Lipid raft microdomains and neurotransmitter signalling

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Abstract | Lipid rafts are specialized structures on the plasma membrane that have an altered lipid composition as well as links to the cytoskeleton. It has been proposed that these structures are membrane domains in which neurotransmitter signalling might occur through a clustering of receptors and components of receptor-activated signalling cascades. The localization of these proteins in lipid rafts, which is affected by the cytoskeleton, also influences the potency and efficacy of neurotransmitter receptors and transporters. The effect of lipid rafts on neurotransmitter signalling has also been implicated in neurological and psychiatric diseases.

The classic Singer–Nicolson fluid mosaic model of the cell membrane describes the lipid bilayer as a neutral two-dimensional solvent in which proteins diffuse freely[1]. This membrane concept has been modified substantially since it was first proposed, as membrane compartmentalization has been shown to occur through lipid–lipid, lipid–protein and membrane–cytoskeletal interactions[2]. Similarly, the notion that a neurotransmitter receptor, once activated, initiates a signalling cascade by randomly colliding with other membrane proteins has given way to the view that signalling molecules are arranged in stable, possibly preformed, complexes at the membrane[3]. These emerging concepts of membrane organization and mechanisms of signalling indicate that membrane heterogeneity might be important; however, the role of such heterogeneity in regulating neurotransmitter response and responsiveness is just beginning to be explored.

Lipid rafts are a well-studied type of membrane microdomain. Numerous roles have been ascribed to lipid rafts, but this review focuses on the hypothesis that they organize and compartmentalize neurotransmitter signalling components to either increase or dampen signalling (FIG. 1). The involvement of lipid rafts as vehicles for endocytosis and trafficking during signalling will also be discussed.

Two common types of lipid raft have been proposed and studied with respect to neurotransmitter signal transduction: planar lipid rafts (which are also referred to as non-caveolar, or glycolipid, rafts) and caveolae (little caves), both of which are estimated to be 25–100 nm in diameter[2,4]. These raft microdomains are often defined by their cholesterol- and sphingomyelin-rich nature, enrichment in glycosylphosphatidylinositol (GPI)-anchored proteins, cytoskeletal association and resistance to detergent extraction. Caveolae are small, flask-shaped invaginations of the membrane that contain caveolin proteins. Caveolins are a major component and marker of caveolae, and are widely expressed in the nervous system in brain microvessels, endothelial cells, astrocytes, oligodendrocytes, Schwann cells, dorsal root ganglia and hippocampal neurons[5]. Caveolins and caveolae are absent from most neurons and neuroblastoma cells; however, neurons possess planar lipid rafts and flotillin, a protein that is analogous, but not homologous, to caveolin[6]. Planar lipid rafts possess many of the features of caveolae (cholesterol- and sphingomyelin-rich cytoskeletal association) but are not invaginated. While caveolae are the most readily-observed structures associated with lipid rafts, the presence of caveolae is not required to assign 'raftness', and we will not attempt to subdivide these structures in this review.

Although lipid rafts have earned wide acceptance among cell biologists as signalling platforms, the field remains controversial. This controversy has stemmed mainly from the technical challenges of studying these submicroscopic, dynamic structures in the membranes of living cells (BOX 1). The field is both developing and employing new techniques to investigate these dynamic membrane domains (as discussed below). Regardless of this debate, data accumulated over the past 10–15 years have suggested that lipid rafts provide both a spatial and a temporal meeting point for signalling molecules, and this review attempts to compile some of these data with a focus on the nervous system.

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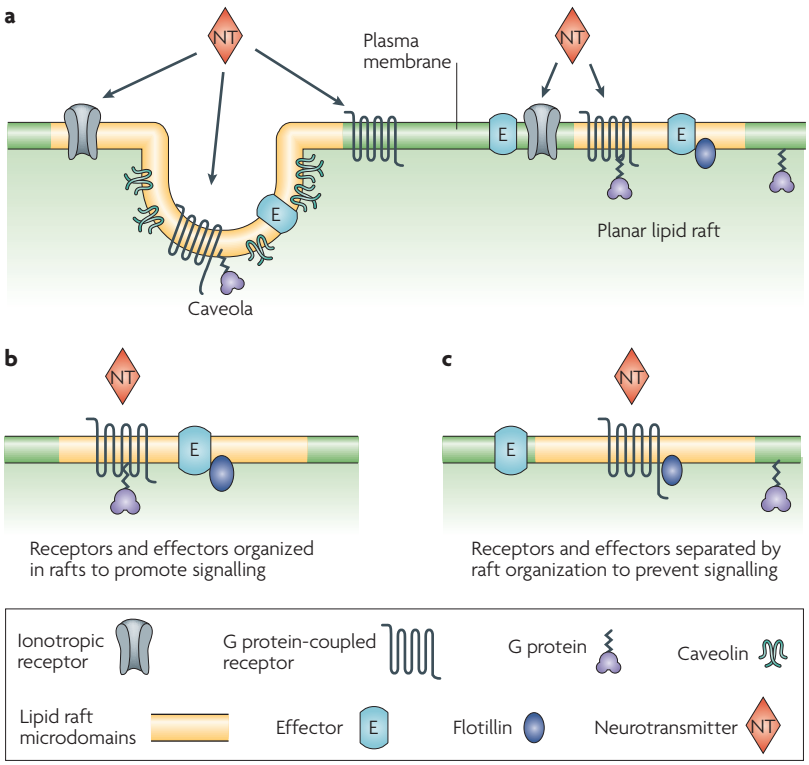

Figure 1 | **Lipid raft microdomains and membrane organization of neurotransmitter signalling molecules.** Lipid rafts are cholesterol- and sphingolipid-enriched, highly dynamic, submicroscopic (25–100 nm diameter) assemblies, which float in the liquid-disordered lipid bilayer in cell membranes[2,4,40]. **a** | There are two common raft domains in mammalian cells: planar lipid rafts and caveolae. Both possess a similar lipid composition. Planar rafts are essentially continuous with the plane of the plasma membrane and lack distinguishing morphological features. By contrast, caveolae are small, flask-shaped membrane invaginations of the plasma membrane that contain caveolins. Caveolin molecules can oligomerize and are thought to be essential in forming these invaginated membrane structures[108]. Caveolins and flotillin can recruit signalling molecules into lipid rafts. Many neurotransmitter receptors (both ionotropic and G-protein-coupled), G proteins, and signalling effectors such as second-messenger-generating enzymes are found in lipid rafts. Neurotransmitters might activate receptors that are located both within and outside lipid rafts. **b** | The lipid raft signalling hypothesis proposes that these microdomains spatially organize signalling molecules at the membrane, perhaps in complexes, to promote kinetically favourable interactions that are necessary for signal transduction. **c** | Alternatively, lipid raft microdomains might inhibit interactions by separating signalling molecules, thereby dampening signalling responses.

Methods for studying lipid rafts

There are several complementary approaches for studying lipid rafts and their putative involvement in signal transduction. The most common definition of a raft-associated protein is one that cannot be extracted by non-ionic detergents (BOX 2). Preparation of membranes that are resistant to Triton X-100 by sucrose density gradient centrifugation is a simple and easy method to assess the potential raft association of a protein. However, this technique is not without pitfalls, as some proteins show a facultative and variable raft association during detergent extraction[4]. In addition, the localization of signalling proteins in fractionated raft membranes does not provide sufficient evidence of raft involvement in a given signalling process, and agreement among biophysical, biochemical and imaging studies has been elusive.

Disruption of lipid rafts can be used to determine the physiological relevance of the localization of proteins to these domains. Agents that sequester, chelate or prevent the synthesis of cholesterol are commonly used to disrupt lipid rafts (BOX 3), and the subsequent effects on neurotransmitter signalling can be observed. In addition, the manipulation of other lipid components — for example, adding exogenous fatty acids or gangliosides — has been effective for disrupting lipid rafts[4]. As with any pharmacological treatment, cholesterol manipulation can result in pleiotropic effects. This can be controlled for by adding cholesterol back to the cells and determining whether this results in a reversal of the observed effects. Recently, genetic approaches to disrupt caveolae have been developed. For example, knockdown of caveolins by small interfering RNA (siRNA) in glial cells disrupts neurotransmitter signalling[7], and caveolin 1-knockout mice possess abnormal behavioural and neurological phenotypes[5].

Although these commonly utilized techniques are informative, they have a number of limitations and the field is developing new and more sophisticated methods to study these domains in living cells. These new techniques rely mainly on biophysical and imaging methodologies including fluorescence correlation spectroscopy[8], single molecule tracking microscopy[9], Laurdan partitioning[10] and fluorescence resonance energy transfer[4,11]. These new techniques are powerful approaches for studying lipid rafts and their influence on signalling in living cells, and they are advantageous as they do not rely on cell disruption. However, they also have limitations, such as often requiring protein labelling, which has the potential to disrupt signalling cascades. Regardless of the individual techniques used, the strongest approach for studying lipid rafts and signal transduction is one that employs several complementary methods.

These methods have been employed, in various combinations, in the studies reviewed here. The results of these studies indicate that lipid rafts influence several components of different neurotransmitter signalling cascades including neurotransmitter receptors and their effectors, as well as transporters. We begin by considering lipid rafts and ion channel receptors.

Ionotropic neurotransmitter receptors

Ionotropic receptors (ligand-gated ion channels) control neurotransmission by facilitating ion conductance and the subsequent generation or inhibition of membrane electrical potentials. Lipid raft microdomains might modulate neuronal excitability by contributing to ionotropic receptor sensitivity and function.

Reported localization in lipid rafts. The lipid raft localization status for nearly all ionotropic receptors that are expressed in the nervous system has been investigated; however, there is some confusion about the raft localization of some of their subunits and subtypes (TABLE 1). Given the lack of consistency in the methods and in the cell and tissue types used for studying rafts, the discrepancies probably result from procedural

Triton X-100
A commonly used non-ionic surfactant detergent that solubilizes proteins and cell membranes.

Sucrose density gradient centrifugation
Separation of cell organelles and subcellular components from crude cellular extracts based on their buoyant density.

Box 1 | The debate regarding lipid rafts

The concept of lipid rafts has received significant attention in recent years, but attendant with that attention has been controversy. Although the lateral heterogeneity of proteins in the plasma membrane is undisputed, the very existence of planar lipid rafts and their biological importance has been questioned[103]. These concerns are rooted in the current technology used to study lipid rafts and their biochemical and biophysical limitations (BOXES 2,3). It is noteworthy that whereas the unequivocal existence of caveolae can be defined by the presence of caveolins and confirmed by electron microscopy, planar lipid rafts have been more difficult to both define and study. The nanometre diameter of these microdomains is beyond the resolution capability of light microscopy, making the study of these structures in intact, living cells complex. In addition, the classic approaches for studying and isolating lipid rafts often rely on detergent extraction, a method that is thought by some to introduce undue artefacts[12,103,104]. Some studies have also questioned the biological importance of raft domains. One recent study removed lipid binding capabilities from signalling proteins and concluded that protein clusters are dependent on protein–protein interactions and that lipid rafts and actin are irrelevant to these clusters[105]. However, despite the observed clustering of signalling molecules in this study, there was no indication that the signalling capabilities of these molecules were maintained in the absence of lipid raft association.

Recent investigations have introduced new, more sophisticated techniques for studying lipid rafts and have addressed some of these controversial issues. Biophysical studies of model membranes have confirmed the existence of nanoscale liquid-ordered and liquid-disordered microdomains predicted by the raft hypothesis[11]. Moreover, a recent hypothesis synthesizing current results and differing views about membrane organization suggests a revised model in which rafts are dynamic associating and dissociating nanodomains influenced by protein–protein interactions[11]. Ultimately, better methodology is needed to further elucidate the nature of these nanoscale, dynamic membrane structures.

variations. This is confounded by the fact that relatively few reports describing biochemical raft isolations give sufficient information to precisely replicate the results. For example, reports of detergent to protein ratios are largely absent from methods sections, and this key ratio can greatly influence the outcome of results[12]. In most instances the biochemical isolation of lipid rafts also reveals a population of ionotropic receptors/subunits that is not associated with lipid rafts. This duality might function to compartmentalize different signal transduction pathways that are associated with the same receptor (see below).

Neurotransmitter binding and receptor localization in rafts. Little is known about how agonist binding affects the localization of ionotropic receptors to membrane microdomains and how this localization, in turn, might affect ligand binding. The $P2X_3$ purinergic receptor retains its residence in the lipid rafts of neuronal cells subsequent to agonist stimulation[13], but the consequences for raft localization following agonist stimulation have not been reported for other ionotropic receptor types. Raft localization does seem to affect the binding of neurotransmitter to ionotropic receptors. Caveolin 1 has been shown to indirectly reduce the binding of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) to its receptor in hippocampal synaptosomes[14]. Both depletion and enrichment of cholesterol, which is a defining component of lipid rafts, decrease the potency of GABA (γ-aminobutyric acid) at the $GABA_A$ receptor, which suggests an optimal level of cholesterol for optimal agonist potency, whereas depletion of membrane cholesterol had no effect on the potency of $GABA_A$ receptor antagonists[15]. Similarly, cholesterol is required for the agonist-induced ion-gating function of the nicotinic acetylcholine (nACh) receptor[16]. Exactly how lipid rafts alter neurotransmitter affinity for ionotropic receptors is unclear. Lipid rafts contain a number of proteins that associate with receptors, modifying their affinity for and capacity to bind ligands, as well as the properties of the channels themselves. Coincident localization of these proteins with receptors in lipid rafts is transitory. In addition, the specific lipid–protein interactions that occur in lipid rafts could influence ionotropic receptor conformation and therefore agonist potency or efficacy. Finally, oligomerization of NMDA

Box 2 | Common methods to isolate lipid rafts

Biochemical isolation of lipid raft membranes and their subsequent analysis is a useful and simple method to determine if signalling components are located (or change localization) in raft microdomains. Two common techniques described here demonstrate protein localization to rafts prior to, during and after neurotransmitter signalling.

Preparation of detergent-resistant membranes (DRMs)

Classically, lipid raft membranes are defined as being insoluble in cold non-ionic detergents such as Triton X-100. Detergent-resistant membrane fractions float into the buoyant fractions of sucrose gradients during ultracentrifugation and are distinguished as two or three opaque bands in the lighter density sucrose[106]. The DRM method of isolation has received criticism by some investigators because it is prone to variable, and sometimes inconsistent, results[12,104]. One problem is that Triton X-100 can solubilize proteins that are only weakly associated with lipid raft membranes. Furthermore, although lipid rafts are often cytoskeletal-associated, the methods used to prepare DRMs disrupt cytoskeletal–membrane interactions and compromise this association.

Non-detergent-based isolation of raft membranes

There is also a non-detergent-based procedure for the isolation of lipid rafts based on pH and carbonate resistance[107]. Sodium carbonate, at high pH, separates proteins that are firmly attached to membranes from those that are more peripherally associated. Rafts are isolated by centrifugation and fractions are harvested as for the detergent preparation. As is the case with detergent-resistant preparations, the high pH method requires cell disruption — in this case by sonication. Such treatment of membranes alters the often delicate relationship between membrane, protein and cytoskeletal components that normally exists in cells.

Monitoring of the level of cholesterol present in sucrose fractions obtained from either method confirms that cholesterol-enriched membrane domains are present or absent within given fractions.

P2X purinoreceptor
A plasma membrane channel that is activated by the binding of ATP and is permeant to mono- and divalent cations.

Synaptosome
A preparation of elements of the presynaptic and postsynaptic terminal, isolated after subcellular fractionation. Synaptosomes retain some anatomical integrity and can take up, store and release neurotransmitters.

Box 3 | **Common approaches for disrupting lipid rafts**

Signalling processes that are promoted or inhibited by lipid rafts are often studied by disruption of the microdomains through chemical or genetic manipulation. Loss of regulation subsequent to raft disruption is evidence for a requirement of raft compartmentalization of signalling components. Owing to their enrichment in cholesterol, lipid rafts are sensitive to the removal of or reduction in membrane cholesterol, and cholesterol manipulations can redistribute proteins away from lipid raft membrane domains. Commonly used techniques to achieve these effects include:

- Sequestration of cholesterol using filipin, nystatin, amphotericin
- Depletion and removal of cholesterol using methyl-β-cyclodextrin
- Inhibition of cholesterol synthesis using HMG-CoA reductase inhibitors (statins)
- Genetic approaches including caveolin 1 (*Cav1*)-knockout mice[5] or caveolin RNA interference[7]

These methods of manipulating cholesterol are particularly informative when combined and confirmed with fractionation studies (BOX 2). With respect to the use of cholesterol-disrupting drugs, which can result in pleiotropic effects, the restoration of cholesterol provides an important experimental control. Also of note is that when genetic ablation of specific caveolin gene products is used, compensatory effects can be observed. Studies designed to reveal the roles of lipid rafts in neurotransmitter signalling should ideally combine complementary approaches, including the evaluation of raft localization of components through fractionation, disruption of lipid rafts by biochemical and/or genetic means, assessment of signalling properties and the examination of signalling protein localization by live-cell imaging.

(*N*-methyl-D-aspartate) and nACh receptor subunits modulates agonist affinity[17,18], and raft localization might have a role in the orientation and availability of ionotropic receptors for multimer formation.

Lipid rafts and trafficking. Lipid rafts might also contribute to the trafficking of ionotropic receptors to and from the cell membrane. Raft-depleted cells have fewer nACh receptors on their cell surface, probably as a result of impaired transport from the Golgi complex to the membrane and/or a reduced membrane stability[19]. Similarly, disrupting lipid rafts reduces the stability of surface AMPA receptors, but also increases internalization of unstimulated receptors[20]. Internalized nACh receptors have been shown to colocalize with caveolin[21], suggesting a caveolae-mediated route of internalization. However, so far, a lipid raft/caveolae trafficking mechanism common to all ionotropic receptors has yet to be identified.

Lipid rafts and clustering. Several ionotropic receptors show a clustered distribution at the membrane, and such a distribution is considered integral to the formation and maintenance of synapses. Likewise, intact lipid rafts seem to be necessary for the maintenance of normal synaptic morphology and dendritic spine density[20]. Lipid rafts have also been shown to contribute to both the size and the number of postsynaptic NMDA and $GABA_A$ receptor clusters[20], which further indicates an important role for lipid rafts in synaptic neurotransmission. The integrity of lipid rafts is also necessary for maintaining the stability of nACh receptor (nAChR) clusters[10,22,23], and is required for the clustering of nAChRs that is induced by the heparan sulphate proteoglycan, agrin[24]. Neural agrin also translocates nAChRs into lipid rafts where they are subsequently anchored by rapsyn[23]. The cytoskeleton is intimately linked to lipid raft stability and is also thought to mediate the clustering of nAChRs (through rapsyn)[25]. However, despite the association between actin, nAChRs and lipid rafts, disruption of actin filaments did not prevent clustering of the receptors in rafts[22]. This suggests that either actin monomers are active in this process or that other factors also contribute to receptor clustering; this will be discussed below.

Raft-regulated signalling. Studies exploring the role of lipid rafts in ionotropic receptor signalling events have begun to emerge in recent years. The calcium-mediated inhibition of type VI adenylyl cyclase by nAChRα7 is dependent on raft integrity[26], which supports the idea that lipid rafts can orchestrate specific signalling events downstream of ionotropic receptors by influencing the inclusion or exclusion of receptors and their associated effector molecules. Furthermore, the segregation of an ionotropic receptor into raft and non-raft fractions can be associated with specific signal transduction cascades and cellular responses subsequent to receptor activation. For example, a population of $P2X_7$ receptors in lipid rafts is responsible for activating phospholipase A_2 and generating ceramide, but $P2X_7$ receptors residing outside rafts form a non-selective cation channel that elevates levels of intracellular calcium[27]. Similarly, NMDA receptors (NMDARs) located in lipid rafts are reported to mediate neurotoxicity[28,29], whereas NMDARs outside of lipid rafts are responsible for glutamate-mediated growth cone guidance[30]. The former observation is somewhat confounded by two studies that report conflicting results on the requirement of lipid rafts for calcium conductance by NMDARs[28,29]. These studies used different neuronal cultures (hippocampal versus cortical), raising the possibility that there might be regional variation in raft–receptor interactions.

G-protein-coupled neurotransmitter receptors

G-protein-coupled neurotransmitter receptors (GPCRs) activate heterotrimeric guanine nucleotide binding proteins (G proteins), which act as signalling intermediates between activated receptors and their intracellular effectors. Signalling through GPCRs is complex, requiring a minimum of three protein components: a receptor, a G protein and a signalling effector; these components must physically interact to transmit information. Lipid rafts can influence any one of these components and both localization and trafficking/internalization of receptor components by rafts can modify GPCR signalling.

Stable signalling complexes in microdomains. In the classic model for GPCR signalling, receptors diffuse randomly in the lipid bilayer of cells and activate their cognate G proteins and effectors through 'collision coupling' that is driven by high-affinity protein–protein interactions. However, studies of these molecules in living cells indicate that stable complexes are formed between many GPCRs and G proteins, and between G proteins and effectors[3]. These results are most easily explained by the existence of GPCR signalling

Heparan sulphate proteoglycans
A group of proteoglycans in which heparan sulphate chains are attached near the cell surface or on extracellular matrix proteins. Agrin is one such proteoglycan, named for its involvement in aggregation of acetylcholine receptors during synaptogenesis.

Table 1 | **Localization of ionotropic receptors and neurotransmitter transporters**

Receptor or transporter	Raft	Non-raft	References
nACh receptor	α, α_7	α, α_2, β, β_5	22–24,26,109
GABA receptor	$GABA_A$		110
NMDA receptor	NR1, NR2A, NR2B	NR1, NR2B	20,29,30,111
AMPA receptor	GluR1, GluR2/3, GluR4		20,111
P2X receptor	$P2X_1$, $P2X_3$, $P2X_7$	$P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_7$	13,27,112
5-HT receptor	$5\text{-}HT_{3A}$		94
Serotonin transporter	Yes	Yes	71,78
Norepinephrine transporter	Yes	Yes	76
Glutamate transporter	EAAT1–4	EAAT1–4	73,113

Lipid raft localization was determined by various biochemical methods or by colocalization with lipid raft markers. 5-HT, 5-hydroxytryptamine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; EAAT, excitatory amino acid transporter; GABA, γ-aminobutyric acid; GluR, glutamate receptor; nACh, acetylcholine receptor (nicotinic subtype); NMDA, *N*-methyl-D-aspartate; NR, NMDA receptor subunit; P2X, purinergic receptor 2 class X.

complexes that are constrained in their lateral mobility in the plasma membrane, and confined within membrane microdomains[31].

Ligand binding and localization in rafts. The involvement of lipid rafts in regulating and organizing neurotransmitter signalling through GPCRs has been reported for many of the main receptor classes and subtypes (TABLE 2); however, these raft-regulated processes are just beginning to be elucidated in the nervous system. As is the case with certain ionotropic receptors, lipid rafts influence the binding of neurotransmitters to several GPCRs. Disruption of rafts by cholesterol depletion significantly reduces agonist binding and G-protein coupling to 5-hydroxytryptamine 1A ($5\text{-}HT_{1A}$) serotonin receptors in bovine hippocampal membranes[32]. Similarly, NK1 neurokinin receptors expressed in human embryonic kidney cells (HEK293 cells) bind significantly less agonist after raft disruption[33]. Physical association (coupling) between receptor and G protein represents the high-affinity state of agonist binding. These studies suggest that for some receptor–G protein pairs, the localization of receptor and G protein in lipid rafts might facilitate their coupling, which subsequently influences agonist binding to receptors. By contrast, human κ-opioid receptors stably expressed in Chinese hamster ovary cells (CHO cells) are concentrated in lipid rafts, and cholesterol depletion of these cells significantly increases maximal agonist binding (B_{max}) and G-protein coupling[34]. This indicates that, in this case, receptor and G protein occupy different membrane compartments. Therefore, raft domains differentially influence neurotransmitter binding and the coupling of G proteins to GPCRs. At present, it is not clear whether these differences are due to receptor type or to the cell/tissue type in which the receptors are expressed.

Caveolin–GPCR interactions. Caveolins have been shown to interact with and/or regulate signalling from several GPCRs including adenosine A_1 receptors[35], muscarinic acetylcholine receptors[36], D1 dopamine receptors[37], $5\text{-}HT_{2A}$ receptors[7] and metabotropic glutamate 1a receptors (mGluR1a)[38]. These caveolin–receptor interactions could function to localize receptors in lipid rafts, and caveolins are known to profoundly influence signalling from certain neurotransmitter receptors. For example, stable knockdown of caveolin 1 by RNA interference (RNAi) in C6 glioma cells alters the signalling of only select $G\alpha_q$-coupled GPCRs, as caveolin knockdown abolishes signalling through $5\text{-}HT_{2A}$ and P2Y receptors; however, thrombin signalling through protease-activated receptor 1 (PAR1) is unaffected[7]. By contrast, the constitutive signalling of mGluR1a is inhibited when receptors and caveolin 2 (but not caveolin 1) are heterologously expressed in HEK293 cells[39]. So, although signalling from certain GPCRs ($5\text{-}HT_{2A}$, P2Y) seems to be dependent on caveolin 1, some (for example, PAR1) are unaffected, whereas others (such as mGluR1a) are inhibited by caveolin 2. One explanation for these varied effects is that caveolins can interact with several G proteins and effectors (as discussed below).

Rafts and GPCR trafficking. In addition to acting as organizing centres for signalling molecules, both planar lipid rafts and caveolae can facilitate clathrin-independent endocytosis[40–42]. During neurotransmitter signalling, many GPCRs undergo agonist-induced endocytosis, which can lead to receptor recycling, receptor sequestration and/or receptor downregulation. The agonist-induced trafficking of GPCRs through arrestin-dependent and dynamin-dependent, clathrin-mediated endocytosis is well established[43]. However, clathrin-independent lipid raft mechanisms might also contribute to receptor trafficking. Trafficking by rafts during signalling could involve three distinct mechanisms: endocytosis mediated by caveolae; endocytosis mediated by planar lipid rafts; and/or lateral trafficking into or out of these microdomains (FIG. 2). Internalization of GPCRs through caveolae or rafts has been suggested for several GPCRs (TABLE 2). For example, activated β_1-adrenergic receptors (β_1-ARs), which commonly undergo clathrin-mediated endocytosis, switch their route of endocytosis to caveolae/rafts when phosphorylated

HEK293 cells
A hypotriploid human cell line derived from embryonic kidney epithelial cells; commonly used as an expression system to study signalling and recombinant proteins.

CHO cells
A chinese hamster ovary cell line often used as an expression system for studying cell signalling and recombinant proteins.

B_{max}
A measure of the total number of receptors, determined by the binding of agonist or antagonist ligands. This number will reflect either surface receptors or surface plus internalized receptors depending on the chemical characteristics of the ligand.

RNA interference
(RNAi). A molecular method in which small interfering RNA sequences are introduced into cells or tissues, and subsequently silence the expression of target genes.

C6 glioma cells
A diploid rat cell line cloned from a glial tumour.

Table 2 | **Lipid raft localization, trafficking and regulation of G protein-coupled neurotransmitter signalling molecules**

	Lipid raft localization		Regulation of function by raft association	Neurotransmitter effects on localization in rafts	Endocytosis mediated by rafts	References
	Anatomical methods	Biochemical methods				
GPCR class						
Acetylcholine	Yes (M2)	Yes (M1, M2)	↑ (M3)	↑ (M2)	Yes (M2)	36,47,64,114
Adrenergic	Yes (β_1, β_2)	Yes (β_1, β_2)	↑ (β_1)	↓ (β_2)	Yes (β_1, β_2)	44,115
			↓ (β_2)			47,48,60,115
Cannabinoid	nd	nd	↓ (CB_1)	nd	Yes (CB_1)	45,116
Dopamine	Yes (D1, D2)	Yes (D1)	↑ (D1)	nd	Yes (D2)	37,117
Glutamate	Yes (mGluR1a)	Variable results	↓ (mGluR1a)	nd	nd	38,39,118
GABA	nd	Yes	↓	nd	nd	119
Histamine	Yes (H_1)	nd	none	nd	Yes (H_1)	46
Neurokinin	Yes (NK_1)	Yes (NK_1)	↑	nd	nd	33
Opioid	nd	Yes (κ, μ)	↓ (κ)	none (μ)	nd	34,120
Purinergic	Yes (A_1, A_{2A})	Yes (A_1)	↑ (P2Y)	↑ (A_1)	Yes (A_1)	35,117
		Yes (P2Y)				7,64
Serotonin	Yes (5-HT_{2A})	Yes (5-HT_{2A})	↑ (5-HT_{1A})	nd	nd	32
			↑ (5-HT_{2A})			7,121
GnRH	nd	Yes	↑	nd	nd	122
Oxytocin	nd	Yes	↑	nd	nd	123
G proteins						
$G\alpha_s$	Yes	Yes	↓	↑	Yes	47,48,60
$G\alpha_i$	nd	Yes	nd	nd	nd	53,59
$G\alpha_q$	Yes	Yes	↑	↓	nd	7,58,59
$G\alpha_{12}$	nd	Yes	nd	nd	nd	124
$G\alpha_o$	nd	Yes	nd	nd	nd	125
$G\alpha_t$	nd	Yes	↓	↑	nd	51
Gβγ	Yes	Yes	nd	nd	nd	59
RGS proteins	nd	Yes	nd	↑	nd	51,61
G-protein effectors						
Adenylyl cyclases	nd	Yes	↓	nd	nd	47,62,63
Phospholipase C	nd	Yes	↑	↑	nd	64
G-protein-gated ion channels	nd	Yes (Kir3)	↓ (Kir3)	nd	nd	65
			↓ (N-type-Ca^{2+})			66
cAMP-gated ion channels	nd	Yes	↑	nd	nd	67
cGMP phosphodiesterase	nd	Yes	nd	↑	nd	51,126

↑, increase in; ↓, decrease in; 5-HT, 5-hydroxytryptamine; A_1, adenosine receptor 1; cAMP, cyclic AMP; cGMP, cyclic GMP; CB_1, cannabinoid receptor 1; D1, dopamine receptor 1; GABA, γ-aminobutyric acid; GnRH, gonadotropin releasing hormone receptor; GPCR, G-protein-coupled receptor; H_1, histamine receptor 1; Kir3, G-protein-gated inwardly rectifying potassium channel; M1, acetylcholine receptor muscarinic subtype 1; mGluR1a, metabotropic glutamate receptor 1a; nd, not determined; NK_1, neurokinin receptor 1; P2Y, purinergic receptor 2 class Y; RGS, regulators of G-protein signalling.

by protein kinase A[44]. Similarly, cannabinoid CB_1 receptors have been suggested to undergo both clathrin- and raft/caveolae-induced endocytosis that leads to receptor downregulation[45]. In addition, histamine H_1 receptors undergo clathrin-independent internalization during signalling but do not colocalize with caveolins after internalization, indicating a lipid raft-mediated endocytic mechanism that is distinct from caveolae-mediated internalization[46].

Some receptors also translocate into or out of lipid rafts during signalling. For example, agonist stimulation of adenosine A_1 receptors increases the pool of receptors in lipid rafts, which presumably facilitates the observed clathrin-independent, raft-mediated internalization of

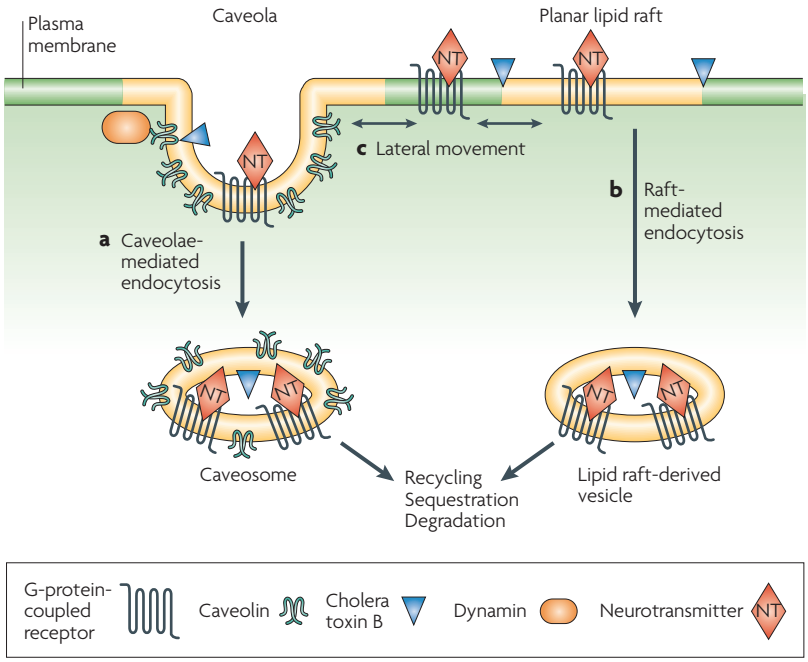

Figure 2 | **Lipid rafts as sites for endocytosis and trafficking of neurotransmitter receptors.** During neurotransmitter signalling, many G-protein-coupled receptors (GPCRs) undergo agonist-induced endocytosis, leading to receptor recycling, receptor sequestration and receptor downregulation. Clathrin-independent lipid raft mechanisms might contribute to this process. Both caveolae and planar lipid rafts can facilitate clathrin-independent endocytosis from the plasma membrane[40,42], although the precise components and intracellular trafficking pathways that are involved have yet to be definitively determined[40,41]. Caveolae or raft endocytosis can be assessed using fluorescence microscopy, and cellular uptake of cholera toxin B is often used to distinguish clathrin-independent, raft-mediated endocytosis; however, this toxin can also be taken up by other pathways[41]. **a** | Several GPCRs have been reported to be internalized through the caveolae pathway (TABLE 2). A signal-dependent event leads to dynamin-dependent fission of the invaginated caveola and subsequent endocytosis and vesicle trafficking to caveolin-containing caveosomes[40]. The molecular mechanisms responsible for GPCR internalization through caveolae are largely undefined and warrant further investigation. **b** | Planar lipid rafts can also facilitate endocytosis; however, less evidence is available for this route of GPCR internalization. **c** | Neurotransmitter signalling can also result in the movement of receptors into or out of lipid rafts. This translocation and lateral movement in the membrane could either activate or diminish neurotransmitter signalling by altering the coupling of receptors with G proteins and/or other signalling effectors.

Palmitoylation
The covalent attachment of a palmitate (16-carbon saturated fatty acid) to a cysteine residue through a thioester bond.

Myristoylation
The covalent (and, in the case of a G protein, cotranslational) attachment of a hydrophobic myristoyl group to the amino-terminal glycine residue of a nascent polypeptide.

Liposome
A lipid vesicle artificially formed by sonicating lipids in an aqueous solution.

the receptors[35]. By contrast, β-ARs are removed from lipid rafts during signalling in cardiac myocytes and glioma cells[47,48]. Also of note, some GPCRs, such as kinin B1 receptors, translocate into lipid raft domains but do not undergo endocytosis[49], indicating that some activated GPCRs move into raft domains and remain there, perhaps to promote or prevent interactions with downstream signalling molecules. These examples indicate that GPCRs undergo agonist-induced endocytosis through lipid rafts and also traffic into or out of these domains — events that could ultimately regulate receptor signalling.

G proteins

Many G proteins, in addition to their cognate receptors, have been reported to localize to lipid rafts, and these associations differentially alter neurotransmitter signalling. Although a definitive consensus about G-protein localization in lipid rafts in the nervous system is lacking, G proteins have been found in lipid raft membranes isolated from whole brain lysates[50], glia[48], photoreceptors[51] and cardiac muscle[52], as well as many other tissues. Various G protein subtypes, including $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, $G\alpha_{12}$, $G\alpha_o$, $G\alpha_t$ and $G\beta\gamma$ are all enriched, to a varying degree, in lipid rafts (TABLE 2).

Raft targeting of G proteins. G proteins could be targeted to raft domains by several mechanisms. The most plausible mechanism is that Gα subunits are subject to fatty acylations such as palmitoylation and/or myristoylation, which might localize the proteins to rafts. For example, in reconstituted membranes or liposomes, palmitoylation or myristoylation of $G\alpha_i$ is required for it to be targeted to lipid rafts, and lipid rafts are sites of increased palmitoyltransferase activity for $G\alpha_i$[53,54]. Interactions of G proteins with caveolins or other scaffolding proteins might also contribute to raft targeting, as $G\alpha_s$, $G\alpha_o$ and $G\alpha_i$ interact directly with the amino (N) terminus of caveolin 1, which could target the proteins to rafts[55]. Furthermore, tubulin, a scaffold protein that is also a component of lipid rafts, shows high-affinity interactions with G proteins (G_s, G_i and G_q)[56,57].

G protein trafficking. As is the case for many receptors, some G proteins also undergo signal-dependent trafficking into or out of lipid rafts. For example, chronic stimulation of thyrotropin-releasing hormone receptors induces $G\alpha_{q/11}$ to exit detergent-resistant membranes[58]. By contrast, during stimulation of β-ARs in glioma cells, $G\alpha_s$ localization in lipid rafts increases and it undergoes agonist-induced endocytosis that is dependent on raft domains[48]. So, rafts can function as sites for G-protein trafficking and internalization, as well as platforms for signalling.

Raft regulation of signalling. Different G proteins probably associate selectively with caveolae or planar lipid rafts and this might help explain the complex regulation of G-protein signalling by raft domains. For example, isolation of caveolae from other lipid raft membranes in endothelial cells indicates that $G\alpha_q$ interacts directly with caveolins, targeting $G\alpha_q$ to caveolae; by contrast, $G\alpha_s$, $G\alpha_i$ and Gβ are targeted to lipid rafts that do not contain caveolins[59]. Stable knockdown of caveolin 1 in glioma cells prevents $G\alpha_q$-coupled signalling from the 5-HT_{2A} receptor, indicating that lipid rafts and/or caveolins are required[7]. By contrast, disrupting raft domains by cholesterol chelation elevates $G\alpha_s$-coupled, β-AR signalling[47,60]. Therefore, lipid raft domains seem to spatially organize specific G proteins at the membrane, which could facilitate or prevent interactions with their receptors and/or effectors, serving to either diminish or promote neurotransmitter signalling.

Rafts and RGS proteins. Regulators of G-protein signalling (RGS) proteins associate with Gα subunits and inactivate them by increasing the hydrolysis of GTP. Palmitoylation of RGS16 targets it to lipid rafts, and this raft localization might be necessary for the involvement of

RGS16 in turning off $G\alpha_i$ or $G\alpha_q$ signalling[61]. Exposure of retinal photoreceptors to light causes a $G\alpha_t$–RGS9–Gβ5 complex to translocate to lipid raft membranes, resulting in the decreased coupling of $G\alpha_t$ to, and the subsequent reduced activation of, its receptor rhodopsin[51]. These reports raise the possibility that the G-protein inactivation cycle could be regulated, in part, by partitioning RGS proteins in lipid raft microdomains.

Downstream effector systems

Common neurotransmitter effector systems include phosphodiesterases, ion channels and enzymes that generate second messengers. Activated G proteins modulate signalling effectors such as adenylyl cyclases, phospholipase C (PLC), ion channels and retinal phosphodiesterase. Several of these effectors are located in lipid rafts (TABLE 2), where these microdomains might facilitate or inhibit interactions among the effectors and their cognate GPCRs and G proteins to propagate or dampen signalling.

Adenylyl cyclases. There is considerable evidence that several adenylyl cyclases, which are regulated by G proteins and calcium to produce cyclic AMP (cAMP) during neurotransmission, are associated with lipid rafts. There are nine membrane-associated adenylyl cyclase isoforms, each of which is uniquely regulated. For example, adenylyl cyclase type VI is enriched in lipid rafts in glial cells, and its inhibition by calcium influx is dependent on raft integrity[62]. Adenylyl cyclase type V can be co-immunoprecipitated with caveolin 3 in cardiac myocytes[52], and N-terminal caveolin peptides inhibit the enzyme activity of purified type V adenylyl cyclase as well as a pool of adenylyl cyclases isolated from brain membranes[63]. Furthermore, forskolin-stimulated adenylyl cyclase activity is significantly increased after disrupting lipid rafts by depleting cholesterol[47,60]. These studies collectively indicate that some adenylyl cyclases are localized in lipid raft domains where their activity is attenuated.

Phospholipases and calcium signalling. Although there is substantial evidence to indicate that lipid rafts spatially and temporally regulate calcium signalling in cardiac myocytes and other tissues, this has yet to be well established in the nervous system. However, one recent study in astrocytes shows that several proteins that form the inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$)-dependent calcium cascade — metabotropic $P2Y_1$, G_q, $Ins(1,4,5)P_3$ receptor 2, PLCβ and protein kinase Cα (PKCα) — are all enriched in lipid rafts[64]. In this study, stimulation of cells with a purinergic agonist recruited PLCβ and PKCα to raft fractions, whereas lipid raft disruption slowed propagation of agonist-evoked calcium waves.

G-protein- and cyclic nucleotide-gated ion channels. The lipid raft compartmentalization of G-protein-gated or cyclic nucleotide-gated (CNG) ion channels also has functional consequences. G-protein-gated inwardly rectifying K^+ (Kir3) channels are localized in lipid rafts and the lipid raft-associated neural cell adhesion molecule (NCAM) impairs the delivery of functional $5\text{-}HT_{1A}$-coupled Kir3 channels to the cell surface[65]. So, lipid rafts and their resident accessory proteins might regulate the functional availability of ion channels at the membrane and also possibly at synapses. Overexpression of caveolins in NG108-15 neuroblastoma/glioma cells prevented the inhibition of N-type calcium channel currents by both the δ-opioid receptor and G proteins, indicating that rafts and caveolins prevent G-protein-coupling to N-type calcium channels[66]. CNG ion channels, effectors of light and olfactory receptors, are located in lipid rafts isolated from olfactory epithelia[67]. When HEK293 cells expressing CNG ion channels are depleted of cholesterol, the channels' affinity for cAMP is significantly reduced and channel activity is abolished. These studies reveal that lipid raft domains influence ion channel effectors in a diverse manner — regulating the targeting of channels to the cell surface, altering the coupling of G proteins to the channels and changing ligand binding affinity.

Neurotransmitter transporters

Neurotransmitter transporters control neuronal signalling by regulating the levels of neurotransmitter in the synapse. A number of antidepressant and psychoactive drugs modulate transporter activity — and thereby also neurotransmitter signalling — underscoring the importance of understanding how transporters are regulated[68]. Several transporters have been found to be localized to lipid rafts (TABLE 1), and these membrane microdomains seem to significantly influence transporter function and availability at the membrane.

Cholesterol and transporter function. Disruption of lipid rafts by cholesterol-interfering agents produces, on average, a 50% decrease in the transport rate (V_{max}) of the GABA transporter[69], 5-HT transporter (5-HTT)[70,71] and glutamate transporters[72,73]. Reconstitution of GABA and glutamate transporter activity in liposomes requires cholesterol[74], and glutamate uptake activity is almost completely confined to lipid raft fractions prepared from mouse forebrain extracts[73]. Cholesterol levels in synaptic plasma membrane vesicles closely parallel the amount of GABA that is bound to its transporter[69] and the rate of GABA and glutamate uptake[69,73]. So, most transporters seem to exert maximal neurotransmitter uptake while residing in lipid rafts. The ratio of raft-associated to non-raft-associated transporter might represent a mechanism for regulating the rate and extent of neurotransmitter uptake. Interestingly, glial-conditioned medium increases glutamate transport in primary neuronal cultures, and cholesterol is implicated as an active factor[75]. These data predict that an external mechanism for altering cholesterol levels in rafts could regulate the amount of a neurotransmitter available to interact with its receptor.

The reduction in the transport rate for 5-HTT after cholesterol depletion occurs with a concomitant reduction in the affinity of the transporter for 5-HT, indicating that lipid rafts might promote a high-affinity state of the transporter[70,71]. Several studies support the idea of specific cholesterol–transporter interactions over the effects of cholesterol on general membrane properties. Optimal 5-HTT and GABA transporter activity is not observed when cholesterol is replaced with sterols that

Scaffolding proteins
Proteins that organize groups of interacting intracellular signalling proteins into signalling complexes.

Second messengers
Small intracellular signalling molecules generated in large numbers in response to the primary message of a hormone or neurotransmitter.

Calcium wave
A phenomena by which calcium released from intracellular stores diffuses within a cell in a pattern of waves.

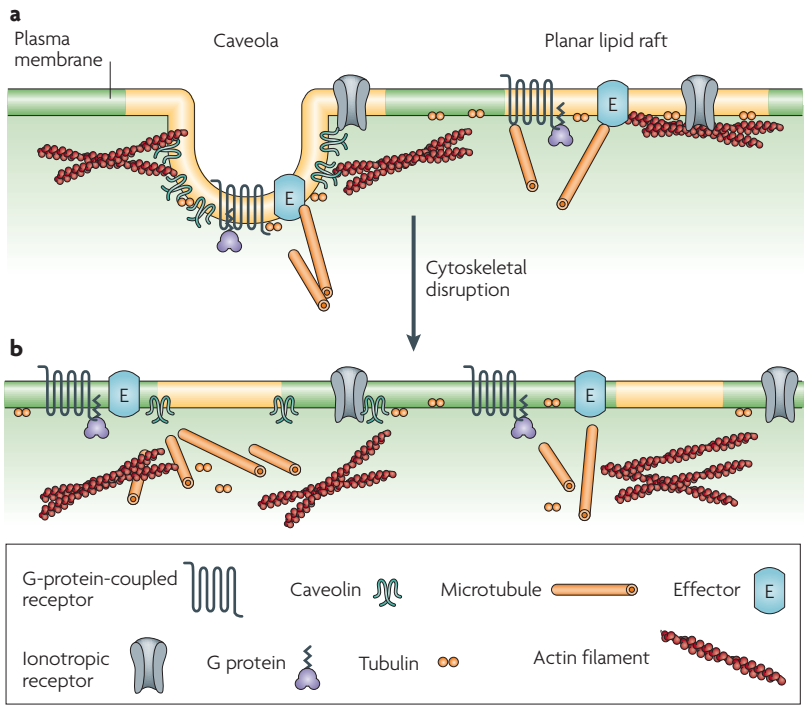

Figure 3 | **Cytoskeletal and lipid raft organization of neurotransmitter signalling molecules. a** | Actin and tubulin associate with lipid rafts and caveolae either as polymerized structures or their building block components (actin monomers or tubulin dimers). These cytoskeletal elements might help to organize lipid raft domains and the neurotransmitter molecules that are present in these structures (G-protein-coupled receptors, ionotropic receptors, effectors, G proteins). **b** | Disruption of actin filaments or microtubules reorganizes certain receptors or G proteins present in lipid rafts, as described in the main text. It is suggested that tubulin-binding or actin-binding drugs could compromise the association between dimeric tubulin and receptors or G proteins. Depending on the system, cytoskeletal elements present in rafts would be expected to facilitate or inhibit neurotransmitter signalling by contributing to the raft organization of the signalling molecules. Adapted, with permission, from REF. 87 © (2006) American Society for Biochemistry and Molecular Biology.

have similar effects on membrane fluidity[70,74], and cholesterol depletion by various distinct mechanisms all reduce the affinity of 5-HTT for its substrate[70]. These data indicate that specific cholesterol–transporter interactions exist independant of the effects of cholesterol on general membrane properties. There is also the possibility that different transporters differentially interact with lipid rafts. For example, the glutamate transporter can function optimally at four- to fivefold lower cholesterol levels than the GABA transporter can[74], and the glia-specific excitatory amino acid transporter 2 (EAAT2) glutamate transporter is more tightly associated with lipid rafts and more sensitive to cholesterol depletion than other glutamate transporter subtypes[73]. Further investigation is needed to determine the molecular mechanisms involved in the regulation of transporter function by lipid rafts and whether these mechanisms are transporter specific.

Regulating transporter levels. One mechanism for controlling the amount of neurotransmitter in the synapse is to alter the amount of functional transporters at the plasma membrane in neurons or glia. PKC-mediated internalization of the noradrenaline transporter is a dynamin- and clathrin-independent process that is blocked by the cholesterol-disrupting agents filipin and nystatin, which is an indication of a lipid raft-mediated process[76]. By contrast, similar experiments reveal that PKC-induced internalization of the dopamine transporter (DAT) is dynamin- and clathrin-dependent, and is independent of lipid rafts[77]. Internalization of 5-HTT in rat midbrain synaptosomes also redistributes 5-HTT from lipid rafts[78]; however, the ramifications of this movement for internalization is unclear, as a defining study for the requirement of lipid rafts in 5-HTT trafficking is lacking. Lipid rafts might also function to insert transporters into the plasma membrane, as demonstrated for the EAAT2 and EAAT3 glutamate transporters[73], and it would be interesting to determine if lipid rafts target transporters to specific regions of the neuronal plasma membrane.

Cytoskeletal components and lipid rafts

Lipid rafts are defined as being both rich in cholesterol and associated with the cytoskeleton. But which cytoskeletal elements are associated with neuronal lipid rafts, and how do they contribute to raft-mediated signalling? And, consequently, how do neurotransmitter signals, emanating from rafts, modify the cytoskeleton? The relationship between cytoskeletal elements and lipid rafts is still emerging; however, it seems that microtubules and actin filaments are the primary interacting partners of lipid rafts. Each is considered below and in FIG. 3.

Rafts and microtubule dynamics. Tubulin, the building block of microtubules, is resident in lipid rafts and can be co-immunoprecipitated with CAV1 in rat forebrain extracts[79]. One possible mechanism for the contribution of lipid rafts to neurotransmitter-induced alterations in microtubules is indicated by experiments in smooth muscle cells. In these cells, caveolins might stabilize microtubules by interfering with the interaction between the microtubule-destabilizing protein stathmin and tubulin[80]. Unfortunately, this model contains no provision for agonist-mediated modifications of the microtubule cytoskeleton. G proteins, specifically $G\alpha_s$, could provide such a link, as they reside in lipid rafts (TABLE 2) and, more importantly, internalize through these rafts in response to agonist activation[48]. Certain G proteins have been shown to promote the GTPase activity of tubulin and to increase the dynamic behaviour of microtubules[81]. Microtubules are particularly dynamic in growth cones[82], and translocation of $G\alpha_s$ to the cytosol through lipid rafts after activation of a GPCR might be a mechanism for neurotransmitter-mediated changes in neurons. Although dendritic spines lack microtubules, the microtubule apparatus reaches the base of the dendritic spine and might participate in neuronal extension and retraction of spines.

Phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) serves as a tubulin anchor on the plasma membrane[83]. Acetylcholine-mediated hydrolysis of $PtdIns(4,5)P_2$ releases tubulin from the plasma membrane and directs its internalization[84] for subsequent re-use in microtubule

Microtubules
Hollow tubes, 25 nm in diameter, formed by the lateral association of 13 protofilaments which are themselves polymers of α- and β-tubulin subunits.

formation. This mechanism could promote microtubule formation in regions subjacent to PtdIns(4,5)P_2-enriched lipid rafts.

Tubulin and neurotransmitter signalling. The role of rafts in mediating microtubule dynamics remains unclear, but the tubulin that is present in lipid rafts might well participate in neurotransmitter signalling. This could occur through the role of tubulin as a scaffolding protein in rafts, through a dynamic interaction of tubulin with signalling proteins, or both. An association between tubulin and heterotrimeric G proteins has been demonstrated[56], and this potentiates signalling of both adrenergic and cholinergic neurotransmitters by directly activating their respective G proteins[57,85]. Curiously, treatment of glial cells or cardiac myocytes with microtubule-disrupting agents such as colchicine results in the loss of many signalling molecules from lipid raft membranes — in particular, those involved in AR signalling[86,87]. Colchicine treatment also significantly increases signalling through the β-AR–Gα_s–adenylyl cyclase system[87,88]. A putative reason for this might be that colchicine disrupts the complexes between Gα_s and tubulin in lipid rafts, releasing Gα_s from raft microdomains. This 'liberated' Gα_s might facilitate increased signalling from GPCRs that couple to Gα_s and activate adenylyl cyclase. So, microtubules and tubulin might help to sequester and anchor signalling molecules in raft domains where their activity is diminished.

Actin and lipid rafts. The actin cytoskeleton seems to have a bidirectional relationship with lipid rafts. Phosphoinositide lipids such as PtdIns(4,5)P_2 and PtdIns(3,4)P_2 have been shown to accumulate in lipid rafts, and these lipids are also known to bind actin and direct actin assembly into filaments[89,90]. Neurotransmitters that induce the hydrolysis of PtdIns(4,5)P_2 promote the removal of actin from these anchoring sites at the membrane[91]. Dendritic spines show actin attachment on their distal end, and rapid remodelling of these spines in living neurons might result from the turnover of PtdIns(4,5)P_2 modulating either the dynamics of actin filaments or their binding sites on the membrane[20].

However, actin also has a role in clustering signalling molecules in lipid rafts. Small G proteins cluster in lipid rafts, and this seems to be dependent on the actin cytoskeleton[92]. Small G proteins influence cell shape at the membrane and various developmental and survival programmes inside the cell by controlling kinase cascades. These GTPases change their raft localization in response to the external signals that modify the actin cytoskeleton[93]. Therefore, agents that modify the raft association of actin can utilize small G proteins and other signalling molecules to further modify the cell shape or to evoke sustained cell signalling pathways in the cell interior[89].

Lipid rafts and disorders of the brain

The burgeoning interest in lipid rafts by neuroscientists has also prompted studies to explore if and how these microdomain components contribute to various neurological and psychiatric diseases. Drugs used to treat mood disorders (for example, unipolar depression, bipolar affective disorder) and schizophrenia accumulate in lipid rafts[94] and affect the signalling of resident proteins. Although chronic antidepressant treatment does not alter membrane cholesterol content, treatment with chemically distinct antidepressants results in the movement of Gα_s (but not other G proteins) out of lipid rafts and into a closer association with adenylyl cyclase[50,86,95]. This might contribute to the increased cAMP tone and synaptic changes that are observed subsequent to chronic antidepressant treatment[96]. The microtubule-disrupting drug colchicine similarly removes Gα_s from lipid rafts[86] and enhances Gα_s-stimulated adenylyl cyclase activity[88]. In concert, these data indicate that antidepressants might exert some of their therapeutic efficacy by altering a component of the membrane or cytoskeleton that is associated with lipid rafts.

There is also evidence in the literature for the involvement of lipid rafts in the pathogenesis of several neurodegenerative diseases. Lipid rafts might function as platforms for the production of neurotoxic proteins, such as amyloid-β in Alzheimer's disease[97] and prion protein in transmissible spongiform encephalopathies[98], which could then be poised to modulate raft-associated signalling cascades. For example, amyloid-β can modulate the activity of the nAChα7 subunit of the nACh receptor[99], both of which are raft-associated proteins; however, a direct relationship has yet to be uncovered. Prion protein can redistribute caveolins from lipid rafts[100], and overexpression of α-synuclein — a protein which aberrantly accumulates in Parkinson's disease — can upregulate caveolin 1 (REF. 101). Furthermore, *Cav1*-knockout mice show abnormal neurological phenotypes including clasping, muscle weakness and reduced activity — results that are consistent with altered motor control[5]. Given the role of caveolins as scaffolding proteins and in the regulation of numerous signalling molecules, disease-related alterations in caveolin localization and expression could globally affect neurotransmitter signalling.

Conclusions

This review has synthesized existing information on lipid rafts and their role in neurotransmitter signalling. We have identified unique instances where lipid rafts regulate the processes of neurotransmitter receptor (ionotropic or GPCR) activation/inactivation or neurotransmitter uptake and have illustrated how lipid rafts modulate the aggregation of signalling components. Curiously, there is no clear definition for the role of rafts in neurotransmitter signalling, as they seem equally likely to be domains in which signalling is enhanced or inhibited. This allows for a rich complexity in signalling, suggesting that different lipid raft compartments might have opposing effects on different neurotransmitters. Furthermore, the unique spatial segregation afforded by raft domains could allow a single neurotransmitter–receptor pair to channel different signalling pathways along different time courses or in limited areas of the same neuron.

This complexity is enhanced when further consideration is given to the relationship between neuronal-specific structures such as the postsynaptic density and

Colchicine
Alkaloid used to inhibit the polymerization of tubulin and cause the depolymerization of microtubules.

Postsynaptic density
An electron-dense thickening underneath the postsynaptic membrane at excitatory synapses that contains receptors, structural proteins linked to the actin cytoskeleton and signalling elements, such as kinases and phosphatases.

lipid rafts. Even though many neurotransmitter receptors are highly enriched and organized in the postsynaptic density, it is unclear whether lipid rafts interact with, or are part of, this structure. The raft marker flotillin is present in dendritic spines of the rat cerebral cortex (which commonly contain postsynaptic densities), and detergent-resistant raft-like fractions have been isolated from preparations of synaptic membranes[102]. A functional relationship between rafts, the postsynaptic density and the cytoskeletal networks comprising them remains to be established. Limitations on our understanding of the process are rooted in experimental methodologies that have failed to deliver a holistic determination of the physiology of rafts as they relate to neurotransmitter signalling (BOXES 1,2). Future studies designed to define the relationship between these unique synaptic structures and lipid raft microdomains with respect to organizing both spatial and temporal aspects of neuronal signalling are essential, and these studies need to combine biochemical, biophysical, genetic and imaging techniques — or develop new ones — to advance our understanding.

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Competing interests statement
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