UNIVERSITY OF TURKU

Interactions and activity of lysergol derivatives at 5-HT_{1A} and 5-$HT_{2A–C}$ receptors

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Master's Thesis

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Traditional use of psychedelics has historically remained restricted between religious ceremonies and countercultural movements. With the pathophysiology of various neuropsychiatric disorders becoming clearer, the world has encountered a raising interest in psychedelic research. Previous studies have shown that 5-HT_{1A} and 5-HT_{2C} receptors play a significant role in neuropsychiatric disorders, whereas 5-HT_{2A} and 5-HT_{2B} are known for their hallucinatory and cardiovascular effects. The main objective of this study is to investigate the binding interactions of lysergol derivatives in serotonin and TrkB receptors through computational methods.

Our findings reveal that the (+)-isomers of lysergol derivatives generally exhibit stronger binding compared to their (−)-isomer counterparts in 5-HT_{1A} and 5-HT_{2C} receptors, without the functional activity in 5-HT_{2A} and 5-HT_{2B} receptors. Instead, both (+)- and (−)-isomers show a comparable binding in TrkB receptors. The results reveal options for further research that could lead to the discovery of novel treatment options for various neuropsychiatric diseases. Our findings suggest that (+)-isomers of lysergol derivatives could be used for treating these disorders, while (−)-isomers could be explored to enhance neuroplasticity and combat depression with a minimal risk of adverse effects.

Keywords: neuropsychiatric disorders, psychedelic therapy, serotonin receptors, lysergol

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1 Introduction

1.1 Background and significance

Unlike classical psychedelics, clavine alkaloids have yet to be thoroughly researched despite them having a pharmacological profile that suggests very potent physiological effects, as well as being often available in nature. Psychedelic compounds have been used for thousands of years to enter a state of consciousness not available without psychotropic substances and are believed to heal various health disorders. Despite being illegal in most countries, they are currently emerging as therapeutics across Europe and North America. Unfortunately, with great success comes great side effects, ranging from visual geometry and other changes in sensory perception, synesthesia, and loss of identity, as well as nausea, hypertension, muscle twitches, and fascinatingly even unity and interconnectedness. From a clinical perspective, these side effects can be distracting from the therapeutic benefits, which has led the research to find non-hallucinatory alternatives for psychedelics. Nevertheless, there is reasonable evidence that psychedelics can be used to treat neuropsychiatric diseases with long-lasting or even permanent effects after the exposure (Doblin *et al*., 2014; Tasker & Wipf, 2022; Tasker *et al*., 2023).

Peter Wipf and colleagues from Pittsburgh University have recently synthesized the (+)- and (−)-isomers of clavine analogues lysergol and isolysergol. The studies show different binding affinities to serotonin receptors depending on the isomer as well as varying levels of agonism. The results show that (+)-isomers of both compounds have a significantly better affinity for all receptor subtypes while (−)-isomers bind poorly to the receptors at 100 nM concentration. When it comes to levels of agonism, (+)-isomers show partial agonism activity at 5-HT_{1A} and 5-HT_{2C} receptors but lack the agonism at 5-HT_{2A} and 5-HT_{2B} receptors, notoriously known for their hallucinatory and cardiovascular side effects, respectively. With (−)-isomers there is no significant agonist activity at the studied receptors. These findings suggest a possibility for discovering new therapeutics with clavine scaffolds, but more research is needed to confirm the results (Tasker & Wipf, 2021; Tasker & Wipf, 2022. Tasker *et al*., 2023).

1.2 The serotonergic system

1.2.1 Serotonin as a neurotransmitter

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a neurotransmitter that is responsible for various physiological and behavioral processes within the central nervous system (CNS). It displays its

effects through a wide range of functions, including the regulation of mood, anxiety, stress response, aggression, as well as eating behavior. Additionally, serotonin contributes to the modulation of circadian rhythm, sexual behavior, and cognitive processes. Beyond its CNS actions, serotonin also modulates peripheral functions, such as gastrointestinal motility, glucose homeostasis, and adiposity, which leads to altered metabolic processes that can result in health problems (Fidalgo *et al.*, 2013; Olivier, 2015; Jones *et al*., 2020).

As illustrated in Figure 1, serotonin is synthetized from L-tryptophan (L-TRP), an essential amino acid available through normal diet in the body, which is then metabolized into 5-hydroxytryptophan (5-HTP) by the enzyme L-tryptophan hydroxylase (TPH). TPH exists in two isoforms in the body, of which TPH1 is expressed in enterochromaffin (EC) cells found in the lumen of the digestive tract, while TPH2 exists in central and enteric neurons. 5-HTP is then metabolized into serotonin by aromatic L-amino acid decarboxylase (AADC) (Olivier, 2015; Jones *et al*., 2020). Upon synthesis, serotonin is rapidly taken up by the serotonin transporter (SERT) for storage in the intestine and blood platelets. There it is internalized into vesicular monoamine transporter (VMAT) where it can be released as needed. Up to 95 % of serotonin is found in the gastrointestinal tract, mainly in EC cells and to lesser extent in enteric neurons. Only the remaining 5 % has an effect in the brain since it cannot cross the blood-brain barrier and must be synthetized locally in the Raphe nuclei of the CNS (Lesurtel *et al*., 2008).

5-HT is mainly metabolized by monoamine oxidase (MAO), an enzyme present in the mitochondrial membrane. MAO catalyzes the deamination of serotonin into 5-hydroxyindole acetaldehyde (5-HIA) which is quickly oxidized forming 5-hydroxyindole acetic acid (5-HIAA) to be excreted in urine (Bortolato *et al*., 2010). Alternatively, 5-HIA can be converted to 5-hydroxytryptophol (5-HTOL) which is then excreted by its glucuronide conjugate or even straight from 5-HT by sulfation. These reactions are enzymatically catalyzed by aldehyde dehydrogenase (ALDH), aldehyde reductase (ALR), UDP-glucuronosyltransferase (UGT), and phenol sulfotransferase. These minor pathways only become active during an exceptionally high concentrations of 5-HT such as in hospitalized serotonin syndrome patients (Lesurtel *et* al., 2008; Suominen *et al*., 2013). Moreover, 5-HT is also the precursor for melatonin production which has a significant role in regulating the circadian rhythm. Conversion happens in the pineal gland of the brain where the enzyme serotonin-N-acetyltransferase (SNAT) catalyzes the production of N-acetyl-serotonin (NAS) which is subsequently methylated by hydroxyindole-O-methyltransferase (HIOMT) into melatonin (Bortolato *et al*., 2010). Together these molecules create the complex group of actions for which serotonin is responsible of in the body.

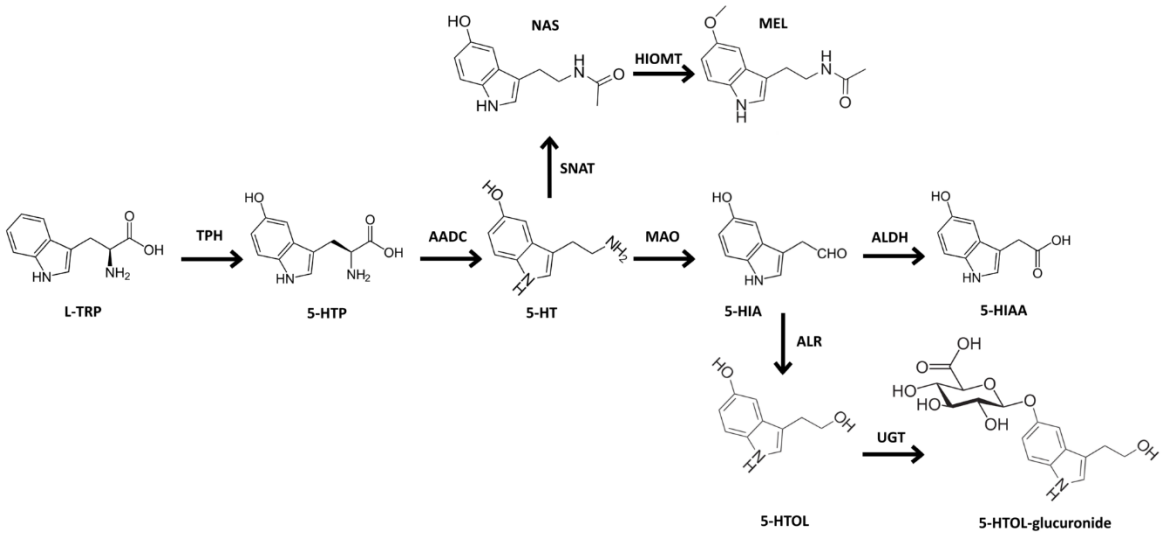

Figure 1. Synthesis and metabolism of serotonin. L-tryptophan (L-TRP) is metabolized into 5-hydroxytryptophan (5-HTP) by L-tryptophan hydroxylase (TPH), and furthermore into serotonin (5-HT) by aromatic L-amino acid decarboxylase (AADC). Serotonin is mainly metabolized by monoamine oxidase (MAO) into 5-hydroxyindole acetaldehyde (5-HIA), which can then be oxidized or reduced by aldehyde dehydrogenase (ALDH) and aldehyde reductase (ALR), forming 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophol (5-HTOL), respectively. Whereas 5-HIAA can be excreted in urine, 5-HTOL is further glucuronidated by UDP-glucuronosyltransferase (UGT) before excretion. Serotonin can also be metabolized by serotonin N-acetyltransferase (SNAT) and subsequently methylated by hydroxyindole O-methyltransferase (HIOMT) to create N-acetylserotonin (NAS) and melatonin (MEL).

1.2.2 Serotonin receptors and neurons

Serotonin receptor family consists of seven different classes of receptors, from 5-HT_1 to 5-HT_7, with a total of 14 different known existing receptors. 5-HT receptors share a common G protein-coupled receptor (GPCR) structure with seven transmembrane helices and binding with a heterotrimeric G protein. The G protein consists of alpha (G_α) and beta-gamma ($G_{\beta\gamma}$) subunits. G_α includes a Ras-like domain that is capable of binding nucleotides such as GTP and GDP. When the 5-HT receptor is in the inactive state, G_α is binding GDP and the $G_{\beta\gamma}$ subunit. Upon ligand activation, G_α-bound GDP is exchanged to GTP by guanine nucleotide exchange factor (GEF) protein, enabling G_α to activate downstream effector proteins. In the active state G_α is susceptible to hydrolyzation by GTPase. GTPase activating proteins (GAP) are recruited to enhance the GTPase autoactivity of the G protein, promoting the termination of signaling that leads to reassociation with the $G_{\beta\gamma}$ subunit, making it inactive. Depending on the type of G protein, the active form of $G_{\beta\gamma}$ subunit is capable of modulating ion channels, for example G protein-coupled inwardly rectifying potassium channels (GIRK), leading to membrane hyperpolarization and reduced neuron excitation, as well as various other effector molecules present in intracellular signaling pathways leading to diverse cellular responses (Nichols & Nichols, 2008; Giulietti *et al.*, 2014). Studies have shown that GIRK can also be activated with PIP_2,

which is the substrate for phospholipase C (PLC), leading to a reduced activity with 5-HT_2 activation (Duncan *et al*., 2020).

As described in chapter 1.2.1., serotonin is formed in the presynaptic axon from L-tryptophan. It is brought into a vesicle by VMAT where it can be excreted into the cytosol through a calcium-dependent process, launching when an action potential reaches the nerve ending. In the cytosol, serotonin can activate GPCRs or ligand-gated ion channels. Signaling is terminated by removing the serotonin from the synaptic junction through a SERT-mediated mechanism, where it can either be metabolized by MAO or recycled again by VMAT for subsequent serotonergic transmission. Additionally, there can also be extraneuronal transporters taking up serotonin from the cytosol where it can be inactivated by MAO (Mohammad-Zadeh *et al*., 2008).

1.2.3 Neural signaling and signaling pathways

5-$HT_{1/5}$ receptor subtypes are inhibitory and therefore bind the G_i protein. The $G_{\alpha,i}$ subunits of these proteins inhibit cyclic AMP (cAMP) synthesis by binding to adenylyl cyclase and limiting its activity. This leads to opening of potassium channels that allows an efflux of K^+ ions from the cell, decreasing the membrane voltage and therefore the chances of nerves firing. This also keeps the calcium channels closed which is required for neuron activation. Additionally, the $G_{\beta\gamma}$ subunit aids in closing ion potassium channels such as GIRK.

Instead, 5-$HT_{2,4,6,7}$ subunits are excitatory, having an opposing effect to 5-$HT_{1,5}$ receptor subtypes. 5-$HT_{4,6,7}$ mechanism of action closely resembles each other with a stimulatory $G_{\alpha,s}$ protein leading to an increase in cAMP concentration and closing of potassium channels. However, 5-HT_2 subunits have a distinct mechanism of action. 5-HT_2 receptors bind $G_{\alpha,q}$ protein that activates PLC. PLC is a membrane-bound protein that catalyzes the cleavage of certain phospholipids, in this case phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) that stays bound to the cell membrane, and inositol-1,4,5-trisphosphate (IP_3) diffusing to cytosol as a soluble molecule. IP_3 activates its receptors in the smooth endoplasmic reticulum which release Ca^{2+} into the cytosol that has multiple effects, such as calmodulin activation. Additionally, DAG and calcium increase protein kinase C (PKC) affinity for its substrate phosphatidylserine which then in turn activates the receptor kinase domain (Newton, 1995; Nichols & Nichols, 2008).

When GPCRs are bound by a ligand for a long time, their C-terminal end is phosphorylated by G protein-coupled receptor kinase (GRK), typically to multiple serine and threonine residues near the intracellular loop 3 (ICL3). This phosphorylated end acts as a binding site to β-arrestin, which can then in turn recruit other adaptor proteins such as AP-2. Clathrin molecules bind to the AP-2 end, leading to the formation of a clathrin coat and subsequent endocytosis by receptor internalization in a clathrin-dependent manner. 5-HT receptors also have a distinct signaling pathway through β-arrestin. It binds a molecule called c-Src that activates the MAP kinase cascade, resulting in various effects all over the body (Marchese *et al*., 2008). There have been multiple drugs available that only activate either the main signaling pathway or β-arrestin pathway, referred to as biased agonists. There has been a lot of research in favor of serotonin-based biased agonists (McCorvy *et al*., 2018; Sałaciak & Pytka, 2021; Pottie *et al*., 2023) as well as theories disputing the effect of biased agonism in treating neuropsychiatric disorders by suggesting an alternative mode of action through tropomyosin receptor kinase B (TrkB) activation (Casarotto *et al*., 2021; Moliner *et al*., 2023).

1.2.4 Serotonin's role in neuropsychiatric disorders

Scientific research has shown the prevalence of various subtypes of serotonin receptors involved in neuropsychiatric disorders (Artigas *et al*., 2013). Out of all, 5-HT_{1A} receptors are the most known to take part in the pathogenesis of anxiety and depression. The effect of 5-HT_{1A} receptor agonists for treatment of depression and anxiety symptoms is well documented in literature. With a diverse set of functions in the body, it is also believed that 5-HT_{1A} receptors play a role in other CNS based disorders such as autism, Alzheimer's disease, obsessive-compulsive behavior (OCD), Parkinson's disease, post-traumatic stress disorder (PTSD), and schizophrenia (Egashira *et al*., 2008; Ohno, 2010; Luo *et al*., 2011; Ohno, 2011; Wang *et al*., 2013a). The exact pathophysiological roles of 5-HT_{1A} receptor involvement in neuropsychiatric disorders still needs more research.

5-HT_{1A} receptors can be found both from pre- and postsynaptic positions, of which presynaptic autoreceptors have not displayed the effects in the body that postsynaptic receptors exert (Kaufman *et al*., 2016). In major depressive disorder, the most commonly used drugs belong to the selective serotonin reuptake inhibitor (SSRI) class. They exhibit their potential by blocking the serotonin transporter which leads to a lower amount of serotonin to be removed from the synapse to the presynaptic neuron, and therefore, increased serotonin concentration present in the synapse. The increased amount of serotonin is initially able to bind to these autoreceptors and inhibit the action potential. However, with long-term usage of SSRIs or any 5-HT_{1A} agonists, autoreceptors have been

shown to display a level of desensitization, not noticed with postsynaptic receptors in the hippocampus (Celada *et* al., 2004). After desensitization happens, the increased level of serotonin is more likely to activate postsynaptic 5-HT_{1A} receptors and induce anti-depressant as well as anti-anxiety effects of SSRI medication.

To a lesser extent, also 5-HT_{2A} receptors are believed to play a role in anxiety and depression pathophysiology (Zięba *et al*., 2022). Even though the focus in depression research is the hippocampus, the prefrontal cortex is also a major factor in its pathophysiology. We can see from multiple brain imaging studies that hypoactivity of the prefrontal lobe, as well as stroke, has been associated with a higher incidence of depression (Pizzagalli & Roberts, 2022). 5-HT_{2A} receptors are often downregulated after a long-term antidepressant medication suggesting a role in the pathophysiology of depression. The involvement of 5-HT_{2A} receptors in the prefrontal cortex can also be seen as potential evidence of having an effect similarly to other neuropsychiatric disorders discussed with 5-HT_{1A} receptors.

More prominently, the role of 5-HT_{2A} receptors has been researched in patients with schizophrenia where 5-HT_{2A} activation is much higher compared to reduced serotonin signaling in patients with depression, although this is a very simplified model of the actual complexity of the diseases (Kantrowitz, 2020). Multiple meta-analyses suggest that there is a reduction in 5-HT_{2A} binding and receptor density with schizophrenic patients, although as stated by Selvaraj *et al*. (2014) these findings may be caused by antipsychotics themselves more than the actual disease. Serotonin receptors are likely to influence both dopaminergic pathways in the mesocorticolimbic system as well as glutamatergic systems, especially metabotropic glutamate 2 ($mGlu_2$) receptor, which both have been suggested as model systems for schizophrenia (Kantrowitz & Kawitt, 2009; Kantrowitz, 2020; Liliawati, 2021), and display the variety of pathways that can communicate with serotonin receptors.

A recent hypothesis by Carhart-Harris and Nutt (2017) suggests that serotonin exerts its effect on the brain by a simplified bipartite model that utilizes 5-HT_{1A} and 5-HT_{2A} receptors, the most abundant receptors in different portions of the brain. According to the research, serotonin function is divided into two broad categories of passive and active coping, modulated by SSRIs and psychedelics, respectively. It is known that both the cortical and subcortical regions are associated with the function of serotonin.

Subcortical regions, such as amygdala, hypothalamus, and pituitary gland of the hypothalamic-pituitary-adrenal (HPA) axis, hippocampus, and brain stem, are believed to control most feelings associated with stress. (Carhart-Harris & Nutt, 2017) Amygdala is known to initiate the fight-or-flight

response and it is important in experiencing emotions such as fear, anxiety, aggression, and arousal. While also involved in anxiety, the main function of the hippocampus is the storage and recollection of memories. HPA axis is responsible for releasing stress hormones, and the brain stem participates in bodily feelings and autonomic controls of the body. The cortex is also associated with stress by thought processing, being able to potentiate and relieve stress. Additionally, cortex is the main organ for sensing internal and external sensations of the body.

Passive coping, or tolerating and blunting an emotional reaction to stress, is mediated by inhibitory postsynaptic 5-HT_{1A} receptors. It binds serotonin strongly and therefore is believed to be more active during mild stress in everyday activities like receiving sad news or other basic circumstances faced throughout the day. As seen in magnetic resonance imaging (MRI) and positron emission tomography (PET) scans by Beliveau et al. (2017), 5-HT_{1A} receptors are present in subcortical regions, especially near the hippocampus and amygdala. Also, selective 5-HT_{1A} agonists such as buspirone are known to have antidepression, anti-anxiety, and stress relieving effects.

Instead, active coping is known to be mediated by excitatory 5-HT_{2A} receptors. They are found from cortical regions as its most abundant serotonin receptor, and less in limbic areas. It has an effect of dealing with the source of stress by changing its relation to it with increased cortical entropy. Activity often leads to enhanced functions like neuroplasticity, learning and unlearning, as well as sensitivity to environmental factors, therefore having increased adaptability and change. Compared to 5-HT_{1A} receptors, it needs significantly more serotonin to be active, which may be present only in more extreme cases with an exceptionally high concentrations of 5-HT (Carhart-Harris & Nutt, 2017).

5-HT_{2B} receptor's role is primarily known to influence cardiovascular function. The receptor itself is expressed in the epithelium of heart and blood vessels where they regulate cardiac contractibility and vascular tone (Brea *et al.*, 2010). Similarly to the earliest serotonergic ligands, activation of 5-HT_{2B} receptor leads to vasoconstriction where it can be used to aid in cardiovascular diseases, such as pulmonary hypertension and cardiac valvulopathy, where research has shown to display 5-HT_{2B} receptor overexpression (Rothman *et al*., 2000; Launay *et al.*, 2002; Jaffré *et al.*, 2009). Due to these adverse effects in the body, 5-HT_{2B} agonists are not typically considered as reasonable candidates for treating neurological disorders even though they have been shown effective. Also, most psychedelics are known to activate 5-HT_{2B} receptor, but considering the low amount and infrequent dosing they are believed not to cause any major cardiovascular problems unlike other controlled substances such as substituted serotonergic phenethylamines (Hoyer, 2020; Luethi & Liechti, 2021; Sharp & Barnes, 2020; Wsół, 2023).

5-HT_{2B} receptor is also expressed in the gastrointestinal (GI) tract where it modulates smooth muscle contraction (Brea *et al.*, 2010). In a study by Bassil *et al*. (2009), it is shown that a 5-HT_{2B} antagonist, which had no effect for 5-HT_4 receptor, dose-dependently reduced peristaltic movements and fecal output. The results show that an exceedingly high dose is needed to efficiently regulate colon motility, supporting the claim that small single doses should not display severe side effects in the GI system. In other research, 5-HT_{2B} antagonists contribute to colonic smooth muscle hypersensitivity, which all together suggests that they could be used as treatment for gastrointestinal disorders like irritable bowel syndrome or functional dyspepsia.

During the recent years, the role of 5-HT_{2C} receptors in the body has widely extended from mood disorders to contain also various addictive behaviors, such as food associated disorders and obesity, substance use disorders, obsessive-compulsive disorder (OCD), excessive gambling disorder, as well as hypersexuality (Higgins & Fletcher, 2015; Howell & Cunningham, 2015; Sharp & Barnes, 2020; Flanigan *et al*., 2023). 5-HT_{2C} receptors are expressed in areas that participate in controlling food intake and reward system of the brain, such as hypothalamus, suggesting an implication in diseases like binge eating disorder and anorexia nervosa. As for psychostimulant abuse, 5-HT_{2C} receptors are thought to modulate dopamine signaling and localize to dopaminergic and glutaminergic neurons, which is a reason to believe their role in drug addiction pathophysiology. It has also been discussed whether these changes could be explained by an increase in impulsivity or enhanced reward mechanisms, and not directly caused by serotonergic activation (Higgins *et al*., 2017).

It has been shown that serotonin receptor subtypes 1A and 2A–C readily form homodimers as well as heterodimers with each other. Additionally, they are also known to interact with other receptors, such as μ-opioid receptors, CB_1 cannabinoid receptors, and TrkB receptors (Herrick-Davis, 2014; Mitroshina *et al*., 2023). With clear and up to date data on the interactions between dimers, any modifications or disruptions caused by ligands can lead to influential functional changes in the serotonergic system and may contribute to the symptoms of neuropsychiatric disorders they are involved in.

1.3 Serotonin receptor structure

The common structure of 5-HT_1 and 5-HT_2 receptors is based on seven transmembrane (TM) helices 1–7 and an additional intracellular helix (H8). Helices are connected to each other by short intracellular and extracellular loops (ICL1–2, ECL1/3) as well as a bigger extracellular loop ECL2, also known as “lid”,

being able to cover the ligand binding site, and the intracellular loop ICL3 known for its important ability to bind to the G protein. ECL2 is divided into two parts by a disulfide bridge between a conserved cysteine in ECL2 and $Cys^{3.25}$. Additionally, ICL2 and ECL2 have the possibility to form minor α-helical structures, both permanently and temporarily (McCorvy & Roth, 2015; Kimura *et al*., 2019). Near ICL2 in TM3, a conserved $Arg^{3.50}$ shows to be a part of a DRY motif that is thought to play a crucial role in maintaining the receptor at an inactive or partially active conformation even after ligand binding. It forms a salt bridge to two residues, $Asp^{3.49}$ and $Glu^{6.30}$, where it remains tightly bound. Upon activation, TM6 is allowed to rotate disrupting the salt bridges and allowing the receptor to take an active conformation. Linked by a water network, at the end of TM7 exists a NPxxY motif that is also responsible for stabilizing the receptor at the inactive state (Nichols & Nichols, 2008; Kim *et al*., 2011; Ishchenko *et al*., 2017). Ligands of serotonin receptors have been shown to bind to a conserved $Asp^{3.32}$ residue in the binding site, forming a salt bridge between the negatively charged aspartate side chain and the positively charged nitrogen in the ligands. A conserved hydrogen bond towards $Tyr^{7.43}$ has also been shown to stabilize the TM3 structure (Kimura *et al*., 2019). Moreover, a tight hydrophobic cleft with residues $Ile^{3.33}$, $Cys^{3.36}$, $Trp^{6.48}$, $Phe^{6.51}$, and $Phe^{6.52}$ is observed between most 5-HT receptors, optimal for ergoline structure binding (Wang *et al*., 2013b; Tan *et al*., 2022).

1.3.1 5-HT_1 receptors

Research by Xu *et al*. (2021) showed insights into the 5-HT_{1A} structure, notably that there resides a phospholipid molecule at the interface between 5-HT_{1A} receptor and $G_{\alpha,i}$ protein. The lipid's two acyl chains formed extensive interactions with TM6, TM7, and H8, leading to the conclusion that lipid membrane interactions are important to consider when researching serotonin receptors. There was also an increase in basal activity of 5-HT_{1A}, suggesting phospholipids' role of a positive allosteric modulator. Cryo-EM density maps also show various cholesterol and acyl tails in the vicinity of the receptor, suggesting a big stabilizing role for the receptor, and even led to the conclusion of cholesterol and phospholipids modulating 5-HT_{1A} signaling, and therefore having a role in the pathogenesis of depression. Additionally, studies revealed that palmitoylation on C-terminal Cys_{417} and Cys_{420} residues guides the receptor into cholesterol-rich parts in the membrane (Gorinski *et al*., 2019). Basal activity of serotonin receptors in the apo state was also reasoned by multiple conserved water molecules binding analogously to serotonin, discussed more deeply in the article by Xu *et al*. (2021). Moreover, they mentioned pan-agonism of serotonin for GPCRs, especially due to similar hydrophobic residues in the binding site, having evidence that different serotonin receptors can interact with the same ligand at similar affinities (Xu *et al*., 2021; Zweckstetter, *et al*., 2021).

1.3.2 5-HT$_2$ receptors

In the agonist binding site, there are two distinctive features present in 5-HT$_2$ receptors. Firstly, the intracellular side of the binding pocket (IBP) is covered with conserved hydrophobic residues, including Ile$^{3.40}$ and Phe$^{6.44}$ of the PIF motif, as well as Trp$^{6.48}$ termed as the "toggle switch". These residues have been shown to change conformations upon receptor activation, leading up to TM domain rotations (Wacker *et al*., 2013; Peng *et al*., 2018). Ligand binding has shown to move Trp$^{6.48}$ down, inducing conformational changes in PIF (Ile$^{3.40}$, Phe$^{6.44}$) and DRY (Arg$^{3.50}$) motifs. TM3 and TM6 will release their interactions which leads to up to 9 Å conformational change outwards in TM6, and 5 Å conformational change inwards in TM7. After the movement, Tyr$^{5.58}$ and Tyr$^{7.53}$ from the NPxxY motif can interact with Arg$^{3.50}$. These conformational changes lead to the opening of the cytoplasmic binding pocket for $G_{q/11}$ protein interactions, as well as G_i protein interactions with 5-HT$_1$ receptors (García-Nafría *et al*., 2018; Xu *et al*., 2021). This was also proven in article by Yuan *et al*. (2016) whose research shows that agonist binding stabilizes a water network through the whole receptor, which antagonist binding fails to fully achieve. Both the agonist and antagonist form interactions to Phe$^{6.51}$, but for the antagonist there are no interactions observed to Trp$^{6.48}$ or Phe$^{6.52}$. These residues are blocking the water network, which is likely to cause differences between G protein binding and therefore the level of agonism.

Secondly, close to IBP there is an extended cavity towards TM4/5. In 5-HT$_2$ receptors, presence of the unique Gly$^{5.42}$ allows for the formation of the cavity, unlike other GPCRs where the side chain blocks cavity access. Due to a different rotamer of Phe$^{5.38}$ in 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors, only 5-HT$_{2A}$ receptors have an accessible cavity. Therefore, the cavity acts as an important binding site to 5-HT$_{2A}$ selective drugs that can treat specific diseases such as Parkinson's disease psychosis (Kimura *et al*., 2019).

For 5-HT$_2$ receptors, the ligand binding site can be divided into orthosteric binding pocket (OBP) and extended binding pocket (EBP). Studies have shown that ligands binding only to OBP (e.g., serotonin) exert equal $G_{\alpha,q}$ and β-arrestin activity and therefore do not act as biased agonists. Instead, ligands reaching EBP can result in either higher $G_{\alpha,q}$ or β-arrestin activity. Depending on the size and shape of these binding ligands, their binding mode may have a hydrogen bond to either Thr$^{3.37}$ or Gly$^{5.42}$, for example ergotamine and lysergic acid diethylamide (LSD), respectively, while both residues are conserved across 5-HT$_2$ but not 5-HT$_1$ receptors (McCorvy *et al*., 2018). Also, 5-HT$_{2A}$ receptor has been shown to bind a zinc ion near ICL2 and ICL3, and a sodium ion binding near Asp$^{2.50}$ on all class A GPCRs. While not a part of their native structure or function, GPCRs in general are able to interact with various

small molecules and ions, presence of which can modulate the activity through allosteric mechanisms (Nichols & Nichols, 2008; Katritch *et al*., 2014; Kimura *et al*., 2019).

1.4 Psychedelics

1.4.1 Historical and clinical perspectives

Psychedelics, derived from the Greek words for "mind manifesting," refers to compounds used to induce a psychedelic experience. As stated by Humphrey Osmond (1957), psychedelics are capable of revealing valuable properties of the mind, with strong implications for therapeutic use. With further research, we know that these substances are psychoactive, altering numerous processes associated with mood, perception, thought, and feeling, for instance, but they are usually considered as safe, with very low if any indication for dependence or addiction (Nutt *et al*., 2007; Nutt *et al*., 2010; van Amsterdam *et al*., 2015). Historically, natural psychedelics derived from plants and fungi such as ayahuasca brew, mescaline, and psilocybin have been used for thousands of years in various religious, ritualistic, shamanistic, social, and spiritualistic settings across the world (Schultes & Hoffman, 1979; El-Seedi *et al.*, 2005; Pettigrew, 2011; Carod-Artal, 2015).

The discovery of lysergic acid diethylamide (LSD)-25 by Albert Hoffman in 1938, originally intended for development of a circulatory and respiratory stimulant since it resembled a known analeptic drug, did not raise specific interest for research at the time. Afterwards, with the accidental ingestion of LSD in 1943, as well as the detection of serotonin in humans in 1953, it was thought that LSD along with other psychedelics had a serotonergic mechanism of action in the brain (Twarog & Page, 1953; Hoffman, 1979; Nichols, 2016). LSD was then quickly made available for research and psychiatric use around the world under the name Delysid (Sandoz), and it witnessed extensive research on psychiatric patients already in the early 1950s.

After the discovery, almost 1 000 articles were published in medical journals that were discussing the use of LSD for neurological disorders only during the 1950s (Dyck, 2005). At first the effects of LSD were compared to mescaline that was already at the time a researched molecule, both mostly for simulating mental illnesses, but trials quickly advanced more towards treating psychiatric patients. The number of patients included in a single study increased from tens to thousands within the decade, and research gave hints about treating alcoholism and other addictions. In the 1960s, LSD gained more even non-medical implications for use, but also raised concerns about failed trials and adverse

reactions such as LSD-induced psychosis. Along with increased recreational use, requirement for proof of efficacy in 1962, as well as War against Drugs in the 1970s made it impossible to continue the promising studies any further (Sessa, 2016; Nichols & Walter, 2020). Psychedelic research came to a halt for 30 years.

Between 1970 and 2000, hardly any research was done regarding psychedelics. Alexander Shulgin with his books PIHKAL (1991) and TIHKAL (1997) gained popularity for vigorous self-administration and documentation of hundreds of new pharmaceuticals with potential benefits. A compound that raised special interest was 3,4-methylenedioxymethamphetamine (MDMA). First reported in 1978, the entactogen class of drugs emerged for their unique effects of empathy, bonding, and euphoria, as well as displaying a therapeutic potential in relieving depression and processing emotional trauma associated with PTSD (Sessa, 2016; Sessa, 2017). In the late 1990s, various brain imaging studies were applied to psychedelic research to explain the exact mechanisms of action in the brain, all before the huge rise in interest around 2010. Various proof-of-concept studies started growing bigger, which initially led to a new wave of intense research (Sessa, 2016; Nichols & Walter, 2020). Studies in the field of psychedelic research are currently advancing very rapidly, making their way to become increasingly more accepted all around the world.

1.4.2 Structure and origin

The classical psychedelics include compounds such as (5R,8R)-(+)-lysergic acid-N,N-diethylamide (LSD), N,N-dimethyl-4-phosphoryloxytryptamine (psilocybin), 3,4,5-trimethoxyphenethylamine (mescaline), and N,N-dimethyltryptamine (DMT). LSD is a semisynthetic molecule, closely related to ergotamine that belongs to the ergot alkaloid family. Ergot alkaloids are primarily found in different fungi, most prominently in *Claviceps purpurea*, a species that is known to infect different grains contaminating entire fields. After infection, the fungus starts to harden into a dark mass which then starts producing different ergot alkaloids, infecting other plants with spores, and contaminating common grains such as rye and wheat. Contaminated plants are not suitable for human consumption, and ingestion can lead to ergot toxicity called ergotism. The symptoms can be described as gangrenous and convulsive, including vasoconstrictive effects and as well as hallucinations, twitches, paranoia, and even psychosis, and it has even been suggested as a reason for witchcraft accusation during the Middle Ages (Haarmann *et al.*, 2009; Tasker & Wipf, 2021).

As with ergot alkaloids, the basic structure of LSD is comprised of a bicyclic indole ring system attached to two other rings in the molecule. Indole is a very stable and planar system whereas the two other rings display variation, including two stereocenters and a tertiary amine (pKa = 7.8) susceptible for partial protonation under physiological conditions. The stereochemistry of LSD adds complexity to the molecule itself and its pharmacological profile, when all four isomers display different attributes. The most potent stereoisomer (5R,8R) has been shown to have over 2 500-fold difference in 5-HT_{2A} affinity over (5S,8S), and 30-fold difference over (5R,8S), (+)-isoLSD (Nichols, 2018). Attached to the lysergic acid moiety of LSD are two diethylamide groups that are known to display unique properties as well as significantly extend the residence time when bound to serotonin receptors. They also increase the lipophilicity of LSD (LogP = 2.95) which influences the rapid onset of action and strong psychoactive effects in the CNS. Substitutions to nitrogens in lysergic acid, as well as modifications for the amide group nitrogen, have been shown to produce active compounds with possible therapeutic implications for neuropsychiatric disorders (Passie *et al*., 2008; Coney *et al*., 2017; Nichols, 2018).

While LSD is typically chemically synthetized, psilocybin and mescaline are found in nature, yet still include a similar structure and effects. Psilocybin is primarily found in certain species of mushrooms, most notably in *Psilocybe* genus, often referred as “magic mushrooms” due to their profound psychoactive effects stemming from indigenous use in Central and South America. Structurally, psilocybin shares a similar tryptamine structure with LSD, added with a 4-fosphoryloxy group. This group increases water solubility and absorption, being essential for the prodrug while it is metabolized into the active ingredient psilocin (Nichols, 2016; Lowe *et al.*, 2021). Mescaline is a naturally occurring psychedelic found in various species of cacti, notably peyote (*Lophophora williamsii*) and San Pedro (*Echinopsis pachanoi*). Instead, mescaline’s structure is more closely classified as a phenethylamine than tryptamine, sharing similarities with entactogens such as MDMA and psychedelic 2C compounds such as 2,5-dimethoxy-4-bromophenethylamine (2C-B). Even with structural differences, the effects of mescaline are very closely related to LSD (Nichols, 2018; Ley *et al*., 2023).

DMT, the active ingredient in ayahuasca, is one of the two basic ingredients in the brew and usually harvested from leaves of *Psychotria viridis*. DMT is not orally active due to rapid metabolism, so it must be combined with a MAO inhibitor (MAOI), typically root bark from *Banisteriopsis caapi* vines, that contains a mixture of β-carboline alkaloids such as harmine and harmaline. MAOI activity allows DMT to be diffused from the GI tract and cross the blood-brain barrier, activating 5-HT_{2A} receptors resulting in a psychedelic experience. When compared to LSD, the structure is simple but still contains the indole ring system. The structure of DMT closely resembles tryptophan making it a serotonergic analog

capable of activating serotonin receptors in the brain, also able to be locally synthesized in the body (Barker, 2018).

Structural variations can make a substantial difference in their pharmacological properties such as potency, duration of action, and subjective effects. For example, closely related DMT analogs 5-MeO-DMT and bufotenine (5-HO-DMT) can be seen to modify their psychoactive effects relative to DMT. Apart from plants, 5-MeO-DMT and bufotenine can readily be found in the parotid gland of *Incilius alvarius*, the Sonoran Desert toad which the toad uses for defense. It is also often used in tribal ceremonies and healing rituals in the Caribbean and the Amazonian rainforest (Reckweg *et al*., 2022). Understanding the structural attributes of psychedelics is crucial in investigating their pharmacological properties and possible applications for therapeutic use.

1.4.3 Psychedelics and serotonin receptors

Throughout their history, psychedelics have been believed to display their effects through serotonin receptor signaling, especially 5-HT_{2A} receptors. LSD with its remarkably high affinity for serotonin receptors has additionally shown a very slow dissociation kinetics from the receptor that can be explained by ECL2 movement. After ligand binding, ECL2 lid covers the binding pocket making the ligand unable to dissociate from the receptor, which is thought to cause the long duration of effects despite of its fast elimination from the body. LSD binding seems to also show β-arrestin signaling with a similar level to $G_{q,11}$ signaling at 5-HT_2 receptors. The receptors stabilize in a conformation suitable for β-arrestin binding, thought to be altered by interactions with TM7, notably $Tyr^{7.43}$ and its interactions with the diethylamide moiety (Wacker *et al*., 2013; Wacker *et al*., 2017; Cao *et al*., 2022; Wallach *et al*., 2023).

As discussed in paragraph 1.2.4, 5-HT receptors form heterodimers, resulting in an entirely different mode of signaling. Lisuride and ergotamine, for example, are 5-HT_{2A} agonists but exert their therapeutic effects without causing hallucinations, suggesting an alternative mechanism for psychedelic effects. Interactions with $mGlu_2$ receptor has led scientists to believe it is responsible for the hallucinogenic effects of 5-HT_{2A} agonists. Additionally, 5-HT_{2A} activation has shown to release glutamate into the prefrontal cortex pyramidal neurons. Increased concentration of glutamate activates ionotropic glutamate receptors and therefore displays a strong effect also on various other signaling pathways (Moreno *et al*., 2011; Marek, 2018; Slocum *et al*., 2022). Psychedelics have also been investigated for their increased neuroregeneration and plasticity, and even to the reopening of

sensitivity window towards brain circuit remodeling (Slocum *et al*., 2022; Nardou *et al*., 2023). Altogether, these effects have led users to report profound psychedelic experiences lasting from 6 hours up to 24 hours depending on the dose.

1.4.4 Biological effects and mechanisms

Psychedelic experiences consist of having different effects in the body. The biological effects of psychedelics can be divided into three groups: physical effects, sensory effects, and cognitive effects, as described in Subjective Effect Index (Kins, 2017). The physical effects are divided into enhancements, suppression, and alterations. Typically, tactile sensations across the body are greatly enhanced which can lead to physical euphoria. Additionally, sexual sensations such as hugging and kissing are amplified, but it can also result in oversensitivity that can easily become overwhelming and highly uncomfortable. Libido is also often increased, but instead appetite is heavily suppressed. There is evidence of serotonergic system working alongside orexin to regulate appetite, even to the point that psychedelics have undergone trials for treatment of obesity and eating disorders. Tactile enhancements can be explained with heightened brain activity and sensory processing, as well as disinhibition of sensory pathways via serotonergic mechanisms (Voigt & Fink, 2015; Marek, 2018; Borgland & Neyens, 2022).

Alterations in physical effects can range from neutral and positive, such as yawning, pupil dilation, changes in the perception of the body, and spontaneous physical sensations, to extremely uncomfortable, including increased blood pressure and heart rate, muscle cramps, nausea, dehydration, and vasoconstriction. These happen due to the serotonergic system having countless implications in the body. Psychedelics work as sympathomimetics that lead to effects that mimic the activation of the sympathetic nervous system, explaining the effects (Schlag *et al*., 2022). Very often a psychedelic experience also includes auditory hallucinations, enhancements, and distortions. This can be experienced on its own or it may be a part of synesthetic effect of multiple senses combining, even to the point of each of the senses becoming completely intertwined, but this is only present in extremely high doses (Brogaard & Gatzia, 2016).

Sensory effects are often thought as the most prominent ones during a psychedelic experience (Hoffmann, 1979). Although playing a big part of the perceived effects, internal cognitive effects often tend to be more meaningful for the patient. Visual effects include enhancements in sharpness and clarity, color vividness and brightness, zooming of the visual field of view, and the ability to recognize

different patterns better, both geometric patterns in nature as well as human-like shapes in normal objects. Visual effects can also include distortions, including things morphing into different shapes, breathing motion of stable objects or scenery, as well as creating a fluid-like imagery by melting and flowing. Distortions can also happen without modifying the colors of the original object, by colors being replaced or constantly shifted to assorted colors and shades. Tracers and after images of moving objects are also quite common with psychedelics, possibly creating recursive patterns and changes in perspective and depth perception. Next level of visual effects can be defined as psychedelic geometry, partially of completely filling the person's view with colors, shapes, forms, fractals, and even indescribable complex geometric patterns. These can be included in hallucinatory states which can range from internal to external hallucinations, as well as transformations of different scenarios and landscapes to unspeakable horrors, meeting autonomous entities, and even visualizing your own consciousness.

Why psychedelics display visual effects come down to two reasons, increasing brain activity in areas that are not typically active during visual experiences, and decreased communication between areas used to create spatial representations of the external reality. Studies have shown an increased activity in the visual cortex, prefrontal cortex, involved in sensation and movement, but reduced communication between the hippocampus and the visual cortex. This suggests increased processing of visual information and disturbances in the visual input, which are then replaced with processed information creating hallucinations (Carhart-Harris *et al*., 2016; Preller *et al*., 2018; Domenico *et al*., 2021).

Typically, cognitive effects can be classified as positive therapeutic effects (Liechti, 2017). They can vary from amplified analytic skills, creativity, empathy and affection, immersion and mindfulness, and laughter, all the way to increased appreciation and fascination, introspection, sociability, accelerated thoughts with conceptualization and associating them together with multiple streams of thought. During the psychedelic experience it is also typical to also perceive more negative cognitive effects, including confusion, time distortions and loops, delusions, feelings of impending doom, disorganization of thoughts and memory suppression, which sometimes may lead to a state called ego death, a total dissolution of the sense of identity and personality. The experience can also include other transpersonal states, such as feelings of rebirth, eternity, and predetermined sensations, perception of opposites existing simultaneously, and unity and interconnectedness, which are all key factors in a fascinating mystical experience induced by psychedelics. These effects can be reasoned by the immediate activation of brain areas that are not typically connected to each other.

1.4.5 Psychedelics and TrkB receptors

Several diverse types of drug classes, such as SSRIs, MAOIs, and tricyclic antidepressants, are identified to treat depression, where they exert the classical effect of increasing monoamine levels in the synaptic cleft. Although having proved clinically effective, their effects can be seen only with a long delay, controversially to rapid effect on monoamine levels (Malhi & Mann, 2018). Additionally, new research on ketamine and its metabolites suggests that N-methyl-D-aspartate (NMDA) glutamate receptor inhibition would not be the main mechanism of action for these molecules. The common factor between ketamine and antidepressants is the increased brain-derived neurotrophic factor (BDNF) signaling and the expression of neurotrophic tyrosine kinase receptor 2, also known as the tropomyosin kinase B (TrkB), as its target molecule (Abdallah *et al*., 2015; Casarotto *et al*., 2021).

TrkB, activated by BDNF and to a lesser extent neurotrophin-4, is a membrane-bound glycoprotein that exerts its effects as a ligand-activated dimer. BDNF along with other neurotropic factors are key regulators of neuroplasticity, effects ranging from synapse formation and reactivation of child-like plasticity in adults to atrophic processes such as elimination of inactive neurons (Castrén, 2013; Castrén & Antila, 2017). TrkB signaling promotes cholesterol production in the brain, which has also been shown to influence neuron maturation, plasticity, and synaptic transmission, although the exact mechanism is still unclear. Studies suggest that CARC/CRAC motifs facilitate a direct binding of cholesterol onto TrkB receptor that can influence its function, along with cholesterol having a concentration-dependent effect on the C-terminal distance between TrkB monomers that is related to the ability to bind BDNF. Moreover, cholesterol was found to induce TrkB translocation onto the cell surface, otherwise being inaccessible to BDNF (Cannarozzo *et al*., 2021; Casarotto *et al*., 2021).

BDNF signaling is crucial for displaying effects of antidepressants, either directly or indirectly through SERT or NMDA receptors. Antidepressants have been shown to induce a stable conformation of TrkB as well as membrane translocation, together promoting access to BDNF and subsequent signaling activation (Castrén & Antila, 2017; Casarotto *et al*., 2021). Classic psychedelics LSD and psilocin are also known to activate TrkB receptor, not directly activating but instead working as positive allosteric modulators. Their affinity has been shown to be 1 000-fold higher when compared to antidepressants, promoting BDNF signaling independently of 5-HT_{2A} activation (Moliner *et al*., 2023).

Clinical trials have shown that LSD and psilocybin, precursor to psilocin, can be used as rapidly acting antidepressants. Despite their potential to induce hallucinogen persisting perception disorder (HPPD) as well as triggering psychotic episodes in susceptible population with family history of such incidences,

they hold massive potential for the treatment of neuropsychiatric disorders through neuroplasticity. The article by Moliner *et al*. (2023) also gives evidence that therapeutic effects are possible to separate from hallucinogenic effects.

The binding site of TrkB dimer showed a hydrogen bond between LSD and Tyr_{433}, and π-stacking to Tyr_{433}' of the other monomer. LSD, psilocin, as well as lisuride, all had more favorable dimer conformation of 17 Å between C-terminal residues compared to the SSRI fluoxetine with a 20 Å distance, although occupying a distinct binding site to fluoxetine, and lisuride having a slightly reduced affinity to the receptor. Moreover, LSD and lisuride increased phosphorylation of Tyr_{816} indicating an increased dimerization potential, interestingly related to increased PLC recruitment by $pTyr_{816}$ and downstream signaling with an antidepressant potential. These results from the study suggest a significant role for TrkB in neuroplasticity induced by psychedelics, and possibilities to develop treatment options with higher efficacy and lower side effects than current treatment options for depression (Moliner *et al*., 2023).

1.4.6 Lysergol and isolysergol

Lysergol and isolysergol are both based on the structure of lysergic acid, and they are stereoisomers with each other (Figure 2). With two stereocenters on carbons 5 and 8 they exist in four different conformations, (+)-lysergol, (+)-isolysergol, (−)-lysergol, and (−)-isolysergol. Under physiological conditions, the molecules all contain a charged nitrogen atom that can also have a distinct stereochemistry, termed as N-inversion. The inversion occurs quickly and spontaneously depending on molecular factors as well as in the binding site energetics. Even though the molecules bind to serotonin receptors and closely resemble LSD, they are not thought to have a hallucinogenic effect in humans. Additionally, the slow binding kinetics of LSD should not be seen in lysergol derivatives due to the lack of diethylamide moiety needed for closing the binding site with ECL2.

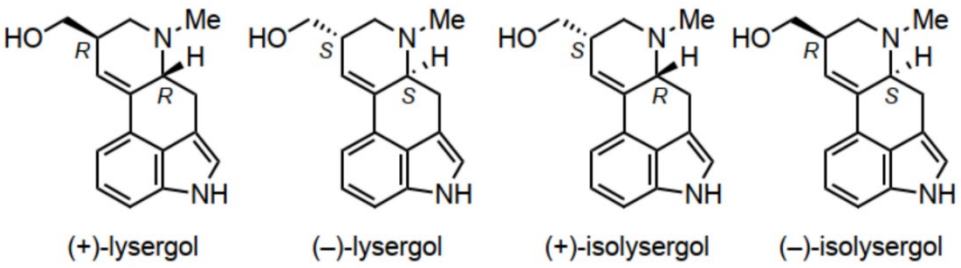

Figure 2. Molecular structures of lysergol and isolysergol isomers. Modified from Tasker *et al*. (2023).

While there is little evidence of lysergol metabolism, it is safe to assume that it closely resembles that of LSD which is a much more widely researched molecule. The metabolism happens mainly in the liver through oxidation and N-dealkylation by cytochrome P450 enzymes CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4, with a minor metabolic pathway producing nor-LSD through the demethylation of nitrogen. Furthermore, LSD undergoes through aromatic hydroxylation, forming 13-OH-LSD and 14-OH-LSD that can be readily excreted in urine as their glucuronide conjugates. The major metabolite of LSD is 2-oxo-3-hydroxy-LSD through its precursor 2-oxo-LSD, which still presents a low activity in 5-HT receptors. Less than 1 % gets eliminated as the unchanged compound (Passie *et al*., 2008; Luethi *et al*., 2019; Marta, 2019).

Lysergol compounds have not yet gone through extensive research on animal models, but cell-based assays suggest that the (+)-isomers have a great binding affinity for all the studied serotonin receptors, while exerting a smaller affinity for (−)-lysergol, and next to no affinity for (−)-isolysergol. Interestingly, (+)-isomers show moderately agonism effect on mood-regulating receptors 5-HT_{1A} and 5-HT_{2C}, but the effects are lacking on 5-HT_{2A} and 5-HT_{2B} receptors, often associated with hallucinogenic and cardiovascular side effects. The (−)-isomers show no agonistic effects on any of the receptors. Out of the studied ligands, the activity of (+)-lysergol resembles the activity of psilocin, although with a lesser potency (Tasker & Wipf, 2021; Tasker & Wipf, 2022. Tasker *et al*., 2023).

As seen in the metabolism route of LSD, it is likely that the lysergol compounds display different drug interactions. Targeted for treatment of patients with severe neuropsychiatric illnesses, other medications with interaction potential with CYP450 enzymes should not be used simultaneously, displaying their effects even weeks away from the discontinuation. Antipsychotics, benzodiazepines, MAOIs, and SSRIs are known to block the effects through different mechanisms, whereas recreational stimulants or dissociative drugs may lead to unpredictable interactions and adverse effects. For regulatory and ethical considerations, it should always be noted that the benefits of psychedelic research in treating neuropsychiatric disorders should always outweigh the risks associated with controlled substances with a high probability of adverse effects (Halman *et al*., 2024).

1.5 Aims of the study

The primary objective of this research is to explore the binding interactions between different stereoisomers of lysergol and isolysergol with 5-$HT_{1A,2A–C}$ receptors through computational methods. We aim to show how these interactions could explain the experimental data obtained from cell-based

assays conducted at the University of Pittsburgh. Additionally, we investigate the functional activity of these compounds to get insights into the interaction results and evaluate whether these findings could be used to further advance drug development projects focusing on neuropsychiatric disorders such as depression and anxiety.

2 Results

2.1 Quantum Mechanics calculations

Quantum mechanics calculations were conducted to elucidate energetically favorable conformations of the N-methyl moiety were calculated for our ligands, and the results were compiled into Table 1. For (+)-lysergol, significant differences were observed in the total potential energy, with the (R)-configuration scoring lower at 183 kJ/mol compared to 206 kJ/mol for the (S)-configuration, resulting in a relative difference of 5.33 kcal/mol. Solvation energies displayed a smaller difference, with the (R)-configuration at −57.86 kcal/mol and (S)-configuration at −58.59 kcal/mol, differing by only 0.73 kcal/mol. Similarly for (−)-lysergol, the (S)-configuration showed a lower potential energy of 181 kJ/mol compared to 206 kJ/mol for the (R)-configuration with, resulting in relative difference of 5.88 kcal/mol. Solvation energies showed a much smaller difference of 0.88 kcal/mol between (S)- and (R)-configurations.

For (+)-isolysergol, the results concluded that the lowest energy for (R)-configuration was 188 kJ/mol, whereas for (S)-configuration it was 192 kJ/mol, resulting in a relative difference of 0.95 kcal/mol. Solvation energies were significantly lower with (S)-configuration at −56.80 kcal/mol, while (R)-configuration was scored at −51.93 kcal/mol, differing by 4.87 kcal/mol. For (−)-isolysergol, the potential energies were similar for both (S)- and (R)-configuration at 191 kJ/mol. However, a significant difference in solvation energy was observed, (R)-configuration scoring −56.82 kcal/mol and the (S)-configuration −51.97 kcal/mol, resulting in a relative difference of 4.85 kcal/mol.

Table 1. Quantum mechanics results for lysergol compounds

Energetics of lysergol compounds are listed according to their N-methyl (R)- and (S)-configurations (Conf.) from Schrödinger Jaguar and Schrödinger MacroModel. The potential energies are listed in kJ/mol, while solvation energies are listed in kcal/mol, a lower score suggesting a more favorable energetic component.

Compound	Conf.	Potential energy (kJ/mol)	Solvation energy (kcal/mol)
(+)-lysergol	(R)	183.24	−57.86
(+)-lysergol	(S)	205.58	−58.59
(−)-lysergol	(R)	205.64	−58.56
(−)-lysergol	(S)	180.97	−57.68
(+)-isolysergol	(R)	187.62	−51.93
(+)-isolysergol	(S)	191.60	−56.80
(−)-isolysergol	(R)	191.59	−56.82
(−)-isolysergol	(S)	191.31	−51.97

2.2 Molecular modeling and binding studies

2.2.1 5-HT$_{1A}$

The 5-HT$_{1A}$ receptor showed a favorable binding for both (+)-isomers (Figure 3A), creating a salt bridge between the charged NH group and Asp116$^{3.32}$. Additionally, a favorable hydrogen bond to Asp116$^{3.32}$ was observed in the ethanol moiety of isolysergol, but not lysergol. Both isomers have edge-to-face π−π-interactions with Phe362$^{6.52}$, and aromatic hydrogen bonds to Ser199$^{5.42}$. The NH moiety of the indole group is oriented towards Ala203$^{5.46}$, also displaying favorable electrostatic interactions with Thr121$^{3.37}$, and to a lesser extent, with Asn386$^{7.39}$ in the case of lysergol. Strong hydrophobic interactions can be seen between the ligands and residues Phe361$^{6.51}$ and Trp358$^{6.48}$, with weaker hydrophobic interactions observed with residues Ile113$^{3.29}$, Val117$^{3.33}$, Cys120$^{3.36}$, Thr121$^{3.37}$, Ile189$^{45.52}$, and Tyr390$^{7.43}$, as well as TM5 backbone, Interestingly, the N-inversions of (+)-lysergol and (+)-isolysergol adopted mirrored binding modes compared to their original configurations (Figure 3E), resulting in two new hydrogen bonds with Asn386$^{7.39}$ and Tyr390$^{7.43}$ side chains for (+)-lysergol, and one hydrogen bond to Asn386$^{7.39}$ backbone for (+)-isolysergol. The conformational difference allows a hydrogen bond also to Ser199$^{5.42}$ and overall, a more favorable electrostatic environment for the indole NH moiety. Moreover, the hydrophobic ethanol moiety of (+)-lysergol clashes with the polar groups of Tyr370$^{7.43}$ and charged Asp116$^{3.32}$, which is not occurring with (+)-isolysergol.

2.2.2 5-HT$_{2A}$

The binding mode of (+)-isomers to the 5-HT$_{2A}$ receptor (Figure 3B) showed a salt bridge to Asp155$^{3.32}$, with the ethanol moiety forming a hydrogen bond to Tyr370$^{7.43}$ in both ligands. Strong hydrogen bonds were observed for both ligands to the Gly238$^{5.42}$ backbone, as well as edge-to-face π−π-interactions to the indole ring system from Phe340$^{6.52}$. Residues Ser242$^{5.46}$ and Thr160$^{3.37}$ facilitate positive electrostatic interactions for the polar part of the indole group. Additionally, strong hydrophobic interactions were noted with residues Phe339$^{6.51}$ and Trp336$^{6.48}$, creating a weak π-π stacking interaction with the charged amine. Lighter hydrophobic interactions were observed with residues Ile152$^{3.29}$, Val156$^{3.33}$, Ser159$^{3.36}$, Leu229$^{45.52}$, Val235$^{5.39}$, and Val366$^{7.39}$, as well as TM5 backbone. Interestingly, the N-inversion of (−)-lysergol adopted a similar binding mode to (+)-lysergol, although creating a collision with the polar head of Ser159$^{3.36}$.

2.2.3 5-HT$_{2B}$

Similarly to 5-HT$_{2A}$ receptor, binding mode of (+)-isomers to the 5-HT$_{2B}$ receptor (Figure 3C) form a salt bridge to Asp135$^{3.32}$, along with a hydrogen bond to Gly221$^{5.42}$ backbone. The ethanol moiety of isolysergol rotated to form a hydrogen bond to Asp135$^{3.32}$, a feature not seen with lysergol. Residues Thr140$^{3.37}$ and Ser222$^{5.43}$ constitute a favorable electrostatic environment for indole NH moiety, with Ser222$^{5.43}$ also forming aromatic hydrogen bonds with the indole group. We can notice edge-to-face π−π-interactions with Phe341$^{6.52}$, as well as strong hydrophobic interactions with Phe340$^{6.51}$ and Trp337$^{6.48}$. Lighter hydrophobic interactions also occurred between residues Val136$^{3.33}$, Ser139$^{3.36}$, Leu209$^{45.52}$, Phe217$^{5.38}$, Met218$^{5.39}$, Ala225$^{5.46}$, Leu362$^{7.35}$, Val366$^{7.39}$, and Tyr370$^{7.43}$, as well as the TM5 backbone. Similarly to 5-HT$_{2A}$ receptor binding, the N-inversions of (−)-lysergol and (−)-isolysergol adopted a binding mode resembling (+)-isomers (Figure 3F) but created a collision with the polar head of Ser139$^{3.36}$.

2.2.4 5-HT$_{2C}$

Binding mode of (+)-isomers to the 5-HT$_{2C}$ receptor (Figure 3D) show a salt bridge to Asp134$^{3.32}$, both with a rotated ethanol moiety that forms a hydrogen bond to Asp134$^{3.32}$, also resembling 5-HT$_{2A}$ receptor binding. For (+)-lysergol, a hydrogen bond was observed between the indole NH group and Gly218$^{5.42}$ with electrostatic interactions towards Ser219$^{5.43}$, whereas with (+)-isolysergol the indole group interacted electrostatically with Thr139$^{3.37}$ and Gly218$^{5.42}$, directed towards Ala222$^{5.46}$ with no hydrogen bonds. Strong edge-to-face π−π-interactions were noted with Phe328$^{6.52}$ and hydrophobic interactions towards Phe327$^{6.51}$ and Trp324$^{6.48}$, whereas softer hydrophobic interactions occur similarly with both ligands for residues Ile131$^{3.29}$, Val135$^{3.33}$, Ser138$^{3.36}$, Leu209$^{45.52}$, Phe214$^{5.38}$, Ala222$^{5.46}$, Val354$^{7.39}$, and Tyr358$^{7.43}$, as well as TM5 backbone. Similarly to 5-HT$_{1A}$ binding mode, the N-inversions of (+)-lysergol and (+)-isolysergol bind with a different pose compared to their original configurations (Figure 3G), difference being the mirrored binding mode that allows a hydrogen bond with Val354$^{7.39}$ backbone but losing the hydrogen bond with Gly218$^{5.42}$ due to conformational change. Additionally, the hydrophobic ethanol moiety of both ligands clashed with the polar groups of Ser138$^{3.36}$ and Tyr358$^{7.43}$, creating an unfavorable electrostatic environment.

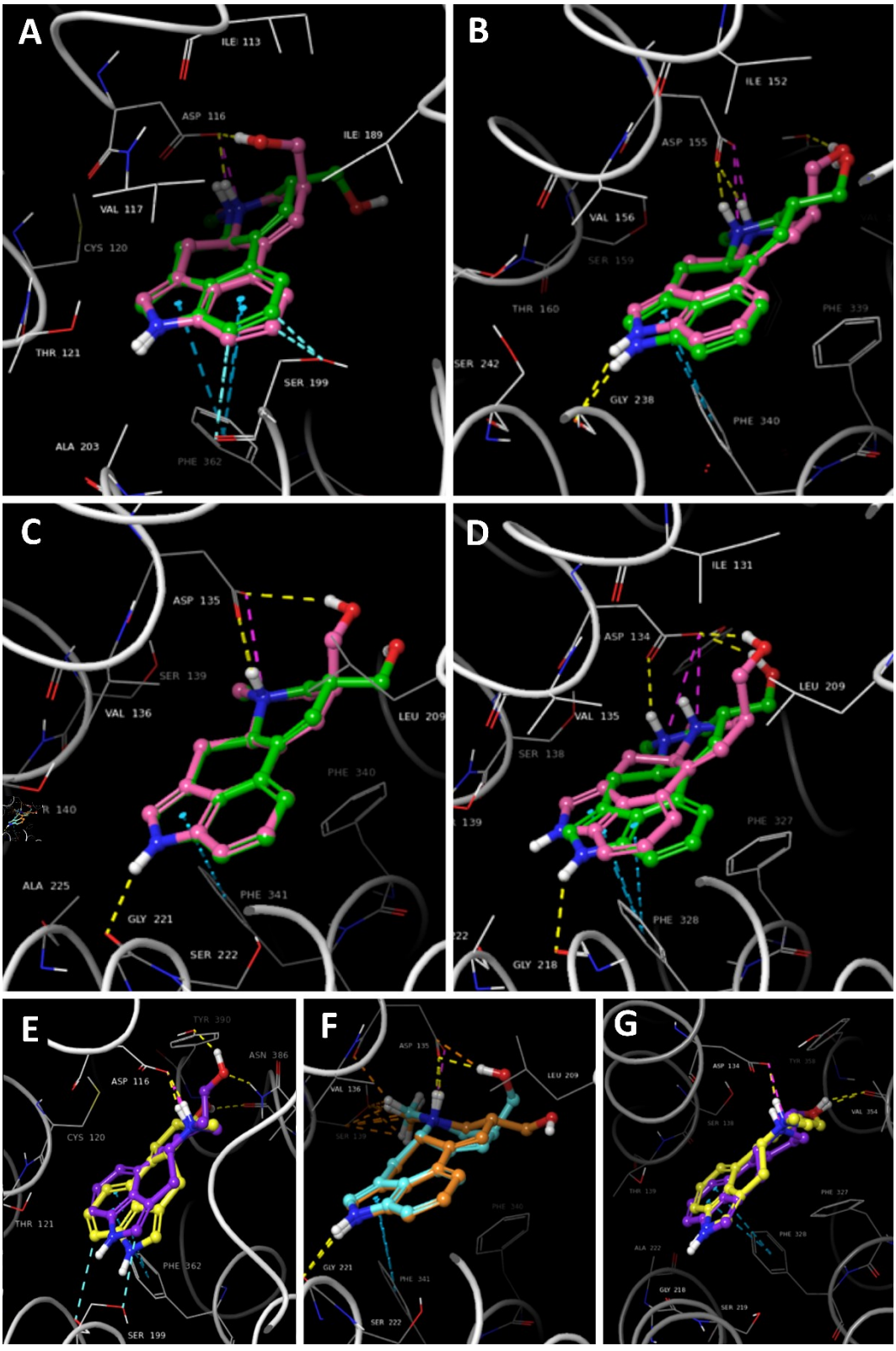

Figure 3. Binding mode of (+)-lysergol (green) and (+)-isolysergol (pink) at 5-HT_{1A} (A), 5-HT_{2A} (B), 5-HT_{2B} (C), and 5-HT_{2C} (D) receptors. Binding mode of (+)-lysergol (purple) and (+)-isolysergol (yellow) with N-inversions at 5-HT_{1A} (E) and 5-HT_{2C} (G) receptors. Binding mode of (−)-lysergol (cyan) and (−)-isolysergol (orange) with N-inversions at 5-HT_{2B} (F) receptor. Ligand interactions are marked in dotted lines for hydrogen bonds (yellow), salt bridges (purple), aromatic and π−π-interactions (cyan) as well as hydrophobic interactions to visible amino acids, for residues described in chapters 2.2–2.5. Constructed using Schrödinger Maestro 13.9.

2.3 Molecular docking studies

Our ligands were docked into the four 5-HT receptors using both 0.6 and 0.8 van der Waals radii. Results from docking studies were compiled into Table 2, where the ligands were scored with added Epik penalties. While SP docking (Appendix 1) yielded inconclusive results for the studied receptors and was left out of the results, XP docking provided valuable insights into the ligand binding. As hypothesized, (+)-isomers scored generally better than (−)-isomers, and the N-inversion impaired the docking of (+)-isomers but acted as an enhancing factor for (−)-isomers. Rigid ligand docking studies and extensive molecular dynamics simulations supported these results. Compared to the results from quantum mechanics studies, it is unlikely that N-inversion ligands function as good binders even with a reasonable docking score, supported by the results from MD simulations where ligands with a mirrored binding mode did not always stay inside the receptor for the whole 500 ns simulation.

2.3.1 5-HT$_{1A}$ receptor

The docking results a strong binding for (+)-lysergol and (+)-isolysergol, while (−)-lysergol and (−)-isolysergol exhibit much poorer binding to the receptor. The N-inversions of (+)-isomers adopt a mirrored binding mode with a good docking score, indicating favorable interactions with the receptor.

Table 2. Docking scores of lysergol derivatives

Compounds (+/−)-lysergol (L) and (+/−)-isolysergol (IL), as well as their (S) and (R)-configurations for the N-methyl group from Schrödinger Glide XP docking. A mirrored docking pose is marked with *, major problems in docking pose with **, − marks that the no output pose was generated for the ligand, and NA that the ligand was not docked. The first two columns mark the target receptor (Rec) along with the van der Waals (vdW) radius scaling factor.

Rec	vdW	(+)-L	(+)-IL	(−)-L	(−)-IL	(+)-L-(S)	(+)-IL-(S)	(−)-L-(R)	(−)-IL-(R)
5-HT$_{1A}$	0.6	−9.292	−8.880	−4.083*	−3.854**	−7.281*	−7.796*	−4.078**	−3.686**
5-HT$_{1A}$	0.8	−9.168	−9.157	−2.290**	−	−7.883*	−8.308*	−3.256**	−
5-HT$_{2A}$	0.6	−7.562	−7.355	−6.540**	−6.028**	−7.293*	−6.522	−6.025**	−3.620**
5-HT$_{2A}$	0.8	−7.763	−7.778	−3.335**	−2.928**	−5.464**	−3.804**	−5.722**	−4.668**
5-HT$_{2B}$	0.6	−8.407	−8.283	−7.656*	−5.007**	−7.758*	−8.652	−9.085	−9.014
5-HT$_{2B}$	0.8	−8.382	−8.965	−5.063**	−5.473**	−8.330*	−5.130	−9.543	−5.411**
5-HT$_{2C}$	0.6	−8.266	−7.973	−5.133	−5.289**	−8.533*	−8.014*	−7.828	−7.330
5-HT$_{2C}$	0.8	−8.121	−8.426	−5.187**	−5.197**	−4.452	−7.694*	−5.348**	−7.010
TrkB	0.8	−4.129	−3.733	−4.442	−3.930	NA	NA	NA	NA

2.3.2 5-HT$_{2A}$ receptor

Similarly, the docking results for the 5-HT2A receptor suggest a favorable binding for (+)-isomers and poor binding for (−)-isomers. The N-inversion of (+)-isolysergol adopts a similar binding pose compared to other (+)-isomers by forming a double hydrogen bond to Asp$^{3.32}$, leaving the charged amine hydrogen to face away from the aspartate residue. Molecular dynamics simulations show a very conserved water molecule near the ligand, suggesting energetically unfavorable interactions in the vicinity of the binding pocket. Rigid ligand docking showed (−)-lysergol with N-inversion binding to resemble (+)-isolysergol binding mode at vdW radius 0.6, but the results were not shown in docking with vdW radius of 0.8 due to heavy clashes with Ser$^{3.36}$.

2.3.3 5-HT$_{2B}$ receptor

Docking scores suggest a strong binding of (+)-isomers to the 5-HT$_{2B}$ receptor, with N-inversions of (−)-lysergol and (−)-isolysergol also binding in similarly to their respective (+)-isomers. However, N-inversions of (+)-isomers seem to bind moderately well to the receptor with normal and rigid ligand docking, but heavily clash with Asp$^{3.32}$ and Phe$^{6.51}$ keeping them from binding strongly to the receptor.

2.3.4 5-HT$_{2C}$ receptor

Similarly, docking scores for the 5-HT$_{2C}$ receptor indicate a strong binding of (+)-isomers, with N-inversions of (−)-lysergol and (−)-isolysergol also adopting a favorable pose. However, the lack of electrostatic interactions, suggests that they may not effectively bind to the receptor. Interestingly, N-inversions of (+)-lysergol and (+)-isolysergol bind in a pose typically seen in (−)-isomer ligands, creating an additional hydrogen bond to the back pocket, and closely resembling 5-HT$_{1A}$ receptor binding. Additionally, (−)-isolysergol adopts a favorable docking pose with vdW radius of 0.6, but the results are not repeated with vdW radius of 0.8.

2.4 TrkB receptor binding mode

In the TrkB binding assessment, our ligands were docked to the receptor where LSD is known to display a strong binding and effects. Notably, our reference (5R,8R)-LSD that is believed to exhibit the most potent interaction, displayed a docking score of −4.370 and a favorable free energy of binding of −33.13 kcal/mol, as determined by MM-GBSA calculations. Another (5R,8R)-LSD docking pose also

exhibited promising results, scoring high on MM-GBSA calculations at −39.85 kcal/mol, but with a lower docking score of −3.434. Evaluation of our ligands with a similar methodology yielded results that very closely resemble the reference ligands, docking scores ranging from −4.442 to −3.733, with free energy of binding spanning from −43.00 to −37.15 kcal/mol. Interestingly, (−)-isomers achieved slightly better docking scores with an average score difference of 0.26, while (+)-isomers outperformed in MM-GBSA results with an average energy difference of −2.92 kcal/mol. Furthermore, lysergol compounds displayed a better docking score by 0.45 units but demonstrated similar average performance in MM-GBSA calculations. Interestingly, (+)-lysergol outperformed isolysergol compounds by 3.92 kcal/mol in free energy binding calculations, while binding of (−)-lysergol did not exhibit a significant difference in binding affinity. The binding modes of LSD and the top-performing lysergol compound are illustrated in Figure 4.

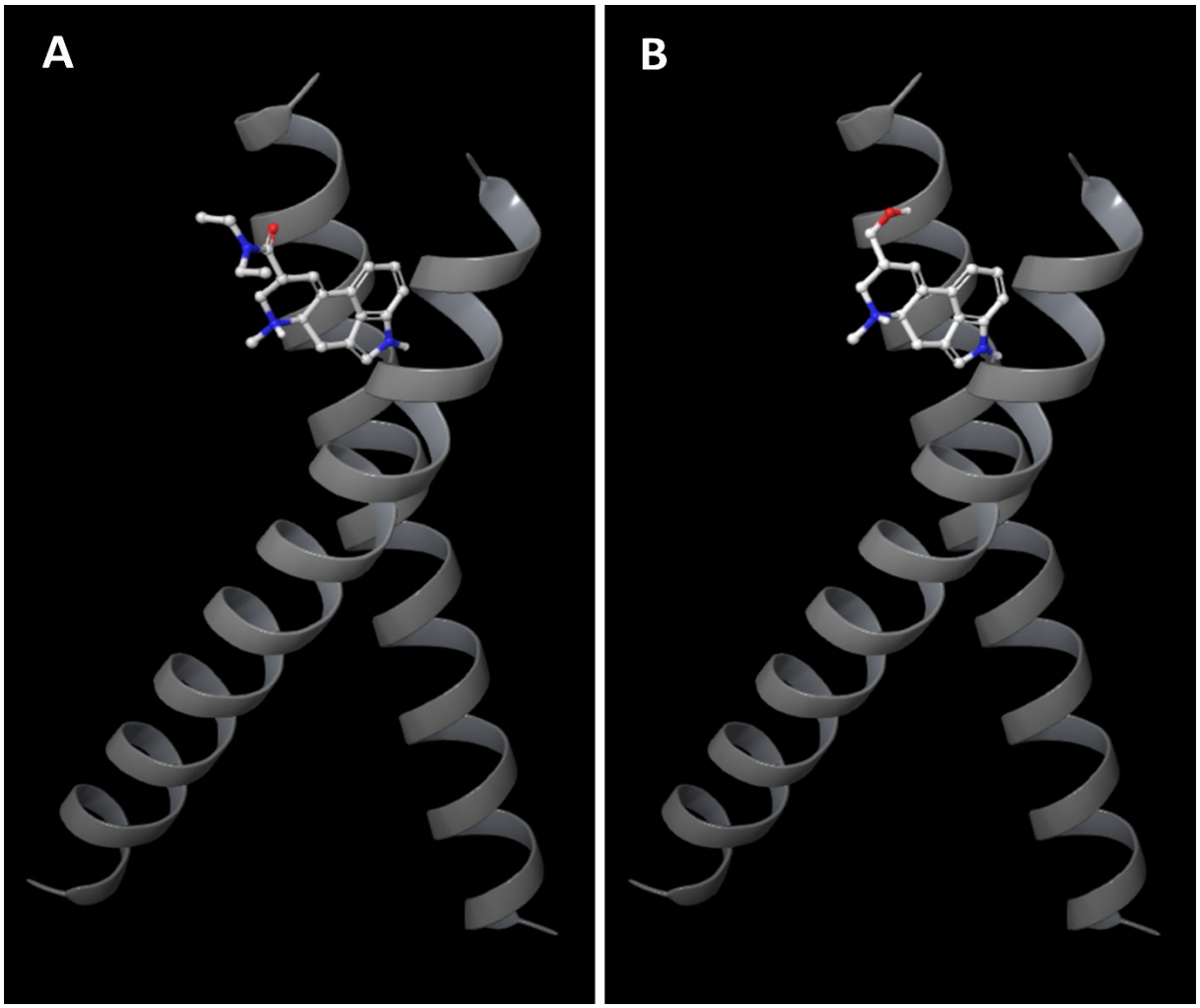

Figure 4. Comparison of the binding modes of LSD and lysergol at the TrkB dimer transmembrane domain (gray). (A) represents the binding mode of (5R,8R)-LSD while (B) illustrates the binding of (+)-lysergol (white). Nitrogen atoms are depicted in blue, while oxygen atoms are shown in red, with only polar hydrogens visible. Constructed using Schrödinger Maestro 13.9.

3 Discussion

3.1 Insights from quantum mechanics calculations

The results of quantum mechanics calculations revealed the preferred configuration of the N-methyl group across all ligands. Specifically, it was observed that lysergol compounds had a distinct preference for a nitrogen configuration where the N-methyl group aligned with the ethanol moiety of the ligands. For (+)-lysergol, the (R)-configuration displayed a significantly lower potential energy compared to its (S)-configuration, with a relative difference of over 5 kcal/mol. Similarly, (−)-lysergol had a strong preference for (S)-configuration over (R)-configuration, with a difference of 6 kcal/mol.

In contrast, the potential energy difference between isolysergol compounds was negligible, differing by less than 1 kcal/mol. However, the difference in solvation energy was almost 5 kcal/mol for both isomers, (S)-configuration being energetically favored for (+)-isolysergol and (R)-configuration for (−)-isolysergol. As observed by Ball (2008), lower solvation energy indicates a higher preference for the ligand being solvated rather than bound to the receptor, resulting in (R)- and (S)-configurations being energetically better for (+)-isolysergol and (−)-isolysergol, respectively. This preference for specific configurations with isolysergol compounds could be explained with the molecular features revealed by quantum mechanics calculations. It is shown that unlike its N-inversion, isolysergol can form an intramolecular hydrogen bond which negatively affects the solvation energy (Kuhn *et al.*, 2010).

3.2 Ligand binding preferences of lysergol derivatives

Our binding mode analysis highlighted a preference for (+)-isomers across all 5-HT receptors. Docking studies showed that all configurations of (+)-lysergol and (+)-isolysergol bind well to 5-HT_{1A} and 5-HT_{2C} receptors, suggesting a high binding affinity and a moderate level of agonism. Current research literature agrees with the results, suggesting the (+)-isomers as potentially effective ligands. Similarly, (+)-isomers show great binding for 5-HT_{2A} and 5-HT_{2B}, receptors often associated with hallucinogenic and cardiovascular side effects. Despite the good binding, findings by Tasker *et al*. (2023) show that these ligands exhibit very low levels of agonism at 5-HT_{2A} and 5-HT_{2B} receptors, suggesting a promising drug candidate for further investigation. These findings are supported by the results of this study. Surprisingly, docking studies indicated promising docking scores for some conformations of (−)-lysergol and (−)-isolysergol at 5-HT_2 receptors, suggesting a good binding to the receptors. However, further analysis revealed that the ligands heavily collide with the polar group of $Ser^{3.36}$, making a novel binding mode very unlikely.

3.3 New therapeutic options with TrkB activation

Our results indicate that lysergol derivatives adopt a similar binding bode to LSD at TrkB receptors, independently of their stereochemistry. Expanding our investigation, recent research by Casarotto *et al*. (2021) and Moliner *et al*. (2023) shows that neither hallucinogenic effects nor 5-HT_{2A} activation is needed for therapeutic potential. The articles discuss possibilities of different compounds activating pathways beyond the classical serotonin receptor activation, such as TrkB receptor activation through enhanced BDNF signaling, that can alter the pathophysiology of various neuropsychiatric disorders. In alignment with our findings, compounds with (−)-lysergol or (−)-isolysergol scaffold could be enough to induce TrkB activation and enhance neuroplasticity similarly to the effects of SSRI antidepressants. With this novel mechanism of action, it could be possible to reduce the hallucinogenic and cardiovascular side effects associated with serotonergic agonists, while still retaining potent therapeutic benefits.

3.4 Considerations and limitations of the study

Despite the promising findings, it is essential to acknowledge the possible limitations of our study. While the computational approach provides valuable insights into the interactions and activity of lysergol compounds, it may oversimplify the complex biological processes involved in ligand binding to serotonin receptors. The results of this study are predictions, that in the end are limited by the accuracy of force field algorithms, as well as the conditions of the crystal structures used. We must remember that a molecular model is still only a model, and it can display an imperfect picture of reality.

3.5 Conclusion and future prospects

In conclusion, the report stated why clavine alkaloids hold potential as promising targets for treating various neurological disorders. Despite the promising results, further research is still needed to validate our computational findings through experimental studies. In the future, the research could be continued with synthesizing new compounds according to the findings of this study, with potential interactions in serotonin receptors. These compounds could be assessed computationally as well as empirically with cell-based assays to determine their binding kinetics and functional activity. This approach would establish a better structure-activity relationship for clavine alkaloids as well as other structurally similar compounds, making the discovery of novel therapeutic options for treating neurological disorders possible. Furthermore, applying structure-based optimization could lead to the identification of potential drug candidates with higher potency and selectivity than current treatment

options, minimizing the risk associated with severe adverse effects. With continued research, I find it extremely plausible for these compounds advancing to clinical studies, and making the world a healthier place for new generations.

4 Materials and methods

4.1 Molecular modeling

Based on release date, resolution, and the cocrystal ligand, crystal structures of serotonin receptors 5-HT_{1A} (8W8B, Liu *et al*., 2023), 5-HT_{2A} (7WC6, Cao *et al*., 2022), 5-HT_{2B} (5TVN, Wacker *et al*., 2017), and 5-HT_{2C} (6BQG, Peng *et al*., 2018) were retrieved from the Protein Data Bank (PDB, Berman *et al*., 2000) and prepared using Schrödinger Protein Preparation Workflow (Schrödinger Release 2023-3: Protein Preparation Workflow; Epik, Schrödinger, LLC, New York, NY, 2023; Impact, Schrödinger, LLC, New York, NY, 2023; Prime, Schrödinger, LLC, New York, NY, 2023). Missing residues, excluding intracellular loop 3 (ICL3), side chains, and hydrogen atoms, were added to the structure. Hydrogen bonds were then optimized at pH 7.0 using PROPKA protonation states, and the whole structure was energetically minimized with OPLS4 force field to reach RMSD of 0.30 Å for heavy atoms. All crystal structure waters were also removed. Quality of the protein structures was checked using Ramachandran plot as well as various computational methods included in the Schrödinger suite (Ramachandran *et al*., 1963; Schrödinger Release 2023-3: Maestro, Schrödinger, LLC, New York, NY, 2023), and receptor docking site was generated around the co-crystallized ligands from the prepared structures. Structure for TrkB receptor was retrieved from Membranome database (Lomize *et al*., 2017), and modeled as above, additionally with Schrödinger SiteMap (Schrödinger Release 2024-1: SiteMap, Schrödinger, LLC, New York, NY, 2024) used to determine a binding pocket for ligand docking.

4.2 Ligand docking

Ligand database was constructed using Schrödinger Maestro's 2D Sketcher and prepared using LigPrep 6.7 (Schrödinger Release 2023-3: LigPrep, Schrödinger, LLC, New York, NY, 2023). The ligands were collected into a Phase database (Schrödinger Release 2023-3: Phase, Schrödinger, LLC, New York, NY, 2023) that was docked into all four receptors. Docking was run using Glide 10.0 (Schrödinger Release 2023-3: Glide, Schrödinger, LLC, New York, NY, 2023) at both Standard Precision (SP) and Extra Precision (XP) modes. The resulting docking pose was restrained to have at least one hydrogen bond interaction with a conserved aspartate residue, $Asp^{3.32}$, in the binding pocket. The van der Waals (vdW) radius for 5-HT_{1A} and 5-HT_{2C} receptors was chosen to be 0.60 due to lower resolution and size difference compared to the original cocrystal ligand, whereas for 5-HT_{2A} and 5-HT_{2B} receptors a normal vdW radius of 0.80 was used. The nitrogen configurations of the ligands were calculated with Jaguar 12.3 (Schrödinger Release 2024-1: Jaguar, Schrödinger, LLC, New York, NY, 2024) by using MacroModel 14.3 energetics (Schrödinger Release 2024-1: MacroModel, Schrödinger, LLC, New York, NY, 2024),

which were used to calculate favorable conformations for our ligands for better docking result interpretation. The most favorable ligands were docked (XP, vdW radius of 0.8) in TrkB receptor using (5R,8R)-LSD with known activity as a reference. The docking results were scored using Glide docking score with Epik penalties as well as molecular mechanics with generalized Born and surface area (MM-GBSA) scoring function calculations with Prime 7.3 (Schrödinger Release 2023-3: Prime, Schrödinger, LLC, New York, NY, 2023). Calculations were performed both only for the ligand as well as using flexible protein residues within 3 Å of the ligand.

4.3 Molecular dynamics simulations

Molecular dynamics (MD) simulations were completed using Desmond 7.6 (Schrödinger Release 2023-4: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2023; Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY, 2023). The system was built by inserting the receptors into the cell membrane according to MemProtMD database (Newport *et al*. 2019) using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) as lipids. Receptors were neutralized using 12, 2, 3, and 10 Cl^- ions, respectively, and dissolved in a 0.15 M NaCl solution using TIP3P water model in an orthorhombic box with a box edge 10 Å away from the receptor. OPLS4 force field was used, and the MD simulations were run using Puhti supercomputer (CSC – IT Center for Science, Finland) for 500 ns with a trajectory recording interval of 200 ps, having five repeats with random seeds for each of the receptor-ligand complexes. The simulation conditions were regulated by Nosé-Hoover chain thermostat and Martyna-Tobias-Klein barostat at 300 K and 1.013 bar, respectively, and the system relaxation protocol is listed in Appendix 2. The full simulations of 2 500 frames were visualized and analyzed using Schrödinger Maestro 13.7 and VMD 1.9.3 (Humphrey *et al*., 1996). Simulation results were compared to docking results using MM-GBSA calculations for both only for the ligand as well as using flexible protein residues within 3 Å of the ligand.

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Abbreviations

5-HT	Serotonin, 5-hydroxytryptamine
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
CYP	Cytochrome P450
DMT	N,N-dimethyltryptamine
ECL	Extracellular loop
GI	Gastrointestinal
GIRK	G protein-coupled inwardly rectifying potassium channel
GPCR	G protein-coupled receptor
G_{α}	G protein, subunit alpha
$G_{\beta\gamma}$	G protein, subunit beta-gamma
ICL	Intracellular loop
LSD	Lysergic acid diethylamide
MAO	Monoamine oxidase
MAOI	Monoamine oxidase inhibitor
MD	Molecular dynamics
MDMA	3,4-methylenedioxymethamphetamine
$mGlu_2$	Metabotropic glutamate receptor 2
MM-GBSA	Molecular mechanics with generalized Born and surface area
NMDA	N-methyl-D-aspartate
PDB	Protein Data Bank
SERT	Serotonin transporter
SSRI	Selective serotonin reuptake inhibitor
TM	Transmembrane
TrkB	Tropomyosin kinase B
vdW	Van der Waals
VMAT	Vesicular monoamine transporter

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Appendices

Appendix 1. Docking results for 5-HT receptors

Rec	vdW	(+)-L	(+)-IL	(−)-L	(−)-IL	(+)-L-N	(+)-IL-N	(−)-L-N	(−)-IL-N
5-HT_{1A}	0.6	−7.427	−7.857	−6.894	−5.950	−6.758	−7.360	−3.844	−5.621
5-HT_{1A}	0.8	−7.826	−7.847	−6.408	−6.500	−7.695	−7.328	−8.182	−5.750
5-HT_{2A}	0.6	−6.660	−7.430	−5.232	−6.569	−6.970	−6.522	−6.878	−5.832
5-HT_{2A}	0.8	−7.073	−7.750	−	−6.291	−6.090	−6.876	−7.022	−5.682
5-HT_{2B}	0.6	−7.396	−7.357	−7.538	−7.092	−8.019	−7.472	−7.902	−7.771
5-HT_{2B}	0.8	−7.556	−7.595	−5.825	−7.274	−7.745	−7.779	−8.158	−8.109
5-HT_{2C}	0.6	−7.840	−7.375	−7.790	−7.384	−7.761	−7.591	−7.647	−6.557
5-HT_{2C}	0.8	−7.368	−7.713	−7.246	−7.676	−7.661	−7.841	−7.586	−6.857

Docking scores of (+/−)-lysergol (L) and (+/−)-isolysergol (IL) compounds and their N-conformers from Schrödinger Glide SP docking. NA marks that the ligand was not docked to the receptor, and − that no output pose was generated for the ligand. The first two columns mark the target receptor (Rec) along with the van der Waals (vdW) radius scaling factor.

Appendix 2. Molecular dynamics simulation relaxation protocol

1. Simulate in the NVT ensemble using Brownian dynamics with a simulation time of 50 ps, a temperature of 10 K, and restraints on the solute with a force constant 50 kcal mol^{-1} $Å^{-2}$, with Nosé-Hoover thermostat and Martyna-Tobias-Klein barostat used throughout the protocol.
2. Simulate in the NVT ensemble using Brownian dynamics with a simulation time of 20 ps, a temperature of 100 K, a pressure of 1000 bar, and restraints on the solute and membrane heavy atoms with force constant 50 kcal mol^{-1} $Å^{-2}$ with a Gaussian biasing force to non-crystal waters.
3. Simulate in the NPγT ensemble with a simulation time of 100 ps, a temperature of 100K, a pressure of 1000 bar, restraints on the solute heavy atoms with force constant 10 kcal mol^{-1} $Å^{-2}$ with a Gaussian biasing force to non-crystal waters, and restraints on the membrane N and P atoms in the z direction with force constant 2 kcal mol^{-1} $Å^{-2}$.
4. Simulate in the NPγT ensemble with a simulation time of 150 ps, heating from a temperature of 100 K to 300 K in a pressure of 100 bar, restraints on the solute heavy atoms with force constant 10 kcal mol^{-1} $Å^{-2}$, and restraints on the membrane N and P atoms in the z direction with force constant 2 kcal mol^{-1} $Å^{-2}$, while restraints are gradually reduced to 0.
5. Simulate in the NVT ensemble with a simulation time of 50 ps, a temperature of 300 K, and restraints on the protein backbone and the ligand heavy atoms with force constant 5 kcal mol^{-1} $Å^{-2}$.
6. Simulate in the NVT ensemble with a simulation time of 50 ps, and temperature of 300 K without any restraints.