Published in final edited form as:

J Neurosci Res. 2016 November ; 94(11): 1019–1024. doi:10.1002/jnr.23750.

THE FLUID LEVITY OF THE CELL: THE ROLE OF MEMBRANE LIPID ARCHITECTURE IN GENETIC SPHINGOLIPIDOSES

Ludovic D'auria¹ and Ernesto R. Bongarzone¹

¹Department of Anatomy and Cell Biology, University of Illinois, Chicago, Illinois, USA.

Abstract

Sphingolipidoses arise from inherited loss-of-function of key enzymes regulating the sphingolipid metabolism, and the accumulation of large quantities of these lipids in affected cells. Most frequently, toxicity is manifested in the nervous system, where survival and function of neurons and glial cells are most impacted. Although detailed information is available about neuroglial alterations during terminal stages of the disease, the initial pathogenic mechanisms triggering neuropathology are largely unclear. Being key components of biological membranes, changes in the local concentration of sphingolipids are likely to impact in the organization of membrane domains and functions. This essay proposes that sphingolipids' toxicity involves initial defects in the integrity of lipid domains, membrane fluidity, and membrane bending, leading to membrane deformation, and deregulation of cell signaling and function. Understanding how sphingolipids alter membrane architecture may provide breakthroughs for more efficient treatment of sphingolipidoses.

Graphical Abstract

Authors for correspondence: Dr Ludovic D'auria, Department of Anatomy and Cell Biology, College of Medicine, University of Illinois at Chicago, 808 S. Wood St M/C 512, Chicago, IL, USA. dauria@uic.edu, Dr Ernesto R. Bongarzone, Department of Anatomy and Cell Biology, College of Medicine, University of Illinois at Chicago, 808 S. Wood St M/C 512, Chicago, IL, USA. ebongarz@uic.edu.

DISCLOSURE OF CONFLICT OF INTEREST

ERB is a consultant with Lysosomal Therapeutics, Inc.

Sphingolipidoses are severe and incurable neurological diseases, where sphingolipids accumulate to high and toxic levels by unknown mechanism. Sphingolipids such as psychosine in Krabbe disease could insert in membrane, decreasing fluidity and disrupting functional lipid domains and/or induce changes in membrane curvature, leading to membrane fragility and cell signaling/function deregulation.

Keywords

lipid rafts; psychosine; sphingolipids; caveolin; fluidity; Krabbe's disease; neurodegeneration

INTRODUCTION AND HYPOTHESIS

Sphingolipidoses represent a large group of neurologically handicapping diseases caused by inherited loss-of-function of key enzymes in lipid catabolism, and the consequent accumulation of undigested lipid material (for a review, Platt 2014). Although promising results are being obtained from gene therapy and hematopoietic replacement studies, the field has remained largely stalled in curing most of these diseases (Cox and Cachon-Gonzalez 2012). This shortage of positive treatments could be in part due to an incomplete understanding of how sphingolipids (SLs) initiate cellular dysfunction (Schulze and

Sandhoff 2011). Emerging roles of SLs in different physiological processes may hold the key to understand their toxicity in non-physiological conditions such as sphingolipidoses. For example, several SLs have been reported to play major roles as bioactive lipids (Bartke and Hannun 2009), suggesting that they are not mere bricks in the big wall of biological membranes, but rather integral, active, and functional constituents. Importantly, the organization of SLs in cellular membranes is not regarded as random and inert anymore (Singer and Nicolson 1972). Rather, we know that SLs are key components arranged into plasma membrane (PM) realms, ranging from nanometric or lipid rafts (Lingwood and Simons 2010; Simons and Ikonen 1997) to submicrometric size (Carquin et al. 2015) domains. Furthermore, it is now accepted that SLs play roles in the macroscopic arrangement and shaping of cell membranes (Cooke and Deserno 2006). These SL features are essential to host proteins and to regulate physiological events such as surface tension, force sensing (Mollinedo and Gajate 2015) or cell signaling (Gomez-Mouton et al. 2004; Iwabuchi et al. 2010).

In pathological conditions, when SLs homeostasis is compromised, specific SLs accumulate one or more orders of magnitude. This alteration in the local stoichiometry of the membrane could lead to rapid changes in lipid aggregation and the disruption of membrane architecture, changes in membrane curvature, stability, and consequently the impairment of vital membrane-dependent cellular processes (Carquin et al. 2015).

This essay postulates that *in sphingolipidoses, the insertion of abnormal quantities of SLs in cell membranes destabilizes lipid domains and impairs the associated functions, inducing membrane changes (e.g. bending, vesiculation, etc) incompatible with normal cellular activities and leading to toxicity and cellular dysfunction.*

EXPERIMENTAL CHALLENGES TO STUDY MEMBRANE LIPID DOMAINS

Largely, three major lipid classes define the lipid organization of the PM (Figure 1A). **Cholesterol** is the most abundant membrane lipid with a non-polar backbone inserted within the membrane bilayer and outwardly exposing a single hydroxyl group. **Glycerophospholipids** (phosphatidylcholine, PC; phosphatidylserine, PS; phosphatidylethanolamine, PE) are abundant lipids in PM, composed by saturated-straight and unsaturated-bended fatty acyl chains and a polar head-group. **Sphingolipids (SLs)** have a hydrophobic core formed by the sphingoid base sphingosine, which in most cases is acetylated with a fatty acyl chain to form a ceramide. Ceramides are bound to different head-groups including choline (i.e. sphingomyelin, SM), sugars (i.e. glycosphingolipids, GSLs, such as in galactosylceramide, GalCer or glucosylceramide, GlucCer) or even more complex and larger head-groups (i.e. sulfatides; gangliosides). There are also **lysolipids**, formed by sphingosine and a sugar (i.e. galactosyl-sphingosine or psychosine).

These lipids spontaneously assemble to form membrane bilayers in a non-random fashion. The biophysical mechanisms and dynamics of membrane lipid organization are essential to understand how SLs modulate lipid domains and the consequent impact during disease. Artificial membrane models provided a major contribution in understanding lipid organization by studying the effects of lipid content in controlled environment. One of the

basic models is the ternary lipid phase diagrams, a chart reporting thermodynamically distinct phases that coexist at equilibrium, namely liquid-disordered (Ld), liquid-ordered (Lo) and solid ordered (So) phases (Goni et al. 2008). Most of mixtures are made of low-melting temperature lipids (Glycerophospholipids) with high-melting temperature lipids (SLs) and cholesterol (de Almeida et al. 2003). The lipid composition is key in the formation and maintenance of membrane domains, particularly to modulate their shape and size (for a review, see Bagatolli et al. 2010). SLs form Lo or So domains in glycerophospholipid-fluid phase (for a review, see Westerlund and Slotte 2009). Studies using simple planar lipid bilayers (Fidorra et al. 2006), giant unilamellar vesicles (GUVs) (Dietrich et al. 2001; Kahya et al. 2003; Pinto et al. 2008) and cell-derived PM (Baumgart et al. 2007; Bernardino de la Serna et al. 2004; Plasencia et al. 2007) have been critical in determining that SLs-cholesterol interactions are essential for domain formation, which is the cornerstone of the lipid-raft theory (Ramstedt and Slotte 2002). Among SLs, ceramide was shown to exert striking effects on domain formation with high lateral separation at low concentration with atypical shape capable to alter the packing of the fluid phase (Castro et al. 2014).

The lipid domain concept is well-established in artificial systems but its occurrence in PM cells has been unclear (Bagatolli 2006; Munro 2003). Innovative imaging approaches are rapidly contributing to address this. Submicrometric domains can be observed with optical techniques such as with high-resolution confocal, two-photon microscopy, and total internal reflection microscopy. These microscopy techniques are versatile and can be combined with other approaches to study dynamics of lipid and the lipid-lipid or lipid-protein interactions such as fluorescence recovery after photobleaching (FRAP), fluorescence lifetime imaging (FLIM) and fluorescence correlation spectroscopy (FCS). The advent of superresolution microscopy techniques have overcome the limitation of the resolution limit (~200nm) to analyze the nanometric lipid rafts. These include 1) photo-activation localization microscopy (PALM), 2) structured illumination microscopy (SIM), 3) stimulated emission depletion microscopy (STED), 4) atomic force microscopy (AFM), 5) near-field scanning optical microscopy (NSOM) and 6) ion mass spectrometry (SIMS) and 7) single dye tracing (SDT). Nanometric molecular interactions can be also measured by Forster resonance energy transfer (FRET) combined with superresolution microscopes. Altogether, these techniques are becoming gold-standards to study micrometric (Carquin et al. 2015) and nanometric (Castro et al. 2013) dynamic parameters of lipid domains in biological membranes.

ALTERATION OF MEMBRANE DOMAINS IN SPHINGOLIPIDOSES

Brain cells are highly polarized and specifically enriched in various types of SLs (*e.g.* gangliosides in neurons; GalCer, sulfatides, and SM in myelin) (for a review, Aureli et al. 2015). A growing body of evidence shows that the pathological accumulation of SLs affects the highly organized architecture of brain membranes. For example, Krabbe's disease is a sphingolipidosis with one the most severe neurodegenerative conditions, caused by the deficiency of the lysosomal enzyme galactosyl-ceramidase and the accumulation of high amounts of psychosine. Psychosine is present in very low levels in healthy conditions but its physiological function remains undetermined, being considered as a biologically irrelevant intermediate (Suzuki 1998). We have actively investigated the physicochemical properties of psychosine, aiming to understand its physiological function in health and disease.

Psychosine accumulates in lipid rafts in central and peripheral nervous tissue from the murine twitcher model of Krabbe's disease as well as in the brain of Krabbe patients (White et al. 2009). Increased levels of psychosine promoted significant changes in rafts, with enrichment of cholesterol, altered flotillin-2 and caveolin-1 distribution, and abnormal activation of protein kinase C (PKC) (White et al. 2009). In neurons, psychosine negatively impacted on fast axonal transport and neurofilament cytoskeleton via deregulation of raft associated protein phosphatase 1 and 2 and GSK3 β (Cantuti-Castelvetri et al. 2012; Cantuti Castelvetri et al. 2013). Further evidence that psychosine alters membrane architecture via destabilization of lipid domains was also recently reported (Hawkins-Salsbury et al. 2013). New evidence from our laboratory underlines that psychosine disrupts endogenous submicrometric lipid domains but promotes the formation of new and likely aberrant high-order submicrometric domains leading to increase of PM rigidity (D'auria & Bongarzone, submitted).

Similar effects are found in other sphingolipidoses. For example, in Niemann-Pick type A disease, the accumulation of SM in lipid rafts impairs membrane RhoA targeting and activation (Galvan et al. 2008). Furthermore, accumulation of SM at synaptic membranes disrupted synaptic plasticity dependent on the phosphoinositides pathway (Trovo et al. 2015). In these studies, the addition of SM in normal neurons reproduced these effects, suggesting that SM membrane accumulation was sufficient to induce the defect. GM1 is another lipid raft component which can lead to major alteration of membranes and cellular processes. For example, neurons from GM1 gangliosidosis (Purpura 1978; Purpura and Baker 1977) or after exogenous administration of GM1 (Byrne et al. 1983) exhibit enlarged neurites. In metachromatic leukodystrophy, the accumulation of sulfatides (sulfated GalCer) decreased the content of MAL in myelin rafts (Saravanan et al. 2004) and affected the association of PDGFR α with lipid rafts, impacting on oligodendrocyte formation (Pituch et al. 2015). A recent work on immune cells analyzed the perturbation of specific SL metabolism on the Toll-like receptor dependent immune signaling, leading to a specific inflammation phenotypes. Furthermore, predictions of inflammatory states in cells of patients affected by lipid storage disorders could be elaborated (Koberlin et al. 2015). As stated previously, ceramide represents a sphingolipid with a particular behavior. Generated via the hydrolysis of SM by sphingomyelinase, ceramide forms submicrometric domains also called ceramide-rich platforms. These platforms participate in membrane fragility (Montes et al. 2008) and changes in normal physiological functions such as in transmembrane signaling, clustering of specific proteins, membrane destabilization by flip-flop diffusion (for reviews, see Castro et al. 2014; Stancevic and Kolesnick 2010) and may be relevant in neuronal deficits in sphingolipidoses (Prinetti et al. 2001).

DESTABILIZATION OF MEMBRANE ARCHITECTURE: A LETHAL KICKOFF FOR SPHINGOLIPIDOSES

The physiological and structural architecture of cell membranes is highly dependent on two key properties: fluidity and membrane curvature. Fluidity is a biophysical parameter of membranes that refers to the average membrane viscosity generated by the rotational and lateral mobility of individual molecules and their interactions with surrounding molecules

(Lenaz 1987). Fluidity is a fundamental property of cell membranes that influences the correct positioning of key proteins, receptors, and even lipids. Fluidity within the PM depends on its composition. For example: short and unsaturated lipid will promote highly fluid membranes while packing of long and saturated fatty acid chains of SLs with cholesterol will decrease membrane fluidity (*i.e.* increase order, rigidity, or viscosity). Most SLs such as GM1 (Nishio et al. 2004), SM (Galvan et al. 2008; Koike et al. 1998; von Einem et al. 2012) and even psychosine (D'auria & Bongarzone, submitted) appear to alter cell membranes by decreasing fluidity. It is then conceivable that one of the first pathological changes in sphingolipidoses results from focal decreases in membrane fluidity, impairing the mobility of proteins and lipids essential for vital cellular functions.

Intimately linked to fluidity, membrane curvature is fundamental to regulate cell shape, endo and exocytosis, process formation, and even synaptic activity. The shapes of different lipids influence how much a membrane can curve (*i.e.* bend). The intrinsic molecular volume of the head-group and composition of the fatty acyl chains (*i.e.* saturated vs unsaturated) in lipids will determine strict steric dimensions and distinct shapes for each lipid species. Based on this consideration, lipids can be classified as cylinders (head-group is similar to the tail, PC, PS), truncated cones (head-group is smaller than the tail, PE), cones or triangles (head-group is minimal, cholesterol), truncated inverted-cones (head-group is larger than the tail, SM), and inverted cones (tail is minimal, GSLs, psychosine and LPC) (Figure 1A). The type of lipid and its abundance are major factors influencing morphological features of cell membranes (McMahon and Boucrot 2015). The degree of membrane curvature of a bilayered membrane is determined by the difference resulting from the combined curvatures of the inner *versus* the outer monolayer leaflets. For example: a monolayer of inverted cone lipids promotes a positive curvature, while conical lipids favors negative curvatures. Cylindrical lipids or associations between cone with inverted cone lipids tend to produce flat monolayers (Cooke and Deserno 2006). The asymmetric distribution of lipid species across the bilayer, (*i.e.* SLs are mainly located in the outer leaflet whereas PS and PE are mainly associated with the inner leaflet) influence the resulting membrane curvature (Devaux et al. 2008). Although specialized proteins play active roles in membrane curvature (for a review McMahon and Boucrot 2015), the strong dependence between shape/composition and membrane curvature underlines the idea that increased SLs in sphingolipidoses change the composition of lipid domains, inducing positive (outward) membrane bending (Figure 1B). This structural alteration is likely to impact on membrane topology, and the activity of associated signaling pathways.

Changes in membrane curvature could explain some of the phenotypic changes such as membrane swelling observed in various sphingolipidoses. For example, neurons from GM1 and GM2 gangliosidoses displayed large axonal and dendritic swellings (Purpura 1978; Purpura and Baker 1977). In Krabbe's disease, our group reported in vivo axonal swellings in spinal cords and peripheral nerves of twitcher mice and also in cultured mutant neurons as well as motoneurons incubated with psychosine (Castelvetri et al. 2011). In sphingolipidoses, where there is a progressive accumulation of SLs in the membrane, increased bending could lead to exacerbated shedding of the membrane, an emerging mechanism with significant roles in pathophysiology (Herring et al. 2013) (see also Scesa et al., this issue). Altogether, these data underline the activation of common pathological

changes of membranes, including increased rigidity, bending, swelling and vesiculation promoted by the presence of SLs (Figure 1B).

SIGNIFICANCE

The successful treatment of sphingolipidoses has been hampered not only by the rapidity and severity of their phenotype but also by an incomplete mechanistic understanding of how SLs inflict damage to brain cells. We have discussed the idea that SLs could impair physiological signals through (1) disruption of functional lipid domains, (2) membrane rigidification, and (3) cellular deformation by changes in membrane curvature (Figure 1B). The consequences that these early changes in membrane architecture impose to brain physiology are major. Decreases of membrane fluidity and curvature may have far reaching effects, from reducing the efficiency of synaptic vesicles docking and their release to alterations in internodal myelin, to the aberrant activation of microglia and formation of the glial scar (Jana and Pahan 2010; Paintlia et al. 2003; Seyfried et al. 1984). Furthermore, it is probable that these early alterations are limiting factors in current treatments (White et al. 2011), which in most cases require long periods of time to elicit the first clinical improvements. Future therapies may take this aspect into consideration by addressing for example protection of membrane curvature, by the use of cone shaped lipids or membrane-stabilizing proteins (for a review, see McMahon and Boucrot 2015). Although our discussion opted for a more simplistic view of singular lipids, future work should complement our analysis by including other more complex lipids and proteins (Koberlin et al. 2015). Decoding toxicity mechanisms in sphingolipidoses also represents an unique opportunity to understand the physiological function and organization of sphingolipids implicated in diseases other than sphingolipidoses (Gulbins and Petrache 2013).

Acknowledgments

This work was partially funded by the National Multiple Sclerosis Society to LD, and by grants from NIH (R01NS065808 and R21NS087474) and the Legacy of Angels Foundation to ERB.

REFERENCES

- Aureli M, Grassi S, Prioni S, Sonnino S, Prinetti A. Lipid membrane domains in the brain. *Biochimica et biophysica acta*. 2015; 1851(8):1006–1016. [PubMed: 25677824]
- Bagatolli LA. To see or not to see: lateral organization of biological membranes and fluorescence microscopy. *Biochimica et biophysica acta*. 2006; 1758(10):1541–1556. [PubMed: 16854370]
- Bagatolli LA, Ipsen JH, Simonsen AC, Mouritsen OG. An outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes. *Prog Lipid Res*. 2010; 49(4):378–389. [PubMed: 20478336]
- Bartke N, Hannun YA. Bioactive sphingolipids: metabolism and function. *Journal of lipid research*. 2009; 50(Suppl):S91–S96. [PubMed: 19017611]
- Baumgart T, Hammond AT, Sengupta P, Hess ST, Holowka DA, Baird BA, Webb WW. Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(9):3165–3170. [PubMed: 17360623]
- Bernardino de la Serna J, Perez-Gil J, Simonsen AC, Bagatolli LA. Cholesterol rules: direct observation of the coexistence of two fluid phases in native pulmonary surfactant membranes at physiological temperatures. *J Biol Chem*. 2004; 279(39):40715–40722. [PubMed: 15231828]

- Byrne MC, Ledeen RW, Roisen FJ, Yorke G, Sclafani JR. Ganglioside-induced neuritogenesis: verification that gangliosides are the active agents, and comparison of molecular species. *Journal of neurochemistry*. 1983; 41(5):1214–1222. [PubMed: 6413653]
- Cantuti-Castelvetri L, Zhu H, Givogri MI, Chidavaenzi RL, Lopez-Rosas A, Bongarzone ER. Psychosine induces the dephosphorylation of neurofilaments by deregulation of PP1 and PP2A phosphatases. *Neurobiology of disease*. 2012; 46(2):325–335. [PubMed: 22326830]
- Cantuti Castelvetri L, Givogri MI, Hebert A, Smith B, Song Y, Kaminska A, Lopez-Rosas A, Morfini G, Pigino G, Sands M, Brady ST, Bongarzone ER. The Sphingolipid Psychosine Inhibits Fast Axonal Transport in Krabbe Disease by Activation of GSK3beta and Deregulation of Molecular Motors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2013; 33(24):10048–10056. [PubMed: 23761900]
- 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]
- Castelvetri LC, Givogri MI, Zhu H, Smith B, Lopez-Rosas A, Qiu X, van Breemen R, Bongarzone ER. Axonopathy is a compounding factor in the pathogenesis of Krabbe disease. *Acta neuropathologica*. 2011; 122(1):35–48. [PubMed: 21373782]
- Castro BM, Prieto M, Silva LC. Ceramide: a simple sphingolipid with unique biophysical properties. *Prog Lipid Res*. 2014; 54:53–67. [PubMed: 24513486]
- Castro BM, Torreno-Pina JA, van Zanten TS, Gracia-Parajo MF. Biochemical and imaging methods to study receptor membrane organization and association with lipid rafts. *Methods Cell Biol*. 2013; 117:105–122. [PubMed: 24143974]
- Cooke IR, Deserno M. Coupling between lipid shape and membrane curvature. *Biophys J*. 2006; 91(2):487–495. [PubMed: 16807230]
- Cox TM, Cachon-Gonzalez MB. The cellular pathology of lysosomal diseases. *J Pathol*. 2012; 226(2):241–254. [PubMed: 21990005]
- de Almeida RF, Fedorov A, Prieto M. Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts. *Biophys J*. 2003; 85(4):2406–2416. [PubMed: 14507704]
- Devaux PF, Herrmann A, Ohlwein N, Kozlov MM. How lipid flippases can modulate membrane structure. *Biochimica et biophysica acta*. 2008; 1778(7–8):1591–1600. [PubMed: 18439418]
- Dietrich C, Bagatolli LA, Volovyk ZN, Thompson NL, Levi M, Jacobson K, Gratton E. Lipid rafts reconstituted in model membranes. *Biophys J*. 2001; 80(3):1417–1428. [PubMed: 11222302]
- Fidorra M, Duelund L, Leidy C, Simonsen AC, Bagatolli LA. Absence of fluid-ordered/fluid-disordered phase coexistence in ceramide/POPC mixtures containing cholesterol. *Biophys J*. 2006; 90(12):4437–4451. [PubMed: 16565051]
- Galvan C, Camoletto PG, Cristofani F, Van Veldhoven PP, Ledesma MD. Anomalous surface distribution of glycosyl phosphatidyl inositol-anchored proteins in neurons lacking acid sphingomyelinase. *Mol Biol Cell*. 2008; 19(2):509–522. [PubMed: 18032586]
- Gomez-Mouton C, Lacalle RA, Mira E, Jimenez-Baranda S, Barber DF, Carrera AC, Martinez AC, Manes S. Dynamic redistribution of raft domains as an organizing platform for signaling during cell chemotaxis. *The Journal of cell biology*. 2004; 164(5):759–768. [PubMed: 14981096]
- Goni FM, Alonso A, Bagatolli LA, Brown RE, Marsh D, Prieto M, Thewalt JL. Phase diagrams of lipid mixtures relevant to the study of membrane rafts. *Biochimica et biophysica acta*. 2008; 1781(11–12):665–684. [PubMed: 18952002]
- Gulbins, E.; Petrache, I. *Sphingolipids in disease*. Wien ; New York: Springer; 2013. p. 478viii
- Hawkins-Salsbury JA, Parameswar AR, Jiang X, Schlesinger PH, Bongarzone E, Ory DS, Demchenko AV, Sands MS. Psychosine, the cytotoxic sphingolipid that accumulates in globoid cell leukodystrophy, alters membrane architecture. *Journal of lipid research*. 2013; 54(12):3303–3311. [PubMed: 24006512]
- Herring JM, McMichael MA, Smith SA. Microparticles in health and disease. *Journal of veterinary internal medicine / American College of Veterinary Internal Medicine*. 2013; 27(5):1020–1033. [PubMed: 23815149]

- Iwabuchi K, Nakayama H, Iwahara C, Takamori K. Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction. *FEBS Lett.* 2010; 584(9):1642–1652. [PubMed: 19852959]
- Jana A, Pahan K. Fibrillar amyloid-beta-activated human astroglia kill primary human neurons via neutral sphingomyelinase: implications for Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2010; 30(38):12676–12689. [PubMed: 20861373]
- Kahya N, Scherfeld D, Bacia K, Poolman B, Schwille P. Probing lipid mobility of raft-exhibiting model membranes by fluorescence correlation spectroscopy. *J Biol Chem.* 2003; 278(30):28109–28115. [PubMed: 12736276]
- Koberlin MS, Snijder B, Heinz LX, Baumann CL, Fauster A, Vladimer GI, Gavin AC, Superti-Furga G. A Conserved Circular Network of Coregulated Lipids Modulates Innate Immune Responses. *Cell.* 2015; 162(1):170–183. [PubMed: 26095250]
- Koike T, Ishida G, Taniguchi M, Higaki K, Ayaki Y, Saito M, Sakakihara Y, Iwamori M, Ohno K. Decreased membrane fluidity and unsaturated fatty acids in Niemann-Pick disease type C fibroblasts. *Biochimica et biophysica acta.* 1998; 1406(3):327–335. [PubMed: 9630707]
- Lenaz G. Lipid fluidity and membrane protein dynamics. *Biosci Rep.* 1987; 7(11):823–837. [PubMed: 3329533]
- Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science.* 2010; 327(5961):46–50. [PubMed: 20044567]
- McMahon HT, Boucrot E. Membrane curvature at a glance. *Journal of cell science.* 2015; 128(6):1065–1070. [PubMed: 25774051]
- Mollinedo F, Gajate C. Lipid rafts as major platforms for signaling regulation in cancer. *Adv Biol Regul.* 2015; 57:130–146. [PubMed: 25465296]
- Montes LR, Lopez DJ, Sot J, Bagatolli LA, Stonehouse MJ, Vasil ML, Wu BX, Hannun YA, Goni FM, Alonso A. Ceramide-enriched membrane domains in red blood cells and the mechanism of sphingomyelinase-induced hot-cold hemolysis. *Biochemistry.* 2008; 47(43):11222–11230. [PubMed: 18826261]
- Munro S. Lipid rafts: elusive or illusive? *Cell.* 2003; 115(4):377–388. [PubMed: 14622593]
- Nishio M, Fukumoto S, Furukawa K, Ichimura A, Miyazaki H, Kusunoki S, Urano T, Furukawa K. Overexpressed GM1 suppresses nerve growth factor (NGF) signals by modulating the intracellular localization of NGF receptors and membrane fluidity in PC12 cells. *J Biol Chem.* 2004; 279(32):33368–33378. [PubMed: 15145933]
- Paintlia AS, Gilg AG, Khan M, Singh AK, Barbosa E, Singh I. Correlation of very long chain fatty acid accumulation and inflammatory disease progression in childhood X-ALD: implications for potential therapies. *Neurobiology of disease.* 2003; 14(3):425–439. [PubMed: 14678759]
- Pinto SN, Silva LC, de Almeida RF, Prieto M. Membrane domain formation, interdigitation, and morphological alterations induced by the very long chain asymmetric C24:1 ceramide. *Biophys J.* 2008; 95(6):2867–2879. [PubMed: 18586849]
- Pituch KC, Moyano AL, Lopez-Rosas A, Marottoli FM, Li G, Hu C, van Breemen R, Mansson JE, Givogri MI. Dysfunction of platelet-derived growth factor receptor alpha (PDGFRalpha) represses the production of oligodendrocytes from arylsulfatase A-deficient multipotential neural precursor cells. *J Biol Chem.* 2015; 290(11):7040–7053. [PubMed: 25605750]
- Plasencia I, Norlen L, Bagatolli LA. Direct visualization of lipid domains in human skin stratum corneum's lipid membranes: effect of pH and temperature. *Biophys J.* 2007; 93(9):3142–3155. [PubMed: 17631535]
- Platt FM. Sphingolipid lysosomal storage disorders. *Nature.* 2014; 510(7503):68–75. [PubMed: 24899306]
- Prinetti A, Chigorno V, Prioni S, Loberto N, Marano N, Tettamanti G, Sonnino S. 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(24):21136–21145. [PubMed: 11264283]
- Purpura DP. Ectopic dendritic growth in mature pyramidal neurones in human ganglioside storage disease. *Nature.* 1978; 276(5687):520–521. [PubMed: 102998]

- Purpura KP, Baker HJ. Neurite induction in mature cortical neurones in feline GM1-ganglioside storage disease. *Nature*. 1977; 266(5602):553–554. [PubMed: 404568]
- Ramstedt B, Slotte JP. Membrane properties of sphingomyelins. *FEBS Lett*. 2002; 531(1):33–37. [PubMed: 12401199]
- Saravanan K, Schaeren-Wiemers N, Klein D, Sandhoff R, Schwarz A, Yaghootfam A, Gieselmann V, Franken S. Specific downregulation and mistargeting of the lipid raft-associated protein MAL in a glycolipid storage disorder. *Neurobiology of disease*. 2004; 16(2):396–406. [PubMed: 15193296]
- Schulze H, Sandhoff K. Lysosomal lipid storage diseases. *Cold Spring Harb Perspect Biol*. 2011; 3(6)
- Seyfried TN, Bernard DJ, Yu RK. Cellular distribution of gangliosides in the developing mouse cerebellum: analysis using the staggerer mutant. *Journal of neurochemistry*. 1984; 43(4):1152–1162. [PubMed: 6470710]
- Simons K, Ikonen E. Functional rafts in cell membranes. *Nature*. 1997; 387(6633):569–572. [PubMed: 9177342]
- Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science*. 1972; 175(23):720–731. [PubMed: 4333397]
- Stancevic B, Kolesnick R. Ceramide-rich platforms in transmembrane signaling. *FEBS Lett*. 2010; 584(9):1728–1740. [PubMed: 20178791]
- Suzuki K. Twenty five years of the "psychosine hypothesis": a personal perspective of its history and present status. *Neurochemical research*. 1998; 23(3):251–259. [PubMed: 9482237]
- Trovo L, Stroobants S, D'Hooge R, Ledesma MD, Dotti CG. Improvement of biochemical and behavioral defects in the Niemann-Pick type A mouse by intraventricular infusion of MARCKS. *Neurobiology of disease*. 2015; 73:319–326. [PubMed: 25251606]
- von Einem B, Weber P, Wagner M, Malnar M, Kosicek M, Hecimovic S, Arnim CA, Schneckenburger H. Cholesterol-dependent energy transfer between fluorescent proteins-insights into protein proximity of APP and BACE1 in different membranes in Niemann-Pick type C disease cells. *International journal of molecular sciences*. 2012; 13(12):15801–15812. [PubMed: 23443094]
- Westerlund B, Slotte JP. How the molecular features of glycosphingolipids affect domain formation in fluid membranes. *Biochimica et biophysica acta*. 2009; 1788(1):194–201. [PubMed: 19073136]
- White AB, Galbiati F, Givogri MI, Lopez Rosas A, Qiu X, van Breemen R, Bongarzone ER. Persistence of psychosine in brain lipid rafts is a limiting factor in the therapeutic recovery of a mouse model for Krabbe disease. *Journal of neuroscience research*. 2011; 89(3):352–364. [PubMed: 21259322]
- White AB, Givogri MI, Lopez-Rosas A, Cao H, van Breemen R, Thinakaran G, Bongarzone ER. Psychosine accumulates in membrane microdomains in the brain of krabbe patients, disrupting the raft architecture. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2009; 29(19):6068–6077. [PubMed: 19439584]

SIGNIFICANCE

Sphingolipidoses are severe and currently, incurable neurological diseases, where sphingolipids accumulate to high and toxic levels. Little is known about the mechanism by which sphingolipids elicit toxicity. This essay proposes that the insertion of sphingolipids in biological membranes disrupts functional lipid domains and/or induce changes in membrane fluidity and curvature, leading to deregulation of cellular signaling and function.

Figure 1. Potential pathological mechanisms initiated by SLs accumulation illustrated in Krabbe disease

A) The plasma membrane is composed by lipid domains as illustrated for the SL domains (rafts and submicrometric domains) and the non-raft phase. Notice the cone shaped cholesterol (brown triangle), truncated cone shaped phosphatidylethanolamine (PE, magenta), cylindrical phosphatidylcholine (PC, light blue) and phosphatidylserine (PS, dark blue) whereas the sphingolipids (grey) such as sphingomyelin (SM; truncated inverted cone shape), galactosylceramide (GalCer), gangliosides and psychosine have inverted cone shaped structures. The sphingolipid tail is composed by a sphingosine base (red polygon in

psychosine) and binds to a long and saturated fatty acyl chain to form ceramide (green square). In contrast, glycerophospholipids contain shorter single or poly unsaturated acyl chains (as illustrated by the bent acyl chain). **B)** Two hypothetical effects of abnormal levels of sphingolipids are presented, taking as an example the increased levels of psychosine observed in Krabbe's disease. The deficiency of the galactosylceramidase (GALC) leads to increasing levels of psychosine over time. In model 1, the accumulation of SLs disrupts the architecture of lipid domains and impairs cellular signaling. The integrity of lipid domains are affected in early stages either by disruption or enlargement of these domains, leading to a decrease of functional signaling and eventually, irreversible toxicity on a variety of potential processes (synapse failure, deficiencies in fast axonal transport impairment (FAT), myelin or autophagy. In model 2, the accumulation of inverted-cone shaped SLs induces an increase of the membrane curvature. The insertion of many molecules of psychosine at the outer leaflet of the plasma membrane promotes a distortion of the membrane and a positive (+) (towards the extracellular space) bending. Ultimately, membrane bending may promote vesiculation, impacting on axonal and myelin membrane integrity. These two models are not mutually exclusive, and likely a combination of both and other alternatives not discussed in this essay could take place during sphingolipidoses.