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3 **9,934 WORDS, 10 FIGURES, 351 REFERENCES**
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Glycans and Glycosaminoglycans in neurobiology: key regulators of neuronal cell function and fate.

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49 **Key words:** glycode; glycan; bikunin; appican; phosphacan; fucose; glycosaminoglycan, lecticans,
50 PNS/CNS.
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52 **Short running head:** Glycans and Neural Function
53

54 **Abstract**

55

56 The aim of this study was to examine the roles of L-fucose and the glycosaminoglycans (GAGs) keratan

57 sulphate (KS) and chondroitin sulphate/dermatan sulphate (CS/DS) with selected functional molecules

58 in neural tissues. Cell surface glycans and GAGs have evolved over millions of years to become cellular

59 mediators which regulate fundamental aspects of cellular survival. The glycocalyx, which surrounds all

60 cells, actuates responses to growth factors, cytokines and morphogens at the cellular boundary

61 silencing or activating downstream signalling pathways and gene expression. In this review we have

62 focussed on interactions mediated by L-fucose, KS and CS/DS in the central and peripheral nervous

63 systems. Fucose makes critical contributions in the area of molecular recognition and information

64 transfer in the blood group substances, cytotoxic immunoglobulins, cell-fate mediated Notch-1

65 interactions, regulation of selectin mediated neutrophil extravasation in innate immunity and CD-34

66 mediated new blood vessel development and the targeting of neuroprogenitor cells to damaged neural

67 tissue. Fucosylated glycoproteins regulate delivery of synaptic neurotransmitters and neural function.

68 Neural KS-proteoglycans were examined in terms of cellular regulation and their interactive properties

69 with neuroregulatory molecules. The paradoxical properties of CS/DS isomers decorating matrix and

70 transmembrane proteoglycans and the positive and negative regulatory cues they provide to neurons

71 is also discussed.

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96	Abbreviations	
97		
98	AD	Alzheimer's disease
99	ADAM	a disintegrin and metalloproteinase domain
100	ADAM-TS	a disintegrin and metalloproteinase domain with thrombospondin motifs
101	ADCC	antibody dependent cellular cytotoxicity
102	AGE	advanced glycation end product
103	Akt	protein-kinase B
104	ALS	amyotrophic lateral sclerosis
105	APP	amyloid precursor protein
106	ATP	adenosine triphosphate
107	BDNF	brain derived neurotrophic factor
108	β 3GlcNAcT	β 1,3-N-acetylglucosaminyltransferase
109	CS	chondroitin sulphate
110	CSPG	chondroitin sulphate proteoglycan
111	CSL	an acronym for CBF-1/RBPJ (recombining binding protein
112		suppressor of hairless)
113	CNS	central nervous system
114	DCC	a receptor named <i>Deleted in Colorectal Cancer</i>
115	DRG	dorsal root ganglion
116	DS	dermatan sulphate
117	DS	dermatan sulphate proteoglycan
118	ECM	extracellular matrix
119	EGF	epidermal growth factor
120	EGFR	epidermal growth factor receptor
121	ER	endoplasmic reticulum
122	ERK	extracellular signal-regulated kinase
123	FGF	fibroblast growth factor
124	FGFR	fibroblast growth factor receptor
125	Fc γ RIIIa	activating Fc receptor specific for IgG Fc region expressed by
126		HNK cells and macrophages
127	FUT	fucosyl transferase
128	GAG	glycosaminoglycan
129	GlcNAc6ST	<i>n</i> -acetylglucosamine-6-O-sulfotransferase
130	GSK	glycogen synthase kinase
131	GTP	guanosine triphosphate
132	HMBG-1	high-mobility group box-1 protein
133	HNK	human natural killer
134	HS	heparan sulphate
135	HA	hyaluronan
136	IGD	interglobular domain
137	IGFBP2	insulin-like growth factor binding protein-2
138	IgG	immunoglobulin G
139	KS	keratan sulphate
140	KSPG	keratan sulphate proteoglycan
141	KSGal6ST	keratan sulfate galactose 6-O-sulfotransferase
142	LAD II	leukocyte adhesion deficiency II
143	LAR	leukocyte common antigen related
144	LC-MS	liquid chromatography-mass spectroscopy
145	LRR	leucine rich repeat
146	MAb	monoclonal antibody
147	MAPK	mitogen-activated protein kinase
148	NCAM	neural cell adhesion molecule
149	NG2	neural/glial antigen 2
150	NMR	nuclear magnetic resonance
151	2D MRS	two dimensional magnetic resonance spectroscopy
152	NG2	neural/glial antigen-2 (CSPG-4)

153	NGF	neural growth factor
154	PKA	cAMP dependent protein kinase
155	POFUT	GDP-fucose protein O-fucosyltransferase 1
156	POFUT	protein - fucosyl transferase
157	PG	proteoglycan
158	PNS	peripheral nervous system
159	PSGL-1	selectin-P ligand (CD162)
160	PTP σ	protein tyrosine phosphatase σ
161	RAGE	receptor for advanced glycation end products
162	RPTP- σ	receptor-like protein tyrosine phosphatase- σ
163	SCI	spinal cord injury
164	SHH	sonic hedge hog
165	sLeX	sialyl Lewis-X antigen
166	Trk B	tyrosine receptor kinase B
167	TGF- β	transforming growth factor- β
168	TNF- α	tumour necrosis factor- α
169	TSRs	thrombospondin repeats
170	SYN	synapsin
171	RPTP- ζ	receptor protein tyrosine phosphatase-zeta
172	Wnt	this is a condensation of terms describing the <i>Winged</i> and <i>Int</i> transcription factor morphogens
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175 1. Introduction

176 1.1 Aim

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

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189

190 1.2 Analysis of glycan and glycosaminoglycan complexity

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

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214 Analysis of the structural complexity of glycans has been considerably aided with the
215 development of software packages which simplify unambiguous representation of glycans and their
216 structural forms. These include GlycanBuilder [35], KCam[36], GlycResoft, a software package for
217 automated recognition of glycans from liquid chromatography-mass spectrometry (LC-MS) data[37],
218 KEGG Carbohydrate matcher (<http://www.genome.jp/ligand/kcam/>), SWEET-DB, annotated
219 carbohydrate data collections[38], DrawRINGS, 2D Glycan structure Drawing Tool
220 (<http://rings.t.soka.ac.jp/java/DrawRings.html>), LINUCS: linear notation for unique description of
221 carbohydrate sequences[39], GLYDE (<http://glycomics.ccr.c.uga.edu/GLYDE-CT/>) [40], EUROCarbDB
222 tools to normalise and convert glycan structures: Glycan builder
223 (<http://www.eurocarbdb.org/applications/structure-tools>) and analysis of MS spectra :
224 GlycoWorkbench (<http://www.eurocarbdb.org/applications/structure-ms-tools>). PROCARB is a
225 database of known and modelled carbohydrate binding protein structures with sequence based
226 prediction tools[41]. Establishment of the Consortium for Functional Glycomics (CFG,
227 <http://functionalglycomics.org/static/consortium/consortium.html>) in 2001 has aided glycan research
228 through the extensive, highly informative reference material readily available on their web-site.
229 Informatics tools are also available for the analysis of GAG structure[42] and conformation [43] and for
230 the determination of interactive GAG sequences [44-49]. Glycomics databases such as EuroCarbDB
231 (<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

232 Determinants [50] and software for the structural determination of GAGs by mass spectrometry [51],
 233 and for automated comparison of low molecular weight heparins from LC/MS data [52] have also
 234 been developed [51]. Nuclear magnetic resonance (NMR) spectroscopy has also been applied to the
 235 structural analysis of sulphated fucose-CS polymers [53]. Furthermore, novel high sensitivity, low
 236 toxicity alkynyl-fucose substrates have been developed for the visualisation of fucose incorporation
 237 into glycopolymers, these alkynyl-fucose substrates are incorporated into N-glycans by a wide range of
 238 fucosyl transferases[54] enabling their visualisation in cells using biotin-steptavidin Alexa-488
 239 histochemistry and they may be extracted, separated by SDS PAGE and identified by Western blotting
 240 [53]. The complexity of glycans surpasses by several magnitudes that of the other major life
 241 biomolecules, proteins, lipids and nucleic acids [9, 10, 21, 55, 56] and their analysis has lagged behind
 242 due to this complexity however with the improvement in glycan analysis now possible with the
 243 methodology outlined above this gap is steadily closing.
 244

245 Glycan biodiversity occurred over at least 500 million years of vertebrate and invertebrate
 246 evolution and an even longer evolutionary period in bacteria leading to their evolution as mediators of
 247 cellular interaction. Glycans occur in the glycocalyx of all cells and they are the first point of contact
 248 between that cell and other cells, with that cell and the extracellular matrix or with any invading
 249 organism. Thus there were heightened evolutionary pressures on these front-line glycans to develop
 250 recognition and effector roles, with this major positive selection stimulus glycans diversified into their
 251 present day level of complexity. The glyco-code could therefore be considered a biodiverse IT
 252 database which nature has developed over a very significant evolutionary period [57]. Thus many
 253 structural permutations were explored and those glycan structures that have persisted to the present
 254 day are ones which offer interactive capability with effector molecules in essential physiological
 255 processes providing improved survival traits. Deciphering this glyco-code using the sophisticated
 256 glycobiological methodology now available is an important research objective and may uncover
 257 invaluable insights as to how glycans regulate cells and be of application in repair biology.
 258

259 **2. The complexity of neural tissues**

260 *2.1 Cell types in the central and peripheral nervous system.*

261 Neurons and glial cells have a common neuro-epithelial origin in the embryonic nervous
 262 system and thus share many structural and molecular characteristics [58, 59]. Neurons and glial cells
 263 display unique properties which distinguish these cell types from others. Approximately 10% of all cells
 264 in the tissues of the central and peripheral nervous systems (CNS/PNS) are neurons. Accessory cell
 265 types also include astrocytes, radial glia, oligodendrocytes, ependymal cells, microglia and
 266 microvascular endothelial cells while neural/glial antigen 2 (NG2) positive glia are also considered to be
 267 a distinct cell type. Microglia are fundamentally distinct from other brain cell types, being derived from
 268 primitive peripheral myeloid progenitors during embryogenesis. Microglia are the resident phagocytic
 269 cells of the brain, taking part in immune-mediated defense processes which clear damaged cell debris
 270 while other glial cells have roles in the nutrition of the neuron and maintenance of axonal structures
 271 [58-61].
 272

273 The CNS/PNS has an extensive blood supply which services its considerable metabolic
 274 demands. Like most cells in the human body, glucose, is also the primary energy source for neurons. The
 275 brain is the most energy-demanding organ in the human body and while it may only constitute ~2% of the
 276 total mass of the human body it uses 20% of the bodies total energy production [62]. Glucose metabolism
 277 is the physiological fuel for brain function and is also required for the generation of ATP and the precursor
 278 compounds required in the synthesis of neurotransmitters needed for cell signalling. Brain functions such
 279 as thinking, memory, and cognitive learning are intricately interlinked to efficient utilisation of glucose in
 280 energy production [63]. However, too much glucose as occurs in type I and II diabetes can also be
 281 detrimental to brain function. Type 2 diabetes accelerates brain aging and accelerates functional decline
 282 in dementia resulting in significant age dependent cognitive changes in brain function.
 283

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

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

311
312 While glial cells are a less excitable cell type than neurons, their membranes nevertheless also
313 contain transporter proteins that facilitate the uptake of ions as well as proteins that remove
314 neurotransmitter molecules from the extracellular space. Thus glial cells act as accessory support cell
315 types to regulate neuronal function and also have roles in the nutrition of neurons and assembly of the
316 myelin sheath. In addition, they undertake running repair processes to ensure the maintenance of
317 neuronal structural integrity (Fig 2). Sophisticated regulatory systems are in place to facilitate neuron-
318 glial cell communication [65-69]. Phosphorylation, ubiquitination, and glycosylation of proteins
319 facilitate weak interactions with multivalent adaptor proteins resulting in the formation of membrane-
320 associated and soluble complexes that mediate information transfer between cells. These systems are
321 dynamic and complex and display remarkable specificity to control signaling pathways and effective
322 communication between neurons and glial cells.

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

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

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

352
 353 CD34 is an important fucosylated endothelial cell surface molecule containing glycan
 354 interactive structures which affect the homing of progenitor cells in microvessels [73, 74]. CD34+
 355 bone marrow haemopoietic stem cells are recruited to sites of brain trauma and differentiate into
 356 microglia which participate in neuronal repair processes. ALS, a complex multifactorial progressive
 357 degenerative disease with numerous intrinsic and extrinsic factors underlying its etiopathogenesis also
 358 displays degenerative vascular pathology underpinned by endothelial cell degeneration [75]. As
 359 discussed more fully later in this review, L-Fucose is a component of many *O*-linked and *N*-linked
 360 glycan modifications in a number of glycoproteins with important functional roles in many
 361 physiological and pathophysiological neural processes[76]. *O*-Fucosylation occurs at consensus
 362 sequences on two small cysteine-rich domains in Epidermal growth factor-like (EGF) repeats and
 363 Thrombospondin Type 1 Repeats (TSRs) in glycoproteins such as Notch-1, CD-34 and thrombospondin-
 364 1 [77]. Mouse Notch-1 contains three *O*-fucosylation sites in EGF repeats 1-5 and thrombospondin-1
 365 has three fucosylation sites in thrombospondin repeats 1-3 [78]. 6-Alkynyl fucose (6AF) is an L-fucose
 366 analogue (Fig 4j) which has been developed to facilitate labelling and tracking of these L-fucose motifs
 367 in physiological processes [79]. Over 100 proteins are predicted to be *O*-fucosylated on the basis of
 368 identified consensus EGF repeat sequences [80]. The Notch receptor family have more predicted *O*-
 369 fucosylation sites than any other protein in the recorded databases [81] (Fig 5). Many groups have
 370 shown that *O*-fucosylation is essential for Notch's functional properties [80, 82-84]. *O*-fucose also has
 371 functional roles in agrin which enables this proteoglycan to cluster acetylcholine receptors in the NMJ
 372 [85]. The precise function of *O*-fucose in the vast majority of these proteins however is unknown.
 373 Thrombospondins produced by astrocytes have roles in the formation of synapses.

374
 375 α -L-fucose is a terminal or core monosaccharide on *N*- and *O*-linked glycan chains on many
 376 glycoproteins (Fig 4d, Fig 5a-g). It also occurs as a capping structure along with sialic acid on the KS-I
 377 and KS-II chains of PGs (Fig 4a-c) and in terminal sLeX motifs in glycoproteins (Fig 4f, Fig 5b, Fig 6f-h).
 378 KS is heavily substituted with fucose and sialic acid in ALS. The prominent terminal locations of L-fucose
 379 points to its role as a molecular recognition site for interacting proteins. Fucose occurs as a terminal
 380 sugar linked to a penultimate galactose residue in glycoconjugates or to core GalNAc residues in N-
 381 glycans (Fig 5b,f). Fucose can also be directly attached to serine or threonine residues by fucosyl
 382 transferases in *O*-linked glycans and can act as an acceptor molecule for the attachment of further
 383 saccharides to form small oligosaccharide side chains (Fig 4e).

385 **3. Functional roles of the glycosaminoglycan components of brain extracellular and cell associated** 386 **proteoglycans in neuroregulation**

387 *3.1 Neural proteoglycans*

388
 389 ECM proteoglycans (PGs) play important directive roles in the growth of axons and in the
 390 navigation, plasticity and regenerative properties of neurons. PGs have paradoxical roles in neuronal
 391 growth and repair processes where they can both promote neuronal growth but in other settings can
 392 inhibit neural repair [86]. The sulphation positions and charge density of the GAG side chains of PGs
 393 can be sources of important signals to the neurons which either inhibit or promote neuronal repair
 394 [86]. Thus the CS-A and CS-C chains of lectican PGs such as aggrecan, versican, neurocan and brevican
 395 are sources of inhibitory signals and a barrier to neural outgrowth in perineural net formations (Fig 3)
 396 which surround areas of axonal damage in glial scar formations [87-90]. CS isomers of higher charge
 397 density such as the CS-D and CS-E motifs of phosphacan, bikunin and appican can actually promote
 398 neuronal repair processes. Thus, collectively, these CS isomers guide axonal growth and repair with
 399 remarkable specificity [91-94]. Another GAG present in some neural PGs is keratan sulphate (KS) and
 400 interesting interactive properties are now emerging for this GAG.

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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 dorsal 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 amyotrophic 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-sulphotransferase (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. β 3GlcNAcT-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- β 1 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 *GlcNAc6ST1(-/-)* 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

515 with over one thousand different pentasaccharide combinations possible [55]. The large number of
 516 structural permutations possible with CS/DS facilitates interactions with a diverse repertoire of
 517 cytokines, chemokines, morphogens and growth factors with regulatory properties in tissue
 518 development and ECM remodelling [55, 138-142]. CS also occurs as a number of isoforms including
 519 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
 520 enriched in the brain transmembrane PGs phosphacan, syndecan-1, syndecan-4, NG2
 521 proteoglycan/CSPG4, neuroglycan-C/CSPG7, and ECM PGs appican (β -APP) and bikunin [143-145]. CS-
 522 A, B, C are abundant in the brain hyalectan proteoglycan family consisting of brevican, neurocan,
 523 versican and aggrecan. The CS-D and CS-E motifs embedded within the CS-A side chains of β -APP,
 524 bikunin and phosphacan convey neuroregulatory properties [108, 145]. While CS-D and CS-E can
 525 promote neural repair the same cannot be said of the CS-A, B, C side-chains of neural net PGs layed
 526 down in the gliotic scar. Perineural nets [146] have been immunolocalised in rat brain tissues using the
 527 MAb 1-B-5 to a non-sulphated aggrecan stub epitope generated by chondroitinase ABC. 1-B-5
 528 reactivity is displayed in extensive extracellular distributions encompassing a large group of neurons
 529 (Fig 3 a, b) as well as pericellularly surrounding single or small numbers of neurons (Fig 3c, d) [147].
 530 Formation of glial scars, seals the injury but also creates a barrier to axonal regrowth. The scar centre is
 531 highly inflammatory and populated by NG2+ glia, astrocytes seal the border of the scar but in so doing
 532 entrap axons attempting to regrow within the scar, thus activated astrocytes and ECM components laid
 533 down in the scar contribute to regenerative failure[148]. The NG2 positive glia are a progenitor cell
 534 type for oligodendrocytes which participate in neural remodelling and repair processes whereas
 535 astrocytes define the boundary of the gliotic scar and do not participate in its repair. PGs in neural
 536 tissues thus have paradoxical modes of action, CS-PGs, of the lectican family hinder axonal regrowth
 537 while the transmembrane CS-PG (NG2/CSPG4) and phosphacan, upon shedding from the cell by ADAM
 538 10 (a disintegrin and metalloproteinase containing protein 10), promote axonal re-growth and
 539 production of synaptic adhesion molecules, promoting synaptic signaling, plasticity and functional
 540 recovery. The positive contribution of CSPG4 to neural repair processes is confirmed from knockout
 541 studies of NG2/CSPG4 mice which display aggravated tissue loss, inflammation and neurologic deficits
 542 after traumatic brain injury. Progranulin, a functional ligand of Notch and Eph2a acts in concert with
 543 NG2/CSPG4 to overcome neuronal inflammation and structural recovery of damaged neuronal tissue.
 544 Progranulin is upregulated after spinal contusion in mice [149]. Progranulin is produced by neurons
 545 and glia and has roles in inflammation and wound repair [150, 151]. Progranulin is proteolytically
 546 processed into peptide fragments (granulins) during tissue remodelling and these display different
 547 biological activity to the native molecule. Progranulin has trophic properties while the granulins act as
 548 inflammatory mediators and contribute to neuroinflammation, dementia and development of AD [151-
 549 153]. Neuronal expression of $\alpha 9\beta 1$ integrin, trkB, and protein tyrosine phosphatase σ (PTP σ), which
 550 are receptors for tenascin-C, brain derived neurotrophic factor (BDNF) and CSPGs respectively, have
 551 also been shown to significantly enhance regeneration of injured axons[154-157]. Thus with the
 552 correct expression of these cell surface receptors, growing axons can respond to appropriate guidance
 553 cues in their extracellular micro-environments by regulating their intracellular signaling pathways to
 554 modify growth cone behaviour and promote intrinsic repair [154, 156, 157]. Neuronal regeneration has
 555 been induced by transgenic integrin expression [158], lentiviral trk-B induced Erk activation [159] or by
 556 modulation of PTP σ expression [157]. PTP σ and the related leukocyte common antigen-related (LAR)
 557 and Nogo receptors 1 and 3 (NgR), bind the inhibitory glycosylated side chains of CSPGs and regulate
 558 synaptic structure and neuroplasticity [160, 161].

559
 560 As already noted, progranulin expressed in mature neurons and microglia, has protective roles
 561 in neurogenerative disorders [162-164] and plays a central role in the regulation of neural
 562 inflammation, enhancing neuronal survival and stimulating neurite outgrowth activity. Progranulin
 563 achieves this through modulation of glycogen synthase kinase (GSK)-3 β . Inhibition of GSK-3 β has
 564 received interest as a therapeutic target in the treatment of traumatic brain injury and is
 565 neuroprotective, promoting functional recovery after intracerebral hemorrhagic stroke [165]. GSK-
 566 3 β inhibitors rescue cognitive impairment in AD, Fragile X syndrome, Down syndrome, Parkinson's
 567 disease and spinocerebellar ataxia type 1 [166]. Levels of phosphorylated tau protein are elevated
 568 following traumatic brain injury and may contribute to pathological structural changes in the CNS
 569 [167]. Misfolded amyloid- β -peptides and hyperphosphorylated tau protein accumulation is a hallmark
 570 of AD [168]. Caspase-3 regulates tau phosphorylation in AD, is mediated by the GSK-3 β pathway and

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

588 *3.4 Contributions from other GAG types in neuroregulation and neural repair processes*

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

606 *3.5 SHH ,HS, CS, and KS interactions model tissue patterning and neural development.*

607 Hedgehog (HH) proteins are highly conserved morphogenetic signaling molecules with
 608 fundamental roles to play in vertebrate and invertebrate embryonic development [183-186]. The HH
 609 signaling pathway plays key roles during embryonic development and remains active in adults. The
 610 GAG chains of cell surface PGs shape HH gradients and signal transduction [119, 134, 187, 188]. Three
 611 HHs have been identified in mammals, Sonic, Indian, and Desert hedgehog, these are typically
 612 expressed in the nervous system, cartilage and testis respectively. SHH is synthesized as a 45-kDa
 613 precursor protein which undergoes autocatalytic cleavage to a 20-kDa N-terminal fragment (residues
 614 24–197 in the human gene sequence) responsible for all known hedgehog biological activity. This is
 615 membrane-associated through a palmitic acid attachment at its N-terminus [189] and cholesterol at its
 616 C -terminus [190-192]. Patched (Ptc), a 12 span transmembrane protein SHH receptor acts as a
 617 negative regulator of SHH signaling. SHH is interactive with glypican and CS GAG isomers and these are
 618 responsible for the production of SHH gradients which are a driving force during tissue morphogenesis.
 619 Surface plasmon resonance studies have demonstrated that corneal KS has interactive properties with
 620 SHH [119]. KS regulates the switch from motor neuron to oligodendrocyte generation during
 621 development of the spinal cord [134]. Glypican and CS participate in SHH mediated cell signaling [187]
 622 regulating tissue patterning and development of the neural system. SHH cell signaling is important in
 623 foetal and postnatal brain development and regulates the proliferation of early cerebral cortex
 624 progenitor and oligodendroglial lineage cells, expansion of their numbers is critical in the development
 625 of the neocortex [183, 185, 193, 194]. SHH guides axonal development during neurogenesis, cellular
 626

628 responses in early brain injury and following demyelination [195]. SHH may represent a therapeutic
 629 target to focus on in neurological disorders [196]. Co-ordinated SHH and Wnt mediated cell signaling
 630 regulates cranial nerve development [197]. SHH has roles in the differentiation of oligodendrocytes
 631 [198] and in glial neural cell communication during brain development which provides neuroprotection
 632 [186] and neuroplasticity. Neurons diversify astrocytes in the adult brain through SHH signaling [199].
 633 SHH is a regulator of extracellular glutamate levels in epilepsy and modulates the release of
 634 gliotransmitters from cultured cerebellar astrocytes [200, 201].
 635

636 *3.6 CS interactions modulate neural cell behaviour.*

637 CS is a prominent CNS GAG and occurs in a number of isomeric forms with differing degrees of
 638 sulphation and interactive properties [19, 20, 22, 202-204]. CS microarrays have proved useful in the
 639 assessment of CS-protein interactions [19, 20, 22] and has detected neurostimulatory and inhibitory CS
 640 species as well as a tumour necrosis factor α (TNF α) antagonist [24, 175]. Interactions of neurons with
 641 CS/DS promotes cellular survival [205]. The CS glycan chains of PGs interact with a diverse collection of
 642 proteins in the CNS to promote neural growth, proliferation, differentiation and long term survival.
 643 Some CS isoforms provide chemorepulsive nerve guidance cues which regulate axonal development
 644 and repair processes following traumatic injury. CSPGs inhibit the growth cone by interaction of CS
 645 chains with laminin, collagen and cell surface integrins. Receptor type protein tyrosine phosphatase- σ
 646 (RPTP- σ) also acts as a neural CS receptor [161] while RPTP- ζ interacts with the NCAM resulting in an
 647 inhibition of neural cell adhesion and growth (Fig 8c, d). The ecto-domain of RPTP- ζ is enzymatically
 648 released from the cell surface by ADAMS 10 generating the soluble phosphacan which can promote
 649 neural outgrowth and repair processes. Highly charged CS isomer side chains such as CS-E on
 650 proteoglycans bind FGFs and present these to FGF receptors (FGFRs) to promote cell signaling, neural
 651 growth and differentiation (Fig 8e). Interaction of the attractive guidance protein Semaphorin 5A with
 652 CS converts this to a repulsive guidance protein (Fig 8h). Semaphorin 3A is a cell membrane bound and
 653 secreted short range repulsive inhibitor guidance protein which interacts with CS-E in lectican
 654 perineural net formations to inhibit nerve regrowth. This effect is mediated by interaction with
 655 neuropilin-1 and neuropilin neural receptors (Fig 8f). Plexin acts as a signal transduction molecule
 656 along with transmembrane neuropilin co-receptors in the neuropilin-plexin receptor complex (Fig 8f)-
 657

658 Eph receptors and ephrins display broad spatial and temporal expression patterns throughout
 659 the nervous system [206, 207]. During early development, these interactions contribute to
 660 neurogenesis (reviewed in [208]) and differentiation [208, 209]. Eph-ephrin signaling influences the
 661 functions of Rho GTPase proteins, which in turn regulate the actin cytoskeleton influencing neuronal
 662 migration during development. Eph-ephrin signalling can generate both attractive and repulsive
 663 interactions and can positively support neurogenesis, axonal guidance and neural repair [208, 209].
 664 EphA2 receptor tyrosine kinase is a functional cell surface receptor for the secreted glycoprotein
 665 progranulin. Fourteen Ephrin receptors have so far been identified. Ephrin-Eph receptor cell signaling
 666 regulates cellular morphology and proliferation influencing the adhesive properties of cells during
 667 cellular migration in embryonic development, vasculogenesis and angiogenesis and has roles to play in
 668 axonal guidance, and synaptogenesis. Progranulin also promotes angiogenesis through the Ephrin
 669 receptors and upregulation of vascular endothelial growth factor (VEGF) production to modulate
 670 neuroinflammation [151, 210]. Phosphorylation of EphA2 by progranulin leads to tyrosine
 671 phosphorylation of other tyrosine kinases such as EphA4, EphB2, and EGFR through extensive cross talk
 672 (Fig 9b) among receptor tyrosine kinases [211]. Progranulin promotes the activation of the mitogen-
 673 activated protein kinase (MAPK) and Akt signaling pathways. Progranulin is secreted as a dimer
 674 containing up to 14 granulin modules per dimer which are available for protein-protein interaction.
 675 This may enable the dimer to bridge several receptors on a cell and serve as a multi-receptor signaling
 676 complex explaining the cross-talk when progranulin binds to EphA2 (Fig 9b).
 677

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

685 [22]. Netrin-1 modulates axonal growth direction and speed and directs F-actin reorganization,
 686 essential for mammalian neural development. The best characterized netrin-1 receptor, *Deleted in*
 687 *Colorectal Cancer* (DCC), is localized to growth cones, but is also observed in neuronal cell bodies [212].
 688 Netrin-1 attracts and repels distinct motor axon populations, according to the spatio-temporal
 689 expression of Netrin receptors [213] in neural tissues. The guidance cues provided by Netrin-1 are
 690 influenced by its interactive properties with ECM PGs, a theme recapitulated by most of the axonal
 691 guidance promoter proteins. These represent complex interplays between multiple components which
 692 regulate spatio-temporal neural growth [100, 214]. Netrin-1 can also synergize with ephrin receptors
 693 to regulate axonal formation [213]. A greater understanding of these axonal guidance cues would be
 694 insightful in therapeutic strategies aimed at producing guided nerve regeneration [215-219].
 695

696 CSPG4 promotes neural repair processes through upregulation of epidermal growth factor
 697 receptor (EGFR) expression [220, 221] and interaction with progranulin [148, 222, 223]. Progranulin is
 698 upregulated after spinal contusion [149]. CSPG4 is highly expressed by macrophages, microglial cells,
 699 tumour, perivascular and oligodendrocytes involved in cell adhesion and migration [224-228]. CSPG4 is
 700 upregulated in glioblastoma, astrocytoma and a number of other human tumours [221, 229, 230].
 701 Activated microglial cells form synapses with neurons to participate in neural repair [224] and re-
 702 organisation of the gliotic scar and improve neural outgrowth [148, 231, 232]. Following traumatic
 703 injury to the brain, the cells in the impacted area upregulate aggrecan, versican, brevican, neurocan in
 704 HA-macroaggregate perineural net structures stabilised by link protein and tenascin-R providing
 705 protection from oxidative stress and further mechanical injury. Astrocytes seal the margins of these
 706 gliotic scars by upregulating the brain matrix proteoglycan abakan. These perineural nets inhibit nerve
 707 outgrowth. Chondroitinase ABC selectively depolymerises the CS side chains of the lectican PGs
 708 improving neural recovery in the gliotic scar [233] and improves spinal cord repair [102, 234-237].
 709 Chondroitinase C also significantly improves repair of peripheral nervous tissue but appears to have a
 710 more specific mode of action [238].
 711

712 ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs 4) is localised
 713 in regions of the spinal cord undergoing spontaneous repair and specifically targeting of the lectican
 714 PGs in the scar tissue [239, 240]. KSPGs are similarly up-regulated in glial scars and inhibit axonal repair
 715 [101, 113, 114, 135, 241, 242]. Mutant mice deficient in the enzyme GlcNAc6ST-1 show improved
 716 functional recovery following spinal cord injury [130]. Therapeutic use of keratanase also improves
 717 axonal repair [103]. The KSPG phosphacan is also upregulated in scar tissues where it promotes mossy
 718 fiber outgrowth and nerve regeneration [107]. Chondroitin-6-sulphate upregulated in scar tissue is
 719 reported to have nerve regenerative potential.
 720

721 3.7 RAGE in the brain

722 Receptor for advanced glycation endproducts (RAGE) is a receptor which binds advanced glycation end
 723 products (AGEs) and CS in brain tissues (Fig 10). RAGE acts as a receptor for oversulphated CS isomers
 724 such as CS-E [243, 244]. AGEs modulate amyloidogenic precursor protein (APP) processing and Tau
 725 protein phosphorylation regulating AD development [245]. AGEs in glioblastoma have a modulatory
 726 role over tumour development [246]. RAGE mediates amyloid β accumulation in a mouse model of AD
 727 by regulation of β - and γ -secretase activity [247]. Targeted inhibition of RAGE reduces amyloid- β influx
 728 across the blood-brain barrier and improves cognitive deficits in mice [248]. High-mobility group box-1
 729 protein (HMGB-1) and β -amyloid oligomers promote neuronal differentiation of adult hippocampal
 730 neural progenitors via RAGE and the NF κ B pathway [249] sustaining neurogenesis counteracting the
 731 hostile AD brain microenvironment [6]. This promotes survival of vulnerable brain cell populations
 732 [249, 250]. AGEs impair NLRP3 inflammasome-mediated innate immune responses in macrophages
 733 and modulates neuroinflammation through the NF κ B pathway [251].
 734

735 4. Roles for L-Fucose in Neuro[?]Processes[?]

736 4.1 O- and N-linked fucosylated proteins in neural tissues

737 While neuronal mitochondria utilize glucose as an obligate primary energy resource in the
 738 tricarboxylic acid glycolytic pathway to generate energy neurons are also responsive to sugars other
 739 than glucose as cell regulatory agents. Positive selection pressure over at least 500 million years of
 740 vertebrate evolution has resulted in sugars which have evolved molecular recognition and information
 741

742 transfer properties equipping them as cellular mediators serving as critical determinants of protein
 743 folding, trafficking, and stability . Glycans are abundant in the brain and are involved in various neural
 744 functions including learning and memory, brain development, and spinal cord injury [102, 252-254].
 745 The precise molecular mechanisms whereby glycans influence these processes is not well understood
 746 but it is clear that synaptic transfer of information between neurons occurs through glycoprotein
 747 mediated interactions. L-fucose exists as a terminal residue on *N*- or *O*-linked glycoproteins attached
 748 to the C-3 and C-6 position of N-acetylglucosamine or the C-2 position of galactose (Fig 4). The fucose
 749 α 1-2 galactose (Fuc α 1-2Gal) linkage has been implicated in cognitive processes such as learning and
 750 memory. Non-invasive two dimensional magnetic resonance spectroscopy (2D MRS) has identified six
 751 Fuc α 1-2 Gal sugars in brain tissue. 2D MRS offers an unprecedented insight into the molecular
 752 mechanisms by which fucosylated sugars contribute to neuronal processes and how they alter during
 753 development, ageing and disease [29]. Fucose is an unusual sugar in that it exists as a 6-deoxy α -L-
 754 galactopyranose configuration and is a prominent functional component of neural tissues such as
 755 synaptic membranes [29]. Addition of 2-deoxy D-galactose, an L-fucose analog to hippocampal
 756 neuronal cultures potently inhibits neural outgrowth activity whereas 3-deoxy D-galactose is inactive,
 757 moreover addition of D-galactose to 2-deoxy-D-galactose treated cultures results in the functional
 758 recovery of normal neuron growth characteristics. Fuc α 1-2 Gal is a non-reducing terminal component
 759 of many glycans [29] and is implicated in neurite outgrowth, synaptogenesis, neuronal development,
 760 learning, and memory [27, 28, 255, 256]. Treatment of animals with 2-deoxy-D-galactose, disrupts the
 761 formation of Fuc α 1-2Gal linkages, and causes reversible amnesia [257] interfering with the
 762 maintenance of long-term potentiation in an electrophysiological model of learning and memory [258].
 763 Furthermore loss of 1, 6-fucosyl transferase activity also decreases hippocampal long term potentiation
 764 [259].

765
 766 α -L-fucose is a terminal or core monosaccharide on *N*-and *O*-linked glycan chains on many
 767 glycoproteins (Fig 4d, Fig 5a-g). It also occurs as a capping structure along with sialic acid on the KS-I
 768 and KS-II chains of PGs (Fig 4a-c) and in terminal sLeX motifs in glycoproteins (Fig 4f, Fig 5b, Fig 6f-h).
 769 KS is heavily substituted with fucose and sialic acid in ALS. The prominent terminal locations of L-fucose
 770 points to its role as a molecular recognition site for interacting proteins. Fucose occurs as a terminal
 771 sugar linked to a penultimate galactose residue in glycoconjugates or to core GalNAc residues in N-
 772 glycans (Fig 5b,f). Fucose can also be directly attached to serine or threonine residues by fucosyl
 773 transferases in *O*-linked glycans and can act as an acceptor molecule for the attachment of further
 774 saccharides to form small oligosaccharide side chains (Fig 4e).

775 776 4.1.1 L-Fucose as a functional component of blood group substances and Immunoglobulins

777 Fucose also occurs as terminal Fuc α 1-2 Gal terminal saccharides in small glycolipids attached
 778 to red blood cells identifying the A, B, O blood group antigens (Fig 6a). Over 95% of circulatory human
 779 IgG antibodies also contain a fucose (core-fucose) residue attached to the first GalNAc in the
 780 glycosylation site of their Fc region. The majority of other plasma proteins are not substituted with
 781 fucose in this manner. Fucosylation dramatically reduces IgG binding to Fc γ R3, an activating Fc
 782 receptor specific for IgG Fc region expressed by immune human natural killer (HNK) cells and
 783 macrophages [260-262]. Fc γ R3 initiates antibody dependent cellular cytotoxicity (ADCC) by HNK cells
 784 and phagocytosis of antigens by macrophages. This core fucose attenuates potentially harmful ADCC
 785 activity. Conversely, ADCC induced by non-fucosylated IgG improves the efficacy of therapeutic
 786 anticancer antibodies. IgG lacking the core-fucose is over 100 times more effective in initiating ADCC
 787 than the fucosylated version (Fig 6b-h).

788 789 4.1.2 Synapsin and Synaptophysin

790 The synapsins are fucosylated proteins [256] which regulate the release of synaptic vesicles to
 791 coordinate release of neurotransmitters within the synaptic vesicles at the synaptic gap [263]. They do
 792 so by tethering the vesicles to cytoskeletal components to prevent the diffusion of vesicles to the
 793 synaptic membrane preventing the un-coordinated release of neurotransmitters at the synaptic gap
 794 [264]. During the transmission of an action potential down the neuron from the cell body the
 795 synapsins are phosphorylated by cAMP dependent protein kinase (PKA). This releases the synaptic
 796 vesicles to the pre-synaptic membrane [265] which depolarizes in response to the action potential
 797 allowing the synaptic vesicle to fuse with the synaptic membrane and release the enclosed
 798 neurotransmitters into the synaptic gap and these are transported across to the post synaptic

799 membrane of a communicating neuron [266]. This results in the transmission of neural signals along
 800 the neural network. There are three synapsin proteins and each occur as two isoforms. Synapsin 1a is
 801 implicated in bipolar disorder and schizophrenia [267]. The synapsin Ia/Ib isoforms are the most highly
 802 expressed hippocampal pre-synaptic vesicle associated phosphoproteins and are implicated in thought
 803 formation and cognitive learning [268-270]. Synapsin is a major neuronal fucosylated glycoprotein
 804 [256, 271, 272]. The synapsin family consists of 3 major isoforms encoded by 3 genes SYN1, SYN2,
 805 SYN3. Each gene occurs as two alternatively spliced forms leading to a total of six isoforms. Mice
 806 lacking synapsin I, II, III are prone to seizures and display learning difficulties and in humans is
 807 associated with bipolar disorder and Schizophrenia[34, 267].

808

809 4.1.3 Fucosylated Glycoproteins and Proteoglycans

810 Fucosylated glycoproteins and PGs have prominent roles in many physiological and
 811 pathological processes including leucocyte adhesion, host-microbe interactions, neuronal development
 812 and neural protection[273-275]. Fucosylated glycolipids on erythrocytes form the ABO blood group
 813 antigens[276]. However, aberrantly fucosylated glycoconjugates are also found in cancer,
 814 inflammation and neoplastic processes [276-279]. The fucosylated sialyl Lewis-X, and sialyl Lewis-Y
 815 antigens are prominently upregulated in some cancers and associated with tumour progression.
 816 Deficient levels of fucose occur in impaired leucocyte interactions with the vascular epithelium in
 817 immunodeficiency. Leukocyte adhesion deficiency II (LAD II) is a rare congenital disease caused by a
 818 defect in fucosylation of glycoconjugates such as P-selectin glycoprotein ligand-1 (PSGL-1) (Fig 5a)
 819 which normally facilitate leucocyte binding to the selectins on the epithelium during inflammation
 820 [280]. This interaction facilitates leucocyte rolling and their extravasation through blood vessels to
 821 tissue sites to combat infection. Leukocyte adhesion deficiency type II (LAD II)-patients show severe
 822 mental and growth retardation indicating an additional essential role for fucose in brain growth and
 823 development [281]. The P-selectin PSGL-1 ligand is a dimeric mucin-like 120-kDa glycoprotein located
 824 on leukocyte surfaces that binds to P-, E- and L-selectin and promotes leucocyte adhesion to the
 825 endothelium facilitating leucocyte rolling during inflammation. PSGL-1 is heavily fucosylated as part of
 826 the branched Lewis X antigen O-glycan structure (NeuAc β 2 \rightarrow 3Gal β 1 \rightarrow 4[Fuca1 \rightarrow 3]GlcNAc β 1 \rightarrow R)[125]
 827 (Fig 5a). High affinity cell adhesion interactions also require the presence of three tyrosine sulfate
 828 residues located near the Lewis-X antigen structure at the N-terminus of PSGL-1 [125, 282, 283].

829

830 4.1.4 Functional role of L-Fucose in Notch Signaling

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

848

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

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

858
 859 Although a relatively minor sugar, its strategic positioning on key functional glycoproteins
 860 points to fucose having a significant role to play in neural pathobiology. As already indicated *O*-
 861 fucosylation is essential for the activity of Notch (Fig 5g) and has significant roles to play in leucocyte
 862 PSGL-1 P-selectin mediated interactions (Fig 5a) with the endothelium in neuro-inflammation [286].
 863 CD-34 is heavily substituted with both *N*- and *O*- linked fucosylated oligosaccharides in microvascular
 864 progenitor cells (Fig 5e). The mode of action of L-Fuc in Notch has been suggested to be due to
 865 induction of conformational changes in the epidermal growth factor (EGF) repeat domains or in the
 866 Notch ligands. Notch signaling is essential for the maintenance of neural progenitors and regulates
 867 cell-fate decisions in neuronal and glial cells to modulate neuronal differentiation and migration [288,
 868 289]. Deletion of *POFUT1* is embryonic lethal causing developmental defects in vasculogenesis,
 869 somitogenesis and neurogenesis similar to those obtained when Notch receptors are deleted. This
 870 reinforces the importance of L-Fuc as a mediator in combination with Notch in neuronal development.

871 872 4.1.5 Roles for L-Fucose in CD-34.

873 Another cell surface protein with cell adhesion and cell regulatory properties in the CNS/PNS
 874 is CD-34 (Fig 5e). CD34 is a heavily fucosylated type I transmembrane sialoprotein, that can be
 875 phosphorylated by a number of kinases including PKC and Tyrosine kinase. The CD-34 proteins are a
 876 family of sialomucin transmembrane adhesion proteins. CD-34 is expressed in early haematopoietic
 877 and vascular tissues and lymph node epithelium. CD-34 interacts with L-selectin expressed by T cells in
 878 the lymph node epithelium. Podocalyxin and endoglycan are related to CD-34 and also facilitate cell
 879 attachment and cell migration during microvessel development in neural tissues [126]. Terminal
 880 fucosylation of these PGs confer unique functional properties in a variety of biological settings. Fucose
 881 is an essential component of the carbohydrate ligands for the selectin family of cell adhesion receptors
 882 [290, 291]. E-, P-, and L-selectin are C-type lectin proteins expressed by platelets (P-selectin),
 883 endothelial cells (E- and P-selectin), and leukocytes (L-selectin). Selectins bind to oligosaccharides
 884 decorating specific cell surface and secreted proteins expressed by leukocytes (E- and P-selectin
 885 ligands) and high endothelial venules (L-selectin ligands). Interaction between selectins and their
 886 ligands enable the rolling of leukocytes on the endothelium, and is an essential requirement for
 887 leukocyte extravasation. The carbohydrate selectin ligands are fucosylated structures related to the
 888 sialyl Lewis-X antigen, an α 1,3-fucosylated glycan structure also known as stage-specific embryonic
 889 antigen-1 (SSEA-1) and CD15 expressed during early embryogenesis [292].

890 4.1.6 L-Fucose as a component of Lewis-X-Antigen

891 Lewis^x epitopes are present in multiple areas of the developmental embryonic brain [293-
 892 296], controlled by FUT 9 expression, an enzyme which is regulated by the transcription factor Pax 6
 893 [297]. The functional role of Lewis^x in the developing brain has yet to be determined, but its dynamic
 894 expression patterns during embryogenesis suggests it may have roles in aspects of molecular
 895 recognition which support the assembly of neural structures [273, 298]. The Lewis^x epitope, an α 1,3-
 896 fucosylated glycan also known as the stage-specific embryonic antigen-1 (SSEA-1) and CD15, is
 897 expressed during early embryogenesis [292]. Exposure of pre-implantation mouse embryos at the
 898 morula developmental stage to Lewis^x oligosaccharides causes decompaction apparently through
 899 disruptive multimeric interactions affecting cell-cell adhesion in early embryos [299, 300].
 900 Oligosaccharides containing L-fucose form part of a recognition signal in sperm-egg attachment in
 901 mammals [301]. At the endometrial surface, adaptations are also required to accommodate the
 902 implanting embryo [302, 303]. These adaptations at the materno-fetal boundary are highly species-
 903 specific. Fucose containing carbohydrate structures in the embryonic-maternal interface have
 904 important molecular recognition roles to play which define the maternofetal glyco-code. Localization
 905 of fucose oligosaccharides at a surface or interface is important in predicting functional roles in cell
 906 recognition. Each mammalian species has its own characteristic materno-fetal glyco-code. This
 907 glycotype permits interbreeding between compatible species like the horse and donkey which have
 908 almost identical patterns of placental glycosylation, whereas the camel has a totally different placental
 909 glycosylation signature and cannot interbreed with either the horse or donkey [304]. Specific
 910 fucosylated glycoconjugates vary in abundance during the receptive and non-receptive phases of

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

917
 918 Defucosylation of the EGF domain from urokinase-type plasminogen activator abolishes its mitogenic
 919 activity despite having no effect on its binding properties at the cell surface , thus *O*-fucosylation can
 920 modulate ligand-receptor interactions necessary for productive signal transduction outcomes. *O*-
 921 fucose residues are also present on EGF domains of the mammalian Notch receptors, a family of
 922 transmembrane cell-fate determining signaling proteins during somite formation, neurogenesis,
 923 angiogenesis, and lymphoid development. Ligand-induced Notch signaling events are impaired in a
 924 fucose-deficient cell line but can be restored by correction of the fucosylation defect, indicating that *O*-
 925 fucosylation of Notch affects its interactions with ligands and is in line with functional roles for L-fucose
 926 in molecular recognition [312, 313]. Such *O*-fucosylation effects are not limited to EGF domains, as
 927 glucose-extended fucose modifications (Glc-Fuc-O -Ser/Thr) have been demonstrated in three
 928 thrombospondin type 1 repeats on thrombospondin-1 [78] and it remains to be determined if the
 929 activity of additional protein modules can also be modified by *O*-fucosylation. Fucosylated glycans
 930 impact on the pathogenesis of several human diseases. Expression of A and B blood group antigens (Fig
 931 8a) is lost in many tumours accompanied by increases in H and Lewis-Y expression associated with poor
 932 clinical prognosis [314, 315]. Up-regulation of sialyl Lewis-X and sialyl Lewis-a occurs in many cancers
 933 associated with advanced tumor grade and poor prognosis. Increased expression of fucosylated serum
 934 immunoglobulins (Fig 6f-h) is evident in juvenile and adult rheumatoid arthritis [260, 261] its
 935 contribution to the pathogenesis of inflammatory arthritis is not known or whether this is a secondary
 936 effect due to an upregulation in fucosylation driven by an autoimmune response. Elevated fucosylation
 937 of mucins has also been observed in cystic fibrosis, accompanied by a decrease in sialylation [278].
 938 Fucosylation impacts on leukocyte recruitment, selectin-selectin ligand interactions and the
 939 development of numerous pathological processes, including atherosclerosis, reperfusion injury
 940 following ischemic events, inflammatory skin diseases, and asthma [316]. A reduction in the density of
 941 cell surface fucosylated glycans in patients with LAD II (also known as congenital disorder of
 942 glycosylation) [280] results in recurrent infections due to defective selectin ligand biosynthesis and an
 943 impairment in the innate immune response. Mental retardation and skeletal abnormalities are also
 944 prominent features in LAD II, but it is not known if these are due directly to fucose-dependent
 945 processes, such as *O*-fucosylation of Notch receptors or are due to Lewis-X mediated interactions in the
 946 embryonic brain.

947 948 4.2 Fucosylated Chondroitin Sulphate as a Therapeutic Agent

949 Investigations over the last 25 years [317-319] on fucosylated CS (Fuc-CS) isolated from the
 950 holothurian echinoderm marine sea cucumber (*Holothuroidea* class) has identified a family of
 951 molecules with great therapeutic potential in a number of physiological processes (Fig 4h). The Fuc-
 952 CSs consist of a core structure consisting of CS-E and CS-A disaccharides with CS-A constituting 10-50%
 953 of the disaccharides. Sulphated fucose side chains are attached through *O*-3 to GlcA of the core
 954 structure. These fucose residues can be monosulphated or disulphated at the 2,4 or 3,4 positions (Fig
 955 4g, h), side chains and disulphated GlcA in the core structure have also been detected adding to the
 956 structural complexity and charge density of the Fuc-CSs. Native Fuc-CS preparations have been
 957 isolated up to 64 kDa in size and for some therapeutic applications these have been depolymerized to
 958 smaller 3-12 kDa forms. The branched sulphated fucose side chains are important features of the Fuc
 959 CSs [320, 321]. Synthetic branched 2,4-di-*O*-sulphated fucosylated CS glycoclusters have been
 960 prepared in order to reproduce this structural feature, these display anti-coagulant properties and
 961 specific inhibition of intrinsic coagulation pathways [322]. Furthermore, the activated partial
 962 thromboplastin time (APTT), prothrombin time (PT) and thrombin times (TT) of these polymers can be
 963 fine-tuned for specific therapeutic applications [322]. The structural features of the Fuc-CSs have been
 964 investigated using high-resolution Fourier transform ion cyclotron resonance mass spectrometry [323]
 965 and to chemical and NMR spectroscopic structural investigations [324, 325]. In NMR and molecular
 966 dynamic simulations, the Fuc-CS repeat unit adopted a similar conformation to the Lewis-X blood
 967 group determinant [326, 327]. This structure accommodates the localization of several sulphate

968 groups in close proximity to one another and these form large negative patches which are distributed
969 along the helical CS backbone of Fuc-CS [326, 327]. Native Fuc-CS preparations display anti-coagulant
970 [323, 328-331] anti-angiogenic [329], anti-inflammatory, blood lipid lowering properties [323, 328-
971 333], and stimulate haemostasis [334], promote neurite outgrowth [335], anti-cancer [336] and potent
972 HIV-1 gp 120 protein binding properties which inhibit HIV replication by preventing viral entry into cells
973 [337]. While neurite outgrowth promoting properties have been noted for native Fuc-CS preparations,
974 a synthetic Fucose-CS trisaccharide (Fig 4j) has been shown to be more potent than CS-E
975 tetrasaccharides (Fig 7) for the outgrowth of DRG hippocampal neurons in monolayer culture. The
976 molecular recognition properties of specific glycan structures can therefore be employed in
977 therapeutic interactions of physiological importance and undoubtedly when further information
978 becomes available on these structures will also be employed in improved applications in repair biology.
979

980 **5. Conclusions**

981 Fucose, CS and KS have evolved properties of molecular recognition and information transfer which
982 equips the proteoglycans and glycoproteins they are attached to with properties as cellular mediators
983 controlling cellular behaviour in a number of physiological processes and in neural development and
984 repair. A greater understanding of this evolved glycode and how it regulates cells may allow a
985 greater understanding of physiological and repair processes and how these might be manipulated in
986 order to improve therapeutic interventions developed in response to altered glycodynamics in neural
987 disorders. These are expected to improve repair responses in cognitively impaired brain tissues.
988

989 **Legends to Figures**

990

991

Figure 1.

992 Morphological features of cultured neurons. Fluorescent images of cultured neural iPSCs (a) and
 993 neuroblastoma cells (b) and IPSCs stimulated with nerve growth factor (c). In (a) cell nuclei were
 994 stained with Hoechst 33258 DNA stain, axons with anti-tubulin Alexa 488 and dendrites and synapses
 995 with anti-F actin phalloidin Alexa 568. In (b) cultured mouse neuroblastoma cells were stimulated with
 996 retinoic acid to induce differentiation. Nuclei (i.e. DNA) are stained yellow; microtubules (anti-tubulin
 997 antibody) are cyan; f-actin (fluorescent phalloidin) is purple. The image was pseudo- coloured,
 998 individual channels were initially recorded with the regular red/green/blue fluorophors (i.e. Alexa 488
 999 and 568 and DAPI) then pseudo coloured as shown. Images a, b, c supplied courtesy of Torsten
 1000 Wittmann, PhD, Dept of Cell and Tissue Biology, University of California, San Francisco, USA. Indirect
 1001 fluorescent immunolocalisation of paraformaldehyde fixed rat hippocampal neurons with anti-
 1002 synapsin-1/2 (red, Alexa 488) raised to a synthetic peptide corresponding to amino acids 2 to 28 from
 1003 rat Synapsin-1 (UniProt Id: P09951)[cat # 106-006] and mouse anti-microtubule associated protein
 1004 (MAP-2, green, FITC) [cat# 188-011], nuclei stained with DAPI (blue) (d). Image d supplied courtesy of
 1005 Synaptic Systems.

1006

1007

Figure 2.

1008 Neural structural organisation and synaptic neurotransmitter transmission. Artistic rendition of a
 1009 synapse with progressive stages of transmission of neurotransmitters across the synaptic gap to a
 1010 communicating neuron (a-c). Diagrammatic representation of SV2A proteoglycan intercalated in the
 1011 plasma membrane of a synaptic vesicle and the Ca²⁺ /neurotransmitter (GABA) smart gel complex
 1012 formed by interaction with the KS side chains of SV2A which forms a neurotransmitter transport
 1013 delivery system (d). Pseudo coloured TEM of a synapse (X 50,000) courtesy Science PhotoLibrary.
 1014 Mitochondria (purple), synaptic vesicles (red), synaptic gap (pink) (e). Diagrammatic depiction of a
 1015 synaptic bouton with 1. microtubular transport system which transports the neurotransmitters
 1016 generated in the neural cell body. 2. mitochondria and 3. synaptic vesicle accumulation in the synaptic
 1017 terminal and 4.the post synaptic neurotransmitter receptors and voltage gated ion-channels on a
 1018 communicating neuron which deliver neurotransmitters such as GABA (as shown) (f). Higher power
 1019 view of the boxed area in (f) showing details of the depolarisation of the synaptic membrane and
 1020 merging of synaptic vesicle plasma membrane and delivery of Ca²⁺ and neurotransmitters across the
 1021 synaptic cleft to neurotransmitter receptors and voltage gated ion channels in a communicating
 1022 neuron (g). Details of synaptic vesicles adjacent to the synaptic membrane of the synaptic cleft viewed
 1023 by TEM (h). Plates a-c from Shutterstock. Plate d modified from [338] and f-h modified from Becker,
 1024 W., Hardin, J., Bertoni, G., Kleinsmith, L. (2012). Becker's World of the Cell (8th ed.). Boston, MA:
 1025 Benjamin Cummings. Source: http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-13/13_16.jpg
 1026 A neuron and surrounding glial cells. (i) H & E stained section of neural tissue depicting
 1027 a central large neuron body with multiple prominent dendritic processes surrounded by numerous
 1028 small glial cells. Image obtained from Pinterest. Cartoon depicting the co-ordinated interplay between
 1029 neurons and blood vessels mediated by astrocytic interconnections (j).

1030

1031

Figure 3.

1032 Immunolocalisation of aggrecan in perineural nets using MAb 1-B-5 following chondroitinase ABC
 1033 digestion of rat brain (a), rat dorsal root ganglion (b) or in isolated neurons (c, d). Images courtesy of
 1034 Caterson archive, Biolumaging Unit, University of Cardiff.

1035

1036

Figure 4.

1037 Fucosylation of KS-I (a) and KS-II from the KS rich region of aggrecan (b) and KS-II chains located within
 1038 the CS2 region of the aggrecan core protein (c) which are detected by MAb 3D12H7. Fucose modified
 1039 glycan structures. The structural diversity of the L- fucose containing glycan chains of O-linked glycans
 1040 of the mucin family (d-f), small glycan chains where L-fucose is linked directly to O-serine residues of
 1041 the glycoprotein or proteoglycan core protein and which acts as an acceptor molecule for subsequent
 1042 additions of additional saccharides (e) and the 6-sulphated Lewis-X epitope which has a widespread
 1043 distribution in glycoproteins (f).Fucosylated chondroitin sulphates (Fuc-CS) of therapeutic potential (g).
 1044 Representative structure of a native Fuc-CS [327] isolated from sea cucumber (g) The structure shown
 1045 is that specified for a Fuc-CS displaying interactive properties with P-and L-selectin which prevented

1046 selectin mediated extravasation of neutrophils in inflammation. The fucose side chains of Fuc-CS
 1047 display some structural variation as shown in the proportion of mono- and disulphated fucose side
 1048 chains (h). Structure of the CS-A and CS-E disaccharide core structures (i). The CS-A content of the
 1049 core varies between 10%-50%. Structure of a fucosyl-CS trisaccharide (j) which displays potent neurite
 1050 outgrowth promoting activity[335].

1051

1052 **Figure 5.**

1053 O-fucosylated PSGL-1, CD-34 and Notch-1. Schematic depiction of the structural organization of PSGL-
 1054 1 of leucocytes which facilitates binding to P-selectin in the endothelium (a). PSGL-1 is heavily
 1055 substituted with O- linked L-fucose oligosaccharides containing the sialyl Lewis X epitope (b). A
 1056 terminal sialyl LeX epitope is associated with sulphated tyrosine residues which are important for the
 1057 interactive properties of PSGL-1 with P-selectin. Fucose modifications on branched glycan structures in
 1058 PSGL-1 (b). The structural diversity of the L-fucose containing glycan chains in N-linked glycans
 1059 displaying tri-antennary, tetra-antennary, high mannose, bi antennary glycan chains and those bearing
 1060 the N-linked 6-sulphated Lewis-X epitope.

1061 O- and N-fucosylation of CD-34. Diagrammatic representation of the CD-34 cell surface receptor that is
 1062 widely expressed by a number of microvascular cells in brain development (c-f). N- and O- linked 6-
 1063 sulphated Lewis-X motifs are prominent interactive components of CD-34 (c, e, f) these are shown
 1064 distributed along the core protein of a model of the transmembrane CD-34 molecule (d).The key
 1065 contribution of O-fucosylation to Notch functional organization (g). Structural representation of the
 1066 Notch-1 receptor modular structural organization and the glycosylation of its epidermal growth factor
 1067 (EGF) repeat units which conveys its interactive properties with a number of ligands (h). The EGF
 1068 repeats are particularly heavily substituted with L-fucose containing glycans as shown, while the other
 1069 glycosylations have a regulatory role to play over the interactive properties mediated by L-fucose
 1070 glycans. Blood vessels can be visualized in human brain tissues using CD-34 immunolocalisation, 5
 1071 week post conception spinal tissue (i). Abbreviations HB , hind brain; OT, otic nerve; SC, spinal cord.
 1072 Image obtained by Open Access from Ilgård K, Dziegielewska KM, Holst CB, Habgood MD, Saunders NR.
 1073 Brain barriers and functional interfaces with sequential appearance of ABC efflux transporters during
 1074 human development. *Sci Rep.* 2017 ;7(1):11603.

1075

1076 **Figure 6.**

1077 Fucosylated blood group antigens and immunoglobulins. Fucose containing glycan chains attached to
 1078 red blood cells which identify the A, B, O blood types (a). Glycan chains attached to serum
 1079 immunoglobulins (b) which determine ADCC toxicity which some IgGs elicit (c-e) and an inflammatory
 1080 response (f-h)

1081

1082 **Figure 7 .**

1083 Neurostimulation by glycans and glycosaminoglycans. The stimulatory effect of CS-E saccharides on
 1084 cultured hippocampal neurons. Immuno-fluorescent localisation of neurons cultured ± CS-E
 1085 saccharides using anti-tau FITC antibodies (a-d). Control (a), CS-E disaccharide (b), unsulphated CS-E
 1086 tetrasaccharide (c), d. CS-E tetrasaccharide (d), Scale bars =45 µm. The minimum size of CS-E required
 1087 for neuronal stimulation was a CS-E tetrasaccharide, the non sulphated CS-E tetra-saccharide and CS-E
 1088 disaccharide were both non-stimulatory. This contrasts with CS-A and CS-C which inhibit neural
 1089 outgrowth. L-fucose and related sugars also have stimulatory properties of on cultured hippocampal
 1090 neurons (e-h). L-fucose (6-deoxy L-galactose), D-galactose and deoxy D-galactose saccharides all
 1091 influence the morphology of cultured hippocampal neurons. Immunofluorescent detection of dendrite
 1092 outgrowth from cultured hippocampal neurons using FITC labeled anti-tau antibodies. Control, L-
 1093 fucose (e), 2-deoxy L-galactose (f); 2-deoxy L-galactose + D-galactose (g); 3 deoxy L-galactose (h). 2-
 1094 deoxy D-galactose has an inhibitory effect on dendrite outgrowths. The structures of these sugars are
 1095 shown in (i)-(m). The structure of an L-Fuc analogue, 6 alkynyl Fuc which is used in the labeling and
 1096 tracking of O-Fucosylation in glycoproteins is also shown (n) [79]. Images a-h reprinted (adapted) with
 1097 permission from Murrey HE, Hsieh-Wilson LC. The chemical neurobiology of carbohydrates.
 1098 *Chem Rev.* 2008 ;108(5):1708-31, and Tulley SE, Mabon CI, Gama CI, Tsai SM, Liu X and Hsieh-Wilson LC.
 1099 *J am Chem Soc* 2004; 126, 7736-7737 copyright (2004 and 2008) American Chemical Society.

1100

1101 **Figure 8.**

1102 Diagrammatic depiction of interactive structures on the surface of neurons and the contributions of CS-
1103 proteoglycans to neural growth arising from interactions with cell surface integrins, laminins and
1104 pericellular collagen and fibronectin fibres and hyaluronan(a) and RPTP- σ (b) which acts as a CS-
1105 receptor (1). Neural outgrowth arising from the interaction of RPTP- ζ with NCAM (neural cell adhesion
1106 molecule) (c,d) (2). Cellular proliferative and cell survival effects stimulated by delivery of fibroblast
1107 growth factors to their receptors (FGFR)(e) (3). Chemorepulsive cues generated by interaction of CS-
1108 proteoglycans in perineural net formations with semaphorin 3A which is normally an attractive
1109 guidance cue. This inhibitory signal is generated by interactions of Sem3A with neuropilin-1 and
1110 neuroplexin (f,g). Semaphorin 5A also interacts with cell surface CS-proteoglycans generating a signal
1111 which inhibits nerve outgrowth (h). Figure modified from L Djerbal, H Lortat-Jacob, JCF Kwok
1112 Chondroitin sulfates and their binding molecules in the central nervous system Glycoconj
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1116 **Figure 9.**

1117 Diagrammatic illustration of the interaction of progranulin dimer with CSPG4 (a) and Eph A2 showing
1118 the resulting phosphorylation of Eph A2 and cross-talk with adjacent receptors leading to their
1119 activation. Figure available under a Creative Commons License (Attribution–Noncommercial–Share
1120 Alike 3.0 Unported license), <http://creativecommons.org/licenses/by-nc-sa/3.0/>. Bateman A.
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1124 **Figure 10.**

1125 Schematic depiction of the structural organization of transmembrane RAGE showing its extracellular,
1126 transmembrane and cytoplasmic portions and the glycan interaction region which acts as a receptor
1127 for AGEs and highly charged CS isomers such as CS-E. Diagram reproduced from Hegab Z, Gibbons S,
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