Archival Report

Biological Psychiatry: GOS

Sex-Specific Concordance of Striatal Transcriptional Signatures of Opioid Addiction in Human and Rodent Brains

Micah A. Shelton, Nicole Horan, Xiangning Xue, Lisa Maturin, Darrell Eacret, Julie Michaud, Navsharan Singh, Benjamin R. Williams, Mackenzie C. Gamble, Joseph A. Seggio, Madeline K. Fish, BaDoi N. Phan, George C. Tseng, Julie A. Blendy, Leah C. Solberg Woods, Abraham A. Palmer, Olivier George, Ryan W. Logan, and Marianne L. Seney

ABSTRACT

BACKGROUND: Opioid use disorder (OUD) has emerged as a severe, ongoing public health emergency. Current treatments for OUD are unsuccessful in leading to lasting abstinence in most users. This underscores the lasting effects of chronic opioid use and emphasizes the need to understand the molecular mechanisms of drug seeking and taking and how those alterations persist through acute and protracted withdrawal.

METHODS: Here, we used RNA sequencing in postmortem human tissue from males (n = 10) and females (n = 10) with OUD and age- and sex-matched control subjects. We compared molecular alterations associated with human OUD in the nucleus accumbens (NAc) to mouse and rat models of nonvolitional (n = 4–5 per group per sex) and volitional (n = 5–6 per group per sex) exposure to opioids across distinct stages of opioid use and withdrawal (acute and prolonged).

RESULTS: We found that the molecular signature in the NAc of females with OUD mirrored effects seen in the NAc of female rodents in a nonvolitional paradigm at all stages of exposure. Conversely, males with OUD showed an expression profile similar to that of rodents with volitional exposure but only during the acute withdrawal phase. Shared coexpression networks were involved in posttranscriptional modification of RNA and epigenetic modification of chromatin state.

CONCLUSIONS: Our results provide fundamental insight into the conserved molecular pathways altered by opioids across species, with evidence suggesting that alterations in females with OUD may be driven by drug exposure, while alterations in males with OUD may be driven by volitional intake.

https://doi.org/10.1016/j.bpsgos.2025.100476

Opioid use disorder (OUD) has emerged as a severe, ongoing public health emergency, with rapidly increasing prevalence rates driven by prescription opioids and recently by a surge in the availability of synthetic opioids, such as fentanyl (1). The rate of overdose deaths due to any opioids has seen a parallel increase over the same period, reaching more than 80,000 overdose deaths per year in the United States alone (2,3). Current treatment strategies have proven inadequate for most users, and rates of relapse are extremely high, underscoring the chronic nature of OUD (4–6). This relapse propensity emphasizes the need to understand not only the mechanisms of drug seeking and taking but also what occurs in the brain during acute and protracted withdrawal. Disentangling withdrawal mechanisms may allow for the development of novel therapies to reduce the negative consequences of withdrawal and promote long-term recovery.

Drug-induced neuroplasticity is thought to involve molecular adaptations to mesolimbic reward regions such as the nucleus accumbens (NAc) and higher-order executive regions such as the dorsolateral prefrontal cortex (DLPFC) (7–9). Chronic opioid use leads to a decrease in drug response. For example, activation of opioid receptors leads to molecular events that promote desensitization and the development of tolerance, including a cascade of receptor adaptations that involve phosphorylation, internalization, and a reduction in signal transduction (10). Opioid exposure also produces an early epigenetic response. The promoter for *OPRM1* is hypermethylated in response to short-term therapeutic use (11). Long-term opioid exposure leads to profound alterations in DNA methylation and gene expression (12), microRNA–messenger RNA network dysregulation (13), DNA damage (14), and dysregulation of angiogenic and cytokine gene networks (15). These epigenetic changes persist long after the drug has been metabolized. However, our knowledge of how these changes evolve from intoxication through withdrawal and into long-term abstinence in human OUD is incomplete.

Previously, we found enrichment of differentially expressed (DE) transcripts involving neuroinflammation and extracellular

ISSN: 2667-1743

matrix (ECM) remodeling in the DLPFC and NAc of people with OUD. Using cell-type deconvolution and weighted gene coexpression network analysis (WGCNA), we identified an accompanying upregulation of microglia markers and increased connectivity of neuroinflammatory and ECM signaling (16). However, all the people in that study had opiates on board at the time of their death, including people who died by overdose. Similarly, preclinical models of molecular mechanisms are typically produced using tissue taken from rodents with drugs on board at the time of sacrifice. Thus, it is difficult to disentangle whether transcriptional changes are a product of acute or chronic drug exposure, and few studies have focused on molecular changes during withdrawal. Furthermore, it is challenging to determine whether alterations in humans with OUD are associated with drug exposure versus volitional intake.

Here, we used a multispecies translational approach to compare transcriptional alterations in the NAc of humans with OUD to rodent models of opioid addiction. We leveraged resources available from the rodent biobank (National Institute on Drug Abuse Center of Excellence for Genetics, Genomics, and Epigenetics of Substance Use Disorders in Outbred Rats) and collaborators to collect and investigate tissues from a rodent model of nonvolitional opioid exposure (17) and a rodent model of volitional exposure (18). Both rodent models resulted in robust behavioral and physiological signs of opioid withdrawal. Rodent models were assessed during distinct stages of opioid use and withdrawal (acute and prolonged) (see Table 1 for details on datasets used for cross-species analyses). This study provides fundamental insight into the converging molecular pathways altered by opioids across species. Furthermore, this work helps to disentangle which alterations observed in humans with OUD are driven by acute versus chronic drug effects, with insights into which are associated with drug exposure versus volitional intake.

METHODS AND MATERIALS

Animals

All procedures were performed in accordance with the National Institutes of Health guidelines and approved by individual Institutional Animal Care and Use Committees at the University of Pennsylvania (C57BL/6J mice) and University of California San Diego (Heterogeneous Stock [HS] rats).

Table 1. Details Associated With Datasets Used in the Cross-Species Analyses

Study	Groups	Sex	
		Male	Female
Human: Volitional, Seney *et al.* (16)	Control	10	10
	Opioid use disorder	10	10
Rodent: Nonvolitional, Eacret *et al.* (17)	Control	4	4
	Intoxication	5	4
	1-day withdrawal	5	4
	14-day withdrawal	5	5
Rodent: Volitional, Carrette *et al.* (18)	Control	6	6
	Intoxicated	5	6
	1-day withdrawal	6	6
	28-day withdrawal	6	6

Humans

Consent for the use of human tissue and data collection were obtained from next of kin, and procedures were approved by the University of Pittsburgh's committee for Oversight of Research and Clinical Training Involving Decedents and Institutional Review Board for Biomedical Research. Details on the postmortem OUD cohort are provided in Figure S1.

Transcriptomics Datasets

We previously published the human OUD data used in the current cross-species comparisons (GEO Accession: GSE174409, ID: 200174409) (16). Details of mouse and HS rat opioid exposure paradigms are included in the Supplement. Behavioral results are reported elsewhere for the mouse nonvolitional exposure (17). Tissue was obtained from the rat oxycodone biobank for the volitional intake dataset (18). In collaboration with the National Institute on Drug Abuse Center of Excellence for Genetics, Genomics, and Epigenetics of Substance Use Disorders in Outbred Rats, we obtained brain samples for RNA sequencing collected from male and female HS rats at different stages of oxycodone self-administration (SA).

Differential Expression Analysis

Differential expression was assessed using limma (19). For humans, we compared control subjects to people with OUD, with every individual having opioids on board at the time of their death. Briefly, for each transcript, we first adjusted the expression differences with covariates (sex, age, race, postmortem interval, pH, and RNA integrity number) by including each in the regression model; at most, 2 covariates were selected to be included in the full model using Bayesian information criteria for each individual transcript. For the mouse nonvolitional dataset, we examined DE in 3 groups compared with controls: 1) chronic forced morphine drinking and intoxicated at the time of sacrifice (intoxication), 2) chronic forced drinking and in withdrawal for 24 hours (1-day withdrawal), and 3) chronic forced drinking and in withdrawal for 14 days (14-day withdrawal). For the rat volitional dataset, we examined DE in 3 groups compared with controls: 1) intravenous SA (IVSA) of oxycodone and intoxicated at the time of sacrifice (intoxication), 2) IVSA and in withdrawal for 24 hours (1-day withdrawal), and 3) IVSA and in withdrawal for 28 days (28-day withdrawal). Transcripts with $p < .01$ and $\log_2$ fold change $> \pm 0.26$ were considered DE. We also used rank-rank hypergeometric overlap (RRHO) to assess the overlap of differential expression patterns between humans and rodent models at each phase of opioid exposure using RRHO2 (20,21). RRHO2 is a threshold-free method that identifies overlap between 2 ranked lists of DE genes, where the ranking is based on the $-\log_{10}(p \text{ value})$ multiplied by the effect size direction.

Identification of Coexpression Networks

Using the human dataset, we performed WGCNA to identify gene modules (22,23) (threshold power = 0.95, soft power = 30,

cut height = 0.25, minimum module size = 30, network type = signed). Fisher's exact tests were used to determine whether DE transcripts were enriched within WGCNA modules. ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks) was used to identify hub and OUD-specific hub genes for network analysis (24), and Cytoscape was used to visualize networks. Pathway overrepresentation categories for each module were assessed using Metascape, with the 5000 WGNCA-analyzed genes as background.

RESULTS

Differential Expression Across Human OUD and Rodent Models of Nonvolitional and Volitional Opioid Exposure

Because corticostriatal circuit dysfunction contributes to the impairments that are a hallmark of substance use disorders (25), we examined the impact of OUD on transcriptional differences in the human NAc. Consistent with our previous work, we found that many more NAc DE transcripts were downregulated than upregulated in OUD when we examined samples from humans (Figure S2A and Table S1). However, sex differences have been described across several domains in OUD including comorbidity with psychiatric symptoms (26), cue-induced craving/neural activity (27), and DNA methylation of OUD-related genes (28). To identify potential sex-specific transcriptional signatures of OUD, we examined males and females separately. Notably, there was a low level of overlap in the patterns of upregulation and downregulation between males and females, with most DE transcripts exhibiting distinct patterns in males and females with OUD (Figure 1A and Table S1). Across analyses, most DE transcripts were protein coding, with a much smaller fraction representing long noncoding RNAs (lncRNAs) and pseudogenes.

Then, we investigated the effect of opioid administration on the NAc transcriptome in a nonvolitional exposure paradigm (mouse; entire NAc) and a volitional exposure paradigm (rat; core and shell separately) at 3 stages of opioid use and withdrawal. For the nonvolitional paradigm (17), we compared controls (not exposed to drug) with 1) 11 days forced administration in drinking water (intoxicated), 2) 1-day withdrawal, and 3) 14-day withdrawal rodents. For the nonvolitional paradigm, opioid exposure was associated with more downregulated than upregulated genes in all 3 opioid-exposed groups (Figure S2B and Table S2). Similar to the sex-specific patterns of OUD effects in humans, male and female mice exhibited distinct alterations for all 3 stages of exposure, with very few genes altered in the same direction in males and females (Figure 1B). Most of the DE transcripts were protein coding genes followed by lncRNAs across the 3 stages of opioid exposure (Figure S2B).

In the volitional paradigm (18), we compared controls (not exposed to drug) with 1) 6 weeks IVSA (intoxicated), 2) 1-day withdrawal, and 3) 28-day withdrawal rodents. More DE transcripts were upregulated than downregulated in the NAc core (Figure S2C) and shell (Figure S2D) across the 3 opioid-exposed groups. When we examined the results separately by sex, we again observed distinct patterns across the 3 stages of exposure for both core (Figure 1C and Table S3) and shell (Figure 1D and Table S4).

Transcriptional Concordance Between Human OUD and the Rodent Model of Nonvolitional Opioid Exposure

Next, we interrogated whether the pattern of gene expression in human OUD mapped onto the signature from our nonvolitional exposure groups using threshold-free RRHO2 analysis. These analyses have the potential to identify which transcriptional changes in human OUD are driven by exposure to opioids that are independent of volitional intake. These comparisons also allow us to determine which alterations in human OUD are a product of the acute effects of opioid intoxication versus the long-term effects of chronic exposure. When the sexes were pooled, there was substantial overlap in both upregulated and downregulated transcripts between humans and intoxicated rodents. However, when the sample was split by sex, OUD females but not males showed strong concordance with sex-matched intoxicated rodents (Figure 2A). The same pattern was true for 1-day (Figure 2C) and 14-day (Figure 2E) withdrawal. Pathway enrichment was performed on shared transcripts between females with OUD and female rodents across the 3 exposure groups (Figure 2B, D, F and Table S5). Top shared pathways included Wnt/β-catenin, myelination, CLEAR (coordinated lysosomal expression and regulation), PDGF-mediated signaling, and synaptogenesis signaling. In intoxicated and 1-day withdrawal rodents, we identified levodopa (L-DOPA), the central precursor in the synthesis of the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine, as an upstream pathway regulator (Figure 2B, D). We also identified the gene for estrogen receptor alpha (ERα), *ESR1*, as a potential upstream regulator in intoxicated and 1-day withdrawal rodents (Figure 2B, D). When activated by its ligands, ERα acts as a transcription factor with roles across the organism (29). The *HNF4A* gene, which also encodes a transcription factor, emerged as an upstream regulator across all 3 opioid-exposed groups.

Transcriptional Concordance Between Human OUD and the Rodent Model of Volitional Opioid Exposure

Then, we turned to our volitional exposure rodent model and compared the transcriptional signature in the NAc shell and core with the NAc of humans with OUD. The pattern of gene expression was discordant between humans and intoxicated rodents when the sexes were pooled for both the shell (Figure 3A) and core (Figure 3E). When the sexes were examined separately, gene expression was still discordant but more strongly in females than in males (Figure 3A, E). Gene expression was concordant between humans and the 1-day withdrawal rodents in both shell (Figure 3B) and core (Figure 3F). However, this effect was driven by males; when the sexes were examined separately, only males with OUD showed concordance with male 1-day withdrawal rodents. The same overall patterns were observed between humans and 28-day withdrawal rodents. Gene expression was weakly concordant between all humans and 28-day withdrawal rodents, but when separated by sex, males were weakly

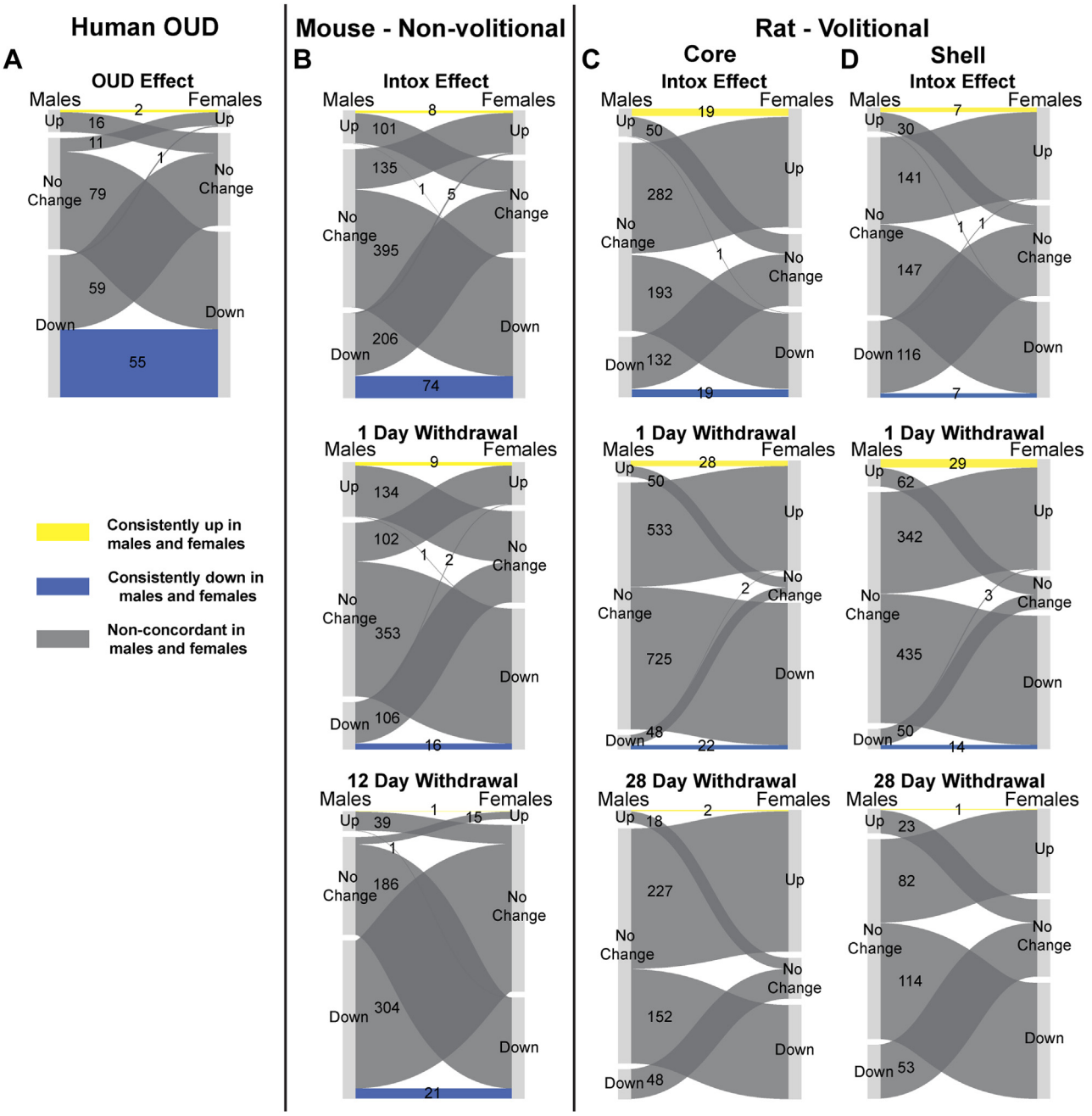


Figure 1. Sex-specific transcriptomic changes in the NAc from human subjects with OUD and 2 rodent models of opioid exposure. **(A)** Alluvial plots showing transcripts that exhibit shared or unique patterns of transcripts that were upregulated, downregulated, or not altered in males and females with OUD. **(B)** Alluvial plots showing transcripts that exhibit shared or unique patterns of transcripts that were upregulated, downregulated, or not altered in male and female mice across 3 stages of opioid exposure in a nonvolitional paradigm. **(C, D)** Alluvial plots showing transcripts exhibiting shared or unique patterns of transcripts that were upregulated, downregulated, or not altered in male and female rats in the NAc core **(C)** and shell **(D)** across 3 stages of opioid exposure in a volitional paradigm. NAc, nucleus accumbens; OUD, opioid use disorder.

concordant, and females were weakly discordant (Figure 3D, H and Table S5). In the shell, top pathways associated with shared transcripts between OUD males and 28-day withdrawal rodent males included protein ubiquitination and mTOR (mechanistic target of rapamycin) signaling (Figure 3C), with *ESR1* and *HNF4A* predicted as upstream regulators. In the core, the 1-day withdrawal comparison in males was associated with estrogen receptor signaling and HIF1a (Figure 3G), while the 28-day withdrawal comparison in males was associated with synaptogenesis and CLEAR signaling (Figure 3I).

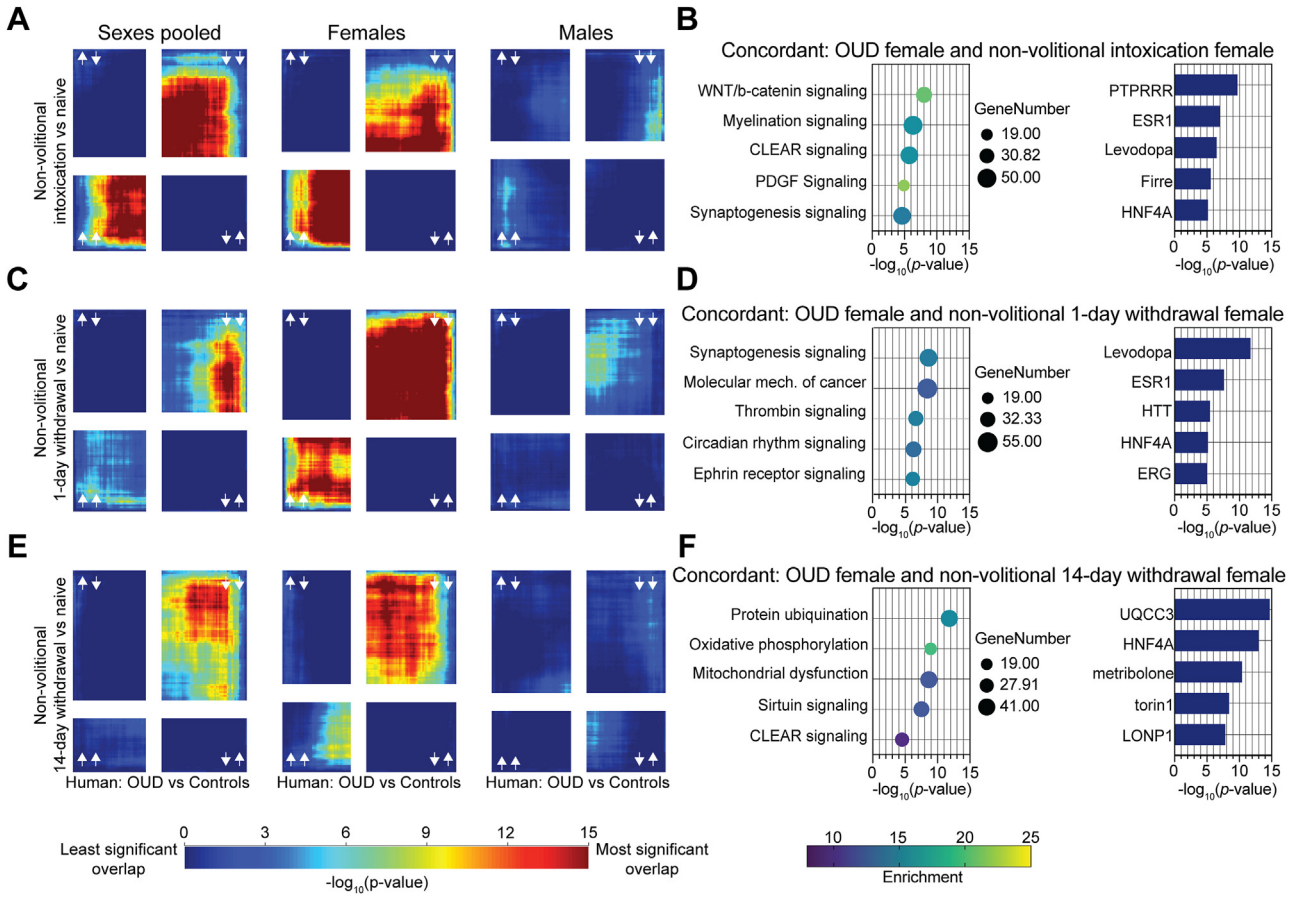
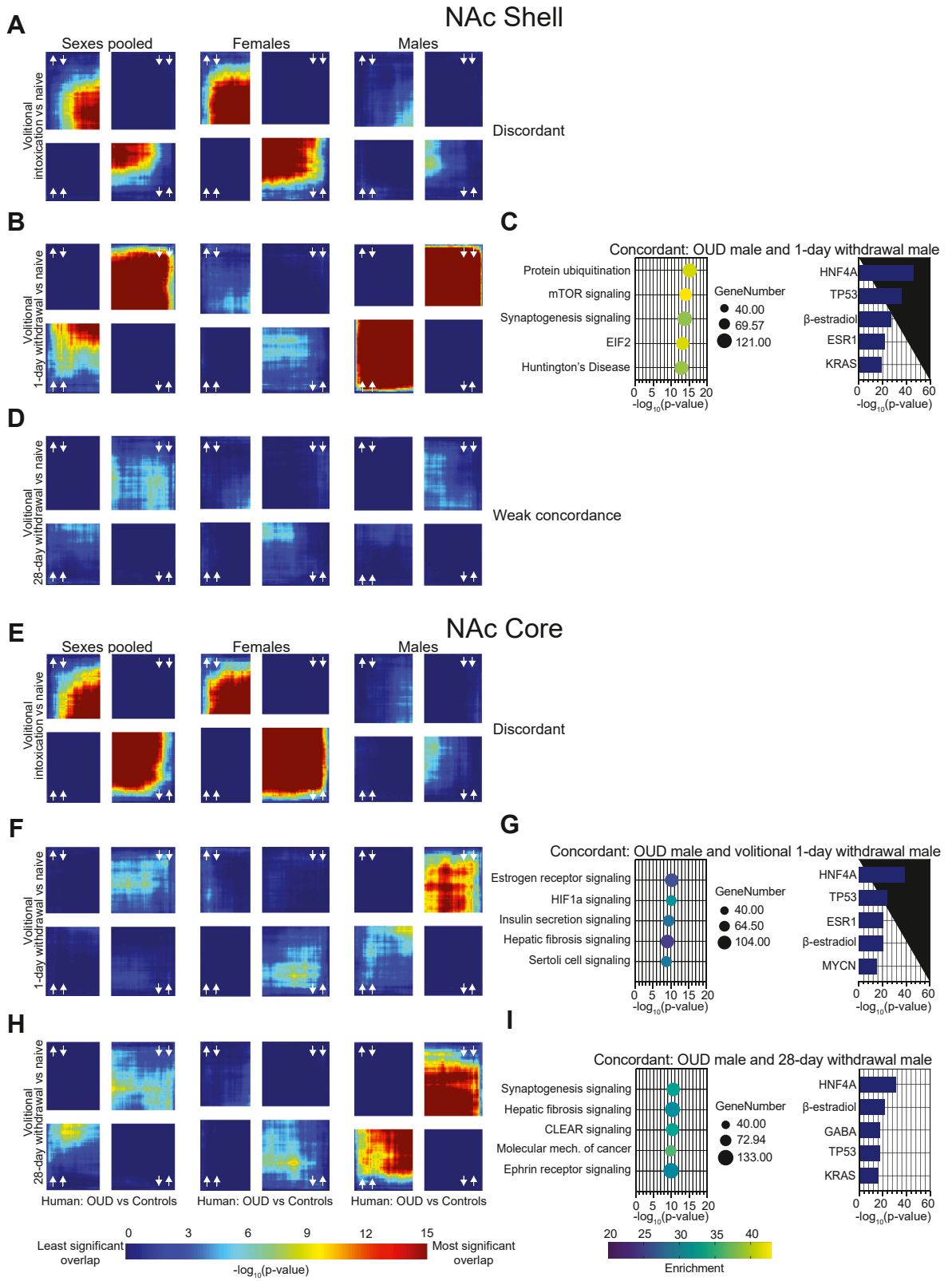


Figure 2. Transcriptional changes in the NAc from female human subjects with OUD overlap with rodent nonvolitional exposure. **(A)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and mice intoxicated ATOD. Increasing color warmth indicates increasing $-\log_{10} p$ value. **(B)** Pathway enrichment analysis of transcripts concordant between females with OUD and female mice intoxicated ATOD (left). Circle size corresponds to gene number, and increasing color warmth indicates enrichment of $-\log_{10} p$ value. The bar graph displays potential upstream regulators with $-\log_{10} p$ value on the x-axis (right). **(C)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and mice at 1-day withdrawal. **(D)** Pathway enrichment analysis of transcripts concordant between females with OUD and female mice at 1-day withdrawal (left). The bar graph displays potential upstream regulators (right). **(E)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and mice at 14-day withdrawal. **(F)** Pathway enrichment analysis of transcripts concordant between females with OUD and female mice at 14-day withdrawal (left). The bar graph displays potential upstream regulators (right). ATOD, at time of death; CLEAR, coordinated lysosomal expression and regulation; NAc, nucleus accumbens; OUD, opioid use disorder; RRHO, rank-rank hypergeometric overlap.

Predicted upstream regulators for both the 1-day and 28-day withdrawal comparisons were *HNF4A, TP53*, and β-estradiol (Figure 3G, I).

Gene Network Analysis Identifies Sex-Specific Hub Genes Associated With Nonvolitional and Volitional Opioid Exposure

To investigate gene correlations in human OUD and our rodent models of nonvolitional and volitional opioid intake, we interrogated gene networks using WGCNA. First, we built the WGCNA modules using the human OUD data and then looked for enrichment in OUD-associated DE transcripts as well as cross-species concordance patterns within each module (see module membership in Table S6). There was strong enrichment of DE transcripts across all human OUD subject groups in the darkorange, yellowgreen (Figure S3), and lightgreen modules (Figure 4A). In addition to being enriched for DE transcripts in all our human OUD subject groups, the lightgreen module showed a high prevalence of DE transcripts that were concordant between human female OUD and rodent female nonvolitional intake as well as human male OUD and rodent male volitional intake groups (Figure 4A). Because this module showed the strongest enrichment for DE transcripts, we focused our attention there. We visualized the pathway network associated with the lightgreen module and identified clusters with significantly enriched terms based on pathways (Figure 4B). The 2 largest clusters were categorized as RNA splicing/spliceosome and acetylation lysine/acetyltransferase. Other network clusters included DNA transcription preinitiation, regulation gene


A
NAc Shell
Sexes pooled
Females
Males
Volitional intoxication vs naive
Discordant
B
Volitional 1-day withdrawal vs naive
C
Concordant: OUD male and 1-day withdrawal male
Protein ubiquitination
mTOR signaling
Synaptogenesis signaling
EIF2
Huntington's Disease
GeneNumber
40.00
69.57
121.00
HNF4A
TP53
β-estradiol
ESR1
KRAS
0 5 10 15 20
-log10(p-value)
0 20 40 60
-log10(p-value)
D
Volitional 28-day withdrawal vs naive
Weak concordance
E
NAc Core
Sexes pooled
Females
Males
Volitional intoxication vs naive
Discordant
F
Volitional 1-day withdrawal vs naive
G
Concordant: OUD male and volitional 1-day withdrawal male
Estrogen receptor signaling
HIF1a signaling
Insulin secretion signaling
Hepatic fibrosis signaling
Sertoli cell signaling
GeneNumber
40.00
64.50
104.00
HNF4A
TP53
ESR1
β-estradiol
MYCN
0 5 10 15 20
-log10(p-value)
0 20 40 60
-log10(p-value)
H
Volitional 28-day withdrawal vs naive
Human: OUD vs Controls
Human: OUD vs Controls
Human: OUD vs Controls
0 3 6 9 12 15
Least significant overlap
Most significant overlap
-log10(p-value)
I
Concordant: OUD male and 28-day withdrawal male
Synaptogenesis signaling
Hepatic fibrosis signaling
CLEAR signaling
Molecular mech. of cancer
Ephrin receptor signaling
GeneNumber
40.00
72.94
133.00
HNF4A
β-estradiol
GABA
TP53
KRAS
0 5 10 15 20
-log10(p-value)
0 20 40 60
-log10(p-value)
20 25 30 35 40
Enrichment

heterochromatin, cytosolic ribosome structural, and transcription coactivator activity. Together, this network analysis underscores the profound effect that opioid exposure has on the regulatory mechanisms of DNA transcription from epigenetic modifiers through posttranscriptional regulation of RNA splicing. Other notable network clusters involved synaptic activity: synaptic vesicle localization and signal release synapse.

To distinguish potential regulators of this coexpression network, we identified highly connected hub genes within the lightgreen module that were predicted to drive the expression of other genes within the module (Figure 4B). Of the 11 identified hub genes, 5 were specific to OUD: *ANKRD11*, *CAMTA2*, *IQSEC1*, *RIPOR1*, and *SKI*. We also identified sex-specific hub genes as part of our network analysis. Three of those genes overlapped with OUD hub genes: *CAMTA2*, *IQSEC1*, and *ANKRD11*, while the other 2, *SEC16A* and *WNK2*, were uniquely sex specific. The central hub gene in our network is *KMT2D*, a histone methyltransferase with a direct relationship with the estrogen receptor (30). This is particularly interesting given that one of the largest clusters in the lightgreen module is acetylation lysine/acetyltransferase, further suggesting that opioids lead to heterochromatin modifications (16,31).

The lightgreen module represents a potential interacting, correlative gene network that is integrated across species and drug administration paradigms. To further deconvolve the cell types represented by the genes in the lightgreen module, we assessed whether module genes were enriched for specific cell types in human striatum (32,33). Overall, the lightgreen module was enriched for genes in neuron subtypes compared with glial cells (Figure 5A), suggesting that the module is composed mainly of genes active in neurons across species in response to opioids. In OUD, module genes were significantly reduced in enrichment within medium spiny neurons relative to interneurons and glial cells (Figure 5B). Enrichment changes were independent of sex (Figure 5B), occurring in both females and males with OUD. Thus, module genes were decreased in expression in medium spiny neurons in OUD, reflecting similar genes and consistent correlative expression across the gene network in medium spiny neurons in rodent models of opioids and individuals with OUD.

DISCUSSION

Here, we demonstrated sex specificity in concordance of the molecular signature of OUD in the human NAc and the molecular signature of opioid exposure in preclinical models. Females with OUD mirrored effects seen in the NAc of female rodents in a nonvolitional paradigm at all stages of opioid exposure, while males with OUD showed overlap in expression profile with male rodents in acute withdrawal (NAc shell, core) and prolonged withdrawal (NAc core) in a volitional paradigm. The top 2 shared pathways, synaptogenesis and CLEAR signaling, were consistently identified in concordant genes across species and opioid exposure, suggesting that opioid addiction is closely linked to synaptic alterations and regulation of lysosomal degradation. WGCNA identified gene coexpression networks involved in posttranscriptional modification of RNA and chromatin state, underscoring the effect of opioid exposure on transcriptional regulation via epigenetic modification.

While epidemiological studies have demonstrated a higher prevalence of illicit drug use in males, evidence suggests that drug use in females transitions more quickly from casual, recreational use to dependence (34–36). Females report higher levels of baseline and cue-induced craving (27,37,38), more physical withdrawal symptoms, greater functional impairment, and higher levels of comorbid psychological suffering (39–41). Preclinical studies have shown that females acquire opioid SA more quickly, self-administer more, reach higher breakpoints during fixed ratio, and exert more behavioral effort during extinction and cue-induced reinstatement (42–48). Our work provides added nuance to understanding sex differences in OUD. When we compared samples from humans to our rodent models of disease, females with OUD were concordant with female rodents that were nonvolitionally exposed to opioids, while males with OUD were concordant with male rodents that were volitionally exposed to opioids. These results help provide potential insight into mechanisms that underlie the OUD-associated alterations that we observed. For example, our results suggest that the pattern of alterations in females with OUD may be due to opioid exposure itself and not necessarily volitional intake, while the pattern in males with OUD is driven more by volitional opioid intake.

Notably, several consistent patterns emerged with regards to top shared pathways in concordant genes and their upstream regulators. Estrogen receptor signaling was the strongest pathway finding in the rat NAc core, and both *ESR1*, the gene for ERα, and a ligand for this receptor, estradiol, were identified as upstream regulators across sex and exposure

Figure 3. Transcriptional changes in the NAc from male humans with OUD overlap with acute and prolonged withdrawal in a rodent volitional exposure paradigm. **(A)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and the NAc shell of rats intoxicated ATOD. **(B)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and the NAc shell of rats at 1-day withdrawal. **(C)** Pathway enrichment analysis of transcripts concordant between males with OUD and the NAc shell of male rats at 1-day withdrawal (left). The bar graph displays potential upstream regulators (right). **(D)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and the NAc shell of rats at 28-day withdrawal. **(E)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and the NAc core of rats intoxicated ATOD. **(F)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and the NAc core of rats at 1-day withdrawal. **(G)** Pathway enrichment analysis of transcripts concordant between males with OUD and the NAc core of male rats at 1-day withdrawal (left). The bar graph displays potential upstream regulators (right). **(H)** RRHO2 plots showing concordant gene expression between human subjects with OUD pooled by sex (left column), human female OUD (center column), human male OUD (right column), and the NAc core of rats at 28-day withdrawal. **(I)** Pathway enrichment analysis of transcripts concordant between males with OUD and the NAc core of male rats at 28-day withdrawal (left). The bar graph displays potential upstream regulators (right). ATOD, at time of death; CLEAR, coordinated lysosomal expression and regulation; mTOR, mechanistic target of rapamycin; NAc, nucleus accumbens; OUD, opioid use disorder; RRHO, rank-rank hypergeometric overlap.

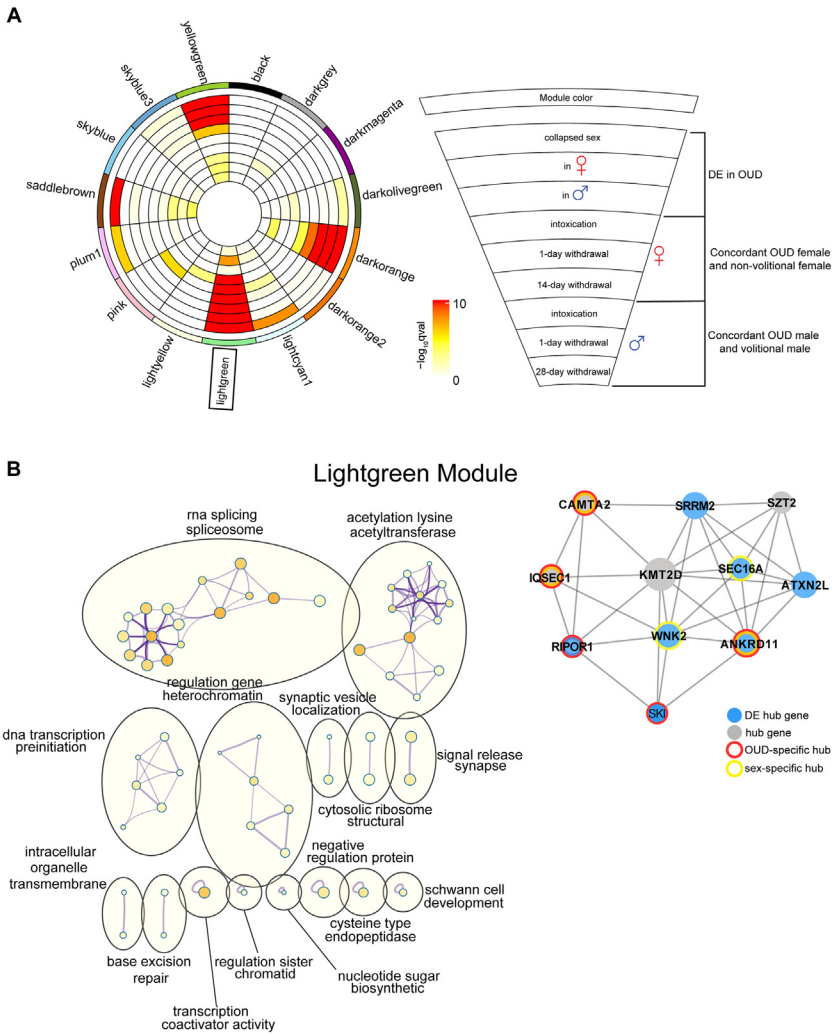


Figure 4. OUD-associated gene networks in the NAc. **(A)** Coexpression modules and network structure of transcription in the NAc were generated using weighted gene coexpression network analysis. Circos plot identified by module names and colors. Enrichment for transcripts DE in OUD, concordant human female and mouse, and concordant human male and rat are indicated by semicircle colors within each module, with increasingly warm colors indicating increasing $-\log_{10} p$ value. **(B)** Left: Network visualization generated using Cytoscape with categorical labels for multiple clusters. Right: Hub gene coexpression networks of the NAc in the lightgreen module. Node size indicates the degree of connectivity for that gene. Blue nodes indicate DE hub genes. Gray nodes indicate hub genes. Red halos indicate OUD-specific hub genes. Yellow halos indicate sex-specific hub genes. DE, differentially expressed; NAc, nucleus accumbens; OUD, opioid use disorder.

paradigm (nonvolitional and volitional). ERα is the predominant estrogen receptor subtype, and estradiol is the primary circulating estrogen hormone. When bound, the receptor undergoes a conformational change, homodimerizes, and together estradiol and ERα form a complex that binds the promoter region of target genes to regulate their expression (29,49). Evidence suggests that gonadal hormones generally and estradiol specifically play a role in guiding behavior related to substance use (50–54). Female rats take a similar number of heroin infusions during estrus, metestrus, and diestrus but self-administer significantly less during proestrus when estradiol is at its peak (46). Ovariectomy (OVX) removes the primary source for estrogens and, in mice, increases morphine conditioned place preference (CPP) at a 10-mg/kg dose, which is reversed by estradiol replacement (55). These findings suggest that estradiol leads to an overall reduction in drug seeking/reward. However, the data are complex. At smaller doses of morphine (2, 5 mg/kg), OVX in female mice decreases CPP, and estradiol replacement increases CPP (55). Following estradiol replacement in OVX rats, animals acquired opioid SA more quickly, self-administered higher levels of opioids, and showed an enhanced sensitivity to opioid-related cues compared with vehicle-treated OVX controls (56,57), which is consistent with estradiol's effect in studies that have used psychostimulants and alcohol (58–63). However, other studies have found no effect of OVX in rat opioid SA models (64,65). Together, this suggests that gonadal hormones play some role in the rewarding effects of drugs of abuse, but the relationship to opioids needs further study. One potential mechanism is through regulation of synaptic density. Estradiol and the primary male gonadal hormone testosterone can modulate spine plasticity in both the NAc core and shell (35,66–69). Notably, synaptogenesis signaling emerged as a consistent pathway finding across sex, species, and opioid exposure group in our analyses. It is interesting to speculate how gonadal hormones may modulate the rewarding effect of drugs of abuse through altering baseline spine density in reward-related cortical regions.

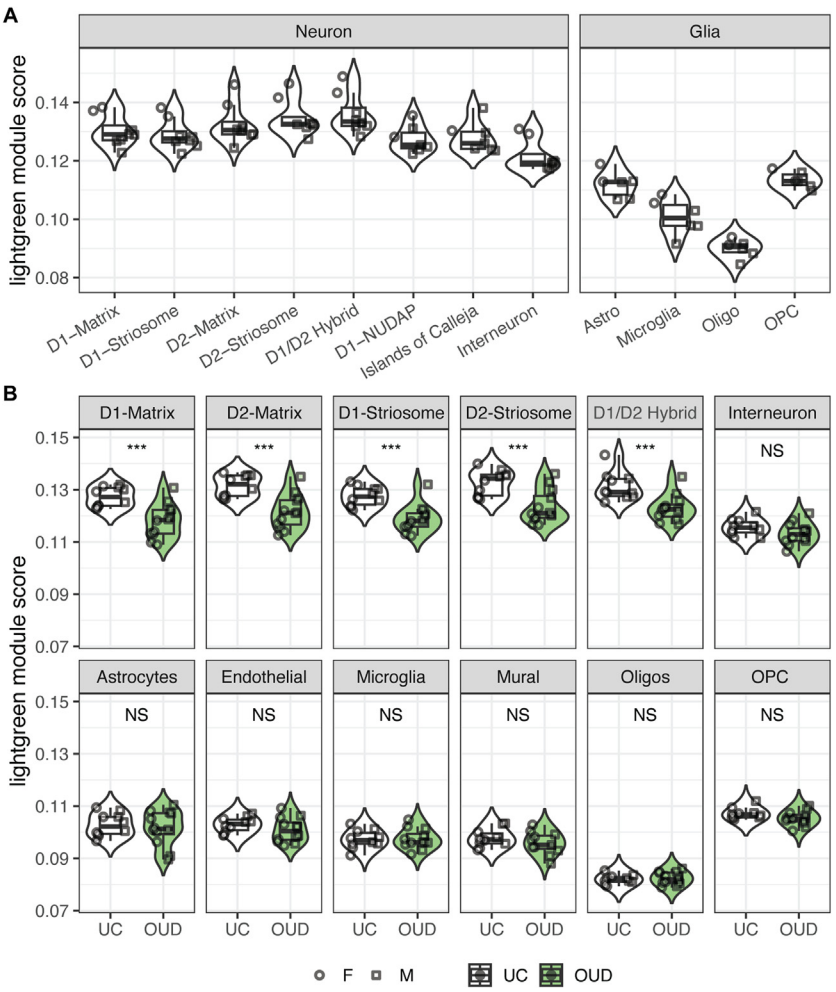


Figure 5. Cell-type enrichments identify opioid-related gene network alterations in striatal medium spiny neurons. **(A)** AUCell of the lightgreen module identified by weighted gene coexpression network analysis derived from human striatum with the y-axis representing the relative expression of module gene score (32). Each major neuronal (left) and glial (right) cell subtype is represented. Higher module scores in neurons reflect preferential enrichment of genes in the lightgreen module within neurons of human striatum relative to glial cells. **(B)** AUCell of the lightgreen module genes are decreased in expression primarily in medium spiny neuron subtypes (matrix and striosome neurons that express dopamine D_1 or D_2 receptor) in individuals with OUD. The y-axis represents the relative expression of module gene score. ***Significantly different between the UC and OUD cohorts at $p < .001$. Astro, astrocyte; AUCell, area under the curve for cell types; D1/D2, D_1/D_2 receptor-expressing neurons; F, female; M, male; NS, nonsignificant; NUDAP, neurochemically unique domains in the accumbens and putamen; Oligo, oligodendrocyte; OPC, oligodendrocyte precursor cell; OUD, opioid use disorder; UC, unaffected control individual.

Consistent with the idea that the long-lasting effects of opioid exposure are mediated through epigenetic modification, the central hub gene that emerged in our network is *KMT2D*, a histone methyltransferase with a direct relationship with ERα. As discussed above, activated ER forms a complex with other transcription factors and binds chromatin at specific sequences. KMT2D actively modulates chromatin, creating a more open chromatin state so that the activated ER complex can be recruited to ER response elements in the genome and promote ER-dependent transcription (30,70). This is interesting because it is a potential link between epigenetic regulation on the one hand and sex specificity on the other. Sex differences have previously been described in the epigenetic regulation of opioid-related genes. In a retrospective study of 250 outpatients who were experiencing chronic noncancer pain, there were sex differences in methylation of the promoter region of *OPRM1*, which was significantly correlated with lower rates of OUD in females (28). We observed a variety of alterations in opioid receptor expression across different patterns of opioid exposure between males and females in rodent models and in individuals with OUD (Table S7). Unpacking the mechanisms by which opioid exposure modulates the ability of transcriptional regulators to interact with chromatin is integral for understanding how environment and experience shape gene expression in the context of addiction. Together, our findings demonstrate how the relationship between opioid use, gene expression, and its regulation through epigenetic modifications is informed by input from gonadal hormones with consequences for synaptic modeling in corticostriatal circuitry.

Regardless of sex or exposure paradigm, HNF4A was identified as an upstream regulator across all concordant pathways. HNF4A is a nuclear transcription factor that binds DNA as a homodimer and plays a role in the development of several major organ systems including the liver, kidneys, and intestines (71–74). Converging lines of evidence implicate HNF4A in the development of several kinds of cancer (75–77). Specifically, HNF4A interacts with the Wnt/β-catenin pathway to guide cell adhesion, cell proliferation, apoptosis, and tumorigenesis (78–80). Interestingly, Wnt/β-catenin was the strongest pathway finding in our female with OUD versus nonvolitional rodent female intoxication comparison, and molecular mechanisms of cancer emerged in several of our

concordance findings. It is currently unclear what this means for the development of dependence, but the consistency of this finding suggests that HNF4A should be a focus of study in future OUD work.

We identified highly connected hub genes within the lightgreen module that were specific to OUD: *ANKRD11*, *CAMTA2*, *IQSEC1*, *RIPOR1*, and *SKI*. Previous evidence has linked the activity and regulation of *CAMTA2*, *IQSEC1*, and *RIPOR1* to OUD. CAMTA2 is a member of the ubiquitous CaMKs (Ca^{2+}/calmodulin-dependent protein kinase family), which are crucial players in producing the synaptic changes that underlie the formation of memory (81–83). Although CAMTA2 has not been directly linked to opioid addiction, this protein family generally, and 1 member, CaMKII specifically, have been linked to the development of opioid tolerance and dependence (84–88). CaMKII activity is increased by morphine treatment, and inhibiting CaMKII in rat hippocampus attenuates morphine tolerance and dependence (89,90). IQSEC1 has been identified as a potential regulator for receptor endocytosis by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of putative targets for responsive NAc microRNAs after morphine SA (91), which has direct relevance to mu opioid receptor desensitization, a molecular correlate of the development of dependence. Lastly, RIPOR1 is a member of the Rho family of GTPases, which regulate cellular processes such as cytoskeleton remodeling and synapse maintenance (92–94). Drugs of abuse, including morphine and heroin, alter the activity of these proteins (95). In addition, we compared the genes from our lightgreen module with genes linked to OUD by existing genome-wide association study (GWAS) work. Two genes stood out as particularly interesting, *GABRG2* and *FURIN*. *GABRG2* encodes a GABA (gamma-aminobutyric acid) receptor subunit. Not only has *GABRG2* been linked to both alcohol use disorder and OUD (96,97), but we previously also demonstrated a microglia-specific upregulation of *GABRG2* in OUD using single-nuclei transcriptomics in human OUD (33). FURIN is a proteolytic enzyme and notably has a single variant association with OUD as identified through GWAS (98–101).

Our data provide crucial nuance to understanding sex-specific mechanisms of OUD in human subjects. However, we acknowledge several limitations to our work. First, due to the nature of human postmortem work, we cannot accurately compare the effects, whether additive or synergistic, of polysubstance use on molecular alterations in the brain. Second, each of the individuals in our human cohort used opioids for years and terminating from drug toxicity and overdose. Thus, it remains difficult to definitively parse the acute versus chronic opioid effects on molecular alterations in human brains. However, the overlap between molecular alterations in humans and rodent models provides some insights. Third, the duration of opioid administration, the type and dose of opioids administered (oxycodone vs. morphine), and the pattern of opioid use, including the duration of withdrawal from opioids (28 vs. 14 days), were different in rats and mice. Nevertheless, our findings support the notion that opioid addiction is driven by large-scale gene alterations in the NAc in both humans and rodents. Furthermore, these results suggest that researchers may consider the relationship between biological sex and exposure paradigm when designing preclinical studies to investigate mechanisms with relevance to human OUD (e.g., use of a nonvolitional paradigm to model female OUD; use of a volitional paradigm to model male OUD). This work deepens our understanding of sex specificity in OUD and opens the door for future research into putative therapies.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the National Institutes of Health (NIH) Helping End Addiction Long-term Initiative under National Heart, Lung, and Blood Institute (Grant No. R01HL150432 [to RWL]), National Institute on Drug Abuse (Grant Nos. R01DA051390 [to MLS and RWL], R21DA058174 [to RWL], and U01DA044451 [to OG and AAP]).

MLS and RWL obtained funding for the project. MLS and RWL conceptualized and managed the project and provided supervision for data analyses and interpretations. MAS, NH, XX, BNP, GCT, MLS, and RWL analyzed the datasets. LCSW, AAP, OG, and RWL provided expertise on the rat experiments. LM and OG provided rat tissues. DE and JAB provided expertise on the mouse experiments and provided mouse tissues. JM, NS, BRW, MCG, and MKF processed rodent tissues and prepared tissues for sequencing. RWL and JAS provided expertise and guidance for tissue processing and sequencing. MAS, NH, JAB, OG, MLS, and RWL wrote the article and created the figures and supplemental information. All authors participated in reviewing and editing the article for publication.

We thank the staff and technicians who work as part of the Brain Tissue Program at the University of Pittsburgh. Human tissue was obtained from the NIH NeuroBioBank and the University of Pittsburgh Tissue Donation Program.

A previous version of this article was published as a preprint on ResearchSquare: https://doi.org/10.21203/rs.3.rs-5006061/v1.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania (MAS, NH, MLS); Center for Neuroscience at University of Pittsburgh, Pittsburgh, Pennsylvania (NH, MLS); Department of Biostatistics, University of Pittsburgh, Pittsburgh, Pennsylvania (XX, GCT); Department of Psychiatry, University of California San Diego, La Jolla, California (LM, AAP, OG); Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (DE, JAB); Department of Biology, Bridgewater State University, Bridgewater, Massachusetts (JM, JAS); Department of Pharmacology, Physiology & Biophysics, Boston University School of Medicine, Boston, Massachusetts (NS, MCG); Department of Psychiatry, University of Massachusetts Chan Medical School, Worcester, Massachusetts (BRW, MCG, MKF, RWL); Medical Scientist Training Program, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania (BNP); Department of Internal Medicine, Section on Molecular Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina (LCSW); Institute for Genomic Medicine, University of California San Diego, La Jolla, California (AAP); and Department of Neurobiology, University of Massachusetts Chan Medical School, Worcester, Massachusetts (RWL).

Address correspondence to Marianne L. Seney, Ph.D., at seneyml@upmc.edu, or Ryan W. Logan, Ph.D., at ryan.logan@umassmed.edu.

Received Dec 20, 2024; revised Jan 23, 2025; accepted Feb 15, 2025.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.bpsgos.2025.100476.

REFERENCES

1. Upp LA, Waljee JF (2020): The opioid epidemic. Clin Plast Surg 47:181–190.
2. Spencer MR, Miniño AM, Warner M (2022): Drug overdose deaths in the United States, 2001–2021. NCHS data brief (457):1–8.
3. Ahmad FB, Cisewski JA, Rossen LM, Sutton P (2024): Provisional drug overdose death counts. National Center for Health Statistics.

Available at: https://www.cdc.gov/nchs/nvss/vsrr/drug-overdose-data.htm. Accessed January 17, 2025.
4. Nunes EV, Gordon M, Friedmann PD, Fishman MJ, Lee JD, Chen DT, *et al.* (2018): Relapse to opioid use disorder after inpatient treatment: Protective effect of injection naltrexone. J Subst Abuse Treat 85:49–55.
5. Smyth BP, Barry J, Keenan E, Ducray K (2010): Lapse and relapse following inpatient treatment of opiate dependence. Ir Med J 103:176–179.
6. Gossop M, Green L, Phillips G, Bradley B (1989): Lapse, relapse and survival among opiate addicts after treatment. A prospective follow-up study. Br J Psychiatry 154:348–353.
7. Strang J, Volkow ND, Degenhardt L, Hickman M, Johnson K, Koob GF, *et al.* (2020): Opioid use disorder. Nat Rev Dis Primers 6:3.
8. Adinoff B, Rilling LM, Williams MJ, Schreffler E, Schepis TS, Rosvall T, Rao U (2007): Impulsivity, neural deficits, and the addictions: The "oops" factor in relapse. J Addict Dis 26(suppl 1):25–39.
9. Koob GF (2020): Neurobiology of opioid addiction: Opponent process, hyperkatifeia, and negative reinforcement. Biol Psychiatry 87:44–53.
10. Koch T, Höllt V (2008): Role of receptor internalization in opioid tolerance and dependence. Pharmacol Ther 117:199–206.
11. Sandoval-Sierra JV, Salgado García FI, Brooks JH, Derefinko KJ, Mozhui K (2020): Effect of short-term prescription opioids on DNA methylation of the OPRM1 promoter. Clin Epigenet 12:76.
12. Liu A, Dai Y, Mendez EF, Hu R, Fries GR, Najera KE, *et al.* (2021): Genome-wide correlation of DNA methylation and gene expression in postmortem brain tissues of opioid use disorder patients. Int J Neuropsychopharmacol 24:879–891.
13. Grimm SL, Mendez EF, Stertz L, Meyer TD, Fries GR, Gandhi T, *et al.* (2022): MicroRNA-mRNA networks are dysregulated in opioid use disorder postmortem brain: Further evidence for opioid-induced neurovascular alterations. Front Psychiatry 13:1025346.
14. Wang Y, Singh A, Li G, Yue S, Hertel K, Wang ZJ (2023): Opioid induces increased DNA damage in prefrontal cortex and nucleus accumbens. Pharmacol Biochem Behav 224:173535.
15. Mendez EF, Wei H, Hu R, Stertz L, Fries GR, Wu X, *et al.* (2021): Angiogenic gene networks are dysregulated in opioid use disorder: Evidence from multi-omics and imaging of postmortem human brain. Mol Psychiatry 26:7803–7812.
16. Seney ML, Kim SM, Glausier JR, Hildebrand MA, Xue X, Zong W, *et al.* (2021): Transcriptional alterations in dorsolateral prefrontal cortex and nucleus accumbens implicate neuroinflammation and synaptic remodeling in opioid use disorder. Biol Psychiatry 90:550–562.
17. Eacret D, Manduchi E, Noreck J, Tyner E, Fenik P, Dunn AD, *et al.* (2023): Mu-opioid receptor-expressing neurons in the paraventricular thalamus modulate chronic morphine-induced wake alterations. Transl Psychiatry 13:78.
18. Carrette LLG, de Guglielmo G, Kallupi M, Maturin L, Brennan M, Boomhower B, *et al.* (2021): The cocaine and oxycodone biobanks, two repositories from genetically diverse and behaviorally characterized rats for the study of addiction. eNeuro 8:ENEURO.0033-21.2021.
19. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015): limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47.
20. Cahill KM, Huo Z, Tseng GC, Logan RW, Seney ML (2018): Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach. Sci Rep 8:9588.
21. Plaisier SB, Taschereau R, Wong JA, Graeber TG (2010): Rank-rank hypergeometric overlap: Identification of statistically significant overlap between gene-expression signatures. Nucleic Acids Res 38:e169.
22. Langfelder P, Horvath S (2008): WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 9:559.
23. Zhang B, Horvath S (2005): A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol 4:Article17.
24. Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Dalla Favera R, Califano A (2006): ARACNE: An algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. BMC Bioinformatics 7(suppl 1):S7.
25. Baldo BA (2016): Prefrontal cortical opioids and dysregulated motivation: A network hypothesis. Trends Neurosci 39:366–377.
26. Hammond CJ, Park G, Kady A, Rathod K, Rahman N, Vidal C, *et al.* (2022): Sex-based differences in psychiatric symptoms and opioid abstinence during buprenorphine/naloxone treatment in adolescents with opioid use disorders. J Subst Abuse Treat 133:108495.
27. Kennedy AP, Epstein DH, Phillips KA, Preston KL (2013): Sex differences in cocaine/heroin users: Drug-use triggers and craving in daily life. Drug Alcohol Depend 132:29–37.
28. Agulló L, Muriel J, Margarit C, Escorial M, Garcia D, Herrero MJ, *et al.* (2023): Sex differences in opioid response linked to OPRM1 and COMT genes DNA methylation/genotypes changes in patients with chronic pain. J Clin Med 12:3449.
29. Arnal JF, Lenfant F, Metivier R, Flouriot G, Henrion D, Adlanmerini M, *et al.* (2017): Membrane and nuclear estrogen receptor alpha actions: From tissue specificity to medical implications. Physiol Rev 97:1045–1087.
30. Castel P, Toska E (2019): Chromatin regulation at the intersection of estrogen receptor and PI3K pathways in breast cancer. Mol Cell Oncol 6:e1625620.
31. Browne CJ, Godino A, Salery M, Nestler EJ (2020): Epigenetic mechanisms of opioid addiction. Biol Psychiatry 87:22–33.
32. Tran MN, Maynard KR, Spangler A, Huuki LA, Montgomery KD, Sadashivaiah V, *et al.* (2021): Single-nucleus transcriptome analysis reveals cell-type-specific molecular signatures across reward circuitry in the human brain. Neuron 109:3088–3103.e5.
33. Phan BN, Ray MH, Xue X, Fu C, Fenster RJ, Kohut SJ, *et al.* (2024): Single nuclei transcriptomics in human and non-human primate striatum in opioid use disorder. Nat Commun 15:878.
34. Westermeyer J, Boedicker AE (2000): Course, severity, and treatment of substance abuse among women versus men. Am J Drug Alcohol Abuse 26:523–535.
35. Knouse MC, Briand LA (2021): Behavioral sex differences in cocaine and opioid use disorders: The role of gonadal hormones. Neurosci Biobehav Rev 128:358–366.
36. Randall CL, Roberts JS, Del Boca FK, Carroll KM, Connors GJ, Mattson ME (1999): Telescoping of landmark events associated with drinking: A gender comparison. J Stud Alcohol 60:252–260.
37. Back SE, Payne RL, Wahlquist AH, Carter RE, Stroud Z, Haynes L, *et al.* (2011): Comparative profiles of men and women with opioid dependence: Results from a national multisite effectiveness trial. Am J Drug Alcohol Abuse 37:313–323.
38. Yu J, Zhang S, Epstein DH, Fang Y, Shi J, Qin H, *et al.* (2007): Gender and stimulus difference in cue-induced responses in abstinent heroin users. Pharmacol Biochem Behav 86:485–492.
39. Giacomuzzi SM, Riemer Y, Ertl M, Kemmler G, Rossler H, Hinterhuber H, Kurz M (2005): Gender differences in health-related quality of life on admission to a maintenance treatment program. Eur Addict Res 11:69–75.
40. McHugh RK, Devito EE, Dodd D, Carroll KM, Potter JS, Greenfield SF, *et al.* (2013): Gender differences in a clinical trial for prescription opioid dependence. J Subst Abuse Treat 45:38–43.
41. Vigna-Taglianti FD, Burroni P, Mathis F, Versino E, Beccaria F, Rotelli M, *et al.* (2016): Gender differences in heroin addiction and treatment: Results from the VEdeTTE cohort. Subst Use Misuse 51:295–309.
42. Carroll ME, Morgan AD, Lynch WJ, Campbell UC, Dess NK (2002): Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: Phenotype and sex differences. Psychopharmacol (Berl) 161:304–313.
43. Cicero TJ, Aylward SC, Meyer ER (2003): Gender differences in the intravenous self-administration of mu opiate agonists. Pharmacol Biochem Behav 74:541–549.

44. Kimbrough A, Kononoff J, Simpson S, Kallupi M, Sedighim S, Palomino K, *et al.* (2020): Oxycodone self-administration and withdrawal behaviors in male and female Wistar rats. Psychopharmacol (Berl) 237:1545–1555.
45. Klein LC, Popke EJ, Grunberg NE (1997): Sex differences in effects of predictable and unpredictable footshock on fentanyl self-administration in rats. Exp Clin Psychopharmacol 5:99–106.
46. Lynch WJ, Carroll ME (1999): Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacol (Berl) 144:77–82.
47. Vazquez M, Frazier JH, Reichel CM, Peters J (2020): Acute ovarian hormone treatment in freely cycling female rats regulates distinct aspects of heroin seeking. Learn Mem 27:6–11.
48. Zanni G, DeSalle MJ, Deutsch HM, Barr GA, Eisch AJ (2020): Female and male rats readily consume and prefer oxycodone to water in a chronic, continuous access, two-bottle oral voluntary paradigm. Neuropharmacology 167:107978.
49. Chen P, Li B, Ou-Yang L (2022): Role of estrogen receptors in health and disease. Front Endocrinol (Lausanne) 13:839005.
50. Hilakivi-Clarke L (1996): Role of estradiol in alcohol intake and alcohol-related behaviors. J Stud Alcohol 57:162–170.
51. Hoffmann S, Gerhardt S, Mühle C, Reinhard I, Reichert D, Bach P, *et al.* (2024): Associations of menstrual cycle and progesterone-to-estradiol ratio with alcohol consumption in alcohol use disorder: A sex-separated multicenter longitudinal study. Am J Psychiatry 181:445–456.
52. Kokane SS, Perrotti LI (2020): Sex differences and the role of estradiol in mesolimbic reward circuits and vulnerability to cocaine and opiate addiction. Front Behav Neurosci 14:74.
53. Mühle C, Barry B, Weinland C, Kornhuber J, Lenz B (2019): Estrogen receptor 1 gene variants and estradiol activities in alcohol dependence. Prog Neuropsychopharmacol Biol Psychiatry 92:301–307.
54. Segarra AC, Agosto-Rivera JL, Febo M, Lugo-Escobar N, Menéndez-Delmestre R, Puig-Ramos A, Torres-Diaz YM (2010): Estradiol: A key biological substrate mediating the response to cocaine in female rats. Horm Behav 58:33–43.
55. Mirbaha H, Tabaeizadeh M, Shaterian-Mohammadi H, Tahsili-Fahadan P, Dehpour AR (2009): Estrogen pretreatment modulates morphine-induced conditioned place preference in ovariectomized mice. Pharmacol Biochem Behav 92:399–403.
56. Roth ME, Casimir AG, Carroll ME (2002): Influence of estrogen in the acquisition of intravenously self-administered heroin in female rats. Pharmacol Biochem Behav 72:313–318.
57. Towers EB, Setaro B, Lynch WJ (2023): Estradiol enhances the development of addiction-like features in a female rat model of opioid use disorder. Neuroendocrinology 113:1099–1111.
58. Ford MM, Eldridge JC, Samson HH (2004): Determination of an estradiol dose–response relationship in the modulation of ethanol intake. Alcohol Clin Exp Res 28:20–28.
59. Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME (2007): Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. Exp Clin Psychopharmacol 15:461–471.
60. Martinez LA, Gross KS, Himmler BT, Emmitt NL, Peterson BM, Zlebnik NE, *et al.* (2016): Estradiol facilitation of cocaine self-administration in female rats requires activation of mGluR5. eNeuro 3:ENEURO.0140-16.2016.
61. Rajasingh J, Bord E, Qin G, Ii M, Silver M, Hamada H, *et al.* (2007): Enhanced voluntary alcohol consumption after estrogen supplementation negates estrogen-mediated vascular repair in ovariectomized mice. Endocrinology 148:3618–3624.
62. Ramôa CP, Doyle SE, Lycas MD, Chernau AK, Lynch WJ (2014): Diminished role of dopamine D1-receptor signaling with the development of an addicted phenotype in rats. Biol Psychiatry 76:8–14.
63. Ramôa CP, Doyle SE, Naim DW, Lynch WJ (2013): Estradiol as a mechanism for sex differences in the development of an addicted phenotype following extended access cocaine self-administration. Neuropsychopharmacology 38:1698–1705.
64. Sedki F, Gardner Gregory J, Luminare A, D'Cunha TM, Shalev U (2015): Food restriction-induced augmentation of heroin seeking in female rats: Manipulations of ovarian hormones. Psychopharmacology 232:3773–3782.
65. Stewart J, Woodside B, Shaham Y (1996): Ovarian hormones do not affect the initiation and maintenance of intravenous self-administration of heroin in the female rat. Psychobiology 24:154–159.
66. Gross KS, Moore KM, Meisel RL, Mermelstein PG (2018): mGluR5 mediates dihydrotestosterone-induced nucleus accumbens structural plasticity, but not conditioned reward. Front Neurosci 12:855.
67. Peterson BM, Mermelstein PG, Meisel RL (2015): Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. Brain Struct Funct 220:2415–2422.
68. Proaño SB, Miller CK, Krentzel AA, Dorris DM, Meitzen J (2024): Sex steroid hormones, the estrous cycle, and rapid modulation of glutamatergic synapse properties in the striatal brain regions with a focus on 17β-estradiol and the nucleus accumbens. Steroids 201:109344.
69. Wallin-Miller K, Li G, Kelishani D, Wood RI (2016): Anabolic-androgenic steroids decrease dendritic spine density in the nucleus accumbens of male rats. Neuroscience 330:72–78.
70. Toska E, Osmanbeyoglu HU, Castel P, Chan C, Hendrickson RC, Elkabets M, *et al.* (2017): PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. Science 355:1324–1330.
71. Hunter AL, Poolman TM, Kim D, Gonzalez FJ, Bechtold DA, Loudon ASI, *et al.* (2022): HNF4A modulates glucocorticoid action in the liver. Cell Rep 39:110697.
72. Lei X, Ketelut-Carneiro N, Shmuel-Galia L, Xu W, Wilson R, Vierbuchen T, *et al.* (2022): Epithelial HNF4A shapes the intraepithelial lymphocyte compartment via direct regulation of immune signaling molecules. J Exp Med 219:e20212563.
73. Ng NHJ, Ghosh S, Bok CM, Ching C, Low BSJ, Chen JT, *et al.* (2024): HNF4A and HNF1A exhibit tissue specific target gene regulation in pancreatic beta cells and hepatocytes. Nat Commun 15:4288.
74. Yoshimura Y, Muto Y, Omachi K, Miner JH, Humphreys BD (2023): Elucidating the proximal tubule HNF4A gene regulatory network in human kidney organoids. J Am Soc Nephrol 34:1672–1686.
75. Besskaya V, Zhang H, Bian Y, Liang J, Bi G, Shan G, *et al.* (2023): Hepatic nuclear factor 4 alpha promotes the ferroptosis of lung adenocarcinoma via transcriptional activation of cytochrome P450 oxidoreductase. PeerJ 11:e15377.
76. Sang L, Wang X, Bai W, Shen J, Zeng Y, Sun J (2022): The role of hepatocyte nuclear factor 4α (HNF4α) in tumorigenesis. Front Oncol 12:1011230.
77. Zhao Y, Tang H, Xu J, Sun F, Zhao Y, Li Y (2024): HNF4A-bridging the gap between intestinal Metaplasia and gastric cancer. Evol Bioinform Online 20:11769343241249017.
78. Ma HM, Zhang Q, Yang XM, Hu Y, Zhang J, Chen L, *et al.* (2022): HNF4A regulates the proliferation and tumor formation of cervical cancer cells through the Wnt/β-catenin pathway. Oxid Med Cell Longev 2022:8168988.
79. Cattin AL, Le Beyec J, Barreau F, Saint-Just S, Houllier A, Gonzalez FJ, *et al.* (2009): Hepatocyte nuclear factor 4alpha, a key factor for homeostasis, cell architecture, and barrier function of the adult intestinal epithelium. Mol Cell Biol 29:6294–6308.
80. Yang M, Li SN, Anjum KM, Gui LX, Zhu SS, Liu J, *et al.* (2013): A double-negative feedback loop between Wnt-β-catenin signaling and HNF4α regulates epithelial-mesenchymal transition in hepatocellular carcinoma. J Cell Sci 126:5692–5703.
81. Giese KP, Mizuno K (2013): The roles of protein kinases in learning and memory. Learn Mem 20:540–552.
82. Nicoll RA, Schulman H (2023): Synaptic memory and CaMKII. Physiol Rev 103:2877–2925.
83. Vigil FA, Giese KP (2018): Calcium/calmodulin-dependent kinase II and memory destabilization: A new role in memory maintenance. J Neurochem 147:12–23.

84. Müller CP, Quednow BB, Lourdusamy A, Kornhuber J, Schumann G, Giese KP (2016): CaM kinases: From memories to addiction. Trends Pharmacol Sci 37:153–166.
85. Andersen JM, Opdal SH, Müller CP, Boix F (2020): CaMKII is activated in opioid induced conditioned place preference, but αCaMKII Thr286 autophosphorylation is not necessary for its establishment. Behav Brain Res 390:112676.
86. Ma SX, Kim HC, Lee SY, Jang CG (2018): TRPV1 modulates morphine self-administration via activation of the CaMKII-CREB pathway in the nucleus accumbens. Neurochem Int 121:1–7.
87. Sadat-Shirazi MS, Ahmadian-Moghadam H, Khalifeh S, Nouri Zadeh-Tehrani S, Farahmandfar M, Zarrindast MR (2019): The role of calcium-calmodulin-dependent protein kinase II in modulation of spatial memory in morphine sensitized rats. Behav Brain Res 359:298–303.
88. Shukla PK, Tang L, Wang ZJ (2006): Phosphorylation of neurogranin, protein kinase C, and Ca2+/calmodulin dependent protein kinase II in opioid tolerance and dependence. Neurosci Lett 404:266–269.
89. Fan GH, Wang LZ, Qiu HC, Ma L, Pei G (1999): Inhibition of calcium/calmodulin-dependent protein kinase II in rat hippocampus attenuates morphine tolerance and dependence. Mol Pharmacol 56:39–45.
90. Tang L, Shukla PK, Wang LX, Wang ZJ (2006): Reversal of morphine antinociceptive tolerance and dependence by the acute supraspinal inhibition of Ca(2+)/calmodulin-dependent protein kinase II. J Pharmacol Exp Ther 317:901–909.
91. Kim J, Im HI, Moon C (2018): Intravenous morphine self-administration alters accumbal microRNA profiles in the mouse brain. Neural Regen Res 13:77–85.
92. Hall A (1998): Rho GTPases and the actin cytoskeleton. Science 279:509–514.
93. Kaibuchi K, Kuroda S, Amano M (1999): Regulation of the cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. Annu Rev Biochem 68:459–486.
94. Mackay DJ, Nobes CD, Hall A (1995): The Rho's progress: A potential role during neuritogenesis for the rho family of GTPases. Trends Neurosci 18:496–501.
95. Ru Q, Wang Y, Zhou E, Chen L, Wu Y (2023): The potential therapeutic roles of Rho GTPases in substance dependence. Front Mol Neurosci 16:1125277.
96. Huang W, Penaherrera EP, Desir DF, Gamarro DL, Cottrell J, Chu T, Chang SL (2019): Bi-directional acceleration of alcohol use and opioid use disorder. J Drug Alcohol Res 2019:236084.
97. Li D, Sulovari A, Cheng C, Zhao H, Kranzler HR, Gelernter J (2014): Association of gamma-aminobutyric acid A receptor α2 gene (GABRA2) with alcohol use disorder. Neuropsychopharmacology 39:907–918.
98. Deak JD, Zhou H, Galimberti M, Levey DF, Wendt FR, Sanchez-Roige S, *et al.* (2022): Genome-wide association study in individuals of European and African ancestry and multi-trait analysis of opioid use disorder identifies 19 independent genome-wide significant risk loci. Mol Psychiatry 27:3970–3979.
99. Gaddis N, Mathur R, Marks J, Zhou L, Quach B, Waldrop A, *et al.* (2022): Multi-trait genome-wide association study of opioid addiction: OPRM1 and beyond. Sci Rep 12:16873.
100. Holen B, Shadrin AA, Icick R, Filiz TT, Hindley G, Rødevand L, *et al.* (2023): Genome-wide analyses reveal novel opioid use disorder loci and genetic overlap with schizophrenia, bipolar disorder, and major depression. Addict Biol 28:e13282.
101. Kember RL, Vickers-Smith R, Xu H, Toikumo S, Niarchou M, Zhou H, *et al.* (2022): Cross-ancestry meta-analysis of opioid use disorder uncovers novel loci with predominant effects in brain regions associated with addiction. Nat Neurosci 25:1279–1287.