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Computational Analysis of GPCR Networks in Opioid Use Disorder: From Transcriptomics to Drug Discovery

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ABSTRACT

Introduction: Opioid Use Disorder (OUD) is a chronic neuropsychiatric condition driven by persistent neuroadaptive changes in reward, motivation, and stress-related circuits. While opioid receptors are central to OUD, the broader role of G protein-coupled receptors (GPCRs) in opioid-induced neuroplasticity remains underexplored. This study systematically characterizes GPCR dysregulation in OUD to identify potential pharmacological targets.

Methods: We performed transcriptomic analysis of RNA sequencing (RNA-seq) datasets from addiction-related brain regions, including the dorsolateral prefrontal cortex (DLPFC) and nucleus accumbens (GSE174409), Brodmann area 9 (GSE182321), and central amygdala (GSE194368). A curated set of ~900 GPCR genes was analyzed for differentially expressed genes (DEG), principal component analysis (PCA), and hierarchical clustering. Drug-targetable GPCRs were identified via DrugBank and ChEMBL, and their behavioral and stress-related roles were determined. Protein-protein interaction (PPI) networks were constructed using STRING.

Results: We identified 58 GPCRs consistently dysregulated across brain regions, reflecting common molecular adaptations in OUD. PCA revealed a clear separation between OUD and control groups, indicating distinct receptor remodeling. Hierarchical clustering identified functional subgroups, including drug-targetable GPCRs and orphan GPCRs. Notably, 17 GPCRs—including DRD1, DRD3, DRD4, HTR1A, HTR2A, OXTR, and CNR1—are involved in behavioral regulation and addiction vulnerability. Network analysis highlighted key receptor hubs, suggesting novel therapeutic targets.

Conclusion: The present study provides a receptor-centric framework for drug repurposing and precision medicine in OUD. Integrating transcriptomic and pharmacological data, we highlight GPCRs with translational potential. Further validation through functional assays and single-cell studies is warranted.

Keywords:

Opioid Use Disorder, GPCR, Bioinformatics, RNA-seq, Drug Repurposing, Addiction Therapy.

1. Introduction

OUD is a chronic relapsing condition characterized by compulsive drug-seeking behavior, neurochemical dysregulation, and significant alterations in molecular signaling. Long-term opioid exposure induces profound neuroadaptive changes, particularly within brain circuits governing motivation, reinforcement, and cognitive control, leading to persistent structural and functional impairments. These neurobiological alterations underpin the cycle of addiction, tolerance, dependence, and heightened relapse vulnerability [1,2].

The ongoing opioid epidemic represents a major public health crisis, contributing to

a sharp increase in morbidity and mortality rates worldwide. The rise of synthetic opioids such as fentanyl has exacerbated opioid-related fatalities, with the World Health Organization (WHO) reporting that opioid overdoses account for over 70% of drug-related deaths globally. While research has extensively focused on opioid receptors—particularly the mu-opioid receptor (OPRM1)—growing evidence suggests that addiction-related neuroplasticity extends beyond classical opioid receptor pathways. Increasingly, studies implicate a broader network of GPCRs in mediating the molecular and cellular mechanisms underlying opioid dependence [1,3].

GPCRs constitute the largest family of membrane receptors and play essential roles in neurotransmission, neuromodulation, and synaptic plasticity. Beyond their conventional roles in neurochemical signaling, they act as key modulators of cellular homeostasis and adaptive responses to chronic drug exposure[4]. GPCR-mediated pathways play a crucial role in opioid-induced alterations in dopaminergic, serotonergic, glutamatergic, and neuroinflammatory signaling, reinforcing their involvement in addiction pathophysiology. Despite their pivotal role, the therapeutic potential of GPCRs in OUD remains underexplored. Current pharmacological treatments—including methadone (opioid agonist), buprenorphine (partial opioid agonist), and naltrexone (opioid antagonist)—are effective in managing withdrawal symptoms and cravings. However, these interventions primarily target opioid receptors and fail to reverse the broader neuroadaptive consequences of prolonged opioid exposure, contributing to high relapse rates [5,6].

Given the pharmacological significance of GPCRs, a targeted investigation of their dysregulation in OUD may yield novel therapeutic strategies[7]. One promising approach is drug repurposing (DR), which involves identifying new applications for FDA-approved drugs, significantly reducing the time and cost associated with conventional drug discovery [8,9]. This study aims to identify clinically actionable targets for precision medicine-based interventions by systematically characterizing GPCR expression alterations across functionally relevant brain regions in OUD. These findings may provide a foundation for developing more effective, mechanism-driven therapeutic strategies to improve treatment outcomes for individuals with opioid addiction.

2. Materials and Methods

2.1. Data acquisition and preprocessing

RNA-seq datasets from opioid use disorder (OUD) and control subjects were obtained from publicly available repositories (GSE174409, GSE182321, GSE194368). The demographic and clinical characteristics of the samples analyzed, including the number of cases, gender distribution, and average age (mean ± SD), are presented in Table 1. These samples encompassed four distinct addiction-related brain regions: Brodmann Area 9, dorsolateral prefrontal cortex (DLPFC), nucleus accumbens (NAc), and central amygdala (CeA).

Table 1: Demographic and clinical characteristics of human postmortem brain samples analyzed in this study.

Brain Section		**Brodmann Area 9**		**Dorsolateral Prefrontal Cortex**		**Nucleus Accumbens**		**Central Amygdala**	
GEO accession		GSE182321		GSE174409		GSE174409		GSE194368	
	Gender	female	Male	female	Male	female	Male	female	Male
Number of Case	OUD	7	20	10	10	10	10	10	11
		27		20		20		21	
	Control	7	7	10	10	10	10	10	11
		14		20		20		21	
Average of Age	OUD	45.3±4.2		46.9±7.1		46.9±7.1		43.6±2.3	
	Control	46.7±5.1		47.3±9.2		47.3±9.3		50.2±6.1	

Raw sequencing reads underwent initial quality assessment using FastQC to detect sequencing artifacts, adapter contamination, and base quality distribution. Adapter sequences and low-quality bases were removed using Trim Galore! to ensure high-quality read alignment. Cleaned reads were aligned to the GRCh38 human genome reference using HISAT2, a fast and sensitive spliced aligner optimized for RNA-seq data. Transcript assembly and quantification of gene expression levels were subsequently carried out using StringTie [10]. For focused analysis, the data were filtered to retain only G protein-coupled receptor (GPCR) genes, totaling approximately 900 genes.

2.2. Principal component analysis (PCA)

PCA was conducted to explore the variance in GPCR expression between OUD and control samples across the different brain regions. This dimensionality reduction approach enabled visualization of global expression patterns and sample clustering, thereby identifying group-specific molecular profiles.

2.3. Differential expression (DEG) analysis

Normalization and statistical testing were conducted using the DESeq2 package. Differentially expressed genes (DEGs) were identified based on an adjusted p-value threshold of < 0.05 and an absolute log2 fold change ($|\log2FC| > 0.5$ (~1.4-fold). This moderate cutoff was chosen to capture subtle expression changes that are nonetheless biologically meaningful, especially for receptors whose signaling output can be amplified. Even relatively small mRNA changes can have significant functional consequences. In the context of GPCRs, minor alterations in receptor abundance may markedly impact downstream signaling cascades due to signal amplification in G protein-coupled pathways. Our cutoff is consistent with prior transcriptomic studies in the brain that employed a similar log2FC threshold [11]. Volcano plots were generated to visualize significantly upregulated and downregulated GPCRs, enabling rapid identification of transcriptionally altered receptors.

2.4. Hierarchical clustering and functional categorization

To identify co-regulated receptors, hierarchical clustering, and heatmap visualization were performed using Ward's method and Euclidean distance metrics. Dysregulated GPCRs were subjected to functional enrichment analysis using Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases. Based on literature mining and pharmacological databases (DrugBank and ChEMBL), the dysregulated GPCRs were classified into three major categories: (1) FDA-approved drug targets, (2) Orphan GPCRs with no known endogenous or synthetic ligands, and (3) GPCRs previously implicated in stress-related and neuropsychiatric processes.

3. Results

3.1. PCA reveals region-specific GPCR dysregulation in OUD

To investigate the transcriptional alterations in GPCR expression associated with OUD, we performed PCA on differentially expressed GPCRs across four addiction-relevant brain regions. Unlike global transcriptomic profiling, our focused analysis exclusively targeted GPCR-encoding genes, enhancing sensitivity to addiction-related molecular changes while reducing noise from unrelated genes. PCA visualizations revealed distinct clustering patterns between OUD and control samples, indicative of region-specific receptor remodeling (Figure 1). In Brodmann Area 9 (PC1: 24%, PC2: 19%), OUD samples segregated clearly from controls, suggesting significant alterations in GPCRs involved in cognitive flexibility and executive function—domains frequently impaired in addiction (Figure 1-A). In the dorsolateral prefrontal cortex (PC1: 23%, PC2: 9%), a strong separation between groups was observed, reflecting extensive GPCR-mediated neuroadaptations related to

impulse control and decision-making (Figure 1-B). The nucleus accumbens (PC1: 24%, PC2: 15%) exhibited transcriptional divergence in GPCR expression patterns linked to reward processing and reinforcement (Figure 1-C), while the central amygdala (PC1: 28%, PC2: 14%) showed pronounced GPCR dysregulation within emotion and stress circuitry (Figure 1-D), implicating these receptors in withdrawal and relapse vulnerability. Collectively, these findings reveal robust region-specific GPCR expression changes in OUD, underscoring their potential as spatially targeted pharmacological nodes for therapeutic intervention.

To ensure that our PCA analysis did not overlook biologically significant variance in higher-order components, we systematically evaluated additional principal components beyond PC1 and PC2. Higher-order components (PC3 and above) explained relatively minor proportions of variance and did not correlate clearly with known biological characteristics or phenotypic distinctions between OUD and control samples. These components were primarily associated with technical or residual noise rather than biologically meaningful signals, a phenomenon commonly observed in similar transcriptomic analyses of neuropsychiatric disorders [12,13]. Consequently, we determined that focusing on the first two principal components sufficiently captured the critical biological variance related to GPCR dysregulation in OUD.

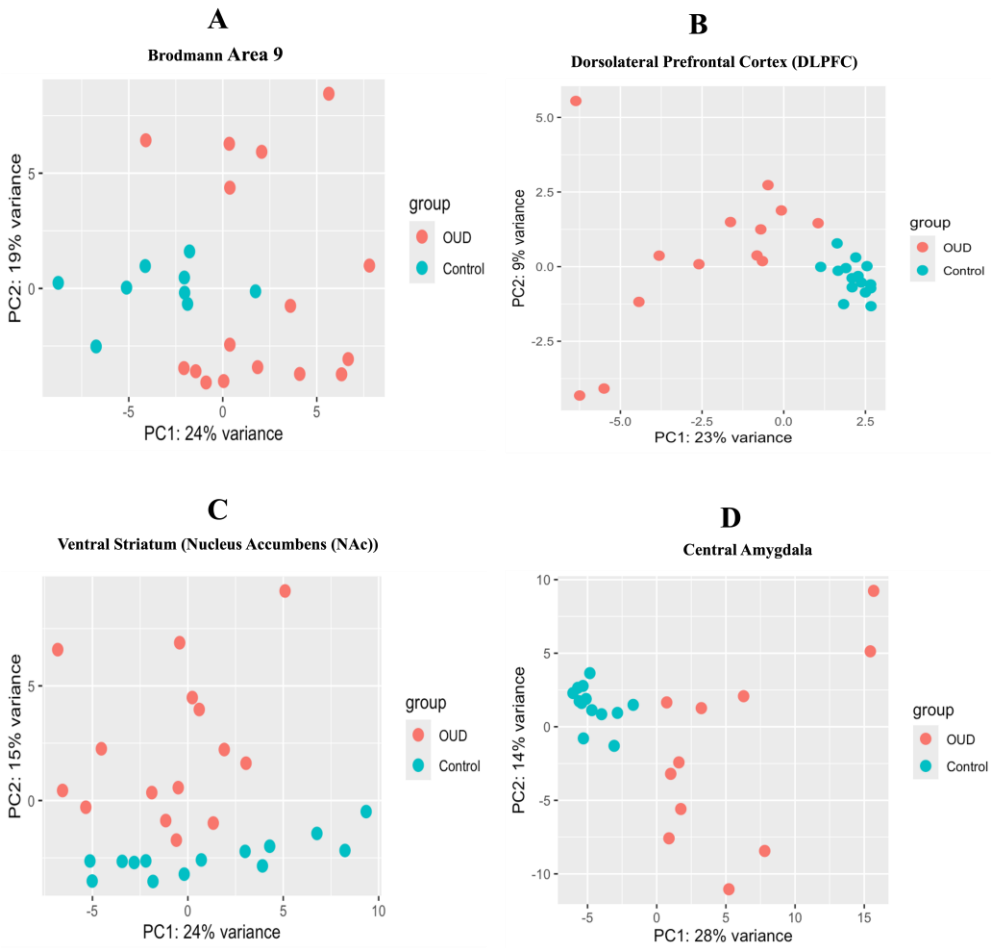


Figure 1: PCA of GPCR expression reveals region-specific neuroadaptations in OUD. This figure presents PCA plots illustrating the variance in GPCR expression across four distinct brain regions affected by OUD: **A)** Brodmann Area 9**, B)** DLPFC, **C)** Nucleus Accumbens, and **D)** Central Amygdala. Each point represents an individual sample, with red indicating OUD cases and blue representing controls. The clear separation between OUD and control groups across all regions highlights significant receptor-specific transcriptional alterations associated with opioid exposure. These findings emphasize the critical role of GPCR genes dysregulation in region-specific neuroadaptations and reinforce their potential as pharmacological targets for addiction treatment.

3.2. Differentially expressed GPCRs reveal receptor-specific adaptations in OUD

To delineate the receptor-level molecular changes associated with OUD, we performed differential expression analysis using DESeq2, focusing exclusively on GPCR-encoding genes across four addiction-relevant brain regions. The resulting volcano plots (Figure 2) revealed numerous GPCRs with significant expression changes, applying a threshold of adjusted p-value < 0.05 and |log2FoldChange| > 0.5. Among the upregulated receptors, several were implicated in dopaminergic, serotonergic, and glutamatergic neurotransmission, consistent with known addiction-related synaptic plasticity and neuroadaptations. Importantly, the key GPCR expression changes we report were also evident under a more stringent cutoff (|log2FC| > 1), confirming that our findings are robust to the choice of fold-change threshold. These alterations may reflect enhanced reward signaling and reinforcement processes driven by opioid exposure. In contrast,

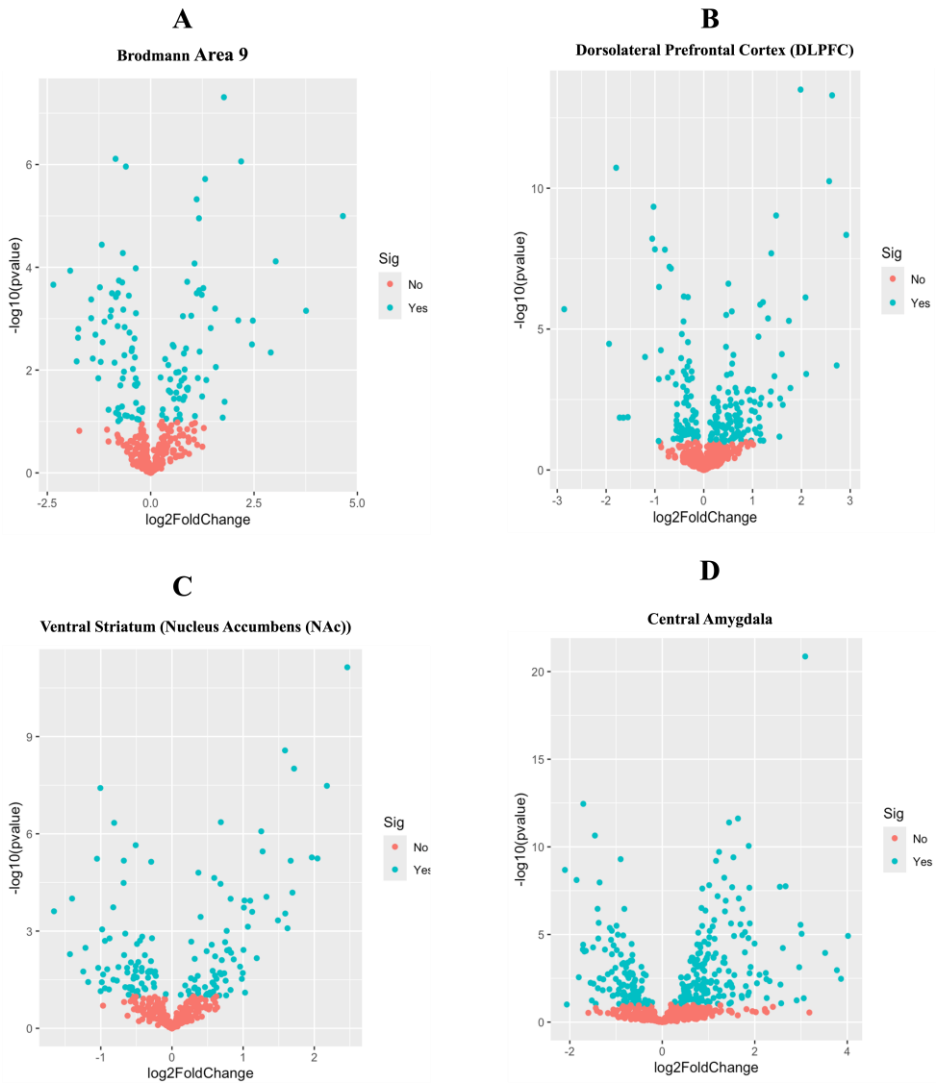


Figure 2: Volcano plot analysis of differentially expressed GPCRs in OUD across brain regions. This figure presents volcano plots depicting the differential gene expression profiles of GPCRs in OUD across four distinct brain regions: A) Brodmann Area 9, B) DLPFC, C) Nucleus Accumbens, and D) Central Amygdala. Each dot represents an individual GPCR, with significantly upregulated genes (p-adjusted < 0.05, |log2FoldChange| > 0.5) shown in blue and non-significant genes in red. The transcriptional shifts observed across brain regions highlight the region-specific dysregulation of GPCRs, reinforcing their potential as molecular targets for pharmacological intervention in opioid addiction. These findings underscore the importance of GPCR signaling in OUD pathology and emphasize their relevance for precision medicine approaches in addiction treatment.

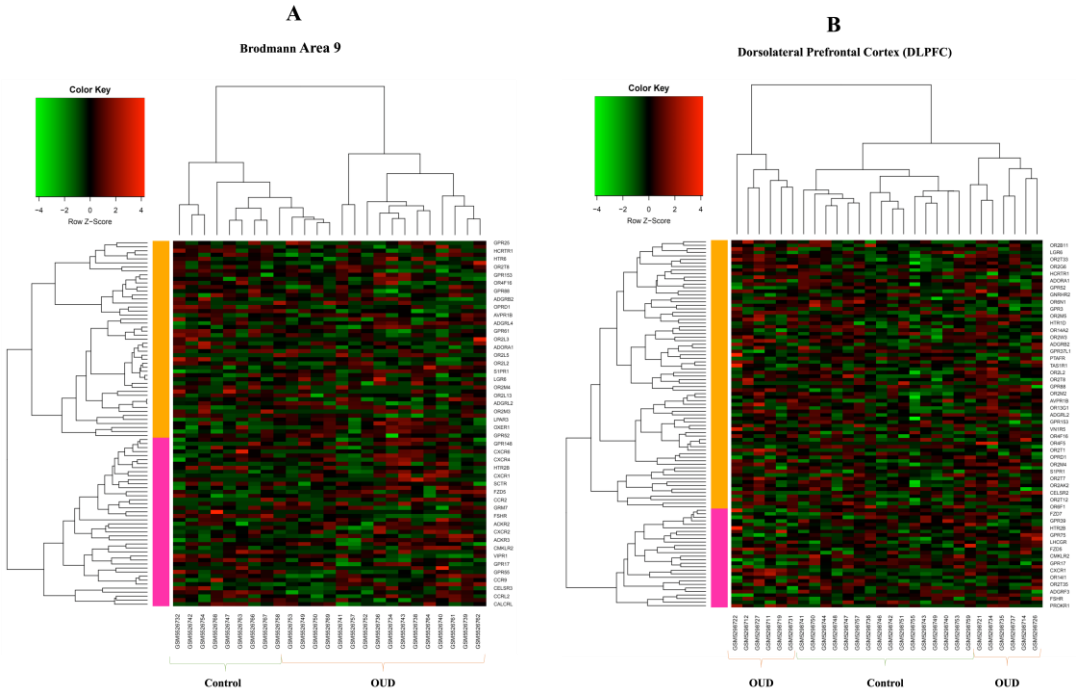
several GPCRs associated with inhibitory neurotransmission and cognitive regulation, such as adenosine and cannabinoid receptors, were downregulated, potentially contributing to impaired executive function, loss of impulse control, and increased vulnerability to relapse.

By isolating GPCRs from the broader transcriptome, our analysis offers a targeted lens to examine receptor-specific dysfunctions in OUD. The consistent dysregulation patterns observed across multiple brain regions not only underscore the central role of GPCRs in addiction neurobiology but also provide a prioritized list of candidate targets for pharmacological modulation. Future studies employing functional assays and cell-type-specific analyses will be essential to validate the mechanistic relevance of these findings.

3.3. Heatmap construction and clustering strategy

To further dissect the transcriptional dysregulation of GPCRs in OUD, we performed hierarchical clustering on differentially expressed GPCRs using heatmap visualization (Figure 3). This approach enabled us to identify functionally related clusters and determine which receptor families exhibit coordinated expression patterns across OUD-affected brain regions. Z-score normalization was applied to standardize gene expression levels across samples. GPCRs were clustered using Ward's method with Euclidean distance as the metric, which grouped genes based on similarity in their expression profiles. Separate heatmaps were constructed for each brain region, including Brodmann Area 9, Dorsolateral Prefrontal Cortex, Nucleus Accumbens, and Central Amygdala. This region-specific approach highlights the functional specificity of GPCR alterations, reinforcing their potential as circuit-selective pharmacological targets in addiction therapy.

To refine our understanding of GPCR dysregulation in OUD, we focused on identifying GPCRs that exhibited differential expression across multiple brain regions. Using a comparative approach, we extracted the set of GPCRs that were consistently dysregulated across the three datasets analyzed (GSE174409, GSE182321, and GSE194368). The intersection of these datasets was visualized using a Venn diagram (Figure 4), highlighting the GPCRs that showed overlapping transcriptional changes across the Brodmann area 9, dorsolateral prefrontal cortex, nucleus accumbens, and central amygdala.



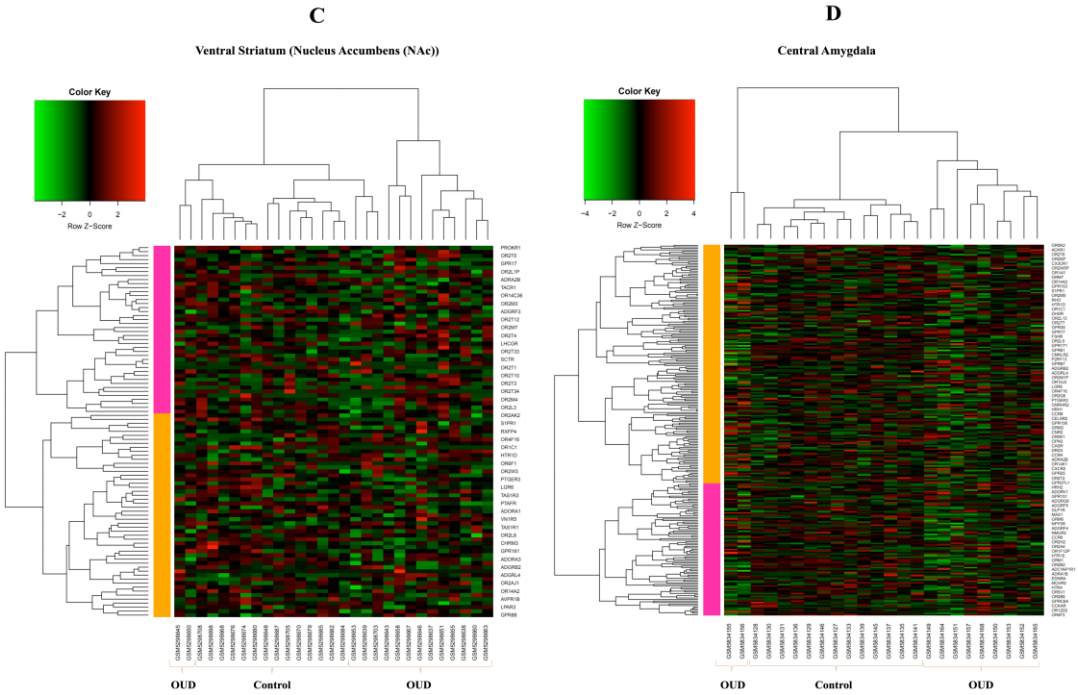


Figure 3: Region-specific GPCR dysregulation in OUD revealed by hierarchical clustering. This figure presents heatmaps illustrating the hierarchical clustering of differentially expressed GPCRs in OUD across four distinct brain regions: **A)** Brodmann Area 9, **B)** DLPFC, **C)** Nucleus Accumbens, and **D)** Central Amygdala. The analysis was performed using Ward's method with Euclidean distance to classify GPCRs based on their transcriptional profiles. Each row represents an individual GPCR, and each column corresponds to a biological sample. The color gradient reflects row-wise Z-score normalization, where red indicates upregulated GPCRs, and green represents downregulated GPCRs. Distinct clustering patterns highlight region-specific GPCR transcriptional alterations, suggesting their involvement in neuroadaptive mechanisms underlying OUD. These findings emphasize the therapeutic potential of targeting GPCR-mediated signaling pathways for pharmacological intervention in opioid addiction.

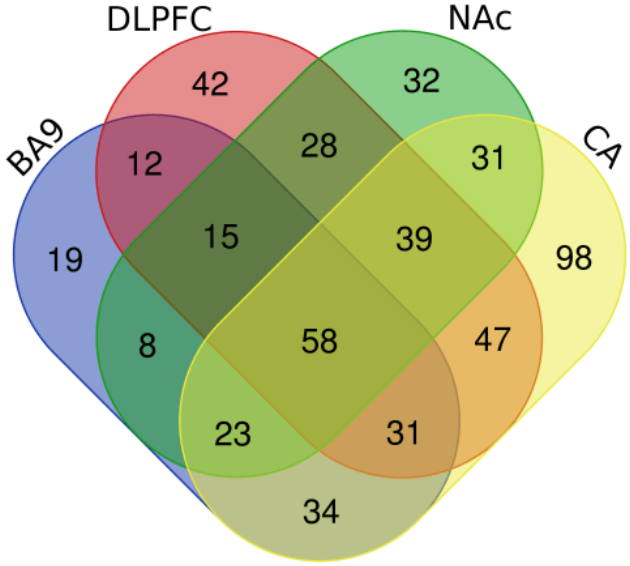


Figure 4: Identification of shared and region-specific GPCR genes dysregulation in OUD. This Venn diagram represents the overlap of differentially expressed GPCRs across four distinct brain regions associated with OUD: Brodmann Area 9 (BA9, blue), DLPFC (red), Nucleus Accumbens (NAc, green), and Central Amygdala (CA, yellow). A total of 58 GPCRs were consistently dysregulated across all regions, representing core molecular mediators of opioid-induced neuroadaptations. The diagram further highlights unique and overlapping receptor sets across brain regions, revealing both widespread and circuit-specific transcriptional alterations. These findings help prioritize candidate GPCRs for future functional validation and therapeutic targeting in addiction treatment.

This intersectional analysis enabled us to prioritize GPCRs that may play a central role in opioid-induced neuroadaptations, as their consistent dysregulation across multiple brain regions suggests a broader functional involvement in OUD pathophysiology. By narrowing our focus to these shared GPCRs, we aimed to enhance the specificity of our findings and identify the most relevant receptor targets for further pharmacological investigation. Subsequent analyses concentrated on these common GPCRs, assessing their functional interactions, ligand availability, and potential as therapeutic targets.

3.4. Functional categorization of dysregulated GPCRs

The hierarchical clustering of differentially expressed GPCRs in OUD-affected brain regions has identified key receptor targets with potential therapeutic implications. These findings reinforce a receptor-centered approach to addiction pharmacotherapy, which could enhance precision medicine strategies for opioid addiction treatment. To refine our analysis and identify the most relevant GPCR targets, we focused exclusively on the 58 GPCRs that were commonly dysregulated across all three datasets (Figure 4). By prioritizing these shared receptors, we aimed to uncover molecular targets that exhibit consistent transcriptional alterations in multiple brain regions implicated in opioid addiction.

We systematically curated a list of GPCRs with known drugs or ongoing drug development using DrugBank and ChEMBL. The results, summarized in Table 2, highlight receptors that present immediate opportunities for drug repurposing in OUD treatment.

3.5. Protein-protein interaction network of dysregulated GPCRs in OUD

To refine our understanding of the molecular mechanisms underlying opioid addiction, we constructed a PPI network focusing on the 62 GPCRs that were consistently differentially expressed across all examined brain regions in OUD. From this subset, we identified 40 GPCRs with known pharmacological ligands or approved drugs, as curated from DrugBank and other pharmacological databases (Table 2). This selection prioritizes receptors that could serve as viable therapeutic targets for drug repurposing in addiction treatment.

To further dissect the functional role of these GPCRs, we performed a network-based clustering analysis (Figure 5). Panel A of Figure 5 illustrates the global interaction map of drug-targetable GPCRs in OUD, constructed using STRING database analysis. This network highlights key receptor hubs such as CNR2, ADORA2A, CXCR4, and DRD3, which exhibit high connectivity and may serve as critical regulators of addiction-related neuroadaptations. These GPCRs, involved in neurotransmitter signaling, neuroinflammation, and synaptic plasticity, provide potential pharmacological targets for mitigating opioid-induced neural dysfunction. Given the established involvement of some of these GPCRs in behavioral, psychological, and stress-related pathways, we extracted a behaviorally relevant subnetwork from the primary interaction map. Panel B of Figure 5 represents this filtered subset, comprising 17 GPCRs that have been extensively documented in mood regulation, cognitive function, stress adaptation, and reward processing. This subset includes major dopaminergic receptors (DRD1, DRD3, DRD4, DRD5), serotonergic receptors (HTR1A, HTR2A, HTR2C), oxytocin receptor (OXTR), cannabinoid receptors (CNR1, CNR2), and adrenergic receptors (ADRB1, ADRB2). The presence of distinct subclusters within this network suggests an underlying regulatory framework through which these receptors collectively influence addiction-related behavioral and psychiatric outcomes.

The network analysis underscores the translational potential of targeting GPCR hubs that are both pharmacologically actionable and behaviorally significant. These findings reinforce the need for a precision medicine approach, leveraging existing drugs targeting GPCRs involved in stress regulation, mood disorders, and cognitive dysfunction as potential adjunct therapies for opioid addiction.

Table 2: GPCRs with known drugs or in drug development

GPCR Gene	Drug	Indications
ADORA1	Caffeine	CNS Stimulant
ADORA2A	Regadenoson	Myocardial perfusion imaging
ADORA2B	Caffeine	CNS Stimulant
ADRA2C	Clonidine	Hypertension, ADHD
ADRB1	Metoprolol	Hypertension
ADRB2	Albuterol	Asthma
AVPR2	Desmopressin	Diabetes insipidus
BDKRB2	Icatibant	Hereditary angioedema
C5AR1	Avacopan	Inflammation
CALCR	Calcitonin	Osteoporosis
CCR5	Maraviroc	HIV
CCR7	CCX771	Autoimmune diseases
CHRM2	Scopolamine	Motion sickness
CNR1	Dronabinol	Nausea, appetite stimulation
CNR2	Nabilone	Nausea, appetite stimulation
CXCR1	Reparixin/Ladarixin	Neutrophil chemotaxis inhibitor/Diabetes
CXCR2	Danirixin	COPD
CXCR4	Plerixafor	Cancer metastasis
DRD1	Levodopa	Parkinson's disease
DRD3	Cariprazine	Schizophrenia
DRD4	Pimavanserin	Parkinson’s psychosis
DRD5	Fenoldopam	Hypertension
F2RL1	Vorapaxar	Thrombosis
F2RL3	Ticagrelor	Antiplatelet
GPR17	Pranlukast	Asthma
GPR183	Navarixin	Asthma
GPR35	Fostamatinib	Immune thrombocytopenia
GPR55	Cannabidiol	Epilepsy
GPR84	E6005	Eczema
HTR1A	Buspirone	Anxiety
HTR2A	Risperidone	Schizophrenia
HTR2C	Olanzapine	Bipolar disorder
MC1R	Afamelanotide	Erythropoietic protoporphyria
MC4R	Setmelanotide	Obesity
OXTR	Oxytocin/Carbetocin	Labor induction/Postpartum hemorrhage
P2RY12	Clopidogrel	Antiplatelet therapy
P2RY13	Cangrelor	Antiplatelet therapy
PTGER2	Misoprostol	Gastroprotection
S1PR1	Fingolimod	Multiple sclerosis
S1PR2	Siponimod/Ozanimod	Multiple sclerosis
S1PR4	Siponimod	Multiple sclerosis

4. Discussion

Opioid Use Disorder (OUD) is a chronic, relapsing neuropsychiatric disorder characterized by compulsive opioid use despite severe consequences. Due to its high prevalence, relapse rates, and socioeconomic burden, understanding its molecular mechanisms is essential. Although classical opioid receptors (OPRM1, OPRK1, OPRD1) have been extensively studied, they do not fully capture the complexity of opioid-induced neuroadaptations [14,15]. Recent research highlights the critical involvement of G protein-coupled receptors (GPCRs), the largest and most diverse receptor family, in addiction-related pathways such as stress response, neuroimmune activation, and reward circuitry [5,6]. Our study systematically identified significant GPCR gene expression dysregulation across multiple addiction-relevant brain regions, demonstrating extensive involvement beyond classical opioid signaling. Particularly, GPCR pathways implicated in HPA-axis stress mechanisms, neuroinflammation, and reward system dysfunction were markedly altered—pathways that are consistently shared across various substance use disorders [16]. These findings support GPCRs as promising therapeutic targets for precision interventions in OUD [17].

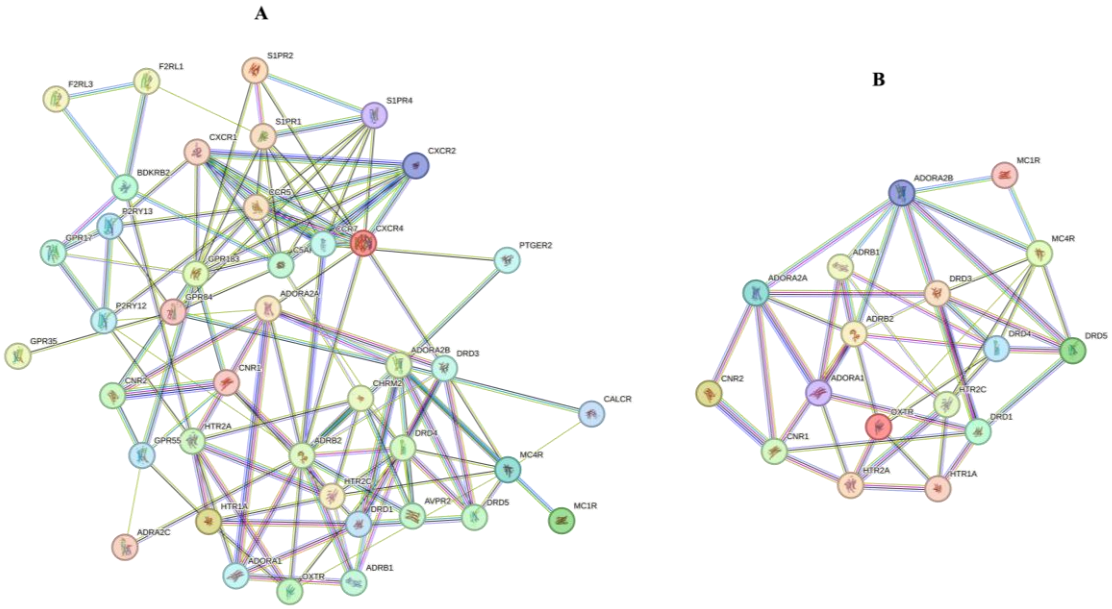


Figure 5: PPI network of GPCRs with known pharmacological ligands and behavioral relevance in OUD. **A**) The global PPI network of 40 GPCRs that have established drug targets, highlighting key hubs such as CNR2, ADORA2A, CXCR4, and DRD3, which may serve as central regulators of addiction-related neuroadaptations. Nodes represent individual GPCRs, and edges indicate known or predicted interactions based on experimental data, co-expression, and curated databases. **B**) A refined behavioral subnetwork extracted from panel A, consisting of 17 GPCRs with documented roles in mood regulation, stress response, cognitive function, and reward processing. Distinct receptor clusters suggest a shared regulatory framework influencing neuropsychiatric aspects of opioid dependence, reinforcing the therapeutic potential of targeting GPCR hubs to modulate addiction-related behavioral outcomes.

In our data, GPCR changes in OUD involve both common addiction-related pathways and opioid-specific neuroadaptations. Notably, neuropeptide systems linked to withdrawal and tolerance were significantly altered, including dysregulation of oxytocin (OXTR), vasopressin, and corticotropin-releasing factor (CRF) receptors in the central amygdala, highlighting opioid-driven activation of stress and neuroendocrine responses [18]. Additionally, suppression of the GnRH pathway—a critical regulator of the hypothalamic-pituitary-gonadal axis—aligns with clinical observations of opioid-induced hypogonadism. These findings emphasize that while OUD shares general mechanisms (stress, immune responses, synaptic plasticity) with other substance use disorders, it also uniquely disrupts opioid peptide and hormonal signaling [5,6]. Differentiating opioid-specific pathways from general drug-induced adaptations is crucial for developing targeted therapeutic interventions.

Our region-specific analysis demonstrates that opioid-induced GPCR gene expression dysregulation affects distinct neural circuits involved in reward, motivation, and stress responses. In frontal cortical areas (dorsolateral prefrontal cortex, Brodmann Area 9), we observed significant upregulation of dopaminergic (DRD1, DRD3, DRD4, DRD5), glutamatergic (GRM2, GRM7), serotonergic (5-HT1A, 5-HT2A), and cannabinoid receptors (CB1, CB2), reflecting known addiction-associated disruptions in cognitive control and mood regulation [19–22]. Similarly, the central amygdala, a critical stress-processing region, showed marked dysregulation of neuropeptide GPCRs (OXTR, AVPR2, CRHR1), aligning with heightened stress reactivity and negative affect during opioid withdrawal [19,23,24]. These patterns underscore that effective OUD interventions must target both shared addiction mechanisms (e.g., stress, neuroimmune responses) and opioid-specific neuroadaptations [25].

A major translational insight is the

identification of druggable GPCR targets, with 40 GPCRs linked to known pharmacological ligands. Notably, a core network of 17 GPCRs emerged as behavioral hubs, including receptors with documented roles in mood, cognition, and reward processing (dopamine D3, serotonin 5-HT1A, cannabinoid CB1/CB2). Several have existing medications suitable for immediate repurposing—such as the anxiolytic buspirone (5-HT1A agonist) and the antipsychotic cariprazine (D3 partial agonist)—highlighting viable therapeutic strategies to mitigate opioid craving, withdrawal anxiety, and relapse risk[24,26]. Another example is the sphingosine-1-phosphate receptor 1 (S1PR1) [26,27], which we found dysregulated and for which the drug fingolimod is available; fingolimod crosses the blood–brain barrier and could modulate neuroinflammatory pathways contributing to opioid-induced neural injury.

The fact that these OUD-linked receptors are already "pharmacologically accessible" – with FDA-approved drugs that reach the central nervous system – is highly advantageous. It means their safety profiles are at least partly characterized and they can be rapidly advanced into clinical trials for OUD, shortening the timeline for potential therapeutic applications. In essence, our receptor-centric network points to a repertoire of medications (from psychiatric and other medical indications) that might be repurposed as adjunct treatments for OUD, targeting dysregulated GPCR systems involved in stress, mood, and cognitive dysfunction.

In assessing the pharmacological potential of these GPCR targets, considerations of brain penetrance, drug safety, and abuse liability are essential. Regarding blood–brain barrier (BBB) penetration, many GPCR-targeting agents like buspirone (5-HT_1A agonist) and cariprazine (D3 partial agonist) readily cross the BBB, supporting their therapeutic applicability in OUD. In contrast, peptides such as oxytocin (OXTR agonist) exhibit poor brain penetration when administered peripherally. However, intranasal oxytocin administration has shown promise in mitigating opioid withdrawal and anxiety, underscoring the potential of innovative delivery systems for peptide-based GPCR therapeutics [28]. In terms of drug safety, identified GPCR targets affect multiple physiological systems, necessitating careful evaluation within the OUD context. Repurposing medications with established safety profiles (e.g., β-blockers, SSRIs, and antipsychotics) provides a strategic advantage by leveraging known tolerability and manageable side effects, thus minimizing risks associated with novel compounds [29]. Evaluating abuse potential is also critical. GPCRs within reward circuits (e.g., CB_1 receptors) pose risks if activated by potent agonists such as tetrahydrocannabinol (THC), highlighting the need for modulatory approaches. Historical experiences with agents like rimonabant (CB_1 antagonist) illustrate psychiatric risks linked to extreme receptor modulation. Therefore, therapies that offer balanced modulation—such as partial agonists (cariprazine) or neuromodulatory agents—are preferred to minimize euphoria and behavioral stimulation. Indeed, established OUD treatments like buprenorphine exemplify how partial receptor modulation effectively manages symptoms with reduced abuse liability [30,31].

Collectively, these considerations inform a cautious yet optimistic approach to developing GPCR-targeted interventions in OUD, emphasizing brain-penetrant medications with proven safety and minimized abuse potential.

Our analysis also identified several orphan GPCRs **(**GPR179, GPR55, PRLHR**)** significantly dysregulated in OUD, which currently has no known ligands or established modulators. These orphan receptors represent intriguing yet challenging targets, potentially involved in novel neuroadaptations linked to opioid dependence [5,6,32,33]. The absence of known ligands limits functional understanding, necessitating foundational

research and dedicated deorphanization efforts—the process of identifying endogenous ligands or pharmacological modulators. Historically, successful deorphanization of receptors such as GPR55 underscores their therapeutic promise upon ligand identification [29]. Given that more than 140 human GPCRs remain orphan, high-throughput and computational approaches combined with reverse pharmacology are crucial to advancing this field [34]. In OUD, orphan GPCRs could modulate currently unexplored dimensions of opioid response (e.g., craving, effect, neuroplasticity), offering selective targets with potentially fewer side effects if their physiological roles and ligands are clarified. We advocate future research to prioritize the deorphanization and functional characterization of these orphan GPCRs, which could open entirely new therapeutic avenues beyond traditional addiction treatments [34].

Finally, we acknowledge the limitations of our study and propose future directions. Our analysis relied on bulk RNA-seq data, providing an averaged view of gene expression without resolving cell-specific changes. Addiction-relevant regions contain heterogeneous neuron (excitatory/inhibitory) and glial (astrocytes, microglia) populations, each potentially responding uniquely to opioids. Recent single-nucleus RNA-seq studies reveal extensive opioid-induced transcriptional changes in glial cells, obscured in bulk analyses [35]. Future studies employing single-cell RNA-seq could clarify GPCR alterations in specific cell types, significantly enhancing our understanding of opioid neuroadaptations. Additionally, integrating spatial transcriptomics or cell-specific proteomics may further elucidate receptor-network dynamics within distinct neural circuits [36]. Another limitation is the observational nature of our study, which precludes establishing causal relationships. Follow-up functional experiments using animal models and in vitro assays are essential. For instance, manipulating chemokine receptors (CXCR4, CCR5) in rodent models could test their causal roles in opioid behaviors. Promisingly, preclinical studies show drugs like maraviroc (a CCR5 antagonist) effectively reduce opioid-related behaviors in animals, supporting our translational approach [37]. Despite these limitations, our integrative approach combining human transcriptomics, network analysis, and pharmacology provides a robust framework for future hypothesis-driven research and targeted therapeutic development.

Clinical Implications and Conclusion: Despite limitations, our study provides a novel, receptor-centric framework with clear clinical implications for opioid use disorder (OUD). We reveal extensive GPCR gene expression dysregulation beyond classical opioid receptors, highlighting receptors with established pharmacological agents, thus facilitating precision medicine strategies. Targeting interconnected neurotransmitters, neuropeptides, and immune GPCR networks may significantly enhance the current standard of care (opioid agonists/antagonists). Moreover, we spotlight innovative biological targets such as orphan GPCRs and immune-related receptors, emphasizing new avenues (e.g., glial GPCR signaling or immune-opioid receptor heterodimers [38]) previously underexplored in addiction research. These findings could significantly expand OUD's therapeutic landscape.

In conclusion, by integrating transcriptomics and pharmacology, our study underscores that OUD involves complex neurochemical networks beyond opioid receptors alone. Targeting this broader GPCR landscape—with careful consideration of specificity, safety, and abuse potential—represents a clinically meaningful and innovative step toward addressing the ongoing opioid crisis.

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Declaration

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