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Mini review

# Lipid–protein interactions, regulation and dysfunction of brain cholesterol

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**Abstract**

The biosynthesis and metabolism of cholesterol in the brain is spatiotemporally and developmentally regulated. Brain cholesterol plays an important role in maintaining the function of neuronal receptors, which are key components in neural signal transduction. This is illustrated by the requirement of membrane cholesterol for the function of the serotonin$_{1A}$ receptor, a transmembrane neurotransmitter receptor. A crucial determinant for the function of neuronal receptors could be the availability of brain cholesterol. The Smith–Lemli–Optiz Syndrome, a metabolic disorder characterized by severe neurodegeneration leading to mental retardation, represents a condition in which the availability of brain cholesterol is limited. A comprehensive molecular analysis of lipid–protein interactions in healthy and diseased states could be crucial for a better understanding of the pathogenesis of psychiatric disorders.


*Keywords:* Brain cholesterol; Lipid–protein interaction; Serotonin$_{1A}$ receptor; Smith–Lemli–Opitz Syndrome

The nervous system characteristically contains a very high concentration of lipids, and displays remarkable lipid diversity [1]. The lipid composition of cells that make up the nervous system is unique and has been correlated with increased complexity in the organization of the nervous system during evolution [2]. Organization and dynamics of cellular membranes in the nervous system play a crucial role in the function of neuronal membrane receptors. Cholesterol is an important lipid in this context since it is known to regulate the function of membrane receptors [3], especially neuronal receptors [4], thereby affecting neurotransmission and possibly giving rise to mood and anxiety disorders [5]. Cholesterol is a predominantly hydrophobic molecule comprising a near planar tetracyclic-fused steroid ring and a flexible isooctyl hydrocarbon tail (see Fig. 1a). The 3β-hydroxyl moiety provides cholesterol its amphiphilic character causing to orient in membrane bilayer with its long axis perpendicular to the plane of the membrane (see Fig. 1b). Interestingly, it has been reported that tail-to-tail cholesterol dimers spanning the two leaflets of the membrane bilayer can be formed under certain conditions [6–11].

Cholesterol is an abundant and essential component of eukaryotic membranes and plays a crucial role in membrane organization, dynamics, function, and sorting [12,13]. Cholesterol is often found distributed nonrandomly in domains or pools in biological and model membranes [10,12–16]. Many of these domains (sometimes termed as 'lipid rafts') are believed to be important for the maintenance of membrane structure and function. The idea of such specialized membrane domains assumes significance in cell biology since physiologically important functions such as membrane sorting and trafficking [17] and signal transduction processes [18], in addition to the entry of pathogens [19,20], have been attributed to these domains.

Interestingly, a strong asymmetry exists even in the manner cholesterol is distributed among various organs in the body of higher eukaryotes. For example, the central nervous system, which accounts for only 2% of the body mass contains ~25% of free cholesterol present in the whole body [21]. Although the brain is highly enriched in

*Abbreviations:* 5-HT$_{1A}$ receptor, 5-hydroxytryptamine$_{1A}$ receptor; 7-DHC, 7-dehydrocholesterol; 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; GPCR, G-protein coupled receptor; MβCD, methyl-β-cyclodextrin; SLOS, Smith–Lemli–Opitz Syndrome.

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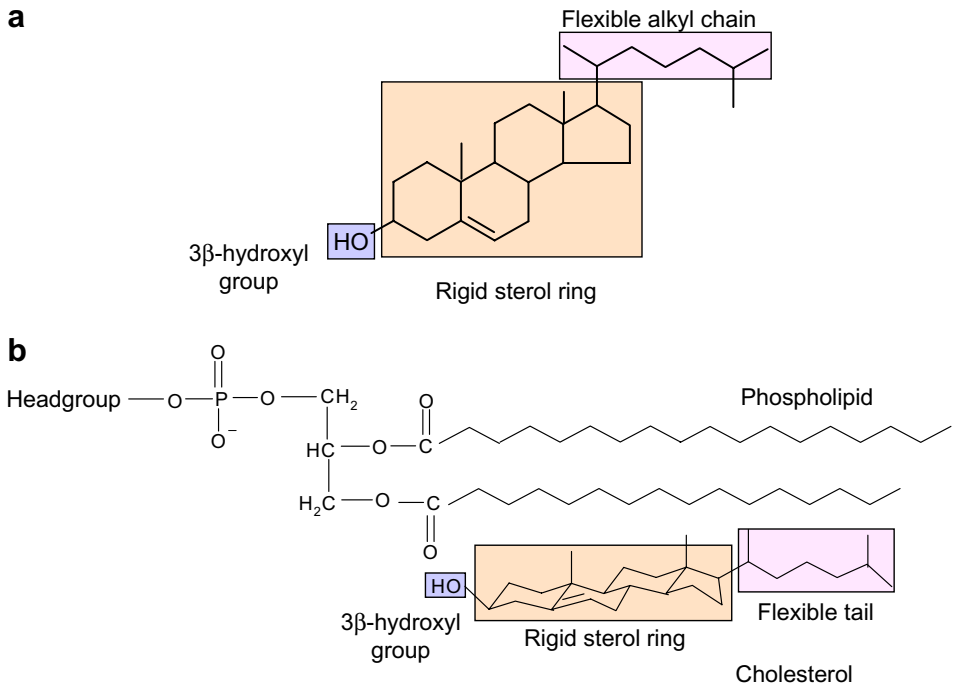


Fig. 1. Chemical structure and membrane orientation of cholesterol. Three structurally distinct regions are shown as shaded boxes in (a): the 3β-hydroxyl group, the rigid steroid ring, and the flexible alkyl chain. The 3β-hydroxyl moiety is the only polar group in these sterols thereby contributing to their amphiphilic character and helps to orient and anchor them in the membrane. (b) The schematic orientation of cholesterol in relation to a phospholipid molecule in a lipid bilayer. The rigid and near planar steroid ring contributes to the ordering effect of cholesterol in phospholipid bilayers due to the restriction in motion imposed by it to adjacent phospholipid fatty acyl chains. The flexible alkyl chain extends into the hydrophobic core of the membrane. Modified from [4].

cholesterol and important neuronal processes such as synaptogenesis require cholesterol [22], the organization and dynamics of brain cholesterol is still poorly understood [23]. Brain cholesterol is synthesized *in situ* [24] and is developmentally regulated [25]. The organization, traffic, and dynamics of brain cholesterol are stringently controlled since the input of cholesterol into the central nervous system is almost exclusively from *in situ* synthesis as there is no evidence for the transfer of cholesterol from blood plasma to brain [21]. As a result, a number of neurological diseases share a common etiology of defective cholesterol metabolism in the brain [26]. The recent increase in the number of studies in understanding the mechanisms by which cholesterol metabolism in the brain is regulated could be attributed to the fact that defects in cholesterol homeostasis in the brain have been linked to development of several neurological disorders such as Alzheimer's disease, Niemann-Pick type C disease and the Smith–Lemli–Opitz Syndrome [27]. In the Smith–Lemli–Opitz Syndrome, for example, the marked abnormalities in brain development and function leading to serious neurological and mental dysfunctions have their origin in the fact that the major input of brain cholesterol comes from the *in situ* synthesis and such synthesis is defective in this syndrome [28; see later]. In view of the importance of cholesterol in relation to membrane domains [10,12–16], the effect of alteration in the cholesterol content of neuronal membranes on membrane dynamics and protein/receptor function represents an important determinant in the analysis of neurogenesis and several neuropathologies. The interaction between cholesterol and other membrane components (such as receptors) in the brain therefore assumes relevance for a comprehensive understanding of brain function.

## Lipid–protein interactions involving brain cholesterol: the hippocampal serotonin$_{1A}$ receptor

Lipid–protein interactions play a crucial role in maintaining the structure and function of biological membranes [29,30]. A possible role of lipids in a variety of neurological disorders is well documented. For example, several epidemiological studies indicate a possible role of lipids in a variety of neurological disorders which have been shown to involve deregulated lipid metabolism [1,26]. Since most of the functions of the membrane are mediated by membrane proteins, monitoring lipid–protein interactions assumes significance. A large portion of any given transmembrane protein remains in contact with the membrane lipid environment. This raises the obvious possibility that the membrane could be an important modulator of protein structure and function. Lipid–protein interactions are implicated in the assembly, stability and function of membrane proteins [29,30]. Such lipid–protein interactions are of particular importance because a cell has the ability of varying the lipid composition of its membrane in response

to a variety of stress and stimuli, thus changing the environment and the activity of the receptors in its membrane. In this context, cholesterol is an important membrane lipid since it has been implicated in the formation and stability of membrane domains [10,12–16], the interaction of cholesterol with membrane proteins [4,31] represents an important determinant in functional studies of such proteins, especially in the nervous system.

Seven transmembrane domain G-protein coupled receptors (GPCRs) constitute one of the largest family of proteins in mammals and account for ~2% of the total proteins coded by the human genome [32]. Signal transduction events mediated by GPCRs are the primary means by which cells communicate with and respond to their external environment [33]. These receptors can be activated by ligands as chemically diverse as biogenic amines, peptides, glycoproteins, lipids, nucleotides, and even photons, thereby mediating diverse physiological processes such as neurotransmission, cellular metabolism, secretion, cellular differentiation, and growth, and inflammatory, and immune responses. As a consequence, GPCRs represent major targets for the development of novel drug candidates in all clinical areas [34]. It is estimated that up to 50% of clinically prescribed drugs act as either agonists or antagonists for GPCRs, which points out their immense therapeutic potential [35]. Since a large number of GPCRs are present in the nervous system and are responsible for neuronal function, the interaction of these transmembrane proteins with the surrounding lipid environment assumes significance in understanding their function.

The serotonin$_{1A}$ (also referred to as 5-HT$_{1A}$) receptor is an important neurotransmitter receptor and is a representative member of the large family of GPCRs. It is the most extensively studied of the serotonin receptors for a number of reasons [36]. Serotonergic signaling plays a key role in the generation and modulation of various cognitive and behavioral functions. Interestingly, mutant (knockout) mice lacking the serotonin$_{1A}$ receptor generated a few years back exhibit enhanced anxiety-related behavior, and represent an important animal model for the analysis of complex traits such as anxiety disorders and aggression in higher animals [37,38]. In addition, the serotonin$_{1A}$ receptor has recently been shown to have a role in neural development [39] and protection of stressed neuronal cells undergoing degeneration and apoptosis [40]. The modulatory role of cholesterol on the ligand binding activity and G-protein coupling of the hippocampal serotonin$_{1A}$ receptor has recently been shown by depleting cholesterol from native membranes using methyl-β-cyclodextrin (MβCD), which selectively extracts cholesterol from membranes by including it in a central non-polar cavity. Removal of cholesterol from hippocampal membranes using various concentrations of MβCD [see Fig. 2a, 41] resulted in a concentration-dependent reduction in specific binding of the agonist 8-OH-DPAT to serotonin$_{1A}$ receptors (Fig. 2b). This is accompanied by alterations in binding sites obtained from analysis of binding data. Importantly, cholesterol depletion affected G-protein coupling of the receptor. Replenishment of membranes with cholesterol using MβCD-cholesterol complex (Fig. 2c), led to recovery of ligand binding activity (Fig. 2d) to a considerable extent. If cholesterol is necessary for ligand binding of the serotonin$_{1A}$ receptor, modulating cholesterol availability by other means could lead to similar effects on the serotonin$_{1A}$ receptor function. This hypothesis was validated by treatment of native hippocampal membranes with the (i) sterol-complexing agent digitonin [42], (ii) sterol-binding antifungal polyene antibiotic nystatin [43], and (iii) cholesterol oxidase which catalyses the oxidation of cholesterol to cholestenone [44]. While treatment with MβCD physically depletes cholesterol from membranes, treatment with these agents modulates the availability of membrane cholesterol without physical depletion. The common finding in all these experiments was reduction in the specific agonist binding, implicating that it is the non-availability of cholesterol rather than the manner in which its availability is modulated, is crucial for ligand binding. Taken together, these results provide evidence that cholesterol is necessary for ligand binding and G-protein coupling of this important neurotransmitter receptor. More importantly, these results could have significant implications in understanding the influence of the membrane lipid environment on the activity and signal transduction of other G-protein coupled transmembrane receptors. The requirement of membrane cholesterol in the function of a related receptor, the serotonin$_{7(a)}$ receptor, has recently been reported [45]. Interestingly, the clinical significance of membrane cholesterol levels resulting in receptor dysfunction has previously been aptly exemplified in the case of cholecystokinin (CCK) receptors [46,47]. Thus, agonist binding and G-protein coupling are reduced in case of CCK receptors isolated from muscle tissues of human gallbladders with cholesterol stones. These effects were found to be reversed upon treatment with cholesterol-free liposomes.

The most important aspect of the above results is that manipulations of membrane cholesterol content can induce significant changes in the activity and G-protein coupling of the serotonin$_{1A}$ receptor. Whether such manipulations in membrane cholesterol content could be induced *in vivo* represents a challenging question. Low serum cholesterol concentration has previously been correlated with an increase in the prevalence of suicide in humans [48] and is partly attributed to an altered serotonin metabolism [49]. The above results on the effect of a reduction in membrane cholesterol levels on the function of the serotonin$_{1A}$ receptor could therefore provide potential insight behind the etiology of psychiatric disorders that are correlated with altered cholesterol metabolism. Interestingly, a previous report has suggested that chronic *in vivo* administration of certain statins, which are cholesterol lowering drugs, specifically reduce brain cholesterol levels leaving the serum cholesterol levels unaffected [50]. Importantly, it has recently been shown that even modest levels of cholesterol reduction achieved using statins can

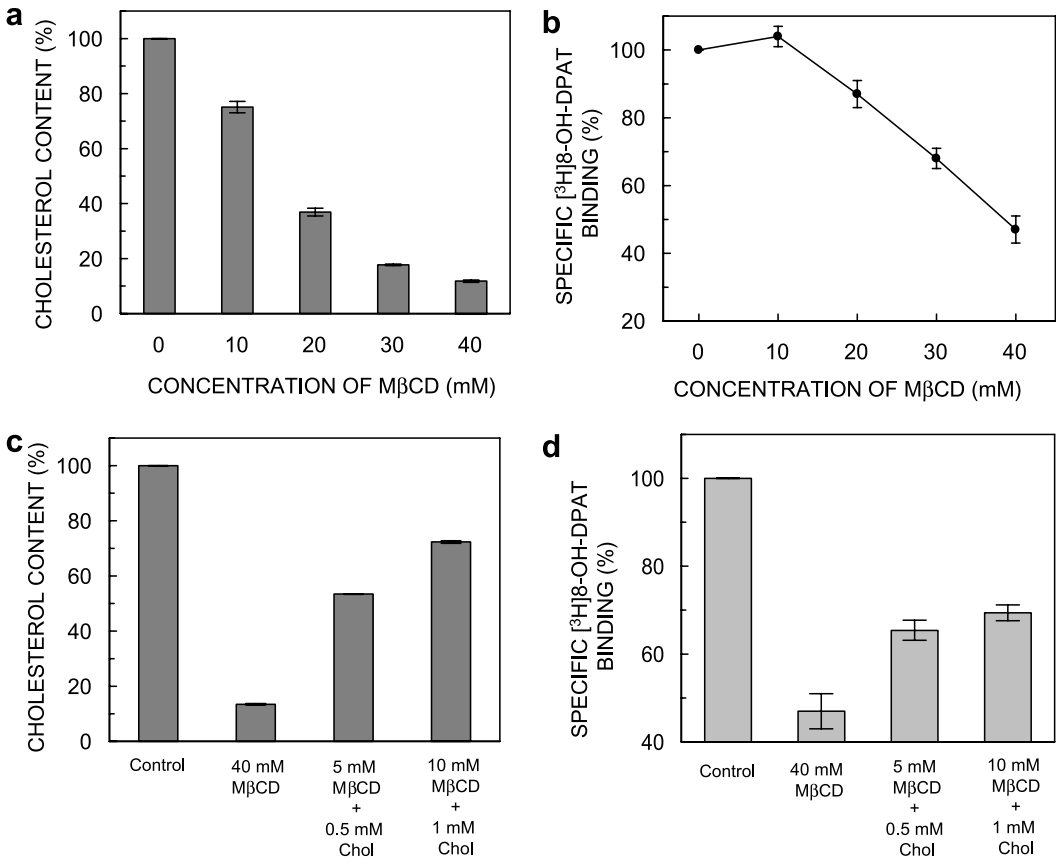


Fig. 2. Cholesterol is required for the specific binding of the agonist [$^3$H]8-OH-DPAT to hippocampal serotonin$_{1A}$ receptors. (a) The effect of increasing concentrations of methyl-β-cyclodextrin (MβCD) on the percentage of cholesterol content of native hippocampal membranes. Increasing concentrations of MβCD leads to a progressive reduction in the cholesterol content. (b) The specific [$^3$H]8-OH-DPAT binding is reduced upon treatment of native hippocampal membranes with increasing concentrations of MβCD. This indicates loss in specific agonist binding of serotonin$_{1A}$ receptors upon reduction in membrane cholesterol levels. Values are expressed as a percentage of specific binding for native membranes without MβCD treatment. (c) Cholesterol replenishment into hippocampal membranes treated with MβCD. The final cholesterol contents (expressed as a percentage of cholesterol content in native membrane without any treatment) when cholesterol-depleted membranes were treated with cholesterol-MβCD complex at a final concentration of 0.5:5 and 1:10 mM (mol/mol of cholesterol:MβCD) are shown. (d) Cholesterol replenishment into hippocampal membranes treated with MβCD and its correlation with specific [$^3$H]8-OH-DPAT binding activity of the hippocampal serotonin$_{1A}$ receptor. Cholesterol depletion resulted in ~50% reduction in specific [$^3$H]8-OH-DPAT binding (see b). Upon replenishment of membrane cholesterol in cholesterol-depleted hippocampal membranes using cholesterol-MβCD complex, a significant recovery in the specific [$^3$H]8-OH-DPAT binding is observed. Values are expressed as a percentage of specific radiolabeled agonist binding in native membranes without any treatment. Adapted and modified from [41].

induce significant alterations in the function of the serotonin$_{1A}$ receptor (T.J. Pucadyil, S. Shrivastava and A. Chattopadhyay, unpublished observations). In addition, a recent report describes the attenuation of the serotonin$_{1A}$ receptor antagonist binding and signaling in brains of suicide victims [51].

Previous clinical studies have identified abnormalities in serum cholesterol levels in patients with mood and anxiety disorders [5]. The corresponding changes in brain cholesterol levels, if any, would be an important determinant in characterizing the extent of these disorders [52]. Importantly, it has been shown that treating humans with cholesterol lowering drugs significantly decreases the incidence of Alzheimer's disease [53,54]. It is possible that more severe deficiency in cholesterol levels in the brain occurs on account of the lower turnover of cholesterol in this tissue. The turnover of brain cholesterol is very low, with a half-life of ~6 months [21]. As a result, the cerebrospinal fluid levels of cholesterol are ~40 to 50-fold lower than the plasma cholesterol [55]. Due to the presence of the blood–brain barrier, alterations in serum levels of cholesterol are believed not to affect the total cholesterol level in the central nervous system. However, under such conditions, the neuronal plasma membrane fractions have not been studied adequately. In addition, regions in the central nervous system (such as the hypothalamic area) that are somewhat weakly protected by the blood–brain barrier may be sensitive to plasma cholesterol fluctuations. Interestingly, there are conditions (such as in the case of the Smith–Lemli–Opitz Syndrome, see later) in which brain cholesterol availability is limited due to defective cholesterol biosynthesis. Whether the function of membrane receptors such as the serotonin$_{1A}$ receptor gets modulated under such condition represents an intriguing question.

## Modulation of cholesterol availability by defective cholesterol biosynthesis: the Smith–Lemli–Opitz Syndrome

The Smith–Lemli–Opitz Syndrome [56] is an autosomal recessive disorder characterized clinically by mental retardation, physical deformities, failure to thrive, and multiple congenital anomalies. SLOS is caused by mutations in 7-dehydrocholesterol reductase, an enzyme required in the penultimate step of cholesterol biosynthesis [see Fig. 3; 57]. The principal route of cholesterol biosynthesis in humans is the Kandutsch-Russell pathway [58]. In this pathway, the immediate precursor of cholesterol is 7-dehydrocholesterol (7-DHC) which differs with cholesterol in its unsaturation at 7th position (between the 7th and 8th carbons) in the sterol ring (highlighted in Fig. 3). SLOS is the second most serious recessive genetic condition after cystic fibrosis. Elevated plasma levels of 7- and 8-dehydrocholesterol and the ratio of their concentration to that of cholesterol are representative parameters for diagnosis of SLOS. The brains of individuals with SLOS are characterized by partial to complete agenesis of the cerebellar vermis and/or corpus callosum, hypoplasia of frontal lobes, and occasionally holoprosencephaly [28,59]. Since SLOS is associated with neurological deformities and malfunction, exploring the function of neuronal receptors and their membrane lipid interactions under these conditions could be relevant. Because cholesterol is often found to be non-randomly distributed in domains in membranes, which are physiologically important for membrane function [13,14], the putative localization of 7-DHC in such domains assumes significance. Interestingly, it has recently been reported that 7-DHC forms more stabilized domains in model membranes compared to cholesterol [60]. In addition, 7-DHC incorporated in model membranes has recently been shown to result in reduced bending rigidity and intrinsic curvature compared to cholesterol [61]. Using skin fibroblasts from SLOS patients, Tulenko and co-workers have recently shown that SLOS is accompanied by general membrane defects [62]. Taken together, these results point out that SLOS could have potential implications in membrane organization and dynamics, and ultimately in the function of membrane proteins due to altered interaction with cholesterol.

## Conclusion and future perspectives

In this review, we have highlighted lipid–protein interactions involving brain cholesterol taking the specific example of the hippocampal serotonin$_{1A}$ receptor, an important transmembrane neurotransmitter receptor, which belongs to the family of GPCRs. These results assume significance in light of the fact that serotonin$_{1A}$ receptor function is greatly modulated, possibly due to the altered membrane lipid environment surrounding this protein, in case of many psychiatric disorders. For example, the serotonin$_{1A}$ receptor levels and function have been shown to be important indices to diagnose several psychiatric disorders such as schizophrenia, major depression and suicide in humans [63–65].

In spite of a number of clinical reports, the link between psychiatric disorders due to altered cholesterol levels [26,27] and the interaction of cholesterol with membrane proteins and receptors (the key components in neural signal transduction) in healthy and diseased conditions, in molecular details is lacking. The diversity of lipids found in natural membranes, combined with the ability of cells

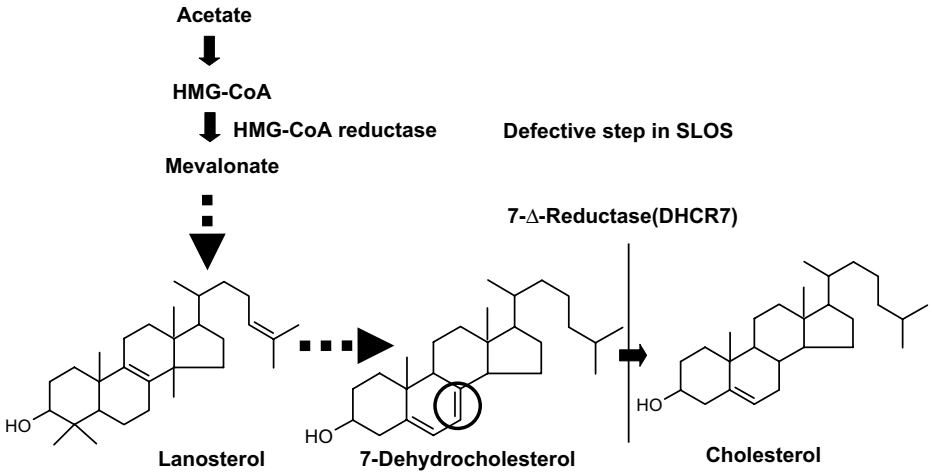


Fig. 3. A schematic representation showing the pathogenesis of the Smith–Lemli–Opitz Syndrome (SLOS) due to metabolic defect in cholesterol biosynthesis. The principal route of cholesterol synthesis in humans is the Kandutsch–Russell pathway [58]. In this pathway, the immediate precursor of cholesterol is 7-dehydrocholesterol (7-DHC) which differs in its unsaturation at 7th position in the sterol ring (highlighted in its chemical structure). The reduction of 7-DHC to yield cholesterol is catalyzed by 3β-hydroxysterol $\Delta^7$-reductase (7-DHCR). Mutations in this enzyme cause SLOS, a severe developmental disorder associated with multiple congenital and morphogenic anomalies. Elevated levels of 7-DHC have been characterized as a diagnostic parameter of the Smith–Lemli–Opitz Syndrome (see text for more details).

to modulate their membrane lipid composition in diseased states, vastly increase the potential by which lipids can exert their influence on membrane receptor and protein function, thereby leading to various psychiatric disorders. The development of newer and more sensitive technologies capable of monitoring receptor interactions at the membrane level would lead to a better understanding of the pathogenesis of these diseases.

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