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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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9  
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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**

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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	<b>Abbreviations</b>	
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	$\beta$ 3GlcNAcT	$\beta$ 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 $\gamma$ 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 $\sigma$	protein tyrosine phosphatase $\sigma$
161	RAGE	receptor for advanced glycation end products
162	RPTP- $\sigma$	receptor-like protein tyrosine phosphatase- $\sigma$
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- $\beta$	transforming growth factor- $\beta$
168	TNF- $\alpha$	tumour necrosis factor- $\alpha$
169	TSRs	thrombospondin repeats
170	SYN	synapsin
171	RPTP- $\zeta$	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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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 $\alpha$  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.

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## 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  $Ca^{2+}$  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.

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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  $Ca^{2+}$   
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



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

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

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

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

#### 3.1 Neural proteoglycans

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

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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 Ca<sup>2+</sup> channel mediated long-term potentiation of non-deprived eye responses after mononuclear deprivation.  $\beta$ 3GlcNAcT-7 and GlcNAc6ST-1, TGF- $\beta$  and FGF-2 in adult mice is elevated in gliotic scars [112]. Fibroblast growth factor 2 (FGF-2) elevates TGF- $\beta$ 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 $\beta$  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  $\alpha$  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 ( $\beta$ -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  $\beta$ -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  $\sigma$  (PTP $\sigma$ ), 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 $\sigma$  expression [157]. PTP $\sigma$  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 $\beta$ . Inhibition of GSK-3 $\beta$  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 $\beta$  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- $\beta$ -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 $\beta$  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 $\beta$  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  $\alpha$  (TNF $\alpha$ ) 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- $\sigma$   
 646 (RPTP- $\sigma$ ) also acts as a neural CS receptor [161] while RPTP- $\zeta$  interacts with the NCAM resulting in an  
 647 inhibition of neural cell adhesion and growth (Fig 8c, d). The ecto-domain of RPTP- $\zeta$  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  $\beta$  accumulation in a mouse model of AD  
 727 by regulation of  $\beta$ - and  $\gamma$ -secretase activity [247]. Targeted inhibition of RAGE reduces amyloid- $\beta$  influx  
 728 across the blood-brain barrier and improves cognitive deficits in mice [248]. High-mobility group box-1  
 729 protein (HMGB-1) and  $\beta$ -amyloid oligomers promote neuronal differentiation of adult hippocampal  
 730 neural progenitors via RAGE and the NF $\kappa$ 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 $\kappa$ 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  $\alpha$ 1-2 galactose (Fuc  $\alpha$ 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  $\alpha$ 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  $\alpha$ -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  $\alpha$  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  $\alpha$  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  $\alpha$ -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  $\alpha$ 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 $\gamma$ R1IIIA, an activating Fc  
 782 receptor specific for IgG Fc region expressed by immune human natural killer (HNK) cells and  
 783 macrophages [260-262]. Fc $\gamma$ R1IIIA 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].

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#### 4.1.3 Fucosylated Glycoproteins and Proteoglycans

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

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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  $\gamma$ -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.

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  $\alpha$ 1-2 Gal containing glycans. FUT3 attaches Fuc via  $\alpha$  1-3 and  $\alpha$  1-4 linkages to Gal and GlcNAc  
 852 residues in glycan chains. FUT4-7 form exclusively  $\alpha$  1-3 linked fucose residues in glycans. FUT8 and  
 853 FUT9 generate Fuc  $\alpha$ 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  $\alpha$  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  $\alpha$  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<sup>x</sup> 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<sup>x</sup> 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<sup>x</sup> epitope, an  $\alpha$  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<sup>x</sup> 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<sup>x</sup> 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 Ca<sup>2+</sup> /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 Ca<sup>2+</sup> 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](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).

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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  
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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- $\sigma$  (b) which acts as a CS-  
1105 receptor (1). Neural outgrowth arising from the interaction of RPTP- $\zeta$  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  
1113 J. 2017; 34(3): 363–37. Open Access.

1114

1115

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  
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1123

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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