CS279 Final Project **Fall 2024**
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Modeling the Relationship Between Dopamine Receptor Mutations to ADHD

1. Introduction

We aim to investigate how mutation in dopamine (DA) receptors contribute to attention-deficit/hyperactivity disorder (ADHD). DA is monoamine that functions both as a neurotransmitter, and a neuromodulator in the brain.[1] It plays a critical role in modulating neuronal activity, and is involved in various functions such as vigilance, reward processing, and motor control.[1], [2] DA can modulate central vestibular neurons by activating large assemblies of neurons, further influencing states of pleasure.[2] Dysregulation of dopaminergic signaling has been linked to ADHD, but the underlying mechanisms remain unclear.[3]

There are several dopaminergic pathways in the central nervous system, notably the nigrostriatal, mesocorticolimbic, and tuberoinfundibular pathways.[1] Our project we will focus on the mesocorticolimbic pathway, as it "projects to limbic structures involved in the regulation of emotional states."[1] More specifically, we will narrow our scope to the mesolimbic pathway, which is defined as the circuitry of dopaminergic neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (NA), as this is the principle reward pathway.[4] This pathway is particularly relevant because it influences reward/motivation which is implicated in attention-deficit/hyperactivity disorder (ADHD).[5] In the mesolimbic pathway –particularly within the NA– DA is associated with the perception of pleasure and reward, playing a significant role in drug dependence.[6]

DA can bind to two classes of G-protein coupled receptors: D1-like receptors (which include D1 and D5 subtypes) and D2-like receptors (which include D2, D3, and D4 subtypes).[1]

The binding of DA initiates a cascade of stimulating reactions.[1] D1-like receptors are coupled to G proteins ($G\alpha_s$ and $G\alpha_{olf}$) that when activated, promote the secondary messenger cyclic adenosine monophosphate (cAMP), which results in plastic changes in the striatum that form the substrate of reward related learning.[7], [8] D2-like receptors are coupled to different G proteins ($G\alpha_i$ and $G\alpha_o$) that when activated, inhibit cAMP formation which can indirectly regulate the release of DA in the NA, controlling the level of reward felt relative to stimulation.[8], [9] As a result, we will focus primarily on modeling the relationship between DA and dopamine receptor mutations.

By creating computational models of DA receptor mutations and simulating their effects on DA binding affinities within the mesolimbic pathway, we aim to understand how genetic variations may contribute to ADHD symptoms. This research could enhance our understanding of the molecular mechanisms of ADHD and potentially inform the development of targeted therapies.

2. Background

ADHD affects approximately 7 million (11.4%) U.S children, according to a national survey of parents in 2022.[10] The most common form of ADHD treatment (53.6% of ADHD patients) is the use of stimulant medications, such as Adderall, Ritalin or Concerta.[10], [11] Due to the widespread consumption of prescription stimulants, there is a shortage of Adderall, which can be associated with an overdiagnosis of ADHD in children.[11], [12] This shortage impacts individuals who rely on Adderall for symptom management and are now unable to refill their prescriptions.[11] Ultimately, underscoring the importance of establishing an objective way to measure ADHD, for example linking ADHD to measurable genetic mutations.

Genetic research has explored DA metabolism and enzymatic breakdown, focusing on genes that encode enzymes such as catechol-O-methyltransferase (COMT) and methylenetetrahydrofolate reductase (MTHFR) which are involved in the breakdown of DA.[13],[14] However, it remains unclear how mutations to these enzymes relate directly to ADHD.[14] Molecular genetic studies have also identified associations between ADHD and specific genes like the DA transporter gene (SLC6A3) and the dopamine D4 receptor gene (DRD4).[15] Further investigation into receptor mutations is important to determine DA's significance in ADHD pathology. As such, we will model DA docking into several DA receptor mutants and compare their function to wild type receptors.

3. Methods

To determine the binding affinities of DA to the mutants that have a suspected role in ADHD, we used DiffDock, a software that simulates docking between ligands and target proteins, in order to return a binding affinity output. For context, we experimented with AutoDock4 and AutoGrid4 but experienced several errors. Specifically, we were able to produce PDBQT files for our ligand and receptor, yet we were not able to get the GUI to produce docking. Ultimately following TA guidance, we settled on using DiffDock to model the dopamine receptor interaction. In accordance with the leading studies on ADHD and dopamine receptor mutations, we investigated all D2-like receptors. We excluded the D1-like receptors, as the literature suggests these mutations to be more ambiguous in their contribution to ADHD, and we wanted to hone in on the physiological response of controlling the level of reward felt relative to stimulation.[8], [9]

We analyzed 3 mutants in the 3 potential D2-like receptors. For the DRD2 mutant, we analyzed a taq1 polymorphism mutation which is caused by a single nucleotide polymorphism (SNP), which converts a methionine to a serine at amino acid position 281 in the DRD2.[16] For the DRD3 mutant, we analyzed a Gly9Ser polymorphism which is commonly associated with ADHD.[15] Finally for the DRD4 mutant, we analyzed the 7-repeat (7R) allele of the Variable Number Tandem Repeat (VNTR). The 7R VNTR mutant is likely the most structurally disruptive, and as such has also been heavily associated with ADHD.[17]

Table 1: Mutations used in AlphaFold

Wild Type	**Mutation Used**
DRD2	281 Methionine > Serine
DRD3	9 Glycine > Serine
DRD4	7-repeat allele of the VNTR

Since these mutants are not available on the PDB, we had to predict the structures. Using the method outlined in assignment 2, we obtained the protein sequence through UniProt, and modeled the protein structure on *Alphafold3* using GoogleCollab. In order to accept the entries from AlphaFold3 instead of using the PDB alone, the DiffDock source code was altered to accept our own files. Upon identifying and isolating our mutants, we performed the DiffDock simulation using Google Collab. We used the PDB to model our wild type proteins structures.

DiffDock software predicts a docking site, by predicting the 3D structure of the receptor ligand complex (termed *pose*), and outputs a confidence score for the predicted structure, denoted *c*.[18] Such that $c > 0$, $0 > c > -1.5$, and $-1.5 > c$ indicates high confidence, moderate confidence, and low confidence, respectively.[18] Once a pose has been predicted using DiffDock, another software, GNINA, was used to evaluate the binding affinity (kcal/mol) between the

ligand and receptor. (GNINA software was embedded in the DiffDock Google Collab) GNINA initially provides a *scored affinity* (kcal/mol) for the predicted pose, which reflects the determined ligand-receptor binding.[19] GNINA then further optimized the pose such that the ligand's position minimizes the energy of the conformation, and outputs the *minimized affinity* (kcal/mol).[19] The *minimized affinity* is what we will primarily investigate, as our basis of measuring the ligand's binding strength.[19]

4. Results and Analysis

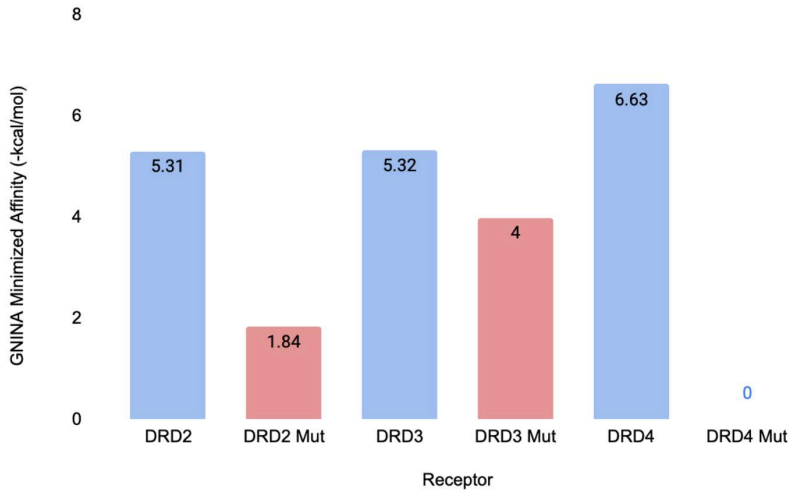
DiffDock uses a diffusion generative model, a type of machine learning model that incrementally adds noise to different poses to determine which is the most optimal solution.[20] The use of diffusion generative models is relatively new, but evidence suggests that it is far more efficient and accurate than traditional molecular docking based prediction models.[20] There are some benefits of using molecular docking approaches to predictions since they are able to capture very weak forces and can even account for other phenomena that affect docking, such as induced fit.[20] However, the trade off of capturing weak forces is a slower prediction.[20] We chose to use DiffDock largely because we were having problems with AutoDock, but also because DiffDock's prediction system is faster and less computationally expensive than AutoDock's molecular docking approach.

DiffDock simulations were performed for the 3 pairs of wild-type and mutant DA and dopamine receptor binding interactions. The completed simulations were then processed using the GNINA software outputting the *scored* and *minimized affinities*. A full table of the results with accompanying graph is shown below.

Table 2: Receptors and respective mutants DiffDock results.

Receptor	PDB ID/rs ID	DiffDock Confidence	GNINA Scored Affinity	GNINA Minimized Affinity
DRD2	6VMS	0.46	-4.04	-5.31
DRD2 Mut	rs1800497	-0.53	-1.11	-1.84
DRD3	3PBL	-0.03	-2.29	-5.32
DRD3 Mut	rs6280	-0.12	-2.53	-4
DRD4	5WIU	-0.01	-0.83	-6.63
*DRD4 Mut**	rs1800955	-4.81*	0	0
*no binding occurred and low DiffDock confidence				

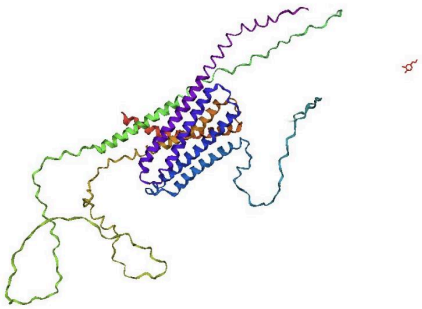
Figure 1: Magnitude of minimized affinity comparison across receptors and respective mutants.



The DRD2 mutant showed a significant drop in binding affinity in the mutated form. The DRD3 mutant showed a slight drop in affinity compared to the wild type. Autodock predicted no binding between DA and the DRD4 mutant.. The DRD4 mutant was unable to bind and had a confidence rating below the -1.5 threshold. We repeated this trial multiple times, and came to the same result. We concluded that this is likely due to the severity of the 7R VNTR mutation and

the large changes in its structure compared to wild type. A figure illustrating this failed binding is below, and the dopamine molecule is in free space on the right hand side of the image.

Figure 2: DRD4 mutant failed docking. DA is in red on the right.



These results align with our initial hypothesis that the mutants selected would show a lower affinity with dopamine. Since ADHD is associated with reduced dopamine activity and signalling, and the mutants have been reported to be associated with ADHD: a lower affinity for the mutants could explain ADHD symptoms. It also is reasonable that the DRD4 7R VNTR mutant was by far the most affected compared to the DRD4 wild type, since its mutation was not an SNP but a large repeat of subsequence. This much larger mutation likely caused a more severe configuration change then the SNPs which is why DiffDock and GNINA reported that dopamine was not able to bind to the 7R VNTR receptor. Interestingly, the literature does not report that there is a complete loss of function with this allele, calling into question the accuracy of DiffDock models for non-SNP mutants.

Throughout this experiment, we got more experience with the PDB for research purposes, as well as structure prediction through *AlphaFold3*. Given that ligand docking software has yet to be tested in this course, we felt an urge to get exposure to it through our project and learned a

lot from the process. Up until recently, understanding a condition like ADHD required imaging, genetic testing, drug response testing, etc.[22] Now, we can computationally model dopamine receptors and gain some basic insights and predictions that can improve the speed of research. The next steps for our project would be to dive deeper into all potential mutations and write a scorer function that can indicate an objective probabilistic measure of how likely an individual given a mutation will develop ADHD. We can further this research by testing our scorer function with real patients, establishing an objective metric for ADHD that is founded on genetic code rather than the subjectivity of a psychiatrist's questions. Additionally, it would be interesting to validate the computational data with wet lab models and experiments that can confirm whether the bringing affinity data is accurate, through calculating dissociation constants.

5. Contributions

For this assignment, we started collaboration right away. We had numerous brainstorming sessions as a group to find an idea everyone was interested in that also seemed attainable given our limited prior knowledge in computational biology. After this, we individually researched topics over Thanksgiving break, until we came back together over the past week to finalize our investigation. All three of us worked to troubleshoot the different parts of the computation as we ran into various roadblocks. After the computation was completed, we worked together to draft and revise this final product. In conclusion, we all participated in equal amounts for the formulation of this final project.

6. References

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