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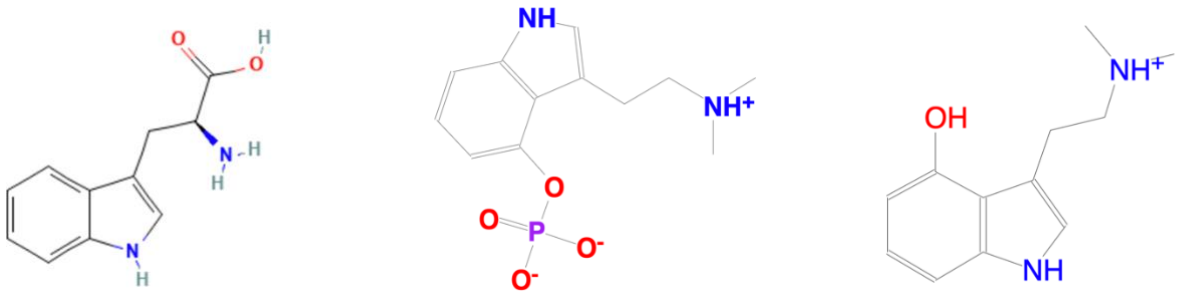
The Biochemistry of Psilocybin & Its Metabolism in the Human Body

I. Introduction

Many organisms evolve to have mechanisms that protect them from external threats. Such is the case for magic mushrooms which synthesize a psychoactive alkaloid, psilocybin (Passie et al. 2002). Its main role in nature is as a hallucinogen that suppresses certain neurotransmitters in insects, which dampens their appetite or poisons them (Blei et al. 2018). In humans, psilocybin is a psychedelic drug that causes euphoria, hallucinations, impaired judgment, and can be lethal when consumed in high amounts (Health Canada c2019). It has also significantly contributed to the worldwide problem of hallucinogenic drug abuse leading to its usage/distribution being internationally controlled under the United Nations Drug Control Conventions and nationally regulated by the Controlled Drugs and Substance Act of Canada (UNODC 2019).

The main reason why psilocybin is such a potent drug lies in its biochemistry and metabolic pathway in humans. To begin, magic mushrooms use L-tryptophan to biosynthesize psilocybin, which is why it is considered a tryptophan derivative (Lenz 2020). The biosynthetic pathway involves phosphorylation of one carbon on tryptophan's indole ring, decarboxylation of its carboxyl group, and methylation of its amino group to form psilocybin as shown in figure 1. When ingested, enzymes like alkaline phosphatase dephosphorylate psilocybin into psilocin, the pharmacologically active metabolite that has a hydroxyl group instead of a phosphate group (Fig. 1, 2A) (Dinis-Oliveira 2017, Patr 2016). Psilocin is then further metabolized by monoamine oxidase A (Fig. 2B). As what will be discussed in the succeeding sections, these modifications to tryptophan enable psilocybin/psilocin to act as strong agonists at serotonin receptors, specifically the 5-HT_{2A} receptor in humans, due to the various non-covalent interactions that their functional groups can form with the receptor's drug binding site (e.g., hydrogen bonding, hydrophobic interactions, and pi-pi stacking). If the hallucinogenic compounds successfully bind to the receptors, a signal cascade is triggered and can result in typical hallucinogenic effects (CAMH c2009).



Tryptophan Psilocybin Psilocin

Figure 1. 2D chemical structure of tryptophan ($C_{11}H_{12}N_2O_2$), psilocybin ($C_{12}H_{16}N_2O_4P$), and psilocin ($C_{12}H_{17}N_2O$). (Images source: MetaCyc: Psilocybin 2017, MetaCyc: Psilocin 2017, PubChem: Tryptophan, 2022)

II. Psilocybin Metabolism

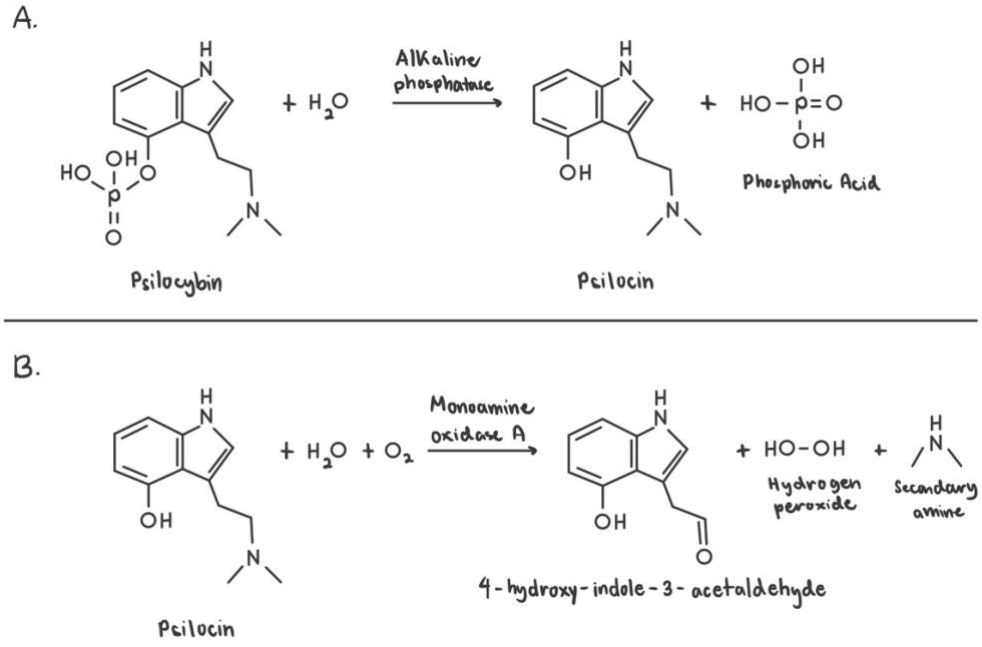


Figure 2. 2D schematic of the metabolism of psilocybin in humans (Adapted from Dinis-Oliveira 2017). (A) Alkaline phosphatase, a hydrolase found in the stomach, intestine, kidneys, and blood, catalyze dephosphorylation of psilocybin via hydrolysis resulting in the formation of psilocin, which can easily cross the blood-brain barrier and induce hallucinogenic effects. (B) To regulate it, monoamine oxidase A, an oxidoreductase found in the outer mitochondrial membrane throughout the brain and in the liver, breaks psilocin down by demethylation and oxidative deamination.

Psilocybin metabolism in the human body involves a series of steps. When digested/ingested, it can interact with specific alkaline phosphatases (ALPs) that dephosphorylate psilocybin with the use of water. ALPs are considered as hydrolytic membrane-bound metalloenzymes that exist as homodimers and have different isoenzymes, including intestinal ALP (IAP) and tissue-nonspecific ALP (TNAP) found in the liver/bone/kidney (Sharma et al. 2014).

Mammalian ALPs are zinc-containing metalloenzymes that have three main metal binding sites, two for Zn^{2+} and one for Mg^{2+}, but TNAP has an additional binding site for Ca^{2+} (Sharma et al. 2014). Mg^{2+} ions stabilize the enzyme and modulate catalytic activity of Zn^{2+} binding, like how Mg^{2+} function in *E. coli* ALP activity (Bosron et al. 1977). For TNAP, One Ca^{2+} binds to another metal-binding site to facilitate proper folding and help stabilize a large area of the enzyme that include loops. However, at high Ca^{2+} concentrations, it can substitute Zn^{2+} at the zinc binding sites resulting in a strongly reduced enzyme activity (Hoylaerts et al. 2015). Ultimately, the binding of these cofactors to ALP allows it to catalyze hydrolytic cleavage of psilocybin's phosphate group which produces psilocin and phosphoric acid (Fig. 2A).

As mentioned, psilocin is the active metabolite of psilocybin that can cross the blood-brain barrier and reach serotonin receptors to elicit hallucinogenic effects. To regulate it, monoamine oxidase A (MAO-A), an oxidoreductase monomer that exists as an outer mitochondrial membrane-bound flavoenzyme found throughout the brain, catalyzes degradation of psilocin by demethylation and oxidative deamination.

MAO-A contains a FAD-binding domain and a substrate-binding domain whose active site is a cavity-shaping loop (De Colibus et al. 2005). When FAD is covalently bounded to MAO-A, it can be reduced by the amine group of psilocin, which drives the oxidative deamination mechanism (Fig. 2B) (Ramsay 2023). This results in the formation of 4-hydroxy-indole-3-acetaldehyde that can be further processed into other products to allow excretion or reduction of the overall psilocin concentration in the brain (Dinis-Oliveira 2017).

III. Target Protein Structure

In humans, the main target protein of psilocybin/psilocin is the 5-HT_{2A} receptor, a serotonin receptor subtype found in various regions of the brain (Passie et al. 2002). It is a G protein-coupled receptor (GPCR) with a 7-transmembrane domain (Fig. 3) and is usually coupled with the Gq protein (Nichols & Sanders-Bush 2004).

The 5-HT_{2A} receptor has a primary structure that is 471 amino acids long (RCSB #6WGT 2020). A hydropathy plot (Fig. 5) shows that somewhere between the 60^{th} and 380^{th} amino acids are where seven separate highly hydrophobic regions can be found, each are ~20 amino acids long that can span the membrane as α-helices. It is also worth noting that the N-terminus is not from the first amino acid and the C-terminus is not from the last amino acid based on the primary sequence (Fig. 3), which can be due to signal sequence cleavage and other post-translational modifications. Thus, the amino acid sequence present in the final protein is what dictates and facilitates specific binding of ligands/molecules.

The primary sequence mostly includes hydrophobic and hydrophilic amino acids, such as M/A/V/L and others, that allows it to fold into α-helices. Thus, the receptor's secondary structures are α-helices with hydrophobic and/or hydrophilic regions that shape its tertiary structure as a GPCR with a barrel-like 7-transmembrane domain with many hydrophobic amino acids that interact with the membrane, and an intracellular domain with many hydrophilic amino acids that can interact with a Gq protein and the intracellular space (Fig. 3). Moreover, Gq has a quaternary structure because it is a heterotrimeric protein with 3 different subunits: α, β, and γ (Nichols & Sanders-Bush 2004).

As labeled in figure 3, some of the ligands/molecules that can bind to the receptor are highly hydrophobic like cholesterols that provide structural support and increase its stability (Sun et al. 2015); and oleic acids or oleamides, a type of fatty acid that can potentiate downstream effects of receptor–ligand binding such as inositol phosphate formation (Thomas et al. 1998). Both are able to interact with the receptor through hydrophobic interactions: between some carbons of a cholesterol and the I/L/M/P/T/V side chains at the cholesterol binding site (Fig. 6), and between some carbons of oleic acid and the A/L/V side chains at the oleic acid binding site (Fig. 7). Overall, it is the amino acid sequence at these different binding sites that results in specificity and is what accommodates the structure and composition of these molecules to allow tight binding at the transmembrane domain (Fig. 3).

One of the main ligands that can bind to the 5-HT_{2A} receptor's drug binding site is lysergic acid diethylamide (LSD) (Fig. 3). As what will be further discussed in section IV, LSD is a stronger hallucinogenic drug than psilocybin. Thus, LSD interacts more strongly with the active site which allowed researchers to better crystallize and generate a 3D model of the receptor's conformation when bound to a hallucinogenic ligand (Fig. 4) than if they were to use psilocybin/psilocin to conduct such procedures. They mainly interact with the receptor through hydrophobic interactions between several hydrocarbon chains, pi-pi stacking between LSD's phenyl ring and the active site's Phe340 phenyl ring, and hydrogen bonding between LSD's N1 and N2 to the receptor's Ser242 hydroxyl group and Asp155 carboxyl group respectively (Fig. 5).

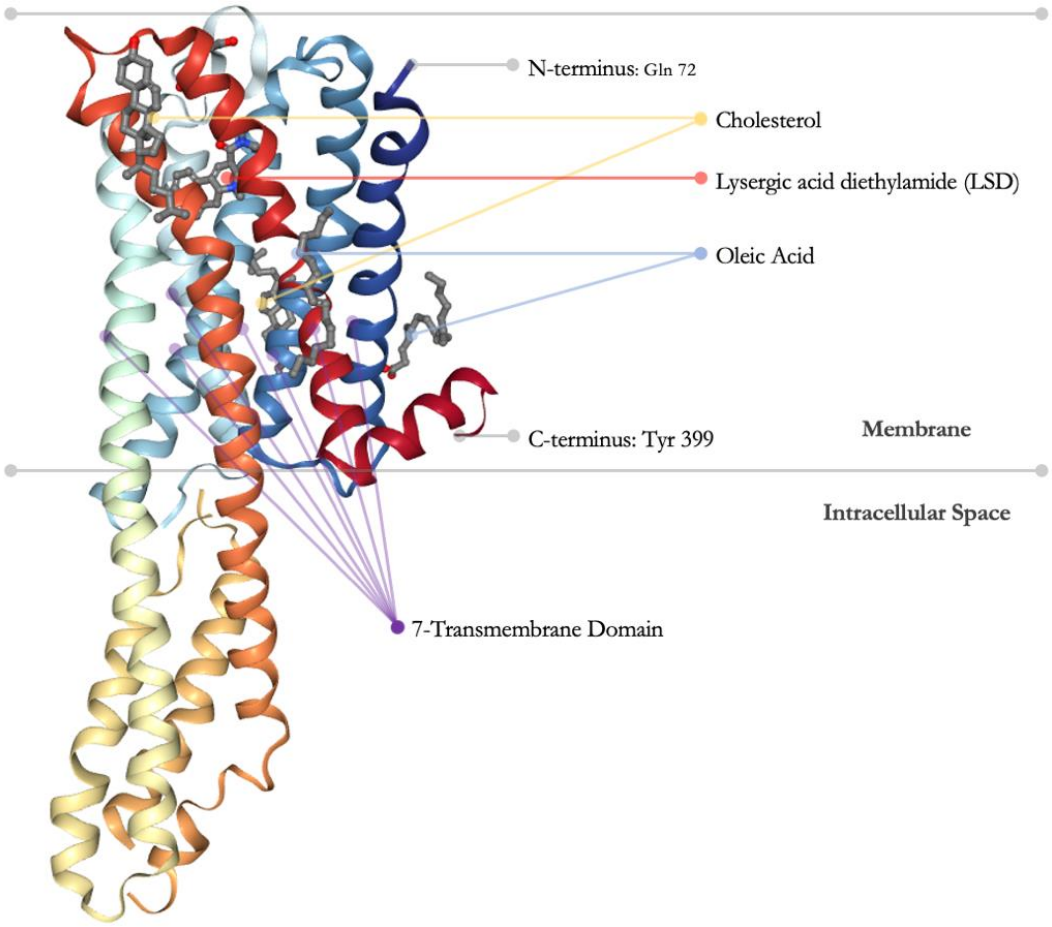


Figure 3. Rainbow/Chainbow structure of human 5-HT_{2A} receptor with labeled ligands, N&C termini, and 7-transmembrane domain (Image source: RCSB #6WGT 2020).

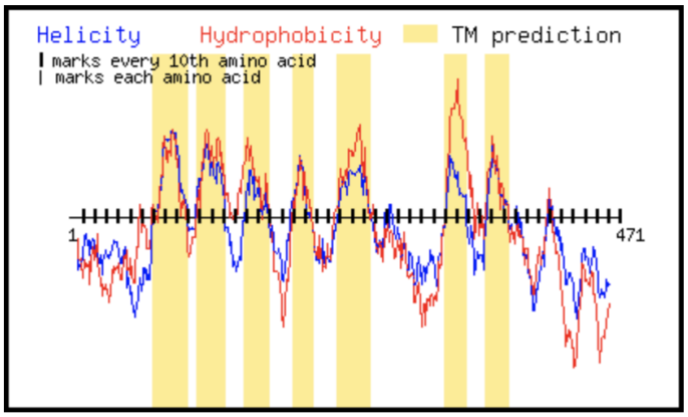


Figure 4. A hydropathy plot depicting the 7-transmembrane domain of human 5-HT_{2A} receptor generated from SickKids TM-Finder (c1999-2010) using the Kyte-Doolittle Hydropathy Scale with a sliding window of 7.

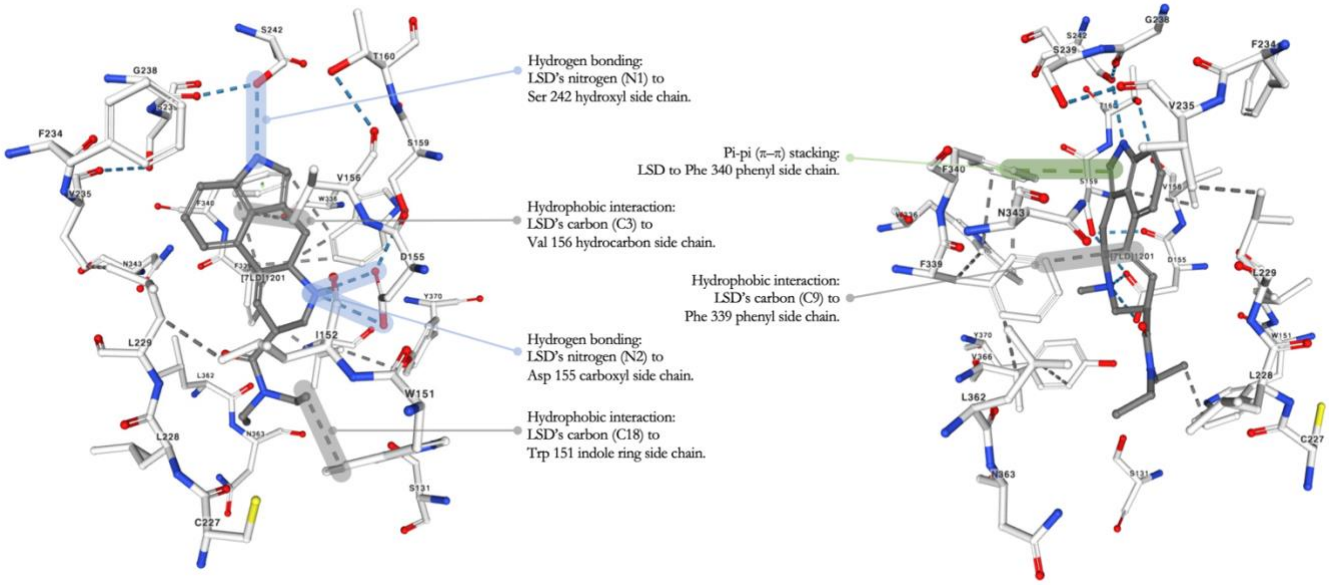


Figure 5. Interaction schematic between lysergic acid diethylamide (LSD) and human 5-HT_{2A} receptor active site with labeled key non-covalent interactions including hydrogen bonding, hydrophobic interactions, and pi-pi stacking (Image source: RCSB #6WGT 2020).

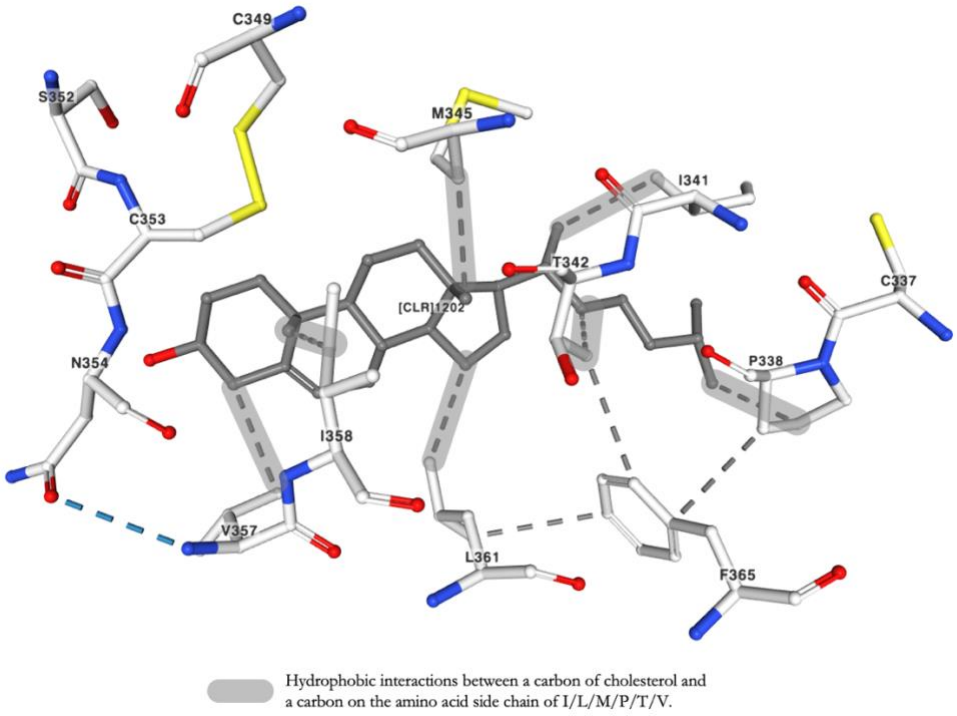


Figure 6. Interaction schematic between cholesterol and human 5-HT_{2A} receptor's cholesterol binding site with labeled hydrophobic interactions (Image source: RCSB #6WGT 2020).

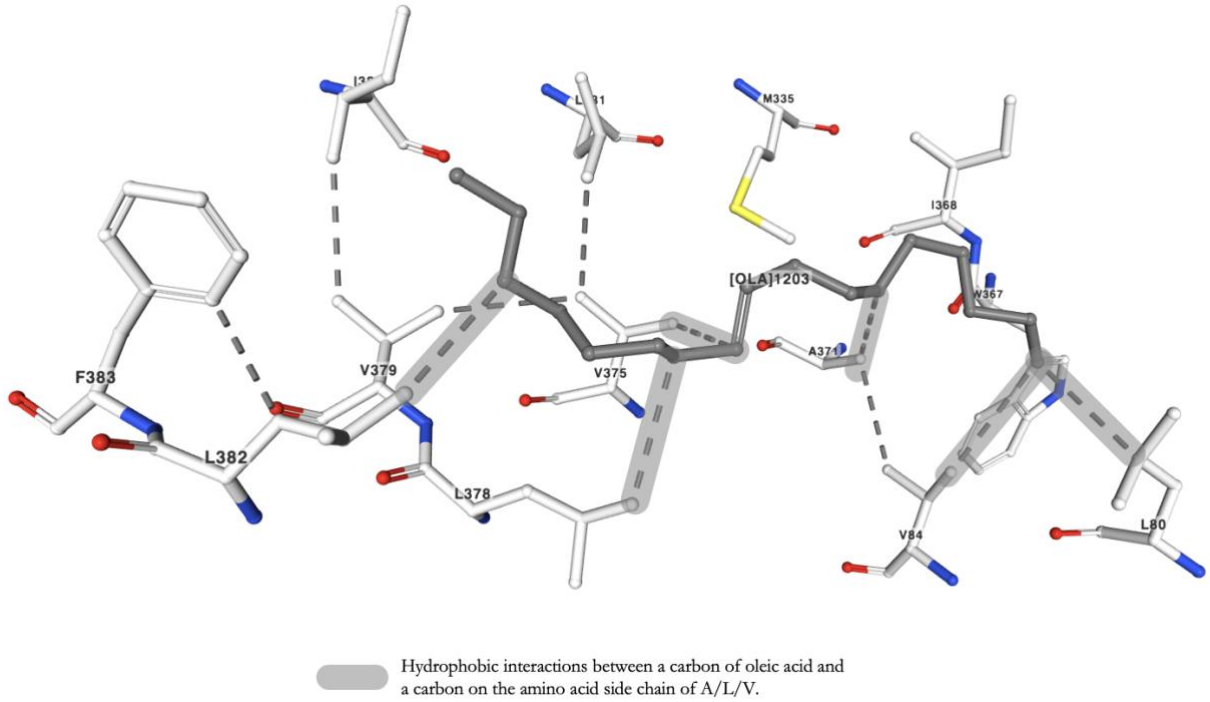


Figure 7. Interaction schematic between oleic acid and human 5-HT_{2A} receptor's oleic acid binding site with labeled hydrophobic interactions (Image source: RCSB #6WGT 2020).

IV. Drug Binding Kinetics

As established in sections I and II, psilocybin is a prodrug that is metabolized by alkaline phosphatase into psilocin, the main active metabolite. Both molecules are structurally similar to serotonin and, thus, can act as agonists at the 5-HT_{2A} serotonin receptor. However, this section will focus on the binding kinetics of psilocin because it is more likely to reach the brain and it has a stronger binding affinity to the receptor than psilocybin based on psilocin's lower K_i value. In experiments using cultured human 5-HT_{2A} receptors, it was determined that psilocin's K_i value is 0.049 μM with a deviation of ± 0.01 μM while psilocybin's K_i value is greater than 10 μM (Rickli et al. 2016, Roth et al. 2000). This suggests that psilocin is more likely to interact strongly at the receptor's drug binding site.

To deduce the factors that influenced the binding kinetics between psilocin and the receptor, a causal inference can be drawn from figure 5 which showcases the interactions between lysergic acid diethylamide (LSD) and the 5-HT_{2A} receptor active site. LSD is more potent than psilocybin and has a K_i value of 0.004 μM with a deviation of ± 0.001 μM (Rickli et al. 2016). This means that it has a higher binding affinity to the receptor than psilocin. The difference in their binding affinity is due to the differences in their structure/composition which affects how they interact with the receptor.

Figure 5 shows that LSD's N2 is capable of hydrogen bonding to the Asp 155 carboxyl side chain. However, psilocin's N2 (as labeled in Fig. 8) is not able to interact in the same manner due to it being a tertiary amine and lack of resonance. Instead, one of the -CH_3 of psilocin's N2 can act similarly to LSD's C18 which has hydrophobic interactions with Trp 151 indole ring side chain. Additionally, psilocin has fewer hydrocarbon rings than LSD which results in less hydrophobic interactions and weaker Van der Waals forces. While

psilocin's B ring may act similarly to LSD's B ring pi-pi stacking with the phenyl side chain of Phe 340 and psilocin's N1 may act like LSD's N1 hydrogen bonding to Ser 242, the differences between their composition and structure results to psilocin having a weaker binding affinity to the 5-HT_{2A} receptor than LSD, but still relatively strong due to their similarities – this explains why their K_i values are close. Overall, these findings entail that the receptor active site is specific for serotonin and serotonin-like molecules, such as LSD and psilocin, because of the amino acid sequence comprising the binding region, and their spatial distribution that can accommodate similarly structured molecules through similar non-covalent interactions.

As an agonist, psilocin binding to the receptor activates the same signaling pathway as serotonin, but at a different/stronger/prolonged degree due to having more non-covalent interactions with the active site (Roth 2011). The signaling pathway starts with ligand-receptor binding that changes the receptor's conformation. This leads to the activation of Gq, the G protein coupled to the receptor, which activates phospholipase C (PLC). PLC is then able to hydrolyze inositol phospholipids into inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), which acts as secondary messengers that further amplify the signal (Fig. 9). Downstream effects of this signal cascade induce neurobehavioral changes creating intense psychedelic experiences that can have direct and indirect negative health outcomes (CAMH c2009). This includes direct neuronal changes and potential addiction, and indirect effects due to the consequences of not being in the right state of mind for long periods, which increases risks for accidents.

In the end, using biochemistry is an important tool to analyze the composition and structure of psilocybin, psilocin, 5-HT_{2A} receptor, and other relevant molecules to gain insights about their functions, psilocybin's metabolism, receptor specificity, receptor-ligand binding kinetics, and how psilocybin can induce its psychoactive effects in humans. This scientific information had been, and will continue to be, used to design pharmaceutical products, to serve as a reference for screening of potential hallucinogenic substances, and for other functional purposes. More importantly, the understanding of the biochemistry of psilocybin's metabolism in the human body and its downstream effects is what will further enable our society to make informed decisions about psilocybin's and other hallucinogenic drugs' use and regulation.

Figure 8. 2D chemical structures of serotonin (5-HT), lysergic acid diethylamide (LSD), and psilocin with labeled rings and nitrogen (Image source: Aghajanian and Marek 1999).

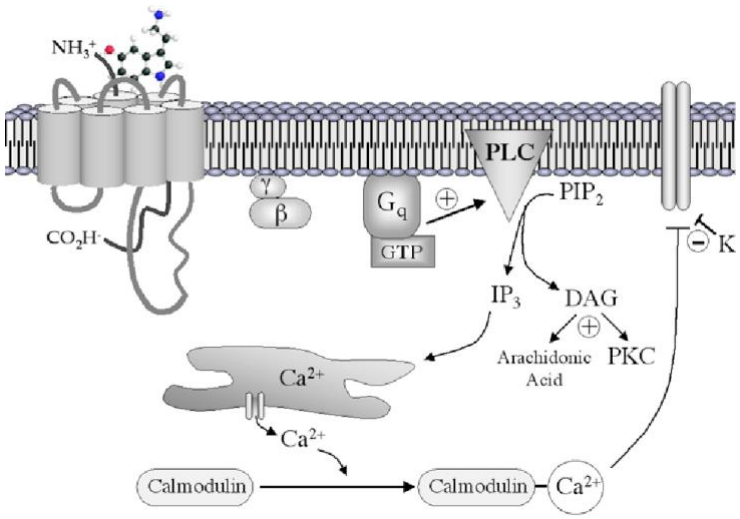


Figure 9. A simple signaling pathway diagram for human 5-HT_{2A} receptor activation by serotonin (Image source: Nichols & Sanders-Bush 2004).

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