Vitamin D and N-Acetylcysteine as Neuroprotective Modulators: A Gene Network Analysis of Opioid Resistance Mechanisms in the Human Brain

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Opioid use disorder (OUD) is a major public health issue in Canada and is associated with altered brain circuits involved in reward, stress, and cognitive control. Although vitamin D and N-acetylcysteine (NAC) have been studied in relation to neurobiological processes such as inflammation, oxidative stress, and glutamatergic signaling, their relevance to opioid-related molecular pathways remains incompletely understood. This study used a literature-based gene curation and GeneMANIA network analysis approach to examine whether genes previously associated with vitamin D or NAC are connected to molecular pathways that overlap with opioid-relevant brain processes. Published studies were reviewed to identify addiction-implicated brain regions and genes linked to vitamin D or NAC in those regions. Curated gene sets were analyzed using GeneMANIA to identify interaction networks and enriched biological functions. Vitamin D-linked gene networks were associated with transcriptional regulation, neurotrophin-related signaling, mitochondrial function, and immune-related pathways. NAC-linked gene networks were associated with glutamatergic synapse-related functions, redox and glutathione metabolism, and inflammatory signaling. These findings do not demonstrate direct effects of vitamin D or NAC in opioid-exposed human brain tissue. Rather, they suggest that vitamin D- and NAC-associated genes may overlap with biological pathways previously implicated in opioid-related neurobiology. This study provides a hypothesis-generating framework for future experimental studies examining whether these pathways are relevant to OUD, treatment response, or neurobiological vulnerability.

Keywords: Addiction-related brain regions, Gene network analysis, Glutamatergic signaling, N-acetylcysteine, Neuroinflammation, Neuroprotection, Opioid use disorder (OUD), Oxidative stress, Vitamin D

Introduction

Canada has experienced a consistent rise in drug overdose mortality over the recent decades primarily due to healthcare practices, evolving drug markets, and social vulnerabilities[1]. Between 1974 and 2023, 80,944 overdose deaths were recorded, with a sharp increase in 2013–2023[1]. Since 2016, more than 30,000 deaths occurred due to opioid-related overdoses in Canada, which is significantly more than from other, major accidental death causes combined; Canada's current drug-poisoning public health epidemic is disproportionately driven by opioids[2]. Beyond mortality rates, Canada has suffered lost productivity due to substance use totaling $15.7 billion in 2014 with opioids accounting for 12% of the economic burden, highest among illegal drugs[3]. Within the opioid-affected population, psychiatric comorbidity was found to be common with 87% of 55,924 individuals enrolled in opioid agonist treatment affected. It was also associated with increased all-cause mortality rates and higher acute care use[4]. To address this issue, the Canadian Government has expanded its initiatives in implementing treatment, recovery, and harm-reduction programs. However, these services are not always accessible to all substance users due to operational barriers or geographical barriers, and they also require a significant expense[5].

Literature Review

Recent studies have examined how certain nutrients and biochemical modulators can influence opioid-related neurobiology. For instance, Vitamin D deficiency amplifies increased vulnerability to opioid use by intensifying reward signaling in vivo models, and supplementation has demonstrated improvements in the cognitive and mental health of patients undergoing methadone maintenance treatment[6–8]. N-acetylcysteine (NAC) has also been shown to reduce cravings, withdrawal symptoms, and physical dependence[9–11]. These findings indicate the possibility that certain over-the-counter (OTC) sup-

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plements, such as Vitamin D and NAC, may have neuroprotective or modulatory properties that are relevant to opioid addiction mechanisms. Given that they are widely available in Canada, relatively low in cost, and generally have mild side effects, they may be possible alternative areas of investigation and merit scientific attention as complementary, low-risk modulators.

Although various supplements such as vitamin D and NAC have been identified to have neuromodulatory roles, current understanding of their mechanisms, specifically in the opioid-exposed human brain, is limited. Existing studies typically focus on vitamin D or NAC-associated gene expression changes within a single brain region, involving a small number of genes, around one to four per study [12,13]. This candidate-gene approach makes it difficult to identify and examine broader molecular mechanisms or to determine how vitamin D- or NAC-linked genes interact within shared gene networks [13]. Most mechanistic gene-expression data also come from non-opioid clinical populations or animal models, limiting their relevance to the opioid-exposed human brain. However, a study has successfully attempted to bridge this gap by pairing epidemiological associations between vitamin D levels and opioid use with in vivo validation using rodent models, showing that vitamin D deficiency may lead to amplification of UV-induced endogenous and exogenous opioid responses [8]. Furthermore, high-throughput postmortem transcriptomic studies have mapped out multi-region molecular landscapes for human OUD, but the exact mechanism in which protective exogenous modulators map onto or interact with broader system-level networks is not well known [14]. For example, a rodent circuit-level study has shown that NAC can reverse heroin-induced structural adaptations, specifically enhanced astrocytic complexity and synaptic association [15]. Although these findings map NAC's capacity to restore synaptic and cellular homeostasis, the fact that they are constrained to isolated pathways limits how these localized effects interact in a system-wide gene interaction network.

Thus, the purpose of this study is to identify and analyze the molecular mechanisms by which vitamin D and NAC may influence neural pathways involved in opioid addiction, focusing on gene expression changes in addiction-implicated human brain regions and evaluating their relevance as potential modulators. Using data from the PubMed database and integrating region-specific findings on gene expression changes associated with vitamin D and NAC from prior studies, gene-network modelling was performed. This study aims to extend to a systems-level understanding of supplement-responsive molecular pathways. The main hypothesis is that genes linked to vitamin D and NAC converge within shared molecular interaction networks related to glutamatergic regulation, neuroinflammatory signaling, and oxidative stress, biological processes known to be disrupted in patients with OUD [5,9].

Methods

Structured literature search strategy

A structured literature review was conducted to identify published studies linking OUD, addiction-relevant human brain regions, vitamin D, N-acetylcysteine, and gene-level molecular pathways. Searches were conducted manually using PubMed, Web of Science, and Google Scholar. The search strategy was designed to identify three categories of studies: studies identifying human brain regions implicated in OUD or opioid-related treatment response, studies describing vitamin D- or NAC-related effects in the brain, and studies reporting gene expression, protein-level changes, or pathway-level molecular findings relevant to vitamin D or NAC in addiction-associated brain regions.

Gene selection from studies for curation of gene sets

From each study, genes that met the study's reported statistical significance criterion for differential expression or association were selected. If multiple-testing–adjusted statistics were reported, we prioritized adjusted thresholds, such as false discovery rate (FDR) /q-value < 0.05. If adjusted statistics were not reported but nominal p-values were provided, we used $p < 0.05$. For targeted, candidate-gene approach studies, genes were selected when the study reported a statistically supported difference or association using the stated threshold of the study. When studies did not provide extractable quantitative statistics for a gene, that gene was not used as a primary input.

GeneMANIA gene network analysis

To investigate the functional interactions among vitamin D and NAC-linked genes implicated in opioid addiction, curated gene sets were analyzed using the GeneMANIA online platform. Genes reported to show expression changes or functional relevance in human brain regions associated with OUD were collectively input into GeneMANIA. The analysis was conducted using default Homo sapiens settings, integrating multiple evidence-based datasets, including physical protein–protein interactions, genetic interactions, co-expression, co-localization, shared pathways, and protein domain similarity. For every network, a maximum of 20 resultant genes and 10 resultant attributes were permitted. The network weighing method was automatically determined by GeneMANIA. The enriched pathways and functional annotations were then examined to identify convergent molecular mechanisms potentially underlying neuroprotection, opioid resistance, or modulation of addiction-related signaling by vitamin D and NAC at a systems-network level. As a simple sensitivity analysis, regional GeneMANIA networks were re-run with resul-

tant genes set to 0 to assess whether enrichment themes persisted without network expansion. For larger curated gene sets, no-expansion runs preserved similar broad functional themes, although term rankings and statistical strength varied. This suggests that the main interpretations in these regions are supported at the level of the curated gene sets. In contrast, smaller curated gene sets yielded substantially different or absent enriched functions without expansion, indicating that broader functional interpretations in these regions depend more strongly on expanded-network structure and should therefore be interpreted cautiously as hypothesis-generating.

Selection process of enrichment functions from generated gene networks

For each network of a brain region, all enriched GeneMANIA functional annotations were considered, and those which met the FDR threshold criteria of < 0.05 were retained. To reduce redundancy while representing key functions, we ranked eligible terms by ascending FDR and selected a non-overlapping set. Overlapping terms are defined as multiple terms describing the same biological process; only the most significant term was retained in a given set of overlapping terms. After the selection process, if there were more than seven terms present, only the top seven were reported.

Key terms

In this paper, opioid resistance is a framework-level term to indicate molecular pathways that are implicated in tolerance, opioid-induced hyperalgesia, withdrawal-related negative affect, and relapse vulnerability. This work is a curated gene set synthesis and network enrichment analysis, not testing of opioid-exposed human outcomes. Therefore, the term opioid resistance is used to refer to potential alignment of gene-network themes with these opioid-relevant processes.

Quality assessment of source evidence and GeneMANIA analysis

Because this study used previously published studies for gene curation and then performed a new GeneMANIA network analysis, quality assessment was conducted in two parts. First, the source evidence used to identify input genes was evaluated for relevance, biological directness, brain-region specificity, gene-level support, statistical reporting, and risk of indirectness. Second, the GeneMANIA analysis performed in this study was evaluated for reproducibility and interpretation control. The quality assessment was not used as a formal meta-analysis or as a reason to automatically exclude all indirect studies. Instead, it was used to guide how cautiously each gene and network result should be interpreted. Studies using human brain tissue, clinical data, specific addiction-relevant brain regions, and statistically supported gene-level findings were considered stronger sources of evidence. Studies using animal models, in vitro systems, non-opioid disease models, review-based evidence, or broad pathway descriptions were considered more indirect and were interpreted only as hypothesis-generating evidence. For the GeneMANIA analysis, quality control focused on whether the analysis was performed consistently and transparently across all brain-region-specific gene networks. Input genes were organized by supplement and brain region before analysis. All networks were analyzed using the Homo sapiens setting in GeneMANIA. Added genes generated by GeneMANIA were interpreted as predicted or database-supported network neighbors, not as experimentally confirmed vitamin D- or NAC-regulated genes. Functional annotations were retained only when they met the FDR < 0.05 threshold. Redundant enriched terms describing similar biological processes were reduced by retaining the most statistically significant non-overlapping term. Therefore, the network results were interpreted as functional associations rather than causal mechanisms or direct evidence of supplement effects in opioid-exposed human brain tissue.

Gene Selection from Source Studies

Genes were selected from source papers using predefined criteria to reduce selective interpretation. First, all genes reported in each paper in relation to vitamin D, NAC, opioid-related biology, or addiction-relevant brain regions were considered as candidate genes. When a paper reported multiple genes, only genes that were explicitly named, relevant to the specific supplement or brain region being analyzed, and supported by gene-level, protein-level, or pathway-level evidence were selected for GeneMANIA input. Statistical significance thresholds from the original studies were applied whenever available; adjusted values such as FDR/q-value < 0.05 were prioritized, and nominal $p < 0.05$ was used when adjusted statistics were not reported. Genes mentioned only in the background or discussion without clear experimental or statistical support were not included as primary input genes. Therefore, the final gene sets represent selected, literature-supported candidate genes rather than all genes mentioned in the source papers.

Results

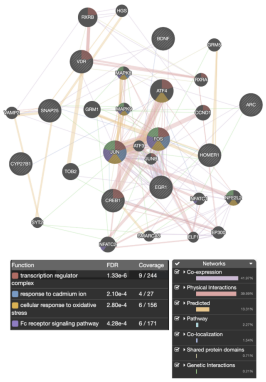
Vitamin D-Associated Gene Network in the Hippocampus

GeneMANIA network analysis of five curated vitamin D-associated hippocampal input genes: SNAP25, BDNF, CREB, VDR, and CYP27B1. The network was generated using the Homo sapiens setting with GeneMANIA network expansion enabled; therefore, the displayed network includes both the five input genes and GeneMANIA-added predicted network

Table 1 Vitamin D-associated genes and pathways identified from hippocampus-related studies. Evidence types were classified to distinguish direct intervention evidence from expression/localization and review-based mechanistic evidence.

Study	Key Genes / Pathways	Evidence Type
Liang et al., 2018 [12]	*SNAP25, BDNF, CREB*	Vitamin D dietary/intake intervention in an animal developmental model; supports vitamin D-associated hippocampal gene-expression changes, but not human opioid-specific treatment regulation
Mirarchi et al., 2023 [16]	*VDR, CYP27B1*	Review-based mechanistic evidence on vitamin D/VDR and microglia; not primary treatment evidence
Eyles et al., 2005 [17]	*VDR, CYP27B1*	Human brain expression/localization evidence; shows presence of *VDR* and 1α-hydroxylase/*CYP27B1* in human brain, not vitamin D treatment regulation.

Note. *VDR* encodes Vitamin D Receptor (VDR). *CYP27B1* encodes 25-hydroxyvitamin D 1α-hydroxylase.



List of input genes: Red
List of added genes: Black

RXRB	*ATF4*	*CCND1*	*ATF3*	*NFATC1*
HGS	*RXRA*	*ARC*	*JUNB*	*NFE2L2*
BDNF	*VAMP2*	*CYP27B1*	*HOMER1*	*NFATC2*
GRM5	*SNAP25*	*TOB2*	*SYT2*	*SMARCA5*
VDR	*GRM1*	*JUN*	*CREB1*	*ELF1*
MAPK8	*MAPK9*	*FOS*	*EGR1*	*EP300*

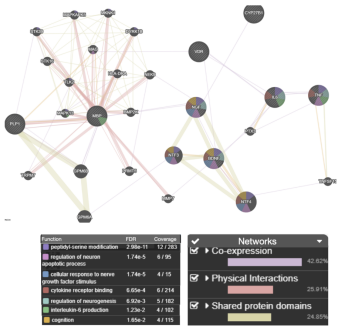
Input genes count	5
Added genes count	25
Node count	30
Has gene addition occurred?	Yes

Figure. 1 Gene network analysis results using curated vitamin D-associated input genes in the hippocampus.

neighbors. GeneMANIA-added genes should be interpreted as predicted or database-supported interaction partners, not as experimentally confirmed vitamin D-associated genes in the human hippocampus.

To identify the key genes associated with vitamin D treatment in the hippocampus, we reviewed four previous studies. Through these studies, the SNAP25, BDNF, CREB, VDR, and CYP27B1 were curated as vitamin D-associated input genes in the human hippocampus (Table 1). To further analyze the gene functions of these gene sets, GeneMANIA identified four key functions through gene network analysis: transcription regulator complex (red), response to cadmium ion (blue), cellular response to oxidative stress (orange), and Fc receptor signaling pathway (purple) (Figure 1). These networks also indicated that co-expression and physical interactions were the dominant relationships within the gene set, accounting for the top two network contribution percentages.

Vitamin D-Associated Gene Network in the Cerebral Cortex



List of input genes: Red
List of added genes: Black

GPM6A	*PRMT5*	*MMP7*	*VDR*
DYRK1B	*NGF*	*NTF3*	*CYP27B1*
PLP1	*BDNF*	*PTX3*	*TNF*
GPM6B	*IL6*	*NTF4*	*TNFSF15*
MAPK13	*MBP*	*BMP2K*	*TPRM7*
STK16	*TLK2*	*HLA-DRA*	*NEK9*
STK39	*MAPKAPK5*	*MKNK1*	*MAG*

Input genes count	8
Added genes count	20
Node count	28
Has gene addition occurred?	Yes

Figure. 2 Gene network analysis results using curated vitamin D-associated input genes in the cerebral cortex.

GeneMANIA network analysis of eight curated vitamin D-associated cerebral cortex input genes: *BDNF*, *NGF*, *VDR*, *CYP27B1*, *MBP*, *PLP1*, *IL6*, and *TNF*. The network was generated with GeneMANIA network expansion enabled; therefore, Enriched functional categories included peptidyl-serine modification, regulation of neuron apoptotic process, cellular response to nerve growth factor stimulus, cytokine receptor binding, regulation of neurogenesis, interleukin-6 production, and cognition. Added genes are interpreted as network-associated genes predicted from existing GeneMANIA evidence rather than direct vitamin D-associated genes in the human cerebral cortex.

To identify the key genes associated with vitamin D treatment in the cerebral cortex, we reviewed five previous studies. Through these studies, the *BDNF*, *NGF*, *VDR*, *CYP27B1*, *MBP*, *PLP1*, *IL6*, and *TNF* were curated as vitamin D-

Table 2 Vitamin D-associated genes/pathways in cerebral cortex-related studies.

Study	Key Genes / Pathways	Evidence Type
Eyles et al., 2013 [7]	*BDNF*	Review-based evidence / deficiency-status and neurodevelopmental context; not primary treatment evidence
Brown et al., 2003 [18]	*NGF*	Experimental vitamin D intervention in embryonic rat hippocampal neurons; supports vitamin D-induced NGF in a neuronal model, but not human cortex or opioid-specific evidence
Eyles et al., 2005 [17]	*VDR, CYP27B1*	Human brain expression/localization evidence; not treatment regulation
Gomez-Pinedo et al., 2020 [19]	*MBP, PLP1*	Direct vitamin D supplementation evidence in an animal demyelination/remyelination model; supports vitamin D-associated myelin protein expression, but not human cortex-specific or opioid-specific treatment regulation
Harms et al., 2011 [20]	*IL6, TNF*	Review-based mechanistic evidence related to vitamin D, inflammation, and brain function; not direct treatment-regulated gene evidence

associated input genes in the human cerebral cortex (Table 2). To further analyze the gene functions of these gene sets, GeneMANIA identified seven key functions through gene network analysis: peptidyl-serine modification (purple), regulation of neuron apoptotic process (pink), cellular response to nerve growth factor stimulus (blue), cytokine receptor binding (red), regulation of neurogenesis (cyan), interleukin-6 production (green), and cognition (orange) (Figure 2). These networks also indicated that co-expression, physical interactions, and shared protein domains were the dominant relationships within the gene set, accounting for the top three network contribution percentages.

Vitamin D-Associated Gene Network in the Amygdala

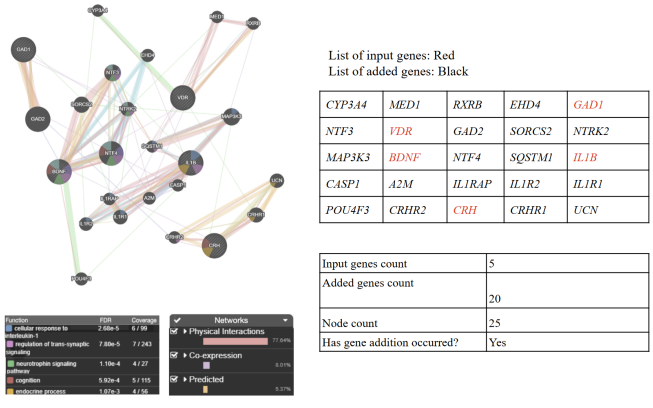


Figure. 3 Gene network analysis results using curated vitamin D-associated input genes in the amygdala.

GeneMANIA network analysis of five curated vitamin D-associated amygdala input genes: *BDNF*, *IL1B*, *GAD1*, *VDR*, and *CRH*. The analysis included GeneMANIA network expansion; therefore, the displayed network contains both the five input genes and GeneMANIA-added predicted network neighbors. Added genes represent predicted interaction partners and should not be interpreted as experimentally validated vitamin D-responsive genes in the amygdala.

To identify the key genes associated with vitamin D treatment in the amygdala, we reviewed three previous studies. Through these studies, the *BDNF*, *IL1B*, *GAD1*, *VDR*, and *CRH* were curated as vitamin D-associated input genes in the human amygdala (Table 3). To further analyze the gene functions of these gene sets, GeneMANIA identified seven key functions through gene network analysis: cellular response to interleukin-1 (purple), regulation of trans-synaptic signaling (pink), neurotrophin signaling pathway (green), cognition (red), and endocrine process (yellow) (Figure 3). These networks also indicated that co-expression and physical interactions were the dominant relationships within the gene set, accounting for the top two network contribution percentages.

NAC-Associated Gene Network in the Nucleus Accumbens

The seven input gene set included *SLC1A2*, *SLC7A11*, *GRM5*, *GRIN2A*, *GRIA1*, *GRIA2*, and *FOS*, which were curated from NAC- and nucleus accumbens-related studies summarized in Table 4. GeneMANIA network expansion was enabled; therefore, the displayed network includes both the seven input genes and GeneMANIA-added predicted network neighbors. GeneMANIA-added genes should be interpreted as predicted or database-supported interaction partners, not as experimentally confirmed NAC-associated genes in the human nucleus

Table 3 Vitamin D-associated genes/pathways in amygdala-related studies.

Study	Key Genes / Pathways	Evidence Type
Harms et al., 2011 [20]	*BDNF, IL1B*	Review-based mechanistic evidence; not direct treatment-regulated evidence
Eyles et al., 2013 [7]	*GAD1, VDR*	Review-based evidence / deficiency-status and neurodevelopmental context; not primary treatment evidence
Kuningas et al., 2009 [21]	*CRH*	Genetic association evidence involving VDR variants and cognitive/depressive phenotypes; not vitamin D treatment evidence and not direct CRH treatment regulation

Table 4 NAC-associated genes and pathways identified from nucleus accumbens-related studies

Study	Key Genes / Pathways	Evidence Type
Reissner et al., 2014 [10]	*SLC1A2* / GLT-1	Direct NAC intervention in an animal cocaine-reinstatement model; supports NAC-linked GLT-1/*SLC1A2* mechanism, but not human opioid-specific evidence
Moussawi et al., 2011 [22]	*SLC7A11* / *xCT*, *SLC1A2* / GLT-1	Direct NAC intervention evidence in a rat cocaine self-administration/extinction model. Chronic NAC restored cocaine-induced cortico-accumbens glutamate-homeostasis abnormalities and reduced relapse-like cocaine seeking. This supports NAC-associated nucleus accumbens glutamate-homeostasis mechanisms, but it remains animal, cocaine-model evidence, not human opioid-specific evidence
Schiavi et al., 2022 [23]	*Fos* / c-Fos	Direct NAC intervention in a rat nucleus accumbens model; supports NAC-related cFos activation, not human opioid-specific evidence
Schiavi et al., 2022 [23]	*GRM5, GRIN2A, GRIA1, GRIA2*	Non-opioid animal NAC intervention / autism-valproic acid (VPA) model; indirect evidence for NAC effects on glutamatergic biology, not opioid-specific or human nucleus accumbens treatment evidence

Note. *SLC1A2* encodes glutamate transporter 1 (GLT-1). *SLC7A11* encodes xCT, the light chain of the cystine/glutamate antiporter. *FOS* encodes the activity-dependent transcription factor c-Fos.

accumbens.

To identify the key genes associated with NAC treatment in the nucleus accumbens, we reviewed five previous studies. Through these studies, the *SLC1A2*, *SLC7A11*, *GRM5*, *GRIN2A*, *GRIA1*, *GRIA2*, and *Fos* were curated as NAC-associated input genes in the human nucleus accumbens (Table 4). To further analyze the gene functions of these gene sets, GeneMANIA identified six key functions through gene network analysis: ionotropic glutamate receptor activity (blue), neurotransmitter receptor complex (purple), extracellular ligand-gated ion channel activity (cyan), postsynapse (orange), synaptic membrane (green), and neuron to neuron synapse (pink) (Figure 4). These networks also indicated that predicted interactions, physical interactions, shared protein domains, and co-expression were the dominant relationships in the gene set, being the top four network contribution percentages.

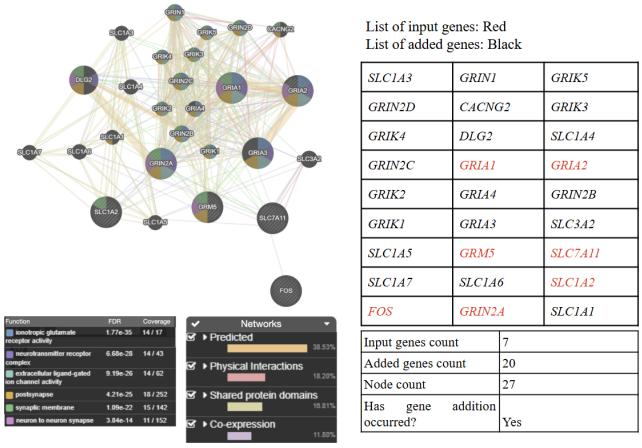
NAC-Associated Gene Network in the Dorsal Striatum

GeneMANIA network analysis of five curated NAC-associated dorsal striatum input genes: *SLC1A2*, *SLC7A11*,

Table 5 NAC-associated genes/pathways in dorsal striatum-related studies.

Study	Key Genes / Pathways	Evidence Type
Fernández-Rodríguez et al., 2023 [24]	*SLC1A2* / GLT-1, *SLC7A11* / xCT	Direct NAC intervention in an animal ethanol-abstinence model; supports NAC-related oxidative stress/neuroinflammation and likely glutamate-homeostasis biology, but not opioid-specific human evidence
Harvey et al., 2008 [25]	*GCLC, GCLM*	Direct NAC intervention evidence in rat striatum; supports NAC-associated striatal antioxidant modulation, but not direct GCLC/GCLM gene regulation
Duty et al., 2011 [26]	*FOSB*	Non-NAC disease/neural activation evidence; likely striatal dopaminergic dysregulation evidence, not NAC treatment evidence. Should be retained only as indirect pathway evidence or removed from NAC-specific tables

Note. *SLC1A2* encodes glutamate transporter 1 (GLT-1). *SLC7A11* encodes xCT, the light chain of the cystine/glutamate antiporter. *FOSB* encodes the FosB transcription factor.

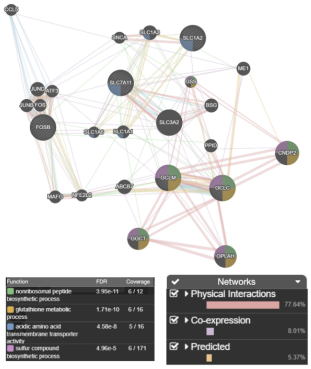


List of input genes: Red
List of added genes: Black

SLC1A3	*GRIN1*	*GRIK5*
GRIN2D	*CACNG2*	*GRIK3*
GRIK4	*DLG2*	*SLC1A4*
GRIN2C	*GRIA1*	*GRIA2*
GRIK2	*GRIA4*	*GRIN2B*
GRIK1	*GRIA3*	*SLC3A2*
SLC1A5	*GRM5*	*SLC7A11*
SLC1A7	*SLC1A6*	*SLC1A2*
FOS	*GRIN2A*	*SLC1A1*

Input genes count	7
Added genes count	20
Node count	27
Has gene addition occurred?	Yes

Figure. 4 Gene network analysis results using curated NAC-associated input genes in the nucleus accumbens.



List of input genes: Red
List of added genes: Black

CCL5	*SNCA*	*SLC1A3*	*SLC1A2*	*ME1*
JUND	*ATF3*	*SLC7A11*	*GSS*	*BSG*
FOS	*SLC1A6*	*SLC1A1*	*SLC3A2*	*PPID*
CNDP2	*NAFG*	*NFE2L2*	*ABCB7*	*GCLM*
GCLC	*GGCT*	*OPLAH*	*PPID*	*JUNB*

Input genes count	5
Added genes count	20
Node count	25
Has gene addition occurred?	Yes

Figure. 5 Gene network analysis results using curated NAC-associated input genes in the dorsal striatum.

GCLC, *GCLM*, and *FOSB*. The network was generated with GeneMANIA network expansion enabled; therefore, the displayed network includes both the five input genes and GeneMANIA-added predicted network neighbors. Added genes represent predicted network neighbors that support functional interpretation but do not provide direct evidence of NAC-associated gene expression in the dorsal striatum.

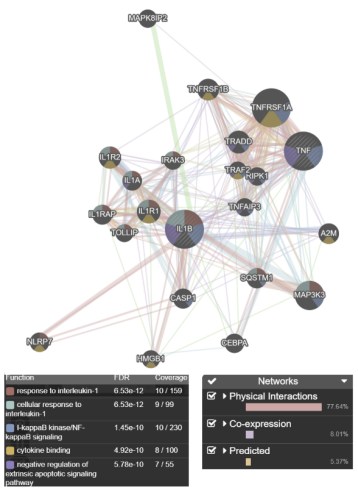
To identify the key genes associated with NAC treatment in the dorsal striatum, we reviewed three previous studies. Through these studies, the *SLC1A2*, *SLC7A11*, *GCLC*, *GCLM*, and *FosB* were curated as NAC-associated input genes in the dorsal striatum (Table 5). To further analyze the gene functions of these gene sets, GeneMANIA identified four key functions through gene network analysis: nonribosomal peptide biosynthetic process (green), glutathione metabolic process (orange), acidic amino acid transmembrane transporter activity (purple), and sulfur compound biosynthetic process (pink) (Figure 5). These networks also indicated physical interactions as the dominant relationships within the gene set, with the top network contributing the highest percentage.

NAC-Associated Gene Network in the Dorsal Anterior Cingulate Cortex (dACC)

GeneMANIA network analysis of eight curated vitamin D-associated cerebral cortex input genes: *BDNF*, *NGF*, *VDR*, *CYP27B1*, *MBP*, *PLP1*, *IL6*, and *TNF*. The network was gen-

Table 6 NAC-associated genes/pathways in dACC-related studies.

Study	Key Genes / Pathways	Evidence Type
Dean et al., 2011 [11]	*TNF, IL1B*	Review-based clinical/mechanistic evidence on NAC in psychiatry, glutathione, glutamatergic, neurotrophic, and inflammatory mechanisms; not dACC-specific and not direct NAC treatment-regulated gene evidence



List of input genes: Red
List of added genes: Black

MAPK8IP2	*TNFRSF1B*
TNFRSF1A	*TNF*
TRADD	*IRAK3*
IL1R2	*IL1A*
TRAF2	*RIPK1*
IL1RAP	*IL1R1*
TOLLIP	*IL1B*
TNFAIP3	*A2M*
CASP1	*SQSTM1*
MAP3K3	*NLRP7*
HMGB1	*CEBPA*

Input genes count	2
Added genes count	20
Node count	22
Has gene addition occurred?	Yes

Figure. 6 Gene network analysis results using curated NAC-associated input genes in the dACC.

erated with GeneMANIA network expansion enabled; therefore, Enriched functional categories included peptidyl-serine modification, regulation of neuron apoptotic process, cellular response to nerve growth factor stimulus, cytokine receptor binding, regulation of neurogenesis, interleukin-6 production, and cognition. Added genes are interpreted as network-associated genes predicted from existing GeneMANIA evidence rather than direct vitamin D-associated genes in the human cerebral cortex.

To identify the key genes associated with vitamin D treatment in the cerebral cortex, we reviewed five previous studies. Through these studies, the *BDNF*, *NGF*, *VDR*, *CYP27B1*, *MBP*, *PLP1*, *IL6*, and *TNF* were curated as vitamin D-associated input genes in the human cerebral cortex (Table 2). To further analyze the gene functions of these gene sets, GeneMANIA identified seven key functions through gene network analysis: peptidyl-serine modification (purple), regulation of neuron apoptotic process (pink), cellular response to nerve growth factor stimulus (blue), cytokine receptor binding (red), regulation of neurogenesis (cyan), interleukin-6 production (green), and cognition (orange) (Figure 2). These networks also indicated that co-expression, physical interactions, and shared protein domains were the dominant relationships within the gene set, accounting for the top three network contribution percentages.

This quality assessment framework was used to guide cautious interpretation rather than to exclude all indirect studies. Source studies with lower directness were retained only as hypothesis-generating evidence, and GeneMANIA results were interpreted as network-level associations rather than proof of causal or therapeutic effects.

Discussion

Vitamin D–associated genes in the human hippocampus mainly fall into two opioid-relevant groups: reduced immune and cellular stress signaling and coordinated activity-dependent transcription linked to circuit plasticity (Figure 1). The network is centred around *VDR* and its nuclear-receptor partner *RXRA*, *RXRB*, *RXRG* (retinoid X receptors) which help gene regulation through vitamin D [27,28]. The enrichment of transcription regulators, including immediate early transcription factors such as *FOS*, *JUN* family members, *EGR1*, and *CREB1*-related regulators suggests the network is involved in lasting synaptic and memory-related adaptations [29,30]. Immediate early genes are also strongly involved in opioid-induced transcriptional changes and have been implicated to long-term neuroadaptations relevant to addiction-related behavior [31,32]. In fact, Kemény et al. (2021) highlights this functional link by demonstrating in vivo that lack of vitamin D signaling led to amplification of behavioural opioid responses, which normalized after VDR activity was restored. In addition, enrichment for oxidative stress response biology that includes *NFE2L2*/NRF2-related regulation is opioid-relevant because morphine exposure can dysregulate NRF2, the transcription factor encoded by *NFE2L2*, and mitochondrial-redox functions in human brain-relevant cells [33]. Finally, enrichment for immune-linked signaling is noteworthy as central immune activation, including microglial mechanisms, is repeatedly implicated in opioid tolerance and opioid-induced hyperalgesia, which can reduce analgesic benefit over time [34,35].

Vitamin D–associated genes in the human cerebral cortex mainly fall into two opioid-relevant groups: reduced cytokine-linked neuroimmune signaling and strengthened trophic and signaling programs that support cortical plasticity and re-

Table. 7 Combined quality assessment framework for source evidence and GeneMANIA analysis.

Category	Quality assessment item	How it was evaluated in this study
Source evidence	Relevance to research question	Whether the study was related to vitamin D, NAC, opioid-related biology, addiction-relevant brain regions, or gene expression
Source evidence	Biological model	Whether the evidence came from human brain tissue, clinical data, animal models, in vitro studies, or non-opioid disease models
Source evidence	Brain-region specificity	Whether the study reported specific brain regions relevant to addiction, such as the hippocampus, amygdala, nucleus accumbens, dorsal striatum, cortex, or dACC.
Source evidence	Gene-level support	Whether the study provided specific genes, gene-expression data, protein-level findings, or pathway-level evidence
Source evidence	Statistical support	Whether the study reported statistical criteria such as p-values, FDR/q-values, or clear significance thresholds
Source evidence	Risk of indirectness	Whether the study directly matched the present topic or was indirect because it used non-human, non-opioid, or non-brain-specific data
GeneMANIA analysis	Input gene transparency	Whether all input genes were listed by supplement and brain region before network analysis
GeneMANIA analysis	Analysis setting	Whether the same Homo sapiens setting and GeneMANIA parameters were used across networks
GeneMANIA analysis	Added-gene control	Whether added GeneMANIA genes were treated as predicted network neighbors, not as experimentally validated supplement-regulated genes
GeneMANIA analysis	Functional annotation control	Whether enriched functions were retained only when they met the FDR < 0.05 threshold
GeneMANIA analysis	Redundancy control	Whether overlapping functional terms were reduced by keeping the most significant non-overlapping terms
GeneMANIA analysis	Interpretation control	Whether results were interpreted as hypothesis-generating network associations, not causal mechanisms or direct evidence from opioid-exposed human brains

silience (Figure 2). The network is anchored by vitamin D biology, including *VDR* and *CYP27B1* (hydroxylase enzyme gene), and it includes inflammatory genes such as *IL6* and *TNF*. The network also showed enrichment for interleukin-6 production and cytokine receptor binding. These are opioid-relevant because cytokine and microglia-related immune signaling have been linked to reduced opioid analgesic effectiveness over time, including opioid tolerance and opioid-induced hyperalgesia[35,36]. Similarly, the network showed enrichment for peptidyl-serine modification and had multiple kinases, suggesting changes in phosphorylation signaling (Figure 2). This is significant as phosphorylation is a major mechanism involved with μ-opioid receptor desensitization and tolerance through phosphorylation-dependent receptor regulation[37,38]. The network also included neurotrophin and growth-factor genes such as *BDNF*, *NTF3*, *NTF4*, and *NGF* as well as enrichment for cellular response to nerve growth factor stimulus, regulation of neurogenesis, regulation of neuron apoptotic process, and cognition. Neurotrophin signaling is well known to modulate nociception and pain-related plasticity, and cortical circuits that support cognition may act as contributors to top-down pain regulation and control processes relevant to addiction. This connects the observed trophic and cognitive enrichments to opioid-relief frameworks[39,40]. In sum, the cortex network suggests that vitamin D signalling may support an opioid-relevant resilience through reducing cytokine-driven immune activity, influencing phosphorylation pathways that intersect with receptor adaptation, and supporting neurotrophin-linked maintenance of cortical function. Combined, these may help limit processes involved in reduced opioid efficacy while supporting cortical function in pain-processing systems[35,41].

Vitamin D–associated genes in the human amygdala mainly fall into two opioid-relevant groups: reduced interleukin-1–linked inflammatory signaling and stabilization of synaptic and stress-response programs that affect the emotional dimension of pain and relapse risk (Figure 3). The network is anchored on *VDR* and is enriched for cellular response to interleukin-1. It includes interleukin-1 pathway genes *IL1B*, *IL1R1*, *IL1R2*, and *IL1RAP* as well as inflammatory processing genes such as *CASP1* and the stress and autophagy adaptor p62, encoded by *SQSTM1*. Interleukin-1 signaling is well known to increase pain sensitivity and may oppose opioid analgesia. Experimental evidence suggests that interleukin-1 activity contributes to morphine tolerance, and blocking interleukin-1 signaling can preserve morphine's analgesic effects and reduce tolerance development[42–44]. More broadly, immune signaling in the brain is repeatedly linked to opioid tolerance and opioid-induced hyperalgesia, connecting this IL-1–enriched network to opioid-relevant pain amplification mechanisms[45]. In addition, the network is enriched for regulation of trans-synaptic signaling and the neurotrophin signaling pathway involving *BDNF*, *NTF3*, *NTF4*, and *NTRK2*, as well as inhibitory markers *GAD1* and *GAD2*, suggesting changes in excitatory–inhibitory balance and plasticity in amygdala circuits. Because the amygdala is a core substrate for the emotional dimension of pain and amygdala plasticity is strongly linked to persistent pain states, these synaptic and neurotrophin findings are relevant to opioid-related negative effects, which can increase drug seeking[46–48]. Lastly, enrichment for the endocrine process is supported by *CRH*, *CRHR1*, *CRHR2*, and *UCN*. This is opioid-relevant because CRH signaling is a central aspect of stress-induced relapse circuitry and has been repeatedly implicated in stress-triggered reinstatement of drug seeking[49,50]. Overall, the amygdala network suggests that vitamin D regulation may reduce interleukin-1–related inflammatory tone while strengthening neurotrophin signaling, inhibitory signaling, and CRH-linked stress regulation. Combined, these may reduce inflammatory pain amplification and weaken stress-driven relapse pressure that contributes to tolerance and relapse vulnerability.

NAC-associated genes in the human nucleus accumbens fall into two opioid-relevant groups: normalization of excitatory synaptic receptor complexes and restoration of extracellular glutamate homeostasis (Figure 4). The network is dominated by synapse-related functions, including ionotropic glutamate receptor activity, neurotransmitter receptor complex, extracellular ligand-gated ion channel activity, postsynapse, synaptic membrane, and neuron to neuron synapse. It also contains many glutamate receptor subunits across α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (*GRIA1*, *GRIA2*, *GRIA3*), N-methyl-D-aspartate (NMDA) receptors (*GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*), and kainate receptors (*GRIK1*, *GRIK2*, *GRIK3*, *GRIK4*, *GRIK5*), as well as postsynaptic organizing and trafficking components such as *DLG2* and *CACNG2*. Overall, this suggests NAC may be associated to molecular changes that affect how strongly glutamatergic inputs activate accumbens neurons. This is opioid-relevant because heroin seeking depends on glutamate release into the nucleus accumbens core, and relapse-like responding is associated with increased extracellular glutamate in this region[51]. These network interactions are consistent with the empirical evidence from Siemsen et al, whose study indicated that heroin self-administration changes prelimbic cortex–nucleus accumbens core circuitry as well as astrocyte-associated and synaptic adaptations that were reversed by repeated NAC treatment during extinction. Furthermore, the presence of many glutamate-handling genes such as *SLC1A2* and *SLC7A11* in the network supports a glutamate homeostasis theme that has been proposed as a key mechanism shaping relapse vulnerability across drugs[52,53]. In opioid models, heroin self-administration can impair glutamate uptake and glutamate spillover in the nucleus accumbens core, and this disruption is mechanistically associated with rein-

stated heroin seeking[54]. This glutamate-homeostasis framing is further strengthened by the same heroin self-administration study that shows how NAC can reverse heroin-related astrocytic and neuronal changes in corticostriatal circuitry, supporting the idea that NAC-linked gene-network themes may align with restoration of relapse-relevant glutamate regulation rather than only general antioxidant effects. Finally, the presence of *FOS* connects this network to activity-dependent recruitment of accumbens ensembles, because Fos expression is commonly used to identify neurons activated during conditioned drug seeking in corticostriatal circuitry[55]. In sum, these findings suggest that NAC may reduce relapse-related signaling by stabilizing excitatory receptor-complex architecture and restoring glial control of extracellular glutamate, which could reduce cue-triggered accumbens overactivation and lower relapse vulnerability driven by glutamatergic dysregulation[56].

NAC-associated genes in the human dorsal striatum mainly fall into two opioid-relevant groups: stronger glutathione-based antioxidant capacity and stabilization of glutamate transport processes that shape corticostriatal signaling (Figure 5). The network is enriched for glutathione metabolic process, sulfur compound biosynthetic process, and nonribosomal peptide biosynthetic process. It contains the core glutathione synthesis and recycling genes, including *GCLC*, *GCLM*, *GSS*, *GGCT*, and *OPLAH*, as well as the redox-responsive transcription factor gene *NFE2L2* and its partner *MAFG*. This matches NAC's well-known role as a cysteine donor that can increase glutathione synthesis when cysteine availability is limited[57]. This is opioid-relevant because morphine exposure and opioid dependence have repeatedly been associated with oxidative stress biology, and redox imbalance is thought to contribute to maladaptive neurobiological changes that can worsen pain processing and drug-related pathology[58,59]. In addition, the network is enriched for acidic amino acid transmembrane transporter activity and includes several glutamate transport and exchange component genes, especially *SLC1A2* and *SLC1A3* (excitatory amino acid transporters) and the cystine–glutamate antiporter module genes *SLC7A11* with *SLC3A2*. The cystine/glutamate antiporter system xC^-, involving xCT/*SLC7A11* and *SLC3A2*, connects antioxidant biology and extracellular glutamate regulation because cystine uptake supports glutathione synthesis while glutamate is exchanged into the extracellular space[60,61]. Disrupted glutamate homeostasis in corticostriatal circuits is a major framework for relapse vulnerability, and restoring glutamate uptake capacity, including GLT-1(*SLC1A2*)–linked mechanisms, is a common therapeutic strategy in substance use models[52,53,56]. Lastly, the presence of activity-dependent transcription factor genes such as *FOS*, *FOSB*, *JUN*, and *ATF3* suggests regulation of neuronal ensemble recruitment, as Fos family markers are commonly used to identify strongly activated corticostriatal neurons during conditioned drug-seeking behaviors[54]. Because the dorsal striatum is strongly implicated in the shift from voluntary drug use to habit-like and compulsive drug seeking, a network-level pattern of improved redox buffering and normalized glutamate handling may help reduce stress-driven amplification of maladaptive corticostriatal activation that supports ongoing opioid seeking[62,63].

NAC-associated genes in the human dACC mainly fall into one opioid-relevant theme: reduced TNF and interleukin-1–linked innate immune signaling with strengthened negative-feedback regulation and stress-control nodes in a pain-affect hub (Figure 6)[64,65]. The network is anchored on the pro-inflammatory cytokine pathway genes *TNF*, *IL1B*, and *IL1A* and includes receptor and proximal signaling components (*TNFRSF1A*, *TNFRSF1B*, *TRADD*, *TRAF2*, *RIPK1*; and *IL1R1*, *IL1R2*, *IL1RAP*). It also contains intracellular regulator-coding genes that typically restrain pathway amplification, including *IRAK3* and *TOLLIP*, and the major negative-feedback regulator-coding gene *TNFAIP3*[64,65]. Consistent with this composition, GeneMANIA enrichments highlight response to interleukin-1, cellular response to interleukin-1, cytokine binding, and negative regulation of extrinsic apoptotic signaling, with physical interactions as the dominant network relationship. Although I-κB kinase and NF-κB signaling appears as an enriched function, core NF-κB transcript nodes are not present in this build. Therefore, this term is best interpreted as reflecting upstream *TNF* and interleukin-1 receptor biology rather than direct NF-κB gene involvement. This inflammatory theme is opioid-relevant because brain immune signaling is repeatedly linked to opioid tolerance and opioid-induced hyperalgesia, in which cytokine activity can erode opioid analgesic effectiveness over time and promote pain sensitization[45]. Similarly, innate immune receptor–linked neuroinflammation has been mechanistically tied to morphine tolerance via soluble TNF–dependent signaling, with accompanying interleukin-1β-related transcriptional changes in pain circuits[66]. The dACC context strengthens this interpretation because anterior cingulate circuitry is strongly implicated in the affective dimension of pain, meaning inflammatory amplification here can plausibly worsen pain aversiveness and negative affect relevant to withdrawal distress[67]. Finally, the presence of *CASP1* and *HMGB1*, alongside the autophagy and stress adaptor *SQSTM1*, links this network to inflammatory processing and stress handling[68]. Overall, the dACC network suggests NAC may reduce opioid-relevant immune amplification primarily by shifting *TNF* and interleukin-1 signaling toward tighter negative feedback and reduced downstream stress signaling.

Interpretive Limitations

Previous studies support the claim that responses to vitamin D and NAC may vary according to participant characteristics,

supplement exposure, and clinical context, including age, sex, dose, treatment duration, baseline nutritional status, concurrent medication use, and comorbid conditions[69–72]. Because this analysis draws information from studies with heterogeneous source designs without testing these factors, the identified gene-network themes should be interpreted as framework-level associations that may vary across populations and individuals.

Conclusion

To investigate the functional interactions among vitamin D and NAC-linked genes implicated in opioid addiction, curated gene sets were analyzed using the GeneMANIA online platform[73–75]. This systems level gene network analysis suggests that vitamin D and NAC align with complementary molecular pathways across key human brain regions implicated in OUD. It also provides a framework for evaluating how the two supplements map onto biological pathways implicated in opioid dependence. Vitamin D–associated networks were mainly associated with the following processes across the hippocampus, cortex, and amygdala. Across these regions, they were associated with transcriptional regulation, neurotrophin signaling, mitochondrial quality control, energy metabolism, and immune modulation. The convergence appeared to be not random. It was directed to key processes of neuroprotection, stress regulation, and tolerance-related biology. On the other hand, NAC-associated networks demonstrated another distinct pattern. In the nucleus accumbens, dorsal striatum, and dACC, they were mostly associated with glutamatergic synapse regulation, redox and glutathione metabolism, and neuroinflammatory signaling. These themes are relevant to craving, withdrawal-related distress, and maladaptive habit circuitry. Combined, these findings appear to suggest that vitamin D and NAC map onto distinct yet complementary biological pathways across multiple brain regions. At framework-level, it supports an interpretation of opioid-relevant processes involving neuroinflammation, oxidative stress, synaptic plasticity, and metabolic stability. The main limitation of the study is the lack of direct experimental validation. The data were sourced from previously published gene expression and the analysis was based on network modeling. Thus, its value is not in providing casual interpretations but rather providing a hypothesis-generating framework to understand how widely accessible supplements may be relevant in opioid-related neurobiology. It also complements high-throughput human postmortem studies that have mapped out the OUD-associated transcriptomic alterations by highlighting specific molecular pathways that warrant experimental and clinical investigation[14].

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Supplementary Table S1. Simplified Quantitative Details of Source Studies Used for Gene Selection.

Study	Model / sample information	Selected gene(s) used in this study	Available quantitative information	Evidence limitation
Liang et al., 2018 [12]	Mouse vitamin D diet study; behavioral testing used 9–15 male mice/group; qRT-PCR used $n = 3$/group	SNAP25, BDNF, CREB	qRT-PCR data reported as mean ± SEM; one-way ANOVA with LSD post hoc test; significance threshold $p < 0.05$	Animal study; not opioid-specific; no human opioid-exposed brain tissue
Brown et al., 2003 [18]	Embryonic rat hippocampal cell/explant culture; neurite assay used 9 explants/treatment group	NGF	BrdU mitosis: $F = 5.65$, $df = 1, 14$, $p = 0.03$; neurite outgrowth: $F = 5.44$, $df = 1, 89$, $p = 0.02$; NGF increase: $p < 0.001$	In vitro rat hippocampal model; not opioid-specific
Eyles et al., 2005 [17]	Human postmortem brain tissue from 5 males, aged 34–58 years	VDR, CYP27B1 / 1α-hydroxylase	Qualitative immunohistochemistry; no gene-expression fold change, p-value, or CI reported for treatment effect	Human brain evidence, but not treatment-based and not opioid-exposed
Kuningas et al., 2009 [21]	Human population-based Leiden 85-plus Study; 563 participants genotyped	VDR-related evidence; CRH used only indirectly if supported by context	Genetic association study; exact gene-expression effect size not applicable	Human association evidence; not brain-region-specific expression and not opioid-specific
Fernández-Rodríguez et al., 2023 [24] / Schiavi-related NAC source [23]	Rat NAC study; NAC doses 30 or 120 mg/kg; cFOS immunoreactivity in nucleus accumbens	Fos / c-Fos, GRM5-related pathway	Experiment 1: $F(5, 27) = 5.807$, $p = 0.0009$; NAC 120 vs. vehicle $p = 0.0019$; NAC 120 vs. NAC 120 + MTEP $p = 0.0127$	Rat alcohol/NAC model; not opioid-specific and not human brain tissue
Fernández-Rodríguez et al., 2023 [24] / Schiavi-related NAC source [23]	Rat VTA ethanol + NAC study; NAC 120 mg/kg, intra-VTA ethanol 150 nmol	Fos / c-Fos	Locomotor activity: Kruskal–Wallis $p = 0.1115$; cFOS experiment: $F(3, 20) = 12.36$, $p < 0.0001$	Rat alcohol activation model; useful for NAC/cFOS pathway only, not opioid-specific
Reissner et al., 2014 [10]	Rat cocaine reinstatement model	SLC1A2 / GLT-1	Quantitative values NR in accessible summary; study conclusion reports GLT-1 as key for NAC inhibition of cocaine reinstatement	Animal cocaine model; indirect for opioid biology
Fernández-Rodríguez et al., 2023 [24]	Long-term ethanol-experienced male rat model; NAC during withdrawal	SLC1A2 / GLT-1, SLC7A11 / xCT	Exact sample size/effect size NR in accessible source; summary reports NAC effects on oxidative stress/neuroinflammation and glutamate transporter-related outcomes	Animal ethanol model; indirect for opioid use disorder
Eyles et al., 2013 [7]	Review article on vitamin D and brain function	BDNF, GAD1, VDR	Not a primary quantitative gene-expression source	Use for background only; not primary gene-selection evidence unless original studies are cited
Harms et al., 2011 [20]	Review article on vitamin D and brain biology	IL6, TNF, BDNF, IL1B	Not a primary quantitative gene-expression source	Use for background only; not sufficient as primary gene-selection evidence
Mirarchi et al., 2023 [16]	Review/mechanistic article on vitamin D, microglia, and brain disorders	VDR, CYP27B1	Not a primary quantitative gene-expression source	Use cautiously as background or pathway rationale
Dean et al., 2011 [11]	Review article on NAC in psychiatry	TNF, IL1B	Not a primary quantitative gene-expression source	Should not be treated as primary gene-expression evidence unless original studies are added
Duty et al., 2011 [26]	Experimental neuroscience study related to FosB expression	FosB	Exact gene-specific sample size/effect size NR from provided source information	Indirect; not NAC-specific unless clearly connected in the original paper
Wang et al., 2001 [76]	Rat neurotoxicity / vitamin D-related neuroprotection model	PARK2-related pathway, if supported	Exact gene-specific p-value/effect size NR from provided source information	Animal model; not opioid-specific

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Table 7 – continued from previous page

Study	Model / sample information	Selected gene(s) used in this study	Available quantitative information	Evidence limitation
Suzuki et al., 2013 [77]	Vitamin D-related dopaminergic neuron/GDNF study	GDNF	Exact sample size/effect size NR from provided source information	Not opioid-specific; likely indirect dopaminergic pathway evidence
Garcion et al., 2002 [78]	Rat astrocyte / vitamin D redox-related study	NQO1-related pathway	Exact sample size/effect size NR from provided source information	In vitro/animal evidence; not opioid-specific