Neuropsychopharmacology www.nature.com/npp

REVIEW ARTICLE

Regulation of monoamine transporters and receptors by lipid microdomains: implications for depression

Joanne J. Liu[1,7], Adrienne Hezghia[1,8], Saame Raza Shaikh[2], Joshua F. Cenido[1,9], Ruth E. Stark[3,4], J. John Mann[1,5,6] and M. Elizabeth Sublette[1,5]

Lipid microdomains ("rafts") are dynamic, nanoscale regions of the plasma membrane enriched in cholesterol and glycosphingolipids, that possess distinctive physicochemical properties including higher order than the surrounding membrane. Lipid microdomain integrity is thought to affect neurotransmitter signaling by regulating membrane-bound protein signaling. Among the proteins potentially affected are monoaminergic receptors and transporters. As dysfunction of monoaminergic neurotransmission is implicated in major depressive disorder and other neuropsychiatric conditions, interactions with lipid microdomains may be of clinical importance. This systematic review evaluates what is known about the molecular relationships of monoamine transporter and receptor regulation to lipid microdomains. The PubMed/MeSH database was searched for original studies published in English through August 2017 concerning relationships between lipid microdomains and serotonin, dopamine and norepinephrine transporters and receptors. Fifty-seven publications were identified and assessed. Strong evidence implicates lipid microdomains in the regulation of serotonin and norepinephrine transporters; serotonin 1A, 2A, 3A, and 7A receptors; and dopamine D1 and β2 adrenergic receptors. Results were conflicting or more complex regarding lipid microdomain associations with the dopamine transporter, D2, D3, and D5 receptors; and negative with respect to β1 adrenergic receptors. Indirect evidence suggests that antidepressants, lipid-lowering drugs, and polyunsaturated fatty acids may exert effects on depression and suicide by altering the lipid milieu, thereby affecting monoaminergic transporter and receptor signaling. The lipid composition of membrane subdomains is involved in localization and trafficking of specific monoaminergic receptors and transporters. Elucidating precise mechanisms whereby lipid microdomains modulate monoamine neurotransmission in clinical contexts can have critical implications for pharmacotherapeutic targeting.

Neuropsychopharmacology (2018) 43:2165–2179; https://doi.org/10.1038/s41386-018-0133-6

INTRODUCTION

Plasma membrane structure–function relationships

A growing area of research over the last two decades concerns structural and functional characteristics conferred by the lipid composition of plasma membranes. The distinct physicochemical properties of different lipid species are accommodated in ways that impose organization on the membrane, with consequences for the distribution and regulation of proteins within the membrane bilayer [1].

Studies in model membrane systems show that lipid domains rich in sphingolipids and cholesterol are highly ordered, existing in a tightly-packed, gel-like state with an aversion to the unsaturated acyl chains of glycerophospholipids, which occur in a loosely-packed, liquid disordered state (reviewed in refs. [2, 3]). These observations support the paradigm that membrane lipid microdomains (lipid rafts) exist as physically and temporally dynamic, nanoscale membrane assemblies enriched in cholesterol, sphingolipids, and glycosylphosphatidylinositol (GPI)-anchored proteins [4].

Lipid microdomains also have been studied in cell membranes [5–9], leading to a proposed categorization into caveolar and non-caveolar, or planar, rafts [10]. Both raft types exhibit high cholesterol and sphingomyelin content, enrichment in GPI-anchored proteins, cytoskeletal association, and resistance to detergent extraction. Most rafts are reported to be of the caveolar type. Caveolae are small, flask-shaped invaginations of the membrane that function in cell signaling, lipid trafficking, and endocytosis, an important mechanism for downregulating cell-surface levels of proteins such as transporters. Key relevant characteristics are the absence of clathrin in caveolar endocytosis and the presence of caveolin and cavin proteins [11, 12], which are present in brain [11]. Another type of raft-related, non-clathrin-mediated endocytotic mechanism involves flotillin proteins (also known as reggies) that are also present in the central nervous

[1]Department of Molecular Imaging & Neuropathology, New York State Psychiatric Institute, New York, NY, USA; [2]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [3]Department of Chemistry and Biochemistry and CUNY Institute for Macromolecular Assemblies, The City College of New York, New York, NY, USA; [4]Ph.D. Program in Biochemistry, The Graduate Center of the City University of New York, New York, NY, USA; [5]Department of Psychiatry, Columbia University, New York, NY, USA and [6]Department of Radiology, Columbia University, New York, NY, USA
Correspondence: M Elizabeth Sublette (sublett@nyspi.columbia.edu)
[7]Present address: Chestnut Hill Hospital, Philadelphia, PA, USA
[8]Present address: Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, NY, USA
[9]Present address: Department of Psychiatry, Charles R. Drew University of Medicine and Science, Los Angeles, CA, USA

Received: 21 December 2017 Revised: 24 May 2018 Accepted: 18 June 2018
Published online: 28 June 2018

system [13, 14] and analogous functionally, but not homologous structurally, to caveolin [15]. Caveolins and flotillins serve a scaffolding/organizing function, anchoring signaling components to lipid microdomains [10]. In addition to stimulating movement into the cell by endocytosis across the membrane, neurotransmitter signaling also can cause movement of signaling proteins into or out of lipid microdomains by translocation or lateral movement within the membrane bilayer [16].

However, countervailing structural models also have been proposed. One alternative is a continuous bilayer with an outer membrane leaflet rich in cholesterol and sphingolipids, with a large ordered fraction of the plasma membrane [17, 18]. In another suggested model, sphingolipids form distinct domains not dependent on cholesterol [19], and a strong role for the actin cytoskeleton is implicated [19–23]. Some investigators propose that lipid domains are larger than nanoscale assemblies and are not organized on the basis of phase-differentiation [24]. The most extreme view suggests that microdomains do not exist but are an artifact of the methods used to study them [21, 24]. Finally, others have attempted to reconcile disparate observations into an integrative model [4].

This controversy is fueled by the uncertain validity of extrapolating information from artificial model membranes, combined with technical difficulties inherent in the study of submicroscopic, temporally dynamic nanostructures [4]. Common methods used to isolate lipid microdomain-containing cellular fractions have drawbacks; notably, the use of nonionic detergent-resistant membranes (DRM) [25, 26] produce results that are inconsistent [27] and are not be adequately representative of in vivo lipid microdomains [28]. Evidence that the detergent itself induces artifacts was presented over a decade ago [29]. Possibly less perturbative methods for isolating raft-like domains include sucrose gradient ultracentrifugation [30], which ostensibly avoids the pitfalls of DRM, but since this technique includes treating the membranes with alkaline buffer, it may cause saponification, essentially retaining a detergent-like effect. Cholesterol depletion is commonly used under the assumption that subsequent functional loss is evidence for lipid microdomain disruption. Disruptive techniques include: (1) sequestration of cholesterol using filipin or nystatin [31]; (2) depletion and removal of cholesterol using methyl-β-cyclodextrin [32]; (3) inhibition of cholesterol synthesis using HMG-CoA reductase inhibitors (statins) [33]; and (4) genetic approaches using caveolin-1 knockout mice [11] or caveolin RNA interference (siRNA) [34]. However, because the use of cholesterol-disrupting, and thus lipid raft-disrupting, drugs can produce pleiotropic effects, including effects on non-raft domains [32] and on the actin cytoskeleton [23], data from cholesterol depletion experiments ideally require validation. The restoration of cholesterol or replacement with its analog, desmosterol (a non-raft promoting sterol) is sometimes used as an experimental control. Depletion experiments can likewise clarify the role of sphingolipids, using Fumonisin B1 [35, 36], and actin, using the actin filament disrupter cytochalasin D [16].

The resolution of these controversies will require continuing development of technologies to more directly study the lipid–protein milieu, such as super-resolution microscopy coupled with fluorescence and bioluminescence techniques [37] harnessed to protein-conjugated quantum dots, which enables nanoscale and microscale quantitation and visualization of receptor clustering in living cells [38]. Sophisticated techniques also include stimulated emission depletion (STED) super-resolution nanoscopy [39], Förster resonance energy transfer (FRET) [40, 41], fluorescence correlation spectroscopy (FCS) [39], k-space image correlation spectroscopy (kICS) [42], and magic-angle spinning nuclear magnetic resonance (HR-MAS NMR) [43, 44], each of which can provide valuable information on the nanometer scale.

Structural characteristics of lipid microdomains are understood to influence plasma membrane proteins in several ways, including (1) providing a scaffolding for spatially co-localizing the membrane proteins with other elements important in cell-cell signaling [45, 46]; (2) affecting lateral movement of proteins [47]; (3) altering molecular trafficking, e.g., endocytosis of proteins from the cell membrane surface to the cell interior [48–50] to achieve receptor recycling, sequestration, and/or downregulation; and (4) directly regulating activity, e.g., modulation of the transport rate or affinity of transporter proteins for substrates by the relative amounts of cholesterol [51, 52].

Lipid microdomains as regulators of monoaminergic signaling

Among membrane proteins affected by lipid microdomains are a variety of monoamine transporters and receptors, important for emotion regulation. Monoamine neurotransmitters comprise a key family of neural signaling proteins, including serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), and norepinephrine (NE). Serotonin modulates mood, aggression, motivation, appetite, sleep, cognition, and sexual activity; and altered 5-HT signaling has been implicated in neuropsychiatric diseases [53–57]. DA systems control motor function, mood, reward, and cognition [58, 59], dysregulation of which is linked to attention deficit hyperactivity disorder (ADHD), schizophrenia, substance use disorders, and Parkinson's disease [60]. NE regulates arousal, mood, attention, and stress-responsiveness [61–64].

Monoaminergic transporters and receptors. Plasma membrane monoamine transporters govern the effects of their respective neurotransmitters within the synaptic cleft by recycling them back into the synaptic boutons, with direct effects on intra-synaptic neurotransmitter concentrations and downstream indirect effects on monoaminergic receptors [65, 66]. Thus, altering the quantity of functional transporters at the neuronal or glial plasma membrane represents one mechanism for regulating synaptic levels of neurotransmitters [67]. These transporters are integral membrane phosphoproteins affected by kinase and phosphatase activities (reviewed in ref. [67]) and are also pharmacological targets of many psychoactive drugs [68–70]. Studies in knockout mice have demonstrated that the mechanisms regulating monoamine neurotransmitter biosynthesis and storage, receptor sensitivity, and transporter expression are interdependent [71–78]. A deeper understanding of lipid microdomain regulation of monoamine transporter function is likely to foster new treatment targets for neuropsychiatric disease.

Monoaminergic receptors exist in multiple subtypes and may be pre-synaptic or post-synaptic. With the exception of 5-HT_3, monoaminergic receptors belong to the G-protein-coupled receptor superfamily, with distinct receptor subtype specificity for G-proteins. Depending on receptor subtype and the concomitant G-protein, agonist binding to monoaminergic receptors activates or inhibits second messengers adenylyl cyclase (AC) and cyclic AMP (cAMP), or inositol triphosphate and diacylglycerol.

We summarize the literature concerning lipid raft involvement in monoaminergic transporter and receptor regulation and depression (Fig. 1), including (1) co-localization of transporters/receptors with lipid microdomains, ganglioside GM1, and raft-associated proteins in detergent-resistant membranes or low-buoyancy sucrose fractions; (2) lateral transporter movement; (3) association of transporters/receptors with caveolar proteins; (4) lipid influences on receptor transport rate; (5) effects on agonist and antagonist receptor binding; (6) downstream effects on second messengers; and (7) sensitivity to effects of cholesterol depletion (Fig. 1).

METHODS

Literature search strategy

Animal and human experimental studies on monoamine neurotransmitters, receptors and transporter proteins, and lipid

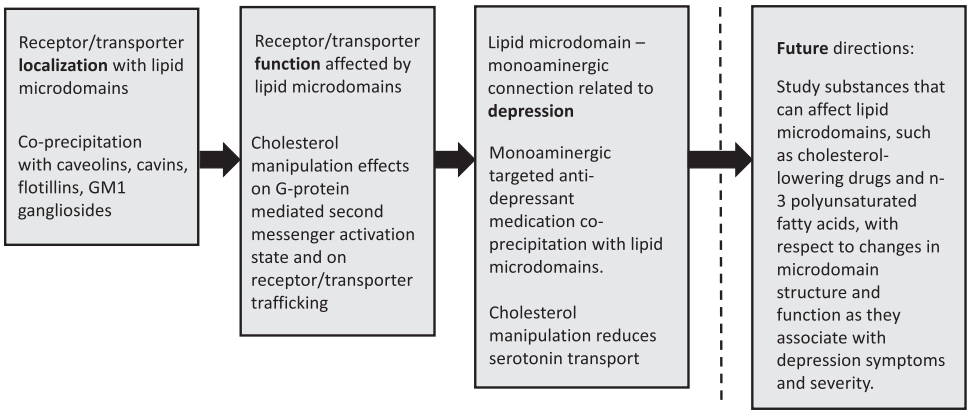

Fig. 1 Trajectory of research on lipid microdomain relationships with monoaminergic receptors/transporters and depression

microdomains published in English through August 2017 were identified by searching with MEDLINE Medical Subject Heading (MeSH) terms. In addition, general PubMed searches were conducted. Duplicate papers were removed, and articles were excluded if not clearly germane. The reference lists of prior reviews and research articles were also manually searched to find other potentially relevant studies (see detailed strategy, Table 1.)

RESULTS

Literature search

Combined PubMed/MeSH searches identified 250 potentially eligible reports, of which 27 review articles were eliminated; 183 were excluded as not directly concerning monoaminergic systems, were focused narrowly on non-brain systems, or were primarily concerned with other elements of the signaling pathways, yielding 37 articles. Twenty additional studies were identified through manual bibliography searching, yielding 57 reports considered in the Results section of this systematic review, which we view as appropriately summarizing the current understanding of lipid microdomain associations with monoaminergic transporters and receptors. Of these investigations into the interactions with lipid microdomains, 21 studies reported on serotonergic systems [16, 33, 34, 36, 51, 52, 90, 93, 117–124, 126–129]; 25 reported on dopaminergic systems [35, 79–81, 99–102, 105–108, 110, 134–136, 138–146]; and 12 reported on noradrenergic systems [46, 113, 147–156].

Monoamine transporters and lipid microdomains

Transporter relationship to plasma membrane is modulated by phosphatidylinositol 4,5-bisphosphate (PIP_2). The monoaminergic transporters for serotonin (SERT), dopamine (DAT), and norepinephrine (NET) are all examples of neurotransmitter:sodium symporters with a 12-transmembrane domain structure that form oligomers in intact cells [82, 83]. One important mechanism whereby these transporters are anchored in lipid microdomains is through binding to PIP_2 [84]. Found in low concentration in lipid microdomains [85, 86], in the cytoplasmic leaflet of the cell membrane [87], PIP_2 nevertheless has an important role in monoaminergic signaling by stabilizing transporter oligomerization [84, 88, 89] and regulating neurotransmitter efflux [88, 89].

SERT. Co-localization of SERT with caveolin and the ganglioside known as GM1 has been demonstrated in rat brain synaptosomes [90]. SERT proteins exist in two states with respect to their degree of lateral mobility: more mobile transporters that can diffuse relatively freely, and less mobile transporters localized to cholesterol and GM1 ganglioside-enriched microdomains [16]. In RN46A serotonergic neuronal cells labeled with quantum dots conjugated to the SERT ligand IDT318, confocal imaging reveals SERT clusters in cholesterol-rich membrane microdomains. Studied before and after cholesterol depletion with methyl-β-cyclodextrin, SERT molecules evince greater lateral movement after reduction of cholesterol, consistent with lipid raft disaggregation. In addition, actin filaments have a role in anchoring SERT molecules via their C terminus, since the actin filament disrupter cytochalasin D also results in increased SERT diffusion rates [16].

Increased SERT mobility after cholesterol depletion or actin disruption is accompanied by decreased transport activity [16]. Functional changes induced by cholesterol depletion result in a mean decrease of 50% in the transport rate (V_{max}) of SERT [51, 52], and a concurrent reduction in SERT affinity (K_m) for 5-HT, suggesting that lipid microdomains may regulate SERT by promoting a high-affinity state [51, 52]. Consistent with this hypothesis, signaling pathways that upregulate SERT activity, particularly p38 MAPK, act on SERT localized within membrane microdomains [16]. Conversely, in synaptosomes, activation of PKC downregulates SERT, causing redistribution from lipid raft to non-raft fractions [90].

Trafficking of SERT appears to be homeostatically regulated, as platelet studies have found that application of 5-HT variously induces SERT upregulation [91, 92] or downregulation [93, 94], which may depend on complex factors including 5-HT concentration and acute vs. chronic exposure [94]. SERT downregulation can be mediated by 5-HT binding to 5-HT2A autoreceptors via activation of $G\alpha_q$ proteins and downstream PKC activation [95, 96]. PKC exerts its effects on SERT by phosphorylating the transporter [97]; under some conditions, the SERT internalization can be blocked by 5-HT [98]. An analogous process has been reported for DAT (see below).

DAT. DAT are equally distributed across raft and non-raft domains [99], and their function is regulated by cholesterol [100]. Cholesterol depletion with methyl-β-cyclodextrin decreases V_{max} and K_m values for DA uptake [101] and efflux [100, 101] via DAT. However, in contrast to the SERT findings, cholesterol effects on DAT may not be mediated by caveolar endocytosis, as DAT downregulation induced by methyl-β-cyclodextrin does not occur by reduction of surface DAT expression [99, 101]. Moreover, although methyl-β-cyclodextrin depletes cholesterol across both raft and non-raft membrane domains [32], cholesterol chelation with nystatin or filipin primarily disrupts raft-localized cholesterol [31] and has no effect on DA uptake and efflux rates [100, 102]. Additionally, supranormal repletion with desmosterol—a sterol that does not form highly ordered domains and thus does not affect lipid rafts [103]—was as effective as cholesterol in restoring DAT transport rates [100]. Finally, DAT is internalized in a clathrin/dynamin-dependent process [104]. As an alternative explanation to lipid raft- mediated effects, studies with DAT mutations suggest that cholesterol may impact DA uptake by promoting an outward-facing conformation [100, 105].

Table 1. Lipid microdomain localization, trafficking, and regulation of monoamine neurotransmitter transporters and receptors

Study biosystem	Lipid raft co-localization	Effects on receptor/ transporter function	Effects of cholesterol manipulation			Lipid raft-mediated mechanism of downregulation
			Cholesterol depletion (methyl-β-cyclodextrin)	Cholesterol sequestration (filipin, nystatin)	Cholesterol repletion (cholesterol, desmosterol, ergosterol, epicholesterol)	
Transporters						
SERT						
•Rat midbrain synaptosomes [90] •RN46A cell line [16] •T-REx [51] •HEK-293 [51, 52] and •RN46A-B14 [51] cells	•Cav-1 and ganglioside GM1 [90] •Raft-associated SERT are confined in terms of lateral mobility [16]	•Localization in lipid rafts is essential for serotonin uptake [51]	•Increased lateral mobility [16] •Decreased 5-HT transport rate (V_{max}) and altered affinity (K_m) [51, 52]	•Treatment with filipin resulted in decreased affinity for agonist and antagonist binding [52]	•Adding cholesterol, but not other sterols, to depleted membranes restored SERT activity [52]	•PKC-mediated movement from lipid microdomains to non-raft membrane domains [90]
DAT						
•Lewis Lung Carcinoma-Porcine kidney (LLC-PK1) cells [99] •HEK-293 cells [100, 105, 107, 109, 110] •Pig kidney epithelial cells (LLC-PK) [100] •Rat striatal synaptosomes and immortalized mouse midbrain neuron MN9D cells [105] •Primary mesencephalic •Dopaminergic neurons [107] •HeLa cells [107] •Neuroblastoma (N2a) cells [101] •Porcine aortic endothelial (PAE) cells [102] •Madin–Darby canine kidney (MDCK) cells [104] •PC12 cells [106] •Human neuroblastoma (SK-N-MC) cells and murine striatal slices [108]	•Equally distributed across raft and non-raft domains [99]	•Outward-facing structural conformation increases DA uptake [100, 105] •Some conflict: •Flotillin depletion does not affect DAT endocytosis but does increase lateral diffusion rates [110] •Flot1 is required for DAT internalization [107]	•Decreased transport rate (V_{max}) and affinity (K_m) for DA uptake [101, 107] and efflux [100] •No change in surface expression [99, 101]	•No effect of nystatin on DA uptake and efflux rates [100, 102]	•Desmosterol restored transport rates [100]	•DAT substrates including DA promote internalization and thus diminishing transport capacity [109] Some conflict: •Clathrin/dynamin-dependent (i.e., non-raft) internalization process [99, 102, 104] •Linked to GTPase (Rin or Rit2) [106] and Flot1 [107] (both localized to lipid rafts) •Different pathways may exist for the "raft" and "non-raft" populations [99] •Basal internalization from raft and non-raft domains. PKC-stimulated internalization only from lipid raft domains [108]
NET						
•Rat [113] and human [46] placental trophoblast cells	•Cholesterol-rich lipid rafts [113]	—	—	•Filipin and nystatin block internalization [113]	—	•Dynamin-, clathrin-, and caveolar independent, cholesterol-rich lipid rafts

Table 1 continued

Study biosystem	Lipid raft co-localization	Effects on receptor/ transporter function	Effects of cholesterol manipulation			Lipid raft-mediated mechanism of downregulation
			Cholesterol depletion (methyl-β-cyclodextrin)	Cholesterol sequestration (filipin, nystatin)	Cholesterol repletion (cholesterol, desmosterol, ergosterol, epicholesterol)	
•Rat brain synaptosomes [46]						mediate PKC-initiated internalization process [113] •NET complexes with NK1R resulting in redistribution to lipid rafts and internalization [46]
Receptors						
Serotonin						
5-HT1A						
•NIH-3T3 and neuroblastoma N1E-115 cells [93] •Amino acid sequences of GPCRs from NCBI database [120]; •Model membrane systems [121] •Bovine hippocampal membranes [118]	•Detergent-resistant fraction [93, 117] along with Cav-1[93] •Exhibits a cholesterol consensus motif indicating a cholesterol binding site [120] •Lipid raft localization dependent on palmitoylation [93]	•Greater membrane order results in increased receptor activity [121]	•Decreased agonist [118] and antagonist [119] binding and signaling •Altered G-protein coupling [118]	—	•Cholesterol replenishment counteracts methyl-β-cyclodextrin effects on ligand binding and membrane order [118]	—
5-HT2A						
•C6 glioma cells, transfected HEK-293 cells, and rat brain synaptic membrane preparations [34] •Rat tail artery devoid of endothelium [122] •H9C2 rat embryonic myoblast-derived cell line [123] •Bovine tracheal smooth muscle [124]	Some conflict: •Co-localizes with Cav-1 [34, 122], and Cav-3 [123], •Non-caveolar [124]	•Inhibits expression of the ANF gene [123] •Inhibits activation of nuclear factor of activated T cells in cardiac myocytes [123] •Stimulates calcium flux in synaptic membranes [34]	•Disrupted physiologic response to stimulation by 5-HT [122, 124]	—	•Cholesterol-loaded methyl-β-cyclodextrin partially restored [124] and exogenous cholesterol restored [122] physiologic response to stimulation	Some conflict: •Caveolar-dependent upon 5-HT stimulation [123] •Co-localized with Cav-1 but 5-HT stimulation did not induce internalization [34]
5-HT3						
•N1E-115 and HEK-5-HT3A cells [126–128] and •Hippocampal neurons [128]	•Localized in low buoyant sucrose gradient fraction along with cholesterol, Cav-2, Flot1, and multiple psychotropic medications [127]	•Psychotropic drug concentrations in microdomain fractions correlate with magnitude of non-competitive inhibition of 5-HT-induced cation currents [127, 128] •Antidepressant antagonism of 5-HT_3 is	•Decreased functioning by reducing peak amplitude and kinetics of 5-HT stimulated cation currents [126]	—	—	•Non-competitive 5-HT_3 functional antagonism does not induce internalization [127]

Table 1 continued

Study biosystem	Lipid raft co-localization	Effects on receptor/ transporter function	Effects of cholesterol manipulation			Lipid raft-mediated mechanism of downregulation
			Cholesterol depletion (methyl-β-cyclodextrin)	Cholesterol sequestration (filipin, nystatin)	Cholesterol repletion (cholesterol, desmosterol, ergosterol, epicholesterol)	
		independent of lipid subdomains [126–128]				
5-HT_7						
•HeLa cells stably transfected with the human 5-HT_7 receptor [33, 36]	•Localized by detergent extraction with Cav-1 [36, 129]	•Decreased 5-HT_7 agonist and antagonist binding; decreased B_{max} without change in 5-HT binding affinity; and decreased 5-HT-induced CREB phosphorylation [33]	•Cholesterol [36, 129] and sphingomyelin [36, 129] depletion affect binding of 5-HT to 5-HT_7	—	—	•Clathrin-independent internalization [36, 129]
Dopamine						
D1-class						
D_1						
•Rat frontal cortex [80]; •HEK-293 [35, 134], •hRPT [136], N2a, and COS-7 [135] cells	•Predominantly localized to lipid microdomains [80] along with Gαs and Gαi_3 as well as Cav-2 and Flot1 [81] •Localization depends on the receptor conformation [35]	•Receptor binding of D1-class agonist increases adenylyl cyclase exclusively in lipid raft domains [136]	•In HEK cells molecular size of adenylyl cyclase was reduced; in hRPT cells, all adenylyl cyclase was shifted to non-lipid rafts [136]	—	—	Some conflict: •Clathrin-dependent [134] and caveolar-dependent [81, 135] endocytosis have been reported. Possible cell line-specific differences
D_5						
•Rat [80] and primate [138] prefrontal cortex; •HEK-293 cells [136]	•Predominantly in cytoplasmic fractions and not present in density gradient buoyant fraction lipid raft-like subdomains [80, 138]	•Enhancement of anti-oxidizing enzyme paraoxonase [139] and adenylyl activity [136] does not take place in lipid raft domains	•Reduced adenylyl cyclase activity in cells heterologously expressing D_5 [136]	—	—	—
D2-class						
D_2						
•Rat prefrontal cortex [80] •Mouse striatum [141]; •HEK-293 [79, 140, 141], •CHO [142, 144], and •COS-7 [143, 144] cells	•Diffusely distributed across membrane domains [80] Some conflict: •Not found in buoyant fractions [80] •Majority found in detergent-insoluble fraction [140, 141]	•Presence of dopamine decreased levels of detergent-insoluble plasma membrane-expressed receptor by >50% [140] •Activation of D_2 causes G-protein-coupled receptor kinase-dependent receptor phosphorylation and robust receptor internalization [144]	•Did not increase the detergent solubility of D_2 [140]	—	—	•Both caveolar-dependency [142] based on glycosylation state [79], and dynamin-dependency [143, 144] have been reported
D_3						
•CHO [144], •HEK-293 [79, 146]	•Co-localizes with G-protein-coupled receptor kinase 4	—	•Redistribution of raft-associated receptor, Cav-	—	—	•Clathrin-dependent (glycosylation of receptor

Table 1 continued

Study biosystem	Lipid raft co-localization	Effects on receptor/ transporter function	Effects of cholesterol manipulation			Lipid raft-mediated mechanism of downregulation
			Cholesterol depletion (methyl-β-cyclodextrin)	Cholesterol sequestration (filipin, nystatin)	Cholesterol repletion (cholesterol, desmosterol, ergosterol, epicholesterol)	
•hRPT [145], and •COS-7 [146] cells	predominately in non-raft fractions but also (10%) in raft fractions with Cav-1 [145]		1, and G-protein-coupled receptor kinase 4 (GRK4) to non-raft fractions [145]			is essential mediator) [79], consistent with dynamin-dependency [144] •Low susceptibility to downregulation through internalization [144, 146]
Norepinephrine						
β1-AR						
•Neonatal myocytes isolated from β1-AR and β2-AR knockout mice [147]	•β1-AR is primarily located in non-raft membrane regions [147]	—	—	•Filipin has no effect on β1-AR [147]	—	•Agonist stimulation does not affect abundance of β1-AR [150]
β2-AR						
•Neonatal mouse [147] and rat [149, 150] myocytes •Adult rat ventricular myocytes [156] •HEK-293 [148, 154, 155] cells •Sf9 insect cell membranes [153] •Rat H9c2(2-1) myoblast cells [152]	•Co-localized into caveolae shown by confocal imaging [147] and co-immunoprecipitation with Cav-3 [147], flotillin and AC [150] and other signaling components [148, 149] •β2-AR exhibits a cholesterol consensus motif indicating a cholesterol binding site [120, 153] Conflicting results: •Confinement of β2-AR does not depend on caveolae but rather on scaffold proteins [152]	•Redistribution of β-AR out of caveolae inhibits cAMP signaling [150] •This is consistent with findings that overexpression of Cav-3 results in increased caveolae, lower β-AR and decreased downstream cAMP signaling; and blocking Cav-3 expression upregulates the cAMP signal [156]	•AC activity and cAMP production are variously reported as inhibited [149], unaffected [148], or enhanced [150, 154, 155] •Decreased β2-AR-induced ERK phosphorylation but this may be non-specific to caveolae [148] •Lateral diffusion of β2-AR was decreased [148]	Filipin reduces co-immunoprecipitation of β-AR with Cav-3 [147]	—	•Agonist stimulation causes β2-AR to redistribute out of caveolae [150]

Articles were identified by searching with the following MEDLINE Medical Subject Heading (MeSH) terms: ("membrane microdomains" OR "caveolae") AND ("biogenic monoamines" OR "catecholamines" OR "tryptamines" OR "serotonin" OR "receptors, serotonin" OR "serotonin plasma membrane transport proteins" OR "dopamine" OR "receptors, dopamine" OR "dopamine plasma membrane transport proteins" OR "norepinephrine" OR "receptors, adrenergic" OR "norepinephrine plasma membrane transport proteins"). In addition, general PubMed searches were conducted using the following search terms as key words in titles/abstracts: ("membrane microdomain" OR "lipid rafts" OR "caveolae" OR "signalosomes" OR "cholesterol-enriched domains" OR "ordered domains") AND ("biogenic monoamines" OR "catecholamines" OR "tryptamines" OR "serotonin receptor" OR "serotonin transporter" OR "dopamine receptor" OR "norepinephrine transporter" OR "norepinephrine receptor" OR "adrenergic receptor")

— Effects have not yet been studied or determined, *β-AR* beta-adrenergic receptors, *5-HT* serotonin, *5-HT$_{1A}$* serotonin receptor subtype 1A, *5-HT$_{2A}$* serotonin receptor subtype 2A, *5-HT$_{3gA}$* serotonin receptor subtype 3A, *5-HT7A* serotonin receptor subtype 7A, *AC* adenylyl cyclase, *ANF* atrial natriuretic factor, *cAMP* cyclic adenosine monophosphate, *C6* rat glioma cell line, *Cav* caveolin, *COS-7* an acronym for fibroblast-like cells derived from simian kidney tissue, **C**v-1 in **O**rigin carrying monkey virus **S**v40 genetic material, *CHO* Chinese hamster ovary cells, *D_1* dopamine receptor subtype 1, *D_2* dopamine receptor subtype 2, *D_3* dopamine receptor subtype 3, *D_5* dopamine receptor subtype 5, *DA* dopamine, *DAT* dopamine transporter, *Flot1* flotillin-1, *HEK-293* human embryonic kidney cells, *hRPT* human renal proximal tubule, *MCF-7* human breast adenocarcinoma cell line, *NE* norepinephrine, *NET* norepinephrine transporter, *NK1R* neurokinin-1 receptor, *RN46A* immortalized serotonergic neural cell line, *SERT* serotonin transporter, *Sf9 Spodoptera frugiperda* insect cells

Contradictory evidence exists concerning a lipid raft role in DAT endocytosis. Lipid raft-mediated DAT endocytosis is linked to the GTPase known as Rin or Rit2 [106], and to flotillin-1 [107], both reportedly localized in raft domains. Different regulatory pathways have been suggested for the "raft" and "non-raft" DAT populations [99], since basal DAT internalization occurs similarly from raft and non-raft domains, while PKC-stimulated DAT internalization occurs only from lipid raft domains [108]. The latter finding does not agree with reports that (1) the clathrin-mediated (i.e., non-raft) endocytosis inhibitor concanvalin A blocks PKC-stimulated loss of surface DAT [99]; (2) coexpression with a dominant-negative mutant of dynamin I inhibits internalization of DAT [109]; and (3) interference with clathrin and dynamin significantly reduces PKC-dependent DAT endocytosis, but flotillin depletion does not [110]. However, depletion of flotillins does decrease lateral mobility of DAT [110], suggesting a limited role for lipid microdomains.

Similar to effects of 5-HT on SERT, DAT substrates, DA and amphetamines, induce internalization and thus downregulation of DA transport [109, 111]. This DA-stimulated trafficking of DAT is accomplished by DA binding to D_2 autoreceptors, triggering the downstream activation of $G\alpha_i$ and protein kinase C [112].

NET. Like SERT, NET is found in lipid microdomains, and NET downregulation is triggered by PKC via a cholesterol-dependent mechanism blocked by filipin and nystatin and accomplished through a lipid raft-mediated internalization that is surprisingly independent not only of dynamin and clathrin but also of caveolae [113]. However, this NET internalization depends on complexing with the neurokinin-1 receptor (NK1R), which occurs in non-raft domains; after agonist binding to NK1R, the NET-NK1R complex translocates to lipid raft membrane domains [46].

Mechanisms of NET inhibition through internalization appear to differ from those described above for SERT and DAT, in that NE does not demonstrate homeostatic regulation of NET [114]. Treatment of PC12 cells with even high concentrations of NE does not alter NET binding properties [115]. Additionally, in 293-hNET cells without the ability to produce NE, NET is down-regulated by desipramine or nisoxetine equally as well as are NE-synthesizing PC12 cells [115].

Monoamine receptors and lipid microdomains

Serotonin (5-HT) receptors

5-HT_{1A} receptor: The 5-HT_{1A} receptor, an ion channel ligated autoreceptor, is coupled to $G\alpha_i$, which mediates inhibitory effects of 5-HT binding on AC and cAMP [116]. Evidence for involvement of lipid microdomains in 5-HT_{1A} regulation includes findings in cell cultures that 5-HT_{1A} receptors distribute to detergent-resistant lipid microdomain fractions together with caveolin-1 [93, 117]. Cholesterol depletion with methyl-β-cyclodextrin inhibits both agonist [118] and antagonist [119] binding and G-protein coupling [118] to 5-HT_{1A} receptors, effects that are reversible with exogenous cholesterol [118]. More direct evidence is the identification of a cholesterol recognition amino acid consensus motif in the 5-HT_{1A} receptor [120]. In a model membrane system, increasing membrane order by manipulating cholesterol, ergosterol, epicholesterol, or sphingomyelin results in increased 5-HT_{1A} receptor activity [121].

5-HT_{2A} receptor: The 5-HT_2 receptor couples to $G\alpha_q$, increasing inositol triphosphate and diacylglycerol levels [116]. 5-HT_{2A} receptors are abundantly expressed in brain and targeted by virtually all atypical antipsychotic agents. Co-localization of 5-HT_{2A} receptors with caveolins occurs in sucrose gradient fractions in a rat tail artery preparation [122]; as co-immunoprecipitated in C6 glioma cells, human embryonic kidney (HEK-293) cells, or rat brain synaptic membrane preparations [34]; and as co-immunoprecipitated in cardiac myocytes [123]. A single study in bovine tracheal muscle, however, found that 5-HT_{2A} co-immunoprecipitated in the non-caveolar fraction [124], although the immunoprecipitation was not studied after 5-HT stimulation. This may be relevant since in cardiac myocytes, 5-HT_{2A} receptors only co-immunoprecipitate with caveolin-3 (a muscle-specific caveolin) upon serotonin stimulation, and are then redistributed into caveolae microdomains, trafficking that is abolished by caveolin-3 knockdown [123]. However, in C6 glioma cells, 5-HT_{2A} and caveolin-1 co-localize, on the cell surface and in intracellular vesicles, exclusively in the unstimulated condition, as 5-HT agonists administration does not induce 5-HT_{2A} receptor internalization [34]. These dissimilarities may be tissue-specific or may reflect methodological differences.

The association of 5-HT_{2A} receptors with caveolae microdomains has functional consequences, inhibiting 5-HT_{2A} receptor-mediated expression of the atrial natriuretic factor (ANF) gene and activation of nuclear factor of activated T cells (NFAT) in cardiac myocytes [123]; and stimulating calcium flux in rat brain synaptic membranes [34]. Physiologic responses to 5-HT stimulation are disrupted by the cholesterol depletion with methyl-β-cyclodextrin and restored by cholesterol repletion in the bovine airway smooth muscle [124] and rat tail artery [122] preparations, and are abolished by caveolin-1 [34] or caveolin-3 [123] knockdown.

5-HT_3 receptor: The only 5-HT receptor that does not bind G-proteins, 5-HT_3 is a member of the Cys-loop pentameric receptor family consisting primarily of five 5-HT_{3A} monomers (although some also contain 5-HT_{3B}). Serotonin binding to 5-HT_3 triggers fast signal transduction across synapses by opening Ca^{2+} channels presynaptically and Ca^{2+}, Na^+, or K^+ channels postsynaptically [125]. Using fluorescence resonance energy transfer technology (FRET), lipid microdomains can be measured on a nanometer scale [40], and ligand binding to 5-HT_{3A} receptors can be quantitated [41]. Cholesterol depletion with methyl-β-cyclodextrin reduces 5-HT_3 function, affecting peak amplitude and kinetics of serotonin-stimulated cation currents [126]. In HEK-293 and neuroblastoma (N1E-115) cells transfected with 5-HT_{3A} receptors, 5-HT_3 receptor proteins localize exclusively in the low buoyant, lipid raft-containing fractions of the sucrose gradient, along with a high content of cholesterol, caveolin-2, and flotillin-1, and a number of antidepressant and antipsychotic medications [127]. In addition to co-localization of these drugs with 5-HT_3 receptors, drug concentrations within lipid microdomain fractions correlated highly with their inhibition of serotonin-induced cation currents. This non-competitive antagonism at the 5-HT_3 receptor was not caused by increased receptor internalization, as demonstrated by immunofluorescence, receptor density in clathrin-coated vesicles, and electrophysiology [127]; the antidepressant antagonism of 5-HT_3 also was shown to be independent of the association of 5-HT_3 with lipid microdomains [127, 128].

5-HT_7 receptor: The 5-HT_7 receptor is coupled to $G\alpha_s$, stimulating cAMP levels [116]. Caveolin-1, cholesterol, sphingomyelin, and gangliosides all regulate 5-HT binding to the 5-HT_7 receptor as well as agonist-induced internalization and signaling. Caveolin-1 and 5-HT_7 receptors co-localize in lipid microdomains [129]. Reduction of cholesterol, by methyl-β-cyclodextrin treatment or cholesterol synthesis inhibition, reduces agonist and antagonist binding to the 5-HT_7 receptor in cell culture, with downstream decreases of 5-HT-induced CREB phosphorylation [33]. Independent of cholesterol-mediated effects, inhibition of sphingolipids and gangliosides attenuates agonist binding at 5-HT_7 receptors [36].

Dopamine (DA) receptors. Dopamine receptors are categorized as D1-class (D_1 and D_5) or D2-class (D_2, D_3, D_4) based on G-protein receptor coupling and associated respective stimulation or inhibition of AC and production of cAMP: stimulating via $G\alpha_s/G\alpha_{olf}$ with D1-class, and inhibiting via $G\alpha_i/G\alpha_o$ with D2-class [130],

although D_3 may also couple to $G\alpha_s$ [131, 132]. In the central nervous system, D1-class receptors are primarily post-synaptic, while D2-class have both pre- and post-synaptic distributions [133].

D1-class (D_1 and D_5) receptors: D_1 receptor: Using both detergent solubilization of rat brain [80] and detergent-free HEK-293 preparations [35, 81], D_1 receptors have been predominantly localized to lipid microdomains. Within the membrane, D_1 localization depends on receptor conformation [35]. As expected, D_1 co-fractionates with Gαs, caveolin-2, flotillins, and other signal transduction proteins [81]. However, D_1 receptor endocytosis can occur via both clathrin-dependent (i.e., non-lipid raft) [134, 135] and caveolar (raft) [81, 135] mechanisms. In heterologously D_1-expressing human renal proximal tubule (hRPT) and HEK-293 cells, a D_1-class receptor agonist fenoldopam increases AC protein in lipid rafts, but not in non-lipid raft domains [136]. Consistent with lipid raft localization, disruption of lipid microdomains with methyl-β-cyclodextrin reduces basal AC activity [136].

D_5 receptor: The D_5 subtype is a high-affinity receptor [137] occurring predominantly in cytoplasmic fractions and not in lipid raft-like subdomains in two studies of rat frontal cortex [80, 138]. Like D_1 receptors, D_5 receptors co-localize with and enhance the activity of anti-oxidizing enzyme paraoxonase in the plasma membrane of hRPT cells and HEK-293 cells heterologously expressing D_5. However, in contrast to D_1 receptors, D_5 agonist binding in HEK-293 cells increases paraoxonase activity [139] and AC protein [136] only in non-rafts although cholesterol depletion with methyl-β-cyclodextrin does reduce AC activity [136].

D2-class (D_2, D_3, D_4) receptors: D_2 receptor: Studies of the D_2 receptor subtype have produced conflicting results. Immunoreactivity assays find a diffuse distribution of the D_2 receptor subtype spanning cytoplasmic, detergent-soluble and detergent-resistant membrane fractions in rat frontal cortex [80]. D_2 receptors are expressed either endogenously in brain or exogenously in HEK-293T cells and localize to lipid microdomains [140, 141], but are unaffected by cholesterol depletion using methyl-β-cyclodextrin [140]. Internalization of D_2 receptors through caveolar endocytosis [142] depends on glycosylation state [79], but dynamin-dependent internalization is also reported [143, 144].

D_3 receptor: Although less abundant than other DA receptors, the D_3 receptor has a much higher affinity for agonists than D_2 receptors. In human proximal tubule cells, D_3 receptors co-localize with G-protein-coupled receptor kinase 4 (GRK4) both in non-raft fractions (90%) and, together with caveolin-1, in lipid raft fractions (10%) isolated using detergent-free sucrose fractionation and co-immunoprecipitation methods [145]. Cholesterol depletion using methyl-β-cyclodextrin causes redistribution of raft-associated D_3 to non-raft fractions [145]. In contrast to D_2 receptors, glycosylation of the D_3 receptor is essential for clathrin-dependent internalization [79]. In brain, D_3 receptors are expressed mainly in limbic regions; they have a low susceptibility to downregulation through internalization, consistent with a role as autoreceptors to monitor DA concentrations [144, 146].

Norepinephrine (NE, adrenergic) receptors. Agonist binding to β1 and β2 adrenergic receptors (β-AR), linked to $G\alpha_s$ proteins and thence to AC, catalyzes the formation of cAMP, with downstream effects such as heart muscle contraction and smooth muscle relaxation. In cardiac myocytes, β1-AR is primarily located in non-raft membrane regions, whereas β2-AR is exclusively localized to caveolae [147] along with signaling components such as AC and $G\alpha_s$ proteins [148, 149]. However, in cardiomyocytes, agonist binding to β2-AR triggers redistribution of the receptor out of caveolae [150], causing sequestration of β2-AR receptors away from the effectors and reducing downstream signaling events [151]. A dissenting view is that localization and signaling of β2-AR do not depend on caveolae, but rather are controlled by protein signaling complexes comprised of scaffold proteins interacting with the receptor carboxy-terminus [152].

Crystallographic studies identify a consensus motif in β2-AR [153] indicating a cholesterol binding site. The effects of β2-AR on cAMP are altered by cholesterol depletion, with disagreement as to whether AC activity and cAMP production are inhibited [148, 149] or enhanced [150, 154, 155]. A disinhibitory mechanism is proposed for enhanced cAMP production, in which β2-AR-induced caveolar endocytosis and concomitant reduction in receptor number is interrupted by the loss of cholesterol [150].

Studies with caveolin-3 dominant-negative mutants in rat cardiomyocytes find that caveolin-3 directly regulates β2-AR functioning. Caveolin-3 loss leads to β2-AR-cAMP signaling that mimics heart failure, which is rescued by caveolin-3 overexpression [156].

DISCUSSION

Summary of findings

Monoaminergic transporters. The evidence supports an association of lipid microdomains with SERT and NET, but is less clear with respect to DAT. SERT co-localizes with caveolin and GM1 and is visualized in cholesterol-rich membrane microdomains [16, 90], and its functioning is adversely affected by cholesterol depletion [51, 52]. NET demonstrates cholesterol-dependent trafficking [46, 113] and internalization that is mediated neither by clathrin nor caveolin [113]. In contrast, DAT occurs across raft and non-raft domains [99], and, although DAT are affected by changes in cholesterol [100], these effects are not clearly lipid raft-mediated.

Monoaminergic receptors. There is strong evidence for lipid microdomain regulation of serotonin function for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors, although leading to very different downstream effects: 5-HT_{1A} couples to $G\alpha_i$, with inhibitory effects on AC and cAMP; 5-HT_7 couples to $G\alpha_s$, stimulating cAMP levels; and 5-HT_2 couples to $G\alpha_q$, increasing inositol triphosphate and diacylglycerol levels [116]. The 5-HT_{1A} receptors co-localize with caveolin-1 in a palmitoylation-dependent manner [93], and cholesterol depletion affects binding to 5-HT_{1A} receptors and signaling [118, 119], effects that are reversed by replenishing cholesterol [118]. Most studies of 5-HT_{2A} [34, 122, 123] but not all [124] find co-localization with caveolins. Findings also are conflicting with regard to caveolin association in agonist-stimulated [123] vs. unstimulated [34] conditions; however, in all studies responses to agonist binding to 5-HT_{2A} receptors were affected by cholesterol depletion or caveolin knockdown [34, 122–124]. Lipid rafts regulate 5-HT_7 binding, the ensuing functional sequelae, and receptor internalization, although it should be noted that all studies have been performed by one group [33, 36, 129]. Lipid microdomain associations with 5-HT_3 have been studied primarily in the context of psychotropic medications, many of which co-localize with the 5-HT_3 receptor in lipid rafts (see section "Lipid microdomains and antidepressant treatment" below).

The role of lipid microdomains in the regulation of both D1- and D2-class dopaminergic receptors is complex. D_1 receptors localize in raft domains [80, 81], and agonist binding to AC protein increases in lipid rafts, but raft localization has only been observed in certain receptor conformations [35]. Both caveolar [81, 135] and clathrin-dependent [134, 135] endocytosis have been reported. The D_5 receptor, on the other hand, does not localize in lipid microdomains [80, 138]; its effects on paraoxonase activity are seen only in non-rafts [139], yet it does mediate effects on AC activity that are attenuated with cholesterol depletion [136]. Also poorly understood are lipid raft effects on D_2 receptors, which are diffusely distributed across membrane domains [80, 140, 141], but

are unaffected by cholesterol depletion [140]. Endocytosis of D_2 receptors can be both caveolar [142] and dynamin-dependent [143, 144].

The evidence strongly supports a key role of lipid microdomains with regard to β2-AR but not β1-AR adrenergic (norepinephrine) receptors: β2-AR are localized to caveolar lipid microdomains [147] along with relevant signaling components [148, 149], and are dependent on cholesterol [150, 153] and caveolin-3 [156], with downstream effects on AC and cAMP [148–150, 154, 155].

One caveat in interpreting the possible importance of these findings for depression is that studies of lipid microdomains with respect to 5-HT_7 and D_3 receptors were carried out only in cells derived from kidney, and likewise β-AR were studied exclusively in cardiomyocytes. Therefore, assuming that lipid microdomain effects on 5-HT_7, D_3, and β-AR have ramifications for brain function and depression, is premature.

Mechanisms of lipid microdomain effects in monoaminergic receptor signaling. Early studies focused on establishing co-localization of receptor and transporter molecules in lipid microdomains. More recent research has expanded the field of study to include related elements of signaling complexes and to begin exploration of structure–function relationships related to lipid microdomains. For instance, a salient characteristic of most monoaminergic receptors is coupling to G-proteins at the cytoplasmic membrane leaflet, and it turns out that lipid microdomains have regulatory effects on G-proteins, impacting localization, trafficking, and signaling. Among the G-protein isoforms associated with monoaminergic receptors, studies in rat lung homogenate find that $G\alpha_s$ (with which 5-HT_7 and β2-AR associate) and $G\alpha_i$ (5-HT_{1A}) tend to localize in lipid microdomains, while $G\alpha_q$ (5-HT_{2A}) more than $G\alpha_s$ or $G\alpha_i$, are enriched in caveolae [157]. As an example of lipid microdomain-mediated trafficking of G-proteins related to monoaminergic signaling, in C6 glioma and MCF-7 adenoma cells that express only endogenous β2-AR, $G\alpha_s$ undergoes internalization within vesicles in response to agonist binding [158], a process blocked by caveolin knockdown or cholesterol depletion [159]. Furthermore, lipid microdomains can have effects downstream from agonist-receptor binding, e.g., in C6 cells, caveolin knockout or knockdown interferes directly with $G\alpha_s$ stimulation of adenylyl cyclase [159].

A growing body of research shows that monoaminergic receptors in the membrane not only interact with G-proteins, but also form complexes with a variety of other molecules in association with lipid microdomains, including pre-synaptic receptors [46], membrane proteins involved in exocytosis [45, 160, 161], and hundreds of adaptor proteins that modulate maturation, internalization, targeting for degradation, and recycling of monoamine receptors [162].

Indirect effects on monoaminergic function via lipid microdomains also have been observed. For example, the raft-associated cellular prion protein (PrP^C) inhibits GSK3β kinase in serotonergic cells in the raphe region, with the downstream effects of suppressing 5-HT_{1B} receptor activity [163]. As 5-HT_{1B} receptors are inhibitory, the net effect is an increase in serotonergic activity.

Lipid microdomains and antidepressant treatment

Although selective serotonin re-uptake inhibitors (SSRI) act acutely on SERT in pre-synaptic neurons, the therapeutic effects of these antidepressants occur after chronic exposure and are believed to occur via downstream effects on post-synaptic 5-HT receptors [164]. Consistent with this hypothesis, accumulation of the active *S*- but not the inactive *R*- enantiomer of citalopram triggers $G\alpha_s$ translocation from raft to non-raft regions in C6 glioma cells that do not express SERT, and is not enhanced in cells that contain transfected SERT [165].

Studies of postmortem brain tissue from suicide decedents with confirmed unipolar major depression find increased accumulation of $G\alpha_s$ in lipid microdomains [166]. Treatment with several chemically distinct antidepressants facilitates translocation of $G\alpha_s$, but not other G-proteins, from lipid microdomains to non-raft fractions and into closer association with AC, potentiating its activity [165, 167–170]. This translocation might contribute to the increased cAMP tone and synaptic changes observed subsequent to chronic antidepressant treatment [171]. These studies are also in agreement with observations that rolipram, a cAMP-phosphodiesterase inhibitor, has antidepressant effects [172]. On the other hand, mood stabilizers lithium and valproate have the opposite effect, causing increased $G\alpha_s$ in lipid microdomains [173], thus suggesting a biphasic effect at the molecular level for treatment of the two "poles" of bipolar illness. Among monoaminergic receptors, D_1 and D_3, 5-HT_7, and β1-AR and β2-AR are known to couple with $G\alpha_s$ and cause increased cAMP, although only D_1, 5-HT_7, and β2-AR co-localize predominantly in lipid raft fractions. Possible connections between these elements and antidepressant mechanisms could be a promising area of future investigation.

In cell culture, the 5-HT_3 receptor protein localizes exclusively to fractions containing lipid microdomains, which are also the same fractions robustly enriched in antidepressants (desipramine, fluoxetine, and reboxetine) and antipsychotics (fluphenazine, haloperidol, and clozapine) [127]. Concentrations of these psychotropic medications correlate with the magnitude of non-competitive inhibition of 5-HT_3 serotonin-induced cation currents [127]. However, the ability of lipid raft co-localizers desipramine and fluoxetine to counter 5-HT_3 effects in N1E-115 cells was not affected by cholesterol depletion [126]. Thus, although lipid rafts have an impact on 5-HT_3 function, antidepressant antagonism of 5-HT_3 appears to be independent of lipid microdomain status [126–128].

In addition to studies in postmortem brain and in vitro cell cultures, ex vivo studies of human tissue such as platelets [44] and B-cells [174] are now feasible approaches to the study of lipid microdomains in humans. Outcome measures of these approaches are physicochemical microdomain properties such as membrane order [44] and microdomain clustering [174] that could be tested alone or in combination with monoaminergic parameters as potential biomarkers for depression or as predictors of antidepressant response.

Lipid microdomains as a target for polyunsaturated fatty acid (PUFA) effects in depression

Lipid raft molecular organization and raft-associated protein distribution are highly susceptible to modulation by long chain *n*-3 PUFAs, dietarily essential fatty acids that are generally low in Western diets [175], and very low in major depressive disorder [176] and in patients at high risk for suicide [177–179]. There is strong evidence that *n*-3 PUFAs, upon incorporation into the plasma membrane as acyl chains of glycerophospholipids, can either prevent or promote lipid raft formation, depending on several factors: cell type, the relative concentrations of eicosapentaenoic acid (EPA, 20:5,*n*-3), docosahexaenoic acid (DHA, 22:6,*n*-3), and docosapentaenoic acid (DPA, 22:5,*n*-3), and the phosphatidyl moiety to which the *n*-3 PUFA is bound [180–183].

It has been hypothesized that *n*-3 PUFA effects on depression [184–186] are mediated by their ability to modify lipid rafts, with downstream effects on G-protein signaling [187]. If correct, monoaminergic transporters and receptors are possible candidates as mechanistic intermediaries. Membrane *n*-3 PUFAs affect oligomerization kinetics of adenosine A_{2A} and D_2 receptors via effects on biophysical membrane properties [188], and in rodent models in a cardiovascular context, α-linolenic acid (ALA, 18:3,*n*-3) enhances caveolin-3 expression [189] and prevents cardiac

damage due to β-adrenergic overstimulation, an effect blocked by preadministration with methyl-β-cyclodextrin [190]. Other possible lipid microdomain-associated targets for PUFA effects in depression relate to the anti-inflammatory and pro-resolving properties of *n*-3 PUFA, as inflammation likely contributes to depression etiology in a subset of patients [191, 192]. For example, in B-cell cultures, DHA inhibits Toll-like receptor (TLR) dimerization and recruitment into lipid microdomains [193].

Antihyperlipidemic drugs and suicide risk

Lower cholesterol is linked to suicide risk [194–196], and in nonhuman primates, low cholesterol is associated with lower CSF levels of the 5-HT metabolite 5-HIAA, blunted prolactin response to fenfluramine-stimulated 5-HT release, and increases in aggressive behaviors [197].

The aggregate evidence presented herein supports a hypothesis that effects of cholesterol-lowering therapies on suicide risk are mediated by influencing lipid raft regulation of plasma membrane serotonergic proteins, since cholesterol depletion decreases the transport rate (V_{max}) and affinity (K_m) of 5-HT for SERT [51, 52], agonist and antagonist binding to 5-HT_{1A} and 5-HT_7 [33, 118, 119], and physiologic responses of 5-HT_{2A} to 5-HT stimulation [122, 124].

CONCLUSIONS

Our literature review suggests that lipid microdomains are a significant modulator of monoaminergic neurotransmission, influencing co-localization of transporters/receptors with other signaling proteins and adaptor molecules; trafficking by endocytosis; agonist and antagonist binding; and downstream second messenger-mediated events. Limitations on knowledge reflect the limitations of current experimental methodologies for nanoscale discrimination of spatial and temporal aspects of neuronal signaling. In addition, there is a paucity of clinical studies, which are needed to advance understanding of lipid microdomain effects on monoaminergic signaling with respect to etiology, diagnosis, and treatment of major depression. Thus, the next wave of research should seek to move toward the study of monoaminergic–lipid microdomain interactions in humans with and without depression. One approach would be to measure physicochemical effects on microdomain structure, using ex vivo techniques, of substances already known to be safe for human use that affect lipid microdomains, such as cholesterol-lowering drugs and *n*-3 PUFAs.

FUNDING

This study was supported in part by the following grants: K08 MH079033 (PI, MES), NIH R01AT008375 (PI, SRS), NIH 8G12 MD007603 (Area Leader, RES). Dr. JJM receives royalties for commercial use of the Columbia-Suicide Severity Rating Scale (C-SSRS) from the Research Foundation for Mental Hygiene.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Lorent JH, Levental I. Structural determinants of protein partitioning into ordered membrane domains and lipid rafts. Chem Phys Lipids. 2015;192:23–32.
2. Levental I, Veatch SL. The continuing mystery of lipid rafts. J Mol Biol. 2016;428:4749–64.
3. Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. Nat Rev Neurosci. 2007;8:128–40.
4. Hancock JF. Lipid rafts: contentious only from simplistic standpoints. Nat Rev Mol Cell Biol. 2006;7:456–62.
5. Pralle A, Keller P, Florin EL, Simons K, Horber JK. Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. J Cell Biol. 2000;148:997–1008.
6. Varma R, Mayor S. GPI-anchored proteins are organized in submicron domains at the cell surface. Nature. 1998;394:798–801.
7. Wilson BS, Pfeiffer JR, Oliver JM. Observing FcepsilonRI signaling from the inside of the mast cell membrane. J Cell Biol. 2000;149:1131–42.
8. Friedrichson T, Kurzchalia TV. Microdomains of GPI-anchored proteins in living cells revealed by crosslinking. Nature. 1998;394:802–5.
9. Harder T, Scheiffele P, Verkade P, Simons K. Lipid domain structure of the plasma membrane revealed by patching of membrane components. J Cell Biol. 1998;141:929–42.
10. Head BP, Patel HH, Insel PA. Interaction of membrane/lipid rafts with the cytoskeleton: impact on signaling and function: membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. Biochim Biophys Acta. 2014;1838:532–45.
11. Trushina E, Du Charme J, Parisi J, McMurray CT. Neurological abnormalities in caveolin-1 knock out mice. Behav Brain Res. 2006;172:24–32.
12. Kovtun O, Tillu VA, Ariotti N, Parton RG, Collins BM. Cavin family proteins and the assembly of caveolae. J Cell Sci. 2015;128:1269–78.
13. Kokubo H, Helms JB, Ohno-Iwashita Y, Shimada Y, Horikoshi Y, Yamaguchi H. Ultrastructural localization of flotillin-1 to cholesterol-rich membrane microdomains, rafts, in rat brain tissue. Brain Res. 2003;965:83–90.
14. Lang DM, Lommel S, Jung M, Ankerhold R, Petrausch B, Laessing U, et al. Identification of reggie-1 and reggie-2 as plasma membrane-associated proteins which cocluster with activated GPI-anchored cell adhesion molecules in non-caveolar micropatches in neurons. J Neurobiol. 1998;37:502–23.
15. Glebov OO, Bright NA, Nichols BJ. Flotillin-1 defines a clathrin-independent endocytic pathway in mammalian cells. Nat Cell Biol. 2006;8:46–54.
16. Chang JC, Tomlinson ID, Warnement MR, Ustione A, Carneiro AM, Piston DW, et al. Single molecule analysis of serotonin transporter regulation using antagonist-conjugated quantum dots reveals restricted, p38 MAPK-dependent mobilization underlying uptake activation. J Neurosci. 2012;32:8919–29.
17. Munro S. Lipid rafts: elusive or illusive? Cell. 2003;115:377–88.
18. Owen DM, Williamson DJ, Magenau A, Gaus K. Sub-resolution lipid domains exist in the plasma membrane and regulate protein diffusion and distribution. Nat Commun. 2012;3:1256.
19. Frisz JF, Klitzing HA, Lou K, Hutcheon ID, Weber PK, Zimmerberg J, et al. Sphingolipid domains in the plasma membranes of fibroblasts are not enriched with cholesterol. J Biol Chem. 2013;288:16855–61.
20. Frisz JF, Lou K, Klitzing HA, Hanafin WP, Lizunov V, Wilson RL, et al. Direct chemical evidence for sphingolipid domains in the plasma membranes of fibroblasts. Proc Natl Acad Sci USA. 2013;110:E613–22.
21. Kraft ML. Plasma membrane organization and function: moving past lipid rafts. Mol Biol Cell. 2013;24:2765–68.
22. Kusumi A, Fujiwara TK, Chadda R, Xie M, Tsunoyama TA, Kalay Z, et al. Dynamic organizing principles of the plasma membrane that regulate signal transduction: commemorating the fortieth anniversary of Singer and Nicolson's fluid-mosaic model. Annu Rev Cell Dev Biol. 2012;28:215–50.
23. Kwik J, Boyle S, Fooksman D, Margolis L, Sheetz MP, Edidin M. Membrane cholesterol, lateral mobility, and the phosphatidylinositol 4,5-bisphosphate-dependent organization of cell actin. Proc Natl Acad Sci USA. 2003;100:13964–9.
24. Sevcsik E, Brameshuber M, Folser M, Weghuber J, Honigmann A, Schutz GJ. GPI-anchored proteins do not reside in ordered domains in the live cell plasma membrane. Nat Commun. 2015;6:6969.
25. Brenner B, Harney JT, Ahmed BA, Jeffus BC, Unal R, Mehta JL, et al. Plasma serotonin levels and the platelet serotonin transporter. J Neurochem. 2007;102:206–15.
26. Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell. 1992;68:533–44.
27. Babiychuk EB, Draeger A. Biochemical characterization of detergent-resistant membranes: a systematic approach. Biochem J. 2006;397:407–16.
28. Lichtenberg D, Goni FM, Heerklotz H. Detergent-resistant membranes should not be identified with membrane rafts. Trends Biochem Sci. 2005;30:430–6.
29. Heerklotz H. Triton promotes domain formation in lipid raft mixtures. Biophys J. 2002;83:2693–701.
30. Song KS, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti MP. Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains. J Biol Chem. 1996;271:9690–7.

31. Schnitzer JE, Oh P, Pinney E, Allard J. Filipin-sensitive caveolae-mediated transport in endothelium: reduced transcytosis, scavenger endocytosis, and capillary permeability of select macromolecules. J Cell Biol. 1994;127:1217–32.
32. Zidovetzki R, Levitan I. Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies. Biochim Biophys Acta. 2007;1768:1311–24.
33. Sjogren B, Hamblin MW, Svenningsson P. Cholesterol depletion reduces serotonin binding and signaling via human 5-HT(7(a)) receptors. Eur J Pharmacol. 2006;552:1–10.
34. Bhatnagar A, Sheffler DJ, Kroeze WK, Compton-Toth B, Roth BL. Caveolin-1 interacts with 5-HT2A serotonin receptors and profoundly modulates the signaling of selected Galphaq-coupled protein receptors. J Biol Chem. 2004;279:34614–23.
35. Mystek P, Dutka P, Tworzydlo M, Dziedzicka-Wasylewska M, Polit A. The role of cholesterol and sphingolipids in the dopamine D1 receptor and G protein distribution in the plasma membrane. Biochim Biophys Acta. 2016;1861:1775–86.
36. Sjogren B, Svenningsson P. Depletion of the lipid raft constituents, sphingomyelin and ganglioside, decreases serotonin binding at human 5-HT7(a) receptors in HeLa cells. Acta Physiol. 2007;190:47–53.
37. Gaus K, Inoue T. New biological frontiers illuminated by molecular sensors and actuators. Biophys J. 2016;111:E01–02.
38. Shaikh SR, Boyle S, Edidin M. A high fat diet containing saturated but not unsaturated fatty acids enhances T cell receptor clustering on the nanoscale. PLEFA. 2015;100:1–4.
39. Mueller V, Honigmann A, Ringemann C, Medda R, Schwarzmann G, Eggeling C. FCS in STED microscopy: studying the nanoscale of lipid membrane dynamics. Methods Enzymol. 2013;519:1–38.
40. Zacharias DA, Violin JD, Newton AC, Tsien RY. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science. 2002;296:913–6.
41. Ilegems E, Pick H, Deluz C, Kellenberger S, Vogel H. Ligand binding transmits conformational changes across the membrane-spanning region to the intracellular side of the 5-HT3 serotonin receptor. Chembiochem. 2005;6:2180–5.
42. Abu-Arish A, Pandzic E, Goepp J, Matthes E, Hanrahan JW, Wiseman PW. Cholesterol modulates CFTR confinement in the plasma membrane of primary epithelial cells. Biophys J. 2015;109:85–94.
43. Polozov IV, Gawrisch K. NMR detection of lipid domains. In: McIntosh TJ, editor. Lipid rafts. Totowa: Himana Press; 2007. p. 107–26.
44. Cenido JF, Itin B, Stark RE, Huang YY, Oquendo MA, John Mann J, et al. Characterization of lipid rafts in human platelets using nuclear magnetic resonance: a pilot study. Biochem Biophys Rep. 2017;10:132–6.
45. Butler B, Saha K, Rana T, Becker JP, Sambo D, Davari P, et al. Dopamine transporter activity is modulated by alpha-synuclein. J Biol Chem. 2015;290:29542–54.
46. Arapulisamy O, Mannangatti P, Jayanthi LD. Regulated norepinephrine transporter interaction with the neurokinin-1 receptor establishes transporter subcellular localization. J Biol Chem. 2013;288:28599–610.
47. Triantafilou M, Morath S, Mackie A, Hartung T, Triantafilou K. Lateral diffusion of Toll-like receptors reveals that they are transiently confined within lipid rafts on the plasma membrane. J Cell Sci. 2004;117:4007–14.
48. Nichols B. Caveosomes and endocytosis of lipid rafts. J Cell Sci. 2003;116:4707–14.
49. Rajendran L, Simons K. Lipid rafts and membrane dynamics. J Cell Sci. 2005;118:1099–102.
50. Le Roy C, Wrana JL. Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling. Nat Rev Mol Cell Biol. 2005;6:112–26.
51. Magnani F, Tate CG, Wynne S, Williams C, Haase J. Partitioning of the serotonin transporter into lipid microdomains modulates transport of serotonin. J Biol Chem. 2004;279:38770–8.
52. Scanlon SM, Williams DC, Schloss P. Membrane cholesterol modulates serotonin transporter activity. Biochemistry. 2001;40:10507–13.
53. Mann JJ. The serotonergic system in mood disorders and suicidal behaviour. Philos Trans R Soc Lond B Biol Sci. 2013;368:20120537.
54. Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem. 1994;40:288–95.
55. Sellers EM, Higgins GA, Tompkins DM, Romach MK. Serotonin and alcohol drinking. NIDA Res Monogr. 1992;119:141–5.
56. Coccaro EF. Central serotonin and impulsive aggression. Br J Psychiatry. 1989;155:52–62.
57. Compagnon P, Ernouf D, Narcisse G, Daoust M. Serotonin in animal models of alcoholism. Alcohol Alcohol Suppl. 1993;2:215–9.
58. Carlsson A. Perspectives on the discovery of central monoaminergic neurotransmission. Annu Rev Neurosci. 1987;10:19–40.
59. Koob GF, Sanna PP, Bloom FE. Neuroscience of addiction. Neuron. 1998;21:467–76.
60. Bannon M, Granneman J. The dopamine transport. Potential involvement in neuropsychiatric disorders. In: Bloom F, Kupfer D, editors. Psychopharmacoogy: the fourth generation of progress. New York, NY: Raven Press; 1995. p. 179–87.
61. Ressler KJ, Nemeroff CB. Role of norepinephrine in the pathophysiology and treatment of mood disorders. Biol Psychiatry. 1999;46:1219–33.
62. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry. 1965;122:509–22.
63. Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, et al. Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J Neurosci. 1997;17:8451–8.
64. Leonard BE. The role of noradrenaline in depression: a review. J Psychopharmacol. 1997;11:S39–47.
65. Blier P, de Montigny C, Chaput Y. A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. J Clin Psychiatry. 1990;51:14–20.
66. Gray NA, Milak MS, DeLorenzo C, Ogden RT, Huang YY, Mann JJ, et al. Antidepressant treatment reduces serotonin-1A autoreceptor binding in major depressive disorder. Biol Psychiatry. 2013;74:26–31.
67. Ramamoorthy S, Shippenberg TS, Jayanthi LD. Regulation of monoamine transporters: Role of transporter phosphorylation. Pharmacol Ther. 2011;129:220–38.
68. Barker EL, Blakely RD. Norephinephrine and serotonin transporters: molecular targets of antidepressant drugs. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York, NY: Raven Press; 1995. p. 321–33.
69. Richelson E. Interactions of antidepressants with neurotransmitter transporters and receptors and their clinical relevance. J Clin Psychiatry. 2003;64:5–12.
70. Jayanthi LD, Vargas G, DeFelice LJ. Characterization of cocaine and antidepressant-sensitive norepinephrine transporters in rat placental trophoblasts. Br J Pharmacol. 2002;135:1927–34.
71. Li Q, Ma L, Innis RB, Seneca N, Ichise M, Huang H, et al. Pharmacological and genetic characterization of two selective serotonin transporter ligands: 2-[2-(dimethylaminomethylphenylthio)]-5-fluoromethylphenylamine (AFM) and 3-amino-4-[2-(dimethylaminomethyl-phenylthio)]benzonitrile (DASB). J Pharmacol Exp Ther. 2004;308:481–6.
72. Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A, et al. Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. Mol Pharmacol. 1998;53:649–55.
73. Rioux A, Fabre V, Lesch KP, Moessner R, Murphy DL, Lanfumey L, et al. Adaptive changes of serotonin 5-HT2A receptors in mice lacking the serotonin transporter. Neurosci Lett. 1999;262:113–16.
74. Sora I, Hall FS, Andrews AM, Itokawa M, Li XF, Wei HB, et al. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc Natl Acad Sci USA. 2001;98:5300–05.
75. Adriani W, Boyer F, Gioiosa L, Macri S, Dreyer JL, Laviola G. Increased impulsive behavior and risk proneness following lentivirus-mediated dopamine transporter over-expression in rats' nucleus accumbens. Neuroscience. 2009;159:47–58.
76. Gainetdinov RR, Caron MG. Monoamine transporters: from genes to behavior. Annu Rev Pharmacol Toxicol. 2003;43:261–84.
77. Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science. 1999;283:397–401.
78. Hall FS, Li XF, Sora I, Xu F, Caron M, Lesch KP, et al. Cocaine mechanisms: enhanced cocaine, fluoxetine and nisoxetine place preferences following monoamine transporter deletions. Neuroscience. 2002;115:153–61.
79. Min C, Zheng M, Zhang X, Guo S, Kwon KJ, Shin CY, et al. N-linked glycosylation on the N-terminus of the dopamine D2 and D3 receptors determines receptor association with specific microdomains in the plasma membrane. Biochim Biophys Acta. 2015;1853:41–51.
80. Voulalas PJ, Schetz J, Undieh AS. Differential subcellular distribution of rat brain dopamine receptors and subtype-specific redistribution induced by cocaine. Mol Cell Neurosci. 2011;46:645–54.
81. Yu P, Yang Z, Jones JE, Wang Z, Owens SA, Mueller SC, et al. D1 dopamine receptor signaling involves caveolin-2 in HEK-293 cells. Kidney Int. 2004;66:2167–80.
82. Torres GE, Carneiro A, Seamans K, Fiorentini C, Sweeney A, Yao WD, et al. Oligomerization and trafficking of the human dopamine transporter. Mutational analysis identifies critical domains important for the functional expression of the transporter. J Biol Chem. 2003;278:2731–9.
83. Kocabas AM, Rudnick G, Kilic F. Functional consequences of homo- but not hetero-oligomerization between transporters for the biogenic amine neurotransmitters. J Neurochem. 2003;85:1513–20.

84. Anderluh A, Hofmaier T, Klotzsch E, Kudlacek O, Stockner T, Sitte HH, et al. Direct PIP2 binding mediates stable oligomer formation of the serotonin transporter. Nat Commun. 2017;8:14089.
85. Liu Y, Casey L, Pike LJ. Compartmentalization of phosphatidylinositol 4,5-bisphosphate in low-density membrane domains in the absence of caveolin. Biochem Biophys Res Commun. 1998;245:684–90.
86. Pike LJ, Casey L. Localization and turnover of phosphatidylinositol 4,5-bisphosphate in caveolin-enriched membrane domains. J Biol Chem. 1996;271:26453–6.
87. Khelashvili G, Weinstein H. Functional mechanisms of neurotransmitter transporters regulated by lipid–protein interactions of their terminal loops. Biochim Biophys Acta. 2015;1848:1765–74.
88. Buchmayer F, Schicker K, Steinkellner T, Geier P, Stubiger G, Hamilton PJ, et al. Amphetamine actions at the serotonin transporter rely on the availability of phosphatidylinositol-4,5-bisphosphate. Proc Natl Acad Sci USA. 2013;110:11642–7.
89. Hamilton PJ, Belovich AN, Khelashvili G, Saunders C, Erreger K, Javitch JA, et al. PIP2 regulates psychostimulant behaviors through its interaction with a membrane protein. Nat Chem Biol. 2014;10:582–9.
90. Samuvel DJ, Jayanthi LD, Bhat NR, Ramamoorthy S. A role for p38 mitogen-activated protein kinase in the regulation of the serotonin transporter: evidence for distinct cellular mechanisms involved in transporter surface expression. J Neurosci. 2005;25:29–41.
91. Whitworth TL, Herndon LC, Quick MW. Psychostimulants differentially regulate serotonin transporter expression in thalamocortical neurons. J Neurosci. 2002;22:RC192.
92. Carneiro AM, Blakely RD. Serotonin-, protein kinase C-, and Hic-5-associated redistribution of the platelet serotonin transporter. J Biol Chem. 2006;281:24769–80.
93. Renner U, Glebov K, Lang T, Papusheva E, Balakrishnan S, Keller B, et al. Localization of the mouse 5-hydroxytryptamine(1A) receptor in lipid microdomains depends on its palmitoylation and is involved in receptor-mediated signaling. Mol Pharmacol. 2007;72:502–13.
94. Jorgensen TN, Christensen PM, Gether U. Serotonin-induced down-regulation of cell surface serotonin transporter. Neurochem Int. 2014;73:107–12.
95. Myers CL, Lazo JS, Pitt BR. Translocation of protein kinase C is associated with inhibition of 5-HT uptake by cultured endothelial cells. Am J Physiol. 1989;257: L253–8.
96. Qian Y, Galli A, Ramamoorthy S, Risso S, DeFelice LJ, Blakely RD. Protein kinase C activation regulates human serotonin transporters in HEK-293 cells via altered cell surface expression. J Neurosci. 1997;17:45–57.
97. Sorensen L, Stromgaard K, Kristensen AS. Characterization of intracellular regions in the human serotonin transporter for phosphorylation sites. ACS Chem Biol. 2014;9:935–44.
98. Ramamoorthy S, Blakely RD. Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. Science. 1999;285:763–6.
99. Foster JD, Adkins SD, Lever JR, Vaughan RA. Phorbol ester induced trafficking-independent regulation and enhanced phosphorylation of the dopamine transporter associated with membrane rafts and cholesterol. J Neurochem. 2008;105:1683–99.
100. Jones KT, Zhen J, Reith ME. Importance of cholesterol in dopamine transporter function. J Neurochem. 2012;123:700–15.
101. Adkins EM, Samuvel DJ, Fog JU, Eriksen J, Jayanthi LD, Vaegter CB, et al. Membrane mobility and microdomain association of the dopamine transporter studied with fluorescence correlation spectroscopy and fluorescence recovery after photobleaching. Biochemistry. 2007;46:10484–97.
102. Sorkina T, Hoover BR, Zahniser NR, Sorkin A. Constitutive and protein kinase C-induced internalization of the dopamine transporter is mediated by a clathrin-dependent mechanism. Traffic. 2005;6:157–70.
103. Vainio S, Jansen M, Koivusalo M, Rog T, Karttunen M, Vattulainen I, et al. Significance of sterol structural specificity. Desmosterol cannot replace cholesterol in lipid rafts. J Biol Chem. 2006;281:348–55.
104. Daniels GM, Amara SG. Regulated trafficking of the human dopamine transporter. Clathrin-mediated internalization and lysosomal degradation in response to phorbol esters. J Biol Chem. 1999;274:35794–801.
105. Hong WC, Amara SG. Membrane cholesterol modulates the outward facing conformation of the dopamine transporter and alters cocaine binding. J Biol Chem. 2010;285:32616–26.
106. Navaroli DM, Stevens ZH, Uzelac Z, Gabriel L, King MJ, Lifshitz LM, et al. The plasma membrane-associated GTPase Rin interacts with the dopamine transporter and is required for protein kinase C-regulated dopamine transporter trafficking. J Neurosci. 2011;31:13758–70.
107. Cremona ML, Matthies HJ, Pau K, Bowton E, Speed N, Lute BJ, et al. Flotillin-1 is essential for PKC-triggered endocytosis and membrane microdomain localization of DAT. Nat Neurosci. 2011;14:469–77.
108. Gabriel LR, Wu S, Kearney P, Bellve KD, Standley C, Fogarty KE, et al. Dopamine transporter endocytic trafficking in striatal dopaminergic neurons: differential dependence on dynamin and the actin cytoskeleton. J Neurosci. 2013;33:17836–46.
109. Saunders C, Ferrer JV, Shi L, Chen J, Merrill G, Lamb ME, et al. Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. Proc Natl Acad Sci USA. 2000;97:6850–5.
110. Sorkina T, Caltagarone J, Sorkin A. Flotillins regulate membrane mobility of the dopamine transporter but are not required for its protein kinase C dependent endocytosis. Traffic. 2013;14:709–24.
111. Chi L, Reith ME. Substrate-induced trafficking of the dopamine transporter in heterologously expressing cells and in rat striatal synaptosomal preparations. J Pharmacol Exp Ther. 2003;307:729–36.
112. Chen R, Daining CP, Sun H, Fraser R, Stokes SL, Leitges M, et al. Protein kinase Cbeta is a modulator of the dopamine D2 autoreceptor-activated trafficking of the dopamine transporter. J Neurochem. 2013;125:663–72.
113. Jayanthi LD, Samuvel DJ, Ramamoorthy S. Regulated internalization and phosphorylation of the native norepinephrine transporter in response to phorbol esters. Evidence for localization in lipid rafts and lipid raft-mediated internalization. J Biol Chem. 2004;279:19315–26.
114. Mandela P, Ordway GA. The norepinephrine transporter and its regulation. J Neurochem. 2006;97:310–33.
115. Zhu MY, Ordway GA. Down-regulation of norepinephrine transporters on PC12 cells by transporter inhibitors. J Neurochem. 1997;68:134–41.
116. Fantini J, Barrantes FJ. Sphingolipid/cholesterol regulation of neurotransmitter receptor conformation and function. Biochim Biophys Acta. 2009;1788:2345–61.
117. Kalipatnapu S, Chattopadhyay A. Membrane organization of the human serotonin(1A) receptor monitored by detergent insolubility using GFP fluorescence. Mol Membr Biol. 2005;22:539–47.
118. Pucadyil TJ, Chattopadhyay A. Cholesterol modulates ligand binding and G-protein coupling to serotonin(1A) receptors from bovine hippocampus. Biochim Biophys Acta. 2004;1663:188–200.
119. Sjogren B, Csoregh L, Svenningsson P. Cholesterol reduction attenuates 5-HT1A receptor-mediated signaling in human primary neuronal cultures. Naunyn Schmiede Arch Pharmacol. 2008;378:441–6.
120. Jafurulla M, Tiwari S, Chattopadhyay A. Identification of cholesterol recognition amino acid consensus (CRAC) motif in G-protein coupled receptors. Biochem Biophys Res Commun. 2011;404:569–73.
121. Gutierrez MG, Mansfield KS, Malmstadt N. The functional activity of the human serotonin 5-HT1A receptor is controlled by lipid bilayer composition. Biophys J. 2016;110:2486–95.
122. Dreja K, Voldstedlund M, Vinten J, Tranum-Jensen J, Hellstrand P, Sward K. Cholesterol depletion disrupts caveolae and differentially impairs agonist-induced arterial contraction. Arterioscler Thromb Vasc Biol. 2002;22:1267–72.
123. Mialet-Perez J, D'Angelo R, Villeneuve C, Ordener C, Negre-Salvayre A, Parini A, et al. Serotonin 5-HT2A receptor-mediated hypertrophy is negatively regulated by caveolin-3 in cardiomyoblasts and neonatal cardiomyocytes. J Mol Cell Cardiol. 2012;52:502–10.
124. Sommer B, Montano LM, Carbajal V, Flores-Soto E, Ortega A, Ramirez-Oseguera R, et al. Extraction of membrane cholesterol disrupts caveolae and impairs serotonergic (5-HT2A) and histaminergic (H1) responses in bovine airway smooth muscle: role of Rho-kinase. Can J Physiol Pharmacol. 2009;87:180–95.
125. Wu ZS, Cheng H, Jiang Y, Melcher K, Xu HE. Ion channels gated by acetylcholine and serotonin: structures, biology, and drug discovery. Acta Pharmacol Sin. 2015;36:895–907.
126. Nothdurfter C, Tanasic S, Di Benedetto B, Rammes G, Wagner EM, Kirmeier T, et al. Impact of lipid raft integrity on 5-HT3 receptor function and its modulation by antidepressants. Neuropsychopharmacology. 2010;35:1510–9.
127. Eisensamer B, Uhr M, Meyr S, Gimpl G, Deiml T, Rammes G, et al. Antidepressants and antipsychotic drugs colocalize with 5-HT3 receptors in raft-like domains. J Neurosci. 2005;25:10198–206.
128. Nothdurfter C, Tanasic S, Rammes G, Rupprecht R. Modulation of ligand-gated ion channels as a novel pharmacological principle. Pharmacopsychiatry. 2011;44:S27–34.
129. Sjogren B, Svenningsson P. Caveolin-1 affects serotonin binding and cell surface levels of human 5-HT7(a) receptors. FEBS Lett. 2007;581:5115–21.
130. Beaulieu JM, Espinoza S, Gainetdinov RR. Dopamine receptors—IUPHAR review 13. Br J Pharmacol. 2015;172:1–23.
131. Obadiah J, Avidor-Reiss T, Fishburn CS, Carmon S, Bayewitch M, Vogel Z, et al. Adenylyl cyclase interaction with the D2 dopamine receptor family; differential coupling to Gi, Gz, and Gs. Cell Mol Neurobiol. 1999;19:653–64.
132. Ilani T, Fishburn CS, Levavi-Sivan B, Carmon S, Raveh L, Fuchs S. Coupling of dopamine receptors to G proteins: studies with chimeric D2/D3 dopamine receptors. Cell Mol Neurobiol. 2002;22:47–56.

133. Jaber M, Robinson SW, Missale C, Caron MG. Dopamine receptors and brain function. Neuropharmacology. 1996;35:1503–19.
134. Vickery RG, von Zastrow M. Distinct dynamin-dependent and -independent mechanisms target structurally homologous dopamine receptors to different endocytic membranes. J Cell Biol. 1999;144:31–3.
135. Kong MM, Hasbi A, Mattocks M, Fan T, O'Dowd BF, George SR. Regulation of D1 dopamine receptor trafficking and signaling by caveolin-1. Mol Pharmacol. 2007;72:1157–70.
136. Yu P, Sun M, Villar VA, Zhang Y, Weinman EJ, Felder RA, et al. Differential dopamine receptor subtype regulation of adenylyl cyclases in lipid rafts in human embryonic kidney and renal proximal tubule cells. Cell Signal. 2014;26:2521–9.
137. Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, et al. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature. 1991;350:614–9.
138. Paspalas CD, Goldman-Rakic PS. Microdomains for dopamine volume neurotransmission in primate prefrontal cortex. J Neurosci. 2004;24:5292–300.
139. Yang S, Yang Y, Yu P, Yang J, Jiang X, Villar VA, et al. Dopamine D1 and D5 receptors differentially regulate oxidative stress through paraoxonase 2 in kidney cells. Free Radic Res. 2015;49:397–410.
140. Sharma M, Celver J, Octeau JC, Kovoor A. Plasma membrane compartmentalization of D2 dopamine receptors. J Biol Chem. 2013;288:12554–68.
141. Celver J, Sharma M, Kovoor A. D(2)-dopamine receptors target regulator of G protein signaling 9-2 to detergent-resistant membrane fractions. J Neurochem. 2012;120:56–69.
142. Genedani S, Guidolin D, Leo G, Filaferro M, Torvinen M, Woods AS, et al. Computer-assisted image analysis of caveolin-1 involvement in the internalization process of adenosine A2A-dopamine D2 receptor heterodimers. J Mol Neurosci. 2005;26:177–84.
143. Iwata K, Ito K, Fukuzaki A, Inaki K, Haga T. Dynamin and rab5 regulate GRK2-dependent internalization of dopamine D2 receptors. Eur J Biochem. 1999;263:596–602.
144. Kim KM, Valenzano KJ, Robinson SR, Yao WD, Barak LS, Caron MG. Differential regulation of the dopamine D2 and D3 receptors by G protein-coupled receptor kinases and beta-arrestins. J Biol Chem. 2001;276:37409–14.
145. Villar VA, Jones JE, Armando I, Palmes-Saloma C, Yu P, Pascua AM, et al. G protein-coupled receptor kinase 4 (GRK4) regulates the phosphorylation and function of the dopamine D3 receptor. J Biol Chem. 2009;284:21425–34.
146. Kim KM, Gainetdinov RR, Laporte SA, Caron MG, Barak LS. G protein-coupled receptor kinase regulates dopamine D3 receptor signaling by modulating the stability of a receptor-filamin-beta-arrestin complex. A case of autoreceptor regulation. J Biol Chem. 2005;280:12774–80.
147. Xiang Y, Rybin VO, Steinberg SF, Kobilka B. Caveolar localization dictates physiologic signaling of beta 2-adrenoceptors in neonatal cardiac myocytes. J Biol Chem. 2002;277:34280–6.
148. Oner SS, Kaya AI, Onaran HO, Ozcan G, Ugur O. beta2-Adrenoceptor, Gs and adenylate cyclase coupling in purified detergent-resistant, low density membrane fractions. Eur J Pharmacol. 2010;630:42–52.
149. Ostrom RS, Bundey RA, Insel PA. Nitric oxide inhibition of adenylyl cyclase type 6 activity is dependent upon lipid rafts and caveolin signaling complexes. J Biol Chem. 2004;279:19846–53.
150. Rybin VO, Xu X, Lisanti MP, Steinberg SF. Differential targeting of beta -adrenergic receptor subtypes and adenylyl cyclase to cardiomyocyte caveolae. A mechanism to functionally regulate the cAMP signaling pathway. J Biol Chem. 2000;275:41447–57.
151. Ostrom RS, Gregorian C, Drenan RM, Xiang Y, Regan JW, Insel PA. Receptor number and caveolar co-localization determine receptor coupling efficiency to adenylyl cyclase. J Biol Chem. 2001;276:42063–9.
152. Valentine CD, Haggie PM. Confinement of beta(1)- and beta(2)-adrenergic receptors in the plasma membrane of cardiomyocyte-like H9c2 cells is mediated by selective interactions with PDZ domain and A-kinase anchoring proteins but not caveolae. Mol Biol Cell. 2011;22:2970–82.
153. Hanson MA, Cherezov V, Griffith MT, Roth CB, Jaakola VP, Chien EY, et al. A specific cholesterol binding site is established by the 2.8 A structure of the human beta2-adrenergic receptor. Structure. 2008;16:897–905.
154. Pontier SM, Percherancier Y, Galandrin S, Breit A, Gales C, Bouvier M. Cholesterol-dependent separation of the beta2-adrenergic receptor from its partners determines signaling efficacy: insight into nanoscale organization of signal transduction. J Biol Chem. 2008;283:24659–72.
155. DiPilato LM, Zhang J. The role of membrane microdomains in shaping beta2-adrenergic receptor-mediated cAMP dynamics. Mol Biosyst. 2009;5:832–7.
156. Wright PT, Nikolaev VO, O'Hara T, Diakonov I, Bhargava A, Tokar S, et al. Caveolin-3 regulates compartmentation of cardiomyocyte beta2-adrenergic receptor-mediated cAMP signaling. J Mol Cell Cardiol. 2014;67:38–48.
157. Oh P, Schnitzer JE. Segregation of heterotrimeric G proteins in cell surface microdomains. G(q) binds caveolin to concentrate in caveolae, whereas G(i) and G(s) target lipid rafts by default. Mol Biol Cell. 2001;12:685–98.
158. Allen JA, Yu JZ, Donati RJ, Rasenick MM. Beta-adrenergic receptor stimulation promotes G alpha s internalization through lipid rafts: a study in living cells. Mol Pharmacol. 2005;67:1493–504.
159. Allen JA, Yu JZ, Dave RH, Bhatnagar A, Roth BL, Rasenick MM. Caveolin-1 and lipid microdomains regulate Gs trafficking and attenuate Gs/adenylyl cyclase signaling. Mol Pharmacol. 2009;76:1082–93.
160. Muller HK, Wiborg O, Haase J. Subcellular redistribution of the serotonin transporter by secretory carrier membrane protein 2. J Biol Chem. 2006;281:28901–9.
161. Chamberlain LH, Burgoyne RD, Gould GW. SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis. Proc Natl Acad Sci USA. 2001;98:5619–24.
162. Bjork K, Svenningsson P. Modulation of monoamine receptors by adaptor proteins and lipid rafts: role in some effects of centrally acting drugs and therapeutic agents. Annu Rev Pharmacol Toxicol. 2011;51:211–42.
163. Hernandez-Rapp J, Martin-Lanneree S, Hirsch TZ, Pradines E, Alleaume-Butaux A, Schneider B, et al. A PrP(C)-caveolin-Lyn complex negatively controls neuronal GSK3beta and serotonin 1B receptor. Sci Rep. 2014;4:4881.
164. Fakhoury M. Revisiting the serotonin hypothesis: implications for major depressive disorders. Mol Neurobiol. 2016;53:2778–86.
165. Erb SJ, Schappi JM, Rasenick MM. Antidepressants accumulate in lipid rafts independent of monoamine transporters to modulate redistribution of the G protein, Galphas. J Biol Chem. 2016;291:19725–33.
166. Donati RJ, Dwivedi Y, Roberts RC, Conley RR, Pandey GN, Rasenick MM. Postmortem brain tissue of depressed suicides reveals increased Gs alpha localization in lipid raft domains where it is less likely to activate adenylyl cyclase. J Neurosci. 2008;28:3042–50.
167. Menkes DB, Rasenick MM, Wheeler MA, Bitensky MW. Guanosine triphosphate activation of brain adenylate cyclase: enhancement by long-term antidepressant treatment. Science. 1983;219:65–7.
168. Toki S, Donati RJ, Rasenick MM. Treatment of C6 glioma cells and rats with antidepressant drugs increases the detergent extraction of G(s alpha) from plasma membrane. J Neurochem. 1999;73:1114–20.
169. Zhang L, Rasenick MM. Chronic treatment with escitalopram but not R-citalopram translocates Galpha(s) from lipid raft domains and potentiates adenylyl cyclase: a 5-hydroxytryptamine transporter-independent action of this antidepressant compound. J Pharmacol Exp Ther. 2010;332:977–84.
170. Donati RJ, Rasenick MM. Chronic antidepressant treatment prevents accumulation of gsalpha in cholesterol-rich, cytoskeletal-associated, plasma membrane domains (lipid rafts). Neuropsychopharmacology. 2005;30:1238–45.
171. Donati RJ, Rasenick MM. G protein signaling and the molecular basis of antidepressant action. Life Sci. 2003;73:1–17.
172. Fleischhacker WW, Hinterhuber H, Bauer H, Pflug B, Berner P, Simhandl C, et al. A multicenter double-blind study of three different doses of the new cAMP-phosphodiesterase inhibitor rolipram in patients with major depressive disorder. Neuropsychobiology. 1992;26:59–64.
173. Donati RJ, Schappi J, Czysz AH, Jackson A, Rasenick MM. Differential effects of antidepressants escitalopram versus lithium on Gs alpha membrane relocalization. BMC Neurosci. 2015;16:40.
174. Guesdon W, Kosaraju R, Brophy P, Clark A, Dillingham S, Aziz S, et al. Effects of fish oils on ex vivo B-cell responses of obese subjects upon BCR/TLR stimulation: a pilot study. J Nutr Biochem. 2018;53:72–80.
175. Simopoulos AP. Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. Mol Neurobiol. 2011;44:203–15.
176. Lin PY, Huang SY, Su KP. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. Biol Psychiatry. 2010;68:140–7.
177. Sublette ME, Hibbeln JR, Galfalvy H, Oquendo MA, Mann JJ. Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk. Am J Psychiatry. 2006;163:1100–2.
178. Huan M, Hamazaki K, Sun Y, Itomura M, Liu H, Kang W, et al. Suicide attempt and n-3 fatty acid levels in red blood cells: a case control study in China. Biol Psychiatry. 2004;56:490–6.
179. Lewis MD, Hibbeln JR, Johnson JE, Lin YH, Hyun DY, Loewke JD. Suicide deaths of active-duty US military and omega-3 fatty-acid status: a case-control comparison. J Clin Psychiatry 2011;72:1585–90.
180. Shaikh SR, Kinnun JJ, Leng X, Williams JA, Wassall SR. How polyunsaturated fatty acids modify molecular organization in membranes: insight from NMR studies of model systems. Biochim Biophys Acta. 2014;1848:211–9.
181. Shaikh SR, Wassall SR, Brown DA, Kosaraju R. N-3 polyunsaturated fatty acids, lipid microclusters, and vitamin E. Curr Top Membr. 2015;75:209–31.

182. Williams JA, Batten SE, Harris M, Rockett BD, Shaikh SR, Stillwell W, et al. Docosahexaenoic and eicosapentaenoic acids segregate differently between raft and nonraft domains. Biophys J. 2012;103:228–37.
183. Harris M, Kinnun JJ, Kosaraju R, Leng X, Wassall SR, Shaikh SR. Membrane disordering by eicosapentaenoic acid in B lymphomas is reduced by elongation to docosapentaenoic acid as revealed with solid-state nuclear magnetic resonance spectroscopy of model membranes. J Nutr. 2016;146:1283–9.
184. Martins JG. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials. J Am Coll Nutr. 2009;28:525–42.
185. Martins JG, Bentsen H, Puri BK. Eicosapentaenoic acid appears to be the key omega-3 fatty acid component associated with efficacy in major depressive disorder: a critique of Bloch and Hannestad and updated meta-analysis. Mol Psychiatry. 2012;17:1144–9.
186. Sublette ME, Ellis SP, Geant AL, Mann JJ. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiatry. 2011;72:1577–84.
187. Czysz AH, Rasenick MM. G-protein signaling, lipid rafts and the possible sites of action for the antidepressant effects of n-3 polyunsaturated fatty acids. CNS Neurol Disord Drug Targets. 2013;12:466–73.
188. Guixa-Gonzalez R, Javanainen M, Gomez-Soler M, Cordobilla B, Domingo JC, Sanz F, et al. Membrane omega-3 fatty acids modulate the oligomerisation kinetics of adenosine A2A and dopamine D2 receptors. Sci Rep. 2016; 6:19839.
189. Carotenuto F, Minieri M, Monego G, Fiaccavento R, Bertoni A, Sinigaglia F, et al. A diet supplemented with ALA-rich flaxseed prevents cardiomyocyte apoptosis by regulating caveolin-3 expression. Cardiovasc Res. 2013;100:422–31.
190. Folino A, Sprio AE, Di Scipio F, Berta GN, Rastaldo R. Alpha-linolenic acid protects against cardiac injury and remodelling induced by beta-adrenergic overstimulation. Food Funct. 2015;6:2231–9.
191. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 2006;27:24–31.
192. Raison CL, Miller AH. Do cytokines really sing the blues? Cerebrum. 2013;2013:10.
193. Wong SW, Kwon MJ, Choi AM, Kim HP, Nakahira K, Hwang DH. Fatty acids modulate Toll-like receptor 4 activation through regulation of receptor dimerization and recruitment into lipid rafts in a reactive oxygen species-dependent manner. J Biol Chem. 2009;284:27384–92.
194. Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. BMJ. 1990;301:309–14.
195. Muldoon MF, Manuck SB, Mendelsohn AB, Kaplan JR, Belle SH. Cholesterol reduction and non-illness mortality: meta-analysis of randomised clinical trials. BMJ. 2001;322:11–15.
196. Wu S, Ding Y, Wu F, Xie G, Hou J, Mao P. Serum lipid levels and suicidality: a meta-analysis of 65 epidemiological studies. J Psychiatry Neurosci. 2016;41:56–69.
197. Kaplan JR, Shively CA, Fontenot MB, Morgan TM, Howell SM, Manuck SB, et al. Demonstration of an association among dietary cholesterol, central serotonergic activity, and social behavior in monkeys. Psychosom Med. 1994;56:479–84.