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Review

Serotonin Signaling through Lipid Membranes

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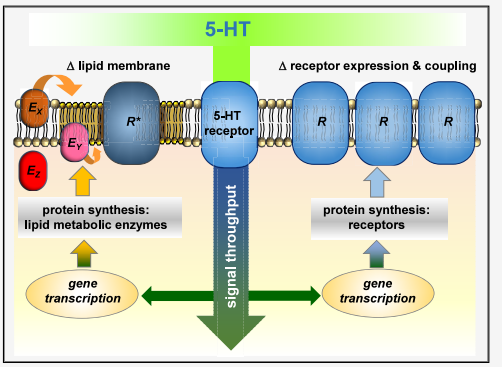
ABSTRACT: Serotonin (5-HT) is a vital modulatory neurotransmitter responsible for regulating most behaviors in the brain. An inefficient 5-HT synaptic function is often linked to various mental disorders. Primarily, membrane proteins controlling the expression and activity of 5-HT synthesis, storage, release, receptor activation, and inactivation are critical to 5-HT signaling in synaptic and extra-synaptic sites. Moreover, these signals represent information transmission across membranes. Although the lipid membrane environment is often viewed as fairly stable, emerging research suggests significant functional lipid−protein interactions with many synaptic 5-HT proteins. These protein−lipid interactions extend to almost all the primary lipid classes that form the plasma membrane. Collectively, these lipid classes and lipid−protein interactions affect 5-HT synaptic efficacy at the synapse. The highly dynamic lipid composition of synaptic membranes suggests that these lipids and their interactions with proteins may contribute to the plasticity of the 5-HT synapse. Therefore, this broader protein−lipid model of the 5-HT synapse necessitates a reconsideration of 5-HT's role in various associated mental disorders.



KEYWORDS: *serotonin, synaptic throughput, lipids, cholesterol, sphingolipids*

INTRODUCTION

Serotonin (5-HT) is a significant neurotransmitter in vertebrates with a long evolutionary history.[1] Its system in the brain impacts nearly all behaviors and states of mind[2] up to human consciousness.[3−5] While only a small number of neurons use 5-HT as a neurochemical signal, synapses and release sites are found throughout the brain.[6,7] A functional 5-HT innervation is necessary for expressing behavioral responses.

Early models suggested that depression could result from reduced 5-HT activity, while an increase might contribute to happiness. This basic model explains the impact selective 5-HT interventions have on our emotional states, such as those exerted by antidepressants[8] or mood-altering drugs.[9] Such interactions highlight 5-HT's significant role in brain function and dysfunction.

Past studies and research on 5-HT have established a traditional view of the 5-HT synapse, involving proteins that coordinate synthesis, storage, release, receptor interactions, and degradation of the neurotransmitter. Their combined efforts guide the synaptic throughput, i.e., the chemically coded information flow from one neuron to several others at 5-HT synapses in the brain.

However, this protein-centric model of the serotonergic synapse, though commonly accepted, overlooks key aspects of synapse topography, providing an incomplete representation of synapse regulation. Recent advancements in lipid analysis tools have challenged the long-held view that synaptic membranes are stable, inert, and inflexible barriers. Rather, these membranes have proven to be highly adaptable in their composition and physical properties.

These revelations strongly influence our understanding of the synapse's protein structure. Membrane-bound proteins specific to 5-HT synapses, such as synthesis and degradation enzymes, receptors, and transporters, rely on lipid proximity for their location and function. Indeed, emerging evidence now suggests that these proteins' actual function is actively regulated by their lipid environment.

In this regard, we propose and investigate the theory that the 5-HT synapse should no longer be seen merely as a protein concert but as a protein−lipid network. Both groups of structural compounds exhibit independently regulated molecular adaptability, which nevertheless interacts closely with the other group. We hypothesize that combining a protein−lipid

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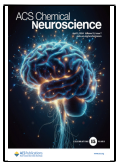

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synapse model could allow for more effective characterization and prediction of holistic synapse function and adaptability.[10]

In this context, we will examine the present state of understanding of the protein concept of the 5-HT synapse and how it influences behavior. Next, we will explore synaptic membrane features and their systemic relevance. Finally, we will discuss how lipids affect the protein network, presenting the serotonergic synapse as the first instance of a protein−lipid adaptability network.

PROTEINS AT THE 5-HT SYNAPSE

Proteins that are characteristic for 5-HT synapses are those involved in 5-HT synthesis. Initially, the precursor amino acid L-tryptophan is hydroxylated to produce L-5-hydroxytryptophan (5-HTP). This process is facilitated by the enzyme tryptophan-5′-monooxygenase, also known as tryptophan hydroxylase (TPH).[11] There are two known forms of TPH which are uniquely distributed. TPH-1 is found in blood cells and the body periphery, while TPH-2 is located within the brain.[12,13] In the following step, 5-HTP is quickly decarboxylated to create 5-hydroxytryptamine (5-HT). This step is completed by the enzyme tryptophan decarboxylase. Both enzymes must be bound to the membrane in the presynaptic cell to be enzymatically active.[1] Newly synthesized 5-HT appears in the cytoplasm and can be readily metabolized to 5-hydroxyindole acetic acid (5-HIAA), a widespread but inactive metabolite.[14−16] This indicates that most newly synthesized 5-HT is quickly degraded.[17,18] Some synthesis products are actively transported to storage vesicles by the vesicular monoamine transporter (vMAT), a protein found in the vesicle membrane which is not specific to 5-HT neurons. As a result, the synthesized 5-HT is preserved until its release into different storage pools within the synaptic cleft.

Once 5-HT is released, it may disperse in the extracellular space, where it reaches synaptic and nonsynaptic membranes at postsynaptic sites. Some of the released 5-HT is likely also absorbed at presynaptic membranes. At all these sites, it is incorporated into the membrane via salt-bridge interactions with the lipid bilayer's polar head groups.[19−21] This can result in changes to physical membrane properties and its interactions with receptors.[22] Such direct 5-HT interaction has been proposed as a receptor-independent signaling pathway with the ability to systemically modulate cell function. Besides altering lipid acyl chain order and instigating the nucleation of liquid-disordered domains within raft-like liquid-ordered domains, it can also modify the membrane's affinity for extracellular proteins and peptides.[23,24] This mechanism might also influence the affinity of neuropeptide receptors, like the neuropeptide Y type 4 receptor, for its ligand.[25]

When there is a tonic firing of 5-HT neurons and quantal release,[26] it is plausible to consider an equilibrium-like distribution of both membrane-bound and free extracellular 5-HT on pre- and postsynaptic membranes. This dynamic process may create a "signal-buffer" at the 5-HT synapse that maintains the concentration of extracellular 5-HT in check under extremely low or high activity levels. Nonetheless, if tonic activity becomes disrupted or in a disease condition that chronically lowers 5-HT synthesis/release, the membrane-bound fraction might decrease. Consequently, this activity buffer may lose flexibility, potentially contributing to a dysfunctional 5-HT system.[27−29] This theory aligns with 5-HT's antioxidant properties in the membrane, which also defend lipids against oxidation.[30] In a normally functioning synapse, membrane-bound 5-HT eventually interacts with selective 5-HT-receptors (5-HT-R), along with freshly released 5-HT, which prompts receptor activation and, ultimately, signal throughput.

Current research identifies 17 unique 5-HT-Rs, categorized into seven receptor subfamilies due to their structural similarities in amino acid composition (5-HT_1-5-HT_7-Rs). Primarily, 5-HT-Rs are a part of the G-protein-coupled metabotropic receptors (GPCR) family.[31] However, the ionotropic 5-HT_3 receptor acts uniquely as a ligand-dependent ion channel.[32−34] They all function within the lipid environment of pre- and/or postsynaptic membranes.[7,9]

The 5-HT_1-R family includes the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptors. The brain's 5-HT_{1A}-Rs are connected through G_i/G_o-proteins[35] and can inhibit adenylate cyclase while simultaneously activating an inwardly rectifying K^+ conductance.[34,36] The opening of K^+ channels decreases membrane resistance and leads to hyperpolarization. Stimulation of 5-HT_{1A}-R may also reduce Ca^{2+} conductance in 5-HT neurons. Collectively, the activation of 5-HT_{1A}-Rs by 5-HT results in the inhibition of 5-HT neuron firing.[37−40]

5-HT_{1A} autoreceptors and postsynaptic receptors have been found in the brain. As inhibitory autoreceptors, they are located at the soma and dendrites of 5-HT neurons,[41,42] while postsynaptic 5-HT_{1A}-Rs are found in the terminal regions of 5-HT projections.[34] The activation of 5-HT_{1A} receptors in the visual cortex affects synaptic excitation in this brain region.[35] Auditory evoked potentials in the electroencephalogram (EEG) are influenced by postsynaptic 5-HT_{1A}-Rs.[43]

However, the behavior-related role of the 5-HT_{1A}-Rs significantly varies depending on its area of expression in the brain[5,36,44] and the formation of heteroreceptors.[45]

The 5-HT_{1B}-Rs have been identified in rats, mice, and hamsters. In humans, the equivalent is the 5-HT_{1D}-R.[46] Both 5-HT_{1B}-Rs and 5-HT_{1D}-Rs function as presynaptic autoreceptors at 5-HT terminals. Furthermore, they serve as heteroreceptors at non-5-HT terminals,[47] regulating the release of other transmitters.[48,49] Notably, activating the 5-HT_{1B}-Rs suppresses the release of terminal 5-HT.[50]

The 5-HT_2-R family comprises 5-HT_{2A}-Rs, 5-HT_{2B}-Rs, and 5-HT_{2C}-Rs. These members are structurally similar and share transduction pathways but are distinctly localized in the brain.[51] They couple with G-protein to activate phospholipase C (PLC), triggering phosphatidyl inositide hydrolysis, which in turn diminishes K^+ conductivity and activates intracellular Ca^{2+}.[52] Overall, activation of 5-HT_2 receptors induces an excitatory effect on postsynaptic neurons. Despite having a relatively low affinity for 5-HT, postsynaptic 5-HT_2-Rs are critical for regulating auditory evoked potential in the EEG[43] and have a significant role in sensorimotor gating.[53,54] The 5-HT-induced increase in excitatory postsynaptic potentials in medial prefrontal cortex (mPFC) pyramidal cells is mediated by 5-HT_{2A}-Rs.[55,56] Notably, activating 5-HT_{2A}-Rs heightens the sensitivity to glutamate in mPFC neurons.[57,58]

The 5-HT_3-R, a ligand-dependent ion channel, is composed of five transmembrane domains. Its opening facilitates the entry of monovalent cations, thus inducing a postsynaptic depolarization. It regulates a wide variety of sensory functions and behaviors.[59−61]

The 5-HT_4-R is a seven-domain GPCR that connects to adenylyl cyclase through a G-protein link. Activation of 5-HT_4-R prompts a gradual membrane depolarization.[62] Stimulating postsynaptic 5-HT_4-Rs can enhance 5-HT activity in terminal

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regions using a feedback loop from the dorsal raphe nucleus.[62] This process may potentially facilitate cognitive performance.[63,64]

The 5-HT$_5$-Rs, 5-HT$_6$-Rs, and 5-HT$_7$-Rs are also part of the G-protein-coupled receptors class found in the brain. The 5-HT$_6$-Rs and 5-HT$_7$-Rs, in particular, encourage cAMP formation. These receptors tend to form a variety of 5-HT GPCR heteromers with other 5-HT-Rs.[31] This formation depends on the immediate lipid environment, which has the potential to alter their function.[65]

Active transport via the serotonin transporter (SERT)[66] removes 5-HT from the extracellular space. SERT is located in the plasma membrane of not only 5-HT neurons but also brain astrocytes, typically ending 5-HT's role in synaptic activity. Once 5-HT re-enters the presynaptic cytoplasm, monoamine oxidases (MAO) convert it into 5-hydroxyindolacetaldehyde, which is then quickly oxidized by aldehyde dehydrogenase to the inactive metabolite 5-HIAA. The mitochondrial membrane-bound MAO-A has greater specificity for 5-HT than MAO-B, although MAO-B is the primary isozyme found in 5-HT neurons.[67] Overall, a variety of protein enzymes form the basis of the 5-HT synapse. These enzymes function within dynamic lipid membrane environments that consistently influence their localization and ultimate structure, thereby affecting protein function.

5-HT ACTIVITY AND SIGNAL THROUGHPUT

5-HT cells typically fire spontaneously at a rate of 0.5−2.5 spikes per second due to oscillations in the membrane potential.[68] However, their activity fluctuates depending on the organism's state. For instance, 5-HT neuron activity significantly escalates during active waking and physical movement and moderately increases during periods of wakefulness with no physical activity. It subsides during slow-wave sleep and is virtually absent during rapid eye movement (REM) sleep.[69]

The behavior of the organism, rather than the light−dark cycle of the environment, mainly influences the activity of 5-HT neurons.[70−72] Drug-induced reductions in general 5-HT activity can temporarily reduce spontaneous and induced locomotor activity. However, the complete exhaustion of neuronal 5-HT can cause a lasting decrease in spontaneous behavioral activity.[73−75]

Apart from these fluctuations, certain distinct behaviors correspond with changes in 5-HT neuron firing. Specifically, the firing of 5-HT neurons increases during repetitive movements like running, chewing, or cleaning but quickly drops when the organism must react to a new sensory stimulus. This change in frequency usually happens a few seconds before the behavior starts.[69]

Likewise, sensory cues predicting rewards or punishments stimulate an increase in 5-HT neuron firing. This increased firing often boosts the extracellular 5-HT concentration in terminal areas, potentially enhancing signal throughput at the 5-HT synapse in these regions.[76−78] Nonetheless, this process is highly organized and usually limited to functional circuits related to processing the respective stimulus modality.

Novel visual stimuli have been seen to increase the activity of 5-HT neurons and 5-HT terminal release.[79] A new white light, as a form of visual stimulation, has been shown to raise the extracellular 5-HT levels selectively in the secondary visual occipital cortex (OccC) and the mPFC. However, it does not affect other high-order integration areas, such as the entorhinal or perirhinal cortex.[80,81] An increase in 5-HT was only noticed with a light intensity that positively induced locomotor activity.[82] Even in anesthetized animals with no locomotor activity, there was an enhanced 5-HT signaling, signifying that the response was not triggered by locomotor feedback.[81] On applying 5-HT to the visual cortex, it inhibited the neuronal responses invoked by the visual stimulus.[83] It was inferred that the increase in local 5-HT might function as a modality-specific "gating signal", making the behavioral response toward a particular stimulus more sensitive. This idea was supported by 5-HT's role in modulating cortical neuronal activation post-whisker-stimulation in the somatosensory cortex of rats.[84,85]

A new auditory stimulus caused a specific drop in extracellular 5-HT activity within the temporal, secondary auditory cortex (TempC). This change had no impact on the 5-HT levels in the OccC or mPFC regions. Accompanying this 5-HT response, there was a minor reduction in locomotor activity and an observed orientation response toward the source of the stimulus.[80,81] A separate study noted a similar decrease in 5-HT neuron firing.[86]

Overall, the influence of 5-HT synaptic activity in the brain on various behaviors is incredibly significant.[87] It has been shown that 5-HT plays a vital role in behaviors related to feeding,[88−90] anxiety, fear and aggression,[91,92] learning and memory,[93,94] reinforcement,[95−97] performance monitoring, adjustment to errors,[98] and reproductive behavior.[99] Moreover, 5-HT is important in dealing with stress,[100,101] and in processes that govern consciousness.[5]

THE LIPID LANDSCAPE AT THE 5-HT SYNAPSE

The lipid bilayer of cellular membranes serves as a vital barrier between the cell and its extracellular environment. Besides this structural role, lipids in biological membranes can function as signaling molecules, form the basis for protein−lipid modification after translation, and facilitate the formation of protein recruitment platforms.[102] The main lipids in mammalian membranes are glycerophospholipids (over 50%) and sphingolipids (15−25%). Characteristics such as chain length, position and number of double bonds, and hydroxylation levels greatly vary among these lipids, creating a significant variety. Cholesterol additionally constitutes 25−35% of these membranes.

It is worth noting that the lipid composition is quite unique in different organelles and the plasma membrane. For instance, mammalian plasma membranes have a markedly higher sphingolipid content compared to membranes of other organelles.[102,103] The lipid composition of the brain, and particularly of neuronal membranes, is markedly rich in cholesterol, ceramides, and polyunsaturated fatty acids (PUFAs).[104,105] Synaptic membranes, on the other hand, are laden with cholesterol, sphingomyelins, gangliosides, and phospholipids, with phosphatidylethanolamines (PE) and phosphatidylcholines (PC) being the most prevalent phospholipids.[106,107]

Glycerophospholipids are sophisticated, dual-natured molecules that comprise a glycerol molecule, two fatty acids, a phosphate, and usually one additional small molecule. They are divided into several categories, with the highest prevalence found in mammalian cells noted among glycerophosphocholines, glycerophosphoethanolamines, glycerophosphoserines, and phosphatidylinositol. Glycerophospholipid synthesis primarily occurs via the de novo pathway. This process generates various glycerophospholipids with distinct polar heads at the

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sn-3 position on the glycerol backbone. The various types include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and cardiolipin. These are all developed from phosphatidic acid and either diacylglycerol or cytidine diphosphate-diacylglycerol. The next stage involves the remodeling of glycerophospholipid acyl chains via the Lands' cycle, a process involving phospholipase A, acyl-CoA synthases, transacylases, and lysophospholipid acyltransferases. The outcome is the creation of more complex glycerophospholipids, specifically those containing PUFAs. PUFA-rich neural membrane glycerophospholipids play several critical roles in membrane functionality.[108−110]

PUFAs are fatty acids with two or more double bonds. Most PUFAs utilized in the synthesis of neural membrane glycerophospholipids in the brain are transported from the gastrointestinal tract. These fatty acids, with the exception of mead acid, are not endogenously produced in mammals. Instead, they can be obtained through dietary intake or conversion from other PUFAs. In the liver, PUFAs can be synthesized from linoleic and α-linolenic acids, followed by further esterification in neural membranes. Essential PUFAs, such as arachidonic acid and docosahexaenoic acid, serve multiple functional roles. They not only maintain membrane stability and fluidity but also influence the orientation of internal membrane domains. Furthermore, they act as second messengers and precursors to eicosanoids and other crucial biological molecules.[102,108]

Cholesterol, a sterol, is one of the most prevalent lipids found in biological membranes. It is composed of four hydrocarbon rings that are highly hydrophobic. The molecule also has a hydrocarbon tail attached to one end of the steroid and a hydroxyl group linked to its other end. Cholesterol can be synthesized de novo in the endoplasmic reticulum from acetate, with the liver acting as the primary site for this process. The enzymes 3-hydroxy-3-methyl-glutaryl CoA reductase and squalene monooxygenase limit the cholesterol biosynthesis rate.[111,112] It is noteworthy that approximately 30% of cholesterol intake comes from dietary sources.[113,114] The brain is unique in that it has limited cholesterol exchange with the periphery across the blood−brain barrier, and it can carry out its own cholesterol synthesis.[115]

Sphingolipids, another group of common lipid molecules found in brain membranes, include ceramides, sphingomyelins (SMs), and glycosphingolipids like gangliosides, cerebrosides, and sulfatides.[110,112] Over 300 distinct molecules, all featuring a sphingosine base, comprise sphingolipids. To generate ceramides, a fatty acid group is added to the sphingosine base, while the production of sphingomyelin (SM) requires a further addition of choline or ethanolamine residues. Factors such as saturation, stereochemical variations, and the addition of hydroxyl groups contribute to the immense variety of ceramides, SMs, and their derivatives in the ceramide system.[116]

Ceramides are synthesized primarily via three pathways: the de novo, sphingomyelinase, and salvage pathways. The de novo pathway commences with the reaction of serine and palmitoyl-coenzyme A, a process catalyzed by serine palmitoyl transferase, leading to the eventual transformation into dihydroceramide. On the other hand, in the salvage pathway, sphingosine-1-phosphate (S1P) and sphingosine are broken down into ceramide, a process catalyzed by sphingosine-1-phosphatase and ceramide synthases (CerS). The sphingomyelinase pathway involves SM degradation into ceramide by acid (ASM) and neutral sphingomyelinase (NSM).[110,116−118]

Complex sphingolipids, particularly gangliosides, abundant in brain gray matter and neurons, are formed from the ceramide backbone through the addition of sugar residues.[117] It has been observed that the composition of sphingolipids in the plasma membrane can influence the function of numerous neurotransmitter receptors located in the membrane.[119,120]

The high heterogeneity and diversity of main membrane lipid classes significantly affect the membrane's properties and function. They enable a flexible orientation, order, and high mobility of the membrane lipids and proteins.

Lipid Domains in Synaptic Membranes. Lipids in biological membranes are now acknowledged to have more than a structural role; they also determine the membrane's signaling function.[121] In neurons, lipid-enriched domains known as "lipid rafts" are particularly abundant in the lipid bilayer, characterized by reduced fluidity.[107,122] These domains, ranging between 5 and 200 nm, can form larger stable "platforms" through various interactions. Abundant phosphatidylcholines lend these structures lower fluidity and greater orderliness.

According to the lipid raft model, interaction between sphingolipids (SLs) and cholesterol is what enables raft formation. Hydrophilic carbohydrate head groups of SLs enhance associations among these lipids through hydrophobic van der Waals interactions with the saturated side chains. Cholesterol further solidifies the structure by occupying the space between the glycosphingolipids and fostering tight interactions within. Cholesterol is also known to interact with gangliosides within the lipid rafts.[123]

These microdomains can modify membrane fluidity, adjust membrane shape, and trigger transbilayer flip-flop transport.[124,125] They freely float within lipid-disordered regions of the membrane.[126−128] Raft assembly begins at the endoplasmic reticulum stage, where preliminary structures are created. The process continues at the Golgi complex, where cholesterol and glycosphingolipids converge, linked to specific proteins.

Additionally, stress conditions can escalate the secretion of sphingomyelinases, inducing sphingomyelin (SM) to degrade into ceramide.[129−131] Elevated ceramide levels in lipid domains are linked to cholesterol depletion.[132] This chemical transition significantly influences the plasma membrane properties, affecting orderliness, topology, and curvature.[133,134] Interestingly, ceramides can link together within the plasma membrane, forming gel-like, ceramide-rich platforms.[135] This leads to a profound restructuring of the lipid domain organization as small domains transform into large ceramide-rich signaling platforms.[136]

The importance of lipid domains in cell function is due to their high protein concentration, especially GPCRs, drawn to these regions.[137,138] Any change in the composition or fluidity of the domains due to lipid composition alterations can directly influence receptor affinity, signaling, and following internalization.[139,140]

Research shows that lipid domains are particularly enriched in synaptic membrane fractions and, specifically, the postsynaptic density (PSD).[141−144] Notably, lipid domain markers like flotilins, known for their interaction with neurotransmitter receptors such as the *N*-methyl-D-aspartate receptor (NMDA-R) subunits, have been identified at the PSD.[144−146] These lipid domains largely determine the protein

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composition of the PSD and are crucial to the signaling of numerous receptor types. Consequently, the structure and arrangement of lipid domains have a significant impact on cell signaling, synaptic transmission, and neuronal plasticity.

This proposal suggests that the creation and structural modification of lipid rafts/platforms under diverse conditions can instigate changes in the functional characteristics of various neurotransmitter receptors, including 5-HT-Rs.

Enzymes Controlling Lipid Landscape and Plasticity. The structure and functionality of lipid domains, which play a key role in cell signaling, are defined by their lipid composition. Several localized mechanisms can manipulate this composition. A key pathway involves alterations in the function of lipid metabolism enzymes, some of which are directly housed in the domains. The plasma membrane accommodates lipid regulatory enzymes such as NSM, which facilitates the conversion of sphingomyelin to ceramide. Other enzymes include sphingomyelin synthases (SMS) that orchestrate the production of sphingomyelin from ceramide, ceramidases that modify ceramide into sphingosine, and sphingosine kinases (SpK) which convert sphingosine to S1P.[133,147,148] These enzymes can tip the balance of sphingomyelin and ceramide within lipid domains, subsequently altering their composition and roles.

NSM, a vital enzyme in ceramide synthesis, is present in the caveolae of fibroblasts in Niemann−Pick disease type A patients. These patients lack functional ASM, positioning NSM as the sole regulator of ceramide production in lipid domains.[149] NSM can also move from other organelles to the plasma membrane under certain stimuli. Specifically, tumor necrosis factor-alpha (TNFα) can instigate this translocation from the Golgi apparatus to the plasma membrane.[150]

NSM plays a crucial role in the swift aggregation or clustering of proteins in lipid rafts, largely through controlling the SM-ceramide balance. After inhibiting NSM, the level of annexin 6 (a lipid rafts' protein marker at synaptic membranes) significantly decreases.[151] Lipid rafts serve as docking sites for glutamate receptors,[144,152] with NSM uniquely modulating synaptic plasticity by managing the membrane insertion of NMDA-Rs.

The inhibition of NSM activity (either pharmacologically or genetically) blocks TNFα-induced generation of ceramide, the phosphorylation and clustering of NR1 subunits, NMDA-triggered Ca^{2+} flux, and excitatory postsynaptic currents in cultured hippocampal neurons.[153] Moreover, inhibiting NSM also impacts the expression of various NMDA-Rs and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-R).[154,155]

ASM activation occurs in response to various stimuli.[156] It can quickly move from an intracellular compartment to the plasma membrane upon Fas stimulation,[157] likely facilitated by Ca^{2+}-dependent lysosome exocytosis.[158,159] Lipopolysaccharide (LPS) prompts ASM activation and ceramide production in lipid domains. This is followed by phosphokinase C (PKC)-zeta phosphorylation, Toll-like receptor 4 (TLR4) assembly, the MAP kinase family activation, and TNFα release in these domains. The ASM functional inhibitor, imipramine, blocks the effects of LPS when used as pretreatment.[160] Similarly, interleukin-1β and nerve growth factor modify the SM/ceramide ratio in the human fibroblasts' caveolae compartment through a zinc-independent mechanism of ASM activity regulation.[161,162] In summary, the SM/ceramide rheostat is mediated by at least two enzymes of ceramide synthesis—ASM and NSM. These enzymes, which are either located in or rapidly translocated to lipid domains upon stimulation, play a vital role in lipid domain formation and further cell signaling and plasticity.

The plasma membrane's sphingomyelin (SM) content could potentially influence its cholesterol content. One of the primary cholesterol reservoirs in a cell is only made accessible when the sphingomyelin's content decreases due to its degradation to ceramide. This reduction prompts the movement of cholesterol from lysosomes to the plasma membrane, facilitated by the Ras-related protein 8 (Rab8). This small GTPase molecule encourages the separation and movement of CD63-positive, cholesterol-rich, lysosome-related organelles from lysosomes to the plasma membrane.[163−166] However, the precise mechanism of cholesterol transport and its potential influencing factors remain undetermined.

The endoplasmic reticulum has been demonstrated to produce the ceramide component of gangliosides. Meanwhile, the enzymes glycosyl transferases and glycosyl hydrolases, which direct the carbohydrate component's breakdown in gangliosides, are located in the plasma membrane. Consequently, changes to the ganglioside content of plasma membranes and lipid domains may occur in response to specific stimuli impacting signal transduction.[167]

LIPID REGULATION OF THE 5-HT SYNAPSE

Cholesterol and 5-HT Signaling Proteins. Multiple cholesterol-binding sites and actual cholesterol binding have been identified for several 5-HT-Rs.[168] Alterations in the cholesterol content within biological membranes significantly influence both their physical attributes and signal transduction processes.[169−171] Particularly, the 5-HT_{1A}-R interfaces directly with cholesterol molecules and clings to phospholipid phosphatidylinositol 4-phosphate.[172] Chronic cholesterol depletion transitions the internalization of 5-HT_{1A}-R from clathrin-led to caveolin-mediated endocytosis. In these circumstances, the internalized receptor pool deviates from endosomal recycling toward lysosomal degradation.[173]

Furthermore, the lysine K101 residue in the CRAC motif of the cholesterol's transmembrane helix plays a vital role in safeguarding 5-HT_{1A}-Rs from thermal deactivation. Any mutation in this residue leads to the loss of 5-HT_{1A}-Rs' thermal stability, a condition independent of membrane cholesterol content.[174] Several studies suggest a direct interaction between cholesterol and 5-HT-Rs impacts receptor functioning. A decrease in membrane cholesterol levels due to the specific inhibitor methyl-β-cyclodextrin (MβCD), or the solubilization of native hippocampal membranes, results in reduced binding of receptor antagonists and agonists to the 5-HT_{1A}-Rs in hippocampal cell culture.[170,175−178] Replenishing cholesterol levels counter this effect.[170,178]

However, an analog of cholesterol, 7-dehydrocholesterol, re-establishes membrane order but fails to restore 5-HT_{1A}-Rs binding.[179] Similarly, cholesterol oxidation inhibits the binding activity of 5-HT_{1A}-Rs without affecting the membrane order.[170] Decreasing the free cholesterol availability using digitonin leads to a corresponding reduction in ligand binding to the 5-HT_{1A}-R.[178]

The cholesterol-binding protein caveolin-1, which is abundant in lipid rafts, interacts with 5-HT_{2A}-Rs and boosts the connection between 5-HT_{2A}-R and G_q proteins.[180]

Simulation data imply that cholesterol significantly influences the dimerization of 5-HT_{2C}-R.[181] Yet, this hypothesis still requires experimental validation.

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Like 5-HT_{1A}-Rs, the function of the 5-HT_3-R is determined by cholesterol. It is been observed that cholesterol molecules next to the M4 α-helices in the 5-HT_{3A}-R's transmembrane domain help stabilize the receptor's configuration and control its transition to an active, open state. The cholesterol sits within a hydrophobic pocket made up of pre-M1, M1, post-M4, and the receptor's Cys loop. Through stabilizing M4, pre-M1, and the Cys loop into a compact conformation attached to the M2−M3 linker, cholesterol can indirectly influence the functioning of the channel. This interaction facilitates a more compact "coupled" conformation of the receptor.

Furthermore, the compression of the transmembrane domain's central axis is determined by cholesterol-enriched domains due to the hydrophobic core thickness of the lipid bilayer. As a result, the 5-HT_{3A}-R assumes an "uncoupled" state resistant to activation.[182] The depletion of cholesterol by MβCD and simvastatin has been observed to reduce both the peak amplitude and the charge of cation currents triggered by 5-HT in N1E0115 mouse neuroblastoma cells and HEK 293 cells that express the human 5-HT_{3A}-R in a stable manner. These findings suggest cholesterol plays a role in extending 5-HT_{3A}-R desensitization and deactivation.[139] Finally, it has been proposed that it is the direct interaction between cholesterol and 5-HT_{1A}- and 5-HT_3-Rs that is crucial to regular functional activity rather than alterations in the membrane's composition and order.

Sjögren and Svenningsson[183] demonstrated that the depletion of cholesterol through combined treatments with mevastatin, FB1, mevalonate, or MβCD can reversibly reduce 5-HT_7-R binding in stably transfected Hela cells. Nonetheless, they suggested that the impact of cholesterol depletion on 5-HT_7-R binding is correlated more with the number of receptors at the cell surface rather than G-protein coupling. They found that silencing caveolin-1, a crucial cholesterol-binding protein, using siRNA led to a decrease in 5-HT binding to 5-HT_7-Rs.

The early experiments on monoamine transporters revealed the stabilizing effect of sterols. These findings were derived from attempts to solubilize, purify, and reconstitute the transporters.[184,185] It was concluded that cholesterol influences SERT activity through both indirect effects in the membrane[186] and direct sterol-protein interaction.[187] The indirect effect is likely achieved through the lipid microdomain localization of SERT.[186] Meanwhile, the direct effect was hypothesized due to findings that sterols such as ergosterol could not restore SERT activity following cholesterol depletion.[187]

Cholesterol depletion in the membrane—achieved via the use of cholesterol-chelating agents like cholesterol oxidase, MβCD, or cholesterol-binding fluorochrome filipin—also impacts SERT activity. This depletion is observed to diminish affinity for the substrate, decrease ligand binding, and reduce the maximum transport rate. It is suggested that a direct molecular interaction between cholesterol molecules and SERT is necessary for stabilizing the transporter in its most active form.[187]

A sterol binding site, CHOL1, between TM1a, TM5, and TM7 was initially proposed for the identification of cholesteryl hemisuccinate binding sites in the first crystal structures of the homologous dopamine transporter from *Drosophila melanogaster* (dDAT).[188−190] Later, additional dDAT crystal structures revealed another cholesterol hemisuccinate binding site, CHOL2, between TM2, TM7, and the cytoplasmic end of TM12, which is adjacent to CHOL1.[189−192] Subsequent SERT crystal structures suggested yet another potential sterol binding site, CHOL3, near the extracellular end of TM11.[193,194] Cholesterol hemisuccinate's presence in CHOL1 was confirmed in cryo-EM structures of SERT.[195]

However, caution should be exercised when interpreting these potential sterol sites as the reaction of the detergent cholesteryl hemisuccinate does not mirror that of cholesterol. The interpretation of the CHOL1 site might also be skewed by the use of cholesteryl hemisuccinate instead of cholesterol. Furthermore, it is noteworthy that the acidic part of cholesteryl hemisuccinate is found to be stabilized by a nearby lysine on TM1 in a favorable ionic interaction, which is irrelevant when it comes to cholesterol.[196,197] It is also possible that these sterol sites hold no functional significance to the transporter.

Regardless, these sterol sites identified in the structures could be suggestive of potential sterol system locations, particularly when considering our understanding of the functional aspects of the SERT.

Further scrutiny was conducted on the CHOL1 site following the observation that it was located between two mobile domains of SERT. It was also discovered that this potential cholesterol site is highly conserved in evolution, suggesting a vital functional role that, when altered, is susceptible to negative selection.[198] Even though molecular dynamics simulations found up to six potential cholesterol sites on SERT,[199] subsequent simulations revealed that cholesterol primarily visited two sites: CHOL1 and CHOL2.[198]

Functional characterization of SERT showed that cholesterol depletion shifts its conformational equilibrium toward a more inward-facing position.[198,200] This finding was replicated in silico in other monoamine transporters. One study found that cholesterol depletion results in partial unwinding of TM5,[66] a trait also found in a similar bacterial transporter structure.[201] This unwinding, in turn, creates an intracellular pathway to the central substrate site.[66]

Then, SERT is cholesterol-depleted; its equilibrium shifts even further toward an inward-facing position. This shift enhances its affinity to the substrate, 5-HT; as well as substrate-like alkaloids, ibogaine and noribogaine. These substances are known to induce the inward-facing conformation in SERT when in native membranes.

Mutational analysis of CHOL1 showed that mutations favoring cholesterol binding to CHOL1 resulted in a more outward-facing SERT. This allows the transporter to overcome the rate-limiting conformational transition from inward to outward-facing more effectively,[202] thus becoming faster.[198] Conversely, mutations that do not favor cholesterol binding to CHOL1 lead to more inward-facing SERT. This hampers the transporter's ability to overcome the rate-limiting conformational transition from inward- to outward-facing, making it slower.[198]

Hence, the dynamic nature of cholesterol binding and unbinding from CHOL1 significantly impacts the conformational balance and turnover rates of SERT. This calls for a new perspective on cholesterol in SERT function.

The findings are in line with the dynamic cofactor role of cholesterol. It disassociates from CHOL1 on SERT to help the transition from a substrate-loaded, outward-facing transporter to a substrate-loaded, inward-facing one. Conversely, cholesterol associates with CHOL1 after intracellular substrate release to assist the rate-limited transition of the apo transporter from an inward to outward-facing orientation.

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Cholesterol's binding to CHOL1 is vital not only for its role in conformational changes related to transport but also in warding off thermal inactivation, likely by stabilizing the SERT structure within the cholesterol-rich cell membrane once correctly folded. Additionally, some evidence points to the necessity of SERT's transitional phase into an inward-facing intermediate state during folding in the endoplasmic reticulum (ER).[203] The ER's low-cholesterol environment may restrict cholesterol's binding to CHOL1, thereby prompting the inward-facing conformation and potentially facilitating the nascent SERT's folding trajectory. Supporting this theory, statin-induced cholesterol reduction partially retrieves some folding-impaired outward-facing mutants (Sinning lab, unpublished data). Regardless of its presence in the ER or cell membrane, cholesterol depletion does not seem to impact SERT oligomerization, indicating that oligomerization is independent of cholesterol.[204]

The crucial role of cholesterol in boosting SERT transport rates raises the question of whether this is a mechanism designed to inhibit SERT activation in the ER's low-cholesterol environment, where transport activity is presumably unnecessary. Similarly, the membrane environment in and around synapses is abundant in cholesterol,[205] which is likely to increase CHOL1 occupancy. In this context, the CHOL1 site serves as an "ON" switch, primarily activated in areas with higher cholesterol content, such as the cell surface and near synapses. This could be a strategy for enhancing SERT activity where it is most needed in the neuron.

Statins, commonly prescribed cholesterol-lowering drugs, can cross the blood−brain barrier and influence brain cholesterol levels. Given the previously discussed inhibitory effect of cholesterol depletion on SERT and 5-HT-R activity, one can consider its impact on serotonergic neurotransmission. Lowering cholesterol can have numerous effects on brain function. Looking at it in isolation, cholesterol depletion can impair SERT function, leading to outcomes like those of antidepressants. Indeed, statins have been found to boost the effects of the antidepressant fluoxetine[206] and change the behavior and activity of SERT in vivo.[207] This implies that reducing cholesterol or targeting the CHOL1 site on SERT with appropriate drugs could offer new treatment strategies for psychiatric disorders, particularly depression. Overall, the evidence emphasizes the significance of cholesterol for 5-HT signaling mediated by 5-HT-Rs and SERT. The specific interactions between cholesterol and these proteins appear to be key to this link.

PUFAs and 5-HT Signaling Proteins. Exogenous PUFAs interact with phosphatidylcholine and phosphatidylethanolamine, the most prevalent phospholipids in lipid rafts. As a result, they influence the interaction, organization, fluidity, and clustering degree of lipid rafts. Supplementation with n-3 PUFAs can enhance hippocampal expression of 5-HT_{1A}-R and reverse depression-like behavior observed in postpartum depression models and during combined chronic mild stress and maternal separation.[208,209] These changes may partly account for the compounded antidepressant effects of n-3 PUFAs and selective serotonin reuptake inhibitors (SSRIs).[210] Contrastingly, treatment with the exogenous n-6 PUFA, arachidonic acid, showed no effect on the density of 5-HT_{1A}-R, its agonist affinity, the coupling efficiency between 5-HT_{1A}-R and G-proteins, or the activation ability of the 5-HT_{1A}-R to interact with G-proteins.[211]

The most recent structure of porcine SERT, derived from native brain tissue, uncovered a lipid-like density that penetrates both TM10 and TM11 and the transporter itself.[212] This density, extending from the in-built membrane portion of the transporter, reaches its headgroup to the extracellular vestibule. It occupies a position near the allosteric S2 site. However, it remains unclear whether this density signifies the presence of a detergent molecule, dodecyl-beta-D-maltoside (DDM), used during the solubilization process, or a native docosahexaenoic acid (DHA) originating from brain tissue. Nevertheless, even if DDM binds permanently to this site, it may suggest a lipid site existing in native tissue. This interaction between a lipid and the S2 site could have potential effects on SERT function. Simulation studies seem to favor DDM as the preferred chemical binding partner, though deprotonated DHA also showed some stability at this site.[212] Future research needs to investigate the existence of this potential fatty acid site in native lipid environments and its potential influence on SERT function.

The impact of direct fatty acid binding on serotonin transporter (SERT) function has remained largely underresearched, and there may still be unseen aspects to discover. Take, for example, the analogous bacterial transporter, Leucine Transporter (LeuT), where it has been demonstrated that cardiolipin, a highly negatively charged lipid, stabilizes the dimer by bridging the dimer interface between two protomers within the TM12 area.[213] Like LeuT, SERT is known to form oligomers,[214−216] and its oligomeric interface is similar to LeuT. However, there could be some differences, possibly attributed to variations in TM12 orientation.[217]

Phosphatidylinositol 4,5-bisphosphate (PIP2) shares a similar high negative charge with cardiolipin and possibly plays a similar role for the SERT as cardiolipin does for LeuT. Simulations indicate that PIP2 indeed impacts the stability of the SERT dimer.[217] Conversely, imaging experiments suggest that PIP2 depletion causes disruption of SERT oligomers at the plasma membrane.[204]

Mutational analysis suggests that PIP2 could potentially form an ionic bridge between positively charged patches on Transmembrane Regions 6 and 9 (TM6 and TM9).[204,218] Thus, Anderluh and colleagues proposed a compelling model[204] demonstrating that the fluid exchange of SERT oligomer subunits in the low PIP2 environment of the ER becomes more static when the transporter reaches the PIP2-rich cell membrane with PIP2 serving as a binder between SERT protomers.

Considering the contribution of oligomerization to numerous functional and pharmacological properties of the SERT,[219] lipids impacting oligomerization can be presumed to have a direct functional effect. In this context, PIP2 is the most extensively studied. However, given the wide variety and complexity of lipids in the cell membrane, many other lipids may also be significant.

The data suggests that PUFAs could play a crucial role in regulating 5-HT signal transmission in the brain. Unlike other lipid raft components, humans cannot synthesize PUFAs from scratch, and they can only be altered from linoleic and α-linoleic acids. Therefore, PUFAs could potentially modify lipid raft structures, which in turn could impact 5-HT function. This could provide a potential treatment method for mental disorders related to 5-HT.

Sphingolipids and 5-HT Signaling Proteins. The main pathways of ceramide metabolism play a crucial role in

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managing 5-HT signaling. The sphingomyelinase pathway, in particular, influences 5-HT_{1A}-R binding. When treated with sphingomyelinase, the membrane order of CHO-5-HT_{1A}-R cells decreases, concurrently increasing the specific ligand-binding activity of the 5-HT_{1A}-R.[220] Conversely, when applied to native hippocampal membranes, sphingomyelinase did not affect membrane order but decreased the binding of the specific 5-HT_{1A}-R agonist, (3H)8-OH-DPAT.[221] This contrasting data suggests a potential for both direct and indirect interactions between the ceramide system and 5-HT_{1A}-Rs. Further, in vivo experiments demonstrated an interaction between enzymes involved in ceramide synthesis and the functioning of 5-HT-Rs and SERT. No detectable changes in the expression of 5-HT-Rs and SERT were found in the ventral striatum of naïve female mice with NSM deficiency. However, when NSM activity was decreased, it blocked the effects of chronic alcohol consumption on 5-HT_{3A}-receptor mRNA while increasing 5-HT_{1A}-R mRNA expression. NSM treatment also lowered 5-HT uptake in the ventral- and dorsal hippocampus synaptosomes but had no impact on the ventral striatum of mice.[222]

Likewise, in male heterozygous knockout mice with reduced NSM activity, there were no observable changes in the expression of 5-HT-Rs and SERT. However, reducing NSM activity did diminish the alcohol-induced increase in mRNA expression of 5-HT_{1A}-R, 5-HT_{2C}-R, and SERT in the ventral striatum of these male mice.[223] Hence, the 5-HT system's response to external stimuli is influenced by NSM in a gender-specific manner.

Ceramide metabolism's de novo pathway, which serine palmitoyltransferase (SPT) mediates, also controls 5-HT signal transduction. A decrease in SPT activity in sphingolipid-depleted B lymphocyte cells lacking a long-chain base 1 unit of SPT correlated with an increase in 5-HT_{1A}-R agonist (3H)-8-OH-DPAT binding. The ceramide content mediates this effect, as adding ceramide to the cell culture reverses changes in 5-HT_{1A}-R binding activity.[224] Inhibition of another enzyme in the de novo pathway, CerS, led to a reduction of 5-HT_{1A}-R ligand binding and signaling in human CHO-K1 cells.[225,226] The CerS inhibitor fumonisin B1 achieved this reduction. Fumonisin B1 increased the mobile fraction of this receptor without altering the diffusion coefficient.[225] However, examining cAMP levels showed a significant decrease in the G-protein coupling and downstream signaling of 5-HT_{1A}-R after administering fumonisin B1.[227] Sjögren and Svenningsson observed similar impacts of fumonisin B1 on 5-HT_7-R binding.[228] Interestingly, the receptor's expression rate remained unaffected by fumonisin B1, indicating the inhibitor's direct effects on receptor signaling rather than on membrane levels.[228]

Complex ceramides, such as glycosylceramides and gangliosides, regulate 5-HT signaling. Evidence includes the use of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), a particular glucosylceramide synthase inhibitor, which decreases the binding of specific 5-HT_{1A}-R- and 5-HT_7-R agonists. This effect likely results from a reduction in total receptor binding sites, with no change in binding affinity or expression rate.[221]

Studies have shown that treatment with glucosylsphingosine activates 5-HT_{2A}-Rs in mouse dorsal root ganglion neurons. This process stimulates PLC through $G_{\alpha q/11}$ and the $G_{\beta\gamma}$ complex. Consequently, PLC activation decomposes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG), leading to further protein kinase C activation and TRPV4 sensitization. This mechanism controls glucosylsphingosine-evoked scratching behavior in mice, which can be significantly reduced by pretreatment with inhibitors of either, the 5-HT_{2A}-R or TRPV4.[229]

The vital ganglioside found in lipid-enriched domains, GM1, has been demonstrated to interact directly with 5-HT_{1A}-Rs. Specifically, the sphingolipid binding domain of 5-HT_{1A}-R binds directly to GM1, which triggers a conformational change in the tryptophan residue, moving it away from the receptor's central lumen. This direct influence of GM1 on the extracellular loop 1 of the 5-HT_{1A}-R could potentially affect ligand binding and the receptor's functionality.[230]

Lipid Rafts/Platforms Interactions with 5-HT Signaling Proteins. Lipid microdomains, also known as lipid rafts, create a dynamic setting for interactions between membrane proteins and lipids. These nanoscale assemblies consist of cholesterol and sphingolipids, which help facilitate protein segregation and interactions between proteins.[231,232] The size and lipid composition of these microdomains are highly diverse. The function of membrane proteins is influenced by their location within the lipid raft, as it allows for direct protein−lipid interactions or offers a distinct physical membrane environment.[233]

The pivotal role of cholesterol in membrane microdomains is emphasized by the disruption of lipid rafts as a result of cholesterol depletion. Sphingolipids, with their long, saturated acyl chains, are drawn to partition into liquid-ordered membrane domains.[234] The existence of sphingolipid-rich lipid microdomains, which do not rely on cholesterol, has also been proposed.[235] Moreover, it is suggested that ceramides organize into ceramide-rich zones and displace cholesterol from lipid rafts, which, in turn, modifies the properties of the membrane microdomain.[236]

Most 5-HT GPCRs, including 5-HT_{1A}-Rs,[205] 5-HT_{2A}-Rs,[239] and 5-HT_7-Rs,[183] are largely found in lipid raft parts of the membrane.[237,238] These receptors, alongside SERTs,[186] tend to cluster primarily in lipid rafts/platforms, potentially involving the cytoskeleton.[240] Double palmitoylation of 5-HT_4-Rs and 5-HT_{1A}-Rs corroborates their recruitment by the lipid domains.[205,241] Additionally, studies demonstrate an association of 5-HT_6-R with Fyn,[242] a crucial Src family member of nonreceptor protein-tyrosine kinases found in lipid raft regions.[243]

The interaction between cholesterol and 5-HT_{1A}-Rs is crucial for ligand binding and the activation of G-proteins.[175,244,245] Three cholesterol-binding motifs known as CRAC have been identified in the 5-HT_{1A}-R. These motifs are found across multiple species.[246] Reducing cholesterol levels or mutating the CRAC sites can lessen the agonist binding of 5-HT_{1A}-R and downstream signaling.[175,244,245] Apart from cholesterol, other membrane lipids, such as cholesterol derivatives and sphingomyelins, can enhance the liquid-ordered properties of the membrane. These lipids increase nucleotide exchange rates induced by 5-HT_{1A}-R in coupled G-proteins.[247] This implies that the functioning of 5-HT_{1A}-R can be affected by cholesterol and other lipids through changes in the receptor's lipid environment. Evidence of this comes from the observation that converting sphingomyelin to ceramide reduces 5-HT_{1A}-R agonist binding.[221] As expected, various model systems and tissues have presented 5-HT_{1A}-R to be associated with lipid microdomains and colocalize with lipid raft marker proteins.[176,205,247] It also seems that receptor

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palmitoylation plays a pivotal role in the receptor's association with the lipid raft.[205]

5-HT_{2A}-Rs are associated with lipid rafts across various cell types.[180,239,248,249] Most studies found the 5-HT_{2A}-R predominantly located in caveolae, which are cholesterol-rich, specialized lipid microdomains abundant in certain cells,[180,239,249] with the exception of one study.[248] The 5-HT_{2A}-Rs also physically interact with Cav-1, Cav-2, and Cav-3.[180,249] Cav-1 enhances the signaling of 5-HT_{2A}-R mediated by promoting the receptor's coupling to $G_{\alpha q}$.[180] Disruptions in Cav-1 are identified as risk factors for schizophrenia, leading to reduced sensitivity to atypical antipsychotics and implicating the 5-HT_{2A}-R as a crucial target.[250]

Conversely, in cardiomyoblasts, the 5-HT_{2A}-R interacts with Cav-3 based on the presence of an agonist, causing the receptor's repositioning into caveolae. This interaction with Cav-3 adversely regulates the activation of the calcineurin/NFAT pathway downstream of 5-HT_{2A}-R in this cell type.[249] Depleting cholesterol disrupts lipid rafts and caveolae, reducing the responses mediated by 5-HT_{2A}-R.[239,248]

Aside from GPCRs, the 5-HT_3-R is the only ligand-gated ion channel among 5-HT-Rs. It coexists in lipid microdomains with adapter proteins such as flotillin-1 and caveolin-2. It is also acted upon by various antidepressant drugs, which serve as noncompetitive antagonists.[139,251,252] 5-HT_3-R-dependent cation currents induced by agonists decrease after cholesterol is depleted. Even so, lipid microdomains do not seem to play a part in the receptor's antidepressant binding.[139] Certain antidepressant and antipsychotic drugs, including desipramine, fluoxetine, reboxetine, fluphenazine, haloperidol, aripiprazole, and clozapine, are found with 5-HT_3-Rs specifically in lipid microdomains. They also alter the phase behavior of complex lipid bilayers.[253] It is interesting to note their concentration in lipid domains correlates with their inhibitory strength against 5-HT-induced cation currents.[251] In conclusion, the specific interaction between the lipids of lipid rafts and 5-HT-Rs and SERTs may be pivotal for 5-HT signaling at the synapse.

Just as with 5-HT_{2A}-Rs, the binding, signaling, and receptor surface expression of the 5-HT_7-R ligand are moderated by lipid rafts and the Cav-1 adaptor protein.[228,254] The attenuation of agonist binding to 5-HT_7-Rs was observed when lipid microdomains were disrupted by either depleting cholesterol, inhibiting SM and ganglioside synthesis, or knocking down Cav-1.[183,254]

The association of SERT with lipid rafts was initially demonstrated two decades ago using sucrose gradient centrifugation to segregate detergent-resistant membranes.[186,255] The use of antagonist-conjugated quantum dots facilitated the colocalization of SERT with cholesterol and GM1 ganglioside-enriched microdomains in vivo.[240] Altered transport activity, antidepressant binding, and lateral mobility of SERT are consequences of cholesterol depletion.[186,187,240] Protein kinase C activation, which results in the down-regulation of SERT activity, is also associated with the transporter's shift from the raft to nonraft fractions.[255]

Our understanding of how sphingolipids and ceramides influence the association of SERT with lipid rafts remains minimal. Nonetheless, recent studies showing that the in vitro application of bacterial sphingomyelinase decreases SERT activity in hippocampal and striatal synaptosomes[222,223] suggest potential interactions. It is plausible that increased ceramide production might alter the lipid environment of SERT or its interaction with cholesterol.

Lipid rafts likely aid in the dynamic regulation of protein−protein interactions within SERT protein complexes, including interactions with the lipid raft marker, flotillin.[256,257] Mice with a flotillin knockout exhibit a heightened likelihood of enduring stress-induced depression-like behavior, possibly due to amplified SERT expression and modified serotonergic neuron activity.[258] Intriguingly, when compared to the wildtype SERT, flotillin's abundance is notably diminished in complexes of the Ala56 SERT variant, which is linked with autism spectrum disorders.[257] It is plausible that changed lipid interactions can contribute to an enhanced functional phenotype in this mutation and thus potentially play a part in autism and related psychiatric disorders.

In summary, 5-HT-Rs and SERTs are associated with lipid rafts, and the integrity of these microdomains can impact protein function. Therefore, conditions that disrupt lipid rafts, like cholesterol depletion, could significantly affect 5-HT signaling and related disorders.[238,259]

LIPID EFFECTS ON 5-HT THROUGHPUT

In addition to pronounced impacts on 5-HT signaling and receptor function, the lipids in biological membranes also influence signal transmission at the 5-HT synapse.

Cholesterol and 5-HT Throughput. Cholesterol, a key lipid of biological membranes, influences the levels of 5-HT in experimental animals. Kaplan et al.[260] introduced the cholesterol-5-HT hypothesis, stating that lowered cholesterol levels from poor diet or inhibited synthesis might result in reduced central 5-HT activity. This theory provides some insight into cholesterol's role in depressive behavior, suicidal tendencies, and aggressiveness.[260−264] It has been suggested that low cholesterol and 5-HT levels make men more prone to violent behavior and risk-taking compared to women.[265]

Statins, medications that hinder cholesterol synthesis and lower plasma levels, reduce the telencephalic 5-HIAA/5-HT ratio in Nile tilapia fish and decrease the 5-HT brain levels in rodents.[261,266] Meanwhile, treatment with antidepressants like paroxetine, clomipramine, sertraline, duloxetine, and fluoxetine, which have strong SERT affinity, leads to higher LDL-cholesterol levels.[266] While there is a definitive link between cholesterol and 5-HT function, the exact process remains unknown.

PUFAs and 5-HT Throughput. A deficiency in n-3 PUFA dietary intake did not appear to shift synaptic 5-HT levels in the brains of rats. Nonetheless, it might influence 5-HT throughput via its impact on MAO-B, albeit not on MAO-A activity.[267,268] A lower density of synaptic vesicles in the hippocampus and frontal cortex in rats subjected to an n-3 PUFA-deficient diet may further contribute to this effect.[268−270] However, a shortage of linoleic (18:2(n-6)) and linolenic (18:3(n-3)) acids triggered a reversible decrease in 5-HT and 5-HIAA tissue concentrations in the frontal cortex of piglets.[271] Alterations in 5-HT signaling owing to n-3 PUFA dietary deficiency could account for heightened anxiety in mice and rats in contrast to rodents on a standard diet.[272,273]

Supplementation with n-3 PUFA may counteract decreases in 5-HT levels in the frontal cortex, striatum, and hippocampus of mice exposed to unpredictable chronic mild stress and may also reduce its physical and behavioral symptoms.[274] Consuming a diet high in very long-chain n-3 PUFAs can boost the turnover of dopamine (DA) and 5-HT in the striatum.[275] Similarly, supplementing with a 1:5 ratio of n-3/n-6 PUFAs has been observed to increase hippocampal 5-HT,

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decrease serum 5-HT levels, and improve autistic behaviors in a rat model of valproic-acid-induced autism.[276]

Sphingolipids and 5-HT Throughput. The sphingolipid system has been revealed to significantly influence 5-HT homeostasis within the central nervous system. ASM, a primary enzyme in ceramide synthesis, appears to be crucial in managing the functioning of the 5-HT system. Functional inhibitors of acid sphingomyelinase (FIASMAs), which include antidepressants like fluoxetine, nortriptyline, sertraline, promazine, paroxetine, and others, primarily affect the 5-HT system.[277,278] In mice, overexpression of ASM causes innate depression and a decrease in 5-HT tissue levels in various brain areas.[279] However, alcohol consumption can restore 5-HT levels in certain regions, contributing to improved depressive behaviors.[279] A negative correlation has been established between ASM activity and 5-HT tissue levels in naïve Wistar rats.[154] Despite this, ASM overexpression does not impact basal extracellular 5-HT levels or the 5-HT response to alcohol exposure in mice.[280] The authors suggest that while the ASM/ceramide system significantly impairs 5-HT synthesis and storage, it probably does not affect release.[280] Furthermore, 5-HT innervation of the dorsal hippocampus in mice with ASM overexpression was found to be preserved.[281]

NSM, another enzyme involved in the synthesis of ceramide, has also been shown to regulate 5-HT throughput, albeit less so than ASM. An in vivo microdialysis study revealed that male mice with reduced NSM activity have increased extracellular 5-HT levels in certain areas but not in the dorsal hippocampus. Reduced NSM activity also decreased the alcohol-induced rise in extracellular 5-HT in the dorsal hippocampus of male mice.[223] However, the same reduction of NSM activity in female mice showed preserved basal extracellular 5-HT levels and response to acute alcohol exposure in two brain areas.[222] However, NSM activity did not correlate with post-mortem 5-HT tissue levels in several parts of naïve male Wistar rats' brains.[154]

NC, an enzyme involved in ceramide breakdown, had a negative correlation with the brain tissue level of 5-HT in one part of naïve male Wistar rats' brains but not in others.[154] This evidence suggests a complex, region and sex-specific relationship between ceramide metabolism enzymes and the 5-HT system.[282]

Myriocin, an inhibitor of ceramide synthesis' de novo pathway, was found to lower 5-HT levels in the cortex and medulla oblongata of mice. Interestingly, the 5-HIAA/5-HT ratio, a measure of 5-HT metabolism intensity, declined in the cerebellum postmyriocin treatment but remained unchanged in the cortex, striatum, and medulla oblongata.[283]

Direct ceramide Cer16:0 infusions into the dorsal hippocampus or basolateral amygdala did not affect 5-HT tissue in the prefrontal cortex (PFC), ventral striatum, and dorsal mesencephalon.[284] These findings suggest a potential specific role for ceramide synthesis enzymes, but not all ceramide species, in regulating 5-HT throughput.

Complex sphingolipids, such as gangliosides, have been demonstrated to regulate the 5-HT system. To date, only limited data support a relationship between gangliosides and 5-HT throughput under normal conditions. However, gangliosides have consistently been shown to restore 5-HT system function following pathological incidents. Specifically, subchronic daily intraperitoneal injections of GM1 reinstated reduced 5-HT and 5-HIAA tissue levels in the ipsilateral hippocampus following electrolytic damage near the nucleus interpeduncularis. Under these circumstances, GM1 did not affect tryptophan or nerve growth factor content.[285,286] Similarly, GM1 restored 5-HT and 5-HIAA levels in the caudal cortex 2 weeks post-transection of monoaminergic cortical innervation, with effects lasting for several weeks.[287] Immediate application of GM1 postocclusion of the left middle cerebral artery reduced the extent of postischemic damage,[288] by restoring 5-HT neurons.[289] 5-HT neuron growth and nerve terminal increase were also promoted by GM1 pretreatment, following infusion of the neurotoxin, 6-hydroxytryptamine.[290,291] All these findings suggest beneficial and restorative impacts of exogenous gangliosides on the 5-HT system. Nonetheless, it remains unclear whether these effects are due to changes in membrane ganglioside composition or their indirect effects via other biological molecules. Evidence of a direct interaction between gangliosides and 5-HT will confirm the beneficial effects of membrane gangliosides on 5-HT system functioning.[292−294] Specifically, the positively charged amino group of 5-HT can engage with the negatively charged sialic acid of GM1, resulting in a low to moderate affinity for the 5-HT/GM1 complex. Additionally, the CH3 from the *N*-acetyl group of GM1's sialic acid points toward 5-HT's aromatic ring, establishing a CH−π interaction.[295] It has been suggested that this GM1-mediated binding and subsequent conformational changes of the 5-HT molecule on the postsynaptic membrane may be the starting point for 5-HT binding, guiding the selection of target 5-HT receptor subsets.[296]

Feedback Loops between Lipid Regulation and 5-HT Throughput. Changes in the functionality of the 5-HT system could alter the lipid composition of membranes, creating a reciprocal lipid-5-HT interaction. Drugs impacting the 5-HT system, specifically numerous antidepressants with FIASMA traits, modify the lipid makeup of the membranes. Paroxetine and desipramine, SSRIs with FIASMA characteristics, when administered consistently, decreased levels of sphingosine and ceramide in the prefrontal cortex and hippocampus (only paroxetine) but not in the striatum or mice's plasma.[297]

Certain antidepressants like escitalopram can accumulate in lipid rafts and subsequently influence the lipid composition.[298] This influence might derive from the disturbance of $G_{\alpha s}$/tubulin complexes and the relocation of $G_{\alpha s}$ from lipid rafts for increased adenylyl cyclase interaction, with no effect on the $G_{\alpha s}$ overall level.[299] Future studies are needed to establish whether 5-HT alone mediates these impacts.

TPH−/− mice experiencing 5-HT depletion exhibited a decrease in phosphocholines, phosphoethanolamines, phosphoinositols, and sphingomyelins and an increase in lysophosphocholines, lysophosphoethanolamines, lysoglycerophosphoinositols, ceramides, glucosylceramides, lactosylceramides, and free fatty acids in the blood.[300] While the analysis was done on peripheral tissue, it is anticipated that changes in the majority of mammalian lipids following 5-HT depletion will impact the entire organism's lipid composition, including the central nervous system's biological membranes.

Direct effects of the 5-HT system on lipid composition have been observed. Upon stable expression of the 5-HT_{1A}-R in transfected dorsal root ganglion cells, an increase was found in the levels of gangliosides GD1A, GD1B, and GT1B. Similarly, when CNS-derived NCB-20T8 cells were treated with the 5-HT_{1A}-R agonist, 8-OH-DPAT, a significant increase in GM3 levels occurred. This suggests that 5-HT_{1A}-R receptors may

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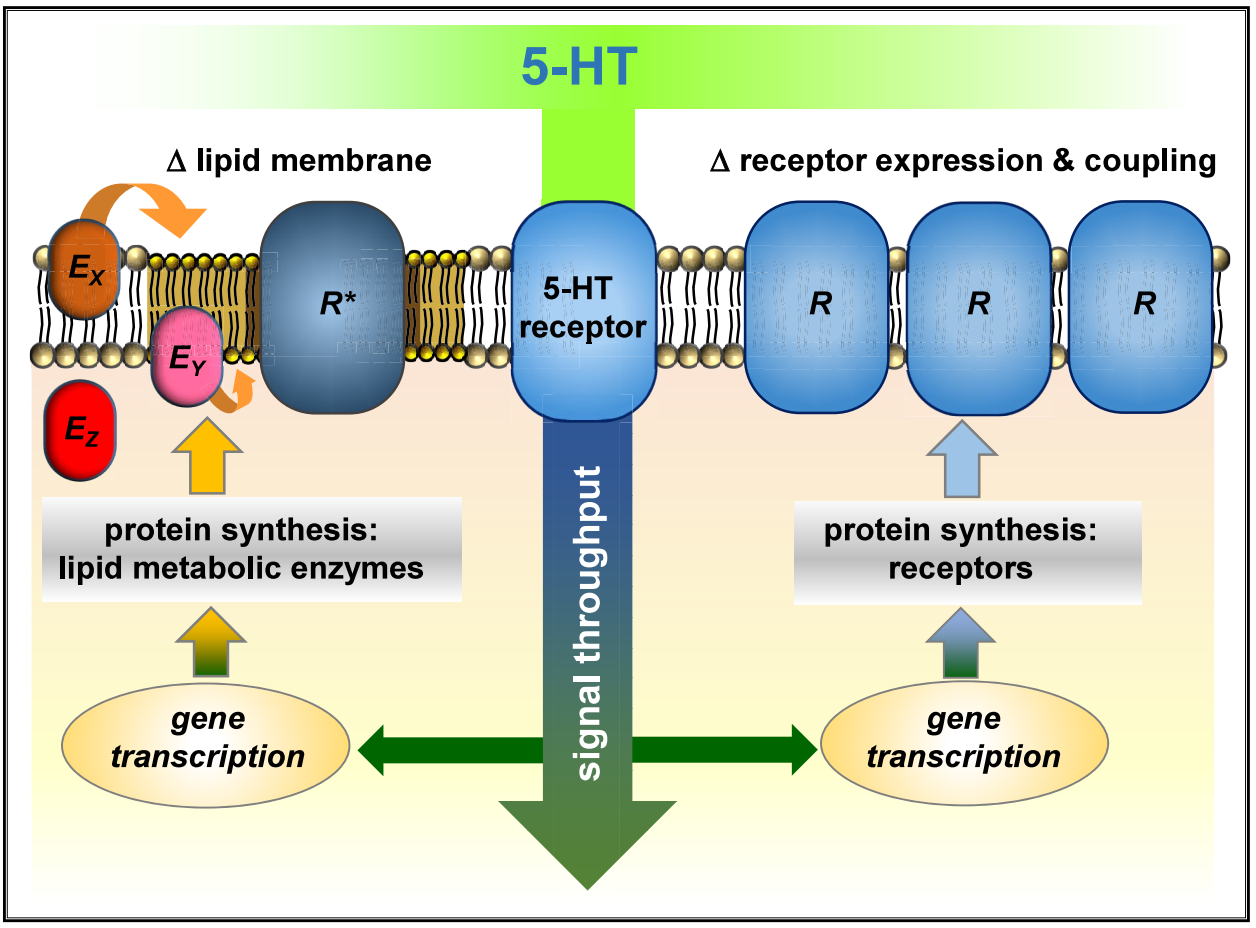

Figure 1. Schematic presentation of signal throughput at the serotonergic (5-HT) synapse and its dynamic regulation. 5-HT activation of metabotropic 5-HT receptors (R) is translated into cascades of intracellular signaling events or into hyperpolarization after ionotropic receptor activation. This affects gene transcription and mRNA production of genes coding for 5-HT-Rs on the one hand, but also for genes coding for lipid rheostat regulating enzymes (E) on the other hand. 5-HT-Rs are formed and integrated into the membrane, which alters future signal throughput. In parallel, lipid enzyme activity in the membrane and beyond changes the composition of the synaptic membrane. The change in the lipid environment of 5-HT-R alters their function (R*) and affects signal throughput. It is assumed that both mechanisms work permanently and interact in their effects on 5-HT synaptic throughput.

influence the synthesis of complex gangliosides in nerve cells.[301]

5-HT impacts membrane composition. It secures itself in the lipid membrane at the synapse, a process allowed by the salt-bridge connecting the primary amine of 5-HT and the lipid phosphate group.[19] Simulation studies reveal that 5-HT's anchoring decreases lipid chain order and membrane elasticity, stability, and permeability. It also enriches lipid domains with phosphatidylcholine, enlarging the latter's domain size.[22,302] In an in vitro study, the introduction of 5-HT to a model membrane composed of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)/N-palmitoyl-D-erythro-sphingosylphosphorylcholine (PSM)/cholesterol in a 4/4/2 molar mixture resulted in a decreased lipid chain order in the disordered phase. Conversely, this increased within the ordered phase. This was followed by altered hydrophobic thickness in the membrane domains, promoting the formation of larger domains due to increased line tension at the domain boundary. Similar effects were observed with the presence of full or partial 5-HT-R agonists such as BRL-54443, BW-723C86, and CP-135807.[253]

Interestingly, 5-HT binds preferentially to the nonraft domain of membranes. This results in compositional changes, enhanced fluidity, decreased phase transition temperature, and increased dissemination of this phase.[303] Dey et al. demonstrated that 5-HT directly binds to an artificial lipid bilayer, resulting in a softer bilayer and inciting the creation of liquid-disordered domains within the raft-like liquid-ordered domains, displaying phase separation.[304] This diffusion of extra-synaptic 5-HT in the lipid membrane enhances its reach to remote receptors or ones with buried ligand-binding sites.[304] Moreover, the rapid binding of 5-HT to the synaptic membrane could trigger synaptic vesicle fusion, serving as a fast mechanism for its removal from the synaptic cleft.[305]

Changes induced by 5-HT in the composition and order of nonraft parts of the membrane may alter the lateral pressure profile of the membrane. This could potentially impact the equilibrium between activated and nonactivated receptor configurations, affecting further 5-HT signaling.[304] These changes could also influence the sorting and oligomerization of 5-HT receptors,[306,307] possibly leading to synaptic vesicle fusion.[20] This underscores the potential of 5-HT to expedite its removal from the synaptic cleft.

In volume transmission, 5-HT diffuses along the membrane to reach extra-synaptic binding sites.[305,308] This process might coincide with extra-synaptic interactions with the plasma

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membrane, leading to local changes in extra-synaptic 5-HT throughput. Overall, 5-HT can significantly modify the phase behavior of the membrane to boost its own signal throughput (Figure 1), potentially impacting the activity of membrane proteins sensitive to the membrane environment.[253]

SEROTONIN EFFECTS ON LIPID REGULATION

While substantial evidence now exists highlighting the role of direct lipid regulation of protein-mediated signaling at the 5-HT synapse, our understanding of how this signaling influences the synapse's lipid composition remains limited. Neuronal plasticity, universally accepted as instrumental for learning, memory, and subsequent behavioral adaptations, incorporates the function of the 5-HT system and its synapses.[94,309−311] Typically, this process is characterized by protein-based modifications. For instance, a synapse's intense activation leads to escalated expression of synaptic proteins such as GPCRs, ion channels, and transporters,[9,310] thereby changing signal throughput. It is widely believed that these adjustments can occur without the need for alterations in synaptic membrane lipids. However, long-term memory-related changes, which involve structural shifts like dendritic arborization and sprouting, do require new cell membrane formation, thereby necessitating lipid formation as well. The emergence of lipid-related mechanisms for 5-HT synaptic plasticity during the learning and memory process has only recently begun to be explored.[116]

The literature suggests that long-chain PUFAs are crucial for efficient 5-HT transmission and serotonin-dependent behavioral responses in *C. elegans*.[312] The direct effects of 5-HT on PUFA regulation in the brain, however, remain relatively understudied. Existing research from other cellular systems indicates an interaction between serotonin and lipids. For instance, continuous administration of the SSRI fluoxetine was found to alter gene expression, including the fatty acid elongase coding gene *ELOVL6*, in the mammary gland cells of mice.[313] Similarly, *ELOVL3* mRNA expression in adipocytes also changed following treatment with the 5-HT receptor antagonist clozapine.[314]

Acute high-fat diets (HFD) are highly satisfying and stimulate an immediate rise in monoaminergic activity within the brain's reward system.[315−317] However, continued HFD consumption has been associated with triggering depression in humans and animal models.[318] This corresponds with a drop in the synthesis and levels of 5-HT,[319,320] while simultaneously increasing the expression of genes responsible for the fatty acid desaturase FADS1 and FADS2.[321]

A study by Jaddoa and colleagues presents indirect evidence of 5-HT's role in regulating lipid enzymes in the brain.[297] They claim that chronic use of SSRI paroxetine, known for enhancing basal 5-HT levels and attenuating acute 5-HT responses,[322] can lower ceramide levels in rat hippocampus. Paroxetine also significantly reduced sphingosine levels in the PFC, hippocampus, and striatum. However, acute use of paroxetine, which increases 5-HT levels in these structures,[323,324] did not affect either sphingolipid. This drug also decreased the mRNA expression of the *SMPD1* gene (which encodes the ceramide-synthesizing enzyme ASM) and possibly *ASAH1*, which codes for acid ceramidase (AC), a sphingosine generating enzyme.[297] This suggests that 5-HT synaptic activity may control the formation of sphingolipids, ceramide, and sphingosine, along with their synthesizing enzymes.

Interestingly, while a strong acute activation of 5-HT with paroxetine was ineffective, modifications in 5-HT synaptic throughput due to chronic paroxetine use showed changes in 5-HT-R activation. This implies that 5-HT could be a key upstream regulator of ceramide and sphingosine synthesis via ASM- and AC mRNA expression. More specifically, alterations in ceramide levels within cells may impact various transduction pathways, such as SAPKs, JNKs, KSR, and PKC zeta. Ceramide might also regulate the activity of protein phosphatases like PP1 and PP2A,[325] hence influencing gene transcription of lipid regulatory enzymes (Figure 1). Still, the exact mechanisms underlying the proposed feedback regulation loops remain unconfirmed, necessitating further investigation.

THE LIPID-5-HT INTERACTION AS SOURCE OF MOLECULAR SEX DIFFERENCES

The 5-HT system is recognized for its sexually dimorphic regulation.[326−331] Estrogen-dependent expression regulation of several genes, including TPH2, SERT, 5HT1A, MAO-A, and MAO-B, has been linked to some sex differences in this system. This likely influences the higher prevalence of depression in women, particularly during periods associated with reduced or fluctuating estrogen levels.[332]

Apart from hormonal regulation, many sex differences only become apparent following pharmacological, genetic, or other types of interventions. These interventions often reveal compensatory mechanisms that maintain physiological activities across both sexes despite genetic differences.[333,334] This has also been observed in the serotonergic system. For example, when the SERT coding gene is knocked out, sex differences in the regulation of 5-HT metabolism, signaling, and the brain's serotonergic neuroanatomy are revealed.[326,329,330,335] Recent studies using knockout mice of $G_{\alpha q}$, a protein interacting with SERT, have demonstrated the sexually dimorphic regulation of SERT itself.[331] Moreover, in animal models of depression, numerous sex differences have been documented in various 5-HT-dependent behavioral paradigms. These differences also extend to the effects of antidepressants.[328] Notably, clinical trials have indicated distinct responses to SSRI antidepressants among male and female patients.[336]

The regulation, or rather the dysregulation, of lipids and lipid metabolism is another documented source of sex differences. For instance, the rate-limiting enzyme in cholesterol biosynthesis, called 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMGR), as well as the low-density lipoprotein receptor (LDL-R), both demonstrate age-dependent sex differences in mouse hippocampus and cortex.[337] The observed reduction in LDL-R expression in female mice is believed to be influenced by estrogen levels.[338] Such sex-dependent dysregulation of cholesterol synthesis could be associated with autism, a condition marked by a significant sex bias, as it more frequently affects males.[339]

Cholesterol levels directly interact with and regulate various 5-HT-Rs and the SERT while also maintaining the integrity of lipid microdomains. This implies that serotoninergic functions could be influenced by cholesterol metabolism in ways that may differ between sexes. Numerous studies have highlighted a potential gender-based connection between low serum cholesterol levels and suicidal behavior, but findings remain inconclusive, as other studies report no significant sex differences.[340] Therefore, further research is necessary to shed light on the sex-specific molecular mechanisms through

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which cholesterol regulates 5-HT neurotransmission and related diseases.

Differences in SM/ceramide metabolism associated with sex and age have been noted in humans.[341−344] In women, variations in the ceramide profile are evident between pre- and postmenopause. However, these effects can be reduced through hormone replacement therapy, implying estrogen's significant role.[341] Likewise, gender-based changes in sphingolipid profiles in the hippocampus have been observed in older mice.[345]

Recent studies have demonstrated a distinct correlation between sphingolipid metabolism and the serotonergic system based on sexual dimorphism. This was achieved through observations of sex-specific changes in 5-HT, 5-HT-R, and SERT levels and different impacts on behaviors linked to depression and anxiety in a mouse model with lowered NSM activity.[222,223] Increased hippocampal ceramide levels were seen only in male mice with overexpression of ASM in the forebrain, presumably due to different compensatory changes in the expression of enzymes tied to ceramide metabolism. This overexpression concurrently triggered depression-like behavior in male mice and social anxiety-like phenotype in females.[346] Although there's still a lot to learn, these studies indicate a crucial role of lipids in the sex-specific regulation of serotonergic functions and serotonin-dependent behaviors.

SYNTHESIS AND OUTLOOK

5-HT is a vital modulatory neurotransmitter responsible for regulating most behaviors in the brain. An inefficient 5-HT synaptic function is often linked to various mental disorders. Primarily, membrane proteins controlling the expression and activity of 5-HT synthesis, storage, release, receptor activation, and inactivation are critical to 5-HT signaling in synaptic and extra-synaptic sites. Moreover, these signals represent information transmission across membranes. Although the lipid membrane environment is often viewed as fairly stable, emerging research suggests significant functional lipid−protein interactions with many synaptic 5-HT proteins. These protein−lipid interactions extend to almost all the primary lipid classes that form the plasma membrane. However, the interaction with specific lipid species still remains unclear. Collectively, these lipid classes and lipid−protein interactions affect 5-HT synaptic efficacy at the synapse. The highly dynamic lipid composition of synaptic membranes suggests that these lipids and their interactions with proteins may contribute to the plasticity of the 5-HT synapse. Therefore, this broader protein−lipid model of the 5-HT synapse necessitates a reconsideration of 5-HT's role in various associated mental disorders.

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Author Contributions

C.P.M. and L.S.K. initiated this review and provided the structure and main hypotheses. C.P.M., L.S.K, J.K., S.S., and J.H. wrote chapters of the review. All authors discussed the results and commented on the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AC, acid ceramidase; AMPA-R, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ASM, acid sphingomyelinase; AUD, alcohol use disorder; BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CerS, ceramide synthase; CPP, conditioned place preference; CRAC, cholesterol binding motifs; DA, dopamine; DAG, diacylglycerol; DAT, dopamine transporter; dDAT, *Drosophila melanogaster* DAT; DDM, dodecyl-beta-D-maltoside; DHA, docosahexaenoic acid; EEG, electroencephalogram; ER, endoplasmic reticulum; 5-HT, 5-hydroxytryptamine, serotonin; 5-HT-R, serotonin receptor; 5-HIAA, 5-hydroxyindole acetic acid; 5-HTP, L-5-hydroxytryptophan; FIASMA, functional inhibitor of acid sphingomyelinase; GPCR, G-protein-coupled metabotropic receptor; HFD, high-fat diet; IP3, inositol trisphosphate; JNK, jun kinase; KSR, kinase suppressor of Ras; LPS, lipopolysaccharide; MAO, monoamine oxidase; MAPK, MAP kinase; MDD, major depressive disorder; mPFC., medial prefrontal cortex; MβCD, methyl-β-cyclodextrin; NAT, norepinephrine transporter; NC, neutral ceramidase; NMDA-R, *N*-methyl-D-aspartate receptor; NSM, neutral sphingomyelinase; OccC, occipital cortex; PC, phosphatidylcholine; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PE, phosphatidylethanolamine; PFC, prefrontal cortex; SAPK, stress-activated protein kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C;

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PKC, phosphokinase C; POPC, 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine; PP, protein phosphatase; PSD, postsynaptic density; PSM, N-palmitoyl-D-erythro-sphingosylphosphorylcholine; PUFA, polyunsaturated fatty acids; REM, rapid eye movement; S1P, sphingosine-1-phosphate; SERT, serotonoin transporter; SL, sphingolipid; SM, sphingomyelin; SMS, sphingomyelin synthase; SpK, sphingosine kinase; SPT, serine palmitoyltransferase; SSRI, selective serotonin reuptake inhibitor; TempC, temporal cortex; TLR4, Toll-like receptor 4; TM, transmembrane helix; TNFα, tumor necrosis factor alpha; TPH, tryptophan-5′-monooxygenase tryptophan hydroxylase; vMAT, vesicular monoamine transporter

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