

Review

Sphingolipids and cell signaling: Relationship between health and disease in the central nervous system

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Abstract

Sphingolipids are lipids derived from an 18-carbons unsaturated amino alcohol, the sphingosine. Ceramide, sphingomyelins, sphingosine-1-phosphates, gangliosides and globosides, are part of this group of lipids that participate in important cellular roles such as structural part of plasmatic and organelle membranes maintaining their function and integrity, cell signaling response, cell growth, cell cycle, cell death, inflammation, cell migration and differentiation, autophagy, angiogenesis, immune system. The metabolism of these lipids involves a broad and complex network of reactions that convert one lipid into others through different specialized enzymes. Impairment of sphingolipids metabolism has been associated with several disorders, from several lysosomal storage diseases, known as sphingolipidoses, to polygenic diseases such as diabetes and Parkinson and Alzheimer diseases. Sphingolipids are mainly located in central nervous system, and their abundance and distribution depend on brain development state and cell type. Although several studies have expanded the knowledge about sphingolipids function both in health and disease, it is still necessary to continue their study to understand the cellular implications of novel sphingolipids. These studies will also contribute to the diagnosis and treatment of diseases in which these molecules are part of their pathophysiology. In this review, we summarize the main sphingolipid characteristics and current knowledge about the synthesis, catabolism, regulatory pathways, participation in action potential and ionic channels, among other relevant functions.

Keywords: Central Nervous System, GM2 Gangliosidoses, Sphingolipids, Sphingolipidosis.

1. Introduction.

Sphingolipids are important structural constituents of cell membrane that participate as signaling molecules in regulatory pathways [1-4]. They are complex phospholipids formed by a sphingosine head and a long fatty acid chain, which could be classified as sphingomyelins (ceramide plus phosphocoline or phosphoethanolamine) and glycosphingolipids (cerebrosides, sulfatides, globosides, and gangliosides) [1, 5].

Sphingolipids are synthesized through a de novo pathway that begins in the endoplasmic reticulum, and are later modified into the Golgi apparatus and transported to the cell membrane

through vesicular transport [1, 2, 5]. On the other hand, their catabolism involve several lysosomal enzymes [6]. Although the sphingolipids are widely distributed in different tissues as part of membranes or vesicles, glycosphingolipids and gangliosides are mainly located in the central nervous system (CNS), where represent almost 20% of the total lipids [7]. In this tissue, sphingolipids participate in physiological and neurodevelopmental processes providing adequate CNS function [3, 8].

It has been described that ganglioside expression change throughout brain development as well as in different brain regions and CNS cell types [4, 9, 10]. For instance, galactolipids and its sulfated derivate sulfatide are found in oligodendrocytes, glia cells and myelin; while gangliosides are mainly located in neurons (axon membrane) and grey matter [11]. Concerning to brain development, at embryonic state gangliosides molecules are simple, but due to formation of neural circuits during neural development, their structures change becoming more complex, which seem to remain in the adulthood [8, 11].

The sphingolipid heterogeneity and its distribution along the membrane of neural cells generate conformational changes and microdomains that participate in the specialized functions of polarized cells as neurons and oligodendrocytes [12-15]. Likewise, gangliosides are related to axonal growth, dendritogenesis, myelination, and modulation and stabilization of ion channels [16-20]. Thus, sphingolipids must play an important role in the establishment of synaptic relationships and connections between neurons, demonstrating their participation in neuronal processes to keep neuroplasticity and stimulate different contexts or types of memory and neural learning [8, 21].

Considering the different roles of sphingolipids into the brain's cells structure and development, is important to note that the impairment of their metabolism may result in cellular changes leading to different metabolic, neuronal, or proliferative disorders. The group of disorders caused by the disfunction of biosynthesis or catabolism of glycosphingolipids are known as sphingolipidoses [22], mainly caused by impaired lysosomal enzymes or by altered transport of cholesterol in the lysosome-cytosol pathway. Due to the broad heterogeneity of this group, it was recently divided into genetic disorders of biosynthesis, defects of degradation, and diseases of lysosomal deposit [22, 23]. All of them can course from asymptomatic presentations to severe neonatal forms, and show organomegaly, bone abnormalities, and CNS progressive impairment conducting to neuronal degeneration [24, 25]. Additionally, it has been described the impact of sphingolipids in other diseases such as cancer, diabetes, and cardiac disorders, among others.

Even though many characteristics and roles of sphingolipids have been described, there are some mechanisms that are not well understood. For this reason, it is important to continue addressing a greater understanding of the synthesis and degradation processes of these compounds, to understand the basis of some neurological disorders and possibly develop new therapeutic alternatives for these disorders. In this review, we provide a compilation of recent information about sphingolipid characteristics, brain distribution and their role in metabolism, neural development, ionic flux and their implications in several disorder

2. Sphingolipids: Structure, metabolism, and central nervous system distribution.

Sphingolipids are complex lipids composed of 18 carbon amino-alcohol backbone representing near 20% of the lipids in the nervous system which acts mainly as structural molecules of the cell membrane. They also participate in essential functions such as brain development, cell recognition, adhesion, and toxin receptors [1-4]. De novo biosynthesis of sphingolipids is a compartmentalized and fine regulated process that begins at the cytosolic leaflet of the endoplasmic reticulum (ER) from non-sphingolipid precursors to synthesize ceramide, in a pathway that involves enzymatic regulated steps in which the serine palmitoyltransferase is the key enzyme [1, 26]. In the luminal face of the ER, this ceramide is modified through the addition of one galactose residue by the ceramide galactosyltransferase to form galactosylceramide (GalCer) and later transported to the Golgi apparatus [5]. The ceramide can also be directly transported from ER to the *trans*-Golgi network by the ceramide-transfer protein (CERT), a protein regulated by phosphorylation in response to cell

sphingomyelin requirements [1, 27, 28]. In this later transport, the sphingomyelin synthase 1 catalyzes the ceramide conversion and phosphatidylcholine into sphingomyelin (SM) [27].

An alternative ceramide transport pathway to *cis*-Golgi from ER through coatomers and vesicular transport has also been described, although its regulation mechanism is not entirely understood [3]. At the *cis*-Golgi, the glucosylceramide synthase transfers glucose from UDP-glucose to the ceramide to form glucosylceramide (GlcCer). Then, the GlcCer is transported to the *trans*-Golgi network by the four-phosphate adaptor protein 2 (FAPP2) [29] to form different glycosphingolipids (GSL) [3, 28, 30]. In the brain, the UDP-Gal:GlcCer β 1-4 galactosyltransferase transfers a galactose unit to the GlcCer to form lactosylceramide (LacCer) which is modified by the addition of sialic acid by the LacCer- α -2-3 sialyltransferase to form the simplest ganglioside GM3 at the *trans*-Golgi [7]. Several sugars attachment on the GM3 allows the synthesis of GM2 and GM1 gangliosides [7] to be finally released in Golgi vesicles to the lipids rafts at the plasma membrane, especially at synaptic surfaces [6], in a fusion membrane-dependent mechanism [31]. Figure 1 summarizes the sphingolipids biosynthetic pathway. GM1 and the GSLs GD1a, GD1b, GT1b, and GQ1b constitute up to 95% of all gangliosides in the brain [7, 31] and represent 20-25% of the outer leaflet of neuron membranes [32]. Although these chemical reactions are usually limited to the membrane organelles, the sialyltransferase has been found as membrane-associated enzymes suggesting pivotal roles in the GSL remodeling [33-35].

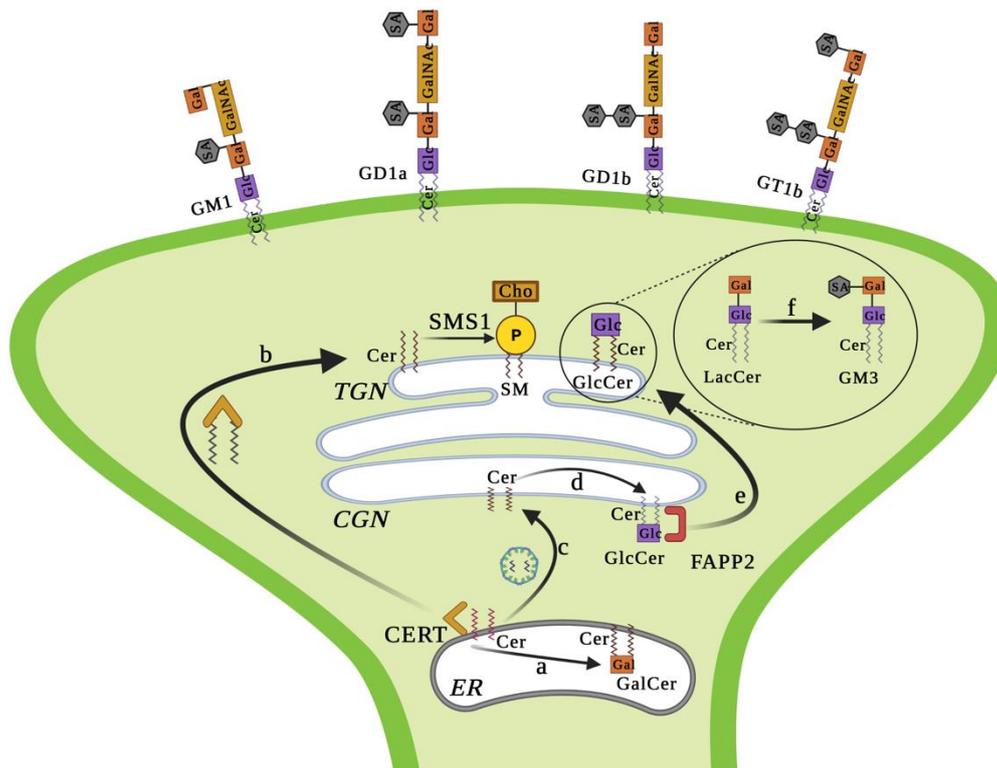


Figure 1. *de Novo* biosynthetic pathway of sphingolipids. Ceramide (Cer) synthesis is the first step in the synthesis of the sphingolipids which is carried out in the cytosolic leaflet of the endoplasmic reticulum (ER). Upon its synthesis, the ceramide is modified by the addition of galactose (a) forming GalCer into the ER lumen, since where it seems to reach the *cis*-Golgi network (CGN). The ceramide can be transported from ER to the *trans*-Golgi network in an independent vesicular transport by the ceramide-transfer protein (CERT) (b), where the sphingomyelin synthase 1 (SMS1) catalyzes the synthesis of sphingomyelin (SM). On the other hand, the ceramide can be coated and transported to the CGN since ER (c). Once there, the glucosylceramide synthase adds glucose to the ceramide to form GlcCer (d). This GlcCer can be transported to the TGN by the four-phosphate adaptor protein 2 (FAPP2) (e). The addition of the galactose and the further sialic acid allows the synthesis of the simplest ganglioside GM3 (f) which is the precursor of major gangliosides. With the GM1

ganglioside, the GD1a, GD1b, GT1b are the most abundant sphingolipids in the brain. This figure was created with BioRender.com.

While the biosynthetic route involves the secretory pathway, the catabolism of sphingolipids requires the endocytosis and the activity of several lysosomal enzymes [6]. Besides, catabolic enzymes like neuraminidase [36], hexosaminidase [37], and galactosidase [38] were early found at the plasma membrane. Respect this, early studies performed by Rodriguez *et al.*, showed that plasma membrane neuraminidase contributes to the regulation of axonal growth and regeneration of hippocampal neurons using *in vitro* approaches [39], suggesting a fine regulation of GSL in the central nervous system. These interesting topics will be discussed later.

2.1 Sphingolipids profile in the Central Nervous System

Even though the sphingolipids are virtually distributed throughout all the brain, differential profiles have been described during the embryonic period, childhood, and adulthood, as well as in different brain regions and CNS cell types [3, 8]. In this respect, it was reported that in rat brains the overall ceramide species are increased since post-natal day 1 (P1) up to P21 and then they are reduced from P30 to adult. This behaviour is believed to stimulate the biosynthesis of several sphingolipids such as the GM1 ganglioside [4]. In this scenario, recently in adult rats, it described the presence of complex GSL such as sulfatides, LacCer, and hexosylceramide in the dorsal hippocampus, a crucial brain structure related to learning, memory, and emotional responses. Enrichment of short ceramides (C16-C20) accompanied by reduced levels of longer ceramide (C22-C26) have been found in the ventral hippocampus [40]. In contrast with this physiological profile, the corticosterone-induced stress in adult rats has shown an increase in the dorsal-ventral sphingolipid catabolism, suggesting that the stress could trigger CNS diseases. Besides, sphingolipid changes have been reported in the brain tissues isolated from autistic patients [41].

GD1a, GD1b, GM1, and GT1b are the major gangliosides in the adult mammalian brain [8] and similar findings have also been reported in human brains (Figure 2) [11, 42, 43]. Consistent with the above, the expression of some galactolipids also changes during murine brain development. In this line, the GM3, GM1, GD1a, GD1b, GT1b, GM2, GD3 gangliosides are enriched in the early developmental stages (primary and secondary follicle), whereas in the blastocyst the GM3 and GT1b are predominant during the mouse embryogenesis [9]. At the embryonic stage (~E15) as well as the P2-P5, the GlcCer is the only monoglycosylceramide present, whereas at P8-P10 begins the synthesis of GalCer which increase up to P21-P25 [4, 10]. These findings suggest that murine brain development is an age-dependent dynamic process, in which sphingolipids seem to be pivotal regulators. Decreased levels of GalCer are frequently found in multiple sclerosis rat brains [4], an autoimmune-dependent demyelinating disease.

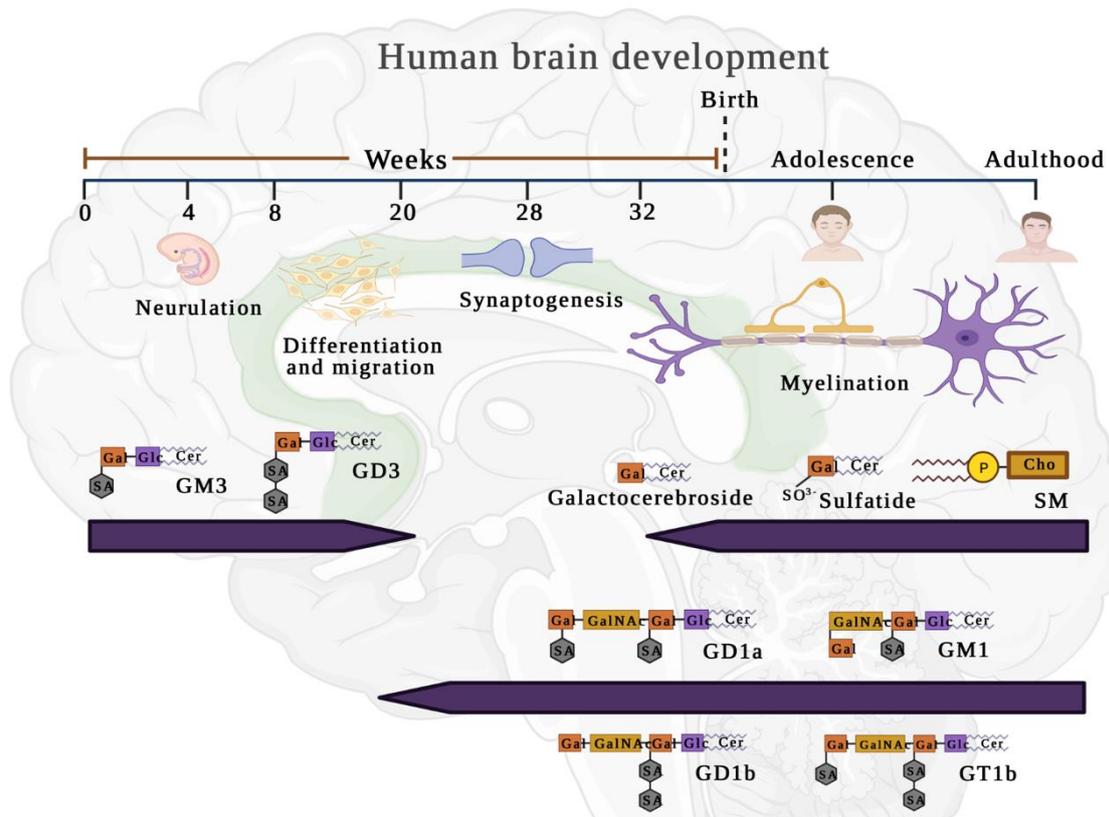


Figure 2. Overall Sphingolipid profile during the human brain development. Note that during the early embryonic stages there are predominantly simple gangliosides, which change to most complex structures in consequence of the neural circuit's formation. This later profile of sphingolipids seems to remain in adulthood. This figure was created with BioRender.com.

As expected, changes in the sphingolipid's arrangement are also found at the different CNS cell types (Figure 3). For instance, neurons are enriched in gangliosides (up to 12% of total lipid content) at the axonal membrane by GD1a and GT1b forming microdomains that promote myelin stabilization through the interaction with the myelin-associated glycoprotein (MAG) [13, 44]. Although GD3 synthesis increase during the early neurogenesis, this is not able to bind MAG, suggesting that GD3 is a key start point to more complex gangliosides without direct interaction with MAG [44]. Concerning glia cells, it is well known that the oligodendrocytes plasma membrane is enriched with GalCer and sulfatide [14]. A recent study provided an interesting finding in P16 rat-derived oligodendrocytes where GlcCer and sphingomyelins represented the major ceramide species [15]. Despite the above, it is clear that myelin lacks GlcCer, and even blocking the biosynthesis of GlcCer-derived sphingolipids does not result in aberrant myelination in the CNS [45]. Therefore, the function of this pool of GlcCer in oligodendrocytes remains to be elucidated. Although myelination in the CNS is driven by the oligodendrocytes, previous reports have suggested that this process seems to depend on the astrocyte-derived lipids rather than from oligodendrocyte lipid synthesis [46]. In astrocytes, in addition to GM3 [47], high amounts of ceramide and sphingosine 1P (S1P) derived from sphingosine metabolism have been found [48, 49], which are well-established as bioactive compounds in health and disease [50-52]. Finally, in microglia different sphingolipids patterns can be observed according to its activation stage. In this aspect, proinflammatory roles have been associated with long-chain ceramides (C16-C24) and S1P, whereas short-chain ceramides exert anti-inflammatory effects in the CNS [53-55].

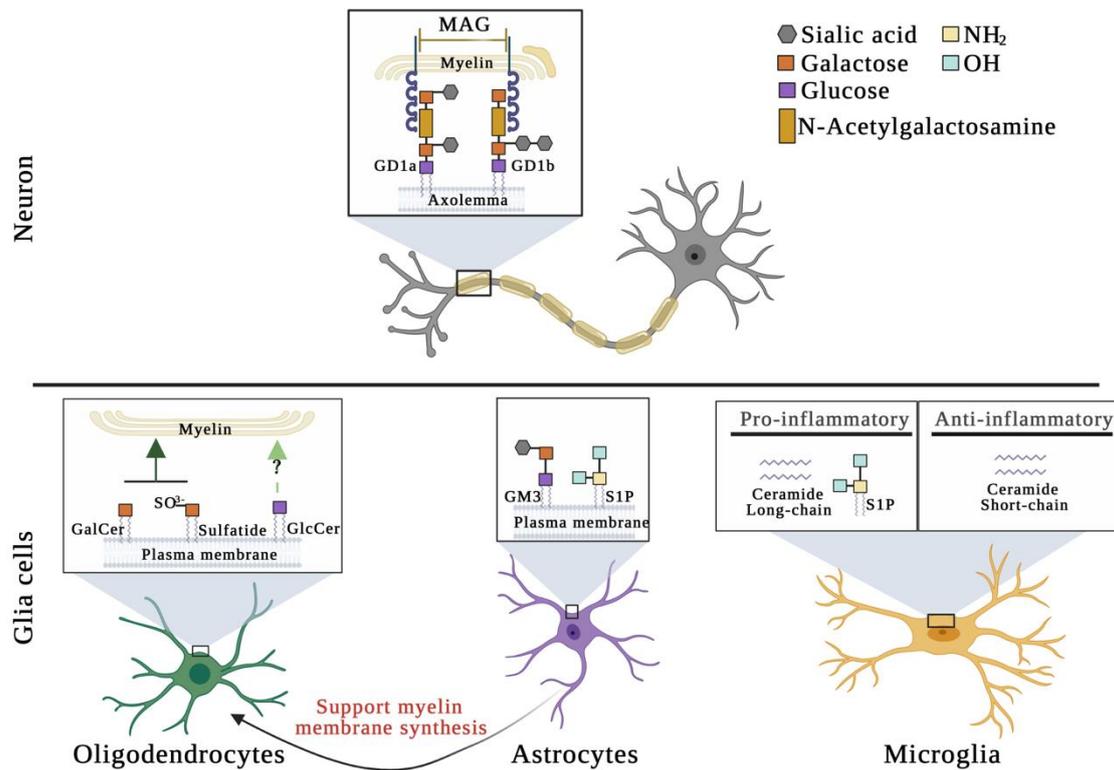


Figure 3. Major sphingolipids in the CNS cells. Complex sphingolipids are present in neuron's axon plasma membrane, which interact with the myelin-associated glycoprotein (MAG), contributing to the myelin stabilization. The GalCer and sulfatides are found in the oligodendrocytes; however, high amounts of GlcCer have also been described [45]. Nevertheless, its role in the myelinating oligodendrocytes has not to be elucidated. Although traditionally it has been accepted that the lipid synthesis by oligodendrocytes is crucial to the myelination of the axon, recently it was established that the astrocytes support this process through the cholesterol and unsaturated fatty acids synthesis [46]. Finally, the pro-inflammatory (M1) and anti-inflammatory (M2) microglia stages are accompanied by different sphingolipids profiles where the ceramide length seems to be crucial for the differential signalling in these immune cells. This figure was created with BioRender.com.

3. Sphingolipids-mediated cellular signaling pathways.

Neurons and oligodendrocytes are highly polarized cells with particular morphology that allow them to carry specialized functions [8]. The particular heterogeneity in sphingolipid (SL) distribution along the cellular membrane of these cells produces microdomain conformations that play important roles in neuronal differentiation, synapse formation, glial-neural interaction, and polarization [8]. In this section, we review the current knowledge about the functions described to the sphingolipids at the CNS, which are summarized in Figure 4.

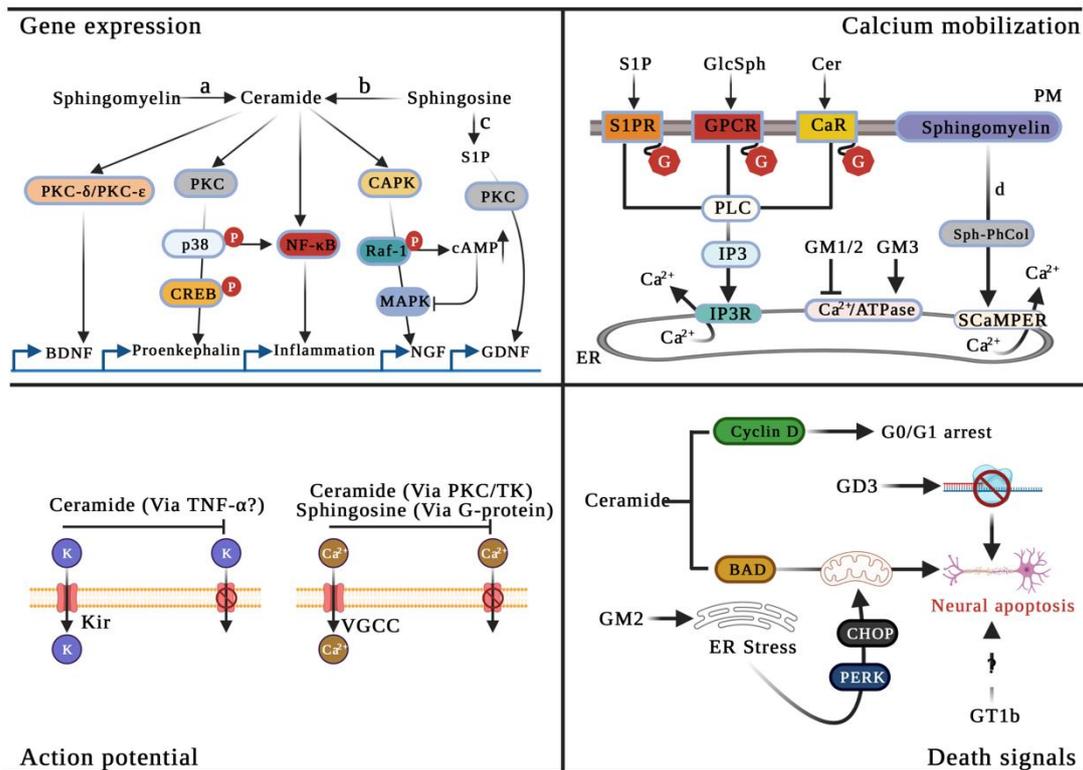


Figure 4. Role of sphingolipids in cell signaling. **Gene expression:** Ceramide and Sphingosine-1-phosphate (S1P) can influence the actions of several signal effectors such as CAPK, nF κ B, PKC, and its isoforms PKC δ /PKC ϵ , to promote the gene expression of some proteins such as BDNF, proenkephalin, NGF, and GDNF. Also, inflammation can be induced by overexpression of different proteins (refer to the text for more details). (a) the enzymatic reaction catalyzed by sphingomyelin phosphodiesterase. (b) the enzymatic reaction catalyzed by sphingosine ceramide synthase. (c) the enzymatic reaction catalyzed by sphingosine kinase 1 and 2. **Calcium mobilization:** S1P, Glucosyl sphingosine (GlcSph), and ceramide (Cer) can activate G protein-coupled receptors to promote calcium release via IP3R activation. GM1/2 and GM3 can both inhibit or activate the endoplasmic reticulum calcium pump Ca²⁺/ATPase, respectively. Sphingosyl phosphorylcholine (Sph-PhCol) acts over the SCaMPER channel and promotes calcium release. **PM:** Plasma membrane. **ER:** Endoplasmic reticulum. (d) the enzymatic reaction catalyzed by sphingomyelin deacetylase. **Action potential:** Ceramide induces a close state in Inwardly Rectifying Potassium (Kir) Channels probably via TNF- α . Also, ceramide and sphingosine act over Voltage activated calcium channels (VGCC) inducing inhibition of these channel-mediated currents. **Death signals:** G0/G1 arrest and caspase-mediated cell death are induced by ceramide build-up. Cell death by GM2 and GT1b build-up can promote cell death by endoplasmic reticulum stress and mitochondria-dependent pathway, and an unknown mechanism, respectively. GD3 increase inhibits DNA synthesis. Although sphingolipids are involved in cell death signals, they also can promote cell survival through PKC, PKC δ /PKC ϵ , NF κ B, and CAPK in the case of ceramide and IP3 dependent or independent mechanism in the case of Sphingosine-1-phosphate (not shown). This figure was created with BioRender.com.

3.1 Sphingolipids in neural differentiation, dendritic formation axonal determination, and growth

During embryonic development, the sphingolipid profile into the brain changes. The changes and distribution of some sphingolipids in membrane microdomains suggest that they are essential for some functions as modulation of signaling transduction, cell adhesion, and neural differentiation [8, 56]. For instance, the GD3 ganglioside is highly expressed in the embryonic mouse brain (particularly in neuronal stem cells - NSC) and decreases dramatically after birth [57], suggesting an important role during embryonic development. In previous studies, it has been shown that GD3 interacts with the epidermal growth factor receptor to support the neural self-renewal of NSCs

through the EGF-induced EGFR signaling [57]. GD3 synthase knockout in mice produces a decrease in NSCs populations proving its essential role in the sustaining of these cells [58].

On the other side, gangliosides have been linked to dendrite and axon growth [16]. In early reports, it was shown that GM2 ganglioside accumulation let abnormal neuronal geometry and induces ectopic dendrites in patients with Tay-Sachs disease, and cat models that mimic GM2 gangliosides [59, 60]. It is thought that GM1 ganglioside is involved in calcium flux between nucleus and cytoplasm through the stabilization of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in the inner membrane of the nuclear envelope during the neural differentiation [17], supporting the previous hypothesis. Likewise, the GD3, GD1b, and GT1b gangliosides are related with dendritogenesis by modulation of the calmodulin kinase II (CaM-KII) pathway in primary cultured neurons, supporting that ganglioside/CaM-KII could be a mechanism to dendritic morphogenesis of growing neurons [18]. This is consistent with early studies suggesting the need for sphingolipids biosynthesis for dendrite growth [19, 20].

Concerning axon growth, it has been observed that ceramide synthesis inhibition reduces the axonal length on cultured hippocampal neurons probably because sphingolipids are used as membrane molecules for axonal elongation [61]. Also, the polarization of neurons, which is triggered by the axon specification and requires the localized destabilization of filamentous actin, seems to be modulated by the G1-mediated downstream signaling in a plasma membrane ganglioside sialidase-dependent mechanism [62]. A similar role has been associated with a GD3 derivative (9-O-acetyl GD3) [63].

3.2 Sphingolipids' role in action potential and ionic channels

Proper myelination is necessary to confer neurons electrical insulation and proper action potential propagation [64]. Due to action potentials are propagated in a saltatory manner from one node of Ranvier (spaces of the axon devoid of myelin) to another due to the opening of voltage-gated sodium channels. The myelination process involves interaction among several molecules like proteins, glycoproteins, and lipids [65]. During the myelination process, some sphingolipids participate in a complex fashion, although the exact mechanism in which they interact is largely unknown [4]. More specifically, Ceramide (Cer)/dihydroceramide (dhCer), Sphingosine (Sph)/dihydrosphingosine (dhSph), and glucosyl/galactosylceramide (Glc/GalCer) participate in myelin formation during mice brain development [4]. GalCer-enriched microdomains seem to co-localize with several proteins of the myelin sheaths and some examples of this are the interaction with myelin basic protein (MBP), 20, 30 -cyclicnucleotide 30-phosphodiesterase (CNP), myelin/oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) [66]. Besides its interaction with different myelin proteins, GalCer is important for myelin stability since GalCer synthesis impairment has been associated with myelin degeneration and encephalopathy [67, 68]. The GM2/GD2 are also important for normal microdomain composition in nodes of Ranvier since the abnormal synthesis of these gangliosides alters its typical microdomain composition as well as the normal K^+ and Na^+ ion channels localization [69, 70].

Synapses are structurally complex and required regulated compartmentalization of membrane components to maintain critical functions such as neural plasticity that is defined as the brain's ability to adapt to different stimuli and undergo learning memory processes and behavior changes [8, 71]. In this context, sphingolipids play a transcendental role as several studies have shown that the balance between SM and ceramide in the hippocampus is associated with post-synaptic excitatory transmission, which is pivotal to spatial memory and episodic-like memory [21, 72]. The so-called Long-Term Potentiation (LTP) is another key phenomenon occurring in neurons of the central nervous system that is associated with the memory and learning process [8]. Among the several mechanisms governing LTP, the proper clustering of glutamate receptor, N-methyl-D-aspartate receptors (NMDARs) seems to be related to sphingolipids microdomain, suggesting that these molecules play an important role in NMDA mediated LTP [71, 73]. Besides, another glutamate receptor (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, also known as AMPAR) mediates

fast synaptic transmission, and its trafficking is a critical component of learning and memory [74]. Prendergast and colleagues report a model in which GluR2 subunits of AMPAR complex and an AMPAR trafficking complex containing Nicalin, Thorase, and NSF are proposed to be sequestered in distinct membrane rafts by association with gangliosides GM1 and GT1b, respectively. In an ATP-dependent process, the ganglioside interactions are released, resulting in lateral migration, the association of AMPA receptors with their trafficking complexes, and receptor endocytosis [75].

Regarding ion channels, a great number are found associated with microdomains, although how sphingolipids influence their organization and function are only well defined for some cases [8]. For instance, SM has been shown to influence Kv2.1, Kv1.3, and Kir1.1 channel activity by interacting with S3b and S4 voltage-sensing domains with positively charged residues through interaction with phospho-heads of membrane lipids to produce energetic stabilization [16, 76]. The overall effect of the hydrolysis of SM into ceramide is the inhibition of K⁺ conductance and hyperpolarization shift in the voltage-conductance relation for Kv 1.5 and Kv2.1 [16, 76]. Microdomains mediate ion channels and kinase proteins co-localization, which is important since phosphorylation is a mechanism of ion channel regulation [8]. It has been proved that phosphorylation suppresses depolarization-evoked currents and disruption of microdomains by cholesterol depletion and inhibition of sphingolipids synthesis produce a hyperpolarizing shift of Kv1.5 channels in Ltk cells [77]. Another mechanism by which sphingolipids may influence ion channel activity is through the regulation of scaffolding proteins as PSD-95, which has been proved to increase in the mouse brain when SM/ceramide balance is altered and is important for Kv 1.4 and NMDA receptor recruitment into microdomains [21, 78].

Finally, it has been described that sphingolipids are involved in calcium mobilization [79]. Particularly, S1P and sphingosine induce Ca²⁺ via Inositol triphosphate (IP3) and PKC inhibition in the case of sphingosine and sphingosylphosphorylcholine (SPC) [80, 81]. A specific SPC-activated calcium channel called SCaMPER has been identified in the endoplasmic reticulum and is proposed to modulate calcium mobilization via SPC signaling [79, 81]. Evidence shows that sphingomyelinase increased activity by tumor necrosis factor- α (TNF α) induction or elevated expression produce transient Ca²⁺ inward currents in neonatal rat dorsal root ganglion (DRG) and fura-2-loaded neurons [79]. In contrast, GlcCer and glucosylsphingosine have been reported to mobilize calcium via the ryanodine receptor [82]. The glucosylsphingosine seems to also regulate Ca²⁺ mobilization in an IP3 and Ca²⁺/ATPase dependent manner [82, 83].

3.3 Sphingolipids and signaling pathways in cell death and survival

Sphingolipids, particularly ceramide, have an important role in cell death [79]. Ceramide can activate both intrinsic and extrinsic cell death pathways by interacting directly or not with different effectors such as Fas-L, TNF α , NGF, MAPK (ERK, JNK, and p38), and Akt [79, 84]. Specifically, ceramide treatment dephosphorylates Akt, releases cytochrome c, and produces caspase 3 release [85]. Akt inactivation is also seen in motor neurons [79]. Early studies have shown that ceramide induces reactive oxygen species (ROS) production by inhibiting the complex III of the respiratory chain [86-88]. There is evidence of a proapoptotic effect of the GD3, GT1b, and GM2. In the case of GM2, the accumulated ganglioside in GM2 gangliosidosis [7], it was recently shown that its accumulation induces PKR-like endoplasmic reticulum kinase (PERK)-mediated apoptosis in N2a cells [89]. In contrast with these proapoptotic sphingolipids, the GM1 ganglioside seems to have a neuroprotective effect on glutamate toxicity through the activation of the Trk receptor [90].

Some sphingolipids can regulate cell proliferation and survival [79]. For instance, evidence suggests that sphingosine kinase1/S1P signaling promotes the proliferation and survival of neurons in a PI3K/Akt-mediated pathway, supporting a key role in neurogenesis [91, 92].

4. Impaired sphingolipids metabolism on the central nervous system.

The diseases that compromise the biosynthesis or catabolism of glycosphingolipids are known as sphingolipidoses. Although the pathogenesis of these diseases is not completely understood, several approaches have been carried out in order to develop treatment strategies and allow a specific and early diagnosis. Figure 5 shows an overview of the significant pathways of sphingolipid catabolism and the related sphingolipidosis.

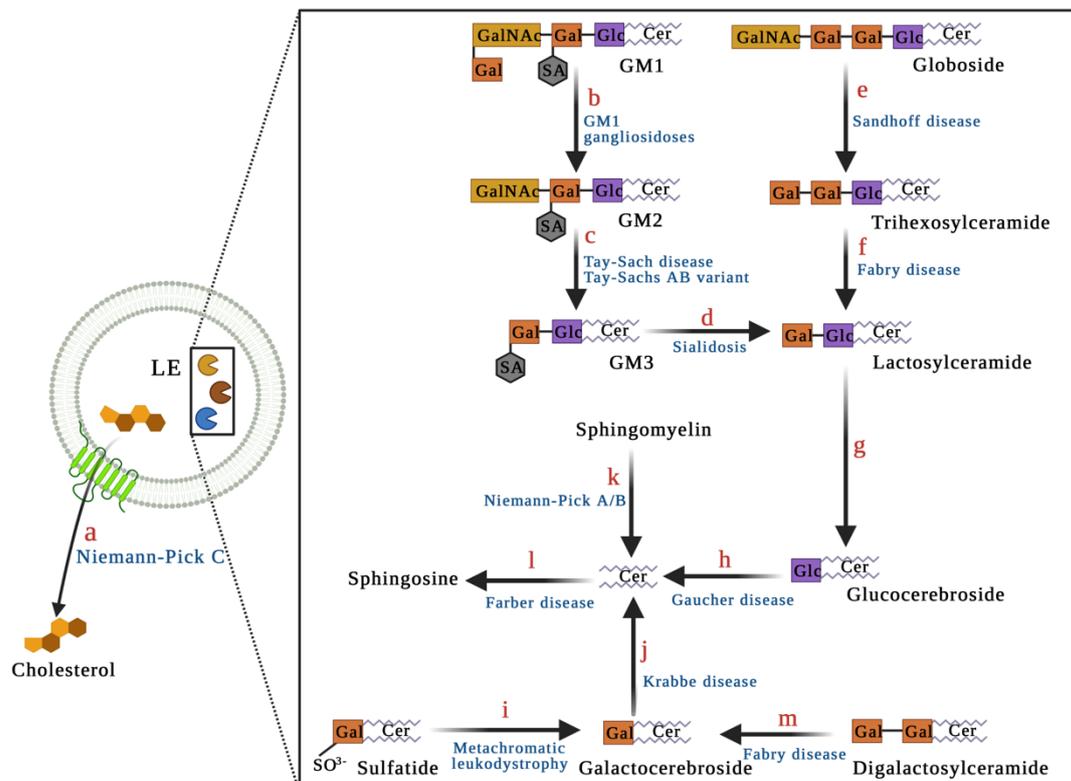


Figure 5. Sphingolipid catabolism and sphingolipidosis. Although most sphingolipidoses are caused by the impaired lysosomal enzyme (LE) activity, Niemann-Pick Type C (NPC) is originated from the altered transport of cholesterol in the lysosome-cytosol pathway allowing its accumulation into the lysosome and affecting the lysosomal function (a). In the case of the ganglioside catabolism during the endocytosis and membrane renewal membrane, some species as GM1 are degraded to GM2 by the removal of Gal in a catabolic step catalyzed by the galactosidase β -1 (b). Upon the resulting GM2 ganglioside is removed a unit of GalNAc by the hexosaminidase A in a GM2 activator protein-dependent mechanism (c). The further removal of the SA in the GM3 ganglioside by the neuraminidase/protective protein/cathepsin A-PPCA (d) allows the formation of the lactosylceramide. On the other hand, the globoside, which differs from the GM1 ganglioside only by the presence of the SA in the latest can be catalyzed by the hexosaminidase B with no participation of the GM2 activator protein (e). The resulting trihexosylceramide is subject to the enzyme activity of the galactosidase alfa/Saposin B (f). In both cases, the degradation of the GM1 ganglioside and the globoside allows the formation of the lactosylceramide which lost a Gal by the action of the galactosidase/Saposin B and C (g) to form glucocerebroside. This simple glycolipid is subject to the catalysis of the glucosylceramidase beta/Saposin C (h) to obtain ceramide. In a parallel catabolic pathway, the sulfatides can form galactocerebroside by the action of the arylsulfatase A/Saposin B (i). The galactocerebroside is additionally catalyzed by the galactosylceramidase/Saposin A and C (j) allowing the formation of ceramide. Likewise, the enzyme activity of the sphingomyelin phosphodiesterase 1/ acid ceramidase on the sphingomyelin (k) from ceramide too. This ceramide is a potent pro-apoptotic molecule by which it must be transformed to sphingosine by the action of the ceramidase/Saposin D (l). An alternative catabolic pathway where the digalactosylceramide is catabolized by the galactosidase alfa/Saposin B (m) to galactocerebroside is shown also. The impaired enzymatic activity of any of these enzymes can produce several sphingolipidoses which are shown in light blue font. Whereas Tay-Sachs disease corresponds to hexosaminidase A deficiency, the Tay-Sachs AB variant refers to the deficiency of the GM2 activator protein. This figure was created with BioRender.com.

Currently, it has been described more than 70 lysosomal diseases related to the catabolism of several substrates, including the sphingolipids, which are accumulated into the lysosome as a consequence of the mutations on the gene encoding to proteins related to the lysosomal function [22]. An interesting review made by Aerts *et al.*, 2019 summarized the main findings of sphingolipidosis using Gaucher disease as a model [25].

In 2020 Pakanova *et al.* expand the concept of sphingolipidosis using the term glycoconjugate metabolism disorders, including glycan biosynthesis disorders, glycoconjugate catabolism, congenital glycosylation diseases, and lysosomal storage diseases [23]. Ryckman *et al.*, 2020, reorganizes the division of glycosphingolipidoses into genetic disorders of biosynthesis, defects of degradation, and diseases of lysosomal deposit, very similar to the initial classification proposed by Platt *et al.*, in 2014 [22, 93]. Table 1 summarizes the current classification of main diseases that affect sphingolipid metabolism and their main characteristics.

Regardless of the classification, sphingolipidoses are characterized by extensive clinical heterogeneity, mainly caused by the accumulation of partially degraded substrates, which can be observed from asymptomatic presentations to severe neonatal forms. It is common to find organomegaly, bone abnormalities, and CNS disorders that usually have a typically degenerative presentation [23, 24]. The sphingolipids can start at very early stages of life, without meaning that they cannot observe later presentations, equally with varying degrees of severity [22]. According to clinical diversity, the diagnostic approach for these disorders is equally controversial and different diagnosis alternatives can be performed from neonatal screening to allow early therapeutic interventions, when these are available, as well as biomarkers identification. In this last point, significant advances have been done using mass spectrometry technology [94, 95], as well as in the improvement of enzyme assays on different biological samples (urine, plasma, saliva, leukocytes, fibroblasts) or dry blood spots on filter paper, and molecular tests that in some cases allow to confirm the diagnostic [96, 97].

Group	Disorder Name	Protein Affected	OMIM number	Main Clinical Features
AD	GM2 activator	GM2 activator	272750	Like to GM2 gangliosidosis. Epileptic fits, macular cherry –spot, muscle weakness
	Prosaposin	Sap precursor	176801	Clinical features are often indistinguishable from the corresponding enzyme defect
	Saposin disorders	Sap A to Sap D	611721	
N/A	Farber Disease	Acid ceramidase	228000	Macular cherry-spot, progressive neurologic disability, hepatosplenomegaly
NPD	Type A, B, and C	AS (A and B) and LTD (C)	257200	Hepatosplenomegaly, progressive pulmonary infiltration, failure to thrive, progressive neurodegenerative course
GD	Type 1, 2 and 3	A β Glu	231000	Hepatosplenomegaly, severe bone compromise, hematological signs
N/A	Krabbe Disease	Gal	245200	Motor deterioration, Cognitive decline, corticospinal tract signs
N/A	ML	ASA	250100	Gross motor deterioration, progressive nervous system compromise
Gangl.	GM1	β -Gal	230600	Progressive neurological deterioration, generalized rigospasticity, sensorimotor
	GM2	Hexo	272800 268800	Seizures, axial hypotonia, intellectual disability, motor regression, psychotic episodes.
N/A	Fabry Disease	α -Gal	301500	Pain and paresthesias in the extremities, hypohidrosis, corneal opacities
N/A	Schindler Disease	NAG	609241	Neuroaxonal dystrophy, cortical blindness, myoclonus, seizures
GDD	α -Mannosidosis	α -Man	248500	progressive mental retardation, hepatosplenomegaly, severe dysostosis multiplex
	β -Mannosidosis	β -Man	248510	Status epilepticus, severe quadriplegia, angiokeratomas
	Fucosidosis	α -Fuc	230000	Psychomotor retardation, coarse facies, growth retardation, dysostosis multiplex
	Sialidosis	Neu	256550	Mucopolysaccharidosis-like phenotype with visceromegaly, dysostosis multiplex, and mental retardation.

Table 1. Main Sphingolipid Disorders Modified from: The Online Metabolic and Molecular Bases of Inherited Disease. 2019 [24]. **AD:** Activator Defects. **NPD:** Niemann-Pick Disease. **GD:** Gaucher Disease. **Gangl:** Gangliosidoses. **GDD:** Glycoprotein degradation disorders. **N/A:** Non-applicable. **AS:** Acid Sphingomyelinase. **ML:** Metachromatic Leukodystrophy. **LTD:** Lipid trafficking disorder. **NAG:** α -N-Acetylgalactosaminidase. **A β Glu:**-Acid β -glucosidase. Gal: Galactocerebrosidase. **ASA:** Arylsulphatase A. β -Gal: Beta-galactosidase. Hexo: Hexosaminidases. **α -Gal:** α -Galactosidase. **α -Man:** α -Mannosidase. **β -Man:** β -Mannosidase. **α -Fuc:** α -fucosidase. **Neu:** Neuraminidase

5. GM2 gangliosidoses as a model of sphingolipidosis

GM2 gangliosidoses are a close group of sphingolipidosis characterized by the dysfunction of the activity of β -hexosaminidases or the GM2 activator protein. The pathology leads to the accumulation, mainly in the neurons, of the GM2 ganglioside with a progressive neuron loss and the subsequent CNS impairment [7].

Since gangliosides are pivotal for membrane structure and critical functions in several CNS cells. Nevertheless, our understanding of the disturbance in the signaling pathway during the GM2 gangliosidoses is limited. In this context, Virgolini *et al.*, recently showed that the GM2 ganglioside overload in neurites activates the mitochondrial-dependent apoptosis in a PERK-mediated mechanism, suggesting prolonged endoplasmic reticulum stress [89]. These findings agree with early reports using microsomes isolated from a Sandhoff disease mouse model (HEXB^{-/-}) where a reduction in the Ca^{2+} -uptake via sarco-endoplasmic reticulum Ca^{2+} -ATPase was observed. This reduction was prevented after the pharmacological inhibition of the ganglioside synthesis by the N-butyldeoxynojirimycin [98]. The molecular mechanisms of ER stress seem to be dependent on the presence of sialic acid in the GM2 ganglioside structure [89, 98, 99]. Further modulators of the PERK-mediated apoptosis pathway could improve the prognostic of this sphingolipidosis in combined therapies. Consequently, several pro-inflammatory cascades can be activated in both innate and acquired immune responses [7]. For instance, microgliosis and infiltration have been observed in GM2 gangliosidoses patients and animal models [100-102]. In this scenario, previous reports of the neurodegenerative progress in a Sandhoff disease mouse model showed that there is an increase in the immunoreactivity for the Major Histocompatibility Complex type II in the brain stem and thalamus and an increase in the proinflammatory cytokines TNF α and IL1 β [103]. Astroglia has also been reported in GM2 gangliosidoses because of the GM2 ganglioside accumulation, with an increase in the secretion of the chemokines CCL2 and CXCL10 [104, 105]. Activated astrocytes may participate in degeneration and death of myelinating oligodendrocytes, promoting active demyelination [106, 107]. In this sense, these findings show the key role of GM2 ganglioside in brain inflammatory process, development, and immunomodulation.

Finally, it was recently reported the association of α -synuclein with lipids (gangliosides) in rat dopaminergic neurons, which was prevented through the overexpression β -hexosaminidases [108]. The authors suggested that the interaction between lipids and α -synuclein may occur in synaptic and vesicular domains due to the presence of gangliosides in lipid rafts located in these domains. These results correlate with previous findings showing that β -hexosaminidases deficiency induce α -synuclein accumulation in a Sandhoff disease mouse model [109, 110]. In addition, the loss of the ability to catabolize GM2 gangliosides led to the formation of high molecular weight α -synuclein aggregates into the brain, as well as an increase in polyubiquitin [108], which suggest that GM2 gangliosides metabolism affects the function of the ubiquitin-proteasome and autophagy-lysosome pathways. These results shown the role of gangliosides in cell homeostasis and may contribute to the understanding of the physiopathology of several CNS disorders.

6. Conclusions and Perspectives

In this review we have described several aspects related to the synthesis, metabolism, and function of sphingolipids. These bioactive lipids are non-structural and non-energetic lipids that participate on cell homeostasis as well as in health and disease. Although they have been studied since late 19th century, the role of most of sphingolipids are not completely understood, and novel structures and modifications are still identified, which shows the importance to continue the identification and characterization of these group of lipids. For instance, it was recently demonstrated that in addition to serine and palmitate, other substrates, such as alanine, glycine, myristate and stearate, can also enter sphingolipid metabolism, and generate novel lipids with potential previously no described cellular functions [111-113]. The synthesis and catabolism of these lipids are carried out

through a highly regulated network of enzymes and transporters, for which their characterization has shed light not only on sphingolipids metabolism but also on most of the cellular regulatory networks. In addition, the presence of tissue-, organelle-, and substrate-specific isoforms of these enzymes adds an extra layer of complexity to the sphingolipid metabolism and correlates with the wide range of cellular functions of these compounds. In this aspect, sphingolipids are involved in almost all cellular functions and networks including cell growth, cell cycle, cell death, inflammation, cell migration and differentiation, autophagy, metabolism, nutrient uptake, angiogenesis, and immune system. One important role of these bioactive lipids is their participation on the regulation of receptor function through the modulation of membrane structure. Although the relation between several metabolic diseases and the loss of function of enzymes directly involve in sphingolipid metabolism has been widely described, growing evidence has shown the impact of sphingolipids on polygenic diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders such as Parkinson and Alzheimer. In this sense, due to the global impact of sphingolipids on cell functions, it is important to continue and extend their study by the combination of several omics' sciences, including genomics, proteomics, transcriptomics, epigenomics, metabolomics, and lipidomics, with a holistic view of cell biology through systems biology approaches. This will complete our understanding of sphingolipids metabolism and function that will impact the diagnosis and treatment of several diseases for which sphingolipids represent a key part of their pathophysiology.

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Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
Cer	Ceramide
CERT	Ceramide-transfer protein
CNS	Central Nervous System
ER	Endoplasmic reticulum
FAPP2	Four-phosphate adaptor protein 2
GalCer	Galactosylceramide
GlcCer	Glucosylceramide
GSL	Glycosphingolipids
IP3	Inositol triphosphate
LacCer	Lactosylceramide
LTP	Long-Term Potentiation
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
NMDARs	N-methyl-D-aspartate receptors
ROS	Reactive oxygen species
S1P	Sphingosine 1P
SM	Sphingomyelin
SPC	Sphingosylphosphorylcholine

TNF α	Tumor necrosis factor- α
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