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Review

Lipid membrane domains in the brain☆

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ABSTRACT

The brain is characterized by the presence of cell types with very different functional specialization, but with the common trait of a very high complexity of structures originated by their plasma membranes. Brain cells bear evident membrane polarization with the creation of different morphological and functional subcompartments, whose formation, stabilization and function require a very high level of lateral order within the membrane. In other words, the membrane specialization of brain cells implies the presence of distinct membrane domains. The brain is the organ with the highest enrichment in lipids like cholesterol, glycosphingolipids, and the most recently discovered brain membrane lipid, phosphatidylglucoside, whose collective behavior strongly favors segregation within the membrane leading to the formation of lipid-driven membrane domains. Lipid-driven membrane domains function as dynamic platforms for signal transduction, protein processing, and membrane turnover. Essential events involved in the development and in the maintenance of the functional integrity of the brain depend on the organization of lipid-driven membrane domains, and alterations in lipid homeostasis, leading to deranged lipid-driven membrane organization, are common in several major brain diseases. In this review, we summarize the forces behind the formation of lipid membrane domains and their biological roles in different brain cells. This article is part of a Special Issue entitled Brain Lipids.

1. Introduction: lateral order in cellular membranes

In the nervous system, the link between the organization of specific membrane subcompartments, at the micron, submicron and nanometer scale, and specialized cellular and tissue functions is probably more evident than in any other tissue. Neurons, oligodendrocytes (and Schwann cells in the peripheral nervous system) and (to a lesser extent) astrocytes are highly polarized cells with different morphologically distinguishable and well characterized membrane "macrodomains": the somatodendritic, axonal, and synaptic membrane macrodomains in neurons; the multi-layered myelin sheath and the nodal domains (the internodal region, the paranodal junction and the juxtaparanodes) in myelinating cells [2]. Even in membrane regions without a morphologically distinguishable architecture, membrane components do not undergo free and continuous lateral diffusion but rather are transiently confined to small domains ("micro-" or "nanodomains") [3]. Stimulated Emission Depletion (STED) microscopy revealed that GPI-anchored proteins were confined together with membrane lipids, including sphingolipids – sphingomyelin (SM) and monosialoganglioside GM1 – and cholesterol into small membrane areas with diameter <20 nm, forming transient and dynamic molecular complexes with an average lifespan of 10–20 ms [4,5].

The high level of lateral organization leading to the organization into specialized macrodomains is reflected by the lateral heterogeneity in membrane structure at the sub-micron and nanometer scale, i.e. by the peculiar molecular composition of the different functional domains in a cell membrane, that is driven and stabilized by the existence of lateral interactions between different membrane components. A striking example is represented by the massive multiprotein complex known as the post-synaptic density in neurons [6,7], which organizes the lateral positioning of neurotransmitter receptors at the post-synaptic site using an incredibly complicated array of scaffolding and adapting proteins, critically influencing the efficiency of synaptic transmission. Certain membrane proteins appear to be specifically designed to serve as scaffolds [8] to organize multiprotein complexes at membrane level (e.g. caveolins, organizing signaling complexes around estrogen receptors and neurotrophin receptors in neurons and oligodendrocytes [9,10], and signaling dependent on the cellular prion protein in serotonergic neurons [11]; reggie/flotillin, regulating the membrane turnover and

Abbreviations ganglioside and glycosphingolipid nomenclature is in accordance with the IUPAC-IUBMB recommendations [1]: AD, Alzheimer's disease; CGT, UDP-galactose ceramide galactosyltransferase; CNS, central nervous system; CST, cerebroside sulfotransferase; GalCer, galactosylceramide; GlcCer, glucosylceramide; GCS, glucosylceramide synthase; GPI, glycosylphosphatidylinositol; *ld*, liquid-disordered; *lo*, liquid-ordered; LPS, lipopolysaccharide; MAG, myelin-associated glycoprotein; MS, multiple sclerosis; NCAM, neural cell adhesion molecule; NF155, neurofascin 155; NgR1, Nogo receptor 1; PC, phosphatidylcholine; PDGFR, PDGFα receptor; PGLRs, phosphatidylglucoside-enriched lipid rafts; PNS, peripheral nervous system; PrP, prion protein; PtdGlc, phosphatidylglucoside; SM, sphingomyelin; STED, stimulated emission depletion

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delivery of specific protein complexes at the neuronal growth cone [12,13]; and membrane-associated guanylate kinases such as PSD95 and MAGI, major components of the postsynaptic density of glutamatergic synapses [14]). In addition, protein-driven limitations to the lateral diffusion of membrane components might not necessarily be due to direct protein-protein interactions. For instance, the "membrane skeleton fence" model explains the lateral compartmentalization of the fluid plasma membrane hypothesizing the formation of compartmental boundaries by actin-based membrane skeleton "fences" that are anchored to the membrane by "pickets" consisting of transmembrane proteins [15].

On the other hand, cellular membranes are characterized by an incredible complexity in terms of their lipid composition, which apparently strongly exceeds the needs for the creation of a fluid bilayer of amphipathic lipids serving as the 2-dimensional solvent for membrane-associated proteins. Literally hundreds of different lipid molecular species are present in a typical biological membrane. This simple fact has obviously been known for a long time, however only recently, thanks to the development of sophisticated and complementary lipidomic approaches [16–18], it has been possible to fully appreciate the molecular complexity of biological membranes (which is the result of a series of events, encompassing the synthesis, trafficking and turnover of membrane components which are tightly regulated along development in different ways in different cell types and tissues). The lipidome is thus no longer a Sphinx enigma, however it is extremely difficult to analyze in details the consequences of the diversity in the lipid composition on the lateral order of biological membranes. This is not surprising, considering that detailed information on the aggregational behavior, coming from the biophysical study of membrane model systems, is available only for relatively simple mixtures of the most common membrane lipids [19–21] and indeed any reductionist approach might be conceptually inadequate to investigate a phenomenon that is based on the collective properties of a complex lipid mixture [22]. Nevertheless, fluid-fluid phase separation due to incomplete miscibility of lipids in complex lipid mixtures could represent a major driving force for the creation of lateral order within cell membranes [23–26], and, as hypothesized by Karnovsky in 1982, the existence of multiple phases in the membrane lipid environment could drive the "organization of the lipid components of membranes into domains" [27]. In simplistic terms, fluid-fluid phase separation in biological membranes is driven by molecular mismatches between the different lipid components [28]. Thus, in principle, the incredible structural heterogeneity observed for the main classes of membrane lipids (glycerophospholipids, and, at a greater extent, sphingolipids) could be functional to the fine modulation of the lateral organization of cellular membranes, i.e., to the formation of specialized membrane domains. Simons and van Meer invoked the notion that lipids can organize domains in cellular membranes (as a consequence of phase separation) to explain the different lipid composition of the apical and basolateral plasma membrane macrodomains of polarized epithelial cells [29]. They proposed that the self-associative properties of the apical lipids (glycosphingolipids and cholesterol), leading to the formation of liquid-ordered (lo) phase [22,30–32] domains ("lipid rafts") in intracellular membranes along the trafficking pathway, might be the driving force underlying the differential sorting of apical and basolateral membrane lipid components [33]. Remarkably, the experimental proof of this hypothesis, i.e. the evidence that sphingolipids and sterols are actually segregated from glycerolipids in the *trans*-Golgi network during trafficking, was obtained 21 years later [34], combining an innovative shotgun lipidomic approach with a novel method for the immunoisolation of post-Golgi secretory vesicles.

Formation of lo phase domains due to spontaneous segregation of certain membrane lipids was subsequently proposed as a general mechanism for sorting and targeting of membrane components, possibly leading to the formation of laterally ordered membrane platforms with specific biological functions, including signal transduction [35]. Since 1997, more than 5,000 papers have been published describing the putative structure and functions of lipid rafts (for a few examples, see [36–44]), and recently a database specifically dedicated to mammalian lipid raft-associated proteins (RaftProt, http://lipid-raft-database.di.uq.edu.au/) has been developed [45]. Recently developed imaging techniques have at least in part overcome the lack of adequate experimental approaches to identify and study lo phases in living cells. The use of order-sensitive probes has demonstrated the existence of GM1- and cholesterol-rich domains with a high degree of lateral order in plasma membrane spheres obtained from A431 cells using a swelling procedure [46,47], and the existence of sphingolipid-enriched ordered membrane domains was supported by high-resolution imaging mass spectrometry studies of the lateral distribution of metabolically labeled ^{15}N-sphingolipids in the plasma membrane of intact fibroblasts [48]. In addition, it has become quite clear that the view of lipid-driven lateral order in cellular membranes exclusively based on phase separation is a partial one. For this reason, we prefer the term "lipid membrane domains" instead of "lipid rafts" to describe laterally organized structures within cell membranes based on lipids. In fact, in cell membranes heterogeneity can be driven by specific lipid-dependent lateral interactions in addition to liquid-liquid immiscibility, e.g., specific interactions between membrane-associated proteins and lipids [49]. In the nervous system, both aspects contribute at a significant extent to the creation of laterally organized membrane domains with specific functions, as discussed in the next sections of this review. The specific binding of glycosphingolipids, and in particular of gangliosides, to membrane receptors (for example, the binding of GM1 to TrkA receptor) has been known for a long time, even if only recently some molecular details underlying these interactions have been unveiled (reviewed in [49]). Several proteins that interact with glycosphingolipids are characterized by the presence of a characteristic aminoacid sequence termed the "sphingolipid binding domain". Amyloidogenic proteins, including α-synuclein and β-amyloid peptide, contain a structurally related loop centered around a tyrosine residue [50], which is essential for the ability of these proteins to bind glycosphingolipids that, in turn, play an essential role in the conformational transition and oligomerization process responsible for fibrillation. Similarly, cholesterol binding domains have been identified in α-synuclein [51], in the amyloid precursor protein [52,53] and in β-amyloid peptide [54,55]. Sphingolipid- and cholesterol- binding domains have also been identified in non-amyloidogenic membrane-associated proteins, such as the human serotonin1A receptor [56], the human β2-adrenergic receptor [57] and the nicotinic acetylcholine receptor [58]. Since sphingolipids and cholesterol are typical lo phase lipids, the presence of sphingolipid- or cholesterol-binding domains in a membrane protein might represent an efficient signal, targeting the protein to lipid rafts. Remarkably, the effective binding of a certain protein to the cellular membrane and its insertion in the correct membrane environment might require a form of "cooperative" binding with both glycosphingolipids and cholesterol, as it is the case for α-synuclein. α-Synuclein interacts with GM3 at the cell surface of astrocytes, or with GM1 at the neuronal surface, and the interaction with the glycosphingolipid induces a conformational change in a specific domain of the protein, allowing a high affinity interaction with membrane cholesterol, which is essential for α-synuclein oligomerization [59]. On the other hand, some proteins lacking a specific lipid-binding motif are nevertheless surrounded by a "shell" or *annulus* of typical raft lipids [60], that could be responsible for a high affinity of a protein for lipid rafts, determining its partitioning to a phase separated membrane domain in cooperation with or even in the absence of a specific raft-targeting signal.

2. Lipid-driven domains in the nervous system: a crucial role for sphingolipids

Usually "A Treatise on the Chemical Constitution of the Brain" published by J. L. W. Thudichum in 1884, the paper that elucidated the structure of sphingosine and of sphingosine-based brain lipids, is

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