Opioid Use and the Gut-Brain Axis: A Literature Review

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Abstract

Opioid use disorder (OUD), formerly known as opioid misuse, is defined as the use of opioids in any way other than prescribed. Opioid use disorder is accompanied by an increased risk of acquiring infectious diseases. The opioid crisis has exhibited a steady increase in severity since the start of opioid prescription in the late 1990s. This substantial increase in opioid use has resulted in increased rates of infectious diseases, posing a significant public health challenge. Many studies have focused research on the psychosocial factors that influence such transmission within this population, yet limited research at the biological level exists on how this behavior increases transmission risk. This review aims to delineate the different mechanisms through which opioid use impacts dopamine concentrations in the body and how this influences immune function and development of pathological diseases. A detailed literature search using OVID search engine was conducted to construct a broad sample of relevant publications for review and to characterize prospective associations between key concepts discussed in the collective work. The review of collective literature revealed multifunctional effects of dopamine in both neuroimmune and gastrointestinal immune systems. Dopamine may be stored, synthesized, and released by many immune cells and acts through dopamine receptors present on these cells to exert immunomodulatory effects. Through dopamine receptor-mediated pathways, dopamine has exhibited the ability to influence immune cell differentiation, mediate cytokine and chemokine secretion, regulate intracellular cAMP production, induce transendothelial migration of

leukocytes, and influence cell survival through pro- or anti-apoptotic functions. This review highlights the multifaceted nature of dopamine and its potential function as an intermediate component between opioid use and the gut-brain axis. The literature reviewed poses implications for future research, practice, and health and disease.

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Preface

My time as a graduate student has provided me with many opportunities to flourish as an approaching public health professional. My passion for public health has only grown since the start of this Master of Public Health program. Thank you to Dr. Jeremy Martinson for his support and encouragement throughout the duration of this program.

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1.0 Introduction

Over the past two decades, the opioid epidemic has only worsened. Approximately 16 million individuals worldwide suffer from opioid use disorder and 2 million reside in the United States (*Understanding the Opioid Overdose Epidemic | Opioids |* CDC, 2023). Opioid use disorder, formerly known as opioid misuse, is defined as the use of opioids in any way other than prescribed. Individuals who engage in chronic drug use are at higher risk of acquisition or transmission of infectious diseases such as HIV, hepatitis, Tuberculosis, and other sexually transmitted infections (Serota et al., 2020). The primary routes of transmission occur via injection drug use or transmission due to risky behaviors such as unprotected sex. The opioid crisis poses a major public health concern due to its impact on society, increasing costs of treatment for opioid use disorder, opioid-related overdoses and deaths, and the supplemental increased rates of infectious diseases. The complexity of dopamine (DA) signaling, the gut-brain axis, and the impact opioid use evokes on these mechanisms marks the large gaps in research that exist as it relates to human populations. This review aims to highlight a major, but less appreciated secondary public health concern that accompanies the opioid crisis.

Understanding the neurobiology of opioid use is important to determine the components of the human body most affected by this behavior. Two classes of opioids exist: endogenous and exogenous opioids. Endogenous opioids consist of those naturally produced in the brain. Exogenous opioids are those introduced from outside the body primarily through oral or nasal ingestion or injection intravenously (Cruz-Lebron et al., 2021). This class of opioids include heroin, morphine, oxycodone, and fentanyl, among other types of opiate-substances (Channer et al., 2023). During opioid use, the exogenous opioid agonist travels through the bloodstream and

crosses the blood brain barrier (BBB) to interact with and stimulate opioid receptors present in the brain. Activation of these receptors stimulates the ventral tegmental area (VTA) of the brain which serves as an important regulator of DA release (Kosten & George, 2002).

Stimulation of the VTA through opioid receptor activation triggers the release of DA into another region of the brain called the nucleus accumbens (NAc) which regulates the distribution of DA to other areas of the brain. Without significant pain, opioids can influence this release and distribution of DA in the brain, likely increasing extracellular DA concentrations, resulting in the individual experiencing euphoric or rewarding effects (Kosten & George et al., 2002; Channer et al., 2023). Opioid withdrawal symptoms are present in individuals who have developed tolerance of opioids which occurs when opioid receptors become less responsive to activation by opioid agonists (Channer et al., 2023).

Withdrawal symptoms are recognized as motivators for continuation of addictive behavior and contribute to the development of opioid tolerance. Tolerance has proven to reduce the release of DA from the VTA and prevent an individual from experiencing the euphoric and pleasurable effects as produced with prior use (Kosten & George et al., 2002; Channer et al., 2023). Dopamine released as a result of VTA stimulation encounters and activates either one of two classes dopamine receptors (DR): D1-like DR or D2-like DR. Neurotransmitters mediate bidirectional communication between the nervous and immune systems. Dopamine acts through either class of receptors to induce immunomodulatory effects on cells expressing such receptors (McKenna et al., 2002).

The gut-brain axis is a network of signals that travel through the vagus nerve and reach components of the nervous and immune systems (Rutsch et al., 2020). Disruption of this communication is associated with altered BBB permeability, neuroinflammation, and changes to

distal organs, such as those of the gut, that may contribute to disease development. The gut microbiome plays a large role in the production of bacterial metabolites, protection against pathogens, maintenance of intestinal epithelial barrier integrity, and food product degradation (Cruz-Lebron et al., 2021; Rutsch et al., 2020). Dopamine is a crucial factor in communication between gut microbes and the CNS and functions as a key modulator of immune functions following opioid use.

2.0 Methods

To cultivate a sample of relevant literature for review, a literature search was conducted using key words in varying combinations. Examples of search terms utilized in the assembly of relevant publications included “opioid use disorder”, “gastrointestinal microbiome”, “dopamine signaling”, “neuroimmunomodulation/immunomodulation” and “immune cells”. Inclusion criteria for this literature search included publications that discussed effects of opioid use on various components of the immune system in the context of dopamine signaling and publications that utilized human subjects in laboratory research or conducted a review of human studies. Publications were also pulled from the reference lists of additional reviews. Table 1 in Appendix A summarizes the overall search strategy used for this review.

3.0 Opioid Use and the Gut Microbiome

The gut microbiome plays an important role in protecting the body from dangerous or harmful pathogens and modulating immune functions primarily through maintaining a diverse bacterial metabolite profile. The core bacterial taxa found in the homeostatic gut microbiome include phyla Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia (Cruz et al., 2021). Diversity of the bacterial metabolite profile in the gut is key to produce important fecal metabolites, such as short-chain fatty acids (SCFAs). Acetate, propionate, and butyrate are the primary SCFAs produced by bacterial metabolites in the gut microbiome. Each serve to regulate gut motility, proliferation of immune cells, barrier integrity maintenance, and activation of sympathetic nervous system functions (Carabotti et al., 2015; Cruz-Lebron et al., 2021).

Several human studies reveal contrasting results when examining the effects of opioid use disorder on the gut microbiome. Some studies have reported an increase in microbial diversity, yet others reported no alterations to diversity in the presence of opioids. In a study that sequenced the V3-V4 region of the bacterial 16S rRNA from human fecal samples, decreased diversity was observed in individuals receiving methadone treatment (Table 2) (Cruz-Lebron et al., 2021). Methadone is a long-acting opioid agonist commonly used in the treatment of opioid use disorders in addition to the attenuation of moderate to severe pain (Cruz-Lebron et al., 2021). In this study, methadone treatment was used to model chronic opioid use. Chronic opioid use causes disruption to the fecal metabolite profile and significantly altered the abundance of two phyla of the core taxa. Opioid use disorder has been found to cause gut dysbiosis leading to several conditions including

opioid-induced bowel dysfunction (OIBD). OIBD is thought to be associated with imbalance of bacterial metabolite profiles and alterations to the integrity of the intestinal epithelial barrier.

In individuals receiving methadone treatment, an overall increase in abundance of Phylum Actinobacteria was observed, specifically *Bifidobacteriaceae bifidum* and *Bifidobacteriaceae longum* (Table 2). The Bifidobacterium genus is a highly expressed bacterial metabolite present in the gut. This genus is responsible for the production of the SCFA acetate. The relative abundance of Phylum Verrucomicrobia was decreased in individuals receiving methadone treatment with a significant decrease observed specifically in the relative abundance of *Akkermansia muciniphila*. *A. muciniphila* is a highly expressed bacterial species in the gut microbiome responsible for stimulating host metabolic and immune responses. This intestinal microbe, characterized as a mucin degrader, is also a major producer of SCFAs and functions as an enhancing factor for intestinal epithelial barrier integrity through adhesion to enterocytes during differentiation suggesting a mechanism through which the microbe mediates its own epithelial translocation (Cruz-Lebron et al., 2021). Lower abundance of this microbe contributes to decreased intestinal barrier integrity and decreased production of SCFAs in the gut.

A significant increase in the relative abundance of Phylum Bacteroidetes was observed in individuals receiving long-term methadone treatment (Table 2.) (Cruz-Lebron et al., 2021). Members of P. Bacteroidetes exert pro-inflammatory effects through enhancing the production of pro-inflammatory cytokines. Elevated levels of P. Bacteroidetes contribute to inflammatory bowel disease (IBD), a term referring to ulcerative colitis and Crohn's disease. The relative abundance of Phylum Firmicutes was significantly decreased in individuals receiving long-term methadone treatment. Members of P. Firmicutes are responsible for inducing anti-inflammatory effects and exhibit the ability to inhibit the progression of IBD (Cruz-Lebron et al., 2021).

Alterations to the bacterial metabolite profile in the gut was accompanied by changes in the abundance of both fecal and plasma SCFAs in individuals receiving methadone treatment. Fecal samples from these individuals revealed decreased abundances of acetate, propionate, and butyrate (Cruz-Lebron et al., 2021). Phyla Actinobacteria and Firmicutes exhibited a positive correlation with fecal acetate while a negative correlation was observed for P. Proteobacteria and fecal acetate. Between fecal butyrate and both P. Bacteroidetes and P. Proteobacteria, a negative correlation was observed. P. Firmicutes was found to have a positive correlation with fecal butyrate in addition to acetate and butyrate from plasma samples of individuals receiving methadone treatment. P. Bacteroidetes is negatively correlated with acetate levels in the plasma. An overall increase in presence of propionate in the plasma and decrease in abundance of all three SCFAs in fecal samples were observed (Cruz-Lebron et al., 2021).

Cytokines and chemokines regulate immune functions and signal immune cells to facilitate an immune response. Disruption to the gut microbiome observed in methadone treated individuals induced changes to cytokine and chemokine production supporting the concept that levels of circulating immune mediators varies depending on bacterial and SCFA metabolite abundance in the gut. Chronic opioid use was found to decrease microbial diversity and increase levels of specific immune mediators including IL-6, TNF-α, C-reactive protein (CRP), lipopolysaccharide binding protein (LBP) and intestinal-type fatty acid-binding protein (IFABP). Levels of each of these immune mediators were significantly elevated in HIV-positive individuals and patients with IBD (Cruz-Lebron et al., 2021).

Opioid agonists include heroin, oxycodone, methadone or prescription opioids like morphine or fentanyl (Channer et al., 2023). In a study that examined the effects on gut microbial diversity in individuals exposed to opioid agonists, decreased diversity of the bacterial metabolite

profile was observed. In individuals exposed to opioid agonists, decreased diversity of P. Bacteroidetes was more prevalent with higher representation of Clostridium cluster XIVa in this group. Genera Prevotella and Bacteroides, both members of Phylum Bacteroidetes, possess a variety of functional properties including the promotion of gut health. Prevotella represents an important bacterial metabolite group present in the gut. Metabolites, such as SCFAs, produced by these genera associate with modulation of the DA synaptic cleft activity (Hamamah et al., 2022). The presence of Prevotella was eliminated in participants exposed to opioid agonists only. The SCFA butyrate is known to reduce inflammation, oxidative stress and promote gut barrier integrity. A decrease in the abundance of butyrate-producing genus Roseburia, was observed in individuals exposed to opioid agonists (Gicquelais et al., 2020).

Intestinal microbes possess the capacity to regulate cytokine functions including cytokine-mediated inflammation. In addition to influencing neuroinflammation, this regulation of cytokine secretion may result in alterations to striatal DA function. When introduced through fecal transplant, *Bacteroides uniformis* increased striatal DAT binding, a DA transporter that induces the reuptake and termination of DA. This striatal binding promotes recycling and storage of DA in vesicles located in the presynaptic terminals. The VTA acts as a meeting point between DA and ghrelin, a gut hormone responsible for regulating satiety through action on the VTA. *Prevotella copri* exhibited the opposite effect while also increasing plasma concentrations of ghrelin which has been shown to recruit dopaminergic neurons by direct activation (Hamamah et al., 2022). This indirectly proportionate relationship observed between DA and ghrelin suggests a connection between components of the gut microbiome and dopamine signaling throughout the body.

Evidence from a study examining the impact of opioid use in patients with type II diabetes (T2D) demonstrated a potential mechanism of opioid interaction with members of

Bifidobacterium, a genus highly represented in the gut microbiome. This genus was negatively impacted by opioid use and its interaction with metformin in T2D patients. In patients taking metformin and using opioids, a decrease in the abundance of the Bifidobacteirum genus was observed compared to the gut metabolite profile of individuals using metformin not using opioids (Barengolts et al., 2018). This study, although focused on a T2D patient population, demonstrates the capacity for opioids to induce changes to gut microbial diversity. Table 2 in Appendix A summarizes changes to the relative abundance of identified bacterial metabolites during chronic opioid use or opioid agonist exposure.

4.0 Dopaminergic Effects on Immune Function

4.1 Dopamine and Immune Cells

Neurotransmitters are key mediators of communication via the gut-brain axis. These chemical messengers activate nerve ganglia in the submucosal and myenteric plexuses of the enteric nervous system. Through the translocation of metabolites and endotoxins from the intestinal lumen to the plasma, intestinal microbes directly interact with the CNS. Vagal sensory nerve terminals are components of the vagus nerve and are widely distributed throughout the gastrointestinal tract. SCFA-producing microbes in the gut exhibit the ability to activate these terminals. In the gut-brain axis, the vagus nerve influences dopamine concentrations to mediate gut-brain cross talk (Carabotti et al., 2015; Hamamah et al., 2022).

Dopamine may be synthesized and released by various types of immune cells. Released DA acts through either class of dopamine receptors to exert its effects on the cells expressing such receptors. The D1-like DR couple to G proteins including $G\alpha_s$ and $G\alpha_{olf}$, and upon activation, stimulate cyclic-adenosine monophosphate (cAMP) production, activation of protein kinase A (PKA) phosphorylation, and modulation of ion channels in specific pathways. The D2-like DR couple to G proteins $G\alpha_i$ and $G\alpha_o$, primarily exerting inhibitory effects on the production of cAMP and decreasing activation of PKA phosphorylation (Baik, 2013). Substantial evidence supports sympathetic innervation of lymphoid tissues indicating neural regulation of immune cells throughout the body. In addition to synthesizing and releasing DA, immune cells encounter the neurotransmitter primarily in the lymph nodes (LNs), spleen, bone marrow, and plasma. Dopamine receptors play a large role in the regulation of numerous immune cell activities including

proliferation, differentiation, cell survival, maintaining immune homeostasis, and modulating cytokine and chemokine secretion, thus altering immune cell function (McKenna et al., 2002).

Dendritic cells are important cells involved in both innate and adaptive immune responses. These cells reside in the skin and mucosa-associated lymphoid tissues (MALT), an immune system component consisting of both the nasopharynx-associated lymphoid tissues and gut-associated lymphoid tissues. Dendritic cells are recognized as professional antigen presenting cells (APCs) that function to present antigen peptides or foreign pathogens to naïve CD4 T cells, resulting in their activation. This activation of T cells is accompanied by differentiation of the naïve CD4 T cell into primarily one of four subsets: Th1, Th2, Th17, and Treg cell lineages. Dendritic cells are one of many immune cells that synthesize and release DA. DC-mediated release of DA influences T helper cell differentiation during the DC-naïve T cell interaction. Following dopaminergic exposure of naïve CD4 T cells expressing D2-like DR, increased production of cAMP in addition to increased IL-4 and IL-5 secretion was observed, suggesting DA acts through D2-like DR to polarize Th2 differentiation (Nakano et al., 2009). Th2 cells respond to extracellular pathogens, contribute to mucus production, and recruit other immune cells involved in the allergic responses. These cells are known to secrete several cytokines including IL-4, IL-5, IL-13, and IL-10. The stimulation of cAMP promotes DA synthesis in monocyte derived DCs (Mo-DCs) contributing to the potential for increased extracellular DA concentrations (Nakano et al., 2009). This bidirectional effect on cAMP production and DA concentration highlights the potential for DA to regulate its own synthesis in immune cells through the activation of D1-like DR. The absence of DA during this interaction may result in Th1 differentiation. Th1 cells are responsible for protection against intracellular pathogens and generally secrete cytokines IFNγ and TNFα (Nakano et al., 2009).

Blocking the activation of D2-like DR in Mo-DCs increased their capacity DA storage. D2-like DR antagonists, such as sulpiride, induce sustained cAMP elevation in Mo-DCs which acts through the cAMP-TH pathway to increase DA synthesis (Nakano et al., 2009). DA increased cAMP production in naïve T cells. This effect was inhibited by antagonizing D1-like DR expressed which indicates dominance of D1-like DR in Mo-DCs and selective activation of D1-like DR by DA to exert its immunomodulatory effects (Nakano et al., 2009). This sustained elevation of cAMP during the priming stage is hypothesized to be partly responsible for the polarization of T cell differentiation toward the Th2 cell lineage.

Regulatory T cells, or Tregs, are responsible for regulating inflammatory CD4 T cells, maintaining homeostasis of the immune systems and notably secrete cytokines IL-10 and TGF-β. $CD4^{+}CD25^{+}$ Tregs have the capacity to synthesize and release substantial amounts of DA. Upon release, this DA acts through D1-like DR, reducing synthesis of both IL-10 and TGF-β by the cells subsequently downregulating the inhibitory effects of Treg effector cells (Sarkar et al., 2010). This observed effect suggests Treg-released DA acts on D1-like DR expressed surrounding Tregs to modulate regulatory effects of these cells.

Activation of T cells typically requires a co-stimulatory signal for proliferation, differentiation into T cell subsets, and cell survival to take place. In the absence of a co-stimulatory signal, DA alone exhibited the ability to activate resting T cells. Through the stimulation of both D2-like DR subtypes D2 and D3 in peripheral T cells, DA activates α4:β1 and α5:β1 integrins, thus promoting adhesion of these cells to fibronectin. Fibronectin is a glycoprotein that serves to regulate motility of adhered cells. This mechanism of adhesion is crucial for T cell trafficking and extraversion across blood vessels and tissue barriers, including the blood-brain barrier (Sarkar et al., 2010; Nasi et al., 2019). Stimulation of the D2-like DR D4 subtype by DA during T cell

receptor activation has been found to induce quiescence, or dormancy, in these cells. This occurs through the inhibition of ERK1/ERK2 pathway and subsequent up-regulation of KLF2 in human T cells.

Several studies have shown higher expression of DR D3 subtype with subsequent increases in IFN-γ secretion in T cells, a result is observed in patients with neurocognitive disorders such as PD or Schizophrenia (Sarkar et al., 2010). This receptor-mediated increase in IFN-γ secretion suggests that dopamine acts through the DR D3 receptor, influencing signaling pathways in the T cells and microglial cells of patients with neurocognitive disorders (Sarkar et al., 2010). Microglial cells are macrophages resident in the CNS. These cells are important for immune function and activated following exposure to infected cells or other forms of stimuli. Once activated, these cells may induce inflammation, cytotoxicity, or regulation of immune responses from T lymphocytes during antigen presentation (Sarkar et al., 2010).

Gut-homing of CD8 T cells is the process of recruiting specific T cells to the gut for an immune response. Evidence supports an important role of DA in the homing of CD8 T cells. Upon DA-mediated stimulation, CD8 T cells adhere to fibronectin and ICAM-1 through integrins. DA acts synergistically with chemokines CCL19, CXCL2, and CCL21 through the DR D3 subtype to induce chemotactic migration of these cells (Sarkar, et al., 2010; Nasi et al., 2019). Acting through various types of receptors, DA induces cytokine secretion in resting T cells. Activation of the DR D1, D3 or D5 subtypes increase secretion of TNFα while stimulation of DR D2 subtype increases secretion of IL-10. DA exerts several additional effects in T cells including the inhibition of T cell proliferation, secretion of IL-2, IFN-γ, and IL-4, and downregulation of signaling molecules lck and fyn through activation of D2-like DR (Sarkar et al., 2010; Nasi et al., 2019).

CD8 T cells express both TH and VMAT2 in the cytoplasmic layer surrounding the nucleus. Expression of these proteins characterizes the capacity for DA synthesis in these cells. Dopamine levels in CD8 T cells are much lower than that of CD4 T cells. Increased expression of mRNA levels for D1, D3, D4, and D5 subtypes stimulates the generation of CD8 T cells. Once activated, D1-like DR inhibit the generation of CD8 T cells and the suppressive activity of existing CD8 T cells in peripheral blood mononuclear cells (PBMC) (Nasi et al., 2019). The DR D3 subtype is primarily expressed in resting CD8 T cells located in the secondary lymphoid tissues, including those of the gut. Dopamine selectively induces homing of naïve CD8 T cells through activation of the D3 receptor subtype. This mechanism occurs via stimulating adhesion of these cells to fibronectin following the activation of β1 integrins VLA-4 and VLA-5 or adhesion to ICAM-1 through the activation of LFA-1 integrin and suggests the involvement of dopamine in the transendothelial migration of CD8 T cells (Watanabe et al., 2006).

Dopamine receptor gene polymorphism plays a major role in the function of CD4 T cells. Evidence supports a strong correlation between D1-like DR and CD4 T cell count in the peripheral blood. D2-like DR were not found to influence the function on these cells (Cosentino et al., 2015). Activation of DR induces polarization of T cell differentiation and alters the expression and activity of metalloproteinases, leading to an increase in clonogenicity of these cells. Action of DA through the D1-like DR may influence CD4 T cell viability by inducing anti-apoptotic effects (Cosentino et al., 2015). Similar to an earlier study, DA exhibited the ability to inhibit Treg function and suppress proliferation of T effector cells. This action is hypothesized to be mediated through DR D5 subtype and may be responsible for inducing increased GATA-3 expression and secretion of IL-4 and IL-5 (Cosentino et al., 2015). GATA-3 is a transcription factor that characterizes Th2 cell differentiation. Although both expressed on CD4 T cells, D1-like DR are

expressed more prominently than D2-like DR. CD4 T cells are highly sensitive to DA as it functions to down-regulate regulatory functions of these cells acting through D1-like DR. In response to DA, these regulatory cells exhibit a pronounced and sustained increase in cAMP as D1-like DR induce stimulatory effects on the second messenger (Cosentino et al., 2015; Cosentino et al., 2017).

Individuals diagnosed with HIV have exhibited subcortical dopamine deficiencies which demonstrates the induction of decreased fronto-striatal functioning and progression or generation of neurocognitive dysfunction. Increased CNS viral load in the presence of DA enhancing agents, including L-DOPA and selegiline was observed in HIV infected individuals as well (Cosentino et al., 2015). Macrophages possess several protective properties in the body, generally promoting homeostasis through trophic, phagocytic, and regulatory functions. Monocyte derived macrophages (MDMs) express both D1-like and D2-like DR, specifically D1 and D2 subtypes, in the cytoplasm and on the surface, respectively. In a study examining HIV-infected macrophages, inoculation of MDMs with HIV in the presence of DA increased the count of infected cells and following activation of D2-like DR, HIV replication was increased in these cells. This effect indicates a potential mechanism through which DA enhances HIV infection and indirectly contributes to the transmissibility of HIV. Agonizing the DR D2 subtype mediated activation of ERK1 suggesting that these receptors are functionally active on the surface of MDMs (Gaskill et al., 2009). Since the development of antiretroviral therapy (ART), HIV-positive individuals on ART are living longer, healthier lives, but their risk of developing HAND has only increased. As DA-rich regions in the brain are susceptible to damage during infection with HIV, increased extracellular DA may contribute to the progression of HAND (Gaskill et al., 2009).

Dopamine receptor activation is associated with different signaling pathways. The primary pathway is mediated by cAMP and is activated by D1-like DR coupling to $G\alpha_s$. This pathway is inhibited by D2-like DR coupling to $G\alpha_i$. A second pathway is mediated by $Ca2^+$ release from the endoplasmic reticulum through the activation of IP3 receptor. This pathway is triggered by activation of DR D2 subtype coupling to G-$\beta\gamma$ or DR D5 subtype coupling to $G\alpha_s$. Dopaminergic activation of both DR subtypes enhances entry of HIV into MDMs (Gaskill et al., 2019). Dopamine exposure in MDMs increases release of $Ca2^+$ but has no effect on cAMP formation in these cells, suggesting the $Ca2^+$-mediated pathway may be the mechanism through which dopamine enhances viral entry. Dopamine mediates the release of $Ca2^+$ in MDMs which has exhibited modulatory effects on protein kinase C phosphorylation through the Gq/11-mediated pathway. This DA-induced migration of $Ca2^+$ is mediated by coupling of DR D5 subtype to Gq/11, suggesting another mechanism through which dopamine acts to increase viral entry of HIV in MDMs (Gaskill et al., 2019).

In addition to expressing D1 and D2 DR subtypes, MDMs also express mRNA for the D3, D4, and D5 DR subtypes. Tyrosine hydroxylase and AADC are proteins required for DA synthesis. In addition to the expression of DAT and VMAT2 in the plasma and cellular membranes, MDMs express TH and AADC in the plasma membrane. This expression suggests the ability to take up extracellular dopamine and store it in a similar manner to Mo-DCs (Gaskill et al., 2012). Activation of any DR expressed on MDMs influences secretion of cytokines including IL-6, CCL2, TNF-α, CXCL8, and IL-10. Each of these cytokines are heavily involved in the regulation of neuroinflammation.

A study examining the effects of dopamine exposure on specific immune cells treated MDMs with LPS to model inflammatory producing cells. The non-LPS treated MDMs serve as

controls or non-inflammatory producing cells. In non-LPS treated MDMs, interaction with high concentrations of DA resulted in a significant increase in IL-6 and CCL2 secretion. LPS treated MDMs induced a significant increase in the secretion of IL-6, CXCL8, and IL-10 while decreasing secretion of TNF-α (Gaskill et al., 2012). TNF-α is an important cytokine with neuroprotective functions. A significant increase in the secretion of CXCL8 was observed when LPS treated MDMs were exposed to high concentrations of DA. Elevated secretion of IL-8 and CCL2 may prompt recruitment of other immune cells including peripheral blood monocytes, T lymphocytes and neutrophils, increasing the permeability of the blood-brain barrier. This effect, not seen in non-LPS treated MDMs, suggests high concentrations of DA is associated with inducing inflammatory responses. These high concentrations of DA were conceptually compared to such concentrations in the brain following opioid use (Gaskill et al., 2012).

The ability of DA to activate NFκB and its impact on MDMs was observed. Dopamine increased the percentage of MDMs with high expression of nuclear NFκB, indicating the capacity for DA to induce nuclear translocation of the transcription factor and elicit inflammatory responses in these cells (Gaskill et al., 2019). Evidence also supports the activation of an intestinal inflammasome as it was discovered that *Salmonella* leucine-rich repeat protein prevents secretion and movement of pro-inflammatory cytokine IL-1β through the inhibition of inflammasome activation. Dopamine exhibits the ability to prime, but not activate, NLRP3 inflammasome. Inflammasomes regulate the production of proinflammatory cytokine IL-1β which is increased by DA. The inflammasome pathway is associated with the development of diseases resulting from neuroinflammation including Multiple Sclerosis, Parkinson's Disease, and Alzheimer's Disease. This pathway has also been linked to development or progression of neuropsychiatric disorders like depression, anxiety, and addiction (Rutsch et al., 2020). Activation of the NLRP3

inflammasome requires a second signal following the DA-induced priming stage. Once fully activated, the inflammasome activates caspase-1 to proteolytically cleave IL-1β (Franchi et al., 2009). The inflammasome is activated upon interaction with pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) which demonstrates an ATP-mediated mechanism. The involvement of dopamine in the activation of NLRP3 inflammasome implicates its role in the development of conditions such as Alzheimer's disease and in the biochemical pathways responsible for producing ATP to drive biological and cellular function within the body.

D2-like DR are preferentially expressed on NK cells. Increased secretion of IL-2 stimulates proliferation and cytotoxicity of NK cells. IL-2 also functions with IL-12 to induce IFN-γ secretion by NK cells, among other immune cells. Significant increase in expression of DR D5 subtype mRNA was observed in IL-2 activated NK cells exposed to DA. IL-2 activated NK cells inhibited the release of IFN-g in a dose-dependent manner which is mediated by upregulation of the D5 subtype through the action of dopamine. (Mikulak et al., 2014). The ability of dopamine to activate NFκB stimulates cytokine release in NK cells following the stimulation of IL-2 (Gaskill et al., 2019; Mikulak et al., 2014). Dopamine-induced inhibition of DNA synthesis and increase in cAMP production in NK cells is mediated through activation of the DR D5 subtype (Mikulak et al., 2014).

4.2 Impact of the Gut Microbiome on Dopamine Concentrations

Many studies have associated gut dysbiosis with the development of pathological conditions including Parkinson's disease (PD), depression, anxiety, HIV-associated neurocognitive disease (HAND), ADHD, and autism. For this association to be true, components

of the gut microbiome must be able to interact with components of the CNS to induce effects that serve as precursors for development of these conditions. Some intestinal microbes can synthesize and influence bioavailability of neurotransmitters including dopamine. Gut-resident microbes produce SCFAs which serve to regulate and protect against systemic inflammation in addition to maintaining intestinal epithelial barrier integrity (Cruz-Lebron et al., 2021; Hamamah et al., 2022). These fecal metabolites incorporate DA from the colon and produce serotonin through GPCR-mediated pathways, primarily through FA3R and FA2R. Butyrate and acetate activate FA3R and FA2R, respectively. Butyrate exerts protective effects through FA3R signaling against SALS-induced toxicity. SALS directly and negatively impacts the abundance of dopaminergic neurons which is key for inhibiting the progression of neurocognitive disorders such as PD (Hamamah et al., 2022).

Alterations to the gut microbiome that result in a reduction of butyrate production induces a reduction in the protective function exerted by butyrate, thus reducing DA concentration or abundance of DA-releasing neurons (Hamamah et al., 2022). Clostridium belongs to the Firmicutes phylum and has shown the ability to degrade dopamine. Clostridia-produced metabolites inhibit DA β-hydroxylase function, thus inhibiting conversion of DA into norepinephrine, resulting in an accumulation of dopamine and norepinephrine deficiency (Hamamah et al., 2022). Accumulation of DA metabolites causes oxidative damage as a result of glutathione depletion in the brain. This DA-induced oxidative stress induces detrimental effects including apoptosis of DA-releasing neurons (Hamamah et al., 2022). L-DOPA is an intermediate in the synthesis of DA. The presence of L-DOPA in the gut is converted to DA by *Enterococcus faecium,* a gut-resident bacterial metabolite. Loss of DA in the periphery presents as

gastrointestinal malfunctions that ultimately result in intestinal dysmotility (Hamamah et al., 2022).

Dopamine impacts immune function in both the brain and the gut, but circulating immune cells have the greatest exposure to dopamine in the plasma. Like most other immune cells, peripheral blood lymphocytes (PBL) express mRNA for dopamine receptors, specifically DR D3, D4, and D5 subtypes (Mckenna et al., 2002). Low concentrations of DA in the plasma induce anti-apoptotic effects on PBL while the opposite is true for high concentrations of DA (Cosentino et al., 2004). Dopamine at high concentrations primarily acts through the DR D5 subtype on PBL to induce oxidative stress and early apoptosis. Cosentino et al. used high concentrations of DA to mimic extracellular DA concentrations in the brain following opioid use. High DA concentrations inhibit proliferation and cytotoxicity of both CD4 and CD8 T cells.

4.3 Impact of Dopamine on Gut Cytokine Secretion

Naïve CD4+ T cells differentiate into distinct functional effector subsets in response to specific cytokine signaling. Upon activation of D2-like DR during DC-T cell interaction, DA led to the production of IL-17, suggesting its ability to polarize T cell differentiation toward the Th17 cell lineage. Th17 cells are best known for their involvement in the maintenance of gut homeostasis, protecting against infections at mucosal surfaces, secretion of cytokines IL-17 and IL-22. When activation of D1-like DR on naïve T cells was blocked, IL-17 production was significantly reduced, thus preventing differentiation to the Th17 subset, indicating DA acts through D2-like DR to polarize such differentiation (Nakano et al., 2008). Decreased IL-17 secretion is accompanied by an increase in IFN-γ suggesting that action through D1-like DR

contributes to polarizing Th1 cell differentiation by decreasing cAMP production and inhibiting DA synthesis in monocyte-derived DCs. An imbalance of Th17 cells is associated with alterations to gut homeostasis (Nakano et al., 2008). Table 3 in Appendix A illustrates effects exerted by DA on immune cells as it acts through specific DR.

Microbial processing in the gut results in an influx of DA which can regulate the production of cytokines including IL-4 and IFN-γ. Levels of these cytokines increase in response to gut dysbiosis or DA deficiency, stimulating NK cell-mediated immune responses. Dopamine released and circulating in the periphery through the activation of D1-like DR on hepatic invariant NKT cells decrease levels of cytokines (Hamamah et al., 2022). Invariant NKT cells are key mediators in liver immunity and homeostasis. For example, patients with Hepatitis C virus (HCV) exhibit decreases in DA synthesis and release in the basal ganglia as a result of increased IFN-α secretion.

5.0 Discussion

The ultimate connection between opioid use and functions of the gut-brain axis at the biological level is dopamine signaling as it is central to gut-brain communication. The collective work reviewed here highlights the multifaceted nature of opioid use, dopamine signaling, and the influential functions on the gut-brain axis.

As discussed throughout this review, opioid use exerts major effects on the health of the CNS, permeability of the blood brain barrier, diversity of the gut microbiome, and the synthesis and release of dopamine. Dopamine has exhibited the ability to influence various immune functions including cytokine and chemokine secretion, influencing immune cell differentiation, determining cell survival, and facilitating specific gut-brain interactions. These functions of dopamine have resulted in altered permeability of intestinal epithelial barrier, promoting translocation of gut bacterial metabolites across the intestinal barrier, and allowing direct interaction of these microbes with cells of the CNS. This dopaminergic effect is one major mechanism through which altered gut diversity may influence immune responses and homeostatic conditions in the brain, placing both the intestinal and blood brain barriers at high risk of infection.

The connection between neuro- and gut-immune communication and pathological disease development exists primarily through dopaminergic mechanisms. Dopamine in the CNS is greatly impacted by opioid use disorder and associates with the development of inflammatory pathologies in the periphery. The collective work suggests multiple mechanisms through which opioid use disorder stimulates inflammation in peripheral tissues as a result of modulating the functions of multiple immune cells. Opioid-induced elevation of DA concentrations in the brain pre-disposes

individuals suffering from opioid use disorder to robust inflammatory responses, modulations to function of the gut-brain axis, and development of pathological disorders.

This review is unique in that it reviews findings from several human studies and aims to identify key associations among those studies. There is a lack of research that identifies a clear path outlining the impact of opioid use on gut-brain-immune communication and what this means for disease development. Based on the collective results from the literature discussed, I speculate that chronic opioid use increases extracellular dopamine concentrations and synthesis of dopamine by various types of cells which allows dopamine to exert its immunomodulatory effects throughout the body, likely increasing the potential of pathological and neurodegenerative disease development discussed throughout this review. I also hypothesize an important role played by opioid-induced dopamine signaling in the alteration of the gut microbiome seen in individuals suffering from opioid use disorder and as a result, contributes to development of neurological conditions that exist as precursors for certain pathological and neurodegenerative diseases.

5.1 Implications

The literature discussed poses significant implications for research, health and disease, and practice. Greater efforts to increase awareness of opioid use disorder, associated risks, and harm reduction behaviors are key to normalizing discussion regarding this topic and addressing the life threatening effects of chronic opioid use. The prevalence of opioid use disorder continues to increase throughout the United States and individuals with opioid use disorder are at increased risk of acquiring infectious diseases. In this context of increased risk and disease burden, it is important for providers and public health practitioners to engage harm reduction approaches and implement

harm reduction programs that may contribute to the reduction of infectious disease transmission and support linkage to and retention in care among this population.

Due to the lack of research that outlines the effects of dopamine concentrations on the gut microbiome in humans, mechanisms behind the bidirectional impact of the gut microbiota and dopamine signaling in this population are poorly understood. Although the effects of opioids on the gut microbiome have been moderately studied, the impact of opioid-induced dopamine signaling on the gut microbiome is a majorly understudied area of research. Continued research focused on such mechanisms in the human body is required to understand the effects dopamine signaling pathways stimulated by opioid use and ultimately reduce the burden of addiction and associated disease.

5.2 Limitations

Several limitations exist for this review. In the collection of relevant publications, studies that focused on human subjects or analyzing other human studies were lacking. Many animal studies have been conducted to examine the effects of opioid use on specific immune cells in addition to determining specific effects of dopamine on the immune system of animal models. Though animal-focused research may serve as an important precursor for research in humans, acquiring a deeper understanding of opioid use, dopamine signaling, and the gut-brain axis at the immunological level requires more research using human subjects.

In human-focused studies, there was a lack of research that explicitly examined the role of the dopamine in the function of the gut microbiome. Many studies included in this review

hypothesized potential effects of dopamine-altered gut microbiome on the CNS, but none of these studies set out to examine this area of research directly.

A systematic review was not within the scope of this review, thus a method more similar to conducting a scoping review was used. This review may serve as a precursor for a future systematic review which may be beneficial for future experimental research.

5.3 Future Directions

As emphasized in previous sections, more research is required to further understand concepts in this topic. Future research should explore the mechanisms through which dopamine acts to modulate immune functions and its contribution to progression of neuro- and gastrointestinal pathologies in individuals suffering from opioid use disorder. Understanding the extent of dopaminergic effects and how these effects associate with neurocognitive or gastrointestinal disorders may provide potential targets for therapeutic intervention to enhance immune function and reduce development of such pathologies for this population.

Future directions for public health practice should continue raising awareness within communities about harm reduction services and the impact of opioid use on the body may contribute to the reduction of infectious disease burden among this population, thus reducing the potential of neurocognitive and gastrointestinal pathology development. Ideal future directions would focus on both continued research in humans and improving upon methods within the practice realm. Collaborative efforts in both areas are crucial to progress in this area of research. Continued research is necessary to improve upon current harm reduction programs and therapeutic

interventions. Likewise, these public health interventions should be practiced for continued work toward reducing addiction and disease burden in this population.

Appendix A

Appendix A.1 Tables

Table 1: Medline Search Strategy

Literature Search Description	
Provider/Interface	Ovid
Database	Medline ALL
Date searched	10 October 2023
Database update	1946 to October 09, 2023
Search developer(s)	Helena M. VonVille; Ireland O'Brien
Limit to English	Yes
Date Range	No date limits specified
Publication Types	No publication types specified
Search filter source	No search filters used

Table 2: Medline Search Strategy (cont.)

Key Search Terminology	
1	Models, Neurological/
2	astrocytes/ or brain/ or exp brain stem/ or basal ganglia/ or exp amygdala/ or basolateral nuclear complex/ or central amygdaloid nucleus/ or corticomedial nuclear complex/ or Hypothalamo-Hypophyseal System/ or Microglia/ or Neurons/ or periamygdaloid cortex/ or cerebral cortex/ or frontal lobe/ or prefrontal cortex/ or broca area/ or dorsolateral prefrontal cortex/
3	Receptors, Opioid/ or Nociceptin Receptor/ or "Receptors, Opioid, delta"/ or "Receptors, Opioid, kappa"/ or "Receptors, Opioid, mu"/ or "Receptors, sigma"/
4	(amygdala or astrocytes or (basal adj1 ganglia) or brain or cortex or microglia or neurobiologic* or neurological or neurons or (opioid* adj2 receptor*)).ti,ab,kf.
5	1 or 2 or 3 or 4
6	models, immunological/
7	Immunomodulation/
8	exp Immune System/ or exp T-Lymphocytes/ or exp Antibodies, Monoclonal/
9	(immune or Immunomodulat* or immunolog* or lymphocyte* or ("t" adj1 (cell or cells))).ti,ab,kf.
10	cytokines/ or exp chemokines/ or interleukins/ or interleukin-6/ or interleukin-8/
11	(chemokine* or cytokine* or interleukin*).ti,ab,kf.
12	microbiota/ or gastrointestinal microbiome/ or microbial consortia/
13	(((gastrointestinal or gut or intestinal) adj3 (epithelial or lymphoid or microbiota or microbiome)) or (microbial adj1 consortia)).ti,ab,kf.
14	im.fs.
15	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16	5 and 15
17	Neuroimmunomodulation/
18	(neuroimmuno* or 28nglish28uroimmunology*).ti,ab,kf.

19	16 or 17 or 18
20	analgesics, opioid/ or codeine/ or fentanyl/ or heroin/ or hydrocodone/ or hydromorphone/ or methadone/ or morphine/ or Narcotic-Related Disorders/ or narcotics/ or exp Opioid-Related Disorders/ or opium/ or oxycodone/ or oxymorphone/ or tramadol/
21	(codeine or fentanyl or heroin or hydrocodone or hydromorphone or methadone or morphine or narcotic* or opioid* or opium or oxycodone or oxymorphone or tramadol).ti,ab,kf.
22	20 or 21
23	19 and 22
24	limit 23 to 29nglish language
25	dopamine.ti,ab,kf.
26	Dopamine/
27	dopamine agents/ or dopamine agonists/ or dopamine antagonists/ or dopamine uptake inhibitors/
28	25 or 26 or 27
29	23 and 28
30	22 and 28
31	Drug Users/
32	substance-related disorders/ or narcotic-related disorders/ or opioid-related disorders/ or heroin dependence/ or morphine dependence/ or opiate overdose/ or opium dependence/ or substance abuse, intravenous/ or substance abuse, oral/
33	(((drug or substance) adj1 (abuse* or dependence or dependent or “use” or user or users)) or addict or addicted or IDU or addiction).ti,ab,kf.
34	31 or 32 or 33
35	23 and 34
36	limit 35 to 29nglish language
37	36 not (exp “Animals”/ not “Humans”/)
38	19 and 38
39	limit 38 to English language
40	((12 or 13) and 28) not (exp “Animals”/ not “Humans”/)
41	limit 40 to 29nglish language
42	((12 or 13) and 34) not (exp “Animals”/ not “Humans”/)
43	limit 42 to 29nglish language
44	((17 or 18) and 34 and 29nglish.la.) not (exp “Animals”/ not “Humans”/)

45	((6 or 7 or 8 or 9 or 10 or 11) and 22 and 30nglish.la.) not (exp "Animals"/ not "Humans"/)
46	((12 or 13) and 22 and 30nglish.la.) not (exp "Animals"/ not "Humans"/)
47	((17 or 18) and 22 and 30nglish.la.) not (exp "Animals"/ not "Humans"/)
48	(22 and 28 and 30nglish.la.) not (exp "Animals"/ not "Humans"/)
49	(22 and 15 and 30nglish.la.) not (exp "Animals"/ not "Humans"/)
50	(34 and 15 and 30nglish.la.) not (exp "Animals"/ not "Humans"/)
51	49 and 50

Table 3: Opioid-Induced Effect on Gut-Resident Microbes in Individuals Receiving Methadone Treatment

Gut-Resident Microbes	Opioid-Induced Effect	Reference
P. Actinobacteria *F. Bifidobacteriaceae* *B. bifidum* *B. longum*	↑ in relative abundance	Cruz-Lebron et al., 2021
P. Verrucomicrobia *F. Akkermasiaceae* *A. muciniphila*	↓ in relative abundance	Cruz-Lebron et al., 2021
P. Bacteroidetes	↑ in relative abundance following long-term opioid use	Cruz-Lebron et al., 2021; Gicquelais et al., 2020
P. Firmicutes	↓ in relative abundance following long-term opioid use; ↑ in relative abundance	Cruz-Lebron et al., 2021; Gicquelais et al., 2020

Table 4: Dopaminergic Effect on Human Immune Cells

Cell Type	Receptor	Dopaminergic Effect	Reference
Peripheral Blood Lymphocytes (PBL)	D1-like DR (D5 subtype)	**Low** DA concentration: ↓ intracellular ROS; induced anti-apoptotic effect **High** DA concentration: ↑ intracellular ROS; induced pro-apoptotic effect	Cosentino at al., 2004
T helper cell	D1-like DR; D2-like DR	D1-like DR: ↓ IL-17 production; ↑ IFN-γ production D2-like DR: ↑ IL-17 production; mediating Th17 differentiation	Nakano et al., 2008
MO-DC	D1-like DR; D2-like DR	D1-like DR: ↑ TH phosphorylation; ↑ cAMP production (promoting DA synthesis) D2-like DR: ↓ TH phosphorylation; ↓ cAMP production (inhibiting DA synthesis); induced transient $Ca2^{+}$ mobilization	Nakano et al., 2009
$CD4^{+}CD45RA^{+}$ naïve T cell	D1-like DR	↑ cAMP production;↑ IL-4 and IL-5 secretion; promoting Th2 differentiation	Nakano et al., 2009
MDM	D2-like DR	↑ HIV replication	=Gaskill et al., 2009
LPS treated MDM	D2-like DR	↑ IL-6, IL-8, and IL-10 secretion; ↓ TNF-α secretion	Gaskill et al., 2012
Non-LPS treated MDM	D2-like DR	↑ IL-6 and CCL2 secretion	Gaskill et al., 2012
$CD4^{+}CD25^{+}$ Tregs	D1-like DR	Regulates autocrine/paracrine loop via regulation of IL-10 and TGF-β secretion	Cosentino et al., 2007
CD4 T cell [Circulating]	D1-like DR	↑ CD4 T cell count; Induced anti-apoptotic effect	Cosentino et al., 2015
CD4 Tregs	D1-like DR	Downregulation of regulatory functions; ↑ CD4 T cell count; ↑ cAMP production	Cosentino et al., 2017
CD8 T cell	D1-like DR	↓ CD8 T cell generation; ↓ CD8 T cell suppressive activity in PBMCs; ↓ CD8 T cell cytotoxicity; Induced β1-integrin-dependent adhesion to fibronectin; ↑ TNF-α production	Nasi, et al., 2019
Macrophage	D1-like DR; D2-like DR	↑ HIV entry in macrophages; Activates $Ca2^{+}$ mediated pathway D1-like DR: Activates cAMP-mediated pathway D2-like DR: Inhibits cAMP-mediated pathway	Gaskill et al., 2019
Macrophages	D1-like DR *and* D2-like DR	Activation of NF-κB; Primes NLRP3; Regulates IL-1β secretion	Gaskill et al., 2019
CD8 T cells	D2-like DR	Activation of β1-integrins and LFA-1; facilitates TEM; ↑ CD8 T cell chemotaxis	Watanabe et al., 2006
T lymphocytes	D2-like DR	↓ T cell proliferation; ↓ ERK1/ERK2 activation; ↑ T cell apoptosis	Sarkar et al., 2006
Keratinocytes	N/A	Stimulates production of IL-6 (adrenal cells) and IL-8 (lung endothelial cells)	Parrado et al., 2012
NK cells	D1-like DR	↑ cAMP production; ↓ function and DNA synthesis; ↓ IFN-γ production	Mikulak et al., 2014

Peripheral T lymphocytes	D1-like DR	↑ IL-10 and TGF-β synthesis	Sarkar et al., 2010
Peripheral T lymphocytes	D2-like DR	Activation of α4:β1 and α5:β1 integrins; ↑ adhesion to fibronectin; ↓ IL-2, IFN-γ, IL-4 secretion; Down-regulation of lck & fyn	Sarkar et al., 2010

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