglobulins*

Fucose also occurs as terminal Fuc α1-2 Gal terminal saccharides in small glycolipids attached to red blood cells identifying the A, B, O blood group antigens (Fig 6a). Over 95% of circulatory human IgG antibodies also contain a fucose (core-fucose) residue attached to the first GalNAc in the glycosylation site of their Fc region. The majority of other plasma proteins are not substituted with fucose in this manner. Fucosylation dramatically reduces IgG binding to FcγRIIIA, an activating Fc receptor specific for IgG Fc region expressed by immune human natural killer (HNK) cells and macrophages [260-262]. FcγRIIIA initiates antibody dependent cellular cytotoxicity (ADCC) by HNK cells and phagocytosis of antigens by macrophages. This core fucose attenuates potentially harmful ADCC activity. Conversely, ADCC induced by non-fucosylated IgG improves the efficacy of therapeutic anticancer antibodies. IgG lacking the core-fucose is over 100 times more effective in initiating ADCC than the fucosylated version (Fig 6b-h).

*4.1.2 Synapsin and Synaptophysin*

The synapsins are fucosylated proteins [256] which regulate the release of synaptic vesicles to coordinate release of neurotransmitters within the synaptic vesicles at the synaptic gap [263]. They do so by tethering the vesicles to cytoskeletal components to prevent the diffusion of vesicles to the synaptic membrane preventing the un-coordinated release of neurotransmitters at the synaptic gap [264]. During the transmission of an action potential down the neuron from the cell body the synapsins are phosphorylated by cAMP dependent protein kinase (PKA). This releases the synaptic vesicles to the pre-synaptic membrane [265] which depolarizes in response to the action potential allowing the synaptic vesicle to fuse with the synaptic membrane and release the enclosed neurotransmitters into the synaptic gap and these are transported across to the post synaptic

membrane of a communicating neuron [266]. This results in the transmission of neural signals along the neural network. There are three synapsin proteins and each occur as two isoforms. Synapsin 1a is implicated in bipolar disorder and schizophrenia [267]. The synapsin Ia/Ib isoforms are the most highly expressed hippocampal pre-synaptic vesicle associated phosphoproteins and are implicated in thought formation and cognitive learning [268-270]. Synapsin is a major neuronal fucosylated glycoprotein [256, 271, 272]. The synapsin family consists of 3 major isoforms encoded by 3 genes SYN1, SYN2, SYN3. Each gene occurs as two alternatively spliced forms leading to a total of six isoforms. Mice lacking synapsin I, II, III are prone to seizures and display learning difficulties and in humans is associated with bipolar disorder and Schizophrenia[34, 267].

*4.1.3 Fucosylated Glycoproteins and Proteoglycans*

Fucosylated glycoproteins and PGs have prominent roles in many physiological and pathological processes including leucocyte adhesion, host-microbe interactions, neuronal development and neural protection[273-275]. Fucosylated glycolipids on erythrocytes form the ABO blood group antigens[276]. However, aberrantly fucosylated glycoconjugates are also found in cancer, inflammation and neoplastic processes [276-279]. The fucosylated sialyl Lewis-X, and sialyl Lewis-Y antigens are prominently upregulated in some cancers and associated with tumour progression. Deficient levels of fucose occur in impaired leucocyte interactions with the vascular epithelium in immunodeficiency. Leukocyte adhesion deficiency II (LAD II) is a rare congenital disease caused by a defect in fucosylation of glycoconjugates such as P-selectin glycoprotein ligand-1 (PSGL-1) (Fig 5a) which normally facilitate leucocyte binding to the selectins on the epithelium during inflammation [280]. This interaction facilitates leucocyte rolling and their extravasation through blood vessels to tissue sites to combat infection. Leukocyte adhesion deficiency type II (LAD II)-patients show severe mental and growth retardation indicating an additional essential role for fucose in brain growth and development [281]. The P-selectin PSGL-1 ligand is a dimeric mucin-like 120-kDa glycoprotein located on leukocyte surfaces that binds to P-, E- and L-selectin and promotes leucocyte adhesion to the endothelium facilitating leucocyte rolling during inflammation. PGSL-1 is heavily fucosylated as part of the branched Lewis X antigen *O*-glycan structure (NeuAc□2→3Galß1→4[Fuca1→3]GlcNAcß1→R)[125] (Fig 5a). High affinity cell adhesion interactions also require the presence of three tyrosine sulfate residues located near the Lewis-X antigen structure at the N-terminus of PSGL-1 [125, 282, 283].

*4.1.4 Functional role of L-Fucose in Notch Signaling*

*O*-fucosylation is essential for the functional properties of Notch [124, 284, 285], a transmembrane receptor that co-ordinates a number of cell-fate decisions in neural development and in neuron-glial cell interactions which determine neuritogenesis, neuronal migration and differentiation[286] (Fig 5g). Fucose knockout causes developmental defects in mice and abnormal vasculogenisis, somitogenisis and neurogenesis [124], Notch is an important mediator in all of these processes. Notch-1 is a member of a family of transmembrane glycoprotein receptors which contains a large number of extracellular epidermal growth factor repeats. These are heavily substituted with fucose providing the extracellular Notch domain with important interactive properties (Fig 5g). Ligand binding to the extracellular domain of Notch-1 by Delta, Jagged or Serrate ligands induces proteolytic cleavage of Notch and the cleaved intracellular domain enters the nucleus to modify gene functions. Upon ligand binding with Notch, ADAM 10 cleaves the extracellular domain and this continues to interact with the ligand in solution. The intracellular portion of Notch is then cleaved by $\gamma$-secretase and it is transported to the nucleus where it regulates gene expression through the transcription factor CSL, an acronym for CBF-1/RBPJ (recombining binding protein suppressor of hairless). CSL acts as a co-repressor negatively regulating Notch signaling to control cell fate decisions[121, 287] in developmental contexts. Notch is widely expressed in many cell types and has fundamental roles in development.

Fucose occurs on structurally diverse *N*- and *O*-linked glycans through the action of over a dozen fucosyl biosynthetic enzymes. Fucosyl transferase 1 (FUT1) and FUT2 attach fucose to galactose in Fuc $\alpha$□1-2 Gal containing glycans. FUT3 attaches Fuc via $\alpha$ 1-3 and $\alpha$ 1-4 linkages to Gal and GlcNAc residues in glycan chains. FUT4-7 form exclusively $\alpha$ 1-3 linked fucose residues in glycans. FUT8 and FUT9 generate Fuc $\alpha$1-6 GlcNAc linkages, FUT8 attaches these to core asparagine residues in N-glycans whereas FUT9 attaches these to the GlcNAc units of polylactosamine chains. FUT10 and FUT11 are

putative fucosyltransferases catalyzing the generation of α 1-3 linked Fuc in glycans. POFUT1 and POFUT2 are *O*-fucosyltransferases which attach Fuc directly to serine and threonine residues in the modular EGF and thrombospondin repeats of glycoproteins.

Although a relatively minor sugar, its strategic positioning on key functional glycoproteins points to fucose having a significant role to play in neural pathobiology. As already indicated *O*-fucosylation is essential for the activity of Notch (Fig 5g) and has significant roles to play in leucocyte PSGL-1 P-selectin mediated interactions (Fig 5a) with the endothelium in neuro-inflammation [286]. CD-34 is heavily substituted with both *N*- and *O*- linked fucosylated oligosaccharides in microvascular progenitor cells (Fig 5e). The mode of action of L-Fuc in Notch has been suggested to be due to induction of conformational changes in the epidermal growth factor (EGF) repeat domains or in the Notch ligands. Notch signaling is essential for the maintenance of neural progenitors and regulates cell-fate decisions in neuronal and glial cells to modulate neuronal differentiation and migration [288, 289]. Deletion of *POFUT1* is embryonic lethal causing developmental defects in vasculogenesis, somitogenesis and neurogenesis similar to those obtained when Notch receptors are deleted. This reinforces the importance of L-Fuc as a mediator in combination with Notch in neuronal development.

*4.1.5 Roles for L-Fucose in CD-34.*

Another cell surface protein with cell adhesion and cell regulatory properties in the CNS/PNS is CD-34 (Fig 5e). CD34 is a heavily fucosylated type I transmembrane sialoprotein, that can be phosphorylated by a number of kinases including PKC and Tyrosine kinase. The CD-34 proteins are a family of sialomucin transmembrane adhesion proteins. CD-34 is expressed in early haematopoietic and vascular tissues and lymph node epithelium. CD-34 interacts with L-selectin expressed by T cells in the lymph node epithelium. Podocalyxin and endoglycan are related to CD-34 and also facilitate cell attachment and cell migration during microvessel development in neural tissues [126]. Terminal fucosylation of these PGs confer unique functional properties in a variety of biological settings. Fucose is an essential component of the carbohydrate ligands for the selectin family of cell adhesion receptors [290, 291]. E-, P-, and L-selectin are C-type lectin proteins expressed by platelets (P-selectin), endothelial cells (E- and P-selectin), and leukocytes (L-selectin). Selectins bind to oligosaccharides decorating specific cell surface and secreted proteins expressed by leukocytes (E- and P-selectin ligands) and high endothelial venules (L-selectin ligands). Interaction between selectins and their ligands enable the rolling of leukocytes on the endothelium, and is an essential requirement for leukocyte extravasation. The carbohydrate selectin ligands are fucosylated structures related to the sialyl Lewis-X antigen, an α 1,3-fucosylated glycan structure also known as stage-specific embryonic antigen-1 (SSEA-1) and CD15 expressed during early embryogenesis [292].

*4.1.6 L-Fucose as a component of Lewis-X-Antigen*

Lewis$^x$ epitopes are present in multiple areas of the developmental embryonic brain[293-296], controlled by FUT 9 expression, an enzyme which is regulated by the transcription factor Pax 6 [297]. The functional role of Lewis$^x$ in the developing brain has yet to be determined, but its dynamic expression patterns during embryogenesis suggests it may have roles in aspects of molecular recognition which support the assembly of neural structures [273, 298]. The Lewis$^x$ epitope, an α 1,3-fucosylated glycan also known as the stage-specific embryonic antigen-1 (SSEA-1) and CD15, is expressed during early embryogenesis [292]. Exposure of pre-implantation mouse embryos at the morula developmental stage to Lewis$^x$ oligosaccharides causes decompaction apparently through disruptive multimeric interactions affecting cell-cell adhesion in early embryos [299, 300]. Oligosaccharides containing L-fucose form part of a recognition signal in sperm–egg attachment in mammals [301]. At the endometrial surface, adaptations are also required to accommodate the implanting embryo [302, 303]. These adaptations at the materno-fetal boundary are highly species-specific. Fucose containing carbohydrate structures in the embryonic–maternal interface have important molecular recognition roles to play which define the maternofetal glyco-code. Localization of fucose oligosaccharides at a surface or interface is important in predicting functional roles in cell recognition. Each mammalian species has its own characteristic materno-fetal glyco-code. This glycotype permits interbreeding between compatible species like the horse and donkey which have almost identical patterns of placental glycosylation, whereas the camel has a totally different placental glycosylation signature and cannot interbreed with either the horse or donkey [304]. Specific fucosylated glycoconjugates vary in abundance during the receptive and non-receptive phases of

implantation [305] and are altered in the infertile endometrium [306]. By analogy with selectin mediated intercellular adhesive interactions during extravasation of leucocytes in the innate immune response a similar process may occur at implantation between endometrial sialyl Lewis$^x$ and trophectodermal selectins [307-309]. Overexpression of FUT 7 in a mouse implantation model promotes embryo adhesion and implantation [310, 311]. Thus fucose oligosaccharides may serve molecular recognition roles both in the fertilization and implantation stages of reproduction.

Defucosylation of the EGF domain from urokinase-type plasminogen activator abolishes its mitogenic activity despite having no effect on its binding properties at the cell surface , thus *O*-fucosylation can modulate ligand-receptor interactions necessary for productive signal transduction outcomes. *O*-fucose residues are also present on EGF domains of the mammalian Notch receptors, a family of transmembrane cell-fate determining signaling proteins during somite formation, neurogenesis, angiogenesis, and lymphoid development. Ligand-induced Notch signaling events are impaired in a fucose-deficient cell line but can be restored by correction of the fucosylation defect, indicating that *O*-fucosylation of Notch affects its interactions with ligands and is in line with functional roles for L-fucose in molecular recognition [312, 313]. Such *O*-fucosylation effects are not limited to EGF domains, as glucose-extended fucose modifications (Glc-Fuc-O -Ser/Thr) have been demonstrated in three thrombospondin type 1 repeats on thrombospondin-1 [78] and it remains to be determined if the activity of additional protein modules can also be modified by *O*-fucosylation. Fucosylated glycans impact on the pathogenesis of several human diseases. Expression of A and B blood group antigens (Fig 8a) is lost in many tumours accompanied by increases in H and Lewis-Y expression associated with poor clinical prognosis [314, 315]. Up-regulation of sialyl Lewis-X and sialyl Lewis-a occurs in many cancers associated with advanced tumor grade and poor prognosis. Increased expression of fucosylated serum immunoglobulins (Fig 6f-h) is evident in juvenile and adult rheumatoid arthritis [260, 261] its contribution to the pathogenesis of inflammatory arthritis is not known or whether this is a secondary effect due to an upregulation in fucosylation driven by an autoimmune response. Elevated fucosylation of mucins has also been observed in cystic fibrosis, accompanied by a decrease in sialylation [278]. Fucosylation impacts on leukocyte recruitment, selectin-selectin ligand interactions and the development of numerous pathological processes, including atherosclerosis, reperfusion injury following ischemic events, inflammatory skin diseases, and asthma [316]. A reduction in the density of cell surface fucosylated glycans in patients with LAD II (also known as congenital disorder of glycosylation) [280] results in recurrent infections due to defective selectin ligand biosynthesis and an impairment in the innate immune response. Mental retardation and skeletal abnormalities are also prominent features in LAD II, but it is not known if these are due directly to fucose-dependent processes, such as *O*-fucosylation of Notch receptors or are due to Lewis-X mediated interactions in the embryonic brain.

*4.2 Fucosylated Chondroitin Sulphate as a Therapeutic Agent*

Investigations over the last 25 years [317-319] on fucosyated CS (Fuc-CS) isolated from the holothurian echinoderm marine sea cucumber (*Holothuroidea* class) has identified a family of molecules with great therapeutic potential in a number of physiological processes (Fig 4h). The Fuc-CSs consist of a core structure consisting of CS-E and CS-A disaccharides with CS-A constituting 10-50% of the disaccharides. Sulphated fucose side chains are attached through *O*-3 to GlcA of the core structure. These fucose residues can be monosulphated or disulphated at the 2,4 or 3,4 positions (Fig 4g, h), side chains and disulphated GlcA in the core structure have also been detected adding to the structural complexity and charge density of the Fuc-CSs. Native Fuc-CS preparations have been isolated up to 64 kDa in size and for some therapeutic applications these have been depolymerized to smaller 3-12 kDa forms. The branched sulphated fucose side chains are important features of the Fuc CSs [320, 321]. Synthetic branched 2,4-di-*O*-sulphated fucosylated CS glycoclusters have been prepared in order to reproduce this structural feature, these display anti-coagulant properties and specific inhibition of intrinsic coagulation pathways [322]. Furthermore, the activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin times (TT) of these polymers can be fine-tuned for specific therapeutic applications [322]. The structural features of the Fuc-CSs have been investigated using high-resolution Fourier transform ion cyclotron resonance mass spectrometry [323] and to chemical and NMR spectroscopic structural investigations [324, 325]. In NMR and molecular dynamic simulations, the Fuc-CS repeat unit adopted a similar conformation to the Lewis-X blood group determinant [326, 327]. This structure accommodates the localization of several sulphate

groups in close proximity to one another and these form large negative patches which are distributed along the helical CS backbone of Fuc-CS [326, 327]. Native Fuc-CS preparations display anti-coagulant [323, 328-331] anti-angiogenic [329], anti-inflammatory, blood lipid lowering properties [323, 328-333], and stimulate haemostasis [334], promote neurite outgrowth [335], anti-cancer [336] and potent HIV-1 gp 120 protein binding properties which inhibit HIV replication by preventing viral entry into cells [337]. While neurite outgrowth promoting properties have been noted for native Fuc-CS preparations, a synthetic Fucose-CS trisaccharide (Fig 4j) has been shown to be more potent than CS-E tetrasaccharides (Fig 7) for the outgrowth of DRG hippocampal neurons in monolayer culture. The molecular recognition properties of specific glycan structures can therefore be employed in therapeutic interactions of physiological importance and undoubtedly when further information becomes available on these structures will also be employed in improved applications in repair biology.

**5. Conclusions**

Fucose, CS and KS have evolved properties of molecular recognition and information transfer which equips the proteoglycans and glycoproteins they are attached to with properties as cellular mediators controlling cellular behaviour in a number of physiological processes and in neural development and repair. A greater understanding of this evolved glycocode and how it regulates cells may allow a greater understanding of physiological and repair processes and how these might be manipulated in order to improve therapeutic interventions developed in response to altered glycodynamics in neural disorders. These are expected to improve repair responses in cognitively impaired brain tissues.

**Legends to Figures**

**Figure 1.**

Morphological features of cultured neurons. Fluorescent images of cultured neural iPSCs (a) and neuroblastoma cells (b) and IPSCs stimulated with nerve growth factor (c). In (a) cell nuclei were stained with Hoechst 33258 DNA stain, axons with anti-tubulin Alexa 488 and dendrites and synapses with anti-F actin phalloidin Alexa 568. In (b) cultured mouse neuroblastoma cells were stimulated with retinoic acid to induce differentiation. Nuclei (i.e. DNA) are stained yellow; microtubules (anti-tubulin antibody) are cyan; f-actin (fluorescent phalloidin) is purple. The image was pseudo- coloured, individual channels were initially recorded with the regular red/green/blue fluorophors (i.e. Alexa 488 and 568 and DAPI) then pseudo coloured as shown. Images a, b, c supplied courtesy of Torsten Wittmann, PhD, Dept of Cell and Tissue Biology, University of California, San Francisco, USA. Indirect fluorescent immunolocalisation of paraformaldehyde fixed rat hippocampal neurons with anti-synapsin-1/2 (red, Alexa 488) raised to a synthetic peptide corresponding to amino acids 2 to 28 from rat Synapsin-1 (UniProt Id: P09951)[cat # 106-006] and mouse anti-microtubule associated protein (MAP-2, green, FITC) [cat# 188-011], nuclei stained with DAPI (blue) (d). Image d supplied courtesy of Synaptic Systems.

**Figure 2.**

Neural structural organisation and synaptic neurotransmitter transmission. Artistic rendition of a synapse with progressive stages of transmission of neurotransmitters across the synaptic gap to a communicating neuron (a-c). Diagrammatic representation of SV2A proteoglycan intercalated in the plasma membrane of a synaptic vesicle and the Ca2+ /neurotransmitter (GABA) smart gel complex formed by interaction with the KS side chains of SV2A which forms a neurotransmitter transport delivery system (d). Pseudo coloured TEM of a synapse (X 50,000) courtesy Science PhotoLibrary. Michondria (purple), synaptic vesicles (red), synaptic gap (pink) (e). Diagrammatic depiction of a synaptic bouton with 1. microtubular transport system which transports the neurotransmitters generated in the neural cell body. 2. mitochondria and 3. synaptic vesicle accumulation in the synaptic terminal and 4.the post synaptic neurotransmitter receptors and voltage gated ion-channels on a communicating neuron which deliver neurotransmitters such as GABA (as shown) (f). Higher power view of the boxed area in (f) showing details of the depolarisation of the synaptic membrane and merging of synaptic vesicle plasma membrane and delivery of Ca2+ and neurotransmitters across the synaptic cleft to neurotransmitter receptors and voltage gated ion channels in a communicating neuron (g). Details of synaptic vesicles adjacent to the synaptic membrane of the synaptic cleft viewed by TEM (h). Plates a-c from Shutterstock. Plate d modified from [338] and f-h modified from Becker, W., Hardin, J., Bertoni, G., Kleinsmith, L. (2012). Becker's World of the Cell (8th ed.). Boston, MA: Benjamin Cummings. Source: http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-13/13_16.jpg A neuron and surrounding glial cells. (i) H & E stained section of neural tissue depicting a central large neuron body with multiple prominent dendritic processes surrounded by numerous small glial cells. Image obtained from Pinterest. Cartoon depicting the co-ordinated interplay between neurons and blood vessels mediated by astrocytic interconnections (j).

**Figure 3.**

Immunolocalisation of aggrecan in perineural nets using MAb 1-B-5 following chondroitinase ABC digestion of rat brain (a), rat dorsal root ganglion (b) or in isolated neurons (c, d). Images courtesy of Caterson archive, BioImaging Unit, University of Cardiff.

**Figure 4.**

Fucosylation of KS-I (a) and KS-II from the KS rich region of aggrecan (b) and KS-II chains located within the CS2 region of the aggrecan core protein (c) which are detected by MAb 3D12H7. Fucose modified glycan structures. The structural diversity of the L- fucose containing glycan chains of *O*-linked glycans of the mucin family (d-f), small glycan chains where L-fucose is linked directly to *O*-serine residues of the glycoprotein or proteoglycan core protein and which acts as an acceptor molecule for subsequent additions of additional saccharides (e) and the 6-sulphated Lewis-X epitope which has a widespread distribution in glycoproteins (f).Fucosylated chondroitin sulphates (Fuc-CS) of therapeutic potential (g). Representative structure of a native Fuc-CS [327] isolated from sea cucumber (g) The structure shown is that specified for a Fuc-CS displaying interactive properties with P-and L-selectin which prevented

selectin mediated extravasation of neutrophils in inflammation. The fucose side chains of Fuc-CS display some structural variation as shown in the proportion of mono- and disulphated fucose side chains (h). Structure of the CS-A and CS-E disaccharide core structures (i). The CS-A content of the core varies between 10%-50%. Structure of a fucosyl-CS trisaccharide (j) which displays potent neurite outgrowth promoting activity[335].

**Figure 5.**

O-fucosylated PSGL-1, CD-34 and Notch-1. Schematic depiction of the structural organization of PSGL-1 of leucocytes which facilitates binding to P-selectin in the endothelium (a). PSGL-1 is heavily substituted with O- linked L-fucose oligosaccharides containing the sialyl Lewis X epitope (b). A terminal sialyl LeX epitope is associated with sulphated tyrosine residues which are important for the interactive properties of PSGL-1 with P-selectin. Fucose modifications on branched glycan structures in PSGL-1 (b). The structural diversity of the L-fucose containing glycan chains in *N*-linked glycans displaying tri-antennary, tetra-antennary, high mannose, bi antennary glycan chains and those bearing the N-linked 6-sulphated Lewis-X epitope.

*O*- and *N*-fucosylation of CD-34. Diagrammatic representation of the CD-34 cell surface receptor that is widely expressed by a number of microvascular cells in brain development (c-f). N- and O- linked 6-sulphated Lewis-X motifs are prominent interactive components of CD-34 (c, e, f) these are shown distributed along the core protein of a model of the transmembrane CD-34 molecule (d).The key contribution of *O*-fucosylation to Notch functional organization (g). Structural representation of the Notch-1 receptor modular structural organization and the glycosylation of its epidermal growth factor (EGF) repeat units which conveys its interactive properties with a number of ligands (h). The EGF repeats are particularly heavily substituted with L-fucose containing glycans as shown, while the other glycosylations have a regulatory role to play over the interactive properties mediated by L-fucose glycans. Blood vessels can be visualized in human brain tissues using CD-34 immunolocalisation, 5 week post conception spinal tissue (i). Abbreviations HB , hind brain; OT, otic nerve; SC, spinal cord. Image obtained by Open Access from llgård K, Dziegielewska KM, Holst CB, Habgood MD, Saunders NR. Brain barriers and functional interfaces with sequential appearance of ABC efflux transporters during human development. Sci Rep. 2017 ;7(1):11603.

**Figure 6.**

Fucosylated blood group antigens and immunoglobulins. Fucose containing glycan chains attached to red blood cells which identify the A, B, O blood types (a). Glycan chains attached to serum immunoglobulins (b) which determine ADCC toxicity which some IgGs elicit (c-e) and an inflammatory response (f-h)

**Figure 7 .**

Neurostimulation by glycans and glycosaminoglycans. The stimulatory effect of CS-E saccharides on cultured hippocampal neurons. Immuno-fluorescent localisation of neurons cultured ± CS-E saccharides using anti-tau FITC antibodies (a-d). Control (a), CS-E disaccharide (b), unsulphated CS-E tetrasaccharide (c), d. CS-E tetrasaccharide (d), Scale bars =45 μm. The minimum size of CS-E required for neuronal stimulation was a CS-E tetrasaccharide, the non sulphated CS-E tetra-saccharide and CS-E disaccharide were both non-stimulatory. This contrasts with CS-A and CS-C which inhibit neural outgrowth. L-fucose and related sugars also have stimulatory properties of on cultured hippocampal neurons (e-h). L-fucose (6-deoxy L-galactose), D-galactose and deoxy D-galactose saccharides all influence the morphology of cultured hippocampal neurons. Immunofluorescent detection of dendrite outgrowth from cultured hippocampal neurons using FITC labeled anti-tau antibodies. Control, L-fucose (e), 2-deoxy L-galactose (f); 2-deoxy L-galactose + D-galactose (g); 3 deoxy L-galactose (h). 2-deoxy D-galactose has an inhibitory effect on dendrite outgrowths. The structures of these sugars are shown in (i)-(m). The structure of an L-Fuc analogue, 6 alkynyl Fuc which is used in the labeling and tracking of O-Fucosylation in glycoproteins is also shown (n) [79]. Images a-h reprinted (adapted) with permission from from Murrey HE, Hsieh-Wilson LC. The chemical neurobiology of carbohydrates. Chem Rev. 2008 ;108(5):1708-31,and Tulley SE, Mabon CI, Gama CI, Tsai SM, Liu X and Hsieh-Wilson LC. J am Chem Soc 2004; 126, 7736-7737 copyright (2004 and 2008) American Chemical Society.

**Figure 8.**

Diagrammatic depiction of interactive structures on the surface of neurons and the contributions of CS-proteoglycans to neural growth arising from interactions with cell surface integrins, laminins and pericellular collagen and fibronectin fibres and hyaluronan(a) and RPTP-σ (b)which acts as a CS-receptor (1). Neural outgrowth arising from the interaction of RPTP-ζ with NCAM (neural cell adhesion molecule) (c,d) (2). Cellular proliferative and cell survival effects stimulated by delivery of fibroblast growth factors to their receptors (FGFR)(e) (3). Chemorepulsive cues generated by interaction of CS-proteoglycans in perineural net formations with semaphorin 3A which is normally an attractive guidance cue. This inhibitory signal is generated by interactions of Sem3A with neuropilin-1 and neuroplexin (f,g). Semaphorin 5A also interacts with cell surface CS-proteoglycans generating a signal which inhibits nerve outgrowth (h). Figure modified from L Djerbal, H Lortat-Jacob, JCF Kwok Chondroitin sulfates and their binding molecules in the central nervous system Glycoconj J. 2017; 34(3): 363–37. Open Access.

**Figure 9.**

Diagrammatic illustration of the interaction of progranulin dimer with CSPG4 (a) and Eph A2 showing the resulting phosphorylation of Eph A2 and cross-talk with adjacent receptors leading to their activation. Figure available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license), http://creativecommons.org/licenses/by-nc-sa/3.0/). Bateman A. Progranulin and the receptor tyrosine kinase EphA2, partners in crime? J Cell Biol. 2016;215(5):603-605.

**Figure 10.**

Schematic depiction of the structural organization of transmembrane RAGE showing its extracellular, transmembrane and cytoplasmic portions and the glycan interaction region which acts as a receptor for AGEs and highly charged CS isomers such as CS-E. Diagram reproduced from Hegab Z, Gibbons S, Neyses L, Mamas MA. Role of advanced glycation end products in cardiovascular disease. World J Cardiol. 2012;4(4):90-102. Under Open Access creative commons attribution Non Commercial (CC BY-NC4.0) licence with permission of Bashideng Publishing Group, CA, USA.

**References**


1 Condomitti, G. and de Wit, J. (2018) Heparan Sulfate Proteoglycans as Emerging Players in Synaptic Specificity. Front Mol Neurosci. **11**, 14

2 Lorente-Gea, L., Garcia, B., Martin, C., Quiros, L. M. and Fernandez-Vega, I. (2017) Heparan sulfate proteoglycans and heparanases in Alzheimer's disease: current outlook and potential therapeutic targets. Neural Regen Res. **12**, 914-915

3 Minge, D., Senkov, O., Kaushik, R., Herde, M. K., Tikhobrazova, O., Wulff, A. B., Mironov, A., van Kuppevelt, T. H., Oosterhof, A., Kochlamazashvili, G., Dityatev, A. and Henneberger, C. (2017) Heparan Sulfates Support Pyramidal Cell Excitability, Synaptic Plasticity, and Context Discrimination. Cereb Cortex. **27**, 903-918

4 Schwartz, N. B. and Domowicz, M. S. (2018) Proteoglycans in brain development and pathogenesis. FEBS Lett

5 Yamaguchi, Y., Inatani, M., Matsumoto, Y., Ogawa, J. and Irie, F. (2010) Roles of heparan sulfate in mammalian brain development current views based on the findings from Ext1 conditional knockout studies. Prog Mol Biol Transl Sci. **93**, 133-152

6 Zhang, G. L., Zhang, X., Wang, X. M. and Li, J. P. (2014) Towards understanding the roles of heparan sulfate proteoglycans in Alzheimer's disease. Biomed Res Int. **2014**, 516028

7 Gagneux, P., Aebi, M. and Varki, A. (2015) Evolution of Glycan Diversity. 253-264

8 Rudd, P., Karlsson, N. G., Khoo, K. H. and Packer, N. H. (2015) Glycomics and Glycoproteomics. 653-666

9 Varki, A. (2017) Biological roles of glycans. Glycobiology. **27**, 3-49

10 Vliegenthart, J. F. (2017) The complexity of glycoprotein-derived glycans. Proc Jpn Acad Ser B Phys Biol Sci. **93**, 64-86

11 Chen, Z., Glover, M. S. and Li, L. (2017) Recent advances in ion mobility-mass spectrometry for improved structural characterization of glycans and glycoconjugates. Curr Opin Chem Biol. **42**, 1-8

12 Hofmann, J. and Pagel, K. (2017) Glycan Analysis by Ion Mobility-Mass Spectrometry. Angew Chem Int Ed Engl. **56**, 8342-8349

13 Perez, S. and de Sanctis, D. (2017) Glycoscience@Synchrotron: Synchrotron radiation applied to structural glycoscience. Beilstein J Org Chem. **13**, 1145-1167

14 Lee, L. Y., Moh, E. S., Parker, B. L., Bern, M., Packer, N. H. and Thaysen-Andersen, M. (2016) Toward Automated N-Glycopeptide Identification in Glycoproteomics. J Proteome Res. **15**, 3904-3915

15 Eliuk, S. and Makarov, A. (2015) Evolution of Orbitrap Mass Spectrometry Instrumentation. Annu Rev Anal Chem (Palo Alto Calif). **8**, 61-80

16 Benz, C., Boomhoff, M., Appun, J., Schneider, C. and Belder, D. (2015) Chip-based free-flow electrophoresis with integrated nanospray mass-spectrometry. Angew Chem Int Ed Engl. **54**, 2766-2770

17 Bennun, S. V., Hizal, D. B., Heffner, K., Can, O., Zhang, H. and Betenbaugh, M. J. (2016) Systems Glycobiology: Integrating Glycogenomics, Glycoproteomics, Glycomics, and Other 'Omics Data Sets to Characterize Cellular Glycosylation Processes. J Mol Biol. **428**, 3337-3352

18 Zamfir, A. D., Flangea, C., Serb, A., Sisu, E., Zagrean, L., Rizzi, A. and Seidler, D. G. (2012) Brain chondroitin/dermatan sulfate, from cerebral tissue to fine structure: extraction, preparation, and fully automated chip-electrospray mass spectrometric analysis. Methods Mol Biol. **836**, 145-159

19 Marson, A., Robinson, D. E., Brookes, P. N., Mulloy, B., Wiles, M., Clark, S. J., Fielder, H. L., Collinson, L. J., Cain, S. A., Kielty, C. M., McArthur, S., Buttle, D. J., Short, R. D., Whittle, J. D. and Day, A. J. (2009) Development of a microtiter plate-based glycosaminoglycan array for the investigation of glycosaminoglycan-protein interactions. Glycobiology. **19**, 1537-1546

20 Rogers, C. J. and Hsieh-Wilson, L. C. (2012) Microarray method for the rapid detection of glycosaminoglycan-protein interactions. Methods Mol Biol. **808**, 321-336

21 Smith, D. F. and Cummings, R. D. (2013) Application of microarrays for deciphering the structure and function of the human glycome. Mol Cell Proteomics. **12**, 902-912

22 Shipp, E. L. and Hsieh-Wilson, L. C. (2007) Profiling the sulfation specificities of glycosaminoglycan interactions with growth factors and chemotactic proteins using microarrays. Chem Biol. **14**, 195-208

23 Takada, W., Fukushima, M., Pothacharoen, P., Kongtawelert, P. and Sugahara, K. (2013) A sulfated glycosaminoglycan array for molecular interactions between glycosaminoglycans and growth factors or anti-glycosaminoglycan antibodies. Anal Biochem. **435**, 123-130

24 Tully, S. E., Rawat, M. and Hsieh-Wilson, L. C. (2006) Discovery of a TNF-alpha antagonist using chondroitin sulfate microarrays. J Am Chem Soc. **128**, 7740-7741
25 Ruff, S. M., Keller, S., Wieland, D. E., Wittmann, V., Tovar, G. E., Bach, M. and Kluger, P. J. (2017) clickECM: Development of a cell-derived extracellular matrix with azide functionalities. Acta Biomater. **52**, 159-170
26 Lopez Aguilar, A., Briard, J. G., Yang, L., Ovryn, B., Macauley, M. S. and Wu, P. (2017) Tools for Studying Glycans: Recent Advances in Chemoenzymatic Glycan Labeling. ACS Chem Biol. **12**, 611-621
27 Chaubard, J. L., Krishnamurthy, C., Yi, W., Smith, D. F. and Hsieh-Wilson, L. C. (2012) Chemoenzymatic probes for detecting and imaging fucose-alpha(1-2)-galactose glycan biomarkers. J Am Chem Soc. **134**, 4489-4492
28 Wibowo, A., Peters, E. C. and Hsieh-Wilson, L. C. (2014) Photoactivatable glycopolymers for the proteome-wide identification of fucose-alpha(1-2)-galactose binding proteins. J Am Chem Soc. **136**, 9528-9531
29 Mountford, C., Quadrelli, S., Lin, A. and Ramadan, S. (2015) Six fucose-alpha(1-2) sugars and alpha-fucose assigned in the human brain using in vivo two-dimensional MRS. NMR Biomed. **28**, 291-296
30 Chiang, A. W., Li, S., Spahn, P. N., Richelle, A., Kuo, C. C., Samoudi, M. and Lewis, N. E. (2016) Modulating carbohydrate-protein interactions through glycoengineering of monoclonal antibodies to impact cancer physiology. Curr Opin Struct Biol. **40**, 104-111
31 Zeng, X., Qu, K. and Rehman, A. (2016) Glycosylated Conductive Polymer: A Multimodal Biointerface for Studying Carbohydrate-Protein Interactions. Acc Chem Res. **49**, 1624-1633
32 Huang, M. L. and Godula, K. (2016) Nanoscale materials for probing the biological functions of the glycocalyx. Glycobiology. **26**, 797-803
33 Cerliani, J. P., Blidner, A. G., Toscano, M. A., Croci, D. O. and Rabinovich, G. A. (2017) Translating the 'Sugar Code' into Immune and Vascular Signaling Programs. Trends Biochem Sci. **42**, 255-273
34 Mueller, T. M., Yates, S. D., Haroutunian, V. and Meador-Woodruff, J. H. (2017) Altered fucosyltransferase expression in the superior temporal gyrus of elderly patients with schizophrenia. Schizophr Res. **182**, 66-73
35 Damerell, D., Ceroni, A., Maass, K., Ranzinger, R., Dell, A. and Haslam, S. M. (2012) The GlycanBuilder and GlycoWorkbench glycoinformatics tools: updates and new developments. Biol Chem. **393**, 1357-1362
36 Aoki, K. F., Yamaguchi, A., Ueda, N., Akutsu, T., Mamitsuka, H., Goto, S. and Kanehisa, M. (2004) KCaM (KEGG Carbohydrate Matcher): a software tool for analyzing the structures of carbohydrate sugar chains. Nucleic Acids Res. **32**, W267-272
37 Maxwell, E., Tan, Y., Hu, H., Benson, G., Aizikov, K., Conley, S., Staples, G. O., Slysz, G. W., Smith, R. D. and Zaia, J. (2012) GlycReSoft: a software package for automated recognition of glycans from LC/MS data. PLoS One. **7**, e45474
38 Loss, A., Bunsmann, P., Bohne, A., Schwarzer, E., Lang, E. and von der Lieth, C. W. (2002) SWEET-DB: an attempt to create annotated data collections for carbohydrates. Nucleic Acids Res. **30**, 405-408
39 Bohne-Lang, A., Lang, E., Forster, T. and von der Lieth, C. W. (2001) LINUCS: linear notation for unique description of carbohydrate sequences. Carbohydr Res. **336**, 1-11
40 Sahoo, S. S., Thomas, C., Sheth, A., Henson, C. and York, W. S. (2005) GLYDE-an expressive XML standard for the representation of glycan structure. Carbohydr Res. **340**, 2802-2807
41 Malik, A., Firoz, A., Jha, V. and Ahmad, S. (2010) PROCARB: A Database of Known and Modelled Carbohydrate-Binding Protein Structures with Sequence-Based Prediction Tools. Adv Bioinformatics, 436036
42 Frey, L. J. (2015) Informatics tools to advance the biology of glycosaminoglycans and proteoglycans. Methods Mol Biol. **1229**, 271-287
43 Rudd, T. R., Skidmore, M. A., Guerrini, M., Hricovini, M., Powell, A. K., Siligardi, G. and Yates, E. A. (2010) The conformation and structure of GAGs: recent progress and perspectives. Curr Opin Struct Biol. **20**, 567-574
44 Chiu, Y., Huang, R., Orlando, R. and Sharp, J. S. (2015) GAG-ID: Heparan Sulfate (HS) and Heparin Glycosaminoglycan High-Throughput Identification Software. Mol Cell Proteomics. **14**, 1720-1730

45 Sankaranarayanan, N. V. and Desai, U. R. (2014) Toward a robust computational screening strategy for identifying glycosaminoglycan sequences that display high specificity for target proteins. Glycobiology. **24**, 1323-1333
46 Sankaranarayanan, N. V., Sarkar, A., Desai, U. R. and Mosier, P. D. (2015) Designing "high-affinity, high-specificity" glycosaminoglycan sequences through computerized modeling. Methods Mol Biol. **1229**, 289-314
47 Sarkar, A. and Desai, U. R. (2015) A Simple Method for Discovering Druggable, Specific Glycosaminoglycan-Protein Systems. Elucidation of Key Principles from Heparin/Heparan Sulfate-Binding Proteins. PLoS One. **10**, e0141127
48 Spencer, J. L., Bernanke, J. A., Buczek-Thomas, J. A. and Nugent, M. A. (2010) A computational approach for deciphering the organization of glycosaminoglycans. PLoS One. **5**, e9389
49 Turnbull, J. E., Miller, R. L., Ahmed, Y., Puvirajesinghe, T. M. and Guimond, S. E. (2010) Glycomics profiling of heparan sulfate structure and activity. Methods Enzymol. **480**, 65-85
50 Sarkar, A., Drouillard, S., Rivet, A. and Perez, S. (2015) Databases of Conformations and NMR Structures of Glycan Determinants. Glycobiology. **25**, 1480-1490
51 Tissot, B., Ceroni, A., Powell, A. K., Morris, H. R., Yates, E. A., Turnbull, J. E., Gallagher, J. T., Dell, A. and Haslam, S. M. (2008) Software tool for the structural determination of glycosaminoglycans by mass spectrometry. Anal Chem. **80**, 9204-9212
52 Wang, X., Liu, X., Li, L., Zhang, F., Hu, M., Ren, F., Chi, L. and Linhardt, R. J. (2016) GlycCompSoft: Software for Automated Comparison of Low Molecular Weight Heparins Using Top-Down LC/MS Data. PLoS One. **11**, e0167727
53 Mourao, P. A., Vilanova, E. and Soares, P. A. (2017) Unveiling the structure of sulfated fucose-rich polysaccharides via nuclear magnetic resonance spectroscopy. Curr Opin Struct Biol. **50**, 33-41
54 Kizuka, Y., Funayama, S., Shogomori, H., Nakano, M., Nakajima, K., Oka, R., Kitazume, S., Yamaguchi, Y., Sano, M., Korekane, H., Hsu, T. L., Lee, H. Y., Wong, C. H. and Taniguchi, N. (2016) High-Sensitivity and Low-Toxicity Fucose Probe for Glycan Imaging and Biomarker Discovery. Cell Chem Biol. **23**, 782-792
55 Cummings, R. D. (2009) The repertoire of glycan determinants in the human glycome. Mol Biosyst. **5**, 1087-1104
56 Klamer, Z., Staal, B., Prudden, A. R., Liu, L., Smith, D. F., Boons, G. J. and Haab, B. (2017) Mining High-Complexity Motifs in Glycans: A New Language To Uncover the Fine Specificities of Lectins and Glycosidases. Anal Chem. **89**, 12342-12350
57 Melrose, J. (2016) The glycosaminoglycan/glycan interactome: a bioinformatic platform. An evolutionary conserved biosensor platform controlling cellular behaviour, tissue morphogenesis, tissue assembly. Scholars Press, Schaltungsdienst Lange OHG., Saarbrucken , Berlin
58 Alvarez-Maubecin, V., Garcia-Hernandez, F., Williams, J. T. and Van Bockstaele, E. J. (2000) Functional coupling between neurons and glia. J Neurosci. **20**, 4091-4098
59 Ehlers, M. D. and Polleux, F. (2010) Neuronal and glial cell biology. Curr Opin Neurobiol. **20**, 529-530
60 Cooper, M. S. (1995) Intercellular signaling in neuronal-glial networks. Biosystems. **34**, 65-85
61 Henn, F. A. and Hamberger, A. (1971) Glial cell function: uptake of transmitter substances. Proc Natl Acad Sci U S A. **68**, 2686-2690
62 Mergenthaler, P., Lindauer, U., Dienel, G. A. and Meisel, A. (2013) Sugar for the brain: the role of glucose in physiological and pathological brain function. Trends Neurosci. **36**, 587-597
63 Gold, P. E. (1995) Role of glucose in regulating the brain and cognition. Am J Clin Nutr. **61**, 987S-995S
64 Poli, D., Pastore, V. P. and Massobrio, P. (2015) Functional connectivity in in vitro neuronal assemblies. Front Neural Circuits. **9**, 57
65 Budnik, V., Ruiz-Canada, C. and Wendler, F. (2016) Extracellular vesicles round off communication in the nervous system. Nat Rev Neurosci. **17**, 160-172
66 Grigoryan, T. and Birchmeier, W. (2015) Molecular signaling mechanisms of axon-glia communication in the peripheral nervous system. Bioessays. **37**, 502-513
67 Lee, H. K., Cording, A., Vielmetter, J. and Zinn, K. (2013) Interactions between a receptor tyrosine phosphatase and a cell surface ligand regulate axon guidance and glial-neuronal communication. Neuron. **78**, 813-826
68 Old, E. A. and Malcangio, M. (2012) Chemokine mediated neuron-glia communication and aberrant signalling in neuropathic pain states. Curr Opin Pharmacol. **12**, 67-73

69 Murai, K. K. and Pasquale, E. B. (2011) Eph receptors and ephrins in neuron-astrocyte communication at synapses. Glia. **59**, 1567-1578
70 Jha, M. K., Kim, J. H., Song, G. J., Lee, W. H., Lee, I. K., Lee, H. W., An, S. S. A., Kim, S. and Suk, K. (2018) Functional dissection of astrocyte-secreted proteins: Implications in brain health and diseases. Prog Neurobiol. **162**, 37-69
71 Ben Haim, L. and Rowitch, D. H. (2017) Functional diversity of astrocytes in neural circuit regulation. Nat Rev Neurosci. **18**, 31-41
72 Schitine, C., Nogaroli, L., Costa, M. R. and Hedin-Pereira, C. (2015) Astrocyte heterogeneity in the brain: from development to disease. Front Cell Neurosci. **9**, 76
73 AbuSamra, D. B., Aleisa, F. A., Al-Amoodi, A. S., Jalal Ahmed, H. M., Chin, C. J., Abuelela, A. F., Bergam, P., Sougrat, R. and Merzaban, J. S. (2017) Not just a marker: CD34 on human hematopoietic stem/progenitor cells dominates vascular selectin binding along with CD44. Blood Adv. **1**, 2799-2816
74 Hidalgo, A. and Frenette, P. S. (2005) Enforced fucosylation of neonatal CD34+ cells generates selectin ligands that enhance the initial interactions with microvessels but not homing to bone marrow. Blood. **105**, 567-575
75 Garbuzova-Davis, S., Kurien, C., Thomson, A., Falco, D., Ahmad, S., Staffetti, J., Steiner, G., Abraham, S., James, G., Mahendrasah, A., Sanberg, P. R. and Borlongan, C. V. (2017) Endothelial and Astrocytic Support by Human Bone Marrow Stem Cell Grafts into Symptomatic ALS Mice towards Blood-Spinal Cord Barrier Repair. Sci Rep. **7**, 884
76 Zhu, X., Zhang, J., Tollkuhn, J., Ohsawa, R., Bresnick, E. H., Guillemot, F., Kageyama, R. and Rosenfeld, M. G. (2006) Sustained Notch signaling in progenitors is required for sequential emergence of distinct cell lineages during organogenesis. Genes Dev. **20**, 2739-2753
77 Leonhard-Melief, C. and Haltiwanger, R. S. (2010) O-fucosylation of thrombospondin type 1 repeats. Methods Enzymol. **480**, 401-416
78 Hofsteenge, J., Huwiler, K. G., Macek, B., Hess, D., Lawler, J., Mosher, D. F. and Peter-Katalinic, J. (2001) C-mannosylation and O-fucosylation of the thrombospondin type 1 module. J Biol Chem. **276**, 6485-6498
79 Al-Shareffi, E., Chaubard, J. L., Leonhard-Melief, C., Wang, S. K., Wong, C. H. and Haltiwanger, R. S. (2013) 6-alkynyl fucose is a bioorthogonal analog for O-fucosylation of epidermal growth factor-like repeats and thrombospondin type-1 repeats by protein O-fucosyltransferases 1 and 2. Glycobiology. **23**, 188-198
80 Rampal, R., Arboleda-Velasquez, J. F., Nita-Lazar, A., Kosik, K. S. and Haltiwanger, R. S. (2005) Highly conserved O-fucose sites have distinct effects on Notch1 function. J Biol Chem. **280**, 32133-32140
81 Moloney, D. J., Shair, L. H., Lu, F. M., Xia, J., Locke, R., Matta, K. L. and Haltiwanger, R. S. (2000) Mammalian Notch1 is modified with two unusual forms of O-linked glycosylation found on epidermal growth factor-like modules. J Biol Chem. **275**, 9604-9611
82 Okajima, T. and Irvine, K. D. (2002) Regulation of notch signaling by o-linked fucose. Cell. **111**, 893-904
83 Sasamura, T., Sasaki, N., Miyashita, F., Nakao, S., Ishikawa, H. O., Ito, M., Kitagawa, M., Harigaya, K., Spana, E., Bilder, D., Perrimon, N. and Matsuno, K. (2003) neurotic, a novel maternal neurogenic gene, encodes an O-fucosyltransferase that is essential for Notch-Delta interactions. Development. **130**, 4785-4795
84 Shi, S. and Stanley, P. (2003) Protein O-fucosyltransferase 1 is an essential component of Notch signaling pathways. Proc Natl Acad Sci U S A. **100**, 5234-5239
85 Kim, M. L., Chandrasekharan, K., Glass, M., Shi, S., Stahl, M. C., Kaspar, B., Stanley, P. and Martin, P. T. (2008) O-fucosylation of muscle agrin determines its ability to cluster acetylcholine receptors. Mol Cell Neurosci. **39**, 452-464
86 Yu, P., Pearson, C. S. and Geller, H. M. (2018) Flexible Roles for Proteoglycan Sulfation and Receptor Signaling. Trends Neurosci. **41**, 47-61
87 Galtrey, C. M. and Fawcett, J. W. (2007) The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res Rev. **54**, 1-18
88 Miyata, S. and Kitagawa, H. (2016) Chondroitin 6-Sulfation Regulates Perineuronal Net Formation by Controlling the Stability of Aggrecan. Neural Plast. **2016**, 1305801
89 Morawski, M., Bruckner, G., Arendt, T. and Matthews, R. T. (2012) Aggrecan: Beyond cartilage and into the brain. Int J Biochem Cell Biol. **44**, 690-693

90 Suttkus, A., Morawski, M. and Arendt, T. (2016) Protective Properties of Neural Extracellular Matrix. Mol Neurobiol. **53**, 73-82

91 Garwood, J., Schnadelbach, O., Clement, A., Schutte, K., Bach, A. and Faissner, A. (1999) DSD-1-proteoglycan is the mouse homolog of phosphacan and displays opposing effects on neurite outgrowth dependent on neuronal lineage. J Neurosci. **19**, 3888-3899

92 Inatani, M., Honjo, M., Otori, Y., Oohira, A., Kido, N., Tano, Y., Honda, Y. and Tanihara, H. (2001) Inhibitory effects of neurocan and phosphacan on neurite outgrowth from retinal ganglion cells in culture. Invest Ophthalmol Vis Sci. **42**, 1930-1938

93 Meyer-Puttlitz, B., Junker, E., Margolis, R. U. and Margolis, R. K. (1996) Chondroitin sulfate proteoglycans in the developing central nervous system. II. Immunocytochemical localization of neurocan and phosphacan. J Comp Neurol. **366**, 44-54

94 Milev, P., Friedlander, D. R., Sakurai, T., Karthikeyan, L., Flad, M., Margolis, R. K., Grumet, M. and Margolis, R. U. (1994) Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neurons, glia, and neural cell adhesion molecules. J Cell Biol. **127**, 1703-1715

95 Gabius, H. J. (2015) The magic of the sugar code. Trends Biochem Sci. **40**, 341

96 Gabius, H. J., Siebert, H. C., Andre, S., Jimenez-Barbero, J. and Rudiger, H. (2004) Chemical biology of the sugar code. Chembiochem. **5**, 740-764

97 Gama, C. I., Tully, S. E., Sotogaku, N., Clark, P. M., Rawat, M., Vaidehi, N., Goddard, W. A., 3rd, Nishi, A. and Hsieh-Wilson, L. C. (2006) Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. Nat Chem Biol. **2**, 467-473

98 Hayes, A., Sugahara, K., Farrugia, B., Whitelock, J. M., Caterson, B. and Melrose, J. (2018) Biodiversity of CS-proteoglycan sulphation motifs: chemical messenger recognition modules with roles in information transfer, control of cellular behaviour and tissue morphogenesis. Biochem J. **475**, 587-620

99 Caterson, B. and Melrose, J. (2018) Keratan Sulphate, a complex Glycosaminoglycan with Unique Functional Capability. Glycobiology

100 Dyck, S. M., Alizadeh, A., Santhosh, K. T., Proulx, E. H., Wu, C. L. and Karimi-Abdolrezaee, S. (2015) Chondroitin Sulfate Proteoglycans Negatively Modulate Spinal Cord Neural Precursor Cells by Signaling Through LAR and RPTPsigma and Modulation of the Rho/ROCK Pathway. Stem Cells. **33**, 2550-2563

101 Zhang, H., Muramatsu, T., Murase, A., Yuasa, S., Uchimura, K. and Kadomatsu, K. (2006) N-Acetylglucosamine 6-O-sulfotransferase-1 is required for brain keratan sulfate biosynthesis and glial scar formation after brain injury. Glycobiology. **16**, 702-710

102 Bradbury, E. J., Moon, L. D., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., Fawcett, J. W. and McMahon, S. B. (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature. **416**, 636-640

103 Imagama, S., Sakamoto, K., Tauchi, R., Shinjo, R., Ohgomori, T., Ito, Z., Zhang, H., Nishida, Y., Asami, N., Takeshita, S., Sugiura, N., Watanabe, H., Yamashita, T., Ishiguro, N., Matsuyama, Y. and Kadomatsu, K. (2011) Keratan sulfate restricts neural plasticity after spinal cord injury. J Neurosci. **31**, 17091-17102

104 Ishikawa, Y., Imagama, S., Ohgomori, T., Ishiguro, N. and Kadomatsu, K. (2015) A combination of keratan sulfate digestion and rehabilitation promotes anatomical plasticity after rat spinal cord injury. Neurosci Lett. **593**, 13-18

105 Krusius, T., Finne, J., Margolis, R. K. and Margolis, R. U. (1986) Identification of an O-glycosidic mannose-linked sialylated tetrasaccharide and keratan sulfate oligosaccharides in the chondroitin sulfate proteoglycan of brain. J Biol Chem. **261**, 8237-8242

106 Fischer, D. C., Haubeck, H. D., Eich, K., Kolbe-Busch, S., Stocker, G., Stuhlsatz, H. W. and Greiling, H. (1996) A novel keratan sulphate domain preferentially expressed on the large aggregating proteoglycan from human articular cartilage is recognized by the monoclonal antibody 3D12/H7. Biochem J. **318 ( Pt 3)**, 1051-1056

107 Butler, C. D., Schnetz, S. A., Yu, E. Y., Davis, J. B., Temple, K., Silver, J. and Malouf, A. T. (2004) Keratan sulfate proteoglycan phosphacan regulates mossy fiber outgrowth and regeneration. J Neurosci. **24**, 462-473

108 Dobbertin, A., Rhodes, K. E., Garwood, J., Properzi, F., Heck, N., Rogers, J. H., Fawcett, J. W. and Faissner, A. (2003) Regulation of RPTPbeta/phosphacan expression and glycosaminoglycan epitopes in injured brain and cytokine-treated glia. Mol Cell Neurosci. **24**, 951-971

109 Burg, M. A. and Cole, G. J. (1994) Claustrin, an antiadhesive neural keratan sulfate proteoglycan, is structurally related to MAP1B. J Neurobiol. **25**, 1-22
110 Fujimoto, H., Ohgomori, T., Abe, K., Uchimura, K., Kadomatsu, K. and Jinno, S. (2015) Time-dependent localization of high- and low-sulfated keratan sulfates in the song nuclei of developing zebra finches. Eur J Neurosci. **42**, 2716-2725
111 Takeda-Uchimura, Y., Uchimura, K., Sugimura, T., Yanagawa, Y., Kawasaki, T., Komatsu, Y. and Kadomatsu, K. (2015) Requirement of keratan sulfate proteoglycan phosphacan with a specific sulfation pattern for critical period plasticity in the visual cortex. Exp Neurol. **274**, 145-155
112 Yin, J., Sakamoto, K., Zhang, H., Ito, Z., Imagama, S., Kishida, S., Natori, T., Sawada, M., Matsuyama, Y. and Kadomatsu, K. (2009) Transforming growth factor-beta1 upregulates keratan sulfate and chondroitin sulfate biosynthesis in microglias after brain injury. Brain Res. **1263**, 10-22
113 Jones, L. L. and Tuszynski, M. H. (2002) Spinal cord injury elicits expression of keratan sulfate proteoglycans by macrophages, reactive microglia, and oligodendrocyte progenitors. J Neurosci. **22**, 4611-4624
114 Geisert, E. E., Jr., Bidanset, D. J., Del Mar, N. and Robson, J. A. (1996) Up-regulation of a keratan sulfate proteoglycan following cortical injury in neonatal rats. Int J Dev Neurosci. **14**, 257-267
115 Kato, Y., Hayatsu, N., Kaneko, M. K., Ogasawara, S., Hamano, T., Takahashi, S., Nishikawa, R., Matsutani, M., Mishima, K. and Narimatsu, H. (2008) Increased expression of highly sulfated keratan sulfate synthesized in malignant astrocytic tumors. Biochem Biophys Res Commun. **369**, 1041-1046
116 Hayatsu, N., Ogasawara, S., Kaneko, M. K., Kato, Y. and Narimatsu, H. (2008) Expression of highly sulfated keratan sulfate synthesized in human glioblastoma cells. Biochem Biophys Res Commun. **368**, 217-222
117 Lindahl, B., Eriksson, L., Spillmann, D., Caterson, B. and Lindahl, U. (1996) Selective loss of cerebral keratan sulfate in Alzheimer's disease. J Biol Chem. **271**, 16991-16994
118 Russo, V. C., Bach, L. A., Fosang, A. J., Baker, N. L. and Werther, G. A. (1997) Insulin-like growth factor binding protein-2 binds to cell surface proteoglycans in the rat brain olfactory bulb. Endocrinology. **138**, 4858-4867
119 Weyers, A., Yang, B., Solakyildirim, K., Yee, V., Li, L., Zhang, F. and Linhardt, R. J. (2013) Isolation of bovine corneal keratan sulfate and its growth factor and morphogen binding. FEBS J. **280**, 2285-2293
120 Conrad, A. H., Zhang, Y., Tasheva, E. S. and Conrad, G. W. (2010) Proteomic analysis of potential keratan sulfate, chondroitin sulfate A, and hyaluronic acid molecular interactions. Invest Ophthalmol Vis Sci. **51**, 4500-4515
121 Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. E. (1995) Notch signaling. Science. **268**, 225-232
122 Cai, Z., Zhao, B., Deng, Y., Shangguan, S., Zhou, F., Zhou, W., Li, X., Li, Y. and Chen, G. (2016) Notch signaling in cerebrovascular diseases (Review). Mol Med Rep. **14**, 2883-2898
123 Shao, L. and Haltiwanger, R. S. (2003) O-fucose modifications of epidermal growth factor-like repeats and thrombospondin type 1 repeats: unusual modifications in unusual places. Cell Mol Life Sci. **60**, 241-250
124 Stanley, P. and Okajima, T. (2010) Roles of glycosylation in Notch signaling. Curr Top Dev Biol. **92**, 131-164
125 Cummings, R. D. (1999) Structure and function of the selectin ligand PSGL-1. Braz J Med Biol Res. **32**, 519-528
126 Nielsen, J. S. and McNagny, K. M. (2008) Novel functions of the CD34 family. J Cell Sci. **121**, 3683-3692
127 Avram, S., Shaposhnikov, S., Buiu, C. and Mernea, M. (2014) Chondroitin sulfate proteoglycans: structure-function relationship with implication in neural development and brain disorders. Biomed Res Int. **2014**, 642798
128 Harris, J. L., Reeves, T. M. and Phillips, L. L. (2011) Phosphacan and receptor protein tyrosine phosphatase beta expression mediates deafferentation-induced synaptogenesis. Hippocampus. **21**, 81-92
129 Meyer-Puttlitz, B., Milev, P., Junker, E., Zimmer, I., Margolis, R. U. and Margolis, R. K. (1995) Chondroitin sulfate and chondroitin/keratan sulfate proteoglycans of nervous tissue: developmental changes of neurocan and phosphacan. J Neurochem. **65**, 2327-2337

130 Ito, Z., Sakamoto, K., Imagama, S., Matsuyama, Y., Zhang, H., Hirano, K., Ando, K., Yamashita, T., Ishiguro, N. and Kadomatsu, K. (2010) N-acetylglucosamine 6-O-sulfotransferase-1-deficient mice show better functional recovery after spinal cord injury. J Neurosci. **30**, 5937-5947

131 Foyez, T., Takeda-Uchimura, Y., Ishigaki, S., Narentuya, Zhang, Z., Sobue, G., Kadomatsu, K. and Uchimura, K. (2015) Microglial keratan sulfate epitope elicits in central nervous tissues of transgenic model mice and patients with amyotrophic lateral sclerosis. Am J Pathol. **185**, 3053-3065

132 Hirano, K., Ohgomori, T., Kobayashi, K., Tanaka, F., Matsumoto, T., Natori, T., Matsuyama, Y., Uchimura, K., Sakamoto, K., Takeuchi, H., Hirakawa, A., Suzumura, A., Sobue, G., Ishiguro, N., Imagama, S. and Kadomatsu, K. (2013) Ablation of keratan sulfate accelerates early phase pathogenesis of ALS. PLoS One. **8**, e66969

133 Zhang, Z., Takeda-Uchimura, Y., Foyez, T., Ohtake-Niimi, S., Narentuya, Akatsu, H., Nishitsuji, K., Michikawa, M., Wyss-Coray, T., Kadomatsu, K. and Uchimura, K. (2017) Deficiency of a sulfotransferase for sialic acid-modified glycans mitigates Alzheimer's pathology. Proc Natl Acad Sci U S A. **114**, E2947-E2954

134 Hashimoto, H., Ishino, Y., Jiang, W., Yoshimura, T., Takeda-Uchimura, Y., Uchimura, K., Kadomatsu, K. and Ikenaka, K. (2016) Keratan Sulfate Regulates the Switch from Motor Neuron to Oligodendrocyte Generation During Development of the Mouse Spinal Cord. Neurochem Res. **41**, 450-462

135 Cole, G. J. and McCabe, C. F. (1991) Identification of a developmentally regulated keratan sulfate proteoglycan that inhibits cell adhesion and neurite outgrowth. Neuron. **7**, 1007-1018

136 Hayashi, M., Kadomatsu, K., Kojima, T. and Ishiguro, N. (2011) Keratan sulfate and related murine glycosylation can suppress murine cartilage damage in vitro and in vivo. Biochem Biophys Res Commun. **409**, 732-737

137 Hasegawa, N., Torii, T., Kato, T., Miyajima, H., Furuhata, A., Nakayasu, K., Kanai, A. and Habuchi, O. (2000) Decreased GlcNAc 6-O-sulfotransferase activity in the cornea with macular corneal dystrophy. Invest Ophthalmol Vis Sci. **41**, 3670-3677

138 Maeda, N., Fukazawa, N. and Hata, T. (2006) The binding of chondroitin sulfate to pleiotrophin/heparin-binding growth-associated molecule is regulated by chain length and oversulfated structures. J Biol Chem. **281**, 4894-4902

139 Pufe, T., Groth, G., Goldring, M. B., Tillmann, B. and Mentlein, R. (2007) Effects of pleiotrophin, a heparin-binding growth factor, on human primary and immortalized chondrocytes. Osteoarthritis Cartilage. **15**, 155-162

140 Miller, R. E., Grodzinsky, A. J., Cummings, K., Plaas, A. H., Cole, A. A., Lee, R. T. and Patwari, P. (2010) Intraarticular injection of heparin-binding insulin-like growth factor 1 sustains delivery of insulin-like growth factor 1 to cartilage through binding to chondroitin sulfate. Arthritis Rheum. **62**, 3686-3694

141 Nandini, C. D. and Sugahara, K. (2006) Role of the sulfation pattern of chondroitin sulfate in its biological activities and in the binding of growth factors. Adv Pharmacol. **53**, 253-279

142 Sugahara, K., Mikami, T., Uyama, T., Mizuguchi, S., Nomura, K. and Kitagawa, H. (2003) Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate. Curr Opin Struct Biol. **13**, 612-620

143 Deepa, S. S., Yamada, S., Zako, M., Goldberger, O. and Sugahara, K. (2004) Chondroitin sulfate chains on syndecan-1 and syndecan-4 from normal murine mammary gland epithelial cells are structurally and functionally distinct and cooperate with heparan sulfate chains to bind growth factors. A novel function to control binding of midkine, pleiotrophin, and basic fibroblast growth factor. J Biol Chem. **279**, 37368-37376

144 Shuo, T., Aono, S., Matsui, F., Tokita, Y., Maeda, H., Shimada, K. and Oohira, A. (2004) Developmental changes in the biochemical and immunological characters of the carbohydrate moiety of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan. Glycoconj J. **20**, 267-278

145 Tsuchida, K., Shioi, J., Yamada, S., Boghosian, G., Wu, A., Cai, H., Sugahara, K. and Robakis, N. K. (2001) Appican, the proteoglycan form of the amyloid precursor protein, contains chondroitin sulfate E in the repeating disaccharide region and 4-O-sulfated galactose in the linkage region. J Biol Chem. **276**, 37155-37160

146 Wang, D. and Fawcett, J. (2012) The perineuronal net and the control of CNS plasticity. Cell Tissue Res. **349**, 147-160

147 Bertolotto, A., Manzardo, E. and Guglielmone, R. (1996) Immunohistochemical mapping of perineuronal nets containing chondroitin unsulfated proteoglycan in the rat central nervous system. Cell Tissue Res. **283**, 283-295
148 Schafer, M. K. E. and Tegeder, I. (2017) NG2/CSPG4 and progranulin in the posttraumatic glial scar. Matrix Biol
149 Naphade, S. B., Kigerl, K. A., Jakeman, L. B., Kostyk, S. K., Popovich, P. G. and Kuret, J. (2010) Progranulin expression is upregulated after spinal contusion in mice. Acta Neuropathol. **119**, 123-133
150 Ahmed, Z., Mackenzie, I. R., Hutton, M. L. and Dickson, D. W. (2007) Progranulin in frontotemporal lobar degeneration and neuroinflammation. J Neuroinflammation. **4**, 7
151 Toh, H., Chitramuthu, B. P., Bennett, H. P. and Bateman, A. (2011) Structure, function, and mechanism of progranulin; the brain and beyond. J Mol Neurosci. **45**, 538-548
152 Mao, Q., Wang, D., Li, Y., Kohler, M., Wilson, J., Parton, Z., Shmaltsuyeva, B., Gursel, D., Rademakers, R., Weintraub, S., Mesulam, M. M., Xia, H. and Bigio, E. H. (2017) Disease and Region Specificity of Granulin Immunopositivities in Alzheimer Disease and Frontotemporal Lobar Degeneration. J Neuropathol Exp Neurol. **76**, 957-968
153 Sun, L. and Eriksen, J. L. (2011) Recent insights into the involvement of progranulin in frontotemporal dementia. Curr Neuropharmacol. **9**, 632-642
154 Andrews, M. R., Czvitkovich, S., Dassie, E., Vogelaar, C. F., Faissner, A., Blits, B., Gage, F. H., ffrench-Constant, C. and Fawcett, J. W. (2009) Alpha9 integrin promotes neurite outgrowth on tenascin-C and enhances sensory axon regeneration. J Neurosci. **29**, 5546-5557
155 Cheah, M. and Andrews, M. R. (2016) Targeting cell surface receptors for axon regeneration in the central nervous system. Neural Regen Res. **11**, 1884-1887
156 Cheah, M., Andrews, M. R., Chew, D. J., Moloney, E. B., Verhaagen, J., Fassler, R. and Fawcett, J. W. (2016) Expression of an Activated Integrin Promotes Long-Distance Sensory Axon Regeneration in the Spinal Cord. J Neurosci. **36**, 7283-7297
157 Lang, B. T., Cregg, J. M., DePaul, M. A., Tran, A. P., Xu, K., Dyck, S. M., Madalena, K. M., Brown, B. P., Weng, Y. L., Li, S., Karimi-Abdolrezaee, S., Busch, S. A., Shen, Y. and Silver, J. (2015) Modulation of the proteoglycan receptor PTPsigma promotes recovery after spinal cord injury. Nature. **518**, 404-408
158 Condic, M. L. (2001) Adult neuronal regeneration induced by transgenic integrin expression. J Neurosci. **21**, 4782-4788
159 Hollis, E. R., 2nd, Jamshidi, P., Low, K., Blesch, A. and Tuszynski, M. H. (2009) Induction of corticospinal regeneration by lentiviral trkB-induced Erk activation. Proc Natl Acad Sci U S A. **106**, 7215-7220
160 Horn, K. E., Xu, B., Gobert, D., Hamam, B. N., Thompson, K. M., Wu, C. L., Bouchard, J. F., Uetani, N., Racine, R. J., Tremblay, M. L., Ruthazer, E. S., Chapman, C. A. and Kennedy, T. E. (2012) Receptor protein tyrosine phosphatase sigma regulates synapse structure, function and plasticity. J Neurochem. **122**, 147-161
161 Shen, Y., Tenney, A. P., Busch, S. A., Horn, K. P., Cuascut, F. X., Liu, K., He, Z., Silver, J. and Flanagan, J. G. (2009) PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. Science. **326**, 592-596
162 D'Alton, S. and Lewis, J. (2014) Understanding the role of progranulin in Alzheimer's disease. Nat Med. **20**, 1099-1100
163 Kortvelyessy, P., Gukasjan, A., Sweeney-Reed, C. M., Heinze, H. J., Thurner, L. and Bittner, D. M. (2015) Progranulin and Amyloid-beta Levels: Relationship to Neuropsychology in Frontotemporal and Alzheimer's Disease. J Alzheimers Dis. **46**, 375-380
164 Minami, S. S., Min, S. W., Krabbe, G., Wang, C., Zhou, Y., Asgarov, R., Li, Y., Martens, L. H., Elia, L. P., Ward, M. E., Mucke, L., Farese, R. V., Jr. and Gan, L. (2014) Progranulin protects against amyloid beta deposition and toxicity in Alzheimer's disease mouse models. Nat Med. **20**, 1157-1164
165 Zhao, Y., Wei, Z. Z., Zhang, J. Y., Zhang, Y., Won, S., Sun, J., Yu, S. P., Li, J. and Wei, L. (2017) GSK-3beta Inhibition Induced Neuroprotection, Regeneration, and Functional Recovery After Intracerebral Hemorrhagic Stroke. Cell Transplant. **26**, 395-407
166 King, M. K., Pardo, M., Cheng, Y., Downey, K., Jope, R. S. and Beurel, E. (2014) Glycogen synthase kinase-3 inhibitors: Rescuers of cognitive impairments. Pharmacol Ther. **141**, 1-12
167 Yang, W. J., Chen, W., Chen, L., Guo, Y. J., Zeng, J. S., Li, G. Y. and Tong, W. S. (2017) Involvement of tau phosphorylation in traumatic brain injury patients. Acta Neurol Scand. **135**, 622-627

168 Chu, J., Lauretti, E. and Pratico, D. (2017) Caspase-3-dependent cleavage of Akt modulates tau phosphorylation via GSK3beta kinase: implications for Alzheimer's disease. Mol Psychiatry. **22**, 1002-1008

169 Whitelock, J. M., Melrose, J. and Iozzo, R. V. (2008) Diverse cell signaling events modulated by perlecan. Biochemistry. **47**, 11174-11183

170 Hikino, M., Mikami, T., Faissner, A., Vilela-Silva, A. C., Pavao, M. S. and Sugahara, K. (2003) Oversulfated dermatan sulfate exhibits neurite outgrowth-promoting activity toward embryonic mouse hippocampal neurons: implications of dermatan sulfate in neuritogenesis in the brain. J Biol Chem. **278**, 43744-43754

171 Nadanaka, S., Clement, A., Masayama, K., Faissner, A. and Sugahara, K. (1998) Characteristic hexasaccharide sequences in octasaccharides derived from shark cartilage chondroitin sulfate D with a neurite outgrowth promoting activity. J Biol Chem. **273**, 3296-3307

172 Kinoshita, A., Yamada, S., Haslam, S. M., Morris, H. R., Dell, A. and Sugahara, K. (1997) Novel tetrasaccharides isolated from squid cartilage chondroitin sulfate E contain unusual sulfated disaccharide units GlcA(3-O-sulfate)beta1-3GalNAc(6-O-sulfate) or GlcA(3-O-sulfate)beta1-3GalNAc. J Biol Chem. **272**, 19656-19665

173 Kinoshita, A., Yamada, S., Haslam, S. M., Morris, H. R., Dell, A. and Sugahara, K. (2001) Isolation and structural determination of novel sulfated hexasaccharides from squid cartilage chondroitin sulfate E that exhibits neuroregulatory activities. Biochemistry. **40**, 12654-12665

174 Miyachi, K., Wakao, M. and Suda, Y. (2015) Syntheses of chondroitin sulfate tetrasaccharide structures containing 4,6-disulfate patterns and analysis of their interaction with glycosaminoglycan-binding protein. Bioorg Med Chem Lett. **25**, 1552-1555

175 Tully, S. E., Mabon, R., Gama, C. I., Tsai, S. M., Liu, X. and Hsieh-Wilson, L. C. (2004) A chondroitin sulfate small molecule that stimulates neuronal growth. J Am Chem Soc. **126**, 7736-7737

176 Matsui, F. and Oohira, A. (2004) Proteoglycans and injury of the central nervous system. Congenit Anom (Kyoto). **44**, 181-188

177 Edgar, D., Timpl, R. and Thoenen, H. (1984) The heparin-binding domain of laminin is responsible for its effects on neurite outgrowth and neuronal survival. EMBO J. **3**, 1463-1468

178 Al-Ahmad, A. J., Lee, B., Saini, M. and Bix, G. J. (2011) Perlecan domain V modulates astrogliosis in vitro and after focal cerebral ischemia through multiple receptors and increased nerve growth factor release. Glia. **59**, 1822-1840

179 Menzel, L., Kleber, L., Friedrich, C., Hummel, R., Dangel, L., Winter, J., Schmitz, K., Tegeder, I. and Schafer, M. K. (2017) Progranulin protects against exaggerated axonal injury and astrogliosis following traumatic brain injury. Glia. **65**, 278-292

180 Gonzalez, E. M., Mongiat, M., Slater, S. J., Baffa, R. and Iozzo, R. V. (2003) A novel interaction between perlecan protein core and progranulin: potential effects on tumor growth. J Biol Chem. **278**, 38113-38116

181 Tang, F., Lord, M. S., Stallcup, W. B. and Whitelock, J. M. (2018) Cell surface chondroitin sulfate proteoglycan 4 (CSPG4) binds to the basement membrane heparan sulfate proteoglycan, perlecan, and is involved in cell adhesion. J Biochem

182 Lee, B., Clarke, D., Al Ahmad, A., Kahle, M., Parham, C., Auckland, L., Shaw, C., Fidanboylu, M., Orr, A. W., Ogunshola, O., Fertala, A., Thomas, S. A. and Bix, G. J. (2011) Perlecan domain V is neuroprotective and proangiogenic following ischemic stroke in rodents. J Clin Invest. **121**, 3005-3023

183 Han, Y. G. (2016) Sonic hedgehog signaling: A conserved mechanism for the expansion of outer radial glia and intermediate progenitor cells and for the growth and folding of the neocortex. Neurogenesis (Austin). **3**, e1242957

184 Jia, J. and Jiang, J. (2006) Decoding the Hedgehog signal in animal development. Cell Mol Life Sci. **63**, 1249-1265

185 Tichy, J., Zinke, J., Bunz, B., Meyermann, R., Harter, P. N. and Mittelbronn, M. (2015) Expression Profile of Sonic Hedgehog Pathway Members in the Developing Human Fetal Brain. Biomed Res Int. **2015**, 494269

186 Ugbode, C. I., Smith, I., Whalley, B. J., Hirst, W. D. and Rattray, M. (2017) Sonic hedgehog signalling mediates astrocyte crosstalk with neurons to confer neuroprotection. J Neurochem. **142**, 429-443

187 Filmus, J. and Capurro, M. (2014) The role of glypicans in Hedgehog signaling. Matrix Biol. **35**, 248-252

188 Whalen, D. M., Malinauskas, T., Gilbert, R. J. and Siebold, C. (2013) Structural insights into proteoglycan-shaped Hedgehog signaling. Proc Natl Acad Sci U S A. **110**, 16420-16425
189 Pepinsky, R. B., Zeng, C., Wen, D., Rayhorn, P., Baker, D. P., Williams, K. P., Bixler, S. A., Ambrose, C. M., Garber, E. A., Miatkowski, K., Taylor, F. R., Wang, E. A. and Galdes, A. (1998) Identification of a palmitic acid-modified form of human Sonic hedgehog. J Biol Chem. **273**, 14037-14045
190 Gritli-Linde, A., Lewis, P., McMahon, A. P. and Linde, A. (2001) The whereabouts of a morphogen: direct evidence for short- and graded long-range activity of hedgehog signaling peptides. Dev Biol. **236**, 364-386
191 Porter, J. A., Young, K. E. and Beachy, P. A. (1996) Cholesterol modification of hedgehog signaling proteins in animal development. Science. **274**, 255-259
192 Porter, J. A., Ekker, S. C., Park, W. J., von Kessler, D. P., Young, K. E., Chen, C. H., Ma, Y., Woods, A. S., Cotter, R. J., Koonin, E. V. and Beachy, P. A. (1996) Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. Cell. **86**, 21-34
193 Alvarez-Buylla, A. and Ihrie, R. A. (2014) Sonic hedgehog signaling in the postnatal brain. Semin Cell Dev Biol. **33**, 105-111
194 Araujo, G. L., Araujo, J. A., Schroeder, T., Tort, A. B. and Costa, M. R. (2014) Sonic hedgehog signaling regulates mode of cell division of early cerebral cortex progenitors and increases astrogliogenesis. Front Cell Neurosci. **8**, 77
195 Ferent, J., Zimmer, C., Durbec, P., Ruat, M. and Traiffort, E. (2013) Sonic Hedgehog signaling is a positive oligodendrocyte regulator during demyelination. J Neurosci. **33**, 1759-1772
196 Patel, S. S., Tomar, S., Sharma, D., Mahindroo, N. and Udayabanu, M. (2017) Targeting sonic hedgehog signaling in neurological disorders. Neurosci Biobehav Rev. **74**, 76-97
197 Kurosaka, H., Trainor, P. A., Leroux-Berger, M. and Iulianella, A. (2015) Cranial nerve development requires co-ordinated Shh and canonical Wnt signaling. PLoS One. **10**, e0120821
198 Wang, L. C. and Almazan, G. (2016) Role of Sonic Hedgehog Signaling in Oligodendrocyte Differentiation. Neurochem Res. **41**, 3289-3299
199 Farmer, W. T., Abrahamsson, T., Chierzi, S., Lui, C., Zaelzer, C., Jones, E. V., Bally, B. P., Chen, G. G., Theroux, J. F., Peng, J., Bourque, C. W., Charron, F., Ernst, C., Sjostrom, P. J. and Murai, K. K. (2016) Neurons diversify astrocytes in the adult brain through sonic hedgehog signaling. Science. **351**, 849-854
200 Feng, S., Ma, S., Jia, C., Su, Y., Yang, S., Zhou, K., Liu, Y., Cheng, J., Lu, D., Fan, L. and Wang, Y. (2016) Sonic hedgehog is a regulator of extracellular glutamate levels and epilepsy. EMBO Rep. **17**, 682-694
201 Okuda, H., Tatsumi, K., Morita-Takemura, S., Nakahara, K., Nochioka, K., Shinjo, T., Terada, Y. and Wanaka, A. (2016) Hedgehog Signaling Modulates the Release of Gliotransmitters from Cultured Cerebellar Astrocytes. Neurochem Res. **41**, 278-289
202 Djerbal, L., Lortat-Jacob, H. and Kwok, J. (2017) Chondroitin sulfates and their binding molecules in the central nervous system. Glycoconj J. **34**, 363-376
203 Properzi, F., Asher, R. A. and Fawcett, J. W. (2003) Chondroitin sulphate proteoglycans in the central nervous system: changes and synthesis after injury. Biochem Soc Trans. **31**, 335-336
204 Sugahara, K. and Mikami, T. (2007) Chondroitin/dermatan sulfate in the central nervous system. Curr Opin Struct Biol. **17**, 536-545
205 Stichel, C. C., Kappler, J., Junghans, U., Koops, A., Kresse, H. and Muller, H. W. (1995) Differential expression of the small chondroitin/dermatan sulfate proteoglycans decorin and biglycan after injury of the adult rat brain. Brain Res. **704**, 263-274
206 Cramer, K. S. and Miko, I. J. (2016) Eph-ephrin signaling in nervous system development. F1000Res. **5**
207 Singh, A., Winterbottom, E. and Daar, I. O. (2012) Eph/ephrin signaling in cell-cell and cell-substrate adhesion. Front Biosci (Landmark Ed). **17**, 473-497
208 Laussu, J., Khuong, A., Gautrais, J. and Davy, A. (2014) Beyond boundaries--Eph:ephrin signaling in neurogenesis. Cell Adh Migr. **8**, 349-359
209 Wilkinson, D. G. (2014) Regulation of cell differentiation by Eph receptor and ephrin signaling. Cell Adh Migr. **8**, 339-348
210 Tang, W., Lu, Y., Tian, Q. Y., Zhang, Y., Guo, F. J., Liu, G. Y., Syed, N. M., Lai, Y., Lin, E. A., Kong, L., Su, J., Yin, F., Ding, A. H., Zanin-Zhorov, A., Dustin, M. L., Tao, J., Craft, J., Yin, Z., Feng, J. Q., Abramson, S. B., Yu, X. P. and Liu, C. J. (2011) The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science. **332**, 478-484

211 Neill, T., Buraschi, S., Goyal, A., Sharpe, C., Natkanski, E., Schaefer, L., Morrione, A. and Iozzo, R. V. (2016) EphA2 is a functional receptor for the growth factor progranulin. J Cell Biol. **215**, 687-703
212 Blasiak, A., Kilinc, D. and Lee, G. U. (2016) Neuronal Cell Bodies Remotely Regulate Axonal Growth Response to Localized Netrin-1 Treatment via Second Messenger and DCC Dynamics. Front Cell Neurosci. **10**, 298
213 Poliak, S., Morales, D., Croteau, L. P., Krawchuk, D., Palmesino, E., Morton, S., Cloutier, J. F., Charron, F., Dalva, M. B., Ackerman, S. L., Kao, T. J. and Kania, A. (2015) Synergistic integration of Netrin and ephrin axon guidance signals by spinal motor neurons. Elife. **4**
214 Mitsogiannis, M. D., Little, G. E. and Mitchell, K. J. (2017) Semaphorin-Plexin signaling influences early ventral telencephalic development and thalamocortical axon guidance. Neural Dev. **12**, 6
215 Chwalek, K., Dening, Y., Hinuber, C., Brunig, H., Nitschke, M. and Werner, C. (2016) Providing the right cues in nerve guidance conduits: Biofunctionalization versus fiber profile to facilitate oriented neuronal outgrowth. Mater Sci Eng C Mater Biol Appl. **61**, 466-472
216 Gopal, A. A., Ricoult, S. G., Harris, S. N., Juncker, D., Kennedy, T. E. and Wiseman, P. W. (2017) Spatially Selective Dissection of Signal Transduction in Neurons Grown on Netrin-1 Printed Nanoarrays via Segmented Fluorescence Fluctuation Analysis. ACS Nano. **11**, 8131-8143
217 Huang, L., Zhu, L., Shi, X., Xia, B., Liu, Z., Zhu, S., Yang, Y., Ma, T., Cheng, P., Luo, K., Huang, J. and Luo, Z. (2018) A compound scaffold with uniform longitudinally oriented guidance cues and a porous sheath promotes peripheral nerve regeneration in vivo. Acta Biomater. **68**, 223-236
218 Jenkins, P. M., Laughter, M. R., Lee, D. J., Lee, Y. M., Freed, C. R. and Park, D. (2015) A nerve guidance conduit with topographical and biochemical cues: potential application using human neural stem cells. Nanoscale Res Lett. **10**, 972
219 Sarker, M., Naghieh, S., McInnes, A. D., Schreyer, D. J. and Chen, X. (2018) Strategic Design and Fabrication of Nerve Guidance Conduits for Peripheral Nerve Regeneration. Biotechnol J
220 Aguirre, A., Dupree, J. L., Mangin, J. M. and Gallo, V. (2007) A functional role for EGFR signaling in myelination and remyelination. Nat Neurosci. **10**, 990-1002
221 Campoli, M., Ferrone, S. and Wang, X. (2010) Functional and clinical relevance of chondroitin sulfate proteoglycan 4. Adv Cancer Res. **109**, 73-121
222 Buss, A., Pech, K., Kakulas, B. A., Martin, D., Schoenen, J., Noth, J. and Brook, G. A. (2009) NG2 and phosphacan are present in the astroglial scar after human traumatic spinal cord injury. BMC Neurol. **9**, 32
223 Petkau, T. L. and Leavitt, B. R. (2014) Progranulin in neurodegenerative disease. Trends Neurosci. **37**, 388-398
224 Eugenin-von Bernhardi, J. and Dimou, L. (2016) NG2-glia, More Than Progenitor Cells. Adv Exp Med Biol. **949**, 27-45
225 Kucharova, K. and Stallcup, W. B. (2017) Distinct NG2 proteoglycan-dependent roles of resident microglia and bone marrow-derived macrophages during myelin damage and repair. PLoS One. **12**, e0187530
226 Lama, G., Mangiola, A., Proietti, G., Colabianchi, A., Angelucci, C., A, D. A., De Bonis, P., Geloso, M. C., Lauriola, L., Binda, E., Biamonte, F., Giuffrida, M. G., Vescovi, A. and Sica, G. (2016) Progenitor/Stem Cell Markers in Brain Adjacent to Glioblastoma: GD3 Ganglioside and NG2 Proteoglycan Expression. J Neuropathol Exp Neurol. **75**, 134-147
227 Yadavilli, S., Hwang, E. I., Packer, R. J. and Nazarian, J. (2016) The Role of NG2 Proteoglycan in Glioma. Transl Oncol. **9**, 57-63
228 Gao, Q., Lu, J., Huo, Y., Baby, N., Ling, E. A. and Dheen, S. T. (2010) NG2, a member of chondroitin sulfate proteoglycans family mediates the inflammatory response of activated microglia. Neuroscience. **165**, 386-394
229 Seyfried, N. T., Huysentruyt, L. C., Atwood, J. A., 3rd, Xia, Q., Seyfried, T. N. and Orlando, R. (2008) Up-regulation of NG2 proteoglycan and interferon-induced transmembrane proteins 1 and 3 in mouse astrocytoma: a membrane proteomics approach. Cancer Lett. **263**, 243-252
230 Pellegatta, S., Savoldo, B., Di Ianni, N., Corbetta, C., Chen, Y., Patane, M., Sun, C., Pollo, B., Ferrone, S., DiMeco, F., Finocchiaro, G. and Dotti, G. (2018) Constitutive and TNFalpha-inducible expression of chondroitin sulfate proteoglycan 4 in glioblastoma and neurospheres: Implications for CAR-T cell therapy. Sci Transl Med. **10**
231 Dimou, L. and Gallo, V. (2015) NG2-glia and their functions in the central nervous system. Glia. **63**, 1429-1451

232 Dimou, L. and Gotz, M. (2014) Glial cells as progenitors and stem cells: new roles in the healthy and diseased brain. Physiol Rev. **94**, 709-737

233 Zuo, J., Neubauer, D., Graham, J., Krekoski, C. A., Ferguson, T. A. and Muir, D. (2002) Regeneration of axons after nerve transection repair is enhanced by degradation of chondroitin sulfate proteoglycan. Exp Neurol. **176**, 221-228

234 Groves, M. L., McKeon, R., Werner, E., Nagarsheth, M., Meador, W. and English, A. W. (2005) Axon regeneration in peripheral nerves is enhanced by proteoglycan degradation. Exp Neurol. **195**, 278-292

235 Li, H. P., Komuta, Y., Kimura-Kuroda, J., van Kuppevelt, T. H. and Kawano, H. (2013) Roles of chondroitin sulfate and dermatan sulfate in the formation of a lesion scar and axonal regeneration after traumatic injury of the mouse brain. J Neurotrauma. **30**, 413-425

236 Massey, J. M., Hubscher, C. H., Wagoner, M. R., Decker, J. A., Amps, J., Silver, J. and Onifer, S. M. (2006) Chondroitinase ABC digestion of the perineuronal net promotes functional collateral sprouting in the cuneate nucleus after cervical spinal cord injury. J Neurosci. **26**, 4406-4414

237 Moon, L. D., Asher, R. A., Rhodes, K. E. and Fawcett, J. W. (2001) Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat Neurosci. **4**, 465-466

238 Graham, J. B. and Muir, D. (2016) Chondroitinase C Selectively Degrades Chondroitin Sulfate Glycosaminoglycans that Inhibit Axonal Growth within the Endoneurium of Peripheral Nerve. PLoS One. **11**, e0167682

239 Lemarchant, S., Pruvost, M., Hebert, M., Gauberti, M., Hommet, Y., Briens, A., Maubert, E., Gueye, Y., Feron, F., Petite, D., Mersel, M., do Rego, J. C., Vaudry, H., Koistinaho, J., Ali, C., Agin, V., Emery, E. and Vivien, D. (2014) tPA promotes ADAMTS-4-induced CSPG degradation, thereby enhancing neuroplasticity following spinal cord injury. Neurobiol Dis. **66**, 28-42

240 Tauchi, R., Imagama, S., Natori, T., Ohgomori, T., Muramoto, A., Shinjo, R., Matsuyama, Y., Ishiguro, N. and Kadomatsu, K. (2012) The endogenous proteoglycan-degrading enzyme ADAMTS-4 promotes functional recovery after spinal cord injury. J Neuroinflammation. **9**, 53

241 Krautstrunk, M., Scholtes, F., Martin, D., Schoenen, J., Schmitt, A. B., Plate, D., Nacimiento, W., Noth, J. and Brook, G. A. (2002) Increased expression of the putative axon growth-repulsive extracellular matrix molecule, keratan sulphate proteoglycan, following traumatic injury of the adult rat spinal cord. Acta Neuropathol. **104**, 592-600

242 Zhang, H., Uchimura, K. and Kadomatsu, K. (2006) Brain keratan sulfate and glial scar formation. Ann N Y Acad Sci. **1086**, 81-90

243 Mizumoto, S. and Sugahara, K. (2013) Glycosaminoglycans are functional ligands for receptor for advanced glycation end-products in tumors. FEBS J. **280**, 2462-2470

244 Mizumoto, S., Takahashi, J. and Sugahara, K. (2012) Receptor for advanced glycation end products (RAGE) functions as receptor for specific sulfated glycosaminoglycans, and anti-RAGE antibody or sulfated glycosaminoglycans delivered in vivo inhibit pulmonary metastasis of tumor cells. J Biol Chem. **287**, 18985-18994

245 Batkulwar, K., Godbole, R., Banarjee, R., Kassaar, O., Williams, R. J. and Kulkarni, M. J. (2018) Advanced Glycation End Products Modulate Amyloidogenic APP Processing and Tau Phosphorylation: A Mechanistic Link between Glycation and the Development of Alzheimer's Disease. ACS Chem Neurosci

246 Jandial, R., Neman, J., Lim, P. P., Tamae, D., Kowolik, C. M., Wuenschell, G. E., Shuck, S. C., Ciminera, A. K., De Jesus, L. R., Ouyang, C., Chen, M. Y. and Termini, J. (2018) Inhibition of GLO1 in Glioblastoma Multiforme Increases DNA-AGEs, Stimulates RAGE Expression, and Inhibits Brain Tumor Growth in Orthotopic Mouse Models. Int J Mol Sci. **19**

247 Fang, F., Yu, Q., Arancio, O., Chen, D., Gore, S. S., Yan, S. S. and Yan, S. F. (2018) RAGE mediates Abeta accumulation in a mouse model of Alzheimer's disease via modulation of beta- and gamma-secretase activity. Hum Mol Genet. **27**, 1002-1014

248 Wang, H., Chen, F., Du, Y. F., Long, Y., Reed, M. N., Hu, M., Suppiramaniam, V., Hong, H. and Tang, S. S. (2018) Targeted inhibition of RAGE reduces amyloid-beta influx across the blood-brain barrier and improves cognitive deficits in db/db mice. Neuropharmacology. **131**, 143-153

249 Bortolotto, V. and Grilli, M. (2016) Every Cloud Has a Silver Lining: Proneurogenic Effects of Abeta Oligomers and HMGB-1 via Activation of the RAGE-NF-kappaB Axis. CNS Neurol Disord Drug Targets

250 Meneghini, V., Bortolotto, V., Francese, M. T., Dellarole, A., Carraro, L., Terzieva, S. and Grilli, M. (2013) High-mobility group box-1 protein and beta-amyloid oligomers promote neuronal

differentiation of adult hippocampal neural progenitors via receptor for advanced glycation end products/nuclear factor-kappaB axis: relevance for Alzheimer's disease. J Neurosci. **33**, 6047-6059
251 Son, S., Hwang, I., Han, S. H., Shin, J. S., Shin, O. S. and Yu, J. W. (2017) Advanced glycation end products impair NLRP3 inflammasome-mediated innate immune responses in macrophages. J Biol Chem. **292**, 20437-20448
252 Holt, C. E. and Dickson, B. J. (2005) Sugar codes for axons? Neuron. **46**, 169-172
253 Rose, S. P. (1995) Cell-adhesion molecules, glucocorticoids and long-term-memory formation. Trends Neurosci. **18**, 502-506
254 Rose, S. P. (1995) Glycoproteins and memory formation. Behav Brain Res. **66**, 73-78
255 Kalovidouris, S. A., Gama, C. I., Lee, L. W. and Hsieh-Wilson, L. C. (2005) A role for fucose alpha(1-2) galactose carbohydrates in neuronal growth. J Am Chem Soc. **127**, 1340-1341
256 Murrey, H. E., Gama, C. I., Kalovidouris, S. A., Luo, W. I., Driggers, E. M., Porton, B. and Hsieh-Wilson, L. C. (2006) Protein fucosylation regulates synapsin Ia/Ib expression and neuronal morphology in primary hippocampal neurons. Proc Natl Acad Sci U S A. **103**, 21-26
257 Bullock, S., Potter, J. and Rose, S. P. (1990) Effects of the amnesic agent 2-deoxygalactose on incorporation of fucose into chick brain glycoproteins. J Neurochem. **54**, 135-142
258 Krug, M., Jork, R., Reymann, K., Wagner, M. and Matthies, H. (1991) The amnesic substance 2-deoxy-D-galactose suppresses the maintenance of hippocampal LTP. Brain Res. **540**, 237-242
259 Gu, W., Fukuda, T., Isaji, T., Hang, Q., Lee, H. H., Sakai, S., Morise, J., Mitoma, J., Higashi, H., Taniguchi, N., Yawo, H., Oka, S. and Gu, J. (2015) Loss of alpha1,6-Fucosyltransferase Decreases Hippocampal Long Term Potentiation: IMPLICATIONS FOR CORE FUCOSYLATION IN THE REGULATION OF AMPA RECEPTOR HETEROMERIZATION AND CELLULAR SIGNALING. J Biol Chem. **290**, 17566-17575
260 Flogel, M., Lauc, G., Gornik, I. and Macek, B. (1998) Fucosylation and galactosylation of IgG heavy chains differ between acute and remission phases of juvenile chronic arthritis. Clin Chem Lab Med. **36**, 99-102
261 Gornik, I., Maravic, G., Dumic, J., Flogel, M. and Lauc, G. (1999) Fucosylation of IgG heavy chains is increased in rheumatoid arthritis. Clin Biochem. **32**, 605-608
262 Pucic, M., Knezevic, A., Vidic, J., Adamczyk, B., Novokmet, M., Polasek, O., Gornik, O., Supraha-Goreta, S., Wormald, M. R., Redzic, I., Campbell, H., Wright, A., Hastie, N. D., Wilson, J. F., Rudan, I., Wuhrer, M., Rudd, P. M., Josic, D. and Lauc, G. (2011) High throughput isolation and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations. Mol Cell Proteomics. **10**, M111 010090
263 Esser, L., Wang, C. R., Hosaka, M., Smagula, C. S., Sudhof, T. C. and Deisenhofer, J. (1998) Synapsin I is structurally similar to ATP-utilizing enzymes. EMBO J. **17**, 977-984
264 Gitler, D., Xu, Y., Kao, H. T., Lin, D., Lim, S., Feng, J., Greengard, P. and Augustine, G. J. (2004) Molecular determinants of synapsin targeting to presynaptic terminals. J Neurosci. **24**, 3711-3720
265 Jovanovic, J. N., Czernik, A. J., Fienberg, A. A., Greengard, P. and Sihra, T. S. (2000) Synapsins as mediators of BDNF-enhanced neurotransmitter release. Nat Neurosci. **3**, 323-329
266 Evergren, E., Benfenati, F. and Shupliakov, O. (2007) The synapsin cycle: a view from the synaptic endocytic zone. J Neurosci Res. **85**, 2648-2656
267 Vawter, M. P., Thatcher, L., Usen, N., Hyde, T. M., Kleinman, J. E. and Freed, W. J. (2002) Reduction of synapsin in the hippocampus of patients with bipolar disorder and schizophrenia. Mol Psychiatry. **7**, 571-578
268 Ferreira, A. and Rapoport, M. (2002) The synapsins: beyond the regulation of neurotransmitter release. Cell Mol Life Sci. **59**, 589-595
269 Gomez-Pinilla, F., So, V. and Kesslak, J. P. (2001) Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus. Brain Res. **904**, 13-19
270 John, J. P., Sunyer, B., Hoger, H., Pollak, A. and Lubec, G. (2009) Hippocampal synapsin isoform levels are linked to spatial memory enhancement by SGS742. Hippocampus. **19**, 731-738
271 Hilfiker, S., Pieribone, V. A., Czernik, A. J., Kao, H. T., Augustine, G. J. and Greengard, P. (1999) Synapsins as regulators of neurotransmitter release. Philos Trans R Soc Lond B Biol Sci. **354**, 269-279
272 Valtorta, F., Benfenati, F. and Greengard, P. (1992) Structure and function of the synapsins. J Biol Chem. **267**, 7195-7198
273 Becker, D. J. and Lowe, J. B. (2003) Fucose: biosynthesis and biological function in mammals. Glycobiology. **13**, 41R-53R
274 Listinsky, J. J., Siegal, G. P. and Listinsky, C. M. (1998) Alpha-L-fucose: a potentially critical molecule in pathologic processes including neoplasia. Am J Clin Pathol. **110**, 425-440

275 Schneider, M., Al-Shareffi, E. and Haltiwanger, R. S. (2017) Biological functions of fucose in mammals. Glycobiology. **27**, 601-618
276 Nasir, W., Frank, M., Koppisetty, C. A., Larson, G. and Nyholm, P. G. (2012) Lewis histo-blood group alpha1,3/alpha1,4 fucose residues may both mediate binding to GII.4 noroviruses. Glycobiology. **22**, 1163-1172
277 Karus, M., Hennen, E., Safina, D., Klausmeyer, A., Wiese, S. and Faissner, A. (2013) Differential expression of micro-heterogeneous LewisX-type glycans in the stem cell compartment of the developing mouse spinal cord. Neurochem Res. **38**, 1285-1294
278 Scanlin, T. F. and Glick, M. C. (1999) Terminal glycosylation in cystic fibrosis. Biochim Biophys Acta. **1455**, 241-253
279 Yagi, H., Saito, T., Yanagisawa, M., Yu, R. K. and Kato, K. (2012) Lewis X-carrying N-glycans regulate the proliferation of mouse embryonic neural stem cells via the Notch signaling pathway. J Biol Chem. **287**, 24356-24364
280 Becker, D. J. and Lowe, J. B. (1999) Leukocyte adhesion deficiency type II. Biochim Biophys Acta. **1455**, 193-204
281 Wild, M. K., Luhn, K., Marquardt, T. and Vestweber, D. (2002) Leukocyte adhesion deficiency II: therapy and genetic defect. Cells Tissues Organs. **172**, 161-173
282 Leppanen, A., Yago, T., Otto, V. I., McEver, R. P. and Cummings, R. D. (2003) Model glycosulfopeptides from P-selectin glycoprotein ligand-1 require tyrosine sulfation and a core 2-branched O-glycan to bind to L-selectin. J Biol Chem. **278**, 26391-26400
283 Moore, K. L. (1998) Structure and function of P-selectin glycoprotein ligand-1. Leuk Lymphoma. **29**, 1-15
284 Stanley, P. (2007) Regulation of Notch signaling by glycosylation. Curr Opin Struct Biol. **17**, 530-535
285 Bakker, H. and Gerardy-Schahn, R. (2017) A sweet development in Notch regulation. J Biol Chem. **292**, 15974-15975
286 Lu, L. and Stanley, P. (2006) Roles of O-fucose glycans in notch signaling revealed by mutant mice. Methods Enzymol. **417**, 127-136
287 Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. J. (1999) Notch signaling: cell fate control and signal integration in development. Science. **284**, 770-776
288 Alexson, T. O., Hitoshi, S., Coles, B. L., Bernstein, A. and van der Kooy, D. (2006) Notch signaling is required to maintain all neural stem cell populations--irrespective of spatial or temporal niche. Dev Neurosci. **28**, 34-48
289 Androutsellis-Theotokis, A., Leker, R. R., Soldner, F., Hoeppner, D. J., Ravin, R., Poser, S. W., Rueger, M. A., Bae, S. K., Kittappa, R. and McKay, R. D. (2006) Notch signalling regulates stem cell numbers in vitro and in vivo. Nature. **442**, 823-826
290 Kansas, G. S. (1996) Selectins and their ligands: current concepts and controversies. Blood. **88**, 3259-3287
291 Vestweber, D. and Blanks, J. E. (1999) Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev. **79**, 181-213
292 Solter, D. and Knowles, B. B. (1978) Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). Proc Natl Acad Sci U S A. **75**, 5565-5569
293 Ashwell, K. W. and Mai, J. K. (1997) Developmental expression of the CD15 epitope in the hippocampus of the mouse. Cell Tissue Res. **289**, 17-23
294 Ashwell, K. W. and Mai, J. K. (1997) Developmental expression of the CD15-epitope in the brainstem and spinal cord of the mouse. Anat Embryol (Berl). **196**, 13-25
295 Ashwell, K. W. and Mai, J. K. (1997) A transient CD15 immunoreactive sling in the developing mouse cerebellum. Int J Dev Neurosci. **15**, 883-889
296 Ashwell, K. W. and Mai, J. K. (1997) Transient developmental expression of CD15 in the motor and auditory cortex of the mouse. Brain Res Dev Brain Res. **100**, 143-148
297 Shimoda, Y., Tajima, Y., Osanai, T., Katsume, A., Kohara, M., Kudo, T., Narimatsu, H., Takashima, N., Ishii, Y., Nakamura, S., Osumi, N. and Sanai, Y. (2002) Pax6 controls the expression of Lewis x epitope in the embryonic forebrain by regulating alpha 1,3-fucosyltransferase IX expression. J Biol Chem. **277**, 2033-2039
298 Kudo, T., Ikehara, Y., Togayachi, A., Kaneko, M., Hiraga, T., Sasaki, K. and Narimatsu, H. (1998) Expression cloning and characterization of a novel murine alpha1, 3-fucosyltransferase, mFuc-TIX, that synthesizes the Lewis x (CD15) epitope in brain and kidney. J Biol Chem. **273**, 26729-26738

299 Bird, J. M. and Kimber, S. J. (1984) Oligosaccharides containing fucose linked alpha(1-3) and alpha(1-4) to N-acetylglucosamine cause decompaction of mouse morulae. Dev Biol. **104**, 449-460

300 Fenderson, B. A., Zehavi, U. and Hakomori, S. (1984) A multivalent lacto-N-fucopentaose III-lysyllysine conjugate decompacts preimplantation mouse embryos, while the free oligosaccharide is ineffective. J Exp Med. **160**, 1591-1596

301 Huang, T., Ohzu, E, Yananagimachi, R. (1982) Evidence suggesting that L‐fucose is part of a recognition signal for sperm‐zona pellucida attachment in mammals. Molecular Reproduction and Development. **5**

302 Aplin, J. D. (1999) MUC-1 glycosylation in endometrium: possible roles of the apical glycocalyx at implantation. Hum Reprod. **14 Suppl 2**, 17-25

303 Aplin, J. D. (2007) Embryo implantation: the molecular mechanism remains elusive. Reprod Biomed Online. **14 Spec No 1**, 49-55

304 Jones, C. J., Wooding, F. B., Abd-Elnaeim, M. M., Leiser, R., Dantzer, V. and Stoddart, R. W. (2000) Glycosylation in the near-term epitheliochorial placenta of the horse, donkey and camel: a comparative study of interbreeding and non-interbreeding species. J Reprod Fertil. **118**, 397-405

305 Jones, C. J., Fazleabas, A. T., McGinlay, P. B. and Aplin, J. D. (1998) Cyclic modulation of epithelial glycosylation in human and baboon (Papio anubis) endometrium demonstrated by the binding of the agglutinin from Dolichos biflorus. Biol Reprod. **58**, 20-27

306 Miller, D. L., Jones, C. J., Aplin, J. D. and Nardo, L. G. (2010) Altered glycosylation in peri-implantation phase endometrium in women with stages III and IV endometriosis. Hum Reprod. **25**, 406-411

307 Aplin, J. D. and Kimber, S. J. (2004) Trophoblast-uterine interactions at implantation. Reprod Biol Endocrinol. **2**, 48

308 Genbacev, O. D., Prakobphol, A., Foulk, R. A., Krtolica, A. R., Ilic, D., Singer, M. S., Yang, Z. Q., Kiessling, L. L., Rosen, S. D. and Fisher, S. J. (2003) Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. Science. **299**, 405-408

309 Harris, L. K., Jones, C. J. and Aplin, J. D. (2009) Adhesion molecules in human trophoblast - a review. II. extravillous trophoblast. Placenta. **30**, 299-304

310 Liu, S., Yang, X., Liu, Y., Wang, X. and Yan, Q. (2011) sLeX/L-selectin mediates adhesion in vitro implantation model. Mol Cell Biochem. **350**, 185-192

311 Zhang, Y., Liu, S., Liu, Y., Wang, Z., Wang, X. and Yan, Q. (2009) Overexpression of fucosyltransferase VII (FUT7) promotes embryo adhesion and implantation. Fertil Steril. **91**, 908-914

312 Chen, J., Moloney, D. J. and Stanley, P. (2001) Fringe modulation of Jagged1-induced Notch signaling requires the action of beta 4galactosyltransferase-1. Proc Natl Acad Sci U S A. **98**, 13716-13721

313 Moloney, D. J., Panin, V. M., Johnston, S. H., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K. D., Haltiwanger, R. S. and Vogt, T. F. (2000) Fringe is a glycosyltransferase that modifies Notch. Nature. **406**, 369-375

314 Kim, Y. J. and Varki, A. (1997) Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glycoconj J. **14**, 569-576

315 Lee, J. S., Ro, J. Y., Sahin, A. A., Hong, W. K., Brown, B. W., Mountain, C. F. and Hittelman, W. N. (1991) Expression of blood-group antigen A--a favorable prognostic factor in non-small-cell lung cancer. N Engl J Med. **324**, 1084-1090

316 Varki, A. (1999) Acquired glycosylation changes in human disease. . In Essentials of glycobiology. (Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., and Marth, J. (Eds), ed.). pp. 565-580, Cold Spring Harbor Laboratory Press, New York

317 Valcarcel, J., Novoa-Carballal, R., Perez-Martin, R. I., Reis, R. L. and Vazquez, J. A. (2017) Glycosaminoglycans from marine sources as therapeutic agents. Biotechnol Adv. **35**, 711-725

318 Pomin, V. H. (2014) Holothurian fucosylated chondroitin sulfate. Mar Drugs. **12**, 232-254

319 Pomin, V. H. (2015) Medical Gains of Chondroitin Sulfate Upon Fucosylation. Curr Med Chem. **22**, 4166-4176

320 Mourao, P. A., Pereira, M. S., Pavao, M. S., Mulloy, B., Tollefsen, D. M., Mowinckel, M. C. and Abildgaard, U. (1996) Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm. Sulfated fucose branches on the polysaccharide account for its high anticoagulant action. J Biol Chem. **271**, 23973-23984

321 Mourao, P. A., Giumaraes, B., Mulloy, B., Thomas, S. and Gray, E. (1998) Antithrombotic activity of a fucosylated chondroitin sulphate from echinoderm: sulphated fucose branches on the polysaccharide account for its antithrombotic action. Br J Haematol. **101**, 647-652

322 Zhang, X., Yao, W., Xu, X., Sun, H., Zhao, J., Meng, X., Wu, M. and Li, Z. (2017) Synthesis of Fucosylated Chondroitin Sulfate Glycoclusters: A Robust Route to New Anticoagulant Agents. Chemistry

323 Agyekum, I., Pepi, L., Yu, Y., Li, J., Yan, L., Linhardt, R. J., Chen, S. and Amster, I. J. (2018) Structural elucidation of fucosylated chondroitin sulfates from sea cucumber using FTICR-MS/MS. Eur J Mass Spectrom (Chichester). **24**, 157-167

324 Ustyuzhanina, N. E., Bilan, M. I., Dmitrenok, A. S., Shashkov, A. S., Nifantiev, N. E. and Usov, A. I. (2017) The structure of a fucosylated chondroitin sulfate from the sea cucumber Cucumaria frondosa. Carbohydr Polym. **165**, 7-12

325 Ustyuzhanina, N. E., Bilan, M. I., Dmitrenok, A. S., Tsvetkova, E. A., Shashkov, A. S., Stonik, V. A., Nifantiev, N. E. and Usov, A. I. (2016) Structural characterization of fucosylated chondroitin sulfates from sea cucumbers Apostichopus japonicus and Actinopyga mauritiana. Carbohydr Polym. **153**, 399-405

326 Borsig, L., Wang, L., Cavalcante, M. C., Cardilo-Reis, L., Ferreira, P. L., Mourao, P. A., Esko, J. D. and Pavao, M. S. (2007) Selectin blocking activity of a fucosylated chondroitin sulfate glycosaminoglycan from sea cucumber. Effect on tumor metastasis and neutrophil recruitment. J Biol Chem. **282**, 14984-14991

327 Panagos, C. G., Thomson, D. S., Moss, C., Hughes, A. D., Kelly, M. S., Liu, Y., Chai, W., Venkatasamy, R., Spina, D., Page, C. P., Hogwood, J., Woods, R. J., Mulloy, B., Bavington, C. D. and Uhrin, D. (2014) Fucosylated chondroitin sulfates from the body wall of the sea cucumber Holothuria forskali: conformation, selectin binding, and biological activity. J Biol Chem. **289**, 28284-28298

328 Ben Mansour, M., Balti, R., Ollivier, V., Ben Jannet, H., Chaubet, F. and Maaroufi, R. M. (2017) Characterization and anticoagulant activity of a fucosylated chondroitin sulfate with unusually procoagulant effect from sea cucumber. Carbohydr Polym. **174**, 760-771

329 Li, X., Luo, L., Cai, Y., Yang, W., Lin, L., Li, Z., Gao, N., Purcell, S. W., Wu, M. and Zhao, J. (2017) Structural Elucidation and Biological Activity of a Highly Regular Fucosylated Glycosaminoglycan from the Edible Sea Cucumber Stichopus herrmanni. J Agric Food Chem. **65**, 9315-9323

330 Ustyuzhanina, N. E., Bilan, M. I., Dmitrenok, A. S., Borodina, E. Y., Stonik, V. A., Nifantiev, N. E. and Usov, A. I. (2017) A highly regular fucosylated chondroitin sulfate from the sea cucumber Massinium magnum: Structure and effects on coagulation. Carbohydr Polym. **167**, 20-26

331 Ustyuzhanina, N. E., Bilan, M. I., Dmitrenok, A. S., Shashkov, A. S., Kusaykin, M. I., Stonik, V. A., Nifantiev, N. E. and Usov, A. I. (2016) Structure and biological activity of a fucosylated chondroitin sulfate from the sea cucumber Cucumaria japonica. Glycobiology. **26**, 449-459

332 Xu, H., Wang, J., Zhang, X., Li, Z., Wang, Y. and Xue, C. (2015) Inhibitory effect of fucosylated chondroitin sulfate from the sea cucumber Acaudina molpadioides on adipogenesis is dependent on Wnt/beta-catenin pathway. J Biosci Bioeng. **119**, 85-91

333 Zhang, Y., Sun, H., Qin, S., Song, Y., Si, Y., Hou, P., Yang, N. and Guo, S. (2017) Fucosylated Chondroitin Sulfate from Sea Cucumber Apostichopus japonicus Retards Atherosclerosis in Apolipoprotein E-deficient Mice. J Agric Food Chem

334 Anisimova, N., Ustyuzhanina, N., Bilan, M., Donenko, F., Usov, A., Kiselevskiy, M. and Nifantiev, N. (2017) Fucoidan and Fucosylated Chondroitin Sulfate Stimulate Hematopoiesis in Cyclophosphamide-Induced Mice. Mar Drugs. **15**

335 Shida, M., Mikami, T., Tamura, J. I. and Kitagawa, H. (2017) A characteristic chondroitin sulfate trisaccharide unit with a sulfated fucose branch exhibits neurite outgrowth-promoting activity: Novel biological roles of fucosylated chondroitin sulfates isolated from the sea cucumber Apostichopus japonicus. Biochem Biophys Res Commun. **487**, 678-683

336 Liu, X., Liu, Y., Hao, J., Zhao, X., Lang, Y., Fan, F., Cai, C., Li, G., Zhang, L. and Yu, G. (2016) In Vivo Anti-Cancer Mechanism of Low-Molecular-Weight Fucosylated Chondroitin Sulfate (LFCS) from Sea Cucumber Cucumaria frondosa. Molecules. **21**

337 Huang, N., Wu, M. Y., Zheng, C. B., Zhu, L., Zhao, J. H. and Zheng, Y. T. (2013) The depolymerized fucosylated chondroitin sulfate from sea cucumber potently inhibits HIV replication via interfering with virus entry. Carbohydr Res. **380**, 64-69

338 Janz, R., Goda, Y., Geppert, M., Missler, M. and Sudhof, T. C. (1999) SV2A and SV2B function as redundant Ca2+ regulators in neurotransmitter release. Neuron. **24**, 1003-1016

2034