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Gangliosides of the vertebrate nervous system

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Abstract

Gangliosides, sialylated glycosphingolipids found on all vertebrate cells and tissues, are major molecular determinants on the surfaces of vertebrate nerve cells. Comprised of a sialylated glycan attached to a ceramide lipid, the same four structures – GM1, GD1a, GD1b and GT1b – represent the vast majority (>90%) of gangliosides in the brains of all mammals and birds. Primarily found on the outer surface of the plasma membrane with their glycans facing outward, gangliosides associate laterally with each other, sphingomyelin, cholesterol, and select proteins in lipid rafts – dynamic functional subdomains of the plasma membrane. The functions of gangliosides in the human nervous system are revealed by congenital mutations in ganglioside biosynthetic genes. Mutations in *ST3GAL5*, which codes for an enzyme early in brain ganglioside biosynthesis, results in an early-onset seizure disorder with profound motor and cognitive decay, whereas mutations in *B4GALNT1*, a gene encoding a later step, result in hereditary spastic paraplegia accompanied by intellectual deficits. The molecular functions of brain gangliosides include regulation of receptors in the same membrane via lateral (*cis*) associations and regulation of cell-cell recognition by *trans* interaction with ganglioside binding proteins on apposing cells. Gangliosides also affect aggregation of Aβ (Alzheimer's disease) and α-synuclein (Parkinson's Disease). As analytical, biochemical and genetic tools advance, research on gangliosides promises to reveal mechanisms of molecular control related to nerve and glial cell differentiation, neuronal excitability, axon outgrowth after nervous system injury, and protein folding in neurodegenerative diseases.

Graphical Abstract

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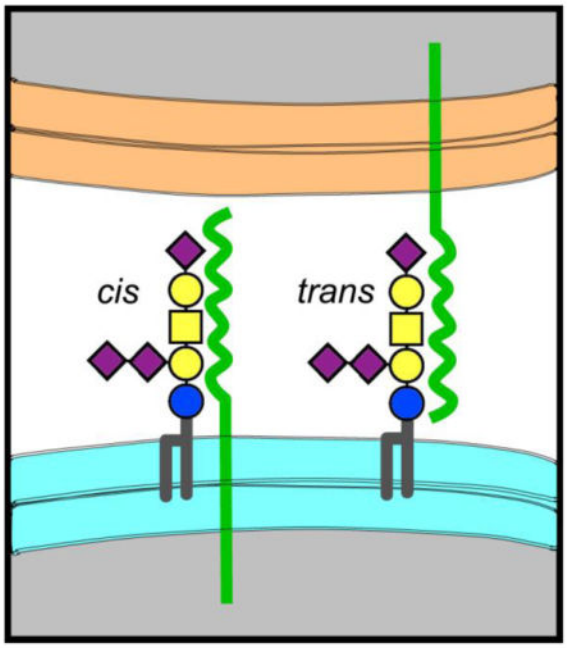

Keywords

glycosphingolipid; sialic acid; lipid raft; receptor tyrosine kinase; glycan binding protein

Introduction

Every cell in nature is endowed with a glycocalyx, a diverse coating of glycans, also referred to as glycoconjugates or complex carbohydrates [1]. The glycocalyx constitutes each cell's distinctive face to its environment, shaping cell surface biophysical properties and providing a biochemical signature that can be read by glycan recognition molecules [2–5]. In animals the glycocalyx is comprised of glycoproteins, glycolipids and proteoglycans, the ratios of which vary greatly among cell types. Most of the cells of the vertebrate nervous system are unusual in that their glycocalyces are dominated by glycolipids, and more specifically by glycosphingolipids consisting of glycans carried on a ceramide lipid embedded in the outer leaflet of the plasma membrane [6]. In nerve cells, these are primarily gangliosides (Figs. 1 & 2), glycosphingolipids with one or more sialic acid residue in their glycan structures [7,8]. Although gangliosides with various glycan structures are found in every vertebrate tissue, they are markedly more abundant in the nervous system. On average, each of the billions of nerve cells in the human brain has ~10 million cell surface gangliosides. Among mammals, the structures of the major brain gangliosides are well conserved, with the same four glycan structures (one pentasaccharide, two hexasaccharides and one heptasaccharide) comprising the vast majority of brain gangliosides from mouse to man (Fig. 2). Advances in biochemistry, cell biology and genetics are revealing the functions of these relatively abundant nerve cell surface molecules and their roles in human physiology and pathology.

This review presents the chemistry of the major mammalian nervous system gangliosides and the biophysical properties that endow them with distinctive behaviors at the plasma membrane. This is followed by review of some of the functions and pathophysiological roles of gangliosides, emphasizing major themes in recent research. Finally, technical advances that have opened new doors to the study of gangliosides and their functions are presented along with pressing technical challenges.

Chemistry and physics of mammalian nervous system gangliosides

Major ganglioside of mammalian nervous systems

Gangliosides are comprised of a sialylated glycan attached to a ceramide lipid core (Fig. 1). The sialoglycan is typically comprised of 3–8 saccharides of which 1–4 are sialic acids, although gangliosides with glycan structures outside these ranges are well known [9]. The ceramide moiety is comprised of sphingosine, a long chain 2-amino alcohol, in which the nitrogen is in amide linkage to a fatty acid. Gangliosides are found in all mammalian tissues, where variations in sialoglycans and in the lipid components of ceramide combine to generate hundreds of distinct ganglioside structures with a variety of functions that range from regulation of receptor tyrosine kinases to directing cell-cell recognition [10]. Mammalian brain gangliosides, however, are quantitatively dominated by just four sialoglycan sequences (Fig. 2), and their ceramide lipid components are quantitatively dominated by a single fatty acid ($C_{18:0}$, stearic acid) attached to one of two sphingosines, 2-amino-4-octadecene-1,3-diol (*d*18:1) and 2-amino-4-dodecene-1,3-diol (*d*20:1) [11]. Whereas the sialic acids in many non-human mammals are found in two abundant N-acylated forms (N-acetylneuraminic acid, Neu5Ac and N-glycolylneuraminic acid, Neu5Gc), brain gangliosides carry almost exclusively Neu5Ac, a portion of which may be O-acetylated (e.g. N-acetyl-9-O-acetylneuraminic acid, Neu5,9Ac2) [12,13]. Although the evolutionary forces that have maintained just four distinct saccharides on just two ceramide lipid cores as the quantitatively dominant gangliosides in the brains of mammals are not known, it is postulated that each structure must provide biological advantages that have yet to be fully elucidated.

The major mammalian brain gangliosides are biosynthesized stepwise by a series of glycosyltransferases that use nucleotide sugar donors to first glucosylate ceramide and then elongate the saccharide chain with a β4-galactose (Fig. 2). The galactose is typically sialylated at its 3-hydroxyl with a single Neu5Ac residue that may be further elongated by addition of a second (or third) α2–8 linked sialic acid. This is a key biosynthetic branch point. The same galactose is also elongated with a β4-N-acetylgalactosamine (GalNAc) at its 4-hydroxyl, but once that GalNAc is added no further addition of sialic acids occur at the 3-hydroxyl position. Gangliosides with no sialic acid on that first galactose residue are termed “0-series”, those with one sialic acid are “a-series”, those with two sialic acids are “b-series” and so on. In adult mammalian brain, 0-series gangliosides are rare, about half of the major gangliosides (GM1, GD1a) are a-series and the other half (GD1b, GT1b) b-series. In adult mammals and birds, c-series (or higher) gangliosides are rare, although they are abundant in fish and amphibians for reasons that aren’t well understood [14]. Beyond the GalNAc residue, major mammalian brain gangliosides carry a β3-linked galactose that may be

further sialylated with one or two sialic acids on the 3-hydroxyl of the terminal galactose. Rare gangliosides, termed "α-series" may carry a sialic acid on the 6-hydroxyl of the GalNAc residue [15,16].

A key to the biological functions of gangliosides is their orientation at the cell surface [17]. Their ceramide lipid is embedded in the outer leaflet of the lipid bilayer of the plasma membrane, which is comprised primarily of phospholipid and cholesterol. Although detailed conformational studies of major brain gangliosides are limited, NMR and modeling indicate that the glucose-ceramide bond of ganglioside GM1 is oriented such that the glycan extends perpendicular to the plane of the lipid bilayer of the plasma membrane [18]. The same conclusion was found with the simpler ganglioside GM3 modeled into a phospholipid bilayer [17]. Studies of GM1 indicated that the centrally located glycan branch point sugars (GalNAc β1–4[Neu5Ac α2–3]Gal) display a single dominant conformation, leading to the conclusion that the branch trisaccharide in major brain gangliosides is a rigid block. The same studies suggest that the linkages of the galactose residues may be more dynamic. The data suggest that gangliosides present distinct structures oriented outward from the plasma membrane.

The glycan moieties of the major brain gangliosides reach 2–2.5 nm outward from the hydrophobic membrane bilayer [19], extending well above the much more abundant phospholipid headgroups (~0.8 nm), and comparable to the lower range of plasma membrane proteins, most of which extend outward in the range of ~5–10 nm. The glycans of gangliosides are sufficiently large to engage proteins laterally on the cell surface, and extend outward sufficiently to engage binding proteins on apposing membranes.

Gangliosides at the plasma membrane

Under physiological conditions biological lipid bilayers behave as two-dimensional fluids dominated by glycerophospholipids [20]. In mammalian nerve cells, gangliosides represent a significant minority population of membrane lipids. In cultured rat cerebellar granule neurons, for example, glycerophospholipids constitute nearly 50 fmol/cell, cholesterol 5 fmol/cell, and sphingolipids 2 fmol/cell represented equally by gangliosides and sphingomyelin (ceramide with a phosphocholine head group) [21]. Phosphatidylcholine, which accounts for more than half the total glycerophospholipids, and sphingolipids are relatively over-represented in the outer leaflet of the plasma membrane compared to other lipids. The natural aggregative properties of glycerophospholipids in an aqueous environment spontaneously drive bilayer membrane formation, whereas the distinct molecular properties of sphingolipids (including gangliosides) compared to glycerophospholipids drive lateral associations within the bilayer. Spontaneously occurring lateral groupings of membrane molecules in which sphingolipids, cholesterol, and select proteins are over-represented have commonly been called lipid rafts or membrane microdomains [22,23]. Gangliosides, in particular, have been proposed as molecular markers for these hypothetical domains. More than thirty years after lateral membrane domains were first proposed [24], controversy persists concerning their existence and composition. Current findings support the view that kinetically dynamic nanometer-scale lipid rafts spontaneously

form on natural eukaryotic cell membranes, and that they have the capability to self-associate into larger and perhaps more stable membrane microenvironments [25].

Because of their ceramide lipid cores, sphingolipids including gangliosides share properties that enhance lateral self-association (Fig. 1) [26]. The sphingosine chain in gangliosides is 18–20 carbons in length with a single double bond at C4–C5. This leaves a long (14–16 carbon) fully saturated chain that inserts into the plasma membrane. In brain gangliosides, the C2 amine is N-acylated almost exclusively with fully saturated stearic acid (C18:0). This generates a ceramide that has two long saturated carbon chains that preferentially associate laterally with each other and other long saturated lipid chains compared to the common glycerophospholipids that typically carry a cis-unsaturated (kinked) lipid chain conducive to enhanced fluidity [20]. In addition ceramide-based lipids are headed by a C3-hydroxyl, a C2-amide nitrogen and an N-acyl (fatty acid) carbonyl, all of which can participate in hydrogen bonding near the aqueous/hydrophobic membrane interface. Together, these properties are believed to contribute to enhanced self-association of sphingolipids in the lateral plane of the membrane. The consequence of dynamic self-association is that multiple gangliosides have a tendency to spontaneously cluster into areas of lower mobility. Functional mechanisms in which gangliosides bind to complementary binding partners that are also clustered benefit entropically, resulting in higher binding avidity [10]. Mechanisms of ganglioside functions are addressed below, following a discussion of genetic disorders of ganglioside metabolism that place ganglioside functions in a biological context.

Genetic dysregulation of ganglioside biosynthesis

The best studied diseases of brain ganglioside metabolism are lysosomal storage diseases, in which mutations in the enzymes of ganglioside catabolism lead to intra-lysosomal accumulation with devastating effects on nerve cells [27]. Although mechanistic insights into ganglioside function can be gleaned from the buildup of a single ganglioside structure behind the catabolic block (e.g. GM2 in Tay-Sachs disease or GM1 in GM1 gangliosidosis [28]), disease outcomes are primarily due to the buildup of intra-lysosomal material.

Much rarer are diseases of ganglioside biosynthesis (Table 1). A single case study published in the 1970’s implied that genetic loss of a ganglioside biosynthetic enzyme resulted in a rapidly fatal developmental disorder [29–32]. Biochemical analysis of postmortem brain homogenates revealed the buildup of GM3 and GD3, the absence of more complex gangliosides, and a ~90% reduction in the enzyme activity of UDP-GalNAc:GM3 β-1,4-N-acetylgalactosaminyltransferase (GM2-synthase, *B4GALNT1* gene product, Fig. 2). The patient had poor physical and motor development at 1-month of age accompanied by frequent seizures, and died 2 months later. Upon autopsy, brain ultrastructure revealed defects in myelination as a notable feature [30,31].

The first genetically confirmed human defect in ganglioside biosynthesis was reported 30 years later when genome wide linkage analysis revealed that a mutation in *ST3GAL5*, the gene for GM3-synthase (CMP-N-acetylneuraminate:lactosylceramide α-2,3-N-acetylneuraminyltransferase, Fig. 2), a ganglioside-specific sialyltransferase, was responsible for a congenital infantile-onset seizure disorder accompanied by profound

developmental stagnation [33]. The same mutation was later found in an unrelated pedigree with affected individuals suffering the same clinical outcome [34]. A separate mutation in the same gene (*ST3GAL5*) resulted in severe cognitive and motor disabilities, seizures in only one of four affected subjects, and survival up to the 4th decade [35]. To date, biochemical analyses of postmortem brain tissue in these rare congenital disorders have not been reported, so relating clinical outcomes to changes in brain ganglioside expression is not yet possible. Studies of gangliosides in the plasma of affected subjects and in subject-derived fibroblasts support the conclusion that GM3 synthesis is compromised. A fascinating inter-species comparison comes from the study of *St3gal5*-null mice [36]. Although these mice display altered insulin sensitivity (see below), no neurological deficits were noted. This may be due to a robust shift from major brain gangliosides (GM1, GD1a, GD1b, GT1b) to quantitatively similar expression of the 0-series gangliosides GM1b and GD1α (Fig. 2). The extent (if any) to which metabolic shifting from a/b- to 0-series gangliosides occurs in human subjects with *ST3GAL5* mutations awaits access to postmortem brain tissue for biochemical studies.

The most recent reports of human mutations in the ganglioside biosynthetic pathway relate to *B4GALNT1* (GM2-synthase), the gene implicated in the case study from the 1970's [37,38]. Unlike the case study above, however, the phenotype was considerably less severe. Studies of >30 affected individuals from 10 families revealed hereditary spastic paraplegia, slowly progressive weakness and loss of motor control of the legs. Most affected individuals remained ambulatory into their 5th or 6th decade, with few progressing to wheelchair use. Notably, all affected individuals suffered from intellectual deficits, although not nearly as profound as reported for subjects with mutated *ST3GAL5*. Reported biochemical studies of ganglioside expression in affected individuals have been limited to skin-derived fibroblasts. A fuller understanding of the relationship between ganglioside expression and clinical outcomes must await access to postmortem brain tissue.

The motor phenotype of subjects with mutated *B4GALNT1* matched that of *B4galnt1*-null mice, which also suffered hindlimb motor disabilities that progressed until at one year of age they were unable to control or support weight on their hindlimbs [39,40]. Biochemical studies of *B4galnt1*-null mice revealed the complete absence of the major mammalian brain complex gangliosides (GM1, GD1a, GD1b, GT1b) with quantitatively equivalent buildup of GM3 and GD3 behind the block [41–43]. Their motor deficits appeared to be downstream of progressive dysmyelination and axonal degeneration [44], at least in part attributed to the function of gangliosides GD1a and GT1b in myelin stabilization (see below).

Functions of nervous system gangliosides in health and disease

Mechanisms of action

The clinical consequences of mutations in ganglioside biosynthetic enzymes and the phenotypes of genetically engineered ganglioside-deficient mice (see above) provide glimpses of the functions of gangliosides in the nervous system. These findings motivate studies of the underlying molecular mechanisms. Clear evidence supports at least two molecular mechanisms by which gangliosides regulate biochemical and cellular functions [10,45]: lateral interactions of gangliosides with proteins on the same cell membrane (*cis*)

and binding of gangliosides on one cell surface to complementary glycan binding proteins on an apposing cell or in the extracellular milieu (*trans*).

A good example of *cis* regulation is the association of the insulin receptor (IR) with the abundant peripheral tissue ganglioside GM3 [46]. Lateral association of GM3 with the IR suppresses insulin-induced autophosphorylation and downstream signaling events *in vitro*. Lateral binding of the IR to GM3 sequesters the receptor, impairing its lateral association with caveolin which is required for insulin-mediated metabolic signaling [47]. Mice engineered to lack GM3 (*St3gal5*-null) displayed enhanced insulin sensitivity and were protected from high fat diet-induced insulin resistance [36]. Ganglioside-mediated lateral sequestration of signaling molecules on the cell surface into active or inactive states may be a general mechanism for ganglioside functions [10].

A good example of *trans* recognition of gangliosides is the interaction of the glycan binding protein myelin-associated glycoprotein (MAG) on the innermost wrap of myelin with gangliosides on the apposing surface of the enwrapped nerve cell axon [48]. Oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system segmentally wrap long nerve axons with myelin, a multilayered membrane that insulates the axon and organizes ion channels along the length of the axon [49]. Myelin segments (internodes) are interrupted by unmyelinated short segments, nodes of Ranvier, where voltage sensitive sodium channels are clustered to propagate action potentials. Axon segments adjacent to the nodes are the site of adhesion molecules and potassium channels, which repolarize the membrane after the action potential.

MAG is found selectively on the innermost myelin wrap, across from the axon [50]. It was found to share sequence similarity with two sialic acid binding proteins, sialoadhesin and CD22 [51], leading to the discovery that MAG (now also called Siglec-4) is part of what is the siglec family (sialic acid-binding immunoglobulin-type lectins) [52,53]. Subsequent studies identified gangliosides GD1a and GT1b as axonal ligands for MAG [54,55]. Mice lacking complex gangliosides (*B4galnt1*-null) fail to maintain appropriate axon-myelin associations leading to disruptions in the structure and molecular distributions at nodes of Ranvier [56], dysmyelination-related axon degeneration [44], and progressive motor neuropathy [39]. Mice engineered to lack MAG (*Mag*-null), complex gangliosides (*B4galnt1*-null) and double-null mice have similar deficits in myelin structure, axon stability, and behavior [40]. These findings support the hypothesis that gangliosides, and particular GD1a and GT1b, are essential for long-term axon-myelin stability and that the loss of MAG-ganglioside binding is responsible for hereditary spastic paraplegia in subjects with mutations in the *B4GALNT1* gene [37,38].

The clinical disorders of humans with ganglioside biosynthetic gene mutations and the phenotypes of mice engineered to have similar genetic deficits indicate the roles of gangliosides in nervous system stability and function, both motor and cognitive. Additional mechanistic insights come from biochemical and cellular studies detailed in the following sections.

Regulation of axon outgrowth

Gangliosides regulate axon outgrowth intrinsically by regulating nerve cell mechanisms that drive growth cone extension [57,58] and extrinsically by conveying outgrowth inhibitory signals from the local environment to the growth cone [59]. The exploration of intrinsic ganglioside regulation of axon outgrowth has focused, in part, on the membrane-associated sialidase Neu3, which readily cleaves terminal sialic acids from GD1a, GD1b and GT1b leaving GM1 [60]. The branching sialic acid on GM1 (or GM2) is resistant to Neu3 as it is to most bacterial and viral sialidases. Therefore, cell surface sialidase Neu3 will locally deplete three of the four major brain gangliosides, increasing the local concentration of GM1. Notably, in cultured embryonic rat hippocampal neurons Neu3 was found to accumulate in one of several membrane protrusions, and that protrusion became the neuron's single axon [57]. Molecular or pharmacologic inhibition of Neu3 blocked axon formation. Data supported a model in which Neu3 conversion of more complex gangliosides to GM1 at the surface of the growth cone resulted in enhanced responsiveness of the neurotrophin receptor tyrosine kinase TrkA, activation of the small GTPase Rac1, inhibition of RhoA and its downstream effectors, loss of F-actin and increased axon outgrowth.

Regulation and responses to gangliosides in the intrinsic control of axon outgrowth are likely to be neuronal cell-type specific. In adult rat dorsal root ganglion (peripheral nervous system) neurons, axon transection resulted in increased Neu3 activity, a shift from GD1a/GT1b to GM1 gangliosides, and axon regeneration [58]. Inhibiting Neu3 reduced axon regeneration, an effect reversed by adding exogenous sialidase. Transection of axons of adult retinal neurons (central nervous system) did not result in increased Neu3 activity or a shift from GD1a/GT1b to GM1, and axon regeneration was weak. Addition of sialidase, however, enhanced axon regeneration in retinal neurons. The data support an intrinsic neuronal mechanism in which Neu3-mediated changes in ganglioside structure result in activation of receptor tyrosine kinases, ERK activation and axon regeneration [58]. Molecular details of how ganglioside structural changes modulate signaling proteins in the neuronal axon and growth cone have not been clearly established and remain a valuable area for future studies.

In addition to intrinsic regulation of axon outgrowth, gangliosides mediate the inhibition of axon outgrowth by extrinsic factors [61–63]. Axon outgrowth in the adult nervous system is highly restricted, in part due to proteins in myelin, including Nogo and MAG, which specifically signal axon outgrowth suppression via cell surface receptors that indirectly activate RhoA [64]. In the injured nervous system, residual myelin is joined by chondroitin sulfate proteoglycans (largely from astrocytes) that also directly inhibit axon outgrowth. Gangliosides are among the key molecules involved in the signaling pathways of axon outgrowth inhibition [61–63]. In some neurons MAG binds to axonal gangliosides GD1a and GT1b resulting in activation of RhoA and axon outgrowth inhibition. In other neurons MAG inhibition is largely independent of gangliosides, instead requiring members of the GPI-anchored NgR (Nogo receptor) family on the axon surface. Whereas evidence supports the independent functions of gangliosides and NgR family members as mediators of MAG inhibition of axon outgrowth, the two types of receptors can also self-associate to form a signaling complex [65]. These mechanisms provide a wealth of regulatory flexibility, presumably to carefully restrict unwanted axon sprouting in the adult nervous system.

However, they also limit axon regeneration after injury, contributing to poor recovery from central nervous system injuries such as spinal cord injury and traumatic brain injury. The potential for ganglioside structural modifications to enhance axon outgrowth in preclinical (rat) models of brachial plexus injury and spinal cord injury were tested by delivering sialidase directly to the site of injury via implanted osmotic pumps resulting in the loss of complex brain gangliosides such as GT1b and sharply increased GM1, the sialidase product [66–68]. Sialidase treatment resulted in significant increases in axon outgrowth accompanied by enhanced functional recovery.

Regulation of receptor function

The regulation of receptors by gangliosides, most notably tyrosine kinase receptors, is well established [10,69]. In addition to GM3 inhibition of the insulin receptor discussed above [46], GM3 inhibits whereas GD1a enhances activation of the EGF receptor [70], GM1 inhibits the PDGF receptor [71], and GM1 activates TrkA [18,72]. For the insulin receptor, GM3 binds directly to the receptor at a site requiring a membrane proximal lysine (K944) and competes with other insulin receptor associations required for function [47]. Similarly, the EGF receptor binds to GM3 directly at a similarly located lysine (K642) [73]. In other cases, regulation of tyrosine phosphorylation by gangliosides may be indirect via alterations in membrane protein organization, especially at the level of lipid rafts [74].

Receptor trafficking is another way in which gangliosides affect receptor function. An unbiased screen for nerve cell proteins that differentially bind to gangliosides GT1b and GM1 revealed a group of proteins that regulate the intracellular trafficking of the GluR2 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptor, the major excitatory neurotransmitter receptor in the brain [75]. AMPA receptors are tetrameric transmembrane proteins composed of dimers of dimers constructed from four gene products (GluR1-GluR4), the most common being GluR1-GluR2 and GluR2-GluR3 [76]. The subunit composition of AMPA receptors is important to their functions, a key being the abundant GluR2 subunit, the presence of which reduces calcium permeability and channel conductance [77]. Synaptic excitability, synaptic plasticity, learning and memory can be driven by quantitative and qualitative changes in synaptic AMPA receptors [78,79]. It is notable in this context that the GluR2 subunit binds selectively to ganglioside GM1, whereas a suite of proteins involved in GluR2 internalization (thorase, NSF, and nicalin) bind selectively to ganglioside GT1b. Modulation of sialoglycans on hippocampal neurons with sialidase resulted in altered distribution of surface GluR2-containing AMPA receptors. The potential for complex gangliosides to regulate the major excitatory neurotransmitter in the brain may explain why congenital deficits in ganglioside biosynthesis invariably result in intellectual deficits and often are associated with seizures [30,33–35,37,38].

Gangliosides in diseases of protein folding

The pathophysiology of major age-related human neurodegenerative disorders involves alterations in protein folding and protein aggregation [80]. The creation of extracellular fibrils of amyloid Aβ is believed to be a primary driver of Alzheimer’s disease pathology, whereas intracellular fibrils of α-synuclein are characteristic of Parkinson’s disease. In both

cases, the highly infrequent generation of an alternatively folded protein seed is believed to induce the same fold in soluble monomers, resulting in feed-forward fibril formation. Gangliosides have been implicated as regulators of both Aβ and α-synuclein aggregation, but in quite different ways [81,82].

A portion of Aβ isolated from Alzheimer brain extract was found associated with ganglioside GM1 [83]. Subsequently, GM1 and other gangliosides in defined liposomes were found to bind to Aβ at sub-micromolar affinity and accelerate Aβ fibril formation in a structurally specific manner (GM1 > GD1b > GD1a, GT1b) [84,85]. Recent work indicates that GM1-Aβ may be a necessary prerequisite to Aβ toxicity. In a study designed to discover the fate of Aβ dimers injected into the cerebrospinal fluid of live mice, the protein was principally recovered bound to membranes [86]. When solubilized, all of the Aβ migrated as a slightly larger molecular weight than expected. The altered molecular weight species, which was stable upon SDS gel electrophoresis, co-stained for GM1 using the highly specific binding reagent cholera toxin B-subunit, indicating very stable spontaneous association of Aβ with GM1 in the living brain. In vitro binding studies indicated that Aβ dimers (or oligomers) are the active GM1-binding species. Detailed Aβ aggregation and structural studies in the presence of GM1-containing liposomes indicate that GM1 can accelerate a shift from α-helical Aβ to toxic fibrils [87]. Functional blocking of GM1 on intact hippocampal neurons with cholera toxin B subunit diminished Aβ dimer-induced pathological electrophysiological changes, and an antibody specific for GM1-bound Aβ detected it in human cerebrospinal fluid [86]. Together, these data imply that brain gangliosides, particularly GM1, may accelerate the conversion of Aβ from a soluble molecule to a toxic fibril in the pathogenesis of Alzheimer's disease [81].

In contrast to its effect on Aβ, GM1 binds to α-synuclein and inhibits fibril formation in vitro [88]. Mice lacking complex gangliosides (*B4galnt1*-null) had fewer tyrosine hydroxylase-positive cells and more α-synuclein positive cells in their substantia nigra pars compacta, consistent with a physiological role for complex gangliosides in the control of α-synuclein deposition [89]. The same mutant mice demonstrated improved behavior after L-dopa administration. Human substantia nigra pars compacta sections from Parkinson tissue donors had fewer dopaminergic neurons, and of those a much lower percent were also GM1 positive as measured by CTB binding. These intriguing data provide the impetus for further exploration of the potential protective role of gangliosides in α-synuclein fibril formation and Parkinson's disease.

Tools and challenges in ganglioside research

Ganglioside analytics

Analytical tools for studying gangliosides have improved significantly, but ganglioside analysis remains largely the purview of specialists in the field [90,91]. The simplest analytical tools to use, ganglioside-specific antibodies and binding proteins (toxins) are widely available and provide enhanced opportunities to study brain ganglioside expression and function in health and disease [92–95]. These techniques are limited, however, to major brain gangliosides and minor gangliosides with well-defined antibodies or toxins, which aren't always available. Furthermore, gangliosides exist in multiple isoforms with varying

ceramide lipid structures and the potential for hydroxyl acetylation that make immuno- or binding protein-detection more challenging and less definitive. In addition, certain routine techniques used in immunohistochemistry, including solvent fixation and the use of certain detergents, either remove gangliosides or worse yet result in anatomical redistribution of gangliosides [96,97], so great care in both experimental execution and interpretation are required.

Beyond antibodies and binding proteins, ganglioside analytical techniques usually require extraction of tissues or cells with organic solvents and partitioning to remove bulk contaminating lipids prior to analysis [98], typically by thin-layer chromatography and/or mass spectrometry (MS). Thin-layer chromatography of gangliosides is relatively simple and sensitive [99], whereas techniques for MS analysis of isolated gangliosides require expensive equipment and advanced expertise, but are much more powerful, providing detailed qualitative and quantitative information by electrospray MS^n techniques on relatively small samples [90,91,100].

An exciting addition to these techniques is spatially-resolved matrix-assisted (MALDI) MS, in which an intact tissue section (e.g. mouse brain) is evenly sprayed with matrix and subjected to laser-induced desorption with raster sampling and time-of-flight mass determination [101–104]. Each laser-struck spot on the tissue ionizes many molecules including brain gangliosides, which have distinctive masses compared to other lipids and small molecules. Anatomic maps of each mass can be generated. MALDI-MS mapping of gangliosides has revealed exciting and unanticipated selective anatomic distributions of different brain gangliosides, especially based on their ceramide structures. The biological significance of these distributions has yet to be understood. Despite its power, MALDI-MS mapping is prone to in-source loss of sialic acids, which are differentially sensitive to the experimental conditions [105]. This can lead to under-representation of major brain gangliosides GT1b, GD1b, and GD1a and over-representation of GM1, for example. Despite current limitations, this technique holds great promise.

Lipid rafts and ganglioside-protein interactions

Gangliosides, along with other sphingolipids, are believed to preferentially reside in lipid rafts [106]. In fact, ganglioside-specific reagents (such as with CTB) are routinely used to identify lipid rafts. A challenge to this area of study was the discovery that under common conditions of lipid raft isolation (1% Triton X-100 detergent in cold aqueous buffers) gangliosides readily enter the soluble phase and redistribute to other membranes [96,97]. Under defined conditions some detergents other than Triton X-100 do not induced ganglioside redistribution, and may provide for more accurate determinations of raft and non-raft ganglioside distributions. Several methods for directly determining ganglioside-protein interactions, including affinity capture and photoaffinity labeling, have been successfully applied. However, the amphipathic nature of gangliosides and their propensity to spontaneously partition into aggregates with proteins and other lipids provide challenges for determination of ganglioside-specific molecular interactions. Careful analysis of the stringency of ganglioside-protein interactions and their structural specificity for related gangliosides can provide the means to overcome background binding limitations.

Ganglioside biosynthesis in human congenital disorders

Some of the most compelling data about ganglioside function has come from genetic linkage analyses relating ganglioside-specific gene mutations to human disorders, including seizure disorders, movement disorders and intellectual disability [33–35,37,38]. To date, access to nervous system tissues from affected patients has been limited or absent. Instead, analysis of ganglioside expression in these disorders has been on blood cells or fibroblasts, which do not have the same concentrations or complexities of gangliosides found in the brain. Related studies in mutant mice have revealed quantitative compensation, with alternative gangliosides building up behind the block [107]. In one case in particular, *St3gal5*-null mice, an over-expression of rare 0-series gangliosides appears to largely functionally compensate for the loss of the major brain gangliosides [36]. Knowledge of the quantitative and qualitative changes in human mutations of ganglioside biosynthesis would provide both enhanced mechanistic insights and perhaps avenues for treatment of ganglioside deficiencies.

Regulated ganglioside expression in animal models

Major brain gangliosides are largely shared, both qualitatively and quantitatively, among mammals (and birds), providing excellent opportunities to develop animal models to explore ganglioside function [108]. There are mouse genetic models that are null for many ganglioside biosynthetic genes (*St3gal5*, *B4galnt1*, *St8sia1*, *St3gal2*, among others) [107]. However, few have been engineered for conditional tissue, cell and developmental regulation of expression. Some of the models suffer from non-neural phenotypes and lack of cell specific ablation in the brain. Recovery of function in *B4galnt1*-null mice by transgenic expression of *B4galnt1* separately in nerve cells or glial cells has been highly revealing, demonstrating that the neurodegenerative phenotype in these mice was due to loss of gangliosides specifically in nerve cells [109]. Similar advances can be anticipated when conditional and multiple ganglioside biosynthetic gene deletions are made in mice. With the advance of rapid gene editing techniques [110], it is timely to invest in a broader set of ganglioside gene regulation models for studying the multiple roles of gangliosides in pathogen binding, cell-cell recognition, and receptor regulation across cells and tissues.

Closing remark

Gangliosides are major cell surface molecules on all nerve cells in the vertebrate nervous system, where they provide molecular signatures for intrinsic (*cis*) and extrinsic (*trans*) molecular interactions. As knowledge of the multiple roles of gangliosides in physiology and pathology continue to emerge, and as the tools to explore the structure-function relationships and mechanisms of ganglioside-mediated cellular regulation continue to evolve, a better understanding of areas as diverse as axon stability and regeneration, synaptic plasticity, and cellular differentiation in health and disease await.

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Abbreviations used

CTB	cholera toxin B subunit
Neu5Ac	N-acetylneuraminic acid (sialic acid)
Siglec	sialic acid-binding immunoglobulin-like lectin. Ganglioside nomenclature is that of Svennerholm [111]

References

1. Varki A. Evolutionary forces shaping the Golgi glycosylation machinery: why cell surface glycans are universal to living cells. Cold Spring Harb Perspect Biol. 2011; 3:a005462. [PubMed: 21525513]
2. Hart GW, Copeland RJ. Glycomics hits the big time. Cell. 2010; 143:672–676. [PubMed: 21111227]
3. Taylor, ME.; Drickamer, K. Introduction to Glycobiology. 3. Oxford University Press; Oxford: 2011.
4. Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P.; Bertozzi, CR., et al. Essentials of Glycobiology. 2. Cold Spring Harbor Laboratory Press; New York: 2009.
5. Gabius, HJ. The Sugar Code. Wiley-VCH; Weinheim: 2009.
6. Schnaar, RL. Neural functions of glycolipids. In: Kamerling, JP.; Boons, GJ.; Lee, YC.; Suzuki, A.; Taniguchi, N.; Voragen, AGJ., editors. Comprehensive Glycoscience. Volume 4: Cell Glycobiology and Development; Health and Disease in Glycomedicine. Elsevier Science; Amsterdam: 2007. p. 323-337.
7. Schnaar RL, Gerardy-Schahn R, Hildebrandt H. Sialic acids in the brain: gangliosides and polysialic acid in nervous system development, stability, disease and regeneration. Physiol Rev. 2014; 94:461–518. [PubMed: 24692354]
8. Schengrund CL. Gangliosides: glycosphingolipids essential for normal neural development and function. Trends Biochem Sci. 2015; 40:406.
9. Yu RK, Tsai YT, Ariga T, Yanagisawa M. Structures, Biosynthesis, and Functions of Gangliosides-an Overview. J Oleo Sci. 2011; 60:537–544. [PubMed: 21937853]
10. Todeschini AR, Hakomori SI. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. Biochim Biophys Acta. 2008; 1780:421–433. [PubMed: 17991443]
11. Jackson SN, Colsch B, Egan T, Lewis EK, Schultz JA, Woods AS. Gangliosides' analysis by MALDI-ion mobility MS. Analyst. 2011; 136:463–466. [PubMed: 21113547]
12. Davies LR, Varki A. Why Is N-Glycolylneuraminic Acid Rare in the Vertebrate Brain? Top Curr Chem. 2015; 366:31–54. [PubMed: 23471785]
13. Mlinac K, Fabris D, Vukelic Z, Rozman M, Heffer M, Bognar SK. Structural analysis of brain ganglioside acetylation patterns in mice with altered ganglioside biosynthesis. Carbohydr Res. 2013; 382C:1–8. [PubMed: 24140892]
14. Greis C, Rosner H. c-pathway polysialogangliosides in the nervous tissue of vertebrates, reacting with the monoclonal antibody Q211. Brain Res. 1990; 517:105–110. [PubMed: 1695858]
15. Ando S, Hirabayashi Y, Kon K, Inagaki F, Tate S, Whittaker VP. A trisialoganglioside containing a sialyl a2–6 N-acetylgalactosamine residue is a cholinergic-specific antigen, Chol-1a-a. J Biochem. 1992; 111:287–290. [PubMed: 1587788]
16. Okajima T, Chen HH, Ito H, Kiso M, Tai T, Furukawa K, et al. Molecular cloning and expression of mouse GD1alpha/GT1aalpha/GQ1balpha synthase (ST6GalNAc VI) gene. J Biol Chem. 2000; 275:6717–6723. [PubMed: 10702226]
17. DeMarco ML, Woods RJ. Atomic-resolution conformational analysis of the GM3 ganglioside in a lipid bilayer and its implications for ganglioside-protein recognition at membrane surfaces. Glycobiology. 2009; 19:344–355. [PubMed: 19056784]

18. Aureli M, Mauri L, Ciampa MG, Prinetti A, Toffano G, Secchieri C, et al. GM1 Ganglioside: Past Studies and Future Potential. Mol Neurobiol. 2015
19. Acquotti D, Sonnino S. Use of nuclear magnetic resonance spectroscopy in evaluation of ganglioside structure, conformation, and dynamics. Methods Enzymol. 2000; 312:247–272. [PubMed: 11070877]
20. Sonnino S, Prinetti A. Membrane domains and the “lipid raft” concept. Curr Med Chem. 2013; 20:4–21. [PubMed: 23150999]
21. Prinetti A, Chigorno V, Prioni S, Loberto N, Marano N, Tettamanti G, et al. Changes in the lipid turnover, composition, and organization, as sphingolipid-enriched membrane domains, in rat cerebellar granule cells developing in vitro. J Biol Chem. 2001; 276:21136–21145. [PubMed: 11264283]
22. Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, et al. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature. 2009; 457:1159–1162. [PubMed: 19098897]
23. Aureli M, Grassi S, Prioni S, Sonnino S, Prinetti A. Lipid membrane domains in the brain. Biochim Biophys Acta. 2015; 1851:1006–1016. [PubMed: 25677824]
24. Simons K, van Meer G. Lipid sorting in epithelial cells. Biochemistry. 1988; 27:6197–6202. [PubMed: 3064805]
25. Carquin M, D’Auria L, Pollet H, Bongarzone ER, Tyteca D. Recent progress on lipid lateral heterogeneity in plasma membranes: From rafts to submicrometric domains. Prog Lipid Res. 2015; 62:1–24. [PubMed: 26738447]
26. Cantu L, Del FE, Sonnino S, Prinetti A. Gangliosides and the multiscale modulation of membrane structure. Chem Phys Lipids. 2011; 164:796–810. [PubMed: 21946022]
27. Sandhoff K, Harzer K. Gangliosides and gangliosidoses: principles of molecular and metabolic pathogenesis. J Neurosci. 2013; 33:10195–10208. [PubMed: 23785136]
28. Walkley SU. Neurobiology and cellular pathogenesis of glycolipid storage diseases. Philos Trans R Soc Lond B Biol Sci. 2003; 358:893–904. [PubMed: 12803923]
29. Fishman PH, Max SR, Tallman JF, Brady RO, Maclaren NK, Cornblath M. Deficient ganglioside biosynthesis: a novel human sphingolipidosis. Science. 1975; 187:68–70. [PubMed: 803227]
30. Max SR, Maclaren NK, Brady RO, Bradley RM, Rennels MB, Tanaka J, et al. GM3 (hematoside) sphingolipodystrophy. N Engl J Med. 1974; 291:929–931. [PubMed: 4213132]
31. Tanaka J, Garcia JH, Max SR, Viloria JE, Kamijyo Y, McLaren NK, et al. Cerebral sponginess and GM3 gangliosidosis; ultrastructure and probable pathogenesis. J Neuropathol Exp Neurol. 1975; 34:249–262. [PubMed: 167132]
32. Maclaren NK, Max SR, Cornblath M, Brady RO, Ozand PT, Campbell J, et al. GM3 gangliosidosis: a novel human sphingolipodystrophy. Pediatrics. 1976; 57:106–110. [PubMed: 813179]
33. Simpson MA, Cross H, Gurtz K, Priestman DA, Neville DCA, Reinkensmeier G, et al. Infantile onset symptomatic epilepsy syndrome caused by homozygous loss of function mutations in GM3 synthase. Nat Genet. 2004; 36:1225–1229. [PubMed: 15502825]
34. Fragaki K, Ait-El-Mkadem S, Chaussenot A, Gire C, Mengual R, Bonesso L, et al. Refractory epilepsy and mitochondrial dysfunction due to GM3 synthase deficiency. Eur J Hum Genet. 2013; 21:528–534. [PubMed: 22990144]
35. Boccuto L, Aoki K, Flanagan-Steet H, Chen CF, Fan X, Bartel F, et al. A mutation in a ganglioside biosynthetic enzyme, ST3GAL5, results in salt & pepper syndrome, a neurocutaneous disorder with altered glycolipid and glycoprotein glycosylation. Hum Mol Genet. 2014; 23:418–433. [PubMed: 24026681]
36. Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proc Natl Acad Sci U S A. 2003; 100:3445–3449. [PubMed: 12629211]
37. Boukhris A, Schule R, Loureiro JL, Lourenco CM, Mundwiller E, Gonzalez MA, et al. Alteration of Ganglioside Biosynthesis Responsible for Complex Hereditary Spastic Paraplegia. Am J Hum Genet. 2013; 93:118–123. [PubMed: 23746551]

38. Harlalka G, Lehman A, Chioza B, Baple E, Maroofian R, Cross H, et al. Mutations in B4GALNT1 (GM2 Synthase) underlie a new disorder of ganglioside biosynthesis. Brain. 2013; 136:3618–3624. [PubMed: 24103911]
39. Chiavegatto S, Sun J, Nelson RJ, Schnaar RL. A functional role for complex gangliosides: motor deficits in GM2/GD2 synthase knockout mice. Exp Neurol. 2000; 166:227–234. [PubMed: 11085888]
40. Pan B, Fromholt SE, Hess EJ, Crawford TO, Griffin JW, Sheikh KA, et al. Myelin-associated glycoprotein and complementary axonal ligands, gangliosides, mediate axon stability in the CNS and PNS: neuropathology and behavioral deficits in single- and double-null mice. Exp Neurol. 2005; 195:208–217. [PubMed: 15953602]
41. Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proc Natl Acad Sci U S A. 1996; 93:10662–10667. [PubMed: 8855236]
42. Liu Y, Wada R, Kawai H, Sango K, Deng C, Tai T, et al. A genetic model of substrate deprivation therapy for a glycosphingolipid storage disorder. J Clin Invest. 1999; 103:497–505. [PubMed: 10021458]
43. Sun J, Shaper NL, Itonori S, Heffer-Lauc M, Sheikh KA, Schnaar RL. Myelin-associated glycoprotein (Siglec-4) expression is progressively and selectively decreased in the brains of mice lacking complex gangliosides. Glycobiology. 2004; 14:851–857. [PubMed: 15175257]
44. Sheikh KA, Sun J, Liu Y, Kawai H, Crawford TO, Proia RL, et al. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. Proc Natl Acad Sci U S A. 1999; 96:7532–7537. [PubMed: 10377449]
45. Hakomori S. The glycosynapse. Proc Natl Acad Sci U S A. 2002; 99:225–232. [PubMed: 11773621]
46. Lipina C, Hundal HS. Ganglioside GM3 as a gatekeeper of obesity-associated insulin resistance: Evidence and mechanisms. FEBS Lett. 2015; 589:3221–3227. [PubMed: 26434718]
47. Kabayama K, Sato T, Saito K, Loberto N, Prinetti A, Sonnino S, et al. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc Natl Acad Sci U S A. 2007; 104:13678–13683. [PubMed: 17699617]
48. Schnaar RL. Brain gangliosides in axon-myelin stability and axon regeneration. FEBS Lett. 2010; 584:1741–1747. [PubMed: 19822144]
49. Nave KA. Myelination and support of axonal integrity by glia. Nature. 2010; 468:244–252. [PubMed: 21068833]
50. Trapp BD, Andrews SB, Cootauco C, Quarles R. The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. J Cell Biol. 1989; 109:2417–2426. [PubMed: 2478568]
51. Kelm S, Pelz A, Schauer R, Filbin MT, Song T, de Bellard ME, et al. Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily. Curr Biol. 1994; 4:965–972. [PubMed: 7533044]
52. Varki A, Angata T. Siglecs--the major subfamily of I-type lectins. Glycobiology. 2006; 16:1R–27R. [PubMed: 16118287]
53. Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol. 2014; 14:653–666. [PubMed: 25234143]
54. Yang LJS, Zeller CB, Shaper NL, Kiso M, Hasegawa A, Shapiro RE, et al. Gangliosides are neuronal ligands for myelin-associated glycoprotein. Proc Natl Acad Sci U S A. 1996; 93:814–818. [PubMed: 8570640]
55. Collins BE, Yang LJS, Mukhopadhyay G, Filbin MT, Kiso M, Hasegawa A, et al. Sialic acid specificity of myelin-associated glycoprotein binding. J Biol Chem. 1997; 272:1248–1255. [PubMed: 8995428]
56. Susuki K, Baba H, Tohyama K, Kanai K, Kuwabara S, Hirata K, et al. Gangliosides contribute to stability of paranodal junctions and ion channel clusters in myelinated nerve fibers. Glia. 2007; 55:746–757. [PubMed: 17352383]

57. Da Silva JS, Hasegawa T, Miyagi T, Dotti CG, Abad-Rodriguez J. Asymmetric membrane ganglioside sialidase activity specifies axonal fate. Nat Neurosci. 2005; 8:606–615. [PubMed: 15834419]
58. Kappagantula S, Andrews MR, Cheah M, Abad-Rodriguez J, Dotti CG, Fawcett JW. Neu3 sialidase-mediated ganglioside conversion is necessary for axon regeneration and is blocked in CNS axons. J Neurosci. 2014; 34:2477–2492. [PubMed: 24523539]
59. Schnaar RL, Lopez PH. Myelin-associated glycoprotein and its axonal receptors. J Neurosci Res. 2009; 87:3267–3276. [PubMed: 19156870]
60. Monti E, Miyagi T. Structure and Function of Mammalian Sialidases. Top Curr Chem. 2015; 366:183–208. [PubMed: 22760823]
61. Vyas AA, Patel HV, Fromholt SE, Heffer-Lauc M, Vyas KA, Dang J, et al. Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. Proc Natl Acad Sci U S A. 2002; 99:8412–8417. [PubMed: 12060784]
62. Vinson M, Strijbos PJ, Rowles A, Facci L, Moore SE, Simmons DL, et al. Myelin-associated glycoprotein interacts with ganglioside GT1b: A mechanism for neurite outgrowth inhibition. J Biol Chem. 2001; 276:20280–20285. [PubMed: 11279053]
63. Mehta NR, Lopez PH, Vyas AA, Schnaar RL. Gangliosides and Nogo receptors independently mediate myelin-associated glycoprotein inhibition of neurite outgrowth in different nerve cells. J Biol Chem. 2007; 282:27875–27886. [PubMed: 17640868]
64. Yiu G, He Z. Glial inhibition of CNS axon regeneration. Nat Rev Neurosci. 2006; 7:617–627. [PubMed: 16858390]
65. Williams G, Wood A, Williams EJ, Gao Y, Mercado ML, Katz A, et al. Ganglioside inhibition of neurite outgrowth requires Nogo receptor function: identification of interaction sites and development of novel antagonists. J Biol Chem. 2008; 283:16641–16652. [PubMed: 18411262]
66. Yang LJ, Lorenzini I, Vajn K, Mountney A, Schramm LP, Schnaar RL. Sialidase enhances spinal axon outgrowth in vivo. Proc Natl Acad Sci U S A. 2006; 103:11057–11062. [PubMed: 16847268]
67. Mountney A, Zahner MR, Lorenzini I, Oudega M, Schramm LP, Schnaar RL. Sialidase enhances recovery from spinal cord contusion injury. Proc Natl Acad Sci U S A. 2010; 107:11561–11566. [PubMed: 20534525]
68. Mountney A, Zahner MR, Sturgill ER, Riley CJ, Aston JW, Oudega M, et al. Sialidase, Chondroitinase ABC, and Combination Therapy after Spinal Cord Contusion Injury. J Neurotrauma. 2013; 30:181–190. [PubMed: 22934782]
69. Kaucic K, Liu Y, Ladisch S. Modulation of growth factor signaling by gangliosides: positive or negative? Methods Enzymol. 2006; 417:168–185. [PubMed: 17132505]
70. Liu Y, Li R, Ladisch S. Exogenous ganglioside GD1a enhances epidermal growth factor receptor binding and dimerization. J Biol Chem. 2004; 279:36481–36489. [PubMed: 15215248]
71. Mitsuda T, Furukawa K, Fukumoto S, Miyazaki H, Urano T, Furukawa K. Overexpression of ganglioside GM1 results in the dispersion of platelet-derived growth factor receptor from glycolipid-enriched microdomains and in the suppression of cell growth signals. J Biol Chem. 2002; 277:11239–11246. [PubMed: 11782461]
72. Farooqui T, Franklin T, Pearl DK, Yates AJ. Ganglioside GM1 enhances induction by nerve growth factor of a putative dimer of TrkA. J Neurochem. 1997; 68:2348–2355. [PubMed: 9166727]
73. Coskun U, Grzybek M, Drechsel D, Simons K. Regulation of human EGF receptor by lipids. Proc Natl Acad Sci U S A. 2011; 108:9044–9048. [PubMed: 21571640]
74. Sekino-Suzuki N, Yuyama K, Miki T, Kaneda M, Suzuki H, Yamamoto N, et al. Involvement of gangliosides in the process of Cbp/PAG phosphorylation by Lyn in developing cerebellar growth cones. J Neurochem. 2013; 124:514–522. [PubMed: 23035659]
75. Prendergast J, Umanah GK, Yoo SW, Lagerlof O, Motari MG, Cole RN, et al. Ganglioside regulation of AMPA receptor trafficking. J Neurosci. 2014; 34:13246–13258. [PubMed: 25253868]
76. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev. 2010; 62:405–496. [PubMed: 20716669]

77. Isaac JT, Ashby MC, McBain CJ. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron. 2007; 54:859–871. [PubMed: 17582328]

78. Liu SJ, Savtchouk I. Ca(2+) permeable AMPA receptors switch allegiances: mechanisms and consequences. J Physiol. 2012; 590:13–20. [PubMed: 21893602]

79. Hanley JG. Subunit-specific trafficking mechanisms regulating the synaptic expression of Ca-permeable AMPA receptors. Semin Cell Dev Biol. 2014; 27:14–22. [PubMed: 24342448]

80. Goedert M. NEURODEGENERATION. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Abeta, tau, and alpha-synuclein. Science. 2015; 349:1255555. [PubMed: 26250687]

81. Yanagisawa K. GM1 ganglioside and Alzheimer's disease. Glycoconj J. 2015; 32:87–91. [PubMed: 25903682]

82. Ariga T. Pathogenic role of ganglioside metabolism in neurodegenerative diseases. J Neurosci Res. 2014; 92:1227–1242. [PubMed: 24903509]

83. Yanagisawa K, Odaka A, Suzuki N, Ihara Y. GM1 ganglioside-bound amyloid beta-protein (A beta): a possible form of preamyloid in Alzheimer's disease. Nat Med. 1995; 1:1062–1066. [PubMed: 7489364]

84. Matsuzaki K, Kato K, Yanagisawa K. Aβ polymerization through interaction with membrane gangliosides. Biochim Biophys Acta. 2010; 1801:868–877. [PubMed: 20117237]

85. Kakio A, Nishimoto S, Yanagisawa K, Kozutsumi Y, Matsuzaki K. Interactions of amyloid beta-protein with various gangliosides in raft-like membranes: importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid. Biochemistry. 2002; 41:7385–7390. [PubMed: 12044171]

86. Hong S, Ostaszewski BL, Yang T, O'Malley TT, Jin M, Yanagisawa K, et al. Soluble Abeta oligomers are rapidly sequestered from brain ISF in vivo and bind GM1 ganglioside on cellular membranes. Neuron. 2014; 82:308–319. [PubMed: 24685176]

87. Ikeda K, Yamaguchi T, Fukunaga S, Hoshino M, Matsuzaki K. Mechanism of amyloid beta-protein aggregation mediated by GM1 ganglioside clusters. Biochemistry. 2011; 50:6433–6440. [PubMed: 21682276]

88. Martinez Z, Zhu M, Han S, Fink AL. GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry. 2007; 46:1868–1877. [PubMed: 17253773]

89. Wu G, Lu ZH, Kulkarni N, Ledeen RW. Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. J Neurosci Res. 2012; 90:1997–2008. [PubMed: 22714832]

90. Zamfir AD. Neurological analyses: focus on gangliosides and mass spectrometry. Adv Exp Med Biol. 2014; 806:153–204. [PubMed: 24952183]

91. Valdes-Gonzalez T, Goto-Inoue N, Hirano W, Ishiyama H, Hayasaka T, Setou M, et al. New approach for glyco- and lipidomics--molecular scanning of human brain gangliosides by TLC-Blot and MALDI-QIT-TOF MS. J Neurochem. 2011; 116:678–683. [PubMed: 21244424]

92. Kotani M, Ozawa H, Kawashima I, Ando S, Tai T. Generation of one set of monoclonal antibodies specific for a- pathway ganglio-series gangliosides. Biochim Biophys Acta. 1992; 1117:97–103. [PubMed: 1627599]

93. Ozawa H, Kotani M, Kawashima I, Tai T. Generation of one set of monoclonal antibodies specific for b- pathway ganglio-series gangliosides. Biochim Biophys Acta. 1992; 1123:184–190. [PubMed: 1371229]

94. Schnaar RL, Fromholt SE, Gong Y, Vyas AA, Laroy W, Wayman DM, et al. Immunoglobulin G-class mouse monoclonal antibodies to major brain gangliosides. Anal Biochem. 2002; 302:276–284. [PubMed: 11878808]

95. Angstrom J, Teneberg S, Karlsson KA. Delineation and comparison of ganglioside-binding epitopes for the toxins of Vibrio cholerae, Escherichia coli, and Clostridium tetani: evidence for overlapping epitopes. Proc Natl Acad Sci U S A. 1994; 91:11859–11863. [PubMed: 7527546]

96. Heffer-Lauc M, Viljetic B, Vajn K, Schnaar RL, Lauc G. Effects of detergents on the redistribution of gangliosides and GPI-anchored proteins in brain tissue sections. J Histochem Cytochem. 2007; 55:805–812. [PubMed: 17409378]

97. Heffer-Lauc M, Lauc G, Nimrichter L, Fromholt SE, Schnaar RL. Membrane redistribution of gangliosides and glycosylphosphatidylinositol-anchored proteins in brain tissue sections under conditions of lipid raft isolation. Biochim Biophys Acta. 2005; 1686:200–208. [PubMed: 15629689]

98. Schnaar RL. Isolation of glycosphingolipids. Methods Enzymol. 1994; 230:348–370. [PubMed: 8139508]

99. Schnaar RL, Needham LK. Thin-layer chromatography of glycosphingolipids. Methods Enzymol. 1994; 230:371–389. [PubMed: 7511203]

100. Sturgill ER, Aoki K, Lopez PH, Colacurcio D, Vajn K, Lorenzini I, et al. Biosynthesis of the major brain gangliosides GD1a and GT1b. Glycobiology. 2012; 22:1289–1301. [PubMed: 22735313]

101. Skraskova K, Claude E, Jones EA, Towers M, Ellis SR, Heeren RM. Enhanced capabilities for imaging gangliosides in murine brain with matrix-assisted laser desorption/ionization and desorption electrospray ionization mass spectrometry coupled to ion mobility separation. Methods. 2016

102. Weishaupt N, Caughlin S, Yeung KK, Whitehead SN. Differential Anatomical Expression of Ganglioside GM1 Species Containing d18:1 or d20:1 Sphingosine Detected by MALDI Imaging Mass Spectrometry in Mature Rat Brain. Front Neuroanat. 2015; 9:155. [PubMed: 26648849]

103. Hirano-Sakamaki W, Sugiyama E, Hayasaka T, Ravid R, Setou M, Taki T. Alzheimer's disease is associated with disordered localization of ganglioside GM1 molecular species in the human dentate gyrus. FEBS Lett. 2015; 589:3611–3616. [PubMed: 26484596]

104. Woods AS, Colsch B, Jackson SN, Post J, Baldwin K, Roux A, et al. Gangliosides and ceramides change in a mouse model of blast induced traumatic brain injury. ACS Chem Neurosci. 2013; 4:594–600. [PubMed: 23590251]

105. de HN, Reiding KR, Haberger M, Reusch D, Falck D, Wuhrer M. Linkage-specific sialic acid derivatization for MALDI-TOF-MS profiling of IgG glycopeptides. Anal Chem. 2015; 87:8284–8291. [PubMed: 26191964]

106. Simons K, Sampaio JL. Membrane organization and lipid rafts. Cold Spring Harb Perspect Biol. 2011; 3:a004697. [PubMed: 21628426]

107. Proia RL. Glycosphingolipid functions: insights from engineered mouse models. Philos Trans R Soc Lond B Biol Sci. 2003; 358:879–883. [PubMed: 12803921]

108. Tettamanti G, Bonali F, Marchesini S, Zambotti V. A new procedure for the extraction, purification and fractionation of brain gangliosides. Biochim Biophys Acta. 1973; 296:160–170. [PubMed: 4693502]

109. Yao D, McGonigal R, Barrie JA, Cappell J, Cunningham ME, Meehan GR, et al. Neuronal expression of GalNAc transferase is sufficient to prevent the age-related neurodegenerative phenotype of complex ganglioside-deficient mice. J Neurosci. 2014; 34:880–891. [PubMed: 24431446]

110. Singh P, Schimenti JC, Bolcun-Filas E. A mouse geneticist's practical guide to CRISPR applications. Genetics. 2015; 199:1–15. [PubMed: 25271304]

111. Svennerholm L. Designation and schematic structure of gangliosides and allied glycosphingolipids. Prog Brain Res. 1994; 101:xi–xiv. [PubMed: 8029441]

112. Varki A, Cummings RD, Aebi M, Packer NH, Seeberger PH, Esko JD, et al. Symbol nomenclature for graphical representations of glycans. Glycobiology. 2015; 25:1323–1324. [PubMed: 26543186]

Highlights

- Gangliosides, sialylated glycosphingolipids, are major cell surface molecular determinants on all vertebrate nerve cells
- The same four ganglioside structures, GM1, GD1a, GD1b, and GT1b comprise the vast majority of brain gangliosides in all mammals.
- Gangliosides regulate membrane receptors via specific molecular interactions with proteins on their own membrane (*cis*) and mediate cell-cell recognition via specific binding to glycan recognition proteins on apposing cells (*trans*).
- Among their functions, gangliosides regulate cell differentiation, modulate cell signaling, stabilize axon-myelin interactions, and affect protein aggregation related to neurodegenerative diseases.
- Inherited mutations in ganglioside biosynthetic genes result in seizures, motor neuropathies, and intellectual deficits.

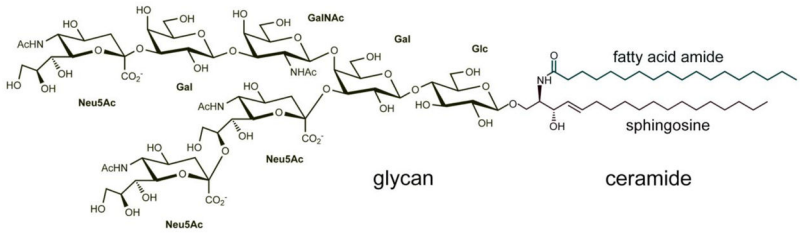

Fig. 1.

Trisialoganglioside GT1b. The structure of the most complex of the four major mammalian brain gangliosides is shown. Other major brain gangliosides and their biosynthetic relationships are shown in Fig. 2.

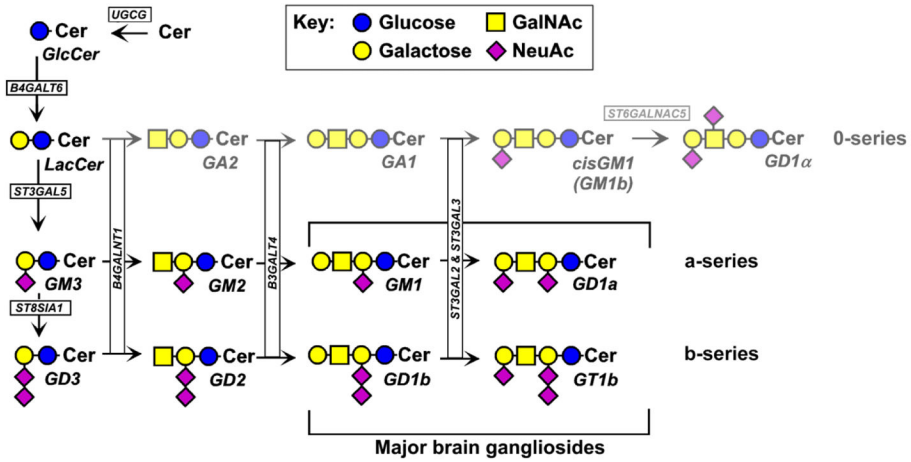

Fig. 2.

Biosynthesis of mammalian brain gangliosides. The step-by-step enzymatic biosynthesis of the major brain gangliosides is shown starting with addition of glucose in β-linkage to ceramide. Each enzyme transfers a single sugar from an activated sugar nucleotide donor (UDP-Glc, UDP-Gal, UDP-GalNAc, or CMP-Neu5Ac) to the end of the growing chain. Linkage positions of each sugar are not shown, but are the same as for GT1b (Fig. 1) with the exception of GD1α, which has a sialic acid linked α2–6 to the GalNAc. Human genes encoding the enzymes responsible for each step are boxed. Note that some enzymes (e.g. *B4GALNT1*) act on multiple acceptors that vary in the number of sialic acids on the inner galactose. The glycan symbol nomenclature shown is widely accepted in the field [112].

TABLE 1

Human diseases of ganglioside biosynthesis

enzyme deficit	mutated gene	ganglioside expression	clinical outcomes	references
GM3 synthase	*ST3GAL5*	unknown	seizures, cognitive and motor decay	[33–35]
GM2/GD2 synthase	*B4GALNT1*	loss of complex gangliosides; increased GM3 and GD3 [a]	spastic paraplegia, intellectual disability	[37,38]

[a] ganglioside analysis from a single case study lacking genetic confirmation [30]