Comparative Analysis of Psychoactive Molecules in Complex with HT2RA Seratonin Receptors

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Abstract

We present a comparative binding analysis of various psychoactive ligands with the human 5-HT2A seratonin transporter to the same receptor protein in complex with the common antipsychotic, risperidone. We find that among tested ligands, mescaline is the most energetically favorable when bound to HT2A. We also induce three simulated mutations in the target protein, finding that L228N stabilizes the mescaline-protein complex.

1 Introduction

Psychedelic compounds have seen increasing attention as potential therapeutic agents in a range of mental disorders from anxiety to Post-Traumatic Stress Disorder (PTSD) [8]. Exploring their therapeutic capabilities requires intimate knowledge of their biochemical interface with the nervous system. In this study, we characterize the molecular interactions between seven psychoactive molecules and the active-state 5-hydroxytryptamine receptor 2A (5HT2A) serotonin receptor to which they bind.

Serotonin and its receptors are essential to the function of the brain and every organ system. Though serotonin's precise role remains poorly characterized, it is believed to affect motor function, respiratory drive, body temperature, and a wide range of additional physiological functions, in addition to aspects of human behavior [1]. There are many classes of serotonin receptor, and activation of specific receptor subtypes is linked to corresponding

moods and behavioral states. Here we focus on 5HT2A receptors, which are located primarily in the frontal cortex, with high density in regions related to emotional response, vision, and motor function [4]. The receptor has been a focus of clinical research on antipsychotic drugs and is the primary binding partner for psychoactive drugs including psilocybin, LSD, DMT, and mescaline, all of which are 5HT2A agonists [8].

In this study we characterize the protein-ligand interactions between a cryogenic electron microscopy-resolved 5HT2A structure and seven binding partners: psilocybin, LSD, DMT, mescaline, psilocin, MDMA, and risperidone. Psilocybin, LSD, DMT, and mescaline are all psychoactive structures that act as 5HT2A agonists. Psilocin is the dephosphorylated form of psilocybin, and the compound which binds 5HT2A receptors to produce a hallucinogenic effect when consumed by a human [7]. MDMA is also believed to bind 5HT2A receptors as treatment with ketanserin, a 5HT2A receptor antagonist, eliminates its psychoactive capabilites [6]. Risperidone is an antipsychotic medication that acts as a 5HT2A agonist [2]. By including risperidone in the study, we hope to compare binding characteristics between the six psychoactive compounds and risperidone, a clinically tested medical treatment, to further explore the therapeutic potential of hallucinogenic molecules.

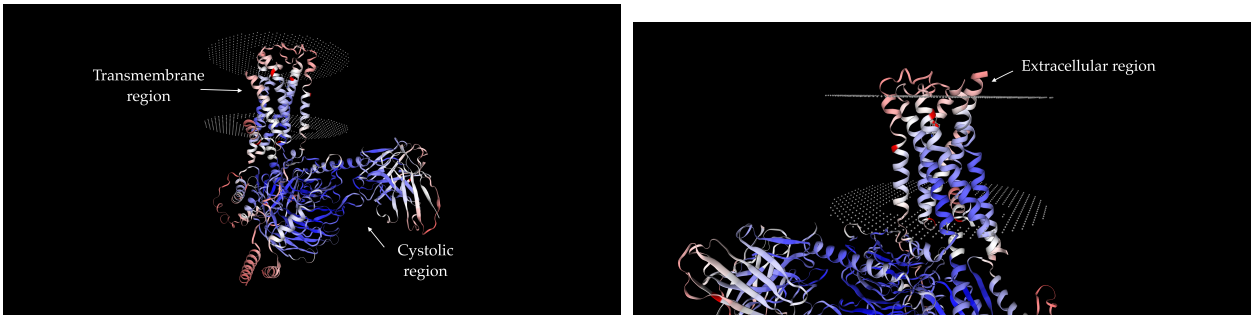
2 Methods

Our analysis sought to compare the known structure of a serotonin transporter in complex with an antipsychotic to the predicted structure of the same transporter in complex with various other psychoactive substances. Doing so required isolating the target receptor, truncating the receptor to a computationally-feasible length, and performing a docking simulation between the isolated receptor structure and various ligands.

2.1 Isolating and Preparing Target Receptor

We began with the crystal structure of the active-state (5-HT2A), a cell-surface human seratonin receptor, in complex with a hallucinogenic agonist. The structure was determined via X-ray crystallography at a resolution of 3.40 Åand is available from the Protein Data Bank (PDB) under the code 6WGT. The hallucinogenic agonist used for this experimentally-determined structure was 25-CN-NBOH, considered a "prototypical hallucinogen" whose conformational effect on HT2A is similar to that of most commonly-used psychadelics. This structure was then uploaded into PyMol where we removed the 25-CN-NBOH hallucinogenic agonist.

For our docking analysis, we used SwissDock, a web-based software developed by the Swiss Institute of Bioinformatics. SwissDock caps the target size at 15,000 atoms, while the ligand-free HT2A was over 18,000 atoms. To address this issue, we examined the regions of the protein that, if truncated for computational purposes, would not affect the ligand-protein conformation. Since HT2A is a membrane-embedded protein with a signaling function, psychoactive ligands will only bind to the non-cystolic or transmembrane regions of the protein. To determine how the protein is situated in the cell membrane, we analyzed the HT2A structure in Phobius, a free software provided by the Stockholm Bioinformatic Center, which predicts transmembrane topology and signal peptides. From this prediction, we used PyMol to truncate an intracellular portion of the protein to meet the size requirement.



Ligands don't reach intracellular region Acessible extracellular region

Figure 1: Membrane Orientation of HT2A Protein

To enhance the docked conformational prediction accuracy, we performed further pre-

processing with ChimeraX, a free software provided by UCSF. We used the Dock Prep tool to:

1. Remove the solvent
2. Remove non-complexed ions
3. Standardize the following residue types
    - selenomethionine (MSE) → methionine (MET)
    - bromo-UMP (5BU) → UMP (U)
    - methylselenyl-dUMP (UMS) → UMP (U)
    - methylselenyl-dCMP (CSL) → CMP (C)
4. Replace incomplete side chains with the Dunbrack rotamer library
5. Add predicted hydrogens
6. Add predicted residue charges

The ligand-free, truncated, and charge and hydrogen-augmented HT2A then served as the final "base structure" for our docking analysis. With this base target, we performed 6 docking analyses in SwissDock, each with the base structure and one of the following psychoactive ligands of interest:

1. Mescaline
2. N,n-Dimethyl-1h-Indole-3-Ethanamine (DMT)
3. Lysergic acid diethylamide (LSD)
4. 3,4-Methylenedioxymethamphetamine (MDMA)
5. Psilocin
6. Psilocybin

These ligand structures were pulled from the ZINC database.

2.2 Docking Simulation and Analysis

SwissDock is an implementation of the EADock Dihedral Space Sampling (DSS) algorithm [3], which features three basic steps: (1) identify cavities within the protein structure to sample as potential binding sites for the small molecule (2) for each cavity, position the small molecule within an allowed spherical volume in which it randomly rotates and estimate energies of each orientation (3) calculate the free energies of the most favorable ligand orientations and cluster by cavity.

Cavities are identified by mapping the receptor onto a 3-dimensional grid. The algorithm them identifies a in the grid that do not contain protein, only solvent. From this grid point, the algorithm searches the x, y, and z axes, as well as the four diagonals, for interactions between the solvent and protein. If more than three but less than seven interactions are found, the grid point is denoted as a cavity point. After all cavity points have been identified, adjacent points are clustered into cavities and compared to the size of the ligand. Only cavities with sufficient space to contain the ligand are considered in the following steps. Energies are estimated in (2) using CHARMM, and precise free energies are found in (3) with the fast analytical continuum treatment of solvation (FACTS) algorithm. The output of SwissDock is a list of possible conformation "clusters" and the associated ΔG values of each conformational state within each cluster [3].

cluster only

To compare the similarity of the experimentally-determined HT2A with risperidone to the predicted structure of HT2A with various ligands, we performed a qualitative structural comparison between the predicted complex structures and the experimentally-determined ones, as well as a quantitative analysis of binding affinity using free energy and full fitness scores.

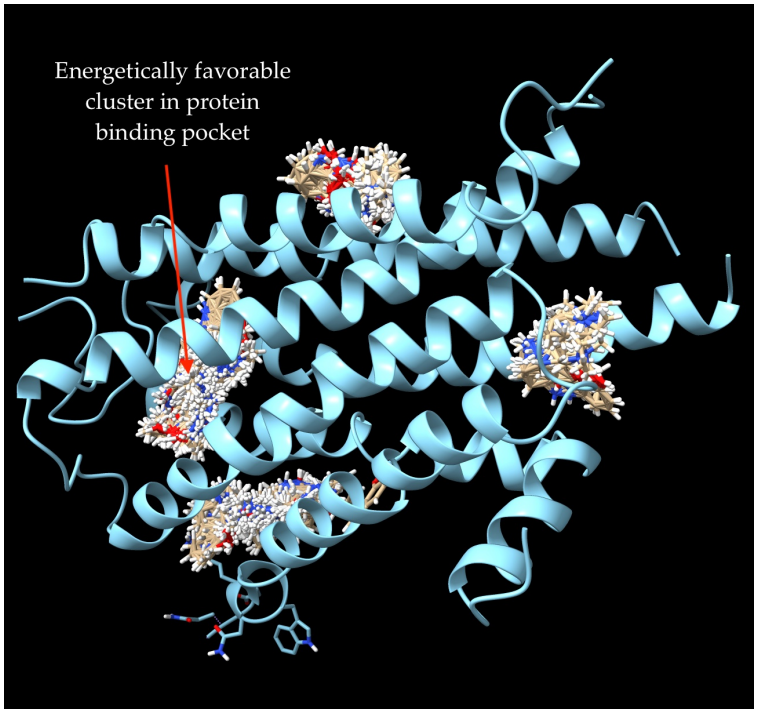
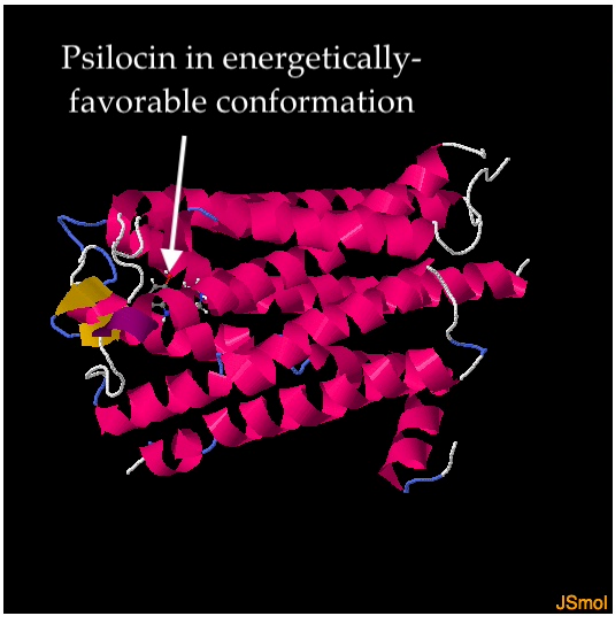


Figure 2: Four possible clusters of conformation states between Psilocin and HT2A

2.3 Simulated Mutation

To further elucidate the binding mechanism between psychoactive substances and the HT2A receptor, we performed a simulated mutation in the target protein and analyzed the resulting binding effects. Using PyMol, we introduced the mutations D155L, W336L and L228N in the binding pocket of the target protein. We then re-ran the docking simulation using the ligand that previously was bound most tightly to the protein — in this case mescaline.

The D115L and W336A mutations were selected to eliminate polar interactions and hydrophobic interactions respectively between the peptide side chains and mescaline. Leucine is of similar shape to aspartic acid but contains a hydrophobic, uncharged side chain, wheras that of aspartic acid is negatively charged. Alanine is substantially shorter than tryptophan (and so further from the core of the binding pocket), and also lacks the benzene ring which we hypothesized was key to the hydrophobic interactions of W336. We posited that these individual mutations would destabilize the interaction and weaken the bond between



Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
◉	0	0	-1180.87	-8.18
○	0	1	-1180.87	-8.18
○	0	2	-1180.84	-8.17
○	0	3	-1178.62	-7.94
○	0	4	-1170.09	-7.41
○	0	5	-1169.51	-7.36
○	0	6	-1168.10	-7.04
○	0	7	-1144.62	-6.03
○	1	0	-1180.64	-7.88
○	1	1	-1179.08	-7.86
○	1	2	-1174.05	-7.26
○	1	3	-1173.43	-7.44
○	1	4	-1168.94	-6.52
○	2	0	-1177.48	-7.80
○	2	1	-1176.87	-7.66
○	2	2	-1176.84	-7.66
○	2	3	-1173.73	-7.20
○	2	4	-1167.24	-6.85
○	3	0	-1177.02	-7.73
○	3	1	-1176.97	-7.72
○	3	2	-1173.85	-7.62
○	3	3	-1173.17	-7.55

Figure 3: One conformation state of Psilocin with HT2A in most favored "cluster" and output of ΔG Values

mescaline and the receptor. The literature suggests both of these residues are essential for binding 25CN-NBOH, with D115 forming a salt bridge to a positively charged nitrogen in the ligand, and W336 interacting hyrophobically. In both cases mutation of the peptide to alanine experimentally eliminated the binding capabilities of the receptor [5].

The L228N mutation was intended to increase inter-molecular forces by substituting a nonpolar residue for a similarly-shaped polar side chain. We hypothesized asparagine would stabilize mescaline's interaction with the binding pocket by introducing hydrogen bonds between the peptide's N-H bonds and a nearby oxygen in mescaline's optimal orientation within the pocket.

Both mutations were performed and analyzed individually. Mutations were introduced using PyMol's mutation wizard. Upon substitution of the residue, the mutated peptide was rotated such that it minimized steric clashes with other side chains in the receptor. Note that we did not perform analysis on the extent to which mutations destabilized the protein itself; we sought only to understand which candidate amino acids in the binding pocket were involved in the receptor-ligand interaction.

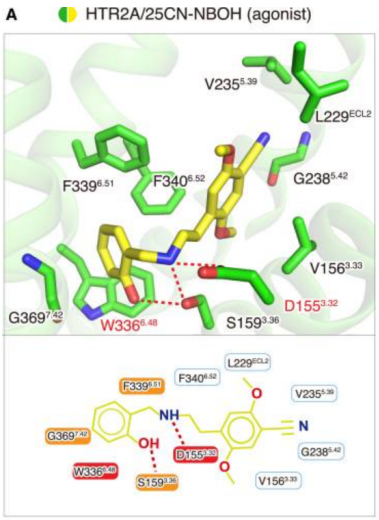
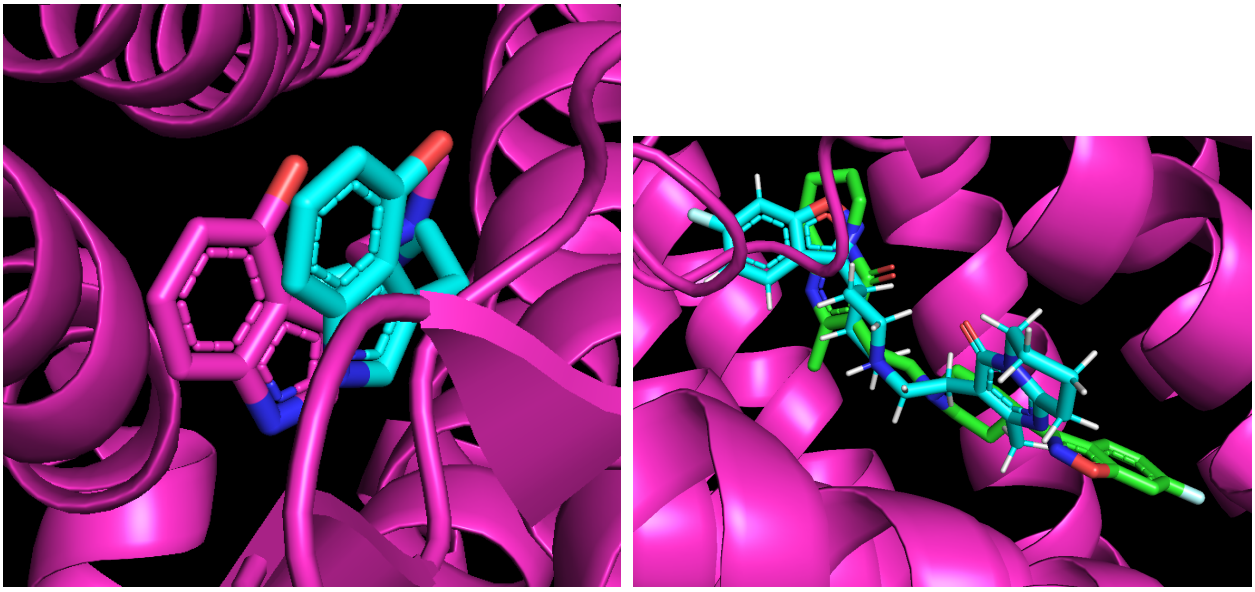


Figure 4: 5HT2A receptor binding interactions with 25CN-NBOH, adapted from [5]

2.4 SwissDock Control

By computationally isolating the HT2A from an experimentally determined structure in complex with the hallucinogenic 25-CN-NBOH agonist, we introduce the risk of negatively biasing the binding affinity between the experimental ligands and the isolated receptor protein. If the hallucinogenic agonist induced significant conformational changes in the receptor protein, this would skew our quantitative evaluations of binding affinities between the receptor and the experimental ligands.

To account for this, we compared the HT2A protein structures in two cases — bound to the hallucinogenic agonist and bound to risperidone — to verify that there was no significant difference between the HT2A structure in these cases. Cryo-Em-determined structures for HT2A bound to risperidone and a prototypical hallucinogenic agonist were attained from PDB and aligned in PyMol. The resulting RMSE of this alignment was just 1.02, giving us confidence that the computationally-isolated HT2A is a viable "base" protein to use with our psychoactive ligands of interest.



Modeled (blue) vs. experimental (purple) psilocin docking to the 5HT2A receptor

Modeled (blue) vs. experimental (green) psilocin docking to the 5HT2A receptor

Figure 5: Orientation comparison between SwissDock output and experimental results

3 Results & Analysis

LSD, DMT, psilocin, and mescaline all bound to the receptor in an energetically favorable pocket that mirrors that of risperidone. The pocket is located near the cell surface but nestled within the cylinder of alpha-helices. A combination of polar and hydrophobic interactions stabilize the ligand, though a search for polar interactions among the most energetically favorable orientations using PyMol revealed the binding to be primarily stabilized by non-polar side chains. Risperdal bound the 5HT2A receptor with the lowest free-energy suggesting it binds more stably than the other tested compounds. The most energetically favorable orientation of risperdal (commercial name for risperidone) bound the pocket near -10 kcal/mol, a greater than 1.5 kcal/mol difference relative to the next most stable binding partner, mescaline.

MDMA and psilocybin were not predicted by the algorithm to dock within the conventional binding pocket. The most favorable clusters for both were liganded to a peripheral portion of the alpha helix cylinder in the extra-cellular space. As psilocybin, the phosphory-

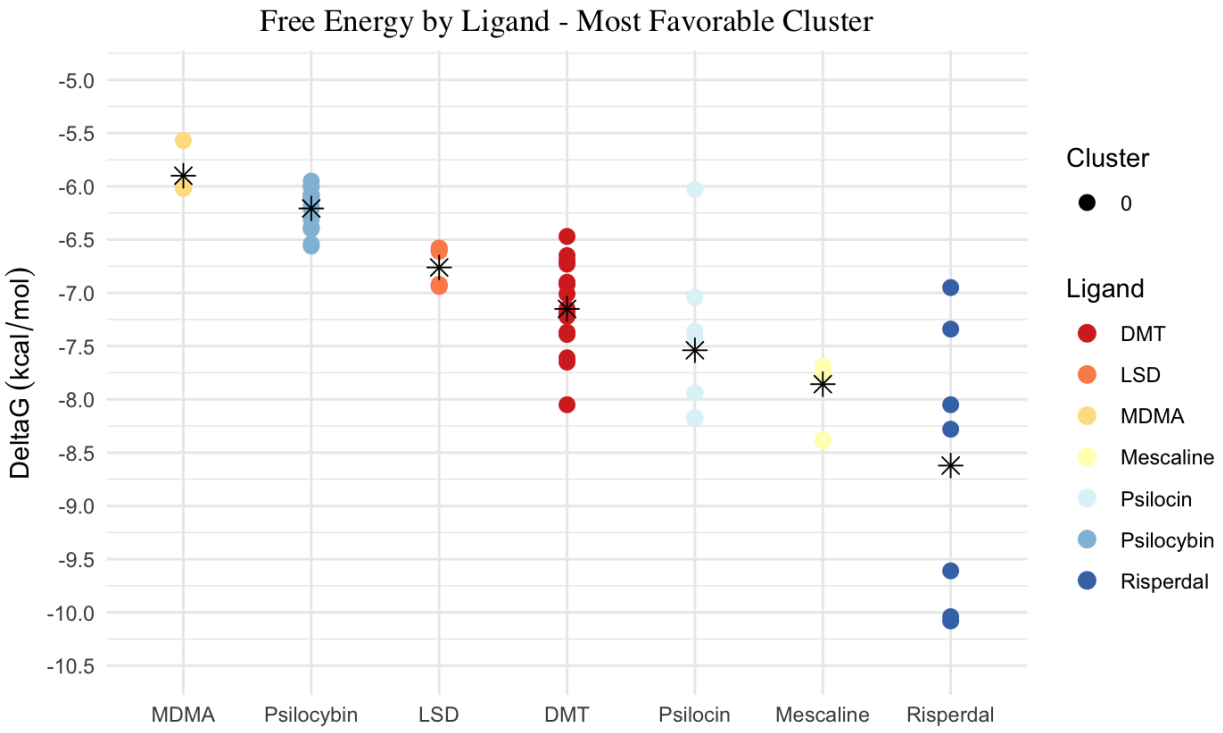


Figure 6: ΔGs by Psychoactive Ligand

lated form of psilocin, does not bind 5HT2A receptors and is hallucinogenically inactive until it has been dephosphorylated, this is a promising negative control. The inability of SwissDock to determine a favorable conformation that fit within the experimentally determined binding pocket suggests the docking algorithm is highly sensitive to the characteristics of the ligand. Note that a phosphoryl group is highly polar and binding may have been destabilized by the large quantity of key hydrophobic residues in the binding pocket.

Beacuse MDMA elicits a psychedelic response similar to that of the other compounds and has been experimentally verified to interact with 5HT2A receptors, we expected this compound to dock in the same pocket as its counterparts. It also shares some structural similarities to mescaline, including a benzene ring, several alkoxy groups, and an amine. Why MDMA did not bind within the pocket remains unclear and is an area of future research, particularly as its interaction with our nervous system receptors is the least well-characterized of the 6 tested compounds.

3.1 Simulated Mutation Analysis

As hypothesized, the L228N mutation stabilized mescaline binding within the binding pocket due to increased polar interactions. The most stable conformation decreased in free energy by slightly more than 1 kcal/mol between the wild-type and L228N simulations, approaching the binding strength of risperidone to the wild-type receptor.

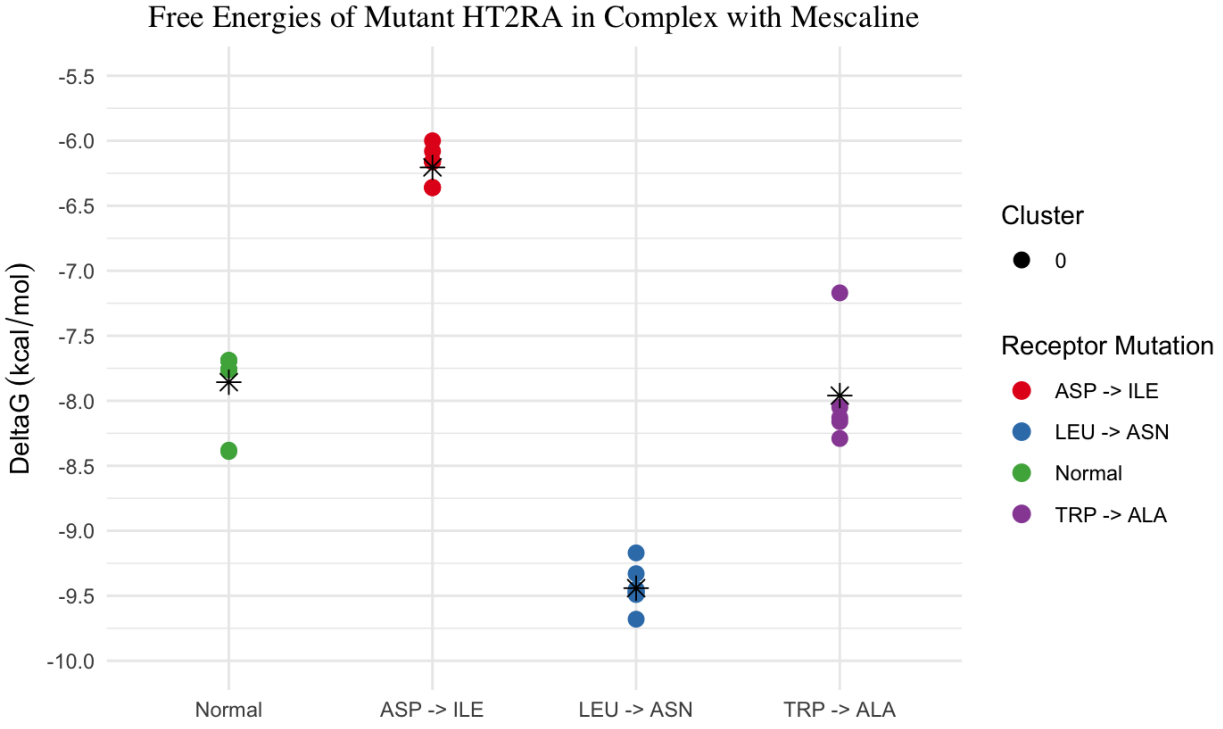


Figure 7: Effects of Induced Mutations on ΔG Binding Energy in Mescaline

In accordance with experimental findings, the D115L mutation greatly destabilized mescaline binding. Due to the aspartic acid's proximity to mescaline's amine group, we suspect a salt-bridge stabilizes the binding interaction in a manner similar to 25CN-NBOH. Removal of that essential interaction prevents binding with strong affinity. The W336A mutation, conversely, had essentially no effect on the free energy of mescaline's binding interaction, unlike what is reported in the literature.

Comparing the the lowest-energy models and cryo-EM-determined structures for psilocin and risperidone confirmed that while SwissDock accurately positioned each ligand within

the binding pocket, the conformation of the molecule was not consistent with experimental results. The lack of synergy with the literature suggests, perhaps, a limitation of SwissDock's algorithm. Cross-referencing using another docking software such as GLIDE would be ideal to validate or disprove these results. Note, however, that ligands may have multiple binding modes, possibly accounting for some of the observed variation [2]

We will also note here the motivation for using free energy values as the basis for our analysis as opposed to a full fitness score, another metric provided by SwissDock. Full fitness scores include the top 30% of favorable clusters in its calculation. As noted above, in certain cases SwissDock predicted unallowable conformation states. This is demonstrated with psilocybin which has a lower total fitness than any other ligand, including risperidone. However, a structural analysis reveals that this statistic is calculated from what appears to be an impossible orientation — with psilocybin liganded outside the HT2A binding pocket. For this reason, we centered our analysis on free energy, however, we included charts with total fitness scores for reference in the appendix.

4 Conclusions

Understanding of the interaction between human seratonin receptors and hallucinogens remains in its infancy, limiting the therapeutic potential of psychoactive substances. This study contributes to our understanding of how hallucinogenic compounds interact with 5HT2A receptors, which are essential to nervous system function. These receptors are therapeutic targets for various anti-psychotic medications, including risperdal. By systematically comparing protein-ligand interactions between risperdal and several common psychoactive molecules, we begin to characterize their biochemical action on the nervous system.

Our mutation analysis highlights the importance of both polar and nonpolar interactions in stabilizing the 5HT2A-ligand bonding. Furthermore, purely from the perspective of binding energies, psilocin and mescaline seem to be the most promising starting candidates for

psychoactive therapeutics.

This study was limited by its computational nature, and experimental techniques should be employed to further examine binding structure of human seratonin receptors to psychoactive ligands. We were also limited by the precision of SwissDock's docking algorithm, which was prone to suggesting physically-unfeasible conformations. More advanced software, such as GLIDE, would be recommended for further computational study.

References

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Appendix

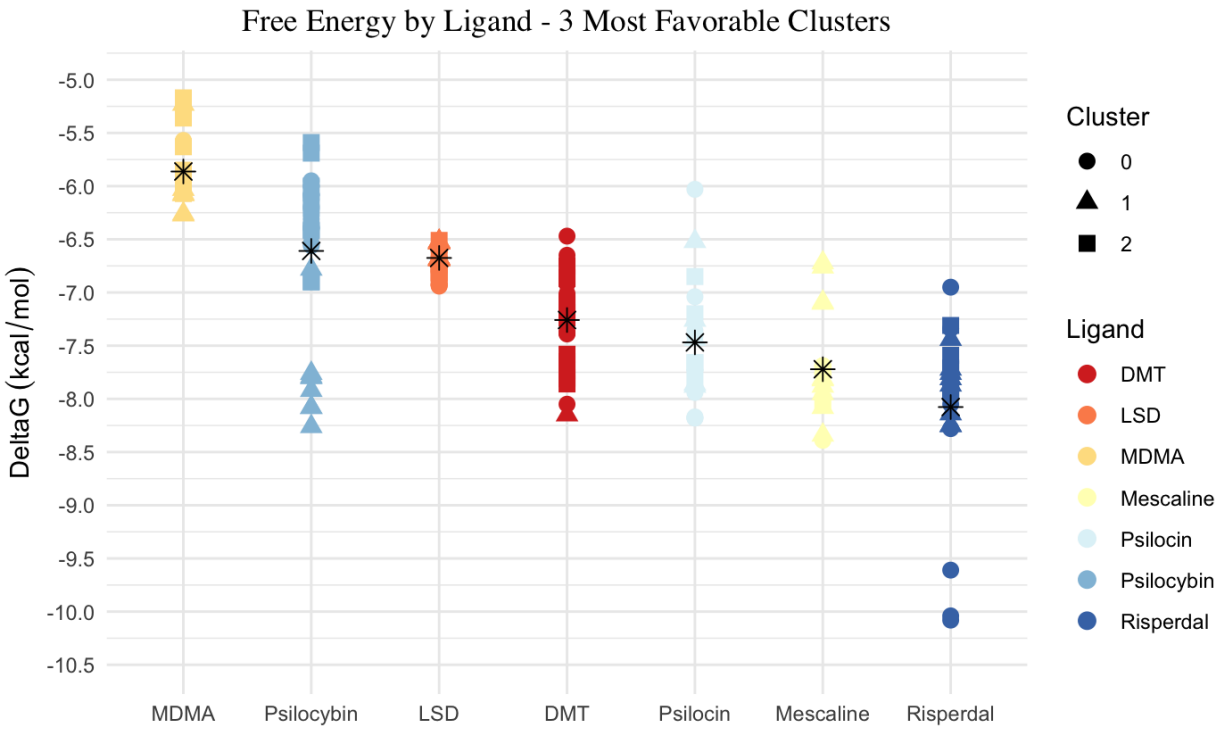

Figure 8: ΔGs by Psychoactive Ligand, Top 3 Clusters Combined

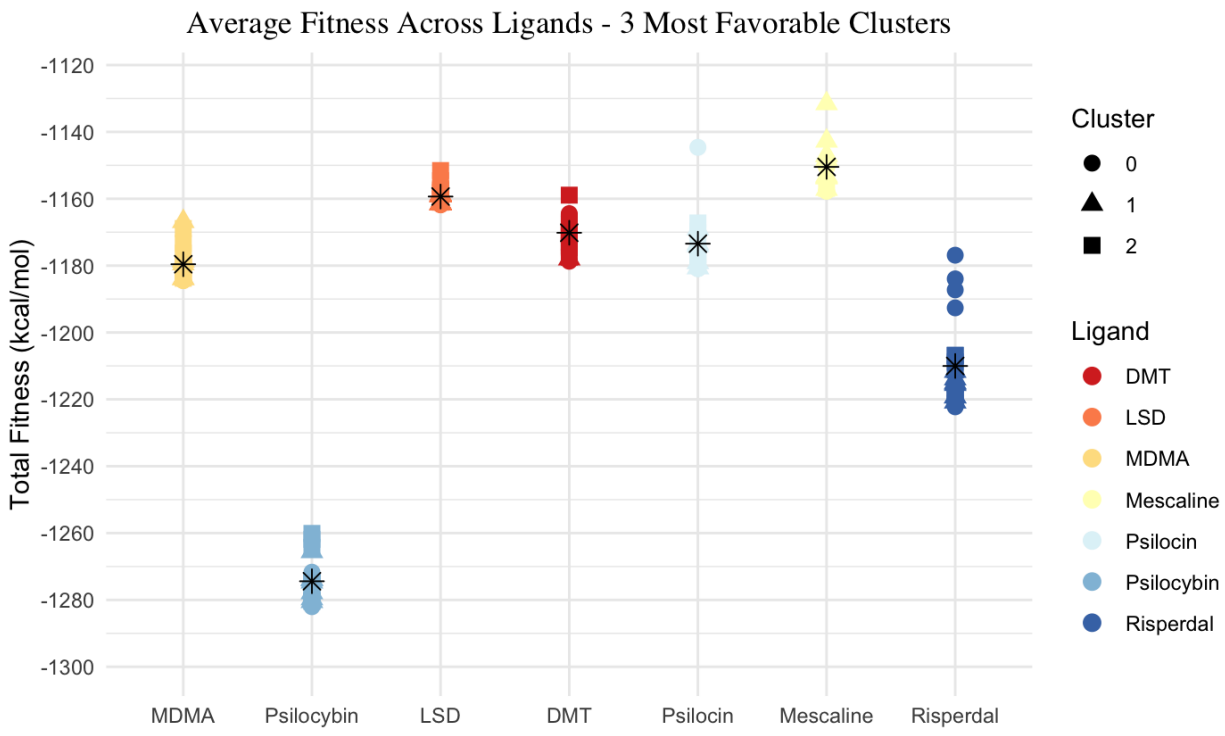

Figure 9: FullFitness by Psychoactive Ligand

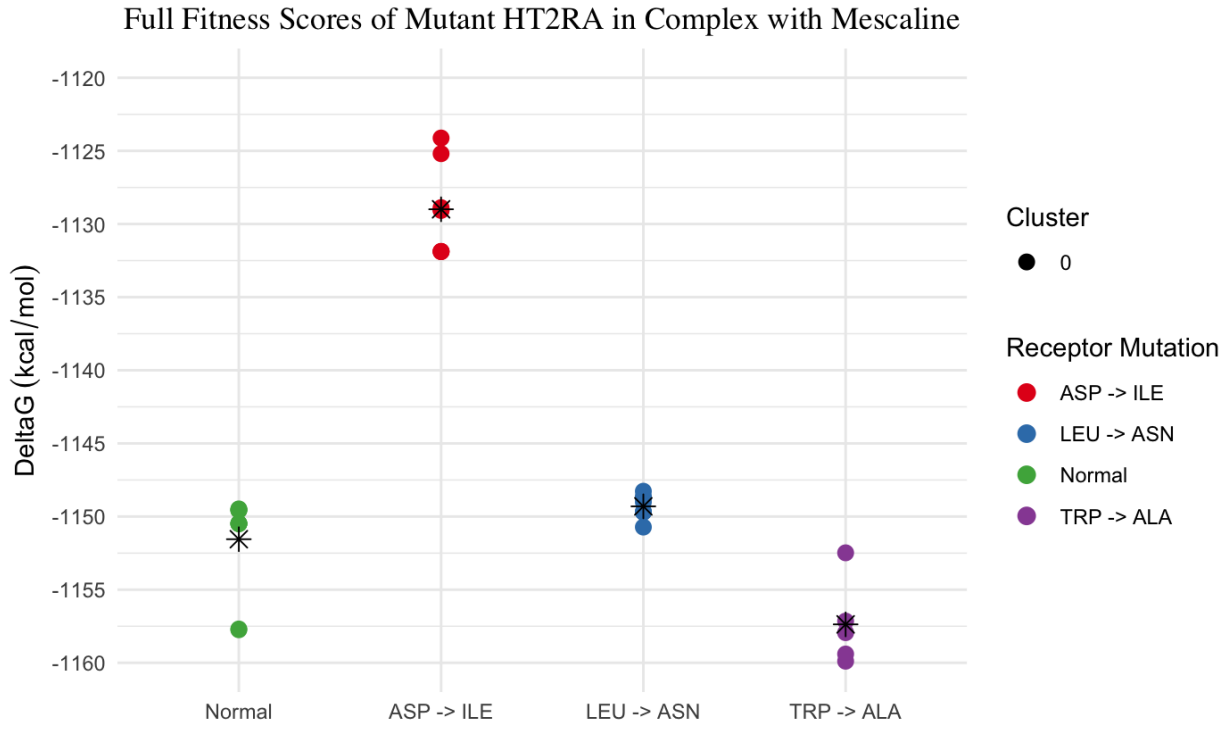

Figure 10: FullFitness Scores for Mutant Receptor