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A neuroscientist's guide to lipidomics

*Daniele Piomelli**‡, *Giuseppe Astarita** *and Rao Rapaka*§

Abstract | Nerve cells mould the lipid fabric of their membranes to ease vesicle fusion, regulate ion fluxes and create specialized microenvironments that contribute to cellular communication. The chemical diversity of membrane lipids controls protein traffic, facilitates recognition between cells and leads to the production of hundreds of molecules that carry information both within and across cells. With so many roles, it is no wonder that lipids make up half of the human brain in dry weight. The objective of neural lipidomics is to understand how these molecules work together; this difficult task will greatly benefit from technical advances that might enable the testing of emerging hypotheses.

Hydrophobic effect
The tendency of hydrophobic molecules to associate in order to diminish contact with water.

Lipidomics
The large-scale analysis of lipid profiles in cells and tissues.

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Neuroscientists have a problem with fat. They know that the greasy substance makes up more than half of the human brain in dry weight, yet they don't seem to pay much attention to it. This peculiar form of cognitive neglect stems from deep-seated memories of university reading assignments that showed cell membranes as groups of interesting-looking proteins floating in a featureless bed of fat, and described 'lipids' in most unflattering terms as "Nucleic acids and proteins are informational macromolecules…On the other hand, polysaccharides and lipids do not have information-carrying function"(REF. 1.) However, references to lipid-related topics are increasing in the neuroscience literature, suggesting that times are changing and that neural lipids may be finally getting the attention they deserve.

This revived interest in the fatty half of the brain has been fuelled by two converging sets of discoveries. On one hand, advances in the fields of cell biology, synaptic physiology and receptor pharmacology have demonstrated that lipids have broad information carrying roles in the CNS. These range from the development of the neocortex to the processing of complex behaviours, covering a territory as vast as those generally ascribed to neurotransmitters, neuropeptides and growth factors. On the other hand, progress in techniques, such as mass spectrometry and atomic-force microscopy, has opened experimental opportunities that were unthinkable ten years ago (FIG. 1). Indeed, we are at a point where the goal of profiling large-scale changes in lipid composition or determining the topographical distribution of individual lipid species in neural cells is no longer beyond reach.

But, as neuroscientists become increasingly aware of the importance of lipid-mediated signalling, they often feel unprepared to grapple with its peculiarities. To begin with, lipids are exceedingly gregarious: forced by hydrophobic effects, they assemble into complex membrane structures. They adapt to the membrane's dynamic and interactive environment by changing their chemical structures (FIG. 2) — a property they also exploit to influence the function of proteins located both inside and outside the membrane. Moreover, when needed, many lipids can leave their water-repelling hubs to act as hormones or paracrine messengers.

In the following pages, we discuss two ways by which lipids affect brain function. We first consider how lipids alter the geometric properties and supramolecular organization of neuronal and glial membranes, and then turn to their role as ligands for proteins, outlining the general rules of engagement in intracellular and transcellular signalling. We will take a critical look at the developing field of neural lipidomics and delineate some of its technical and conceptual challenges.

The geometric properties of lipids

Cell membranes are dynamic entities that require continuous adjustments in the chemical structure and molecular shape of their lipid constituents, particularly of the phospholipids[2,3]. A major factor determining the shape of phospholipids is the nature of their hydrophobic tail, the fatty acid residues linked to the *sn*-1 and *sn*-2 hydroxyl group on the glycerol backbone (FIG. 2b,c). At physiological temperatures, the length of a phospholipid molecule is directly proportional to the number of carbon atoms and inversely proportional to the number of double bonds present in its fatty acid chains. In addition, the molecular shape of a phospholipid within the bilayer, although influenced by the surrounding environment, is ultimately determined by the compatibility between the size of its polar head group and that of its hydrophobic tail: if the two are fully compatible, the lipid has a cylindrical form; if not, it is either conical (when the tail is larger) or inverted conical (when the

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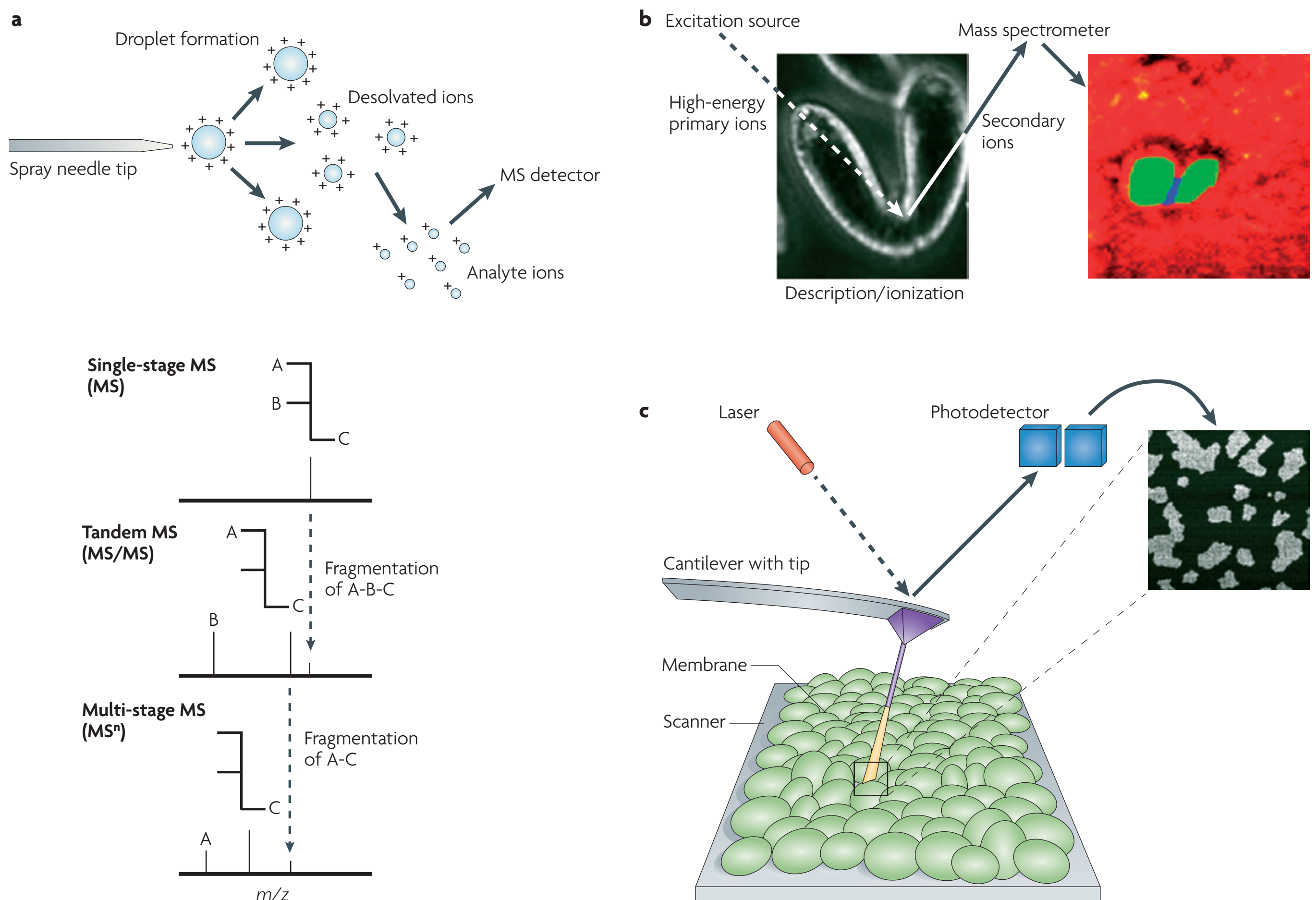

Figure 1 | **Strategies to identify, measure and image lipids in membranes.** The complex nature of lipid signalling and its strict dependence on specific membrane localization imposes structural and topological constraints, which can be tackled using three revolutionary techniques. **a** | In electrospray ionization mass spectrometry (MS) a solution containing the analyte is sprayed through a needle forming charged droplets. After desolvation, these explode forming ions that are detected by MS (top panel). Single-stage MS separates ions according to their mass-to-charge ratio (*m/z*); tandem MS and multi-stage MS (MS^n) allow multiple fragmentation to occur, generating fragments that are used to identify and quantify specific analytes in complex mixtures (bottom panel). **b** | In secondary ion MS, a beam of high-energy ions scans the membrane of a cell that is fixed by freeze-fracture (in this example, two mating *Tetrahymena* cells). The membrane emits 'secondary' ions, which are detected by MS and used to create a topographic map of a specific lipid analyte (in this case, the fusogenic 2-aminoethylphosphonolipid, shown in blue). **c** | In atomic force microscopy, a tip connected to a cantilever is brought into close proximity of a membrane. The force between the tip and the membrane causes a deflection of the cantilever, which is measured by a laser detector (photodetector). As the tip scans across the membrane, a topographic map of its surface is created. The panel on the right shows an artificial membrane containing sphingomyelin, phosphatidylcholine and cholesterol. The patchy appearance is due to the formation of nanosized liquid-ordered domains. Part **b** was modified, with permission, from REF. 5 © (2004) American Association for the Advancement of Science. Part **c** was modified, with permission, from REF. 103 © (2003) Biophysical Society.

head is larger) (FIG. 3a). Conical lipids tend to form negatively curved monolayers, whereas inverted conical lipids tend to form positively curved monolayers[2,3] (FIG. 3b). As we shall see in the following sections, changes in the geometrical attributes of membrane lipids have major consequences on the functional properties of neurons.

Lysophospholipid
A phospholipid containing a single fatty-acid chain; examples include signalling lipids, such as 1-oleoyl-*sn*-glycero-3-phosphate (lysophosphatidic acid).

A role for lipids in exocytosis? Synaptic vesicle exocytosis, the Ca^{2+}-regulated process through which presynaptic vesicles fuse with the plasma membrane to release their neurotransmitter content, is our first example of how lipid geometry may influence neuronal function. Exocytosis proceeds through a series of docking and fusing events that involve a complex interplay among a specialized set of proteins (the soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins), the cytoskeletal framework and lipids[4]. A simplified model posits that the fusion of vesicles with presynaptic membranes occurs in two sequential steps (FIG. 3c). In the first step, the bilayers of the two adjoining structures partially merge forming an intermediate (the 'stalk') in which only the outer leaflets of the two membranes are connected; this requires the

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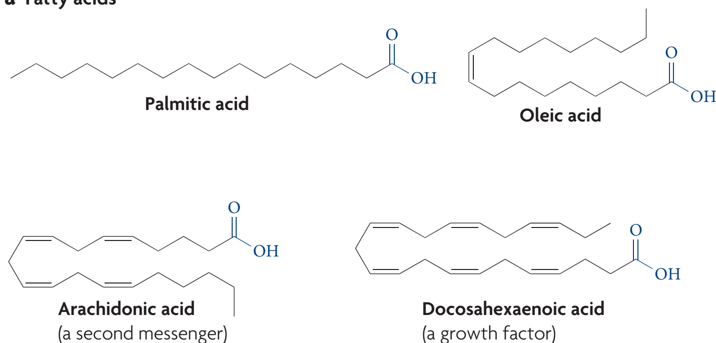

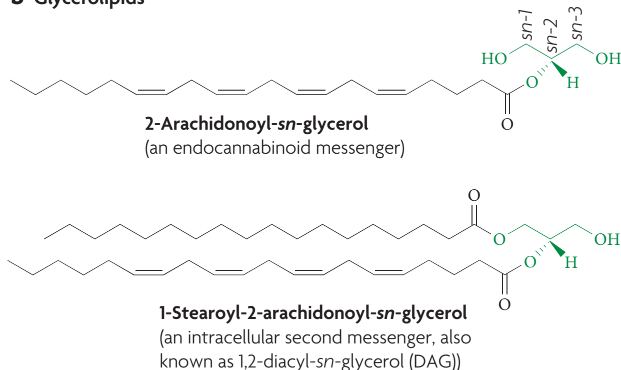

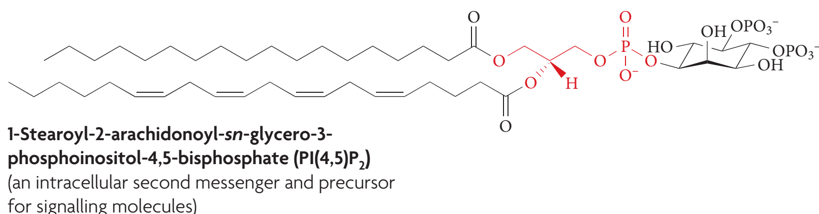

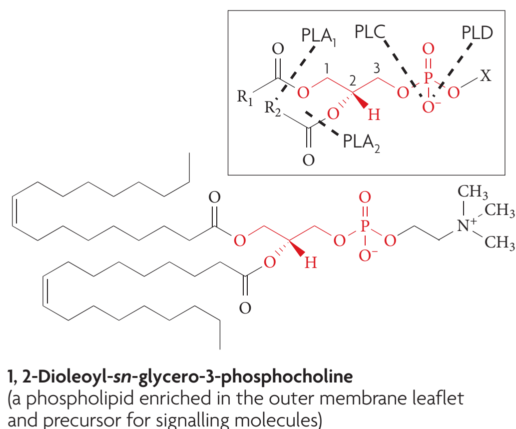

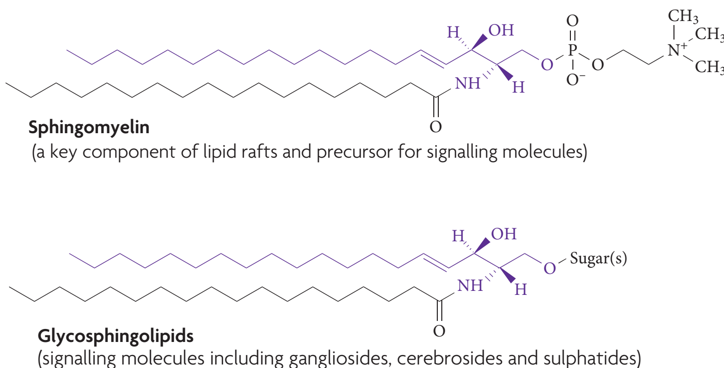

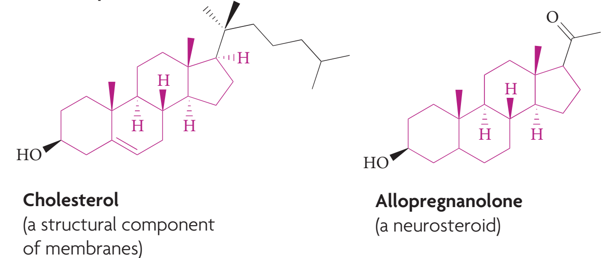

Figure 2 | **Brain lipids. a** | Fatty acids contain a carboxylic group (blue) attached to an aliphatic chain. Saturated fatty acids do not contain double bonds, whereas monounsaturated and polyunsaturated fatty acids contain one or more *cis* double bonds. There are two types of polyunsaturated fatty acids: ω-6 (for example, arachidonic acid) and ω-3 (for example, docosahexaenoic acid), which are named according to the position of the first double bond in the carbon chain, starting from the methyl end of the molecule. **b** | Glycerolipids are fatty acid esters of glycerol (green) and comprise mono-, di- and tri-acylglycerols. Carbons in the glycerol moiety are identified using the stereospecific numbering (*sn*) system[104,105]. **c** | Glycerophospholipids contain phosphoric acid in ester form with a glycerolipid (red). They are subdivided into distinct classes (for example, phosphatidylinositols) based on the nature of the head group linked to the phosphate at the *sn*-3 position of the glycerol backbone. The inset shows the specific cleavage sites of phospholipids for phospholipase A_1 (PLA_1), phospholipase A_2 (PLA_2), phospholipase C (PLC) and phospholipase D (PLD). **d** | Sphingolipids contain a common sphingoid base moiety (violet). They are acylated to form ceramides, which are modified to generate phosphosphingolipids (for example, sphingomyelin) and glycosphingolipids. **e** | Sterol lipids contain a fused four-ring core (magenta) and comprise cholesterol and its steroid derivatives.

Phosphoinositide
Phosphatidylinositol derivative in which the inositol head-group is in ester form with one or more phosphates; examples include signalling lipids, such as $PI(4,5)P_2$.

transient formation of a negatively curved monolayer, which can be promoted by the local accumulation of conical lipids (for example, fatty acids and 1,2-diacylglycerols (1,2-DAG)) — see FIG. 2a,b for chemical structures. In the second step, the stalk widens to generate a fusion pore that joins the aqueous volumes initially enclosed within the two membranes; this involves the generation of a positively curved monolayer, which can be favoured by the presence of inverted-conical lipids (for example, lysophospholipids and phosphoinositides).

These events occur within the lipid matrix but are driven by an evolutionarily conserved set of proteins

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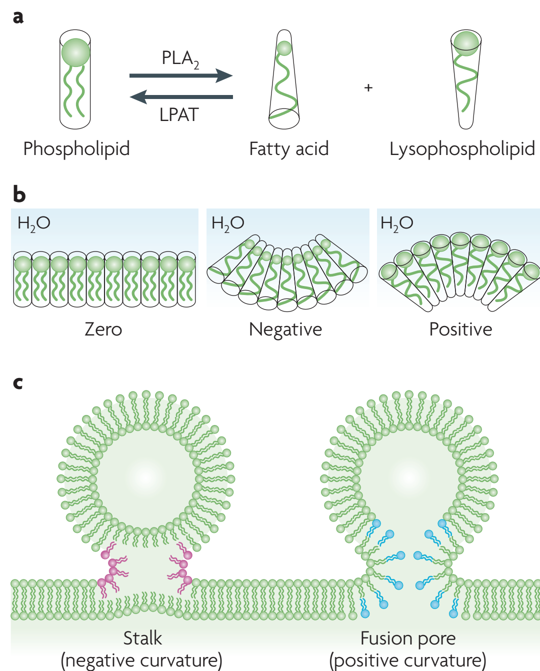

Figure 3 | **Lipid geometry. a** | Phospholipids in which the polar head group and the fatty acid chains have similar sizes are thought to adopt a cylindrical shape in membranes (filled circles symbolize the polar head groups, wavy lines represent the fatty-acid chains). Lipid hydrolases, such as phospholipase A_2 (PLA_2) convert phospholipids into conical (fatty acid) and inverted conical (lysophospholipid) products, whereas fatty acid transferases such as lysophospholipid acyl transferases (LPAT) catalyse the opposite reaction[106]. **b** | In an aqueous environment, cylindrical lipids produce stable planar monolayers, whereas conical and inverted-conical lipids produce monolayers with negative or positive curvature, respectively. **c** | The two steps of membrane fusion: left, during stalk formation, two adjoining membranes merge their outer leaflets producing a negatively curved monolayer region (red) that is facilitated by cone-shaped lipids such as fatty acids; right, widening of the stalk generates a fusion pore that is lined by a positively curved monolayer region (blue), which is favoured by inverted cone-shaped lipids, such as lysophospholipids[3].

clustered around the SNARE proteins, which fasten incoming vesicles to their target membrane and promote the Ca^{2+}-dependent merging of adjoining bilayers[4]. However, if these proteins have such a dominant role in membrane fusion, what place is left for lipids? To begin to address this question, researchers have produced high-resolution images of the microscopic changes in lipid composition that occur at fusion sites between two mating cells of the protozoon *Tetrahymena thermophila,* using a technique called time-of-flight secondary-ion mass spectrometry (FIG. 1b). The results of these experiments show that small membrane regions containing the largest number of fusion pores are highly enriched in the cone-shaped phospholipid 2-aminoethylphosphonolipid, implying that localized changes in lipid geometry are involved in the fusion process[5] .

Phospholipase A_2
An enzyme that hydrolyses the *sn*-2 position of phospholipids, producing fatty acids and lysophospholipids.

Although similar studies have not yet been attempted in neurons or glia, pharmacological evidence suggests that alterations in membrane-lipid composition may contribute to synaptic vesicle exocytosis. For example, muscle-paralyzing poisons from various snake species contain phospholipase A_2 neurotoxins, which bind to the external layer of axon-terminal membranes and catalyse the cleavage of phospholipids to produce conical fatty acids and inverted-conical lysophospholipids (FIG. 3a). Owing to their ability to bend monolayers, these non-cylindrical lipids promote vesicle fusion while inhibiting vesicle fission. The combination of these two effects causes an initial outburst of acetylcholine release at the neuromuscular junction followed by blockade of release and ensuing muscle paralysis[6,7].

It is still unknown whether any of the lipid-modifying enzymes expressed in the mammalian brain (Supplementary information S1 (table)) influence exocytosis by generating a local membrane microenvironment enriched in monolayer-bending lipids. An enticing hint comes from genetic experiments in the fruitfly *Drosophila melanogaster*. Conditional loss of the *rolling black out* gene in temperature-sensitive *Drosophila* mutants suggests that the presynaptic membrane protein encoded by this gene catalyses the hydrolysis of phosphatidylinositol(4,5)bisphosphate ($PI(4,5)P_2$) to produce 1,2-DAG[4,8] (FIG. 2b,c). This change in biochemical phenotype develops in parallel with a rapid and reversible blockade of synaptic transmission, which is rescued by transgenic expression of *rolling black out* in neurons[4,8]. One interpretation of these findings is that conversion of the inverted-conical $PI(4,5)P_2$ to the conical 1,2-DAG is necessary to create a membrane microenvironment conducive to exocytosis. An alternative (or perhaps complementary) possibility, discussed later in this article, is that $PI(4,5)P_2$ and 1,2-DAG bind to proteins required for exocytosis, recruiting them to the multimeric membrane-associated complex that drives the fusion process.

Ion channel regulation. Whereas the role of non-cylindrical lipids in exocytosis is still debated, the ability of these molecules to influence the activity of mechanosensitive ion channels is reasonably well established. The TRAAK (Twik 1-related arachidonic acid-stimulated K^+) family of K^+ channels is a case in point. TRAAK channels are reversibly opened by mechanical pressure as well as by administration of exogenous arachidonic acid or other unsaturated fatty acids[9]. TRAAK channel activation by fatty acids does not reach a plateau even when high concentrations of lipid are applied; this suggests that it may be caused by membrane deformation rather than by the interaction of fatty acids with saturable binding sites on the channel protein. A plausible explanation for these results is that the fatty acids may concentrate preferentially on the outer leaflet of the membrane, possibly owing to electrostatic interactions between their carboxylate groups and the positively charged lipids phosphatidylcholine and sphingomyelin (FIG. 2c,d). These two lipids are enriched in the outer leaflet and can induce a

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transient negative curvature in the monolayer that stretches the TRAAK channel and causes it to open[9].

A similar mechanism probably operates in other instances of lipid regulation of mechanosensitive channels — including transient receptor potential vanilloid-1 (TRPV1) channels[10], Twik-related K^+ 1 (TREK1) channels[11] and glutamate *N*-methyl-D-aspartate (NMDA) receptor channels[12,13]. A recent series of experiments with purified NMDA receptors reconstituted in liposomes has shown that membrane stretch and arachidonic acid application reduce Mg^{2+} blockade of NMDA channel activity and concomitantly enhance ion currents through the channels[14]. As these results were obtained in a minimal system that lacks cellular proteins, they unambiguously demonstrate that mechanical deformation of the lipid bilayer is sufficient to modulate the gating properties of NMDA channels. The data further suggest that administration of exogenous arachidonic acid closely mimics the effects of membrane stretch, probably by bending the liposomal membrane[14]. In fact, a general role for lipid-mediated membrane flexing in mechanosensation has been proposed[15]. However, the available information does not allow us to exclude the possibility that arachidonic acid also binds to the NMDA receptor itself, which contains a lipid-recognition domain that is homologous to those found in intracellular fatty acid-binding proteins[16]. In a later section, we shall discuss how arachidonic acid may use its propensity to bind proteins to regulate voltage-dependent K^+ channels in neurons.

Membrane domain formation. Cone-shaped lipids capture one's imagination because of their striking monolayer-flexing properties; however, cylindrical lipids, such as sphingomyelin, are far from being dull. Together with cholesterol, sphingomyelin promotes the assembly of lateral membrane domains that may have important roles in cells. The standard fluid-mosaic model of biological membranes does not predict this type of heterogeneity because it assigns complete freedom of lateral movement to lipids, and expects them to distribute randomly throughout the membrane[17]. Nevertheless, lateral partitioning has been demonstrated in both artificial and natural lipid bilayers and may constitute an important organizing principle for cell membranes.

Experimental support for this idea comes from studies with model membranes — artificial bilayers with a phospholipid composition similar to that of plasma membranes — in which the formation of lipid assemblies enriched in sphingomyelin and cholesterol can be observed using high-resolution techniques such as atomic-force microscopy (FIG. 1c). Needless to say, natural membranes are much more complex than these man-made systems. Nevertheless, evidence for lipid domain formation has also been obtained in cells by monitoring the lateral movement of transmembrane proteins or the partition of fluorescent membrane probes[18–20]. The entities visualized by these techniques, which are generally thought to correspond to the detergent-resistant 'lipid rafts' that are isolated from tissue extracts[21–23], may regulate cell function by facilitating selective protein–protein interactions within the plasma membrane[21]. In neurons and glia, it has been proposed that they may be implicated in stabilizing clusters of neurotransmitter receptors with proteins involved in intracellular signalling and the promotion of clathrin-independent endocytosis, and influence the activity and localization of neurotransmitter transporters[4,22].

Such proposals have not gone unchallenged, mainly because of the intrinsic difficulties involved in isolating and visualizing putative structures of nanometre-to-micrometre lengths and second-to-minute lifespans[20,23,24]. Despite these concerns, biophysical data support the idea that a cooperative molecular interaction between sphingomyelin and cholesterol drives the generation of lipid assemblies in natural membranes[23].

Sphingomyelin differs from glycerol-containing phospholipids in that it is composed of long, mostly saturated fatty acid chains (FIG. 2d). These straight, hydrophobic tails allow sphingomyelin molecules to adopt a tight cylindrical shape, assemble into a highly ordered solid-like gel phase and separate from surrounding glycerophospholipids, which are more loosely packed owing to their kinked unsaturated chains. In fact, the formation of two distinct phases, one rich in saturated sphingomyelin, called solid ordered (S_o) phase, and another rich in unsaturated glycerophospholipids, called liquid disordered (L_d) phase, is readily seen in artificial membranes that are prepared by mixing the two lipid species. The addition of cholesterol to these mixtures induces, through a yet uncertain mechanism[23], the creation of a third phase in which a high degree of acyl chain ordering (typical of the S_o phase) is associated with an increased lateral mobility (characteristic of the L_d phase). Patches of this hybrid liquid ordered (L_o) material have been found in membranes of non-neuronal cells[18,19,20] where they may be stabilized by cytoskeletal and adaptor proteins to generate domains of varying sizes and lifetimes[23]. Thus, despite current controversies, the idea that mutual interactions between lipids and proteins promote lateral heterogeneity in membranes remains a core hypothesis in cell biology. Its value for neuroscience becomes even more apparent as we turn to consider the central role of membrane partitioning in brain lipid-messenger function.

Lipids as direct effectors

Until now we have focused our discussion on biological events — exocytosis, ion channel regulation and membrane domain formation — in which lipids indirectly regulate protein activity by influencing the curvature or supramolecular organization of membranes. However, lipids can also affect ion channels, receptors and other signal-transduction proteins by directly binding to them. This is, of course, the mechanism of action used by most information-carrying molecules in the brain, but lipids are different in one important way: whereas water-soluble neurotransmitters and second messengers have limited access to the hydrophobic core of the membrane, lipids are amphipathic compounds that are produced within the bilayer and have the option to operate either inside or outside its boundaries — for this

Liposome
An artificial membrane-bound vesicle generally composed of phospholipids and cholesterol.

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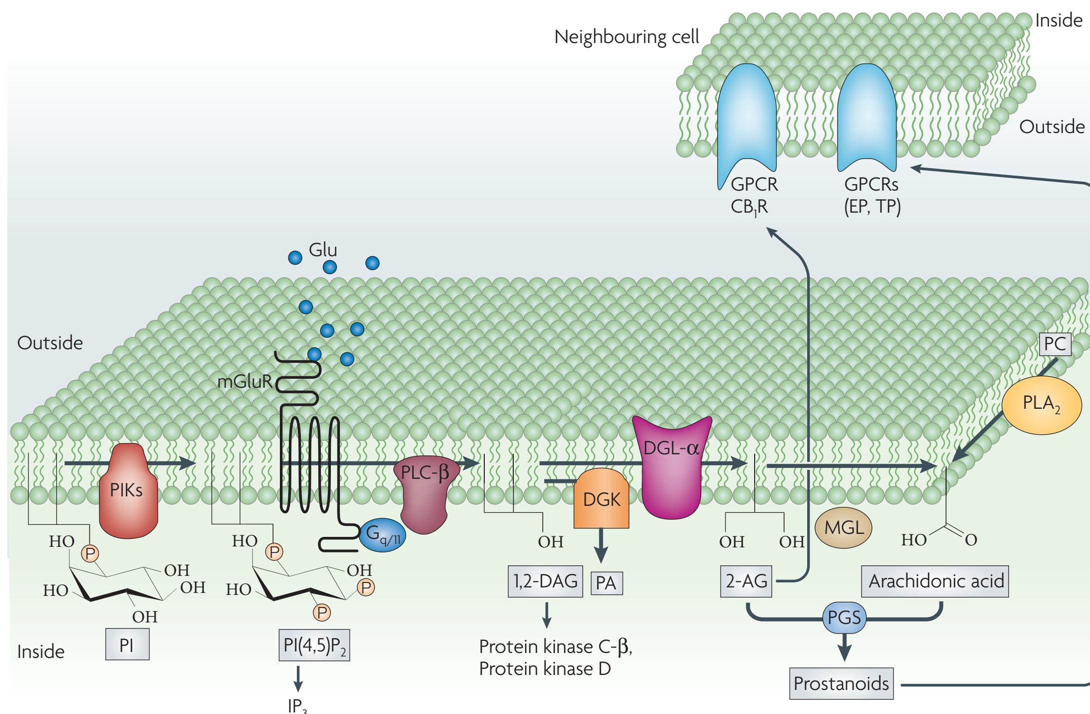

Figure 4 | **Key features of lipid signalling: the example of the phosphatidylinositol (4,5) bisphosphate cascade.** Phosphatidylinositol (PI) is localized in the inner leaflet of neuronal and glial membranes. It is converted to phosphatidylinositol (4,5) bisphosphate ($PI(4,5)P_2$) by phosphatidylinositol kinases (PIKs) associated with membrane raft-like clusters (not shown). Activation of phospholipases C (for example, PLC-β) by $G_{q/11}$-protein-coupled receptors (such as type I metabotropic glutamate receptors (mGluR) converts $PI(4,5)P_2$ to inositol polyphosphate 3 (IP_3) and 1,2-diacylglycerol (1,2-DAG). The actions of 1,2-DAG are terminated by the phosphorylation to phosphatidic acid (PA), which is catalysed by 1,2-DAG kinases (DGK). PA serves as a phospholipid precursor and intracellular signal. Alternatively, DAG lipases (DGL), such as DGL-α, hydrolyse 1,2-DAG to 2-arachidonoyl-*sn*-glycerol (2-AG), an endogenous cannabinoid messenger that activates the G-protein-coupled receptor (GPCR) CB_1R. 2-AG is hydrolysed by monoacylglycerol lipase (MGL) or other lipases to produce arachidonic acid, an intracellular messenger. 2-AG and arachidonic acid are substrates for prostaglandin endoperoxide synthases (PGS), which produce the prostanoids. These transcellular messengers activate GPCRs, such as prostaglandin and thromboxane receptors (EP and TP, respectively) (FIG. 5). Arachidonic acid can also be produced through phospholipase A_2 (PLA_2)-mediated cleavage of phosphatidylcholine (PC) and other phospholipids. Important aspects of membrane heterogeneity, including lateral heterogeneity and fatty acid diversity, are omitted for clarity. Glu, glutamate.

Endocannabinoid
An endogenous lipid that acts as a ligand for G-protein-coupled cannabinoid receptors; examples include 2-arachidonoyl-*sn*-glycerol and anandamide.

Eicosanoid
A signalling lipid that is involved in pain and neuroinflammation. It is derived from the enzymatic oxygenation of arachidonic acid and other polyunsaturated fatty acids. Examples include prostaglandins, prostacyclin, thromboxane, leukotrienes and lipoxins.

Phospholipase D
An enzyme that hydrolyses the distal phosphodiester bond of phospholipids, such as phosphatidylcholine, producing choline and phosphatidic acid.

class of biomolecules the membrane is a conduit rather than a barrier. To explain the functional implications of this property, we need to take a closer look at how neural lipid signalling works.

Lipid signalling in neurons. Let us consider, for example, the pathway that begins with the conversion of phosphatidylinositol to $PI(4,5)P_2$ (FIG. 4). Phosphatidylinositol is primarily localized on the inner leaflet of neuronal and glial plasma membranes[25], where its conversion to $PI(4,5)P_2$ is controlled by two activity-dependent phosphatidylinositol kinases — phosphatidylinositol 4 kinase and phosphatidylinositol(4)P 5 kinase — that are associated with membrane raft-like clusters[26] (Supplementary information S1 (table)). The subsequent cleavage of $PI(4,5)P_2$ by phospholipase C (FIG. 2c, inset), which is also regulated by physiological stimuli, generates the second messengers inositol polyphosphate 3 (which releases Ca^{2+} from the endoplasmic reticulum), and 1,2-DAG, (which activates protein kinase C, protein kinase D and other effector proteins)[27,28]. In addition to acting as a second messenger molecule, 1,2-DAG is also the starting point for two important biochemical transformations: DAG-kinases catalyse the phosphorylation of 1,2-DAG to phosphatidic acid (an intracellular messenger and phospholipid precursor)[29], whereas DAG-lipases cleave 1,2-DAG to yield monoacylglycerol[30,31], which is further hydrolysed by 2-acyl-*sn*-glycerol lipases to produce fatty acid and glycerol[32] (FIG. 4). As the *sn*-2 position of $PI(4,5)P_2$ is generally occupied by arachidonic acid (FIG. 2c), the main biologically active products of 1,2-DAG hydrolysis include 2-arachidonoyl-*sn*-glycerol (2-AG; a transcellular endocannabinoid messenger)[33] and arachidonic acid (an intracellular messenger)[34]. These lipids can be further modified by oxygen-adding enzymes (for example, prostaglandin-endoperoxide synthases) (FIG. 4), forming a family of paracrine signals called eicosanoids[35]. Notably, most of the lipids listed above can also be produced through alternative routes — for example, phosphatidic acid by phospholipase D acting on phosphatidylcholine (FIG. 2c, inset); arachidonic

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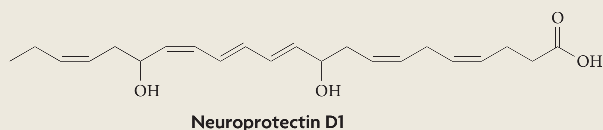
Box 1 | **The docosahexaenoic acid puzzle**

Docosahexaenoic acid (FIG. 2a) is the most abundant polyunsaturated fatty acid found in the mammalian brain. This ω-3 fatty acid is mainly obtained through the diet, although smaller amounts can be produced by chain elongation and desaturation of its precursor, linolenic acid. This process occurs in the liver as well as in brain astrocytes[84,85]. Irrespective of its source, docosahexaenoic acid is rapidly internalized by neurons and incorporated into phospholipids, mainly phosphatidylserine and phosphatidylethanolamine[40,86,87]. High levels of docosahexaenoic acid have been found in growth cones[88], synaptic plasma membranes and synaptic vesicles[89], but the functional significance of this localization is still unclear. Because of its high degree of unsaturation, phospholipids containing this fatty acid may increase membrane fluidity and regulate the functions of membrane-associated proteins[90–92]. Docosahexaenoic acid itself may act as a signalling molecule by binding to retinoid X receptor (RXR), a ligand-activated transcription factor[93] (FIG. 5), or it may be oxygenated to produce various bioactive lipids. Docosahexaenoic acid oxygenation is thought to proceed through two main pathways: a lipoxygenase-mediated pathway, which converts docosahexaenoic acid to resolvins and neuroprotectins (such as neuroprotectin D1 (REF. 94)), two families of lipid signals with marked anti-inflammatory and neuroprotective effects[95–97]; and a free-radical-mediated peroxidation pathway that leads to the production of neuroprostanes, which are involved in oxidative stress[98]. Both mechanisms may be relevant to the alterations in docosahexaenoic acid levels observed in aging and Alzheimer's disease[95–97,99–102].



Neuroprotectin D1

acid (and other fatty acids, such as docosahexaenoic acid; BOX 1) by phospholipase A_2 acting on glycerophospholipids.

The PI(4,5)P_2 cascade underscores three key features of lipid-mediated signalling in the brain. First, the shape-shifting skills used by lipids to control membrane flexibility are also used to generate a host of intracellular and transcellular messengers on demand. Second, such messengers are almost invariably produced through a 'serial' process in which a single biochemical route yields not one, but a sequence of functionally distinct signals. Lastly, adding redundancy to complexity, different biochemical mechanisms can be used to generate the same lipid species. So, how is this multiplicity of informational molecules and pathways coordinated? A plausible answer may be found in the temporal and spatial partitioning of lipid signalling within the heterogenous layout of the membrane bilayer.

Signalling on demand. A large number of physiological signals — electrical activity, neurotransmitters and growth factors — stimulate the formation of lipid messengers by activating enzymes that modify lipid precursors in membranes. There are many variations of this theme, but most of them entail the creation of lipid molecules that remain transiently associated with the membrane in which they originate: two familiar examples are the stimulus-dependent activations of phosphatidylinositol kinases to produce PI(4,5)P_2 and of phospholipases A_2 to mobilize arachidonic acid from glycerophospholipids[34,35].

Since the discovery of its second-messenger functions in neurons[36], the effects of arachidonic acid on ion channels, protein kinases and other signal-transducing proteins have been intensively investigated. Some of these actions depend on its membrane-flexing properties, but others are more likely to require arachidonic acid binding proteins. Pharmacological experiments suggest that arachidonic acid interacts with a family of voltage-sensitive K^+ channels, called Kv channels, and converts them from a slowly inactivated (delayed rectifier) into a rapidly inactivated (A-) type. This effect may have an impact on both action potential propagation and neurotransmitter release from presynaptic terminals[37].

Structural analyses of the bacterial K^+ channel KcsA support the possibility that arachidonic acid binds directly to Kv channels. These studies have revealed that a negatively charged lipid ligand, probably phosphatidylglycerol, interacts with a recognition pocket on the membrane-facing surface of the KcsA channel protein[38]. Notably, this lipid recognizes a site that is distinct from the non-selective lipid-binding grooves that make up the channel's annulus[39], indicating that phosphatidylglycerol may have a signalling rather than structural role. Confirming this idea, the ligand was found to be necessary for ion conduction rather than for normal channel assembly[38].

The results outlined above suggest a model in which newly released arachidonic acid, mobilized within the membrane bilayer by phospholipases A_2 or other lipases, gains access to contiguous Kv channels by lateral diffusion before being rapidly deactivated through phospholipid remodelling[40]. A similar scenario may be invoked to visualize the direct effects of other intracellular lipid messengers on ion channels (FIG. 5). A core feature of this model is that it identifies the membrane as a topological space in which complex sets of lipid signals are produced under precise temporal and spatial constraints. Within this space, three kinds of compartmentalization might guide each lipid messenger to its own signalling fate: lateral heterogeneity, bilayer asymmetry and anatomical specialization.

Lateral heterogeneity. As we noted before, the lipid kinases responsible for converting phosphatidylinositol to PI(4,5)P_2 may be confined to raft-like clusters found in the cytoplasmic leaflet of neuronal and glial membranes[25]. Compared with phosphatidylinositol, PI(4,5)P_2 has a remarkably low lateral mobility in the bilayer[41] and may remain temporarily concentrated at these sites. What is the functional significance of this transient PI(4,5)P_2 segregation? It has been proposed that membrane foci enriched in PI(4,5)P_2 may serve as anchoring points for intracellular proteins that bind to this phosphoinositide with high affinity[26,42] (Supplementary information S2 (box)). This recruitment to specific membrane sites is well suited to guide cellular events that require a high degree of membrane localization, including synaptic vesicle exocytosis[42]. Experiments with permeabilized neuroendocrine cells have shown that two key proteins for PI(4,5)P_2 metabolism — phosphatidylinositol-transfer protein, which transports phosphatidylinositol across the cytosol, and PI(4)P 5-kinase, which converts PI(4)P

Kv channel
Voltage-gated K^+ channel that controls action-potential repolarization, action-potential frequency and interspike interval in excitable cells.

Annulus
A thin ring-shaped sheet of lipids that separates transmembrane proteins from bulk membrane phospholipids.

Phospholipid remodelling
Hydrolytic removal of fatty acids from the *sn*-1 or *sn*-2 positions of phospholipids (catalysed by phospholipases A_1 and A_2, respectively) followed by their replacement with new fatty acids (catalysed by lysophospholipid acyltransferases).

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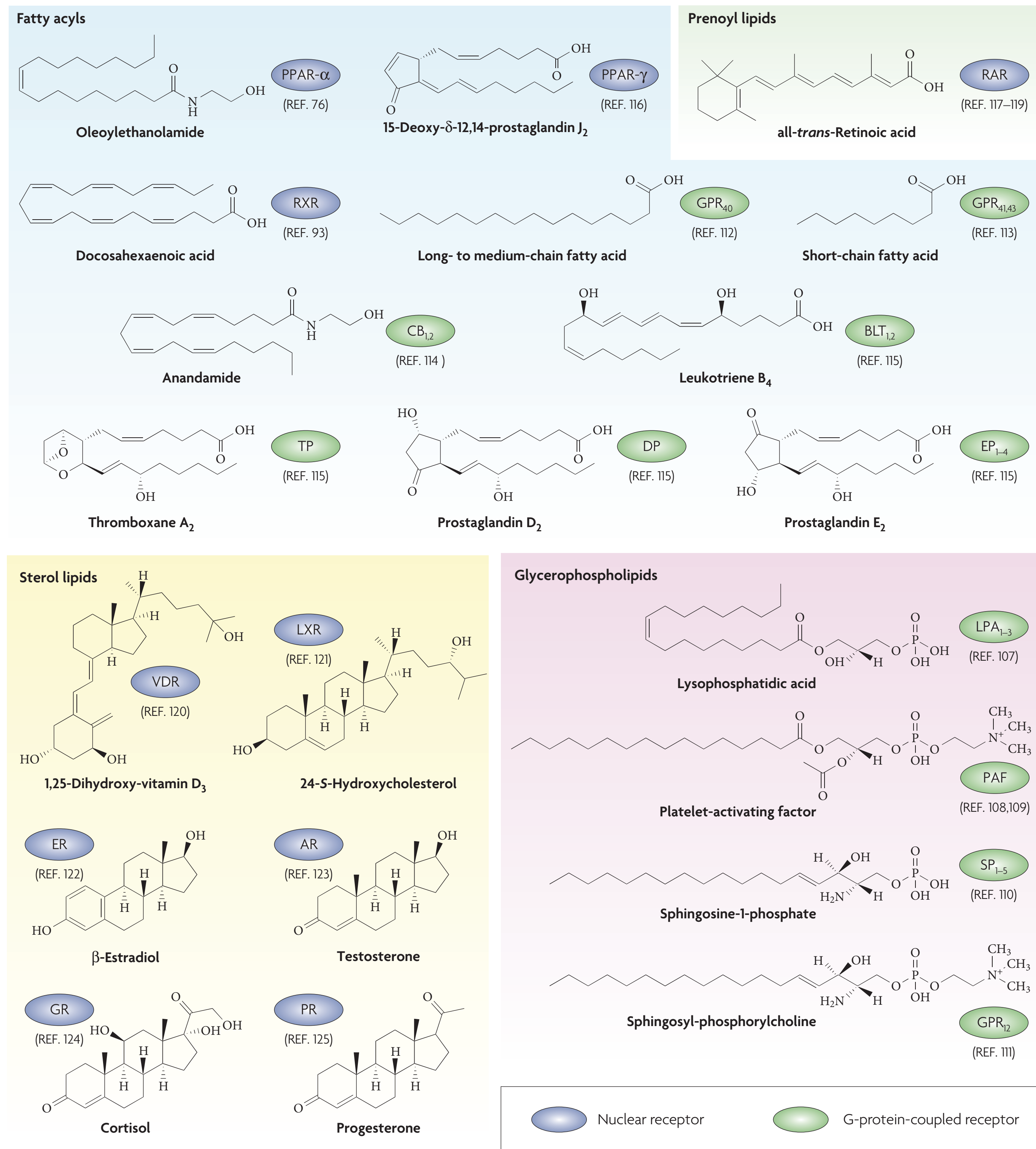

Figure 5 | **Representative bioactive neural lipids and their cellular receptors.** Lipids might act as direct effectors of signal transduction by directly binding to G-protein-coupled receptors (green oval shape) and nuclear receptors (blue oval shape). Lipids have been divided into four colour-coded categories symbolizing different chemically functional backbones: fatty acyls (blue), prenol lipids (green), sterol lipids (yellow) and glycerophospholipids (pink); next to each structure, the receptor for each lipid is indicated in the coloured oval shape. AR, androgen receptor; BLT, leukotriene B receptor; CB, cannabinoid receptor; DP, prostaglandin type D receptor; EP, prostaglandin type E receptor; ER, oestrogen receptor; GPR, G-protein-coupled receptor; GR, glucocorticoid receptor; LPA, lysophosphatidic acid receptor; LXR, liver X receptor; PAF, platelet-activating factor receptor; PPAR, peroxisome proliferator-activated receptor; PR, progesterone receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; SP, sphingosine-1-phosphate receptor; TP, thromboxane A2 receptor; VDR, vitamin D receptor.

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Flippase
The enzyme responsible for the energy-dependent transfer of phospholipids across the membrane bilayer ('flip-flop' process).

to $PI(4,5)P_2$ — are required for the reconstitution of Ca^{2+}-dependent dense-core vesicle secretion[43–45]. Two findings suggest that these results may be directly relevant to synaptic vesicle exocytosis. First, as previously mentioned, *Drosophila* mutants that lack the *rolling black out* lipase have both reduced $PI(4,5)P_2$ hydrolysis and impaired synaptic transmission[4,8]; second, the sequences of many essential proteins in the exocytosis machinery contain a phosphoinositide-binding C2-domain, which recognizes $PI(4,5)P_2$ in a Ca^{2+}-dependent manner[46]. Most lipids have greater lateral mobility in bilayers than $PI(4,5)P_2$ — it has been calculated, for example, that an unhindered lipid molecule could circumnavigate a cell of average size (about 10 micrometres in diameter) in less than 30 seconds[2]. But how many lipids are truly unhindered in neuronal or glial membranes? As evidence for lateral partitioning continues to accumulate, the number may turn out to be less than expected[47].

Bilayer asymmetry. Whereas the functional role of lateral heterogeneity still remains somewhat speculative, the importance of bilayer asymmetry in lipid signalling is clearly documented. In most cells, including neurons and glia, the two plasma membrane monolayers have strikingly different lipid compositions: the inner leaflet is enriched in phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol, whereas the outer leaflet is enriched in phosphatidylcholine and sphingomyelin[2].

The non-random distribution of phosphatidylserine across the membrane bilayer is due to the energy-dependent activity of aminophospholipid translocase, a member of the flippase family of enzymes[48]. During apoptosis or cell injury, a rise in intracellular Ca^{2+} levels triggers two concomitant events that disrupt this normal arrangement: the inhibition of aminophospholipid translocase and the stimulation of scramblases, enzymes that facilitate the transbilayer randomization of phosphatidylserine[49,50]. In addition to membrane reshuffling, Ca^{2+} also stimulates cytosolic enzymes that catalyse the synthesis of this phospholipid[51]. The net result of these convergent reactions is the appearance of phosphatidylserine on the outer membrane leaflet. The phospholipid is recognized by an as-yet-unidentified receptor on phagocytes, allowing the engulfment and elimination of apoptotic neural cells during brain development[52] and neural inflammation[53].

Anatomical specialization. Neurotransmitters and neuropeptides are released at chemical synapses — specialized junctions where these molecules are synthesized, secreted and eliminated. The fact that lipid messengers are produced on demand has led to the assumption that they can be released from virtually any site of a neuronal or glial cell membrane. However, this view appears to be simplistic as evidence for the existance of anatomically definable structures, which may be responsible for the production, release and deactivation of lipid messengers in the brain, accumulates. The endocannabinoid lipid 2-AG (FIG. 2b) offers a compelling illustration of this idea.

When principal neurons in the hippocampus are stimulated by glutamate released from adjacent excitatory terminals, the input received by these neurons is transiently depressed. This depression is initiated by the activation of postsynaptic type I metabotropic glutamate receptors (mGluRs), and results in the presynaptic inhibition of glutamate release. This suggests that a chemical messenger generated by mGluR activation travels backwards across the synapse to modulate excitatory inputs[54–56]. A related form of retrograde signalling is initiated by a voltage-dependent influx of Ca^{2+} into principal hippocampal neurons that leads to the inhibition of glutamate-mediated or GABA (γ-aminobutyric acid)-mediated inputs[54–56]. This unconventional retrograde signalling mechanism appears to be widespread in the CNS, and there is evidence that it is mediated by a diffusible endocannabinoid lipid[57–59].

In particular, the endocannabinoid 2-AG has been directly implicated in mGluR-induced retrograde signalling in the hippocampus, cerebellum and other regions of the brain[60–62]. It is envisaged that 2-AG may be produced in dendritic spines, through activation of type I mGluRs and the transducing G-protein $G_{q/11}$, which are coupled to the phospholipase C/DAG lipase (DGL) pathway (FIG. 4).

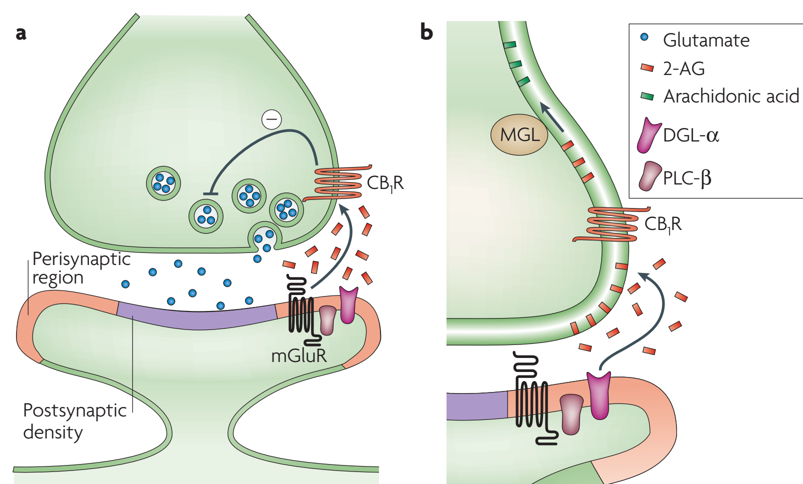

Figure 6 | **Specialized lipid signalling junctions in the brain. a** | The endocannabinoid lipid 2-arachidonoyl-*sn*-glycerol (2-AG) is thought to mediate retrograde signalling in the hippocampus, cerebellum and other brain regions. Glutamate (blue circles) released from excitatory axon terminals activates postsynaptic type I metabotropic glutamate receptors (mGluR), stimulating 2-AG production through the phospholipase C-β (PLC-β)/diacylglycerol lipase (DGL) pathway[60] (FIG. 4). Type I mGluR, PLC-β and DGL-α are localized at the perisynapse (light red), a region of the dendritic spine that borders the postsynaptic density (purple)[63–66]. 2-AG crosses the synaptic cleft and activates presynaptic CB_1 cannabinoid receptors (CB_1R), which suppress glutamate release. **b** | Hypothetical model of a specialized lipid-signalling junction at hippocampal glutamate-containing synapses. Endocannabinoid-synthesizing enzymes (PLC-β and DGL-α) and CB_1R are positioned to optimize the transynaptic actions of 2-AG. These may be further facilitated by the ability of this lipid messenger to reach CB_1R by lateral diffusion through the lipid bilayer. Cleavage of 2-AG by monoacylglycerol lipase (MGL), leading to the production of arachidonic acid, might terminate the effects of this messenger[32]. The flipping of 2-AG across the bilayer, which may be rather slow[126], might occur either before or after the interaction of the lipid with CB_1R.

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The lipid messenger might diffuse across the synaptic cleft to activate CB_1 cannabinoid receptors (CB_1R) on nearby axon terminals, reducing presynaptic Ca^{2+} channel activity and inhibiting glutamate release[55,56] (FIG. 6a).

The fidelity of this signalling sequence appears to depend on the precise anatomical localization of DGL-α, a major biosynthetic enzyme for 2-AG in neural cells[30,31]. Immunogold electron-microscopy studies of hippocampal and cerebellar neurons have shown that this lipid hydrolase is primarily localized in a subdivision of the dendritic spine, called the perisynapse, which forms a thin border (100–200 nm thick) around the postsynaptic density[63,64]. Along with DAG lipase α, this same area also contains type I mGluRs[65] and phospholipase C-β[66], and might, thus, be viewed as a stable perisynaptic site for the receptor-operated release of 2-AG.

Facing DGLα, on the opposite side of the synaptic cleft, are both CB_1Rs[63] and monoacylglycerol lipase[67], a presynaptic enzyme that cleaves 2-AG to terminate its actions[32,61,68]. We do not know precisely how 2-AG crosses the water-filled cleft to reach CB_1R-containing terminals, but its amphipathic nature and/or its association with extracellular lipid-binding proteins[69,70] are likely to be important. Another element that may facilitate the transynaptic movement of 2-AG relates to the molecular structure of the CB_1R. CB_1R belongs to a subgroup of about 60 G-protein-coupled receptors that contain a characteristic alkyl-binding domain that is implicated in the recognition of lipid ligands[71]. This motif is positioned in a region of the receptor protein that is embedded within the membrane, suggesting that an incoming ligand may first enter the bilayer and then reach the receptor-binding site by lateral diffusion[71]. Accessing CB_1R through the bilayer has three potential advantages: first, it would shorten the distance that 2-AG needs to cover in the aqueous medium; second, it would increase the density of 2-AG molecules surrounding the CB_1R binding site; and, third, it would facilitate the subsequent deactivation of 2-AG by monoacylglycerol lipase associated with the cytoplasmic aspect of the presynaptic membrane[32,67] (FIG. 6b).

Do other lipid messengers use stable signalling junctions similar to those described above? We do not know, but it is clear that most lipid signals in the brain operate, like 2-AG, by travelling short distances from their sites of production and engaging G-protein-coupled receptors on neighbouring neurons and glial cells. In addition to those already cited, other important examples include lysophosphatidic acid (a neurotrophic signal also involved in the initiation of neuropathic pain)[72,73], platelet-activating factor (a retrograde messenger implicated in hippocampal long-term potentiation)[74] and anandamide (an endocannabinoid ligand)[33] (FIG. 5). Interestingly, some lipid messengers do not require G-protein-coupled receptors to exert their function. For example, neurosteroids interact with membrane GABA-gated receptor channels to enhance neuronal inhibition[75], whereas oleoylethanolamide and its analogue palmitoylethanolamide engage peroxisome proliferator-activated receptors-α in the cell cytosol and nucleus to regulate feeding[76] and pain[77,78] (FIG. 5).

Anandamide
Endogenous amide of arachidonic acid and ethanolamine produced by the cleavage of *N*-arachidonoyl-phosphatidylethanolamine. It activates CB_1 cannabinoid receptors with nanomolar potency.

Neurosteroid
Steroid paracrine messenger synthesized *de novo* in the brain, which acts by binding neurotransmitter-gated ion channels; examples include the progesterone metabolite 5α-pregnan-3α-ol-20-one.

Oleoylethanolamide
Endogenous amide of oleic acid and ethanolamine produced by cleavage of *N*-oleoyl-phosphatidylethanolamine. It is a nanomolar agonist of peroxisome proliferator-activated receptor-α.

Resolvin
A signalling lipid implicated in the termination of acute inflammation. It is derived from the enzymatic oxygenation of eicosapentenoic and docosahexaenoic acid.

Neuroprotectin
A signalling lipid implicated in neuronal survival. It is derived from the enzymatic oxygenation of docosahexaenoic acid.

Future directions

Time and space acquire special significance in lipid signalling. The time and space scales in which lipid-mediated signalling operates are dictated by transient changes in their chemical structures and the subcellular localization of ensembles of interconnected lipid signals. This *modus operandi* is not unique to lipids — genes and proteins undergo all sorts of rapid modifications and cellular movements that are essential to their functions — but lipids appear to have adopted it as their primary information-carrying mechanism.

Thus, the first challenge that neural lipidomics needs to meet is the development of technologies that can uncover the information hidden beneath complex lipid signalling fluxes. Because the precise membrane localization of lipid-signals is crucial to their function, these molecules must be identified and quantified without altering their spatial organization in membranes. This is not a trivial task, because it requires technological advances in at least four areas: first, the development of tissue-fixation techniques that do not cause artefactual alterations in lipid profile and localization; second, the adaptation of cell-dissection tools, such as laser-capture microdissection[79], and high-sensitivity analytical methods, such as nanoflow liquid chromatography or mass spectrometry[80], for the analysis of neural lipids (FIG. 1a); third, tissue-imaging technologies, such as secondary-ion mass spectrometry or matrix-assisted laser desorption ionization mass spectrometry[81,82], which allow the localization of individual lipids in their native membrane environment (FIG. 1b) to be applied to lipid analysis; and finally, the creation of software that is capable of handling and interpreting large volumes of complex data[83].

Much progress has been recently made in those areas, and more is underway. By contrast, another challenge faced by neural lipidomics has attracted less attention. In the beginning of this Review, we noted that lipids affect neuronal and glial function in two distinct ways: by altering the geometric properties and the supramolecular organization of neural cell membranes, or by acting as ligands for effector proteins. However, the examples of $PI(4,5)P_2$ and arachidonic acid, among others, show that these two modes of action are not mutually exclusive, as many lipid species can both bend membranes and modify protein activities. Are these two properties functionally related? Do lipid-driven changes in membrane shape and organization cooperate with protein-mediated lipid signalling events to affect the functions of neuronal and glial cells? And, if so, how? Answering these questions will require a research programme that is capable of merging the theoretical and experimental approaches of neuroscience with those of membrane biophysics and lipid biochemistry in an interdisciplinary framework.

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Competing interests statement
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