Review essays

Lipids under stress – a lipidomic approach for the study of mood disorders

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The emerging field of lipidomics has identified lipids as key players in disease physiology. Their physicochemical diversity allows precise control of cell structure and signaling events through modulation of membrane properties and trafficking of proteins. As such, lipids are important regulators of brain function and have been implicated in neurodegenerative and mood disorders. Importantly, environmental chronic stress has been associated with anxiety and depression and its exposure in rodents has been extensively used as a model to study these diseases. With the accessibility to modern mass-spectrometry lipidomic platforms, it is now possible to snapshot the extensively interconnected lipid network. Here, we review the fundamentals of lipid biology and outline a framework for the interpretation of lipidomic studies as a new approach to study brain pathophysiology. Thus, lipid profiling provides an exciting avenue for the identification of disease signatures with important implications for diagnosis and treatment of mood disorders.

Introduction

Lipids constitute a diverse group of molecules that are fundamental for the structural organization and signaling regulation of cells. Their vast physicochemical diversity reflects their multiple functions at the cellular level, from modulation of the chemical and mechanical properties of membranes to protein trafficking, ion channel functioning and cell-to-cell communication [1]. While the research areas of genomics and proteomics have been extensively explored in the past few years, the study of the lipidome has been lagging behind essentially due to technical limitations. The recent improvement in analytical techniques, such as mass spectrometry (MS)-based lipidomics, currently allows a comprehensive study of the lipid profile of different systems, from cells to tissues or whole organisms, in an attempt to understand the role of lipids in physiology and pathological signaling events [2].

The study of lipids is particularly challenging, for lipid structure is immensely varied and lipid species can be dynamically interchangeable. The classical definition of a lipid is a molecule soluble in organic solvents. However, certain lipids present such a polarity that they are easily diffused in the aqueous phase when performing organic solvent-based lipid extraction [3]. Consequently, a thorough qualitative and quantitative analysis of the lipidome of a

system requires different analytical approaches directed to each subset of lipid classes [4].

Considering the overwhelming structural diversity of lipids and their role as putative modulators of different aspects of cellular functioning, a tight regulation of its metabolic pathways, both in space and time, is fundamental. Since lipids make up to half of the brain's dry weight, it is not surprising that lipid imbalances are involved in the pathophysiology of different neurodegenerative diseases [5–7]. Indeed, the recent use of MS has allowed the characterization of large-scale changes in lipid profiles both in humans and in animal models of disease [8–10]. This approach has been complemented by studies with genetically modified mice to better understand the role of specific lipids in health and disease [11]. Moreover, the identification of lipidomic signatures as biomarkers for diagnosis and therapeutical responses is an emerging field of study [2].

In this review, we discuss new lipid analytical strategies to study brain pathophysiology in the context of chronic stress and of diseases for which stress is a recognized trigger, such as anxiety and depression [12]. These novel experimental approaches will allow a better understanding of pathophysiological processes and the potential identification of surrogate markers of disease. We outline new ways of interpreting lipidomic data in a disease context and discuss new technical and conceptual challenges for the future.

Understanding the complexity of the brain lipidome

Lipids are crucial structural and functional components of the brain, regulating membrane assembly, vesicle synthesis and trafficking, neurotransmitter release, and signaling propagation [13]. Despite the implication of different lipids in various brain pathologies, mostly through studies focusing on their metabolizing enzymes, we still lack a comprehensive characterization of the brain lipidome and how it is affected by disease. Indeed, a recent MS-based lipidomic study analyzed the composition of different brain regions and non-neural tissues of humans and other animal species and concluded that concentration of lipids in the brain evolved faster when compared to non-neural tissues, with particular acceleration in the cortex [14]. This faster divergence privileges a specific lipid organization of the brain and reinforces the role of lipids in cognition and brain physiology.

Given this plethora of functions, one can be challenged by how lipids are structured and how they relate to other molecules. In mammalian cells, the main lipid categories are sterols (such as cholesterol), glycerophospholipids, and sphingolipids (Fig. 1). Lipids are subdivided on the basis of their chemical structure, namely a hydrophobic tail and a polar head group. The hydrocarbon chain moiety can vary in length, saturation, and hydroxylation, while polar head groups can differ in shape and charge [15]. Theoretically, thousands of lipids may co-exist within the same system and allow cells to organize their internal constituents in discrete organelles, with particular identities

and functions. For instance, the assembly of highly organized lipids in the plasma membrane promotes the existence of a rigid and impermeable barrier between the intra- and extracellular interface, in contrast with the flexible nature of the endoplasmic reticulum (ER), required for biogenic functions, such as synthesis and shuttling of lipids as part of secretory vesicles [16]. These features ultimately determine protein localization, conformation, and function. Finally, since lipids are highly interconvertible molecules and act as substrates to different lipid-modifying enzymes, hydrolytic enzymes are able to breakdown structural membrane components into second messengers [1]. While the hydrophobic product (e.g. diacylglycerol (DG)) is typically sequestered in the membrane of the parent molecule and potentially acts as an anchor signal for protein kinases, the polar structure (e.g. inositol 3-phosphate) is released into the cytoplasm and available to bind receptors and propagate intracellular signaling events, such as signal-induced calcium release [17]. The result of such dynamism is a complex crosstalk between structural components and regulators of cell signaling.

Cholesterol is the most abundant lipid in the brain

Cholesterol is mainly present in myelin sheaths and plasma membranes [18]. There, it resides with other lipid categories in constant molar ratios, namely glycerophospholipids and sphingolipids, such that impairment of this lipid balance is associated with disease [11]. Remarkably, delivery of peripheral lipoproteins across the blood-brain barrier is limited, requiring the cholesterol content of the brain to be mostly dependent on de novo synthesis and shuttling between glial cells and neurons [19, 20]. Despite its enrichment in membranes, cholesterol also plays a role in signaling, serving as a precursor to active steroids, such as glucocorticoids (GCs) [11]. These active molecules act as hormones, and differ from protein signaling factors in the sense that they do not typically bind to membrane receptors but rather diffuse across membranes and complex with cytoplasmic/nuclear receptors [21].

Cholesterol plays an important role in membranar lipid packing and raft assembly, which has implications in protein trafficking and folding. As a result, sterol levels are tightly regulated. Excess free cholesterol is converted to cholesteryl esters by the enzyme cholesterol acyltransferase 1 (ACAT 1) for storage in lipid droplets or shuttling to the extracellular milieu [22]. Alternatively, cholesterol conversion to 24S-hydroxycholesterol is the most efficient mechanism for efflux from the brain [23]. While the isoform 27S-hydroxycholesterol can be incorporated in the brain from the periphery, its levels are comparatively low and presents a very active metabolism, hence its contribution to cholesterol homeostasis and brain function is not yet very clear [24–26]. Additionally, mammalian cells have a feedback system in which release of the transcript factor SREBP2 from the ER under sterol depletion, followed by cleavage in the Golgi apparatus and migration through nuclear pores, activates sterol synthesis, and uptake machinery [27].
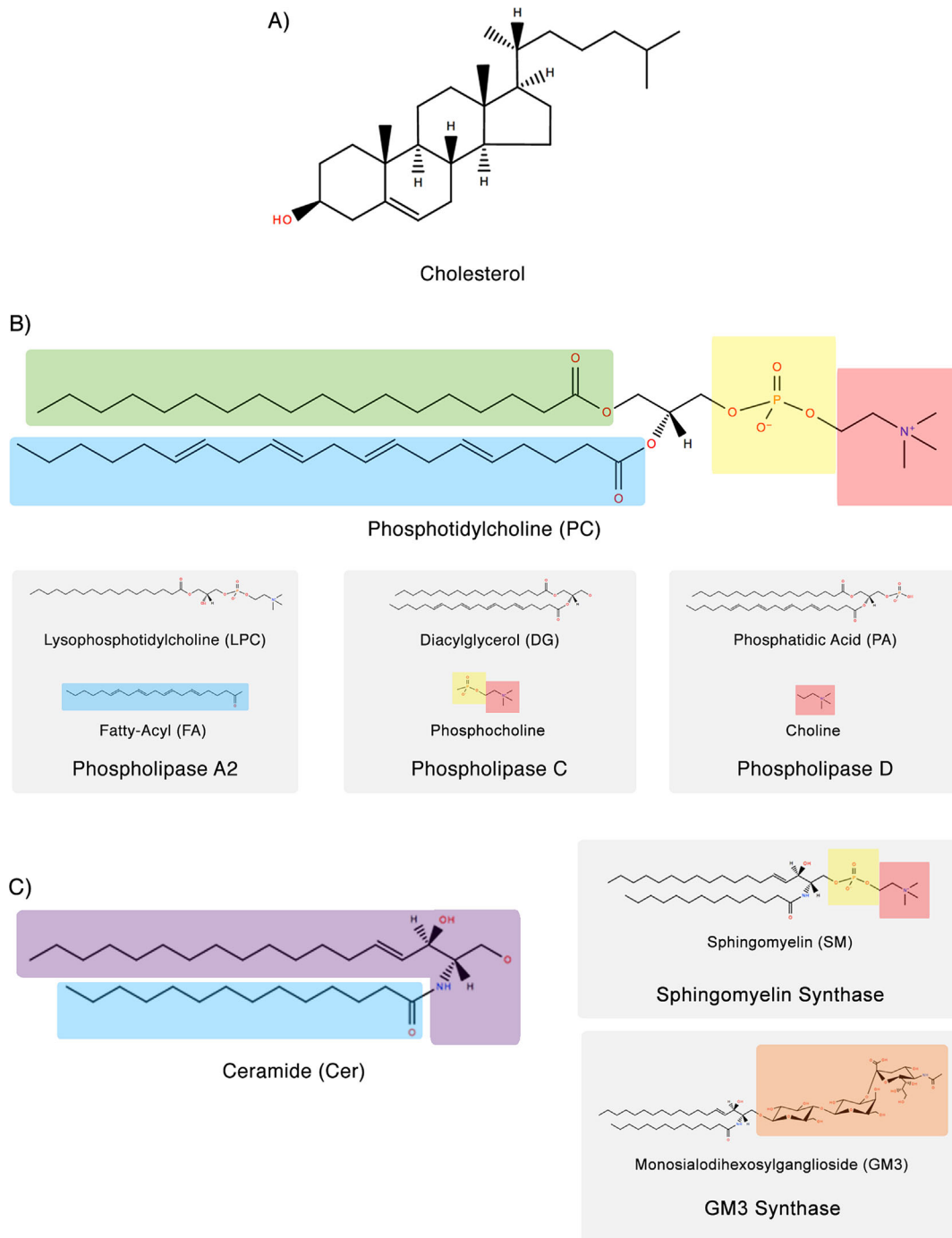
Figure 1. Examples of representative classes of the major lipid categories in mammalian cells. **A:** Cholesterol is the most abundant sterol in mammals. It contains an inflexible four-ring core that confers high hydrophobicity and interferes with acyl packing in membranes, promoting the assembly of lipid microdomains. Cholesterol plays an important role on the physical properties of membranes, namely fluidity, thickness, and permeability. Enzymes, such as ACAT1 and cholesterol 24-hydroxylase, whose activities determine the rate of cholesterol efflux from the brain to periphery, tightly regulate its levels. **B:** Glycerophospholipids are divided in classes according to the nature of the head group linked to the glycerol backbone. These vary in shape and charge, determining their location within membranes in cells. The insight boxes show the site of cleavage by phospholipases A2, C, and D. The respective products contribute to changes in membrane properties, such as induction of curvature (lysoPC and PA), or act as intracellular signaling molecules (fatty acyls and DG). **C:** Sphingolipids contain a sphingoid backbone, which is acylated to form a ceramide. Addition of polar head groups, phosphate or sugars, yields more complex classes, such as phosphosphingolipids and gangliosides. Note the similarity of the building blocks from both categories, suggesting putative steps of crosstalk between lipid metabolizing pathways. Colors: blue and green, fatty-acyl chain; orange, oligosaccharide chain; purple, sphingosine; red, choline; yellow, phosphate group. Lipid structures adapted from LIPID MAPS [114].

Glycerophospholipids are both structural and signaling molecules

Glycerophospholipids rank amongst the most abundant lipids in cells and serve as membrane components and signaling molecules. Their structure is based on a glycerol backbone linked to a polar head group at the position *sn3* and one or two fatty-acyl substituents present at *sn1* and *sn2* positions (Fig. 1B). Examples of different subclasses are phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI), which differ among themselves by the addition of a phosphocholine, phosphoserine, or a phosphoinositol ring as head groups, respectively. Further glycerol units – up to two – can be added to synthesize more complex phospholipids, such as bismonoacylglycerophosphate and cardiolipin [15].

The category of glycerophospholipids is a great example of how physicochemically related lipids differ in abundance and localization. For instance, PC is one of the most common glycerophospholipid subclasses in cells and it is a major component of cellular membranes, given its spontaneous propensity to self-assemble in stable bilayer membranes [16]. PS is also abundant in cell membranes and, because of its negatively charged head group, it plays an important role in determining membrane surface charge in the inner leaflet of membranes [28]. In regard to this specific example of non-random distribution of phospholipids, apoptotic pathways can be modulated by the inhibition of translocases and activation of scramblases that induce a randomization of the transbilayer gradient, resulting in external exposure of PS and clearance of apoptotic bodies by phagocytes [29, 30]. Moreover, other less abundant glycerophospholipids, such as phosphoinositides (PIs), have been reported to provide an identity code to membrane compartments with a major impact in protein recruitment and signaling pathways [31]. Similarly to sterols, glycerophospholipids also display feedback control mechanisms that can respond to specific changes in lipid concentration or physicochemical properties of membranes, such as curvature stress [16].

Sphingolipids regulate the assembly of membrane domains

Sphingolipids differ from the previous lipid category in the substitution of the glycerol backbone by sphingosine. They can be subdivided into sphingosine derivatives, ceramides (Cer) (with the addition of a fatty acid), and more complex sphingolipids with phosphate or carbohydrate head groups. Glycosphingolipids are classified based on the charge and number of carbohydrate head groups [15]. The simplest of these have a single moiety linked to Cer (glucosyl- or galactosylceramide), but more complex structures called gangliosides include oligosaccharide chains containing sialic acid groups [32].

Given their long and saturated fatty acid chains, sphingolipids present a tall, straight, and narrow cylindrical shape. This feature allows the assembly of highly ordered solid-gel phase membrane domains, named by some authors as lipid rafts, structures of higher complexity than initially predicted by the fluid-mosaic model [33]. These detergent-resistant membrane domains are also enriched in cholesterol, which facilitates lateral association and mobility, and modulates membrane thickness, with implications for protein recruitment and sorting [11, 34].

Lipid metabolic networks are intrinsically connected

Understanding the dynamics behind lipid diversity is an essential step to unveil their biological function and relevance. Most de novo lipid synthesis takes place in the ER [35]. Here, phospholipids and sterols are produced and shuttled to the Golgi and plasma membrane through secretory vesicles or pipelines orchestrated by lipid transfer proteins [36, 37]. In the case of sphingolipids, the Cer backbone is synthesized and transported from the ER to the Golgi apparatus where it is then converted to sphingomyelin (SM) by sphingomyelin synthases (SMS). Consistently, sterols preferably accumulate in the Golgi where stabilization with long, saturated acyl chains of SM and derivatives afford protection of the hydrophobic core from the cytosol [38]. The gradient of sterol and sphingolipids along the secretory pathway is conserved by the exclusion of both from coat protein I vesicles that retrogradely transport proteins and lipids back to the ER [39]. Additionally, some lipid metabolizing enzymes show different affinities for fatty acyls and generate distinctive lipid subspecies with a unique fatty acyl length and saturation, e.g. ceramide synthases and phospholipases A. These may present distinct subcellular localizations, and therefore mediate specific functions [40]. Moreover, some lipids present concentrations of different order of magnitude such that minor disturbances in their levels may result in larger impacts in their relative counterparts, within the same lipid category, e.g. relatively small fluctuations in SM and PC breakdown may result in two or more fold-change increases in Cer or PA, respectively [40]. The crosstalk between metabolic pathways of distinct lipid categories, such as the step mediating SM synthesis from the transfer of the polar head group from the glycerophospholipid PC to the sphingolipid Cer, adds another level of complexity to the integrated system of lipid metabolism [40]. These points of connection between multiple-lipid pathways may allow a small disturbance in a specific metabolic branch to propagate to other lipid categories. Such phenomena impair the overall lipid shape equilibrium, in which the interplay between the abundance of each lipid species and acyl chain composition are tightly controlled to ensure the appropriate membrane properties for organelle function. For a more detailed discussion of membrane sensing, cross-talk and compensatory mechanisms between lipid pathways, see [16, 41].

Lipids determine the properties of membranes—from shape to function

Eukaryotic cells have evolved to form a complex membrane system that allows compartmentalization of the cell into

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different organelles, where specific cellular functions occur, such as post-translational modification and maturation of proteins, spatial and temporal sorting, degradation, and recycling of long-lived compounds and damaged organelles [31]. Consistent with their functional complexity relatively to prokaryotes is the sophistication of their lipidomes [42]. Although lipid classes are generally defined by their polar head group, the fatty acids linked to their backbone may still vary in length and saturation, increasing the number of possible subspecies of lipids within the same class exponentially.

We have discussed that different lipids present different shapes and biochemical behaviors when assembled together in the same membranes. In general, the length of a phospholipid is proportional to the number of carbon atoms and inversely proportional to the degree of saturation (presence of double bonds). Taking into consideration the geometry of their polar head, lipids can assume different shapes, such as cylindrical or conical forms. These changes in geometry predict the shape of membranes, inducing planar or curved structures, respectively. Interestingly, a cylindrical phospholipid such as PC can be rapidly catabolized into conical lipids, through hydrolysis to diacylglycerol (DG) or phosphatidic acid (PA), by phospholipase C (PLC) and phospholipase D (PLD), respectively, or converted in an inverted-conical lysophosphatidylcholine (lysoPC) through the release of a fatty acyl chain by phospholipase A (PLA). Accordingly, cells defective in PS upregulate the similarly charged and shaped PI as a compensatory mechanism and another example of possible functional redundancy arises from the similarity between conical phosphatidylethanolamine (PE) and PA [16].

It is, therefore, possible for a membrane to assume different physical properties through the modulation of its lipids, namely thickness, fluidity, raft assembly, or surface charge [43]. Experimental evidence suggests that enrichment of sterols in the plasma membrane, in addition to saturated lipids, causes an increase in its thickness comparatively to other compartments, such as the ER. Not only the transmembrane domains (TMD) of plasma membrane proteins are longer than those located in the ER, but also proteins with smaller TMD are more easily segregated from this compartment when cholesterol is added, suggesting a model of hydrophobic matching [44].

Another key feature of membranes is the propensity to form microdomains, such as lipid rafts [6]. These lipid rafts were reported to be particularly enriched in cholesterol and sphingolipids with long and saturated fatty acids, a feature allowing an ordered sorting and interdigitation of their long chains between both leaflets [45]. These specific lipid microdomain structures function as specialized docks for localized cellular processes and have an important impact in disease physiology [5, 46]. Consistently, perturbation of sphingolipid metabolism results in altered protein compartmentalization through loss of assembly of these domains [43]. However, lipid rafts are not exclusively packed with sphingolipids and sterols but also contain glycerophospholipids, such as PC, its degree of recruitment being dependent on its level of saturation [34]. Indeed, sphingolipid depletion is compensated by synthesis of highly ordered and cylindrical saturated phospholipids [41]. Such planar structures allow a

more ordered state and the likelihood to be recruited and included in rafts. Additionally, the extent of phospholipid saturation determines membrane adsorption of cytosolic proteins by allowing packing defects that anchor hydrophobic motifs [47].

Altogether, manipulation of membrane composition regulates protein trafficking and folding and adjust flexibility to ease the mechanical forces exerted by proteins, such as to induce membrane curvature to facilitate vesicle fusion for neurotransmitter release or vesicle budding for synaptic vesicle recycling [48, 49].

Lipid imbalance is a hallmark of the stressed brain

In modern societies, the exposure to environmental stress is an increasing challenge. While physiological mechanisms to respond to acute stress are fundamental for the survival of an organism, the continuous or prolonged exposure to stress stimuli can have a deleterious impact. This maladaptive response includes functional and morphological impairment in various brain regions, such as the hippocampus and the prefrontal cortex (PFC). Consequently, chronic stress affects brain functions, such as learning and memory, decision making, emotional responses, and underlies the pathophysiology of various brain disorders, namely anxiety, depression and Alzheimer's disease (AD) [50–57].

The response to chronic stress involves a fast activation of the sympathetic nervous system, followed by a slower activation of the hypothalamic-pituitary-adrenal axis that culminates in the release of glucocorticoids (GCs), such as cortisol in primates and corticosterone (CORT) in rodents. GCs are believed to mediate a significant part of the pathologic effects of chronic stress and lead to structural and functional hippocampal impairment, with remarkable behavioral perturbations [52, 58–60]. Considering the multifaceted role of lipids in brain function, their implication in stress-related disorders, such as major depression and anxiety, is not surprising [61]. In fact, lipid-modifying enzymes have been reported to act downstream of GC signaling pathways and to be subject of modulation by antidepressant treatment [62, 63]. Dietary intake of lipids, namely poly-unsaturated fatty acids (e.g. docosahexaenoic acid (DHA)) affects the brain lipid composition in a region specific manner and impacts behavior [64–67]. Glycerolipid metabolism has been implicated in the pathology of depression, from increased turnover of phospholipids to single nucleotide polymorphisms in related enzymes that confer higher risk for bipolar disorder [68]. Increased sphingolipid breakdown and Cer production has also been linked to depressive-like behavior and neuroinflammation, and has been postulated as a pharmacological target of tricyclic antidepressants [61, 62]. Multiple studies suggest a connection between aberrant lipid metabolism and the neurobiology of depression through deregulation of monoaminergic transmission or neurogenesis [68]. In addition, indirect pathways may occur, such as lipid deficiency-induced inflammation and aberrant lipid transport from the periphery to the brain [69, 70].

Taken together, certain specific lipid classes may be directly implicated in brain disorders. Considering the impact of dietary lipid intake and pharmacological intervention in brain lipid metabolism, it was thus timely and informative to conduct a comprehensive lipidomic profile of the brain under conditions of stress.

Lipidomic analysis of the chronic-stressed brain

In order to understand the impact of chronic stress exposure on the rat brain lipidome, we employed a detailed MS-based lipidomic analysis of different brain regions from animals submitted to a 4-week chronic unpredictable stress paradigm (Table 1) [12, 53]. In the next sections, we detail a basic framework to conduct a lipidomic study and dissect the metabolic implications for lipid signaling from this pathological context.

Snapshotting lipid pathways

A possible initial approach to characterize the lipidome of a system is to analyze the abundance of lipid categories referenced to a control group. Given the diverse biochemical properties of the mammalian lipidome, particularly in terms of polarity, different extraction methods need to be employed for a full characterization. The use of a modified Bligh and Dyer method allows an efficient and reproducible lipid extraction of the major lipid classes soluble in organic solvents [71]. After extraction, lipid classes are identified by a combined liquid chromatography-mass spectrometry approach and quantified, in molar values, by spiking with appropriate internal standards. With such a broad analysis, it is possible to integrate multiple lipid metabolic pathways and denote whether changes in a lipid class are in concert with levels of metabolically related products and whether any compensatory mechanisms are activated in a diseased setting.

Table 1. Changes in rat brain lipid composition induced by chronic unpredictable stress exposure [12].

Brain region	Lipid changes
Prefrontal cortex	Decrease in glycerophospholipids (PC, PCe, PE)
	Increase in lysophospholipids (lysoPC[a], lysoPE)
	Decrease in sphingolipids (SM[a] and dhSM)
	Increase in sphingomyelin derivatives (Cer, LacCer)
Hippocampus	Decrease in PC
	Increase in PI
	Decrease in SM
	Increase in sphingomyelin derivatives (Cer, dhSM)
Amygdala	Increase in sulfatide derivatives (Sulf(2OH))
Cerebellum	No significant changes

[a]Lipid changes correlated with serum CORT levels ($R^2 > 0.4$)

While the present method is successful for the isolation of most lipid species, accurate measurement of highly polarized lipids (e.g. complex gangliosides) is hampered by their retention in the aqueous phase or need for alternative extraction methods for their purification [72]. Complementing strategies, such as high-performance liquid chromatography or the use of isotope-tagged derivatives, are required for the characterization of low-abundant lipids and isoform specific phospholipids, e.g. PI(3)P and PI(4)P [73–75]. Even though the use of the present tools only allows the identification of high-order relative fold changes, it has proven successful in the collection of a macro-snapshot of the lipid molecular species involved in disease pathology [8, 76].

Despite the common practice of using tissue homogenates in lipidomic approaches to identify lipid alterations in brain disorders, it is important to consider that whole tissue is composed of multiple cell types, such as neuronal and glial cells, each of which harbors its own lipid composition, enhancing the level of complexity of interpreting large-scale data sets [8, 63, 77, 78]. Indeed, we found significant differences between the lipid compositions of the different brain regions assessed [12]. Consequently, regional cellular heterogeneity is a potential major contribute for lipid disparity among different brain regions. Alternative approaches to complement this tissue analysis include the use of micro-dissection techniques or in vitro cell-type specific studies. Ultimately, a complete spatial characterization of the cell lipidome would require the analysis of the composition of particular organelles or more spatially defined structures in the central nervous system, through different biochemical fractionation and purification methods [79, 80]. This would be of particular relevance considering that bulk lipids determine the physical properties of membranes, modulate cellular protein machinery, and influence organelle functionality. The increase in spatial resolution can also be addressed by imaging lipid distribution in frozen brain sections using matrix-assisted laser desorption/ionization – time of flight/mass-spectrometry imaging (MALDI-TOF/MSI) [81, 82]. Nevertheless, the cost for topographical specificity is the reduced number of lipid species able to be identified, comparatively to other more established MS methods. Finally, the set-up of pulse and chase experiments in vitro, combined with the use of fluorescently tagged lipid binding domains, caged lipids, or direct labeling of lipids (e.g. deuterated lipids), can provide unique information about their exact location and how dynamically they change positions [83–86].

Knowing lipids from the inside: Fatty-acyl profile and metabolizing enzymes

The structural diversity of lipid classes impacts membrane function and interaction with the surrounding environment. This is of particular importance in brain functioning and neuronal processes. Neuronal communication relies on complex mechanisms mediating chemical synapses, from neurotransmitter release to sensing and signal initiation and propagation. Lipids are involved not only in synaptic vesicle fusion, and thus neurotransmitter release, but also in the activity of neurotransmitter receptors, ion channel

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conductivity, and intracellular signaling cascades [87–89]. Another level of complexity on top of the diversity of lipid heads, that define each lipid class, is the variability of the carbon chain length and degree of saturation of the fatty acyls that ultimately determine the membrane biophysical properties [90]. These include lipid packing, fluidity and propensity to raft assembly. While in MS lipid ions are identified by their mass/charge ratio, such analysis is not directly informative about their fatty-acyl composition other than the total number of carbons and saturated bonds in the molecule. Therefore, to determine the nature of the fatty acids in a specific lipid, it is necessary to fragment lipids and detect the resulting products, yielding a head group and one or two fatty acids, whether it is a lyso- or phospholipid, respectively [43]. We have recently characterized the profile of the fatty-acyl chains of phospholipids, sphingolipids, and other neutral lipids in different brain regions and how these are affected by the exposure to chronic stress. In addition to a generalized increase in glycerophospho- and sphingolipid hydrolysis, we reported a decrease in shorter fatty-acyl phospholipids/DG in the PFC and an increase in 38 carbon and four-double bond phospholipids/ DG in the hippocampus [12]. This accumulation of longer, unsaturated species suggests the incorporation of arachidonic acid (AA) – with the following "number of carbons":"double bonds" composition – 20:4 – as part of the fatty acyl composition. AA is a known mediator of inflammation, is positively correlated with age-induced changes and has also been described to be enriched in a different rat model of depressive-like behavior [91, 92]. In contrast, diets rich in polyunsaturated fatty acids, such as fish oil diet, show an increase in short, unsaturated DHA-containing phospholipids, which are proposed to be neuroprotective and anxiolytic [93, 94]. Of note, patients with depression were found to have an altered ratio of n-6/n-3 polyunsaturated fatty acids in peripheral blood and these changes may reflect an altered brain lipid composition, considering these fatty acids are also used as substrates in brain lipid synthesis [95–98].

Another piece of relevant information obtained from depicting lipid subspecies is the possible modulation of their metabolic enzymes. Some lipid modifying enzymes have been extensively implicated in brain disorders and proposed to be modulated at the level of protein expression as well as phosphorylation status [99–101]. Given the observed increase in glycerophospholipid and sphingolipid breakdown after exposure to chronic stress, we quantified the mRNA levels of the lipid enzymes most likely to underlie the observed findings (Fig. 2). We found no changes in the transcript levels of several PLA2, PLD, and sphingomyelinase isoforms [12]. These results suggest a complex and yet to be identified mechanism of post-transcriptional lipid enzyme regulation, which could involve altered protein synthesis, post-translational modifications, altered trafficking, or enzymatic activity.

Specific lipid imbalances are correlated with biological mediators of chronic stress

Depression and anxiety disorders are examples of the impact of the allostatic load caused by chronic stressors. In a recent study, we showed that the long-term consequences of
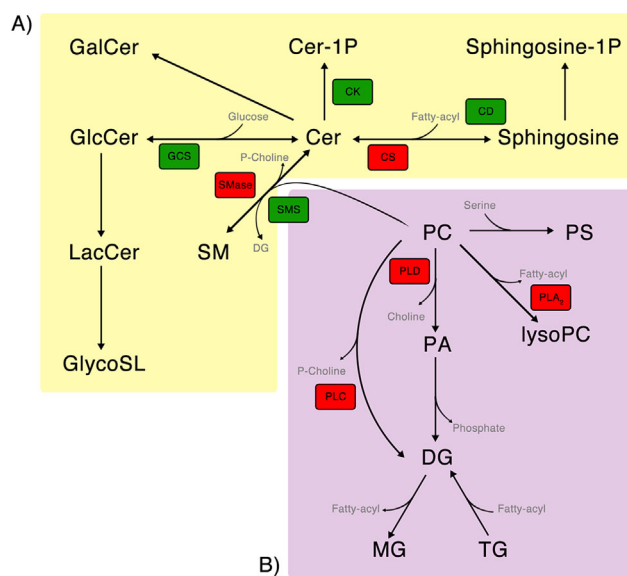
Figure 2. Pathways of sphingolipid and glycerophospholipid metabolism. **A:** Brain Cer levels are increased under conditions of stress [12, 61]. Such flux in sphingolipid metabolism is likely to occur due to increased hydrolysis of SM by sphingomyelinases but can also be related to increased de novo formation (enzymes highlighted in red). To alleviate Cer burden, sphingolipid breakdown may be reduced by pharmacological inhibition of SMases or stimulation of both SM and glycosphingolipid synthesis (highlighted in green). Alternatively, Cer may be hydrolyzed by ceramidases or phosphorylated to ceramide 1-phosphate by ceramide kinases. **B:** Glycerophospholipid metabolism is impaired under chronic stress with concerted PC breakdown and increase in its metabolites LysoPC, PA, and DAG, possibly by increased PLA, PLD, and PLC enzyme activity, respectively (highlighted in red). Decreased PC levels have been reported in animal models of neurodegenerative diseases and genetic ablation of the highlighted enzymes confers protection from synaptic and cognitive impairment. In addition, studies have shown decreased plasma PC levels and increased cerebrospinal fluid PC metabolites in patients with AD [115]. Abbreviations: CD, ceramidase; Cer, ceramide; Cer-1P, ceramide 1-phosphate; CK, ceramide kinase; CS, ceramide synthase; DG, diacylglycerol; GalCer, galactosylceramide; GCS, glucosylceramide synthase; GlcCer, glucosylceramide; GlycoSL, glycosphingolipids; LacCer, lactosylceramide; LysoPC, lysophosphatidylcholine; MG, monoacylglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; P-Choline, phospho-choline; SM, sphingomyelin; SMase, sphingomyelinase; SMS, sphingomyelin synthase; Sphingosine-1P, sphingosine-1-phosphate; TG, triacylglyceride.

exposure to stress leads to an imbalance in lipid homeostasis in specific brain areas [12]. However, chronic stress also affects many other physiological parameters. As previously mentioned, the response to chronic stress in the rodent involves an increase in CORT blood levels, which partly modulates its deleterious effects [102]. We reasoned that specific lipid changes could potentially be correlated with CORT blood levels [103, 104]. To further validate our hypothesis, we performed a full-scale unbiased correlation analysis between the abundance of over 300 quantifiable lipid species and the serum levels of CORT, in each brain region. With such an approach, we extended our analysis to the level of the individual within each group. This is particularly important

considering that individual humans and animals present different responses to stress, hence the concept of resilience [105]. These distinct responses to the same stimulus are linked to specific molecular alterations and genetic susceptibility and should be considered in studies addressing depressive-like behaviors [106–108]. Our results reinforced the initial findings: in the PFC, phospholipid, and sphingolipid breakdown correlated with serum CORT. Although correlation does not imply causation, such strong coherence suggests that these metabolic pathways are involved and possibly mediate stress responses. Further agreement with previous studies implicating these metabolizing enzymes in other cognitive disorders increase the relevance of such observations [62, 76, 109]. Other appealing approaches to screen the role of lipid imbalances in brain dyshomeostasis would be to correlate their levels to other known biological parameters, such as behavioral changes, electrophysiological activity, or morphological remodeling, including synaptic plasticity and neurogenesis. These findings should be followed by validation studies using genetic or pharmacological approaches targeting lipid modulating enzymes.

Lipid signatures as biomarkers for neurodegenerative disorders

The prevalence of neurological and psychiatric disorders is on the rise, and brings with it a considerable economic burden [110]. Continuous efforts have been implemented in order to early diagnose, prevent, halt, or reverse these disorders. Presently, it is still a major challenge to diagnose mood disorders and to predict their disease progression and response to therapy. Moreover, some cognitive disorders, such as AD, still rely on *post-mortem* evaluation for its diagnosis [111]. There is thus the need to develop new diagnostic and disease progression biomarkers. Not surprisingly, lipidomic studies are arising as potential means to find lipid signatures that identify specific disease states. Importantly, while it is still unclear the biological implication of these lipid signatures in disease physiology, their clinical relevance arises from the reproducibility amid independent studies and consistency between human and animal models of disease [8, 73, 78]. In the context of AD, a recent study has reported a blood-based lipid profile for successful detection of preclinical AD and prediction of phenoconversion to either mild cognitive impairment (MCI) or AD within 2–3 years [112]. The untargeted metabolomics and lipidomic profiling resulted in the identification of 10 metabolites, namely PC and acylcarnitine species, which could be used as a diagnostic tool. Therefore, it is now essential to extend the approach of lipid profiling to other neurological and psychiatric diseases, such as chronic stress associated disorders. It would be interesting to understand whether human studies present similar pathological signatures to the ones observed in rodent models. For the time being, magnetic resonance imaging spectroscopy methods have enabled non-invasive measurements of choline-containing molecules, further implicating phospholipid membrane metabolism in mood disorders, such as schizophrenia and depression [113]. Additional studies co-analyzing brain data with blood and CSF samples will not only be useful in setting new diagnostic biomarkers, but also predicting prognosis and therapy response. Given the complexity of the lipidome in terms of lipid categories, classes, and subclasses, only large post-processing and integrative methods of data analysis will allow researchers to uncover the many important roles of these highly modulatable molecules and transform lipidomic profiling in a routine and cost-effective procedure, readily available for patient care.

Conclusions and outlook

Lipidomic MS methods have opened new avenues in the analysis of biological samples. Since lipids are major constituents of the brain, these new analytical techniques provide new insights in the understanding of brain physiology and pathophysiology. Lipidomic studies allow the identification of altered lipid pathways and lipid signatures associated with a disease state. From now on, it will be fundamental to characterize the impact on the lipidome of genetic manipulation, pharmacological modulation, or exposure to different environmental factors, such as diet, exercise, and psychosocial stressors. Since lipids are intrinsically metabolically connected, lipidomic mappings will provide us the framework to correctly apply lipid-modifying therapies and use lipid signatures for diagnosis, prognosis, and therapeutic response in a given disease state.

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