Cell Type-Specific Expression of Molecular Players in Morphine Action

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***Abstract*—Hidden drug abuse has become a concerning issue in Hong Kong, despite an overall decrease in reported cases. The opioid epidemic, fueled by substances like heroin and fentanyl, continues to be a global problem. Understanding the molecular mechanisms of morphine addiction is crucial for developing effective treatments to combat this problem. This study investigates the gene expression patterns of key players in morphine addiction in the Nucleus Accumbens (NAc) region of the mesolimbic dopamine system, a key area in drug-associated reward. The RNA transcripts produced in mice NAc under acute morphine treatment can reveal transcriptional changes that alter the regulation of gene expression related to its molecular mechanisms. By analyzing publicly available single-cell RNA sequencing data, we identified gene expression patterns in specific cell types for genes like BDNF, ΔFosB, CREB, and Npy, all of which were previously studied to be relevant to morphine addiction. The study also highlights novel differentially expressed genes in response to morphine treatment. These findings provide insight into new and relevant genes as well as their cell type-specific expression patterns to glean the pathways that they influence. Understanding these molecular processes can inform personalized pharmaceutical approaches and aid in the development of new therapies.**

***Keywords*—morphine addiction, single-cell RNA sequencing, nucleus accumbens, transcriptomics, cell-type specificity, differential expression analysis**

I. Introduction

Morphine is a potent opioid analgesic drug with a long history of use in managing severe pain. Morphine was first derived from poppy in 1805, making it the ancestor of the opioids used today and therefore a crucial area of investigation for understanding opioid addiction [1]. Worldwide, about 0.5 million deaths are attributable to drug use, of which more than 70% are related to opioids [2]. Despite the overall decreasing trend in reported drug abuse in Hong Kong, a shift towards hidden drug abuse was observed from the age range and drug history of newly reported cases in recent years, constituting the "dark figure" phenomenon [3]. Though it has been almost three decades since the first wave of the opioid epidemic, the severity of the phenomenon has also been extended through new derivatives of opioids, such as heroin and fentanyl.

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The current research on chronic addiction focuses on the pharmacological and neuroadaptive mechanisms in specific neural circuits that underpin the transition from controlled to compulsive drug use. In morphine addiction, the role of the mesolimbic dopamine system is notable for the mediation of drug reward. The mesolimbic dopamine system is comprised of dopaminergic neurons with cell bodies in the Ventral Tegmental Area (VTA) of the midbrain and its projection in the limbic forebrain and the Nucleus Accumbens (NAc) in particular [4]. The activation of mu-opioid receptors in the VTA causes the disinhibition of dopamine neurons, elevating dopamine levels in the NAc. This mechanism appears to result in the rewarding and reinforcing effects of opioids [5]. Prolonged morphine exposure results in transcriptional modifications that influence gene expression patterns, which may play a critical role in linking the rewarding experience of drugs with external and internal cues that trigger craving and relapse, leading to addiction cycle [6]. Fundamentally, the RNA transcripts produced at a specific time under a specific condition can reflect the underlying biological processes. Under the condition of morphine exposure, transcriptional changes alter the regulation of gene expression related to molecular mechanisms, leading to addiction-related consequences. Therefore, the investigation of novel differentially expressed genes is crucial as it reveals the changing variables that are at the core of morphine addiction, thereby highlighting them as potential therapeutic targets.

Since all addictive drugs regulate the mesolimbic pathway of the brain by altering dopamine levels in the NAc, researchers have aimed to elucidate the effects of morphine on gene expression in the NAc. In this investigation, the gene expression patterns of important molecular players were studied, including transcription factors, neuromodulators, protein kinase, glutamate receptor encoding proteins, and Corticotropin-Releasing Hormone (CRH). The different genes each play crucial roles in the molecular processes of morphine addiction. For example, increased levels of transcriptional factors like ΔFosB heighten the sensitivity to the rewarding effects of morphine and decrease sensitivity to its analgesic effects, leading to faster development of physical dependence and analgesic tolerance [7]. Another example would be BDNF, where the removal of the TrkB BDNF receptor on dopamine D1 receptor-containing medium spiny neurons results in reduced GABA-A receptor

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currents in these neurons, and the decreased inhibition ultimately promotes morphine reward [8]. Studying the unique expression patterns of known molecular players and relevant genes can reveal their specific effects in the NAc.

This study seeks to uncover the cell-type specificity of known molecular players and identify the top differentially expressed genes in each characterized cell type of the NAc in response to morphine treatment through bioinformatic analysis of existing scRNA-seq data. A recent study collected scRNA-seq data from the NAc of mice under morphine treatment, providing valuable transcriptional datasets for analysis of molecular players in each different cell type [9]. Investigating the cell-type specificity can corroborate previously reported morphine-related genes that exhibit cell-type specificity in NAc. Additionally, the differentially expressed genes between the control and morphine treatments in each cell type were extracted in search of new genes that may play a role in the cause or process of morphine addiction. By future examining computational analysis of publicly available data sets, we aim to further study the cell type-specific expression patterns of such molecular players and identify top differential expressed genes in each cell type in NAc between control and morphine-treated subjects. The results obtained from the scRNA-seq data analysis are arranged in two sections: cell-type specificity of known molecular players and top differentially expressed genes in each characterized cell type in NAc in response to morphine treatment.

II. Materials and Method

Materials:

(1) The raw single-cell RNA-sequencing data obtained from Gene Omnibus Express database was downloaded from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE118918 and a copy of the dataset was stored in my personal computer for processing.
(2) Personal computer, RStudio software, R Packages (Seurat, Tidyverse, Ggplot, etc.).
(3) Internet access for downloading original datasets and R code sharing. The original codes composed are available below: https://drive.google.com/file/d/1WPS817YxRzHeh1CiuVO2Jo4cPHw5ECE2/view?usp=sharing.
(4) Notebook to keep track of research progress.

Research Procedures:

(1) Retrieving scRNA datasets. Publicly available scRNA seq datasets from mouse NAc samples in saline and morphine treatment were obtained from the GEO database before undergoing pre-processing and downstream analysis. The scRNA datasets retrieved from the NCBI GEO database include 8 matrix datasets, 4 of which were treated with saline, and the other 4 with morphine.
(2) Data preprocessing and cell clustering. Main preprocessing steps include Principal Component Analysis (PCA) and cell clustering, all of which relied primarily on Seurat in RStudio. Specifically, each of the 8 gene expression matrices was merged into 1 SeuratObject, the cells were then filtered and batch effects were removed by specific criteria. 28,976 cells and the top 3000 genes with the highest variability between control and morphine treatment groups were used in PCA and UMAP dimensionality reduction. 15 broad clusters were created using the function FindClusters (Fig. A1(a)), and classified into various cell types based on specific cell markers and clustering outcomes.
(3) Cell type classification. Clusters were identified using the following cell marker genes, Snap25, Drd1a, Adora2a, Resp18, Top2a, Gja1, C1qa, Cldn5, Mog, and Pdgfra. The UMAP dimensional reduction graph was the main focus of the analysis. To refine the clusters, preliminary clusters showing the same marker gene expressions were merged. We generated dot plots for different cell-type specific gene markers, presenting their expression rate and level in each of the 15 clusters in Fig. 1(a). Initially, clusters were annotated based on the expression of known marker genes. Some clusters were identified as double droplets (clusters 13 and 14) and were subsequently excluded from the analysis. By verifying marker gene expressions in each cluster, we categorized them into nine distinct cell types: Astro, Drd1, Drd2, Endo, IN, Micro, NB, Oligo, and OPC.
(4) The expression levels of gene candidates in morphine treated samples v.s. controls were measured in the 9 identified cell clusters. The cell type specific expression pattern of gene candidates in broad cell clusters was visualized through dot plots.
(5) To search for highly differentially expressed genes in each cell type, we identified the marker genes in morphine treated samples v.s. saline control samples. Dot plots were made to show the top 6 differentially expressed gene levels between two groups of samples.

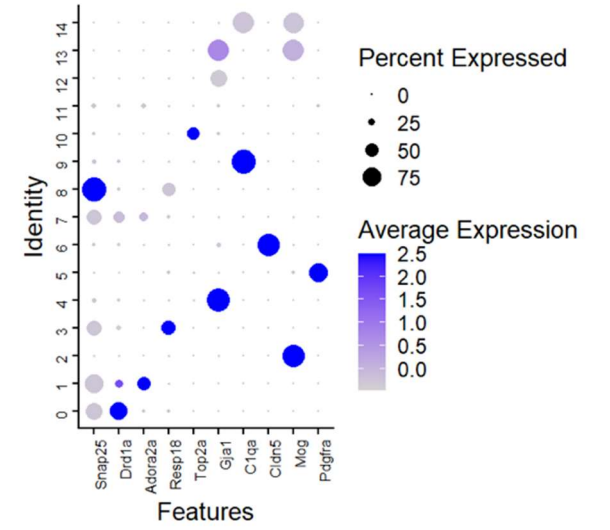
III. Results

A. Cell Type Classification

We utilized cell type-specific markers to categorize our initial cell clusters based on their specific cell types. A dot plot was then created to illustrate the gene expression levels and frequencies for multiple clusters. Each cell type was assigned to its corresponding category, allowing us to observe distinct gene expression patterns within each cluster. This visualization (Fig. 1(a)), clearly demonstrates the validity of our findings, as it showcases nine distinct cell types under the UMAP plot (Fig. 1(b)). We carefully examined the following cell markers: Snap25 (a cell marker of neuronal cells), Drd1a (cell marker of D1 MSN), Drd2a (cell marker of D2 MSN), Adora2a (cell marker of D2 MSN), Resp18 (cell marker of interneurons), Top2a (cell marker of neuroblasts), Gja1 (cell marker of astrocytes), C1qa (cell marker of microglial), Cldr5 (cell marker of endothelial cell), Mog (cell marker of oligodendrocytes), and Pdgfra (cell marker of

oligodendrocyte progenitor cell). In the dot plot, the larger the dots are, the more percentage of cells that express that cell marker. Therefore, clusters that display higher expression levels of cell markers (i.e., larger dots) are grouped together as the same cell type. FeaturePlot of each marker was generated to verify the validity of cell type classification (Fig. A1(b)).

In Fig. 1(a), Snap25 is a cell marker expressed in neuronal cells, Drd1a is cell marker of D1 MSN (Drd1), Drd2a is cell marker of D2 MSN (Drd2), Adora2a is cell marker of D2 MSN (Drd2), Resp18 is cell marker of interneurons (IN), Top2a is cell marker of neuroblasts (NB), Gja1 is cell marker of astrocytes (Astro), C1qa is cell marker of microglial (micro), Cldr5 is cell marker of endothelial cells (endo), Mog is cell marker of oligodendrocytes (oligo), Pdgfra is cell marker of Oligodendrocyte Progenitor Cell (OPC).



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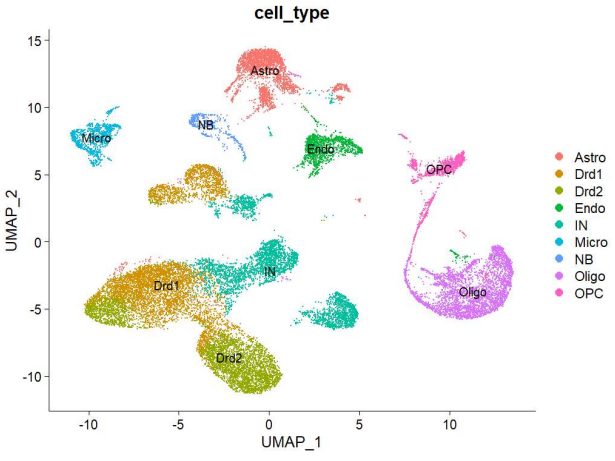


(b)

Astro, astrocytes. Drd1, Drd1 expressing cells. Drd2, Drd2 expressing cells. Endo, endothelial cells. IN, interneurons. Micro, microglia. NB, newborn. Oligo, oligodendrocyte. OPC, oligodendrocyte progenitor cell

Fig. 1. Cell type classification through cell markers. (a) Dot plot showing the expression levels of gene markers in specific cell clusters. (b) Identification of individual cell clusters as the corresponding cell types on the UMAP.

B. Cell Type Specific-Expression of Morphine Addiction-Related Genes

Cell-type specificity refers to the gene being only actively transcribed and translated into proteins in specific cell types, while it remains inactive or expressed at low levels in other cell types. The pattern of gene expression can be gleaned through the analysis of a large quantity of samples, and there is an indication of clear cell-type specificity in terms of expression breadth and expression level (Fig. 2). The violin plot presents a more qualitative representation while the dot plot is more quantitative in its representation. When both are combined, the expression specificity of known influential genes can be gleaned with more conspicuity. Although some candidate genes are expressed chiefly in two to three cell types, others seem to be exclusively expressed in certain cell types. Specifically, BDNF and Npy are both observed to be expressed only in IN, while Pdyn shows exclusive expression in Drd1 and Grin2c in Astro.

In Fig. 2, Fosb is primarily expressed in Endo and micro, Creb3l4 is primarily expressed in Endo and Astro, BDNF is clearly expressed in IN, Npy is also primarily expressed in IN, Cdk5rap2 is dominantly expressed in NB, Pdyn is dominantly expressed in Drd1, Gria2 is mostly expressed in Drd2 and Drd2, Grin3a is mostly expressed in IN and OPC, Grin2c is dominantly expressed in Astro, Grik1 in OPC and IN, Crh in IN and Drd1, Crhbp in IN.

A detailed description of the expressions of candidate genes is as follows:

FosB is expressed in both Micro and Endo. Around 20% of Micro expresses FosB at around 1.0–1.5 and < 20% of endo expresses fosB to around 2.0. The percent expressed and expression levels are similar between the two cell types. Creb3l4 is expressed in both astro and endo to around < 20%.

BDNF is expressed only in IN at around 20% at the average expression of 2.5. The significant difference in expression level of BDNF also corroborates the exclusive nature.

Npy is expressed in between 40% and 60% of interneurons at the average expression of 1.5. Similarly, the violin plot shows that Npy is expressed in higher numbers and to a larger degree in interneurons.

Cdk5rap2 is expressed in NB, OPC, Oligo, and Astro in similar levels according to the violin plot. However, the dot plot shows Cdk5rap2 to be expressed in ~40% of NB to the average expression of 1.5–2.0, ~20%–40% of OPC to 0.0–0.5, and around 20% of Oligo to around 0.0.

Pdyn is clearly expressed in 40%–60% of Drd1 cells to the average expression level of about 1.0–1.5. In the violin plot, there is a larger number of Pdyn expressions in Drd1, as well as to a higher level.

Gria2 is primarily expressed in Drd1 and Drd2, but also in IN with a lower percentage of expression but higher average expression level. Specifically, From the dot plot, over 60% (~80%) of Drd2 cells express Gria2 to about 1.0–1.5. In the violin plot, expression levels seem to be lower due to the different y-axis scale. In IN, about 40%–60% of Gria2 is expressed to about 2.0, which corroborates with the relatively higher expression level in violin plot.

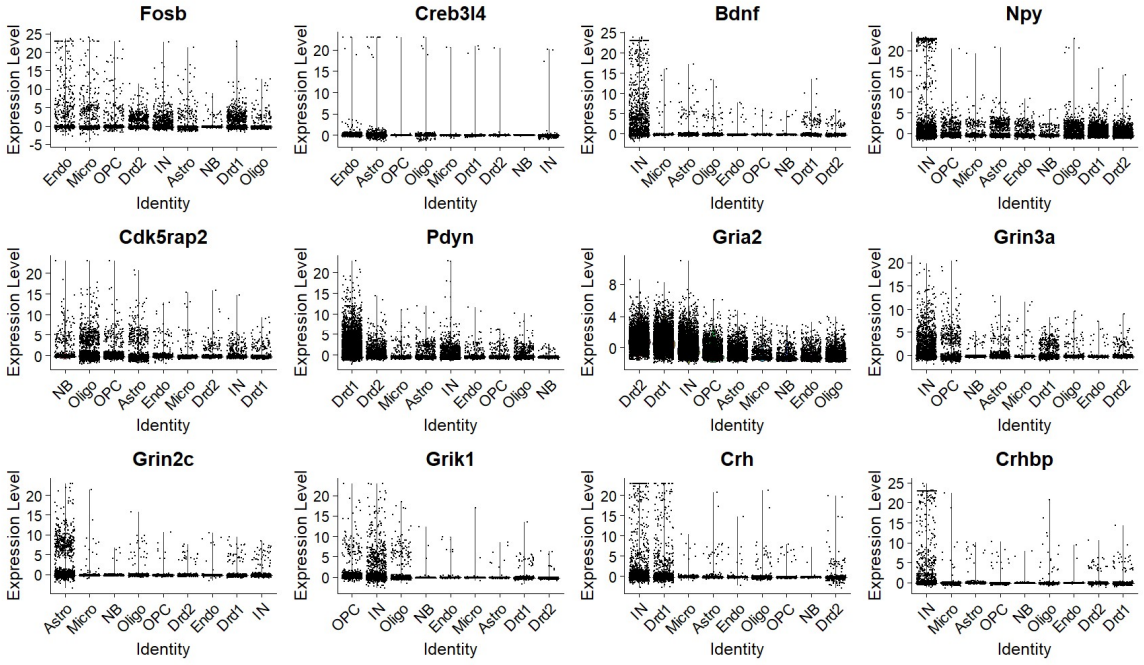
From the dot plot, about 20%–40% of IN express Grin3a to about 0.0–0.5, which is consistent with the relatively high expression level shown in the violin plot.

Grin2c is almost exclusively expressed in Astro, with around 20% of expression and an average expression of about 2.0–2.5. In the violin plot, Grin2c also has distinctly more points and a higher expression level.

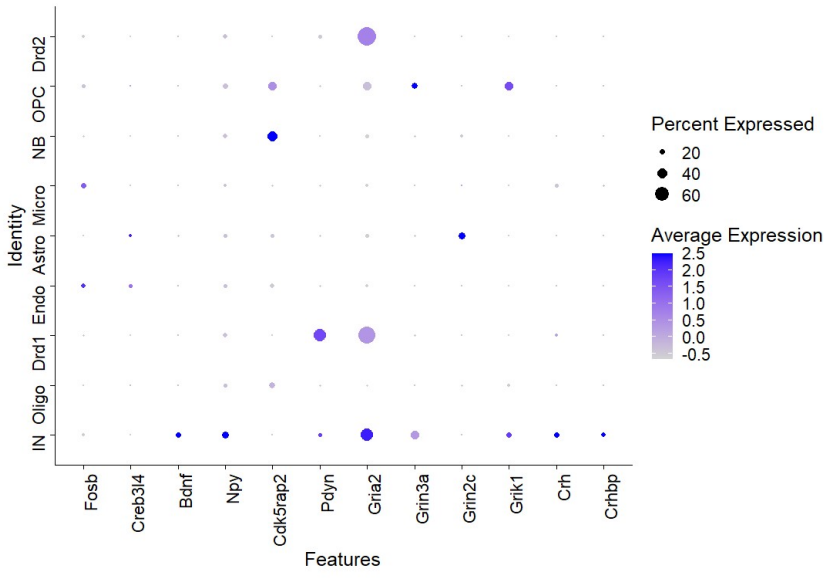
Grik1 is expressed in both OPC and IN, with higher percentage expressed in OPC (20%–40%) and higher average expressed in IN (~1.5–2.0).

Crh is expressed clearly in IN, with around 20% of the cells expressing it to around 2.5. Crh is also expressed in Drd1it at a lower level, which is less obvious in the dot plot but demonstrated by the violin plot.

Lastly, Crhbp is exclusively expressed in IN, with less than 20% expressing at around 2.0–2.5. This corresponds to the distinctly higher expression level observed in the violin plot.



(a)



(b)

Fig. 2. Cell type specific expression of candidate genes. (a) Violin plots of expression levels of candidate genes in each cell type. (b) Frequency and average expression levels of candidate genes in each cell type.

The difference in percentage of expression and average expression level of candidate genes also changes drastically between the mock and morphine groups (Fig. 3) within each cell type. This alteration in expression patterns of certain genes may affect the molecular mechanism of its addiction. Notably, the genes with the largest increase in

percent expressed include Cdkrap2 in OPC (from ~0% to ~60%), Grik1 in OPC (~0% to ~80%), Cdkrap2 in NB (~20% to 60%), Grin2c in astro (~0% to ~50%), and Grin3a in IN (~20% to ~60%). This increase in percent expressed suggests an upregulation of activity or production of these genes in specific cell types as a result of exposure to morphine. Additionally, selective genes have also changed in their average expression level in specific cell types. For example, slightly increased expression of Gria2 in Drd2, increased expression of Grin3a in OPC, decreased expression of Grik1 in OPC, increased expression of FosB in micro, decreased expression of Creb 3l4 in Endo, decreased expression of Pdyn in Drd1, slightly increased expression of Gria2, slightly decreased expression of BDNF in IN, decreased expression of Gria2 in IN, decreased expression of Grik1 in IN, decreased expression of Crh in IN. The change in average expression level means that there is a higher amount of the gene's mRNA or protein present compared to the mock, which could indicate the gene's involvement in the response to morphine, potentially contributing to effect, tolerance, or addiction to morphine.

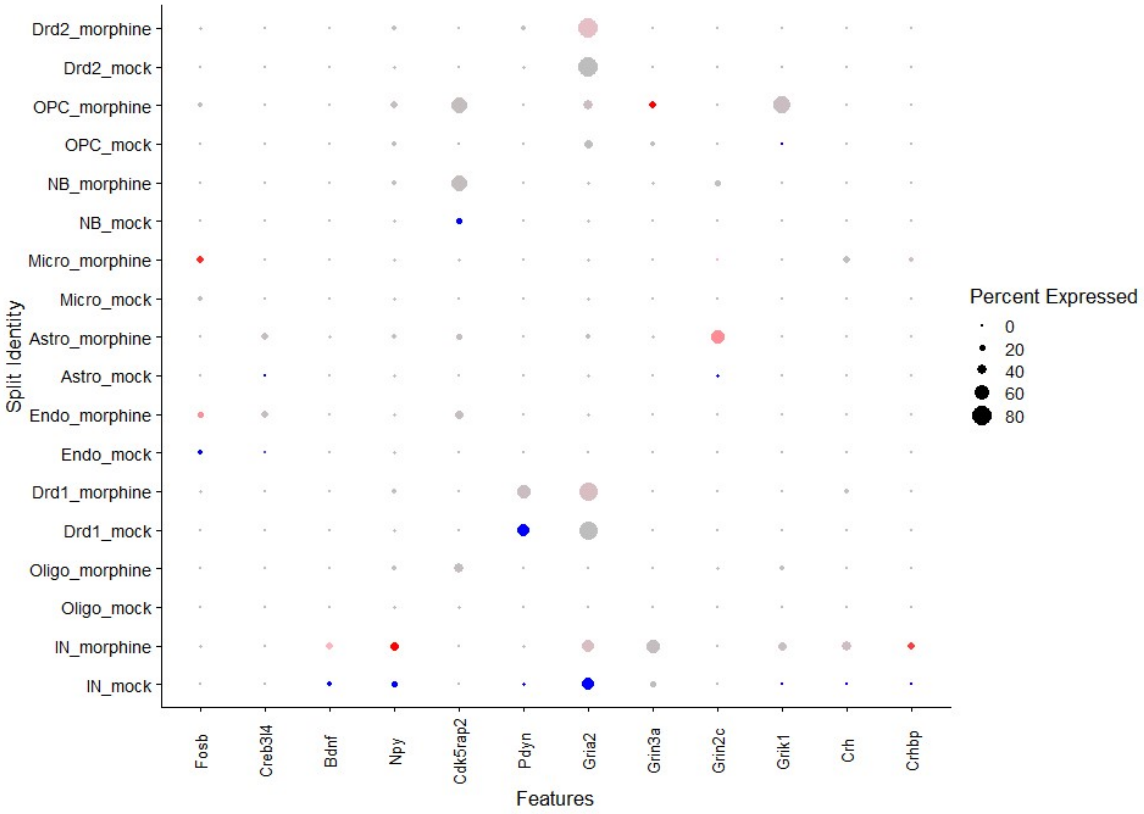


Fig. 3. Change in expression of candidate genes in each cell type in mock and morphine samples.

C. *Differentially Expressed Genes in Each Cell Type*

To search for highly differentially expressed genes in each cell type, identified marker genes in control and morphine-treated groups were visualized by dot plots and violin plots to show the top 6 differentially expressed genes between the two groups. The top 6 most varied genes over the morphine treatment of each of the 9 cell types were identified and summarized in Table I and the degree of difference in expression is represented in Fig. 4. From the dot plots, it is evident that the six genes with highest differential expression all have a very low percentage of expression in the mock samples and a dramatically higher expression percentage in the morphine samples.

In Table I, the difference in percent expressed and average expression between the mock and morphine groups is also represented in the dot plots below. There is a consistent and significant increase in expression of all of the genes from mock to morphine.

TABLE I. TOP 6 MOST VARIED GENES IN RESPONSE TO MORPHINE TREATMENT IN EACH CELL TYPE

Cell Type	Top 6 most varied genes in response to morphine treatment in each cell type					
IN	Samd 3	Pf4	Dusp2	Adamts5	H2-Eb1	Blnk
Oligo	Srpk3	Defb42	Rhoc	Hes6	Lcp1	Cpxm2
Drd1	Gpr101	Perp	Trhr	Arhgap35	Tec	Ill6
Endo	Egflam	Stab1	Itga4	Kcnk6	Rcn3	Alpk1
Astro	Pdgfrl	Otx2	Rdh5	Tmem3	4930506M 07Rik	Ranbp31
Micro	Trim21	Rg13	Tlr4	Mdfi	Ifi35	Gsx2
NB	Smoc1	Rcn3	Tnfrsf1a	Stk17b	Gm216	E2f8
OPC	Pgf	Gpr179	Gm26512	Ttll3	Rg13	Casp1
Drd2	Fbln5	Cenph	H2-DMb1	Jag1	Gpr101	Gm26735

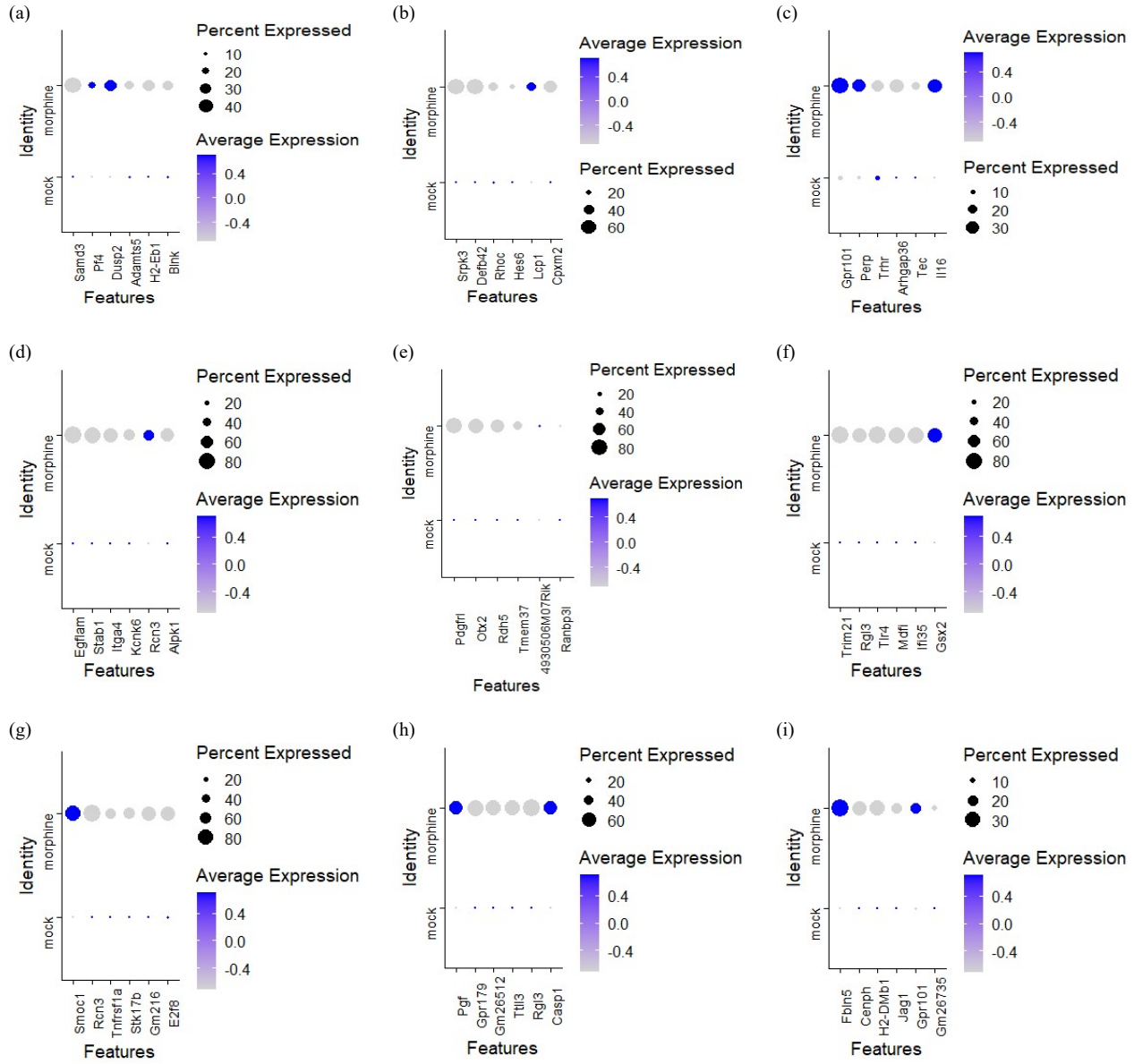


Fig. 4. Expression levels and frequencies of 6 most varied genes after morphine treatment in each cell type. (a) IN, (b) Oligo, (c) Drd1, (d) Endo, (e) Astro, (f) Micro, (g) NB, (h) OPC, (i) Drd 2.

Although most genes listed have relatively novel hold little current citations on its impact on morphine addiction, some do have preliminary studies that suggests relevance in the field of substance use research.

IV. Discussion

A. Cell-Type Specificity Analysis

By completing the computational analysis of gene expression mouse NAc in morphine treatment, we found the majority of addiction regulating genes are expressed in a cell-type specific manner, with most genes having clear tendency towards a higher expression in two or three cell types. However, BDNF, Npy, Pdyn, and Grin2c are expressed in an exclusive manner, which prompted a further investigation into possible reasons for such preferences.

1) Cell-type specificity of immediate early genes: BDNF and FosB

Immediate Early Genes (IEGs) are a cluster of genes that rapidly and transiently become active when exposed to different external signals. The name "immediate early" stems from the fact that their expression commences within minutes of cellular stimulation. The activation of immediate early genes plays a vital role in signal transduction pathways, serving as a primary reaction to external stimuli. Our investigation focuses on exploring the expression of specific IEG components, such as BDNF and FosB, in distinct cell types, aiming to identify the actively responsive cell populations to morphine.

Immediate Early Genes (IEGs) are a group of genes that respond rapidly and transiently to various extracellular stimuli when activated, as they can be expressed within minutes of cellular stimulation. The activation of immediate early genes is an essential step in signal transduction pathways and serves as a primary response to external stimuli [10]. In our study, examining cell-type specific expression of IEGs components such as BDNF

and FosB can reveal the cell types that respond actively to morphine. From the dot plot in Fig. 4(b), around 20% of interneurons express BDNF at the average expression of 2.5. Similarly, the violin plot shows that BDNF is expressed in comparatively higher numbers and to a larger degree.

As an immediate early gene, BDNF responds rapidly to external triggers such as a morphine dose in various brain regions. Studies have proposed that BDNF-deficiency may cause abnormal cellular adaptations to opioid exposure due to a dysregulation of cAMP-mediated excitation in the mutant noradrenergic neurons [11]. In addition to previous detections on dopaminergic neurons, recent studies also explored the presence of BDNF in cortical GABAergic interneurons [12], and it is known that such interneurons play a role in regulating the mesolimbic dopamine system in the VTA [13]. At the end, this paper also suggested that dopamine-independent mechanisms (such as cortical GABAergic interneurons) may be involved in the reinforcing properties of drugs of abuse. Taken together, BDNF is not only an important molecule in dopaminergic neurons, but the exclusive nature of its high expression in interneurons in morphine-treated samples of this study further highlights its potential roles in dopamine-independent mechanisms that also contribute to drug addiction.

Another IEG examined is FosB, which encodes for ΔFosB, a transcriptional factor that accumulates in the nuclear accumbens after repeated administration of various drugs of abuse. It has been hypothesized that ΔFosB may be a molecular mechanism that can initiate and sustain changes in gene expressions after the cessation of drug exposure [14]. It is reported ΔFosB in the nucleus accumbens mediates several major features of opiate addiction [7]. However, in which specific cell type FosB is expressed in the NAc is yet known. Through our computational analysis, we found FosB is highly expressed in both Micro and Endo, revealing the cell-type specific nature of its expression and further implying essential roles of these two cell types in morphine action.

2) Cell-type specificity of Pdyn

Prodynorphins are precursor proteins that are cleaved to produce the neuropeptide dynorphin. Pdyn is clearly expressed in 40%–60% of Drd1 cells to the average expression level of about 1.0–1.5. In the violin plot, there is a larger number of Pdyn expressions in Drd1, as well as to a higher level. This pattern is supported by a previous study, which also identified the cell-type specificity shown towards Drd1 by Pdyn [15]. The importance of Pdyn in the reward system has been shown through studies conducted on inbred mice, where higher Pdyn was shown to result in less sensitivity to morphine reward [16]. The aversive state ascribed to dynorphins' inhibition of morphine-elicited dopamine transmission may be due to the activation of κ opioid receptors located presynaptically on dopaminergic terminals in the NAc [17–19]. This finding correlates with its specific expression pattern in Drd1 cells found in this analysis.

3) Cell-type specificity of Npy

The expression of Npy in interneurons is also distinguishable, being expressed in about 40% and 60% of interneurons at the average expression of 1.5. Similarly, the violin plot shows that Npy is expressed in higher numbers and to a larger degree in interneurons. Previous studies have investigated primarily its role in morphine withdrawal symptoms in mice, such as morphine-reduced feeding and body weight reduction [20]. Another study demonstrated that the concomitant administration of NPY or [Leu31, Pro34]-NPY with chronic morphine for 7 days prevented the morphine withdrawal hyperalgesia [21]. These findings suggest the possible involvement of the NPY Y1/Y5 receptors in the development of morphine tolerance and dependence. The link to these previous findings to the cell-type specific expression of interneurons occurs at the NAc, where they are not only found to be highly sensitive to μ-opioid receptors, but also strongly connect to D1 MSNs and D2 MSNs [22]. Therefore, NAc NPY interneurons may directly regulate D1 MSNs and D2 MSNs to modulate anxiety as well as the withdrawal symptoms presented in previous findings.

Overall, the difference in gene expression in each cell type between the mock and morphine groups reveals the cell-type specific nature of their expression, pinpointing the potential location of where previously studied transcriptional changes occur. This finding may contribute to both the observable characteristics of morphine addiction and possible target cells of pharmacological therapy.

B. Differentially Expressed Genes Study

To further the characterization of cell-type specific gene expression, we found the top 6 most varied genes in each cell type between mock and morphine groups. The cell-type specificity of these novel genes implicates its potential role in modulating cell-type specific morphine response pathways. With the top 6 most differential genes from each specific cell type, we can further examine which differentially expressed genes are correlated to the specific traits of opioid addiction. A few of these genes have already been investigated for links to drug addiction.

For example, Srpk3, Stab1, Eglam, and Otx2 have also been identified as addiction-related genes in previous studies in opioid dependence and alcohol dependence [23–26]. Notably, binge-like ethanol drinking by mice increased Otx2 mRNA and protein in the ventral tegmental area of mice [26], which may provide a reference for the transcriptional changes in the reward system during acute morphine administration. Another study highlighted the effect of oxycodone on chemotherapy-induced neuropathic pain and the potential role of Pf4 [27]. The presence of Smoc1 has also been identified to be highly correlated with amyloid β peptide in Alzheimer's disease, though with little obvious relevance to addiction [28]. These findings underscore the significance of considering cellular heterogeneity and the necessity to investigate cell type-specific responses to gain a comprehensive understanding of the effects of opioids on the brain. Further studies are needed to confirm the involvement of these genes in drug addiction.

Although differential expression analysis was performed in this experiment, whether any gene pathways are involved is still unknown. In future studies, we could use gene pathways that can be analyzed in the context of each cell type as new candidate genes to gain potential insight into the impact of its expression significance of the gene and the cell type it acts on.

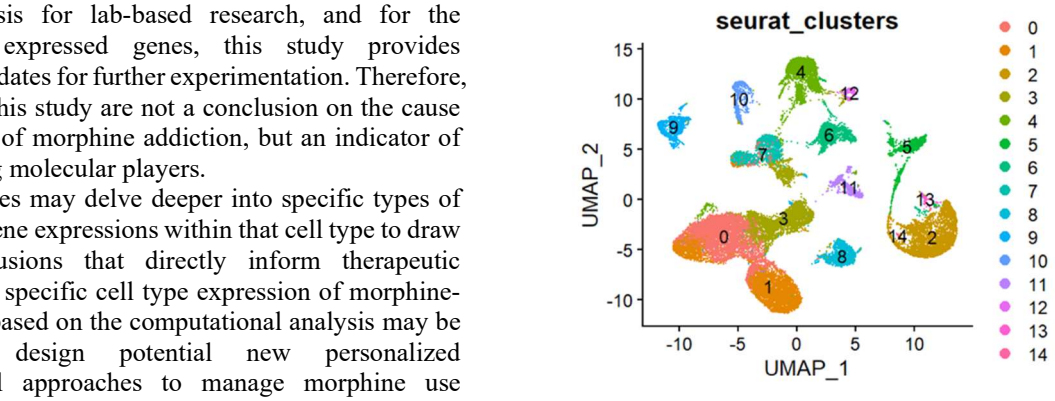
V. Conclusion

This analysis uncovered the cell type-specific expression patterns of several morphine-related genes, as well as the most differentially expressed genes in response to morphine treatment. We identified which known and new genes are most influenced by morphine treatment in specific cell types, which offers valuable starting points for future investigations into the functional roles and mechanisms underlying the effects of morphine at the cellular level.

Despite the insight of this analysis, the limits lie within the computer-based nature of the study. In the search of cell type specificity, this study looks for patterns that can become a basis for lab-based research, and for the differentially expressed genes, this study provides potential candidates for further experimentation. Therefore, the results of this study are not a conclusion on the cause or mechanism of morphine addiction, but an indicator of its contributing molecular players.

Future studies may delve deeper into specific types of cells and the gene expressions within that cell type to draw precise conclusions that directly inform therapeutic strategies. The specific cell type expression of morphine-related genes based on the computational analysis may be relevant to design potential new personalized pharmaceutical approaches to manage morphine use disorder, and high variance genes may also become new targets in therapeutic development. Future investigations may uncover the highest variable genes that can be a potential therapeutic target in the development of pharmaceutical solutions. The findings can inform potential therapeutic strategies that are more effective and efficient by targeting molecular-level players that are directly influenced by substance abuse, thereby resulting in higher efficacy of treatment due to the direct action taken to alter the neuronal response that triggers dependence and other effects that lead to addiction. Additionally, this study examined samples after acute morphine administration, and the results can only reflect the transcriptional responses to acute morphine. However, this excludes the real-life scenarios of chronic morphine administration that also contributes to morphine addiction. We can further this study of transcriptional programs on the mechanism of chronic morphine abuse if such datasets are available for analysis.

Appendix A Supplementary Figure



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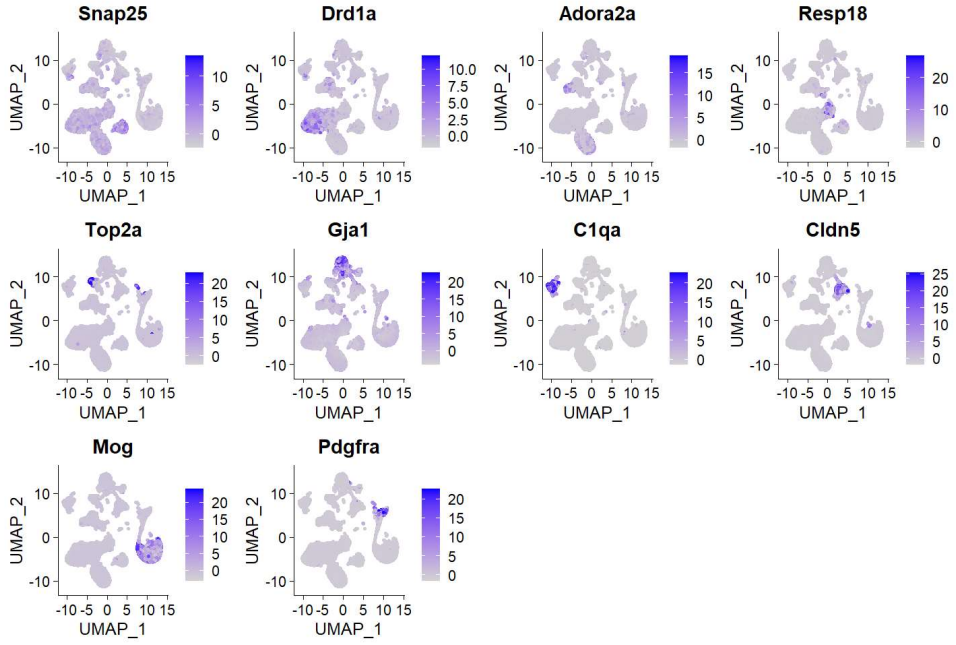

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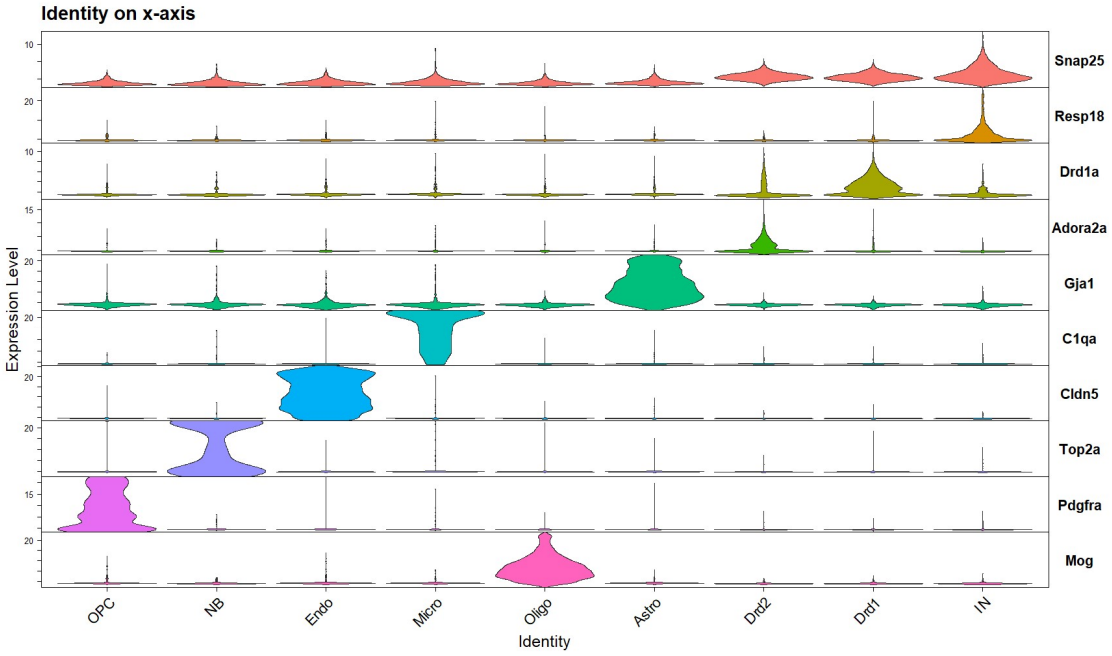

(c)

Fig. A1. (a) UMAP plot visualizing the 15 broad clusters of NAc cell types. (b) Feature plot of gene markers of each identified cell type. The expression level of each gene marker in a particular cluster on the UMAP. (c) Violin Plot representing the expression level of each gene marker in the cell types. Snap25 is primarily expressed in IN, Drd1, and Drd2, Resp18 in IN, Drd1a in Drd1, Adora2a in Drd2, Gja1 in Astro, C1qa in Micro, Top2a in NB, Pdgfra in OPC, and Mog in Oligo.

Conflict of Interest

The author declares no conflict of interest.

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