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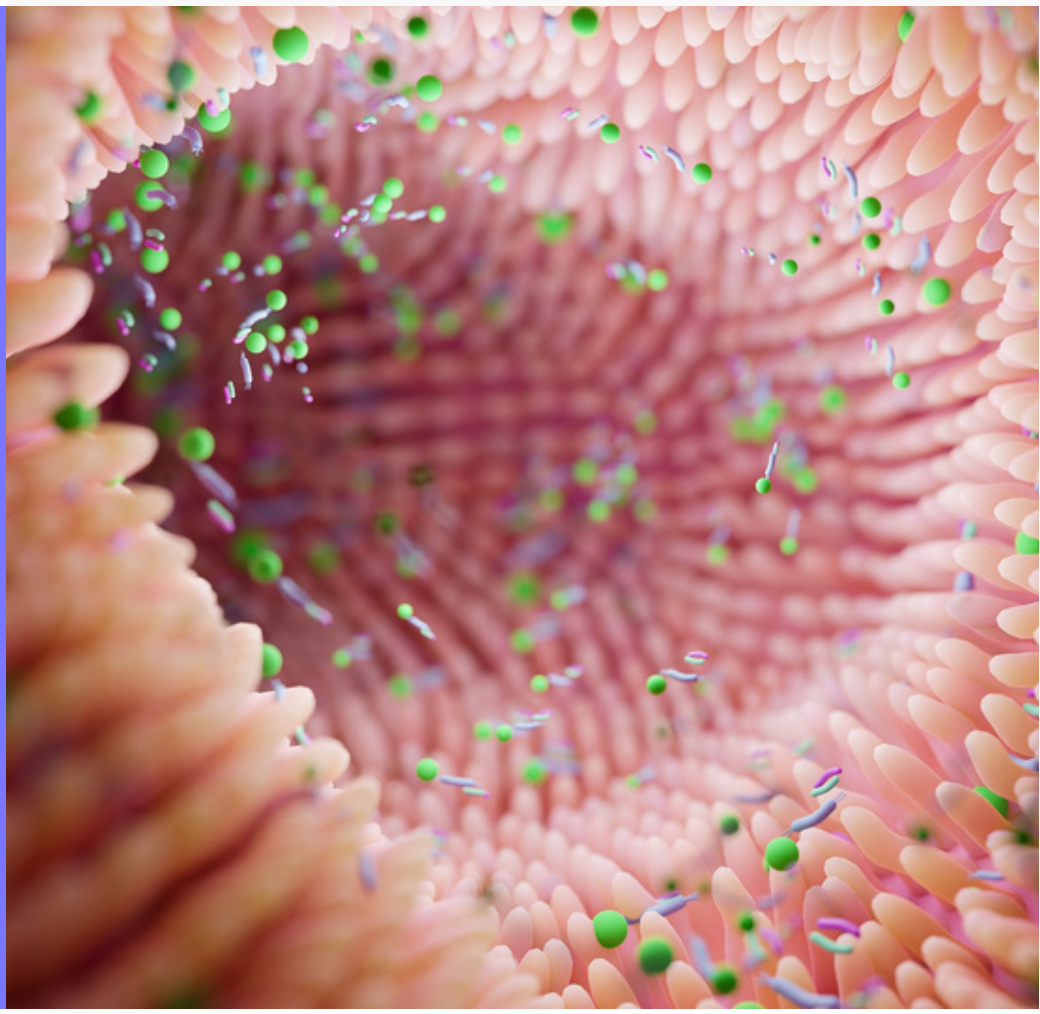
Substance Abuse and the Microbiome

Issue Editors

Prakash Nagarkatti
University of South Carolina, United States

Mitzi Nagarkatti
University of South Carolina, United States

Shilpa Buch
University of Nebraska Medical Center, United States



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ISSN 2674-0001
ISBN 978-2-8325-4637-6
DOI 10.3389/978-2-8325-4637-6

Substance abuse, also known as drug abuse, refers to excessive use of agents that cause harm to self, society, or both. Some of the commonly used substances of abuse include: Opioids, Cocaine, Lysergic acid diethylamide (LSD), marijuana and alcohol. Substance abuse can lead to addiction thereby triggering significant negative effects on the brain and the body. The gastrointestinal (GI) tract harbors a complex array of microorganisms, called the gut microbiota which play a critical role in regulating homeostasis in the body and disease. Extensive use of next-generation sequencing technology has enabled the discovery of how dysbiosis caused by environmental factors can impact the development and severity of many clinical disorders. Such studies have also led to the detection of gut-brain axis, a bidirectional communication pathway between the Central Nervous System and the Gastrointestinal System in which the gut microbiota play a critical role. The gut microbiota can regulate the brain functions through microbial metabolites, including the short-chain fatty acids which can cross the blood-brain barrier. Additionally, the brain can regulate the gut microbiota through neural, endocrine and cytokine pathways. How does substance abuse fit into the complex cross-talk between the gut microbiota and the brain? This is an exciting area of research that is drawing a lot of attention. Some substances of abuse such as alcohol have already been shown to cause dysbiosis which may influence alcoholic liver disease through leaky gut. Such findings also raise an exciting possibility of using probiotics, reversing dysbiosis, or fecal microbiota transplantation as a therapeutic modality to reverse the negative impacts of substance abuse.

This Special Issue is focused on how substance use disorder can alter gut microbiota and impact functions of the Central Nervous and other organ systems. It is also the goal to explore how stabilization of gut microbiota can prevent the negative effects of substance abuse.

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TYPE Special Issue Editorial
PUBLISHED 06 March 2024
DOI 10.3389/adar.2024.12734

OPEN ACCESS

EDITED BY
Sabita Roy,
University of Miami, United States

*CORRESPONDENCE
Prakash Nagarkatti,
prakash@mailbox.sc.edu

RECEIVED 25 January 2024
ACCEPTED 28 February 2024
PUBLISHED 06 March 2024

CITATION
Nagarkatti P, Nagarkatti M and Buch S (2024), Editorial: Substance abuse and the microbiome.
Adv. Drug Alcohol Res. 4:12734.
doi: 10.3389/adar.2024.12734

Editorial: Substance abuse and the microbiome

Prakash Nagarkatti[1]*, Mitzi Nagarkatti[1] and Shilpa Buch[2]

[1]Department of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC, United States, [2]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, United States

KEYWORDS

drug abuse, microbiota, cannabinoids, opioids, dysbiosis

Editorial on the Special Issue
Substance abuse and the microbiome

The gastrointestinal (GI) tract harbors a highly complex array of microorganisms that play a critical role in regulating homeostasis in the body. Thus, any perturbations or imbalance, termed dysbiosis, can trigger disease. While the direct effects of abused substances on various organ systems such as the brain, have been well-recognized, whether drug misuse can also lead to dysbiosis, resulting in clinical disorders remains an interesting possibility that is actively researched. This Special Issue was therefore focused on how substance use disorders (SUD) are linked to dysbiosis, and how altered gut microbial composition can influence the functions of the Central Nervous System (CNS) and the immune system. It was also the goal of this Special Issue to explore whether the stabilization of gut microbiota could lead to the restoration of clinical disorders that are triggered by drugs of abuse.

In this Special Topic Issue, we present four articles: In the first article, Varsha et al. describe how the ability of cannabinoids to suppress inflammation could likely be mediated, in part, through the alterations in the gut microbiome and metabolome. There is significant evidence demonstrating that cannabinoids such as delta-9-tetrahydrocannabinol (THC), and Cannabidiol (CBD) act as anti-inflammatory agents. Also, these cannabinoids have been approved by the FDA to treat several clinical disorders. For example, THC has been approved by the FDA to treat HIV/AIDS-induced anorexia as well as chemotherapy-induced nausea and vomiting in cancer patients undergoing chemotherapy. CBD has also been approved by the FDA to treat certain types of epilepsy syndromes. Moreover, several states in the US have legalized cannabis for medicinal and/or recreational use. Thus, it is important to understand whether the anti-inflammatory effects of cannabinoids are mediated via the regulation of dysbiosis and, if so, the impact of this on health. This review captures the mechanism(s) that trigger dysbiosis following exposure to cannabinoids. Additionally, it highlights how cannabinoids can induce microbial secondary bile acids, short-chain fatty acids (SCFA), and indole metabolites, that can have an immunoregulatory role even in distant organs.

The second review by Ellermann is closely related to the first article in that the review focuses on endocannabinoids. An important highlight of this article is the demonstration

that changes in the gut microbiome caused by external factors such as diet or disease can have a significant impact on the endocannabinoid tone. This is critical inasmuch as endocannabinoids regulate a wide array of important bodily functions such as memory, sleep, temperature control, pain control, appetite, and immune functions. The article also highlights endocannabinoid-mediated regulation of naturally occuring bacteria within the gut microbiome. Additional exciting areas covered by this article include the preclinical studies demonstrating that engineered gut bacteria synthesizing the host N-acylethanolamides could be potentially used to treat diseases that involve aberrant lipid signaling, including obesity and inflammatory bowel diseases.

Drug abuse by HIV-1/AIDS patients has been shown to increase viral load and accelerate the disease progression. The third article by Ray et al. highlights the evidence that the gut microbiome plays an important role in the pathogenesis of HIV-1-linked drug abuse and subsequent neuroinflammation and neurodegeneration. It is well documented that drug abuse can disrupt the gut-brain axis resulting in dysbiosis, and altered expression of neurotransmitters, bile acids, and metabolites, including SCFA. Such alterations can activate a wide range of pro-inflammatory signaling pathways, which, in turn, can impact the CNS through the hypothalamic-pituitary axis, ultimately resulting in pain, stress, and anxiety. The article highlights how understanding the mechanism(s) underlying how drugs of abuse alter the microbiota in HIV-1/AIDS patients could aid in the development of better treatment modalities for drug abuse-related disorders.

The fourth article by Herlihy and Roy focuses on opioid-mediated microbial dysbiosis and its impact on behavior. The review highlights how drug-induced dysbiosis can lead to an increased prevalence of pathogenic bacteria, in turn, manifesting as a compromised gut barrier with consequent systemic translocation of bacteria that trigger proinflammatory cytokine release. The microbiome also communicates with the brain by sending signals through the vagus nerve. The article also discusses how the microbiome can increase microglial activation in the brain as well as dysregulation of brain-derived neurotrophic factor (BDNF) signaling during drug use. All such alterations in the microbiome also impact the behavioral consequences of drug use. Together, these studies suggest that preventing dysbiosis could likely attenuate behavioral symptoms associated with drug use.

In summary, this Special Issue consisting of four review articles comprehensively discusses the complex interactions between drug use and microbial dysbiosis. It also highlights how dysbiosis is closely associated with the endocannabinoid and immune system while communicating with the brain through the gut-brain axis, thereby regulating pain, anxiety, and behavior. The articles highlight the challenges and opportunities to advance this research to better understand and control drug use and behavioral disorders.

Author contributions

PN wrote the original draft and MN and SB edited the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported in part by NIH grants R01ES030144, P20GM103641, and R01AI160896 awarded to PN and MN).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ADAR

MINI REVIEW
published: 14 July 2022
doi: 10.3389/adar.2022.10550

Role of Gut Microbiota in Cannabinoid-Mediated Suppression of Inflammation

*Kontham Kulangara Varsha, Mitzi Nagarkatti and Prakash Nagarkatti**

Department of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC, United States

Cannabinoids and the endocannabinoid system have been well established to play a crucial role in the regulation of the immune response. Also, emerging data from numerous investigations unravel the imperative role of gut microbiota and their metabolites in the maintenance of immune homeostasis and gut barrier integrity. In this review, we concisely report the immunosuppressive mechanisms triggered by cannabinoids, and how they are closely associated with the alterations in the gut microbiome and metabolome following exposure to endogenous or exogenous cannabinoids. We discuss how cannabinoid-mediated induction of microbial secondary bile acids, short chain fatty acids, and indole metabolites, produced in the gut, can suppress inflammation even in distal organs. While clearly, more clinical studies are necessary to establish the cross talk between exo- or endocannabinoid system with the gut microbiome and the immune system, the current evidence opens a new avenue of cannabinoid-gut-microbiota-based therapeutics to regulate immunological disorders.

Keywords: inflammation, cannabinoids, microbiota, drug abuse, endocannabinoids

OPEN ACCESS

Edited by:
Emmanuel Onaivi, William Paterson University, United States

Reviewed by:
Georgianna G. Gould, The University of Texas Health Science Center at San Antonio, United States
Declan Ali, University of Alberta, Canada
Anna Bukiya, University of Tennessee Health Science Center (UTHSC), United States

****Correspondence:***
Prakash Nagarkatti
Prakash@mailbox.sc.edu

Received: *04 April 2022*
Accepted: *10 June 2022*
Published: *14 July 2022*

Citation:
Varsha KK, Nagarkatti M and Nagarkatti P (2022) Role of Gut Microbiota in Cannabinoid-Mediated Suppression of Inflammation. Adv. Drug. Alco. Res. 2:10550. doi: 10.3389/adar.2022.10550

INTRODUCTION

Cannabis sativa, otherwise known as marijuana, has a rich history of being used for medical and recreational purposes. The complex biochemical metabolism of cannabis leads to the production of over 550 chemical constituents of which over 100 are identified as phytocannabinoids (1). The chemical structure of the non-psychoactive cannabinoid compound, cannabidiol (CBD) was first deduced in 1963 followed by the identification of the psychoactive cannabinoid, δ9-tertrahydrocannabinol (THC), in 1964 (2). Cannabinoids accomplish their physiological and behavioral consequences *via* their binding to the cannabinoid G-protein-coupled receptors (GPCRs), CB1 and CB2 (CBRs). The existence of these receptors and their endogenous ligands, named endocannabinoids (eCBs), in the human system was discovered in the 1990s thus revealing the functions of eCB system in neuronal and immunomodulatory functions. eCBs such as anandamide (AEA) and 2-arachydonoylglycerol (2-AG), which are native lipid-based retrograde neurotransmitters, as well as exogenous cannabinoids such as THC, act as strong agonists of CBRs. CBD, unlike THC, is not psychoactive and considered to be a negative allosteric modulator of the CB1 receptors (3). Fatty acid amide hydrolase (FAAH) and monoacylglycerol acid lipase that break down AEA and 2–AG, respectively, are the two other important participants of the eCB signaling system (4-6). The CB1 and CB2 receptors are expressed primarily in the brain and immune cells respectively and mediate nearly all the effects of both endogenous and exogenous cannabinoids (6, 7).

The downstream signaling initiated by CBRs involves the inactivation of protein kinase A following inhibition of adenyl cyclase activity and a decrease in cyclic adenosine monophosphate (cAMP) levels. In addition, CBRs trigger an array of various mechanisms in the wake of activation. This includes multiple effector protein kinase signaling cascades related to cell proliferation and survival such as phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/AKT), p38 mitogen-activated protein (p38 MAP) kinases, extracellular signal-regulated kinase (ERK) as well as focal adhesion kinase (FAK) (8). Coupling to ion channels, phospholipase-cb activation, and ceramide biosynthesis are certain other pathways activated by CBRs (7–11). It is demonstrated that eCBs can also bind to non-CBRs, of which the most investigated are transient receptor potential vanilloid 1 (TRPV1) channel along with peroxisome proliferator-activated receptor and orphan GPCRs (9, 12). Most of these pathways mentioned above are entwined with maintenance of immune homeostasis. For example, certain subsets of effector $CD4^+$ T cells depend on the PI3K signaling activation for their differentiation and steering of effector functions. Additionally, PI3K and p38MAPK signaling are involved in the production of inflammatory cytokines (13, 14), ERK signaling plays a role in resistance to immunomodulatory drugs (15), and nuclear translocation of FAK is important to regulate inflammatory gene expression of chemokines and cytokines (16). Such studies demonstrate that the downstream signaling pathways initiated by CBRs can lead to immunomodulation.

It is well documented that the gut microbiome plays a crucial role in host metabolism as well as the balance between pro- and anti-inflammatory responses, thereby controlling disease pathogenesis (17). Short-chain fatty acids (SCFAs), lipopolysaccharides (LPS), and other biologically active metabolites generated by various microbial species contribute toward immune regulation, activation or suppression (18). Thus, dietary and medical interventions that manipulate the composition of the microbiome have been shown to cause pro- or anti-inflammatory milieu (19). There is emerging evidence that the gut microbiome and eCB system communicate via signaling pathways involved in nutrient processing and energy metabolism (17). In this mini-review, we briefly recite an account of the effects and mechanisms of endogenous and exogenous cannabinoids on immunosuppression via microbiome-mediated activities.

MECHANISMS AND NATURE OF IMMUNOMODULATION CAUSED BY CANNABINOIDS

Cannabinoids are well established as anti-inflammatory agents with a significant and wide range of immunosuppressive properties that have been meticulously reviewed before (20–25). CBD, the nonpsychotic cannabinoid, was shown to induce myeloid-derived suppressor cells (MDSCs) which suppressed T cell proliferation *in vitro* and *in vivo* (26). MDSCs mostly express CD11b and Gr-1 and represent a heterogeneous population of immature myeloid cells which produce arginase 1 and inducible nitric oxide synthase that enables them to suppress T cell proliferation (27). Cannabinoid-induced MDSCs upon adoptive transfer were shown to attenuate LPS-induced acute inflammation *in vivo* (26, 28). The psychoactive THC was also shown to induce MDSCs independent of TLR4. THC mobilized MDSCs from bone marrow and caused their expansion in the periphery (29). In addition to the generation of MDSCs, cannabinoids have also been shown to induce regulatory T cells (Tregs) (30–33). Such T cells express FoxP3, a transcription factor that plays a critical role in their differentiation and functions, and secrete immunosuppressive cytokines such as interleukin (IL)-10 and transforming growth factor β (TGF-β) (34). Additionally, cannabinoids can also induce apoptosis of immune cells such as T and B lymphocytes, macrophages, and dendritic cells (DCs) leading to immunosuppression (35). THC triggered DC apoptosis via reduction of mitochondrial membrane permeability, cleavage of Bid, activation of caspase cascade, and release of cytochrome-c (36). THC treatment caused phosphorylation of IkappaB-alpha and augmented apoptotic gene transcription regulated by NF-kappaB (35–38). Moreover, agonists of CBRs can disrupt the balance of pro and anti-inflammatory cytokines. THC exposure restrained the production of IL2, IL-12, and interferon-gamma (IFN-γ), and altered the equilibrium of T helper 1 (Th1)/T helper 2 (Th2) cytokines in a CB2R dependent manner (39, 40). Also, THC and AEA were shown to suppress inflammatory Th1 and Th17 response during delayed-type hypersensitivity response (41, 42).

Epigenetic modulations are additional mechanisms of immunosuppression triggered by cannabinoids (43). The eCB system undergoes epigenetic modifications and such variations are observed in pathological disorders such as Diabetes, Parkinson's, Alzheimer's, and colorectal cancer. The main targets of these modifications are the genes CNR1 and CNR2 that encode for CB1R and CB2R along with FAAH (44–46). Recent investigations provide insights into cannabinoid-mediated epigenome modifications and their impact on the suppression of the immune system. THC treatment increased methylation of the promoter region of DNA methyl transferases, DNMT3a and DNMT3b in MDSCs leading to subsequent reduction in DNMT3a and DNMT3b expressions in C57BL/6 mice. Moreover, a decrease in the methylation of Arg1 and STAT3 promoter regions was observed that led to over-expression of Arg1 and STAT3 (47). THC was shown to activate or suppress the expression of genes via histone modifications. THC treatment led to histone modifications that led to increases in Th2 cytokine genes while suppressing Th1 cytokine genes, thereby switching the immune response from Th1 to Th2 (48).

Up-regulation or down-regulation of microRNAs (miRNAs) are another major route of epigenetic alterations prompted by cannabinoids. Treatment of C57BL/6 mice with THC elevated miR-21 while lowered miR-29b expression that was associated with a corresponding increase in SMAD7 and decrease in IFN-γ expressions. This in turn inhibited Th1/Th17 activation in delayed-type hypersensitivity reaction (47). The eCB, AEA

mitigated Staphylococcal enterotoxin B (SEB)-induced acute respiratory distress syndrome (ARDS) in mice via down-regulation of miRNA-23a-3p, which up-regulated arginase and TGF-β2, and miRNA-34a-5p that prompted FoxP3 induction. A reduction in pro-inflammatory cytokines such as IL-2, TNF-α, and IFN-γ, while an increase in MDSCs was detected following AEA treatment (49). THC administration into SEB-injected C3H/HeJ mice was reported to down-regulate miR-17/92 and miR-374b/421 clusters while up-regulating miR146a leading to the release of PTEN thus acting as an AKT inhibitor leading to a reduction in IFN-γ production (31). Another study reported that THC altered expressions of the members of miR-17-92 cluster, particularly miR-18a that directed the release of PTEN (50). A detailed description of cannabinoid-mediated epigenetic modulations pertaining to immune suppression has been recently published (43). It is interesting to note that the intestinal microbiota and their metabolites have been shown to regulate several epigenetic pathways (51). This raises the question of whether the cannabinoid-mediated changes in the epigenetic pathways are linked to the gut microbiota.

GUT MICROBIOTA, ECB SYSTEM, AND GUT-BRAIN AXIS

The diverse intestinal microbial population found in the gut shares a mutual symbiotic relationship with the host. The microbiota benefits the host by modulation of gut motility, intestinal barrier function, and nutrient absorption. Moreover, gut microbiota plays a major role in host metabolism and is associated with regulation of the inflammatory status of the host not only in the gut but also in other organs such as the brain (52, 53). Thus, alterations in the microbiota, called dysbiosis, caused by nutrition, stress, environmental factors, and drugs, can have either beneficial or deleterious effects on the inflammatory status of the host. Gut-brain axis, which is the bidirectional crosstalk between the central and enteric nervous systems is influenced by gut microbiota via neural, endocrine, and immune networks (54). Emerging data establishes the influence of gut microbiota in anxiety and depression-like behavior (55). Clinical studies denote the abundance of pro-inflammatory and reduced SCFA producing bacterial species in these disease conditions, and this pathophysiology relates to the transmission of peripheral inflammation to the brain (56). There are recent reviews which have discussed the effects of cannabinoids, including CBD and alcohol in the microbiota-gut-brain axis (57) and therefore not further discussed this topic in this review.

It has been widely recognized that eCB is dynamically involved in the regulation of glucose and energy metabolism. It is also important to note that the immunomodulatory effects of eCBs are not always mediated via CBRs. Metabolism of 2-AG and AEA generates lipid components and hence acts as a source of arachidonic acid in the biosynthesis of additional pro-inflammatory lipids (58). Advanced research in the field of eCB system and immune modulation indicates the contribution of bio lipid members of eCB system in the onset or progression of various diseases such as obesity, diabetes, inflammatory bowel disease (IBD), and multiple sclerosis (MS) which are also reported to be augmented by alterations in the microbiota (59). Elevated eCBs level impedes excitatory and inhibitory neurotransmitters release which affects immune homeostasis and energy balance while increasing gut permeability (59, 60). Direct evidence of intestinal microbe-mediated eCB system manipulation comes from a recent study where *Candida albicans* manifestation altered the levels of lipid and eCBs in the brain and gastrointestinal (GI) tract leading to increased anxiety-like behavior in mice (61). Considering the relevance of eCB system and gut microbiota in the manipulation of the immune system, it is inevitable to explore the possible relationships and mechanisms between both systems from the perspective of inflammatory diseases.

ALTERATION OF THE GUT MICROBIOTA BY CANNABINOIDS

Various lines of ongoing research have connected the gut microbiota with metabolic and neurological disorders (52). Dietary interventions with specific fatty acids have been reported to increase the level of eCBs in human observational studies. These changes in eCBs have been attributed to variations in Peptostreptococcaceae, Veillonellaceae, and Akkermansiaceae (62). Cannabis consumption has been demonstrated to alter eCB tone and induce mucosal healing in ulcerative colitis (UC) patients in addition to improving quality of life (63). Modulation of eCB system using cannabinoids has been demonstrated to favor immune suppression *in vivo* (64). Present-day research targets to unravel the role of exogenous as well as endogenous cannabinoids in gut microbiota modulations and their impact on neurological and inflammatory conditions. Our lab has published multiple research articles on the alterations of microbiome and inflammation employing endogenous and exogenous cannabinoids (64, 65). A recent study demonstrated that the eCB, AEA, reversed the adverse microbiota perturbations instigated by SEB-mediated ARDS in mice. AEA treatment increased the abundance of beneficial bacteria producing SCFAs such as butyrate. In addition, AEA treatment curbed inflammation in the lungs and in the gut-associated mesenteric lymph node (MLN). Production of antimicrobial peptides (AMPs) and tight junction proteins (TJPs) which are key molecules sustaining epithelial barrier integrity in lung epithelial cells, as validated by single-cell RNA (Sc-RNA) sequencing were reported to attenuate the inflammation. Also, in this study, pathogenic Enterobacteriaceae and *Pseudomonas* were seen in the lungs of mice with ARDS while treatment with AEA led to their disappearance. Furthermore, the relative abundance of butyrate producing Lachnospiraceae and Clostridia were enhanced with AEA treatment (64). Emphasizing this observation, the abundance of butyrate-producing Firmicutes compared to *Bacteroides* was discovered following THC treatment of mice with diet-induced obesity (DIO) (66). In a similar line of study, the efficacy of THC to ease SEB-induced ARDS was examined. THC treatment

improved the abundance of beneficial bacteria, *Ruminococcus gnavus*, while reducing pathogenic *Akkermansia muciniphila* in the lungs and gut. THC administration enriched SCFAs, specifically propionic acid, which attenuated the inflammatory response and protected mice from fatality. This study concluded that THC-induced reversal of microbial dysbiosis played a central role in the diminution of SEB-induced ARDS (65).

Colitis is another noteworthy disease model where the influence of cannabinoids on microbiota has been effectively demonstrated (67, 68). One study from our lab explored the effects of CBR activation following administration of THC and CBD either alone or in combination, in a chemically-induced murine colitis model. THC improved colonic barrier integrity as a result of higher mucus, AMPs, and TJPs production. Albeit alteration of the gut microbiota towards gram-negative bacteria was observed, the authors noted that the favorable effects of THC were not associated with microbiome modulation (67). A recent study presented the synergistic effect of fish oil and CBD treatment in the murine model of colitis. Co-administration of fish oil and CBD reduced inflammatory markers and ameliorated intestinal permeability in dextran sulfate sodium (DSS) model of mouse colitis. However, independent treatment with either of these failed to generate a favorable effect. The colonic inflammation was alleviated independent of the increased abundance of *A. muciniphila*. Of note is that the combination therapy reduced the abundance of Marinifilaceae, Desulfovibrionaceae, and Ruminococcaceae. Interestingly, Desulfovibrionaceae abundance has been reported in IBD and UC patients suggesting the functional role these microbial families play in GI diseases (68, 69). Another study investigated the role of gut microbiota in tempering clinical symptoms of paralysis and inflammation following cannabinoids treatment in an experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. A combination of THC and CBD alleviated the symptoms of EAE and decreased pro-inflammatory cytokines while enhancing anti-inflammatory cytokine production. The EAE disease model showed abundant mucin degrading *A. muciniphila* which was considerably decreased following treatment with THC and CBD. A higher level of LPS was found in the brains of EAE mice while this scenario was reversed with cannabinoids treatment (70). The potential of cannabis extract to improve gut barrier function was investigated in the poultry industry where necrotic enteritis caused by *Clostridium perfringens* caused mortality in birds leading to economic loss along with the potential hazard of pathogen transmission to the consumer via the food chain. A combination of cannabis extract and selenium nanoparticles altered the response of chickens towards *C. perfringens*. This treatment upregulated the expression of genes involved in gut barrier function and improved collagenase activity. However, the extract alone could not generate significant beneficial effects (71).

Synthetic cannabinoids have also been extensively studied for their anti-inflammatory properties and their ability to alter the gut microbiota. Treatment with the CB2R agonist, JWH133 alleviated overgrowth of bacteria, bacterial translocation, and bacterial peritonitis, up-regulated intestinal TJPs, and reduced intestinal oxidative stress in cirrhotic rats. Furthermore, the treatment considerably diminished the levels of TNFα and inflammatory facilitators, intestinal mucosal impairment, and infection (72). Blockade of CB1R using the antagonist, Rimonabant reduced DIO and inflammatory cytokines. Trafficking of M1 macrophages and decreased intestinal permeability were also observed with CB1R blocking. Further metagenomics analysis demonstrated an elevated relative abundance of *A. muciniphila* and reduced abundance of Lanchnospiraceae and Erysipelotrichaceae in the gut (73). Nabilone, a CB1R agonist, was found useful in the treatment of post-traumatic stress disorder (PTSD), nausea, and vomiting associated with chemotherapy and pain management (74, 75). Administration of nabilone for 3 months improved health and alleviated diarrheal symptoms in patients. Although microbial dysbiosis was not investigated in this study, it encourages further clinically-oriented investigations on the effect of cannabinoids on such disease models as related to microbial dysbiosis (76).

IMMUNOMODULATORY MECHANISMS OF GUT MICROBIOTA

Endogenous, as well as exogenous cannabinoids, have been widely recognized to regulate inflammation and mucosal permeability of the GI tract where they possibly interact with the gut microbiome. In this section, we have tried to pull together the known mechanisms through which cannabinoids control microbial dysbiosis and accompanying inflammation. The indispensable role of gut microbiota on immune regulation has been excellently validated by multiple investigations. For example, one such study demonstrated the ability of commensal segmented filamentous bacterium (SFB) to induce $CD4^+$ T cells to produce IL-17 and IL-22 in the lamina propria of mice. SFB adhered to the Th17 cells and induced inflammation and production of antimicrobial defensins (77). In the gut, under normal circumstances, eCB system is regulated by CB1R. However, both CB1R and CB2R get activated during inflammation leading to anti-inflammatory cytokine production that suppresses inflammation and intestinal damage (78).

The crosstalk between eCB system and gut microbiota has been established with murine models of obesity where low-grade inflammation and increased eCB system tone are reported. Obese mice exhibited higher colonic CB1R colonic mRNA and the modulation of gut microbiota with the use of prebiotics reduced this scenario. Moreover, prebiotic treatment alleviated CB1R mRNA and concentration of AEA in genetically obese mice explaining the involvement of the gut microbial community on CB1R and eCB expression. The same study disclosed the maintenance of intestinal barrier integrity by eCB system (79). Reduction in the number of TJPs increases the space between epithelial cells promoting paracellular translocation of microbial metabolites from the intestinal lumen to circulation and other organs, and elevated LPS levels impair adipogenesis and promote inflammation (79). THC administration has been reported to reduce LPS levels in mice while increasing TJPs. THC mediated

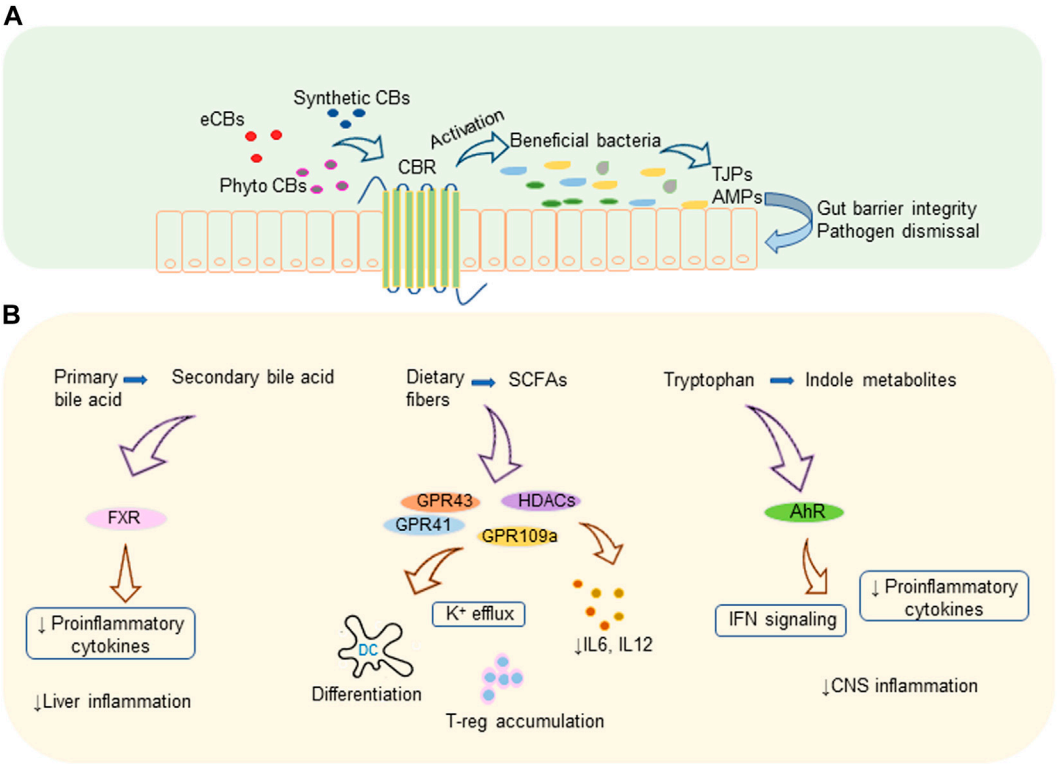

FIGURE 1 | Cannabinoids and gut microbiota. **(A)** Cannabinoid mediated microbiome modulation: endogenous or exogenous cannabinoids increase the beneficial bacteria which produce TJPs that improve gut barrier integrity and AMPs that eliminate pathogens. **(B)** Immunomodulatory mechanisms of microbial metabolites: microbiota generated secondary bile acids, SCFAs, and indole metabolites modulate various receptors leading to decreased pro-inflammatory cytokines and immune suppression. AhR, aryl hydrocarbon receptor; AMP, antimicrobial protein; CBR, cannabinoid receptor; CBs, cannabinoids; CNS, central nervous system; eCBs, endocannabinoids; FXR, farnesoid X receptor; GPR, G-protein-coupled receptors; HDACs, histone deacetylases; IFN, interferon; IL, interleukin; K, potassium; TJP, tight junction proteins; T-reg, regulatory T cell.

CBR modulation reduced plasma LPS levels by altering the distribution and localization of TJPs which led to improved gut barrier function (65, 70).

Microbial-derived SCFAs, neurotransmitters, and amino acids take part in the immune, endocrinal, and neuronal signaling pathways via binding to host receptors (80, 81). Multiple investigations have validated the potential of AEA and THC to enhance the levels of SCFAs and AMPs in murine models of inflammation (64–67). SCFAs are produced in the colon by fermentation and subsequent degradation of undigested dietary fibers by gut harboring bacteria and they contribute to the regulation of both innate and adaptive immunity of the host. Acetate and propionate, produced by Bacteroidetes, and butyrate, produced by Firmicutes are the major SCFAs involved in host-bacterial communications (82). Blocking of histone deacetylases (HDAC) and activation of GPCRs are two main signaling pathways modulated by microbial SCFAs (80–82). Interestingly, GPCRs such as GPR43 and GPR109A are expressed by adipose tissue macrophages and dendritic cells (DCs). The binding of SCFAs to these receptors induces K^+ efflux and membrane hyperpolarization which in turn stimulates NLRP3 inflammasome in primed macrophages to produce IL-18 (83, 84). Butyrate-dependent activation of GPR109A induces apoptosis of colon cancer cells. Furthermore, these receptor/ligand complexes inhibit nuclear factor-kappaB (NF-κB) activation in the colon of mice (85). Butyrate enhanced the function of human TGFβ1 in the intestinal epithelial cells (IECs) which in turn directed the accumulation of Treg cells in the lumen, and the study suggested inhibition of HDAC as the major mechanism behind this activity. Butyrate-induced HDAC inhibition down-regulated the generation of LPS-triggered pro-inflammatory cytokines such as IL-6 and IL-12 (86, 87). Another study demonstrated that butyrate enhanced the expression of AMPs, LL-37, and CAP-18 by IECs in rabbits (88). In a similar manner, activation of genes encoding host defense peptides in HD11 macrophages and monocytes has been observed in chickens following butyrate consumption (89). Succinate, another SCFA produced by the gut bacteria, *Prevotella copri* was shown to be involved in gut gluconeogenesis and improved glucose homeostasis (90). Once transported into circulation, SCFAs exert their effect on distant organs as well. For example, circulating propionate modified bone marrow hematopoiesis by increasing levels of macrophages and DCs precursors. The phagocytic DCs invaded the lung but lacked Th2 effector cell differentiation ability and controlled inflammation (91). SCFAs, as evident from numerous studies, represent the most important connecting link between gut microbiome and host immune homeostasis.

Bile acid metabolism is another activity implemented by a variety of gut microbes harboring the gut. Microbes convert primary bile acid to secondary and tertiary bile acids *via* various mechanisms that include deconjugation of glycine and taurine by bile salt hydrolase, de-hydroxylation as well as dehydrogenation and epimerization of cholesterol core (92). Members of the genera *Bifidobacterium*, *Clostridium*, and *Lactobacillus* are reported to efficiently metabolize primary bile acids (92, 93). Secondary bile acid metabolism and prevention of bile acid production in the liver by activating nuclear receptor farnesoid X receptor (FXR) in the ileum by gut microbiota controls liver inflammation. Also, intestinal microbiota decreases the levels of pro-inflammatory cytokines which are involved in reducing the transcription of FXR target genes (94). Proteins and peptides in the diets are digested to free amino acids as a result of microbial fermentation and major amino acid of such kind is tryptophan. Tryptophan metabolites are another set of biologically active metabolites generated by intestinal bacteria that affect intestinal epithelial barrier integrity as well as the organogenesis of intestinal lymphoid follicles. Members of the phylum Firmicutes convert tryptophan to tryptamine and other indole derivatives (95, 96). A recent study showed that tryptamine can attenuate neuroinflammation in the murine model of MS (97). *Lactobacillus* strains were found to efficiently metabolize Tryptophan to its derivatives which act as aryl hydrocarbon receptor (AhR) ligands in the colitis mouse model (98). These metabolites, mostly indoles, act as AhR agonists and regulate type-1 IFN signaling in astrocytes leading to suppression of central nervous system (CNS) inflammation (99). AhR signaling mediates IL-22 production in the gut by activating innate lymphoid cell 3 (ILC3) (100). In addition, AhR has been shown to play a vital role in the development of ILC and intraepithelial lymphocytes (100, 101). How AhR activation leads to suppression of inflammation has been the topic of recent reviews (102, 103). **Figure 1** illustrates a summary of cannabinoid mediated microbiome modulation and the immunomodulatory mechanism of microbial metabolites.

While most of the studies that we have reviewed above have shown an association between the administration of cannabinoids and suppression of inflammation to changes in the microbiota, one can question whether these studies merely indicate a relationship between these events or whether the cannabinoid-mediated alterations in the microbiota are actually responsible for inducing attenuation of inflammation. The association between microbial changes seen following exposure to cannabinoids and the consequent impact of such changes in immunomodulation can only be proven through fecal microbiota transplants (FMT).

There is evidence to suggest the role of microbiota on eCB signaling through use of FMT. Multiple studies reported that FMT-mediated microbial dysbiosis can modify eCB signaling (104, 105). One study clearly investigated the impact of FMT from conventionally raised mice to germ-free mice. Endocannabinoidome gene expression and lipidomics were analyzed by transcriptomics and LC-MS/MS before and after FMT. Age-dependent endocannabinoidome gene expression and lipid variations in the germ-free mice were reversed following FMT from age-matched conventionally raised donor mice (106). In another study, FMT from murine models of EAE disease treated with THC and CBD, into antibiotics treated, microbe depleted mice demonstrated that the recipient mice showed decreased EAE disease severity (70). A similar kind of study was conducted in a murine model of SEB-mediated ARDS. The microbiota transplanted from THC-treated ARDS mice into antibiotic-treated, microbiome-depleted recipient mice showed better survival from ARDS than those that received FMT from the control group. FMT from THC-treated groups caused a decrease in inflammatory $CD4^+$ and $CD8^+$ T cells and an increase in immune suppressive MDSCs and Tregs in the lungs (65). Such studies clearly demonstrate that endogenous and exogenous cannabinoids can promote beneficial microbiota in the gut that can attenuate inflammatory diseases even in distal organs.

CONCLUSION

The communications among eCB system, immune regulation, and gut microbiota are intricately interconnected. CBRs agonists/antagonists have been pre-clinically validated to be useful in the treatment of metabolic conditions, such as obesity and diabetes as well as in disease models of colitis and cardiometabolic malfunctions. Also, well-established is the role of intestinal microbial community in the onset or progression of these disorders. The numerous groups of microbial clusters and the myriad of biologically active metabolites produced by them along with their receptors trigger extensive signaling pathways that affect the energy balance and immune homeostasis of the host. The microbiome-eCB signaling modulation exploiting exo- or endogenous cannabinoids opens a new avenue of cannabinoid-gut microbiota-based therapeutics to curb metabolic and immune-oriented conditions. However, more clinical investigations are essential to validate this concept.

AUTHOR CONTRIBUTIONS

KKV prepared the draft, PN and MN provided the concept and edited the manuscript.

FUNDING

This work was supported in part by NIH grants: R01ES019313, R01MH094755, R01AI123947, R01AI129788, R01AT006888, P01AT003961, and P20GM103641.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Boggs DL, Nguyen JD, Morgenson D, Taffe MA, Ranganathan M. Clinical and Preclinical Evidence for Functional Interactions of Cannabidiol and Δ9-Tetrahydrocannabinol. *Neuropsychopharmacol.* (2018) 43(1):142–54. doi:10.1038/npp.2017.209
2. Pertwee RG. Cannabinoid Pharmacology: the First 66 Years. *Br J Pharmacol* (2006) 147(Suppl. 1):S163–71. doi:10.1038/sj.bjp.0706406
3. Tham M, Yilmaz O, Alaverdashvili M, Kelly MEM, Denovan-Wright EM, Laprairie RB. Allosteric and Orthosteric Pharmacology of Cannabidiol and Cannabidiol-Dimethylheptyl at the Type 1 and Type 2 Cannabinoid Receptors. *Br J Pharmacol* (2019) 176(10):1455–69. doi:10.1111/bph.14440
4. Munro S, Thomas KL, Abu-Shaar M. Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature* (1993) 365(6441):61–5. doi:10.1038/365061a0
5. Devane WA, Hanuš L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and Structure of a Brain Constituent that Binds to the Cannabinoid Receptor. *Science* (1992) 258(5090):1946–9. doi:10.1126/science.1470919
6. Marzo VD, Bifulco M, Petrocellis LD. The Endocannabinoid System and its Therapeutic Exploitation. *Nat Rev Drug Discov* (2004) 3(9):771–84. doi:10.1038/nrd1495
7. Demuth DG, Molleman A. Cannabinoid Signalling. *Life Sci* (2006) 78(6):549–63. doi:10.1016/j.lfs.2005.05.055
8. Derkinderen P, Toutant M, Burgaya F, Le Bert M, Siciliano JC, de Franciscis V, et al. Regulation of a Neuronal Form of Focal Adhesion Kinase by Anandamide. *Science* (1996) 273(5282):1719–22. doi:10.1126/science.273.5282.1719
9. McAllister SD, Glass M. CB(1) and CB(2) Receptor-Mediated Signalling: a Focus on Endocannabinoids. *Prostaglandins Leukot Essent Fatty Acids* (2002) 66(2-3):161–71. doi:10.1054/plef.2001.0344
10. Piomelli D. The Molecular Logic of Endocannabinoid Signalling. *Nat Rev Neurosci* (2003) 4(11):873–84. doi:10.1038/nrn1247
11. Derkinderen P, Valjent E, Toutant M, Corvol J-C, Enslen H, Ledent C, et al. Regulation of Extracellular Signal-Regulated Kinase by Cannabinoids in hippocampus. *J Neurosci* (2003) 23(6):2371–82. doi:10.1523/jneurosci.23-06-02371.2003
12. Pertwee RG, Ross RA. Cannabinoid Receptors and Their Ligands. *Prostaglandins Leukot Essent Fatty Acids* (2002) 66(2):101–21. doi:10.1054/plef.2001.0341
13. Cuenda A, Rousseau S. p38 MAP-Kinases Pathway Regulation, Function and Role in Human Diseases. *Biochim Biophys Acta (Bba) - Mol Cell Res* (2007) 1773(8):1358–75. doi:10.1016/j.bbamcr.2007.03.010
14. Han JM, Patterson SJ, Levings MK. The Role of the PI3K Signaling Pathway in CD4+ T Cell Differentiation and Function. *Front Immun* (2012) 3:245. doi:10.3389/fimmu.2012.00245
15. Liu J, Hideshima T, Xing L, Wang S, Zhou W, Samur MK, et al. ERK Signaling Mediates Resistance to Immunomodulatory Drugs in the Bone Marrow Microenvironment. *Sci Adv* (2021) 7(23). doi:10.1126/sciadv.abg2697
16. Canel M, Taggart D, Sims AH, Lonergan DW, Waizenegger IC, Serrels A. T-cell Co-stimulation in Combination with Targeting FAK Drives Enhanced Anti-tumor Immunity. *eLife* (2020) 9. doi:10.7554/eLife.48092
17. Iannotti FA, Di Marzo V. The Gut Microbiome, Endocannabinoids and Metabolic Disorders. *J Endocrinol* (2021) 248(2):R83–r97. doi:10.1530/joe-20-0444
18. Al Bander Z, Nitert MD, Mousa A, Naderpoor N. The Gut Microbiota and Inflammation: An Overview. *Int J Environ Res Public Health* (2020) 17(20). doi:10.3390/ijerph17207618
19. Bolte LA, Vich Vila A, Imhann F, Collij V, Gacesa R, Peters V, et al. Long-term Dietary Patterns Are Associated with Pro-inflammatory and Anti-inflammatory Features of the Gut Microbiome. *Gut* (2021) 70(7):1287–98. doi:10.1136/gutjnl-2020-322670
20. Nagarkatti P, Pandey R, Rieder SA, Hegde VL, Nagarkatti M. Cannabinoids as Novel Anti-inflammatory Drugs. *Future Med Chem* (2009) 1(7):1333–49. doi:10.4155/fmc.09.93
21. Giacobbe J, Marrocu A, Di Benedetto MG, Pariante CM, Borsini A. A Systematic, Integrative Review of the Effects of the Endocannabinoid System on Inflammation and Neurogenesis in Animal Models of Affective Disorders. *Brain Behav Immun* (2021) 93:353–67. doi:10.1016/j.bbi.2020.12.024
22. Dong C, Chen J, Harrington A, Vinod KY, Hegde ML, Hegde VL. Cannabinoid Exposure during Pregnancy and its Impact on Immune Function. *Cell Mol Life Sci* (2019) 76(4):729–43. doi:10.1007/s00018-018-2955-0
23. Joshi N, Onaivi ES. Endocannabinoid System Components: Overview and Tissue Distribution. *Adv Exp Med Biol* (2019) 1162:1–12. doi:10.1007/978-3-030-21737-2_1
24. Young AP, Denovan-Wright EM. The Dynamic Role of Microglia and the Endocannabinoid System in Neuroinflammation. *Front Pharmacol* (2021) 12:806417. doi:10.3389/fphar.2021.806417
25. Zhu D, Gao F, Chen C. Endocannabinoid Metabolism and Traumatic Brain Injury. *Cells* (2021) 10(11). doi:10.3390/cells10112979
26. Elliott DM, Singh N, Nagarkatti M, Nagarkatti PS. Cannabidiol Attenuates Experimental Autoimmune Encephalomyelitis Model of Multiple Sclerosis through Induction of Myeloid-Derived Suppressor Cells. *Front Immunol* (2018) 9:1782. doi:10.3389/fimmu.2018.01782
27. Karin N. The Development and Homing of Myeloid-Derived Suppressor Cells: From a Two-Stage Model to a Multistep Narrative. *Front Immunol* (2020) 11:557586. doi:10.3389/fimmu.2020.557586
28. Hegde VL, Singh UP, Nagarkatti PS, Nagarkatti M. Critical Role of Mast Cells and Peroxisome Proliferator-Activated Receptor γ in the Induction of Myeloid-Derived Suppressor Cells by Marijuana Cannabidiol *In Vivo*. *J.I.* (2015) 194(11):5211–22. doi:10.4049/jimmunol.1401844
29. Hegde VL, Nagarkatti M, Nagarkatti PS. Cannabinoid Receptor Activation Leads to Massive Mobilization of Myeloid-Derived Suppressor Cells with Potent Immunosuppressive Properties. *Eur J Immunol* (2010) 40(12):3358–71. doi:10.1002/eji.201040667
30. Becker W, Alrafas HR, Wilson K, Miranda K, Culpepper C, Chatzistamou I, et al. Activation of Cannabinoid Receptor 2 Prevents Colitis-Associated Colon Cancer through Myeloid Cell De-activation Upstream of IL-22 Production. *iScience* (2020) 23(9):101504. doi:10.1016/j.isci.2020.101504
31. Rao R, Nagarkatti PS, Nagarkatti M. Δ9Tetrahydrocannabinol Attenuates Staphylococcal Enterotoxin B-Induced Inflammatory Lung Injury and Prevents Mortality in Mice by Modulation of miR-17-92 Cluster and Induction of T-Regulatory Cells. *Br J Pharmacol* (2015) 172(7):1792–806. doi:10.1111/bph.13026
32. Berg BB, Soares JS, Paiva IR, Rezende BM, Rachid MA, Cau SBd A, et al. Cannabidiol Enhances Intestinal Cannabinoid Receptor Type 2 Receptor Expression and Activation Increasing Regulatory T Cells and Reduces Murine Acute Graft-Versus-Host Disease without Interfering with the Graft-Versus-Leukemia Response. *J Pharmacol Exp Ther* (2021) 377(2):273–83. doi:10.1124/jpet.120.000479
33. Angelina A, Pérez-Diego M, Maldonado A, Rückert B, Akdis M, Martín-Fontecha M, et al. The Cannabinoid WIN55212-2 Suppresses Effector T-cell Responses and Promotes Regulatory T Cells in Human Tonsils. *Allergy* (2022) 77(3):1029–32. doi:10.1111/all.15160
34. Schmidt A, Oberle N, Krammer PH. Molecular Mechanisms of Treg-Mediated T Cell Suppression. *Front Immun* (2012) 3:51. doi:10.3389/fimmu.2012.00051
35. Lombard C, Nagarkatti M, Nagarkatti P. CB2 Cannabinoid Receptor Agonist, JWH-015, Triggers Apoptosis in Immune Cells: Potential Role for CB2-Selective Ligands as Immunosuppressive Agents. *Clin Immunol* (2007) 122(3):259–70. doi:10.1016/j.clim.2006.11.002
36. Mohammed A, F K Alghetaa H, Miranda K, Wilson K, Cai G, P Singh N, et al. Δ9-Tetrahydrocannabinol Prevents Mortality from Acute Respiratory Distress Syndrome through the Induction of Apoptosis in Immune Cells, Leading to Cytokine Storm Suppression. *Int J Mol Sci* (2020) 21(17). doi:10.3390/ijms21176244
37. Do Y, McKallip RJ, Nagarkatti M, Nagarkatti PS. Activation through Cannabinoid Receptors 1 and 2 on Dendritic Cells Triggers NF-kappaB-dependent Apoptosis: Novel Role for Endogenous and Exogenous Cannabinoids in Immunoregulation. *J Immunol* (2004) 173(4):2373–82. doi:10.4049/jimmunol.173.4.2373
38. Singh UP, Singh NP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Cannabinoid Receptor-2 (CB2) Agonist Ameliorates Colitis in IL-10−/− Mice by Attenuating the Activation of T Cells and Promoting Their

Apoptosis. *Toxicol Appl Pharmacol* (2012) 258(2):256–67. doi:10.1016/j.taap.2011.11.005
39. Yuan M, Kiertscher SM, Cheng Q, Zoumalan R, Tashkin DP, Roth MD. Delta 9-Tetrahydrocannabinol Regulates Th1/Th2 Cytokine Balance in Activated Human T Cells. *J Neuroimmunol* (2002) 133(1-2):124–31. doi:10.1016/s0165-5728(02)00370-3
40. Klein TW, Newton CA, Nakachi N, Friedman H. Delta 9-tetrahydrocannabinol Treatment Suppresses Immunity and Early IFN-Gamma, IL-12, and IL-12 Receptor Beta 2 Responses to *Legionella pneumophila* Infection. *J Immunol* (2000) 164(12):6461–6. doi:10.4049/jimmunol.164.12.6461
41. Sido JM, Jackson AR, Nagarkatti PS, Nagarkatti M. Marijuana-derived Δ-9-tetrahydrocannabinol Suppresses Th1/Th17 Cell-Mediated Delayed-type Hypersensitivity through microRNA Regulation. *J Mol Med* (2016) 94(9):1039–51. doi:10.1007/s00109-016-1404-5
42. Jackson AR, Nagarkatti P, Nagarkatti M. Anandamide Attenuates Th-17 Cell-Mediated Delayed-type Hypersensitivity Response by Triggering IL-10 Production and Consequent microRNA Induction. *PloS one* (2014) 9(4):e93954. doi:10.1371/journal.pone.0093954
43. Holloman BL, Nagarkatti M, Nagarkatti P. Epigenetic Regulation of Cannabinoid-Mediated Attenuation of Inflammation and its Impact on the Use of Cannabinoids to Treat Autoimmune Diseases. *Int J Mol Sci* (2021) 22(14). doi:10.3390/ijms22147302
44. D'Addario C, Di Francesco A, Arosio B, Gussago C, Dell'Osso B, Bari M, et al. Epigenetic Regulation of Fatty Acid Amide Hydrolase in Alzheimer Disease. *PloS one* (2012) 7(6):e39186. doi:10.1371/journal.pone.0039186
45. Meccariello R, Santoro A, D'Angelo S, Morrone R, Fasano S, Viggiano A, et al. The Epigenetics of the Endocannabinoid System. *Int J Mol Sci* (2020) 21(3). doi:10.3390/ijms21031113
46. Gomes TM, Dias da Silva D, Carmo H, Carvalho F, Silva JP. Epigenetics and the Endocannabinoid System Signaling: An Intricate Interplay Modulating Neurodevelopment. *Pharmacol Res* (2020) 162:105237. doi:10.1016/j.phrs.2020.105237
47. Sido JM, Yang X, Nagarkatti PS, Nagarkatti M. Δ9 -Tetrahydrocannabinol-Mediated Epigenetic Modifications Elicit Myeloid-Derived Suppressor Cell Activation via STAT3/S100A8. *J Leukoc Biol* (2015) 97(4):677–88. doi:10.1189/jlb.1a1014-479r
48. Yang X, Hegde VL, Rao R, Zhang J, Nagarkatti PS, Nagarkatti M. Histone Modifications Are Associated with Δ9-Tetrahydrocannabinol-mediated Alterations in Antigen-specific T Cell Responses. *J Biol Chem* (2014) 289(27):18707–18. doi:10.1074/jbc.m113.545210
49. Sultan M, Alghetaa H, Mohammed A, Abdulla OA, Wisniewski PJ, Singh N, et al. The Endocannabinoid Anandamide Attenuates Acute Respiratory Distress Syndrome by Downregulating miRNA that Target Inflammatory Pathways. *Front Pharmacol* (2021) 12:644281. doi:10.3389/fphar.2021.644281
50. Yang X, Bam M, Nagarkatti PS, Nagarkatti M. RNA-seq Analysis of δ9-Tetrahydrocannabinol-treated T Cells Reveals Altered Gene Expression Profiles that Regulate Immune Response and Cell Proliferation. *J Biol Chem* (2016) 291(30):15460–72. doi:10.1074/jbc.m116.719179
51. Wu Y, Wang CZ, Wan JY, Yao H, Yuan CS. Dissecting the Interplay Mechanism between Epigenetics and Gut Microbiota: Health Maintenance and Disease Prevention. *Int J Mol Sci* (2021) 22(13). doi:10.3390/ijms22136933
52. Wu H-J, Wu E. The Role of Gut Microbiota in Immune Homeostasis and Autoimmunity. *Gut microbes* (2012) 3(1):4–14. doi:10.4161/gmic.19320
53. Dopkins N, Nagarkatti PS, Nagarkatti M. The Role of Gut Microbiome and Associated Metabolome in the Regulation of Neuroinflammation in Multiple Sclerosis and its Implications in Attenuating Chronic Inflammation in Other Inflammatory and Autoimmune Disorders. *Immunology* (2018) 154(2):178–85. doi:10.1111/imm.12903
54. Carabotti M, Scirocco A, Maselli MA, Severi C. The Gut-Brain axis: Interactions between Enteric Microbiota, central and Enteric Nervous Systems. *Ann Gastroenterol* (2015) 28(2):203–9.
55. Tian P, Wang G, Zhao J, Zhang H, Chen W. Bifidobacterium with the Role of 5-hydroxytryptophan Synthesis Regulation Alleviates the Symptom of Depression and Related Microbiota Dysbiosis. *J Nutr Biochem* (2019) 66:43–51. doi:10.1016/j.jnutbio.2019.01.007
56. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The Gut Microbiota in Anxiety and Depression - A Systematic Review. *Clin Psychol Rev* (2021) 83:101943. doi:10.1016/j.cpr.2020.101943
57. Karoly HC, Mueller RL, Bidwell LC, Hutchison KE. Cannabinoids and the Microbiota-Gut-Brain Axis: Emerging Effects of Cannabidiol and Potential Applications to Alcohol Use Disorders. *Alcohol Clin Exp Res* (2020) 44(2):340–53. doi:10.1111/acer.14256
58. Khan RN, Maner-Smith K, A Owens J, Barbian ME, M Jones R, R Naudin C. At the Heart of Microbial Conversations: Endocannabinoids and the Microbiome in Cardiometabolic Risk. *Gut microbes* (2021) 13(1):1–21. doi:10.1080/19490976.2021.1911572
59. Mestre L, Carrillo-Salinas FJ, Mecha M, Feliú A, Guaza C. Gut Microbiota, Cannabinoid System and Neuroimmune Interactions: New Perspectives in Multiple Sclerosis. *Biochem Pharmacol* (2018) 157:51–66. doi:10.1016/j.bcp.2018.08.037
60. Cani PD, Plovier H, Van Hul M, Geurts L, Delzenne NM, Druart C, et al. Endocannabinoids--at the Crossroads between the Gut Microbiota and Host Metabolism. *Nat Rev Endocrinol* (2016) 12(3):133–43. doi:10.1038/nrendo.2015.211
61. Markey L, Hooper A, Melon LC, Baglot S, Hill MN, Maguire J, et al. Colonization with the Commensal Fungus Candida Albicans Perturbs the Gut-Brain axis through Dysregulation of Endocannabinoid Signaling. *Psychoneuroendocrinology* (2020) 121:104808. doi:10.1016/j.psyneuen.2020.104808
62. Castonguay-Paradis S, Lacroix S, Rochefort G, Parent L, Perron J, Martin C, et al. Dietary Fatty Acid Intake and Gut Microbiota Determine Circulating Endocannabinoidome Signaling beyond the Effect of Body Fat. *Scientific Rep* (2020) 10(1):15975. doi:10.1038/s41598-020-72861-3
63. Tartakover Matalon S, Azar S, Meiri D, Hadar R, Nemirovski A, Abu Jabal N, et al. Endocannabinoid Levels in Ulcerative Colitis Patients Correlate with Clinical Parameters and Are Affected by Cannabis Consumption. *Front Endocrinol* (2021) 12:685289. doi:10.3389/fendo.2021.685289
64. Sultan M, Wilson K, Abdulla OA, Busbee PB, Hall A, Carter T, et al. Endocannabinoid Anandamide Attenuates Acute Respiratory Distress Syndrome through Modulation of Microbiome in the Gut-Lung Axis. *Cells* (2021) 10(12). doi:10.3390/cells10123305
65. Mohammed A, Alghetaa HK, Zhou J, Chatterjee S, Nagarkatti P, Nagarkatti M. Protective Effects of Δ(9) -tetrahydrocannabinol against Enterotoxin-Induced Acute Respiratory Distress Syndrome Are Mediated by Modulation of Microbiota. *Br J Pharmacol* (2020) 177(22):5078–95. doi:10.1111/bph.15226
66. Cluny NL, Keenan CM, Reimer RA, Le Foll B, Sharkey KA. Prevention of Diet-Induced Obesity Effects on Body Weight and Gut Microbiota in Mice Treated Chronically with Δ9-Tetrahydrocannabinol. *PloS one* (2015) 10(12):e0144270. doi:10.1371/journal.pone.0144270
67. Becker W, Alrafas HR, Busbee PB, Walla MD, Wilson K, Miranda K, et al. Cannabinoid Receptor Activation on Haematopoietic Cells and Enterocytes Protects against Colitis. *J Crohn's colitis* (2021) 15(6):1032–48. doi:10.1093/ecco-jcc/jjaa253
68. Silvestri C, Pagano E, Lacroix S, Venneri T, Cristiano C, Calignano A, et al. Fish Oil, Cannabidiol and the Gut Microbiota: An Investigation in a Murine Model of Colitis. *Front Pharmacol* (2020) 11:585096. doi:10.3389/fphar.2020.585096
69. Rowan F, Docherty NG, Murphy M, Murphy B, Calvin Coffey J, O'Connell PR. Desulfovibrio Bacterial Species Are Increased in Ulcerative Colitis. *Dis colon rectum* (2010) 53(11):1530–6. doi:10.1007/dcr.0b013e3181f1e620
70. Al-Ghezi ZZ, Busbee PB, Alghetaa H, Nagarkatti PS, Nagarkatti M. Combination of Cannabinoids, delta-9-tetrahydrocannabinol (THC) and Cannabidiol (CBD), Mitigates Experimental Autoimmune Encephalomyelitis (EAE) by Altering the Gut Microbiome. *Brain Behav Immun* (2019) 82:25–35. doi:10.1016/j.bbi.2019.07.028
71. Konieczka P, Szkopek D, Kinsner M, Fotschki B, Juśkiewicz J, Banach J. Cannabis-derived Cannabidiol and Nanoselenium Improve Gut Barrier Function and Affect Bacterial Enzyme Activity in Chickens Subjected to *C. perfringens* challenge. *Vet Res* (2020) 51(1):141. doi:10.1186/s13567-020-00863-0
72. Yang YY, Hsieh SL, Lee PC, Yeh YC, Lee KC, Hsieh YC, et al. Long-term Cannabinoid Type 2 Receptor Agonist Therapy Decreases Bacterial

Translocation in Rats with Cirrhosis and Ascites. *J Hepatol* (2014) 61(5): 1004–13. doi:10.1016/j.jhep.2014.05.049
73. Mehrpouya-Bahrami P, Chitrala KN, Ganewatta MS, Tang C, Murphy EA, Enos RT, et al. Blockade of CB1 Cannabinoid Receptor Alters Gut Microbiota and Attenuates Inflammation and Diet-Induced Obesity. *Scientific Rep* (2017) 7(1):15645. doi:10.1038/s41598-017-15154-6
74. Cameron C, Watson D, Robinson J. Use of a Synthetic Cannabinoid in a Correctional Population for Posttraumatic Stress Disorder-Related Insomnia and Nightmares, Chronic Pain, Harm Reduction, and Other Indications: a Retrospective Evaluation. *J Clin Psychopharmacol* (2014) 34(5):559–64. doi:10.1097/jcp.0000000000000180
75. Tsang CC, Giudice MG. Nabilone for the Management of Pain. *Pharmacotherapy* (2016) 36(3):273–86. doi:10.1002/phar.1709
76. Pellesi L, Verga MC, De Maria N, Villa E, Pini LA, Guerzoni S. Nabilone Administration in Refractory Chronic Diarrhea: a Case Series. *BMC Gastroenterol* (2019) 19(1):105. doi:10.1186/s12876-019-1024-y
77. IvanovII, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* (2009) 139(3):485–98. doi:10.1016/j.cell.2009.09.033
78. Jansma J, Brinkman F, van Hemert S, El Aidy S. Targeting the Endocannabinoid System with Microbial Interventions to Improve Gut Integrity. *Prog neuro-psychopharmacology Biol Psychiatry* (2021) 106: 110169. doi:10.1016/j.pnpbp.2020.110169
79. Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, et al. The Endocannabinoid System Links Gut Microbiota to Adipogenesis. *Mol Syst Biol* (2010) 6:392. doi:10.1038/msb.2010.46
80. Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, et al. The Orphan G Protein-Coupled Receptors GPR41 and GPR43 Are Activated by Propionate and Other Short Chain Carboxylic Acids. *J Biol Chem* (2003) 278(13):11312–9. doi:10.1074/jbc.m211609200
81. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain Fatty Acids Induce Both Effector and Regulatory T Cells by Suppression of Histone Deacetylases and Regulation of the mTOR–S6k Pathway. *Mucosal Immunol* (2015) 8(1):80–93. doi:10.1038/mi.2014.44
82. Hosseinkhani F, Heinken A, Thiele I, Lindenburg PW, Harms AC, Hankemeier T. The Contribution of Gut Bacterial Metabolites in the Human Immune Signaling Pathway of Non-communicable Diseases. *Gut microbes* (2021) 13(1):1–22. doi:10.1080/19490976.2021.1882927
83. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, et al. Metabolite-sensing Receptors GPR43 and GPR109A Facilitate Dietary Fibre-Induced Gut Homeostasis through Regulation of the Inflammasome. *Nat Commun* (2015) 6(1):6734. doi:10.1038/ncomms7734
84. Nakajima A, Nakatani A, Hasegawa S, Irie J, Ozawa K, Tsujimoto G, et al. The Short Chain Fatty Acid Receptor GPR43 Regulates Inflammatory Signals in Adipose Tissue M2-type Macrophages. *PloS one* (2017) 12(7):e0179696. doi:10.1371/journal.pone.0179696
85. Thangaraju M, Cresci GA, Liu K, Ananth S, Gnanaprakasam JP, Browning DD, et al. GPR109A Is a G-Protein-Coupled Receptor for the Bacterial Fermentation Product Butyrate and Functions as a Tumor Suppressor in colon. *Cancer Res* (2009) 69(7):2826–32. doi:10.1158/0008-5472.can-08-4466
86. Chang PV, Hao L, Offermanns S, Medzhitov R. The Microbial Metabolite Butyrate Regulates Intestinal Macrophage Function via Histone Deacetylase Inhibition. *Proc Natl Acad Sci United States America* (2014) 111(6):2247–52. doi:10.1073/pnas.1322269111
87. Martin-Gallausiaux C, Béguet-Crespel F, Marinelli L, Jamet A, Ledue F, Blottière HM, et al. Butyrate Produced by Gut Commensal Bacteria Activates TGF-Beta1 Expression through the Transcription Factor SP1 in Human Intestinal Epithelial Cells. *Scientific Rep* (2018) 8(1):9742. doi:10.1038/s41598-018-28048-y
88. Raqib R, Sarker P, Bergman P, Ara G, Lindh M, Sack DA, et al. Improved Outcome in Shigellosis Associated with Butyrate Induction of an Endogenous Peptide Antibiotic. *Proc Natl Acad Sci United States America* (2006) 103(24): 9178–83. doi:10.1073/pnas.0602888103
89. Sunkara LT, Achanta M, Schreiber NB, Bommineni YR, Dai G, Jiang W, et al. Butyrate Enhances Disease Resistance of Chickens by Inducing Antimicrobial Host Defense Peptide Gene Expression. *PloS one* (2011) 6(11):e27225–e. doi:10.1371/journal.pone.0027225
90. De Vadder F, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis. *Cell Metab* (2016) 24(1):151–7. doi:10.1016/j.cmet.2016.06.013
91. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut Microbiota Metabolism of Dietary Fiber Influences Allergic Airway Disease and Hematopoiesis. *Nat Med* (2014) 20(2):159–66. doi:10.1038/nm.3444
92. Guzior DV, Quinn RA. Review: Microbial Transformations of Human Bile Acids. *Microbiome* (2021) 9(1):140. doi:10.1186/s40168-021-01101-1
93. Lucas LN, Barrett K, Kerby RL, Zhang Q, Cattaneo LE, Stevenson D, et al. Dominant Bacterial Phyla from the Human Gut Show Widespread Ability to Transform and Conjugate Bile Acids. *mSystems* (2021) 2021:e0080521. doi:10.1128/msystems.00805-21
94. Jia W, Xie G, Jia W. Bile Acid–Microbiota Crosstalk in Gastrointestinal Inflammation and Carcinogenesis. *Nat Rev Gastroenterol Hepatol* (2018) 15(2):111–28. doi:10.1038/nrgastro.2017.119
95. Taleb S. Tryptophan Dietary Impacts Gut Barrier and Metabolic Diseases. *Front Immunol* (2019) 10:2113. doi:10.3389/fimmu.2019.02113
96. Gao J, Xu K, Liu H, Liu G, Bai M, Peng C, et al. Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. *Front Cell Infect Microbiol* (2018) 8:13. doi:10.3389/fcimb.2018.00013
97. Dopkins N, Becker W, Miranda K, Walla M, Nagarkatti P, Nagarkatti M. Tryptamine Attenuates Experimental Multiple Sclerosis through Activation of Aryl Hydrocarbon Receptor. *Front Pharmacol* (2020) 11:619265. doi:10.3389/fphar.2020.619265
98. Lamas B, Richard ML, Leducq V, Pham HP, Michel ML, Da Costa G, et al. CARD9 Impacts Colitis by Altering Gut Microbiota Metabolism of Tryptophan into Aryl Hydrocarbon Receptor Ligands. *Nat Med* (2016) 22(6):598–605. doi:10.1038/nm.4102
99. Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Kenison JE, Mayo L, et al. Type I Interferons and Microbial Metabolites of Tryptophan Modulate Astrocyte Activity and central Nervous System Inflammation via the Aryl Hydrocarbon Receptor. *Nat Med* (2016) 22(6):586–97. doi:10.1038/nm.4106
100. Abdulla OA, Neamah W, Sultan M, Alghetaa HK, Singh N, Busbee PB, et al. The Ability of AhR Ligands to Attenuate Delayed Type Hypersensitivity Reaction Is Associated with Alterations in the Gut Microbiota. *Front Immunol* (2021) 12:684727. doi:10.3389/fimmu.2021.684727
101. Gao J, Xu K, Liu H, Liu G, Bai M, Peng C, et al. Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. *Front Cell Infect Microbiol* (2018) 8:13. doi:10.3389/fcimb.2018.00013
102. Cannon AS, Nagarkatti PS, Nagarkatti M. Targeting AhR as a Novel Therapeutic Modality against Inflammatory Diseases. *Int J Mol Sci* (2021) 23(1). doi:10.3390/ijms23010288
103. Puccetti M, Pariano M, Costantini C, Giovagnoli S, Ricci M. Pharmaceutically Active Microbial AhR Agonists as Innovative Biodrugs in Inflammation. *Pharmaceuticals (Basel, Switzerland)* (2022) 15(3). doi:10.3390/ph15030336
104. Hua D, Li S, Li S, Wang X, Wang Y, Xie Z, et al. Gut Microbiome and Plasma Metabolome Signatures in Middle-Aged Mice with Cognitive Dysfunction Induced by Chronic Neuropathic Pain. *Front Mol Neurosci* (2021) 14:806700. doi:10.3389/fnmol.2021.806700
105. Chevalier G, Siopi E, Guenin-Macé L, Pascal M, Laval T, Rifflet A, et al. Effect of Gut Microbiota on Depressive-like Behaviors in Mice Is Mediated by the Endocannabinoid System. *Nat Commun* (2020) 11(1):6363. doi:10.1038/s41467-020-19931-2
106. Manca C, Boubertakh B, Leblanc N, Deschênes T, Lacroix S, Martin C, et al. Germ-free Mice Exhibit Profound Gut Microbiota-dependent Alterations of Intestinal Endocannabinoidome Signaling. *J lipid Res* (2020) 61(1):70–85. doi:10.1194/jlr.ra119000424

GLOSSARY

2-AG 2-arachydonoylglycerol

AEA anandamide

AhR aryl hydrocarbon receptor

AMPs antimicrobial peptides

ARDS acute respiratory distress syndrome

cAMP cyclic adenosine monophosphate

CBD cannabidiol

CNS central nervous system

CBR cannabinoid receptors

DCs dendritic cells

DIO diet-induced obesity

DNMT DNA methyl transferases

DSS dextran sulfate sodium

EAE experimental autoimmune encephalomyelitis

ERK extracellular signal-regulated kinase

FAAH fatty acid amide hydrolase

FAK focal adhesion kinase

FMT fecal microbiota transplants

FXR farnesoid X receptor

GPCRs G-protein-coupled receptors

GI gastrointestinal

HDAC histone deacetylase

IBD inflammatory bowel disease

IECs intestinal epithelial cells

IFN-γ interferon-gamma

IL interleukin

ILC3 innate lymphoid cell 3

LPS lipopolysaccharides

MDSCs myeloid-derived suppressor cells

miRNAs microRNAs

MLN mesenteric lymph node

MS multiple sclerosis

PI3K-PKB/AKT phosphoinositide-3-kinase–protein kinase B/Akt

p38 MAP p38 mitogen-activated protein

PTSD post-traumatic stress disorder

SCFAs short-chain fatty acids

Sc-RNA single-cell RNA

SEB staphylococcal enterotoxin B

SFB segmented filamentous bacterium

TGF-β transforming growth factor β

Th1 T helper 1

Th2 T helper 2

THC δ9-tertrahydrocannabinol

TJPs tight junction proteins

Tregs regulatory T cells

TRPV1 transient receptor potential vanilloid 1

UC ulcerative colitis

ADAR | Publishing Partnerships

TYPE Mini Review
PUBLISHED 08 December 2023
DOI 10.3389/adar.2023.11359

OPEN ACCESS

EDITED BY
Shilpa Buch,
University of Nebraska Medical Center, United States

REVIEWED BY
Gagan Deep,
Wake Forest University, United States
Sangeetha Senthil Kumar,
University of Arizona, United States

*CORRESPONDENCE
Melissa Ellermann,
mellermann@sc.edu

RECEIVED 14 March 2023
ACCEPTED 28 November 2023
PUBLISHED 08 December 2023

CITATION
Ellermann M (2023), Emerging mechanisms by which endocannabinoids and their derivatives modulate bacterial populations within the gut microbiome.
Adv. Drug Alcohol Res. 3:11359.
doi: 10.3389/adar.2023.11359

Emerging mechanisms by which endocannabinoids and their derivatives modulate bacterial populations within the gut microbiome

Melissa Ellermann*

Department of Biological Sciences, University of South Carolina, Columbia, SC, United States

Bioactive lipids such as endocannabinoids serve as important modulators of host health and disease through their effects on various host functions including central metabolism, gut physiology, and immunity. Furthermore, changes to the gut microbiome caused by external factors such as diet or by disease development have been associated with altered endocannabinoid tone and disease outcomes. These observations suggest the existence of reciprocal relationships between host lipid signaling networks and bacterial populations that reside within the gut. Indeed, endocannabinoids and their congeners such as *N*-acylethanolamides have been recently shown to alter bacterial growth, functions, physiology, and behaviors, therefore introducing putative mechanisms by which these bioactive lipids directly modulate the gut microbiome. Moreover, these potential interactions add another layer of complexity to the regulation of host health and disease pathogenesis that may be mediated by endocannabinoids and their derivatives. This mini review will summarize recent literature that exemplifies how *N*-acylethanolamides and monoacylglycerols including endocannabinoids can impact bacterial populations *in vitro* and within the gut microbiome. We also highlight exciting preclinical studies that have engineered gut bacteria to synthesize host *N*-acylethanolamides or their precursors as potential strategies to treat diseases that are in part driven by aberrant lipid signaling, including obesity and inflammatory bowel diseases.

KEYWORDS

host microbe interactions, gut microbiome, endocannabinoid, bioactive lipids, bacteria

Introduction

Host-associated microbial communities and their functional capabilities, collectively referred to as the host microbiome, play integral roles in modulating the health of their hosts and susceptibility to disease. Germ-free experimental models have elegantly demonstrated the dramatic consequences that result from the absence of microbes on

host development, metabolism, anatomy, physiology, and behavior. The clear impacts of endogenous microbes on host biology have been further substantiated by gnotobiology, where the introduction of known populations or communities of microbes into germ-free animals promotes defined host responses and health outcomes [1]. Powerful 'omics approaches such as 16S rRNA sequencing, metagenomics, metatranscriptomics, and metabolomics have been instrumental in correlating specific compositional and functional changes to the host microbiome with certain disease states. These studies have further inspired hypothesis-driven investigations aimed at defining the microbial functions and interactions that contribute to disease pathogenesis. Taken together, studies within the microbiome field have unequivocally demonstrated the importance of these complex and fascinating microbial communities to host biology.

In recent decades, the endocannabinoid system has emerged as an important modulator of gut physiology and homeostasis through its effects on immunity, motility, barrier function, and host metabolism [2]. The endocannabinoid system is comprised of G-protein coupled receptors that are activated by endogenous lipid hormones known as endocannabinoids [3]. The two most well-studied endocannabinoids are 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (AEA)—commonly referred to as anandamide [4, 5]. The structure of 2-AG is a monoacylglycerol (MAG) comprised of an arachidonic acid moiety esterified to a glycerol backbone. AEA is an *N*-acyl ethanolamide (NAE) comprised of an arachidonic acid moiety esterified to an ethanolamine backbone. NAEs and MAGs of other acyl lengths and saturation states are also produced by the host including 2-palmitoyl glycerol (2-PG), 2-oleoyl glycerol (2-OG), palmitoyl ethanolamide (PEA), and oleoyl ethanolamide (OEA) [6]. These compounds also act as bioactive lipids that regulate diverse host functions. This mini review will discuss how these two classes of bioactive lipids may also impact the growth and functions of bacteria within the host microbiome, thus expanding the potential effects of these lipids on host physiology.

Endocannabinoid activity is modulated by biosynthetic and degradative enzymes that alter tissue concentrations of these hormones, which function as ligands at cannabinoid receptors through which they exert their physiological effects [7]. Tissue expression profiles of cannabinoids receptors and these biosynthetic and degradative enzymes also impact endocannabinoid tone. Numerous factors have been linked with altered endocannabinoid tone such as diet, stress, and inflammation status [8–12], although the precise molecular mechanisms remain to be elucidated. The gut microbiome is an additional factor that may modulate the endocannabinoid system [13]. Compositional changes to the gut microbiome triggered by dietary interventions or antibiotic treatment correlate with differential expression of endocannabinoid system components and altered profiles of bioactive lipids in the blood stream and in intestinal tissues [14–17]. Moreover, endocannabinoid tone in intestinal tissues is significantly altered in germ free mice compared to conventional mice colonized with a microbiome, suggesting that microbes somehow impact the degradation and/or biosynthesis of NAEs and MAGs [18, 19]. Conversely, pharmacological and genetic interventions that alter host endocannabinoid activity is correlated with an altered gut microbiome [20–27]. Together, these findings suggest that incompletely defined reciprocal relationships exist between the host endocannabinoid system and the gut microbiome. Moreover, the effects of these relationships on host health and susceptibility to disease remain to be fully elucidated.

More recently, experimental evidence has emerged demonstrating that endocannabinoids and their congeners can modulate bacterial functions, physiology, and behaviors (Figure 1). These findings introduce the exciting possibility that these host lipid hormones may directly modulate bacterial populations within host-associated microbial communities such as the gut microbiome. The mini review will summarize literature that exemplifies how endocannabinoids and their derivatives impact bacterial populations *in vitro* and within rodent models. This mini review will also highlight a collection of preclinical studies that have designed genetically engineered bacteria to modulate host NAE levels to treat metabolic and inflammatory diseases. Included research articles were located using the following search terms in the PubMed database: endocannabinoid + bacteria; endocannabinoid + gut microbiome; *N*-acylethanolamide + bacteria; 2-arachidonoyl glycerol + bacteria; anadamide + bacteria. The mechanisms by which the gut microbiome modulates the host endocannabinoid system and the consequent effects on disease development have been reviewed in a companion article for this special issue [24].

Effects on bacterial growth and metabolism

Numerous studies have correlated compositional changes to the gut microbiome with altered host endocannabinoid tone [20–27]. These ecological changes to the microbial community are likely driven by multiple factors including the indirect effects of host cannabinoid signaling on the gut environment and the direct effects of endocannabinoids on endogenous bacteria. Untargeted metabolomics and metagenomics analyses on fecal samples collected from an IBD patient cohort revealed that NAEs including the endocannabinoid AEA were increased in Crohn's disease patients relative to ulcerative colitis patients and non-IBD controls [28, 29]. Further experimentation in a murine T-cell transfer colitis model revealed that NAEs are also increased following induction of disease relative to the pre-colitic state [28]. The increased concentrations of luminal NAEs in the inflamed gut corresponded with community-wide changes in the relative abundances of diverse bacterial taxa [28, 29]. These

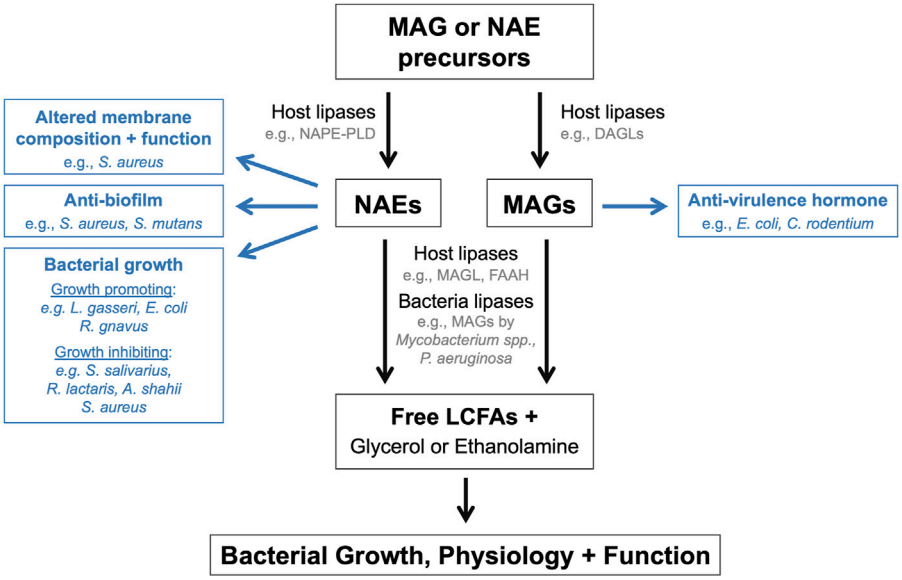

FIGURE 1
Endocannabinoids and their derivatives directly modulate bacterial populations. Schematic summarizing the mechanisms by which endocannabinoids and their derivatives or breakdown products can influence the growth, physiology, and function of endogenous bacteria within the microbiome.

observations prompted the authors to test whether NAEs directly modulate the growth kinetics of gut bacteria [28]. *In vitro* monocultures revealed that NAEs—in particular, OEA and linoleoyl ethanolamine (LEA)—enhanced the growth rates and population densities of several bacterial taxa that are elevated in Crohn's disease patients, including *Escherichia coli, Lactobacillus gasseri*, and *Ruminococcus gnavus* [28]. In contrast, NAEs generally exerted growth inhibitory effects on bacterial taxa depleted in Crohn's disease patients, including *Streptococcus salivarius, Ruminococcus lactaris,* and *Alistipes shahii* [28]. In a separate collection of studies that sought to investigate the antimicrobial properties of AEA on clinical *Staphylococcus aureus* isolates, AEA exerted bacteriostatic effects on strains grown planktonically and within biofilms [30, 31]. Further analyses via scanning electron microscopy revealed that AEA arrested *S. aureus* replication during late-stage cell division, resulting in larger cells with fully formed septa [30]. Notably, all studies reported strain level variations when evaluating the effects of NAEs on bacterial growth [28, 30–32], therefore suggesting that bacterial strains harbor distinct capabilities in responding to NAEs within their environments.

To evaluate the effects of NAEs on bacterial populations residing within complex microbial communities, Fornelos et al. introduced various combinations of NAEs into an *in vitro* model of the gut microbiota [28], which eliminates any effects of NAEs on the host that may also alter the microbial community. The addition of NAEs to the chemostat cultures altered community composition within 12 h. This was characterized by an increase in several taxa including *Escherichia*, *Enterococcus*, and *Veillonella* species and the depletion of several taxa including *Bacteroides*, *Allistipes*, *Ruminococcus*, and *Clostridium* species. Notably, several of these compositional changes recapitulated putative pathological features of the gut microbiome in Crohn's disease patients [28, 29, 33, 34]. Together, these findings support the idea that changes in NAE availability within the intestinal lumen during inflammation may promote and/or sustain the ecological changes that drive microbiome dysfunction. To our knowledge, similar studies focused on MAGs have not yet been published. Considering that altered gut endocannabinoid tone and microbiome dysfunction are both associated with numerous disease states including obesity, cardiovascular diseases, metabolic dysfunction, and neurological diseases [14, 24, 35–39], it will be interesting to learn whether the direct effects of NAEs and other endocannabinoid-like molecules on bacterial growth contribute to the pathogenesis of these complex diseases.

The chemical structures of NAEs and MAGs contain potential bacterial nutrients (i.e., long-chain fatty acids—LCFAs, ethanolamine, glycerol) and antimicrobial agents (i.e., LCFAs). This introduces the possibility that NAEs and MAGs may be hydrolyzed into their constituent components to exert their growth inducing or inhibitory effects. In support of this hypothesis, functional bacterial lipases with structural homology to mammalian monoacylglycerol lipases have been reported in environmental bacteria and in *Mycobacterium* species [40–44]. Similarly, *Pseudomonas aeruginosa* encodes an ABHD6-like lipase that can hydrolyze MAGs [45].

Together, these findings demonstrate the potential for bacterial metabolism of endocannabinoids and their congeners within the gut microbiome. In an *in vitro* chemostat model of the gut microbiota, metatranscriptomics analyses revealed that NAEs significantly alter the community transcriptome, which notably included the differential expression of genes involved in LCFA and ethanolamine metabolism [28]. These transcriptional responses suggest that NAEs may be metabolized by certain members within the bacterial community to liberate free LCFAs and ethanolamine. These nutrients may then be consumed by other bacterial taxa, thus exemplifying a putative cooperative interspecific interaction driven by NAE metabolism.

In contrast, transcriptomics studies performed with the gut bacterium *Bacteroides fragilis* cultivated *in vitro* in monocultures revealed that exposure to LEA and AEA induced the upregulation of efflux pumps and the downregulation of the LCFA transporter FadL [28]. Notably, both NAEs inhibited *B. fragilis* growth, suggesting that this bacterium may respond to NAEs by limiting their import and increasing their export to counteract their toxic effects. Notably, free linoleic acid and arachidonic acid can both exert growth inhibitory effects on various bacterial taxa [46, 47]. This introduces the possibility that NAE hydrolysis within the gut microbiome may also be disadvantageous for bacteria that are susceptible to these poly-unsaturated fatty acids.

To summarize, the few studies that have evaluated the effects of endocannabinoids and their congeners on bacterial growth have demonstrated that their effects on microbial ecology are likely complex. Further studies are clearly needed to investigate how NAEs and MAGs impact the growth of bacterial populations within a complex community, both *in vitro* and within a host. Moreover, it will be interesting to investigate how the effects of these bioactive lipids on microbial ecology ultimately modulates host physiology and susceptibility to disease.

Effects on bacterial physiology and multi-cellular behaviors

The cellular membranes of host-associated bacteria are generally composed of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and cardiolipin as the major phospholipid species [48]. The fatty acids that are esterified to these phospholipids vary between bacterial strains, but usually range between 14 and 18 carbons and are typically in saturated or mono-unsaturated states. Environmental conditions including stressors that alter membrane function and exogenous lipid availability can modify the relative abundances of specific phospholipids and their fatty acid content within cellular membranes [49, 50]. These structural changes to the membrane can then impact several bacterial functions that including growth, susceptibility to extracellular stressors, and biofilm formation—all of which can subsequently influence host-microbial interactions.

In vitro studies performed on bacterial monocultures have demonstrated that host-derived fluids rich in LCFAs—such as bile and serum—impact acyl-LCFA content within bacterial membranes [51–53]. For example, when grown in a nutrient rich medium, the *Enterococcus faecalis* membrane is dominated by vaccenic acid, which comprises approximately 40% of all fatty acid species present [52]. However, when bile is supplemented into this same medium, the percentage of vaccenic acid decreases to about 3%. This corresponds with significant increases in several LCFA species present within the bile including palmitic acid, oleic acid, and stearic acid [52]. These observations suggest that LCFAs within bile are imported by *E. faecalis* and incorporated into phospholipids during membrane biosynthesis. Indeed, when supplied individually, each LCFA dominates fatty acid content within the membrane [52, 54, 55] Similarly, the membrane lipid profile of the nosocomial pathogen *Acinetobacter baumannii* is significantly altered following recovery from pleural lavage fluid in the lungs compared to growth in standard laboratory media [56]. In particular, *A. baumannii* growth within the lungs corresponds with an increase in polyunsaturated LCFA content within membrane PEs. Notably, *de novo* synthesis of polyunsaturated LCFAs was not detected following *in vitro* cultivation in LCFA-free media, suggesting that *A. baumannii* utilizes host-derived PUFAs for membrane biosynthesis during *in vivo* growth. Supporting this hypothesis, genetic inactivation of the main exogenous LCFA transporter FadL conferred a growth defect in *A. baumannii* within several host microenvironments [56]. Notably, studies performed in diverse bacterial taxa—including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*, and *Lactobacillus* species—have demonstrated that exogenous LCFA availability influences membrane structure [54, 55, 57–65]. Taken together, these studies clearly demonstrate that LCFAs within host environments can be utilized by bacterial organisms to modify their membrane structures, which in turn may impact host-microbial interactions.

The molecular structure of endocannabinoids and their congeners include an LCFA moiety, and therefore, it is conceivable that these molecules may also impact bacterial membrane physiology and function. In a recent collection of studies, the authors demonstrated that exposure to the endocannabinoid AEA alters lipid content, fluidity, bioenergetics, and permeability of the cytoplasmic membrane in clinical *Staphylococcus aureus* isolates [30–32, 66]. AEA exposure corresponded with increased cardiolipin content within the *S. aureus* membrane [30]. Cardiolipins can form microdomains within bacterial membranes, which in turn can impact the functionality of membrane proteins such as transporters [48]. The addition of AEA to *in vitro* cultures also resulted in decreased efflux of various toxic compounds, including antibiotics, from *S. aureus* cells [30, 31]. These observations corresponded with lower membrane potential,

decreased membrane ATPase activity, and increased cardiolipin content, all of which can modulate the functionality of efflux pumps. Decreased efflux associated with AEA also corresponded with the differential expression of various efflux pump genes, which may also contribute to this response [31]. AEA also sensitized clinical *S. aureus* isolates to various antibiotics including beta-lactams, gentamicin, tetracycline, and fluoroquinolones through a mechanism that likely involves compromising efflux pump function [30–32]. The addition of AEA and LEA to *in vitro* cultures also modulated the expression of efflux genes in *B. fragilis* [28]. Together, these studies demonstrate that AEA impacts several compositional and functional aspects of the bacterial cellular membrane. Outstanding questions include whether other NAEs impart similar effects on *S. aureus* growth and membrane function. More broadly, it will be interesting to investigate the effects of NAEs and MAGs on membrane composition and function in other bacterial taxa present within host associated microbial communities and whether these alterations in bacterial physiology impact host disease development.

Within host environments, commensal bacteria and invading pathogens can grow within multicellular structures such as cellular aggregates and biofilms. The formation of these structures involves the biosynthesis and export of components that comprise the eventual extracellular matrix, which serves to adhere bacterial cells together while also acting as a thick protective barrier against environmental insults including the host immune response and antimicrobials. Recent studies have demonstrated that NAEs can exert anti-biofilm effects against *S. aureus* and the oral commensal bacterium *Streptococcus mutans* [30–32, 66, 67]. AEA and arachidonoyl serine both synergized with several types of antimicrobial agents to inhibit biofilm formation in clinical *S. aureus* isolates [30–32]. When supplied individually, both compounds inhibited several *S. aureus* behaviors associated with enhanced biofilm formation including surface motility and cell-to-cell aggregation [66]. AEA also altered the gene expression of several biofilm-associated genes and decreased extracellular matrix production in *S. aureus* [31]. Notably, efflux pumps can contribute to biofilm formation by exporting components needed to construct the extracellular matrix. Therefore, it is possible that the inhibitory effects of AEA on efflux pumps as described above may also explain its anti-biofilm effects in *S. aureus*. In *S. mutans,* exposure to either OEA or AEA exacerbated the anti-biofilm effects of the antimicrobial compound poly-L-lysine [67]. In contrast, the NAEs PEA and stearoylethanolamide (SEA) did not impact *S. mutans* biofilm formation, therefore suggesting that this inhibitory effect is unique to NAEs with a monounsaturated LCFA moiety. Taken together, in addition to their effects on membrane physiology, NAEs can also antagonize bacterial behaviors that lead to the formation of biofilms and other multi-cellular structures. Future studies are warranted to investigate how NAEs and other endocannabinoid-like molecules modulate these behaviors within host environments.

Effects on bacterial signaling

Bioactive lipids function as signaling molecules that are sensed by bacterial organisms to elicit a particular cellular response. Nutrients such as sugars, amino acids, and fatty acids are also sensed by membrane-bound and intracellular receptors that couple nutrient availability with the transcriptional regulation of metabolic pathways and other functions. Bacterial sensing of these environmental cues plays a central role in bacterial pathogenesis and in host-microbial interactions within the gut [68].

A collection of studies over the past decade have demonstrated that free LCFAs act as both nutrients and signals that can modulate a variety of bacterial functions—recently reviewed here [47, 69, 70]. More recently, the endocannabinoid 2-AG was shown to function as a host-derived hormone that is directly sensed by several gut bacterial pathogens including *Citrobacter rodentium* and enterohemorrhagic *E. coli* (EHEC) [71]. *In vitro* functional and biochemical approaches revealed that 2-AG inhibits the membrane-bound bacterial receptor QseC, which functions to stimulate intracellular signaling cascades that activate virulence programs in response to the catecholamines epinephrine and norepinephrine and to the quorum sensing hormone autoinducer-3 [71–75]. In a mouse model of intestinal infection, Magl-deficient mice with elevated levels of 2-AG developed attenuated disease in response to *C. rodentium* challenge [71]. These protective effects were no longer observed when Magl-deficient mice were challenged with *qseC*-deficient *C. rodentium*, suggesting that 2-AG exerts its anti-virulence effects in the gut by inhibiting QseC-dependent virulence. Interestingly, free arachidonic acid—a product released following 2-AG hydrolysis—also exerts anti-virulence effects on EHEC [76]. When imported into EHEC, arachidonic acid is esterified to coenzyme A and then allosterically inhibits the lipid-responsive transcription factor FadR, which in turn represses the expression of virulence genes [76, 77]. Notably, QseC and FadR homologues are present in other bacterial pathogens and commensals [78–81], which introduces the possibility that endocannabinoids and their derivatives may directly modulate the behaviors of many other bacterial organisms.

Engineering bacteria to modulate host lipid signaling

The first three sections of this mini review summarized experimental evidence that demonstrates how bioactive lipids

may directly modulate bacterial populations. Many bacterial taxa within the gut microbiome are currently genetically tractable, therefore introducing the possibility of designing probiotics that specifically target these lipid signaling networks. This last section will summarize two collections of studies that apply this concept to diseases that are in part driven by aberrant NAE signaling—obesity and inflammatory bowel diseases.

Certain NAE species and their *N*-acyl-phosphatidylethanolamine (NAPE) precursors function as satiety signals that are synthesized in the small intestines to regulate host feeding behaviors [82–84]. In rodent models, chronic administration of exogenous NAEs exerts various anorexigenic effects including decreased food consumption and improved host metabolic parameters in rodent models [85]. In one collection of studies, the probiotic *E. coli* strain Nissle was engineered to synthesize and secrete NAEs or their *N*-acyl-phosphatidylethanolamine (NAPE) precursors as a novel therapeutic strategy to treat obesity [86–89]. Chronic administration of NAPE-producing Nissle resulted in decreased weight gain and adiposity in diet-induced and genetic obesity models in a NAPE-PLD dependent manner and in a cardiometabolic disease model [86, 88, 89]. These results corresponded with increased hepatic NAE levels, decreased lipid accumulation and inflammation markers in the liver, and improved metabolic parameters such as glucose tolerance and insulin sensitivity [88]. Similar anti-obesogenic effects were observed with the NAE-producing Nissle strain [89].

In addition to their effects on central host metabolism, certain NAE species such as PEA also exhibit anti-inflammatory properties in experimental colitis models through incompletely defined mechanisms [90–96]. As a strategy to augment local levels of PEA, a second group genetically engineered the probiotic *Lactobacillus paracasei* substrain *Paracasei* F19 to synthesize and secrete PEA when the strain was supplied exogenous palmitic acid [97]. Using a chemically induced model of colitis, the authors demonstrated that co-administration of the PEA-producing *L. paracasei* with palmitate significantly increased intestinal concentrations of PEA and resulted in attenuated colitis development. These protective effects were no longer apparent in mice lacking the PEA receptor PPAR-alpha. In a follow-up study, the authors also demonstrated that this PEA-producing probiotic protected against colitis development induced by the TcdA toxin from *Clostridioides difficile* in a PPAR-alpha dependent manner [98].

Taken together, these studies demonstrate how intestinal probiotic strains can serve as bacterial platforms for delivering NAEs or their precursors to host tissues to treat metabolic and inflammation driven diseases that are characterized by low NAE tone. Because endogenous bacteria within the microbiome likely also synthesize and metabolize NAEs [28, 99], it will be interesting to investigate whether the endogenous microbial production of these bioactive lipids also serve as inputs into host lipid signaling networks, which in turn may also impact the disease development.

Discussion

This mini review has highlighted the numerous ways in which endocannabinoids and their derivatives directly impact bacterial growth, physiology, and behaviors (Figure 1). These effects have primarily been investigated using *in vitro* single bacterial populations, rather than within polymicrobial communities and/or host environments. Bacterial growth and behaviors are substantially different within host environments and complex microbial community in comparison to *in vitro* conditions. Therefore, future studies are warranted to investigate how these bioactive lipids impact bacteria populations within the gut microbiome in animal models to evaluate whether the effects observe *in vitro* also occur *in vivo*. Approaches to address this question could include microbial sequencing, metabolomics, bacterial and mouse genetics, and gnotobiology. The application of these approaches to established animal models of disease would also begin to address how the regulation of bacterial growth and behaviors by these bioactive lipids may impact disease pathogenesis and susceptibility. Finally, while not the focus of this mini review, it is important to acknowledge that endocannabinoid activity also impacts the function of host cell populations within the intestines, which in turn can modulate gut physiology and microbiome function. However, it remains unclear how the distinct effects of endocannabinoid activity on host tissues and microbial populations, and on the reciprocal interactions between host and microbe, together ultimately impact the establishment and maintenance of gut homeostasis and the development of disease. This represents an additional exciting avenue of research highly worth exploring.

Author contributions

ME wrote and edited the manuscript.

Funding

This work was supported by a target faculty grant awarded to ME from an NIH COBRE grant (5P20GM103641-09; awarded to Drs. Mitzi and Prakash Nagarkatti).

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Basic M, Bleich A. Gnotobiotics: past, present and future. *Lab Anim* (2019) 53: 232–43. doi:10.1177/0023677219836715

2. Hasenoehrl C, Taschler U, Storr M, Schicho R. The gastrointestinal tract – a central organ of cannabinoid signaling in health and disease. *Neurogastroenterol Motil* (2016) 28:1765–80. doi:10.1111/nmo.12931

3. Mackie K. Cannabinoid receptors as therapeutic targets. *Annu Rev Pharmacol* (2006) 46:101–22. doi:10.1146/annurev.pharmtox.46.120604.141254

4. Devane W, Hanus L, Breuer A, Pertwee R, Stevenson L, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* (1992) 258:1946–9. doi:10.1126/science.1470919

5. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* (1995) 50:83–90. doi:10.1016/0006-2952(95)00109-d

6. Hansen HS, Vana V. Non-endocannabinoid N-acylethanolamines and 2-monoacylglycerols in the intestine. *Br J Pharmacol* (2019) 176:1443–54. doi:10.1111/bph.14175

7. Ahn K, McKinney MK, Cravatt BF. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev* (2008) 108: 1687–707. doi:10.1021/cr0782067

8. Hillard CJ. Stress regulates endocannabinoid-CB1 receptor signaling. *Semin Immunol* (2014) 26:380–8. doi:10.1016/j.smim.2014.04.001

9. Morris G, Sominsky L, Walder KR, Berk M, Marx W, Carvalho AF, et al. Inflammation and nitro-oxidative stress as drivers of endocannabinoid system aberrations in mood disorders and schizophrenia. *Mol Neurobiol* (2022) 59: 3485–503. doi:10.1007/s12035-022-02800-y

10. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* (2010) 13:951–7. doi:10.1038/nn.2601

11. Kuipers EN, Kantae V, Maarse BCE, van den Berg SM, van Eenige R, Nahon KJ, et al. High fat diet increases circulating endocannabinoids accompanied by increased synthesis enzymes in adipose tissue. *Front Physiol* (2019) 9:1913. doi:10.3389/fphys.2018.01913

12. Bisogno T, Maccarrone M. Endocannabinoid signaling and its regulation by nutrients. *BioFactors* (2014) 40:373–80. doi:10.1002/biof.1167

13. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci* (2013) 110:9066–71. doi:10.1073/pnas.1219451110

14. Guida F, Turco F, Iannotta M, Gregorio DD, Palumbo I, Sarnelli G, et al. Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain Behav Immun* (2018) 67:230–45. doi:10.1016/j.bbi.2017.09.001

15. Lacroix S, Pechereau F, Leblanc N, Boubertakh B, Houde A, Martin C, et al. Rapid and concomitant gut microbiota and endocannabinoidome response to diet-induced obesity in mice. *Msystems* (2019) 4:e00407–19. doi:10.1128/msystems.00407-19

16. Tagliamonte S, Laiola M, Ferracane R, Vitale M, Gallo MA, Meslier V, et al. Mediterranean diet consumption affects the endocannabinoid system in overweight and obese subjects: possible links with gut microbiome, insulin resistance and inflammation. *Eur J Nutr* (2021) 60:3703–16. doi:10.1007/s00394-021-02538-8

17. Hussein HM, Elyamany MF, Rashed LA, Sallam NA. Vitamin D mitigates diabetes-associated metabolic and cognitive dysfunction by modulating gut microbiota and colonic cannabinoid receptor 1. *Eur J Pharm Sci* (2022) 170: 106105. doi:10.1016/j.ejps.2021.106105

18. Manca C, Boubertakh B, Leblanc N, Deschênes T, Lacroix S, Martin C, et al. Germ-free mice exhibit profound gut microbiota-dependent alterations of intestinal endocannabinoidome signaling. *J Lipid Res* (2020) 61:70–85. doi:10.1194/jlr.ra119000424

19. Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, et al. The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol* (2010) 6:392. doi:10.1038/msb.2010.46

20. Cani PD, Plovier H, Hul MV, Geurts L, Delzenne NM, Druart C, et al. Endocannabinoids — at the crossroads between the gut microbiota and host metabolism. *Nat Rev Endocrinol* (2016) 12:133–43. doi:10.1038/nrendo.2015.211

21. Mehrpouya-Bahrami P, Chitrala KN, Ganewatta MS, Tang C, Murphy EA, Enos RT, et al. Blockade of CB1 cannabinoid receptor alters gut microbiota and attenuates inflammation and diet-induced obesity. *Sci Rep* (2017) 7:15645. doi:10.1038/s41598-017-15154-6

22. Al-Ghezi ZZ, Busbee PB, Alghetaa H, Nagarkatti PS, Nagarkatti M. Combination of cannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), mitigates experimental autoimmune encephalomyelitis (EAE) by altering the gut microbiome. *Brain Behav Immun* (2019) 82:25–35. doi:10.1016/j.bbi.2019.07.028

23. Dione N, Lacroix S, Taschler U, Deschênes T, Abolghasemi A, Leblanc N, et al. Mgll knockout mouse resistance to diet-induced dysmetabolism is associated with altered gut microbiota. *Cells* (2020) 9:2705. doi:10.3390/cells9122705

24. Varsha KK, Nagarkatti M, Nagarkatti P. Role of gut microbiota in cannabinoid-mediated suppression of inflammation. *Adv Drug Alcohol Res* (2022) 2:10550. doi:10.3389/adar.2022.10550

25. Sultan M, Wilson K, Abdulla OA, Busbee PB, Hall A, Carter T, et al. Endocannabinoid anandamide attenuates acute respiratory distress syndrome through modulation of microbiome in the gut-lung axis. *Cells* (2021) 10:3305. doi:10.3390/cells10123305

26. Rodríguez-González A, Vitali F, Moya M, Filippo CD, Passani MB, Orio L. Effects of alcohol binge drinking and oleoylethanolamide pretreatment in the gut microbiota. *Front Cell Infect Mi* (2021) 11:731910. doi:10.3389/fcimb.2021.731910

27. Paola MD, Bonechi E, Provensi G, Costa A, Clarke G, Ballerini C, et al. Oleoylethanolamide treatment affects gut microbiota composition and the expression of intestinal cytokines in Peyer's patches of mice. *Sci Rep* (2018) 8: 14881. doi:10.1038/s41598-018-32925-x

28. Fornelos N, Franzosa EA, Bishai J, Annand JW, Oka A, Lloyd-Price J, et al. Growth effects of N-acylethanolamines on gut bacteria reflect altered bacterial abundances in inflammatory bowel disease. *Nat Microbiol* (2020) 5:486–97. doi:10.1038/s41564-019-0655-7

29. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* (2019) 4:293–305. doi:10.1038/s41564-018-0306-4

30. Sionov RV, Banerjee S, Bogomolov S, Smoum R, Mechoulam R, Steinberg D. Targeting the achilles' heel of multidrug-resistant *Staphylococcus aureus* by the endocannabinoid anandamide. *Int J Mol Sci* (2022) 23:7798. doi:10.3390/ijms23147798

31. Banerjee S, Sionov RV, Feldman M, Smoum R, Mechoulam R, Steinberg D. Anandamide alters the membrane properties, halts the cell division and prevents drug efflux in multidrug resistant *Staphylococcus aureus*. *Sci Rep* (2021) 11:8690. doi:10.1038/s41598-021-88099-6

32. Feldman M, Smoum R, Mechoulam R, Steinberg D. Potential combinations of endocannabinoid/endocannabinoid-like compounds and antibiotics against methicillin-resistant *Staphylococcus aureus*. *Plos One* (2020) 15:e0231583. doi:10.1371/journal.pone.0231583

33. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* (2014) 15:382–92. doi:10.1016/j.chom.2014.02.005

34. Pascal V, Pozuelo M, Borruel N, Casellas F, Campos D, Santiago A, et al. A microbial signature for Crohn's disease. *Gut* (2017) 66:813–22. doi:10.1136/gutjnl-2016-313235

35. Tsiantas K, Konteles SJ, Kritsi E, Sinanoglou VJ, Tsiaka T, Zoumpoulakis P. Effects of non-polar dietary and endogenous lipids on gut microbiota alterations: the role of lipidomics. *Int J Mol Sci* (2022) 23:4070. doi:10.3390/ijms23084070

36. Khan RN, Maner-Smith K, Owens JA, Barbian ME, Jones RM, Naudin CR. At the heart of microbial conversations: endocannabinoids and the microbiome in cardiometabolic risk. *Gut Microbes* (2021) 13:1911572. doi:10.1080/19490976.2021.1911572

37. Suriano F, Manca C, Flamand N, Hul MV, Delzenne NM, Silvestri C, et al. A lipidomics- and transcriptomics-based analysis of the intestine of genetically obese (ob/ob) and diabetic (db/db) mice: links with inflammation and gut microbiota. *Cells* (2023) 12:411. doi:10.3390/cells12030411

38. Mestre L, Carrillo-Salinas FJ, Mecha M, Feliú A, Guaza C. Gut microbiota, cannabinoid system and neuroimmune interactions: new perspectives in multiple sclerosis. *Biochem Pharmacol* (2018) 157:51–66. doi:10.1016/j.bcp.2018.08.037

39. Iannotti FA, Marzo VD. The gut microbiome, endocannabinoids and metabolic disorders. *J Endocrinol* (2021) 248:R83–R97. doi:10.1530/joe-20-0444

40. Dhouib R, Laval F, Carrière F, Daffé M, Canaan S. A monoacylglycerol lipase from Mycobacterium smegmatis involved in bacterial cell interaction. *J Bacteriol* (2010) 192:4776–85. doi:10.1128/jb.00261-10

41. Côtes K, Dhouib R, Douchet I, Chahinian H, de Caro A, Carrière F, et al. Characterization of an exported monoglyceride lipase from *Mycobacterium tuberculosis* possibly involved in the metabolism of host cell membrane lipids. *Biochem J* (2007) 408:417–27. doi:10.1042/bj20070745

42. Aschauer P, Zimmermann R, Breinbauer R, Pavkov-Keller T, Oberer M. The crystal structure of monoacylglycerol lipase from *M. tuberculosis* reveals the basis for specific inhibition. *Sci Rep* (2018) 8:8948. doi:10.1038/s41598-018-27051-7

43. Rengachari S, Bezerra GA, Riegler-Berket L, Gruber CC, Sturm C, Taschler U, et al. The structure of monoacylglycerol lipase from Bacillus sp. H257 reveals unexpected conservation of the cap architecture between bacterial and human enzymes. *Biochim Biophys Acta Bba - Mol Cell Biol Lipids* (2012) 1821:1012–21. doi:10.1016/j.bbalip.2012.04.006

44. Riegler-Berket L, Leitmeier A, Aschauer P, Dreveny I, Oberer M. Identification of lipases with activity towards monoacylglycerol by criterion of conserved cap architectures. *Biochim Biophys Acta Bba - Mol Cell Biol Lipids* (2018) 1863:679–87. doi:10.1016/j.bbalip.2018.03.009

45. Bleffert F, Granzin J, Gohlke H, Batra-Safferling R, Jaeger K-E, Kovacic F. *Pseudomonas aeruginosa* esterase PA2949, a bacterial homolog of the human membrane esterase ABHD6: expression, purification and crystallization. *Acta Crystallogr Sect F* (2019) 75:270–7. doi:10.1107/s2053230x19002152

46. Casillas-Vargas G, Ocasio-Malavé C, Medina S, Morales-Guzmán C, Valle RGD, Carballeira NM, et al. Antibacterial fatty acids: an update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Prog Lipid Res* (2021) 82:101093. doi:10.1016/j.plipres.2021.101093

47. Tchoupa AK, Eijkelkamp BA, Andreas P. Bacterial adaptation strategies to host-derived fatty acids. *Trends Microbiol* (2021) 30:241–53. doi:10.1016/j.tim.2021.06.002

48. Strahl H, Errington J. Bacterial membranes: structure, domains and function. *Annu Rev Microbiol* (2017) 157:367. doi:10.1038/157367a0

49. López-Lara IM, Geiger O. Bacterial lipid diversity. *Biochim Biophys Acta Bba - Mol Cell Biol Lipids* (2017) 1862:1287–99. doi:10.1016/j.bbalip.2016.10.007

50. Chautrand T, Souak D, Chevalier S, Duclairoir-Poc C. Gram-negative bacterial envelope homeostasis under oxidative and nitrosative stress. *Microorg* (2022) 10:924. doi:10.3390/microorganisms10050924

51. Giles DK, Hankins JV, Guan Z, Trent MS. Remodelling of the *Vibrio cholerae* membrane by incorporation of exogenous fatty acids from host and aquatic environments. *Mol Microbiol* (2011) 79:716–28. doi:10.1111/j.1365-2958.2010.07476.x

52. Saito HE, Harp JR, Fozo EM. Incorporation of exogenous fatty acids protects *Enterococcus faecalis* from membrane-damaging agents. *Appl Environ Microb* (2014) 80:6527–38. doi:10.1128/aem.02044-14

53. Harp JR, Saito HE, Bourdon AK, Reyes J, Arias CA, Campagna SR, et al. Exogenous fatty acids protect *Enterococcus faecalis* from daptomycin-induced membrane stress independently of the response regulator LiaR. *Appl Environ Microb* (2016) 82:4410–20. doi:10.1128/aem.00933-16

54. Saito HE, Harp JR, Fozo EM. *Enterococcus faecalis* responds to individual exogenous fatty acids independently of their degree of saturation or chain length. *Appl Environ Microb* (2017) 84:e01633–17. doi:10.1128/aem.01633-17

55. Brewer W, Harrison J, Saito HE, Fozo EM. Induction of daptomycin tolerance in *Enterococcus faecalis* by fatty acid combinations. *Appl Environ Microb* (2020) 86:e01178–20. doi:10.1128/aem.01178-20

56. Adams FG, Trappetti C, Waters JK, Zang M, Brazel EB, Paton JC, et al. To make or take: bacterial lipid homeostasis during infection. *Mbio* (2021) 12:e0092821–21. doi:10.1128/mbio.00928-21

57. Moravec AR, Siv AW, Hobby CR, Lindsay EN, Norbash LV, Shults DJ, et al. Exogenous polyunsaturated fatty acids impact membrane remodeling and affect virulence phenotypes among pathogenic Vibrio species. *Appl Environ Microb* (2017) 83:e01415-17. doi:10.1128/aem.01415-17

58. Hobby CR, Herndon JL, Morrow CA, Peters RE, Symes SJK, Giles DK. Exogenous fatty acids alter phospholipid composition, membrane permeability, capacity for biofilm formation, and antimicrobial peptide susceptibility in *Klebsiella pneumoniae*. *Microbiologyopen* (2019) 8:e00635. doi:10.1002/mbo3.635

59. Eder AE, Munir SA, Hobby CR, Anderson DM, Herndon JL, Siv AW, et al. Exogenous polyunsaturated fatty acids (PUFAs) alter phospholipid composition, membrane permeability, biofilm formation and motility in Acinetobacter baumannii. *Microbiology* (2017) 163:1626–36. doi:10.1099/mic.0.000556

60. Herndon JL, Peters RE, Hofer RN, Simmons TB, Symes SJ, Giles DK. Exogenous polyunsaturated fatty acids (PUFAs) promote changes in growth, phospholipid composition, membrane permeability and virulence phenotypes in *Escherichia coli*. *Bmc Microbiol* (2020) 20:305. doi:10.1186/s12866-020-01988-0

61. Baker LY, Hobby CR, Siv AW, Bible WC, Glennon MS, Anderson DM, et al. *Pseudomonas aeruginosa* responds to exogenous polyunsaturated fatty acids (PUFAs) by modifying phospholipid composition, membrane permeability, and phenotypes associated with virulence. *Bmc Microbiol* (2018) 18:117. doi:10.1186/s12866-018-1259-8

62. Zang M, Adams FG, Hassan KA, Eijkelkamp BA. The impact of omega-3 fatty acids on the evolution of acinetobacter baumannii drug resistance. *Microbiol Spectr* (2021) 9:e0145521–21. doi:10.1128/spectrum.01455-21

63. Parsons JB, Frank MW, Subramanian C, Saenkham P, Rock CO. Metabolic basis for the differential susceptibility of gram-positive pathogens to fatty acid synthesis inhibitors. *Proc Natl Acad Sci* (2011) 108:15378–83. doi:10.1073/pnas.1109208108

64. Brinster S, Lamberet G, Staels B, Trieu-Cuot P, Gruss A, Poyart C. Type II fatty acid synthesis is not a suitable antibiotic target for gram-positive pathogens. *Nature* (2009) 458:83–6. doi:10.1038/nature07772

65. Johnsson T, Nikkila P, Toivonen L, Rosenqvist H, Laakso S. Cellular fatty acid profiles of lactobacillus and lactococcus strains in relation to the oleic acid content of the cultivation medium. *Appl Environ Microb* (1995) 61:4497–9. doi:10.1128/aem.61.12.4497-4499.1995

66. Feldman M, Smoum R, Mechoulam R, Steinberg D. Antimicrobial potential of endocannabinoid and endocannabinoid-like compounds against methicillin-resistant *Staphylococcus aureus*. *Sci Rep* (2018) 8:17696. doi:10.1038/s41598-018-35793-7

67. Feldman M, Sionov R, Smoum R, Mechoulam R, Ginsburg I, Steinberg D. Comparative evaluation of combinatory interaction between endocannabinoid system compounds and poly-L-lysine against Streptococcus mutans growth and biofilm formation. *Biomed Res Int* (2020) 2020:7258380. doi:10.1155/2020/7258380

68. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. *Nature* (2016) 535:85–93. doi:10.1038/nature18849

69. Mitchell MK, Ellermann M. Long chain fatty acids and virulence repression in intestinal bacterial pathogens. *Front Cell Infect Mi* (2022) 12:928503. doi:10.3389/fcimb.2022.928503

70. Prasun K, Jin-Hyung L, Haluk B, Jintae L. Fatty acids as antibiofilm and antivirulence agents. *Trends Microbiol* (2020) 28:753–68. doi:10.1016/j.tim.2020.03.014

71. Ellermann M, Pacheco AR, Jimenez AG, Russell RM, Cuesta S, Kumar A, et al. Endocannabinoids inhibit the induction of virulence in enteric pathogens. *Cell* (2020) 183:650–65. doi:10.1016/j.cell.2020.09.022

72. Moreira CG, Russell R, Mishra AA, Narayanan S, Ritchie JM, Waldor MK, et al. Bacterial adrenergic sensors regulate virulence of enteric pathogens in the gut. *Mbio* (2016) 7:e00826–16. doi:10.1128/mbio.00826-16

73. Hughes DT, Clarke MB, Yamamoto K, Rasko DA, Sperandio V. The QseC adrenergic signaling cascade in enterohemorrhagic *E. coli* (EHEC). *Plos Pathog* (2009) 5:e1000553. doi:10.1371/journal.ppat.1000553

74. Marcie BC, David TH, Chengru Z, Edgar CB, Vanessa S. The QseC sensor kinase: a bacterial adrenergic receptor. *Proc Natl Acad Sci* (2006) 103:10420–5. doi:10.1073/pnas.0604343103

75. Reading NC, Rasko DA, Torres AG, Sperandio V. The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *Proc Natl Acad Sci* (2009) 106:5889–94. doi:10.1073/pnas.0811409106

76. Ellermann M, Jimenez AG, Pifer R, Ruiz N, Sperandio V. The canonical long-chain fatty acid sensing machinery processes arachidonic acid to inhibit virulence in enterohemorrhagic *Escherichia coli*. *Mbio* (2021) 12:e03247–20. doi:10.1128/mbio.03247-20

77. Reed P, Regan MR, Aman K, Meredith MC, Vanessa S. Redox, amino acid, and fatty acid metabolism intersect with bacterial virulence in the gut. *Proc Natl Acad Sci* (2018) 115:E10712–E10719. doi:10.1073/pnas.1813451115

78. Rasko DA, Moreira CG, Li DR, Reading NC, Ritchie JM, Waldor MK, et al. Targeting QseC signaling and virulence for antibiotic development. *Science* (2008) 321:1078–80. doi:10.1126/science.1160354

79. Zhu Y, Dou Q, Du L, Wang Y. QseB/QseC: a two-component system globally regulating bacterial behaviors. *Trends Microbiol* (2023) 31:749–62. doi:10.1016/j.tim.2023.02.001

80. Iram SH, Cronan JE. Unexpected functional diversity among FadR fatty acid transcriptional regulatory proteins. *J Biol Chem* (2005) 280:32148–56. doi:10.1074/jbc.m504054200

81. Cronan JE. The *Escherichia coli* FadR transcription factor: too much of a good thing? *Mol Microbiol* (2021) 115:1080–5. doi:10.1111/mmi.14663

82. Gillum MP, Zhang D, Zhang X-M, Erion DM, Jamison RA, Choi C, et al. N-Acylphosphatidylethanolamine, a gut- derived circulating factor induced by fat ingestion, inhibits food intake. *Cell* (2008) 135:813–24. doi:10.1016/j.cell.2008.10.043

83. Schwartz GJ, Fu J, Astarita G, Li X, Gaetani S, Campolongo P, et al. The lipid messenger OEA links dietary fat intake to satiety. *Cell Metab* (2008) 8:281–8. doi:10.1016/j.cmet.2008.08.005

84. Hansen HS. Role of anorectic N-acylethanolamines in intestinal physiology and satiety control with respect to dietary fat. *Pharmacol Res* (2014) 86:18–25. doi:10.1016/j.phrs.2014.03.006

85. Sihag J, Jones PJH. Oleoylethanolamide: the role of a bioactive lipid amide in modulating eating behaviour. *Obes Rev* (2018) 19:178–97. doi:10.1111/obr.12630

86. May-Zhang LS, Chen Z, Dosoky NS, Yancey PG, Boyd KL, Hasty AH, et al. Administration of N-Acyl-phosphatidylethanolamine expressing bacteria to low density lipoprotein receptor−/− mice improves indices of cardiometabolic disease. *Sci Rep* (2019) 9:420. doi:10.1038/s41598-018-37373-1

87. Dosoky NS, Guo L, Chen Z, Feigley AV, Davies SS. Dietary fatty acids control the species of N-Acyl-phosphatidylethanolamines synthesized by therapeutically modified bacteria in the intestinal tract. *Acs Infect Dis* (2018) 4:3–13. doi:10.1021/acsinfecdis.7b00127

88. Chen Z, Guo L, Zhang Y, Walzem RL, Pendergast JS, Printz RL, et al. Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. *J Clin Invest* (2014) 124:3391–406. doi:10.1172/jci72517

89. Chen Z, Zhang Y, Guo L, Dosoky N, de Ferra L, Peters S, et al. Leptogenic effects of NAPE require activity of NAPE-hydrolyzing phospholipase D. *J Lipid Res* (2017) 58:1624–35. doi:10.1194/jlr.m076513

90. Alhouayek M, Bottemanne P, Subramanian KV, Lambert DM, Makriyannis A, Cani PD, et al. N-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis. *Faseb J* (2015) 29:650–61. doi:10.1096/fj.14-255208

91. Alhouayek M, Ameraoui H, Muccioli GG. Bioactive lipids in inflammatory bowel diseases – from pathophysiological alterations to therapeutic opportunities. *Biochim Biophys Acta Bba - Mol Cell Biol Lipids* (2021) 1866:158854. doi:10.1016/j.bbalip.2020.158854

92. Otagiri S, Ohnishi S, Ohara M, Fu Q, Yamamoto K, Yamamoto K, et al. Oleoylethanolamide ameliorates dextran sulfate sodium-induced colitis in rats. *Front Pharmacol* (2020) 11:1277. doi:10.3389/fphar.2020.01277

93. D'Antongiovanni V, Pellegrini C, Antonioli L, Benvenuti L, Salvo CD, Flori L, et al. Palmitoylethanolamide counteracts enteric inflammation and bowel motor dysfunctions in a mouse model of alzheimer's disease. *Front Pharmacol* (2021) 12:748021. doi:10.3389/fphar.2021.748021

94. Esposito G, Capoccia E, Turco F, Palumbo I, Lu J, Steardo A, et al. Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-α activation. *Gut* (2014) 63:1300–12. doi:10.1136/gutjnl-2013-305005

95. Borrelli F, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, et al. Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. *Br J Pharmacol* (2015) 172:142–58. doi:10.1111/bph.12907

96. Lama A, Provensi G, Amoriello R, Pirozzi C, Rani B, Mollica MP, et al. The anti-inflammatory and immune-modulatory effects of OEA limit DSS-induced colitis in mice. *Biomed Pharmacother* (2020) 129:110368. doi:10.1016/j.biopha.2020.110368

97. Esposito G, Corpetti C, Pesce M, Seguella L, Annunziata G, Re AD, et al. A palmitoylethanolamide producing lactobacillus paracasei improves *Clostridium difficile* toxin A-induced colitis. *Front Pharmacol* (2021) 12:639728. doi:10.3389/fphar.2021.639728

98. Esposito G, Pesce M, Seguella L, Lu J, Corpetti C, Re AD, et al. Engineered lactobacillus paracasei producing palmitoylethanolamide (PEA) prevents colitis in mice. *Int J Mol Sci* (2021) 22:2945. doi:10.3390/ijms22062945

99. Igarashi M, Hayakawa T, Tanabe H, Watanabe K, Nishida A, Kimura I. Intestinal GPR119 activation by microbiota-derived metabolites impacts feeding behavior and energy metabolism. *Mol Metab* (2023) 67:101649. doi:10.1016/j.molmet.2022.101649

ADAR | Publishing Partnerships

TYPE Review
PUBLISHED 03 March 2023
DOI 10.3389/adar.2023.11092

OPEN ACCESS

EDITED BY
Emmanuel Onaivi,
William Paterson University,
United States

REVIEWED BY
Yuri Persidsky,
Temple University, United States
Laura Orio,
Complutense University of Madrid,
Spain

*CORRESPONDENCE
Palsamy Periyasamy,
palsamy.periyasamy@unmc.edu
Shilpa Buch,
sbuch@unmc.edu

†These authors have contributed equally to this work

RECEIVED 01 December 2022
ACCEPTED 23 February 2023
PUBLISHED 03 March 2023

CITATION
Ray S, Sil S, Kannan M, Periyasamy P and Buch S (2023), Role of the gut-brain axis in HIV and drug abuse-mediated neuroinflammation. *Adv. Drug. Alco. Res.* 3:11092. doi: 10.3389/adar.2023.11092

Role of the gut-brain axis in HIV and drug abuse-mediated neuroinflammation

Sudipta Ray, Susmita Sil†, Muthukumar Kannan†, Palsamy Periyasamy* and Shilpa Buch*

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, United States

Drug abuse and related disorders are a global public health crisis affecting millions, but to date, limited treatment options are available. Abused drugs include but are not limited to opioids, cocaine, nicotine, methamphetamine, and alcohol. Drug abuse and human immunodeficiency virus-1/acquired immune deficiency syndrome (HIV-1/AIDS) are inextricably linked. Extensive research has been done to understand the effect of prolonged drug use on neuronal signaling networks and gut microbiota. Recently, there has been rising interest in exploring the interactions between the central nervous system and the gut microbiome. This review summarizes the existing research that points toward the potential role of the gut microbiome in the pathogenesis of HIV-1-linked drug abuse and subsequent neuroinflammation and neurodegenerative disorders. Preclinical data about gut dysbiosis as a consequence of drug abuse in the context of HIV-1 has been discussed in detail, along with its implications in various neurodegenerative disorders. Understanding this interplay will help elucidate the etiology and progression of drug abuse-induced neurodegenerative disorders. This will consequently be beneficial in developing possible interventions and therapeutic options for these drug abuse-related disorders.

KEYWORDS

microbiome, drug abuse, neuroinflammation, HIV-1, gut-brain axis

Introduction

Drug abuse is a significant global problem prevalent in those infected with Human Immunodeficiency Virus-1 (HIV-1). The most commonly abused drugs in HIV-1 infected individuals are opioids, alcohol, cocaine, cannabis, methamphetamine (Meth), and nicotine. Among all the drugs used, opioid abuse is a growing problem since opioids are often the mainstay of pain management in infected individuals. While these drugs effectively control the pain associated with HIV-1, their long-term use is associated with addiction, tolerance, and neurocognitive impairment, adding to the burden of behavioral deficits in HIV-1-infected individuals. When HIV-1-affected individuals use morphine, it may cause a loss of functional connectivity between the amygdala and the frontal cortex of the brain, insula, and striatum leading to neurodegenerative effects (1). Alcohol

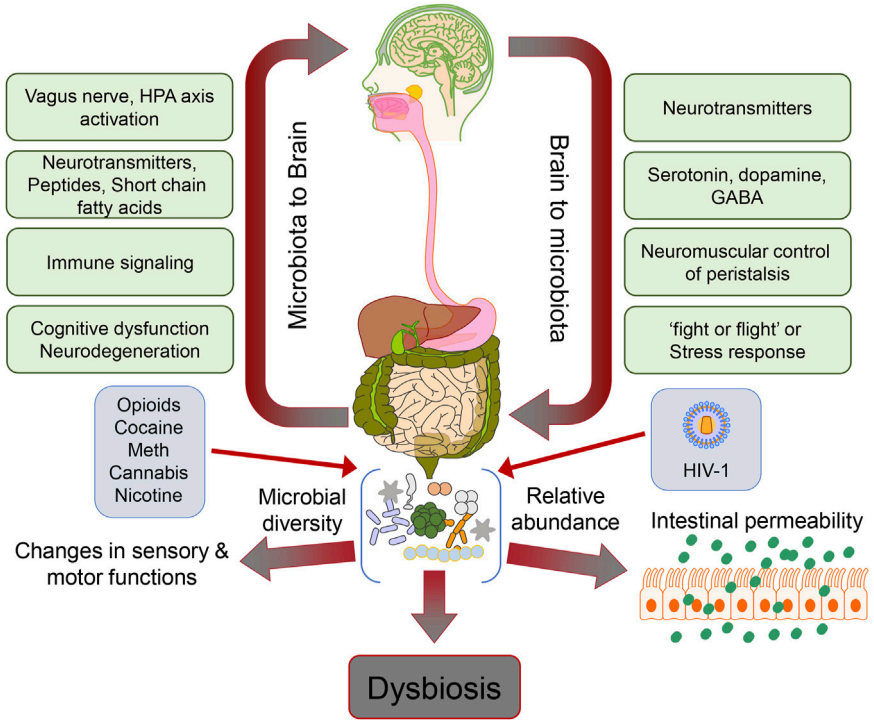

FIGURE 1
Schematic depicting the gut microbiome dysbiosis and its effects on the gut-brain axis in the context of HIV-1 and drug abuse.

consumption in the form of ethanol is both toxic and has metabolic and addictive effects on the brain, accumulating over time with age, dose, and duration of exposure. Severe debilitating diseases of the central nervous system (CNS) and the peripheral nervous system are known to manifest due to alcohol consumption. For example, it is well established that prenatal alcohol exposure paves the way for lifelong behavioral, cognitive, and psychological problems, which account for a range of cognitive dysfunctions referred to as fetal alcohol spectrum disorders (2). Prolonged heavy alcohol abuse has been shown to lead to neurodegeneration and proportionate loss of cerebral white matter. The affected regions in chronic alcohol-related metabolic injury and degeneration include the cerebellum (especially the vermis), cortical-limbic circuits, skeletal muscle, and peripheral nerves (3). Specifically, alcohol impairs neuronal and glial cell functionality (3). Also, alcohol exerts prolonged effects at the cellular and systemic levels of the neurological networks, leading to neurodegeneration. Excess alcohol exposure is associated with specific diseases such as dementias, ataxias, and Niemann-Pick disease (4). Both excess and heavy alcohol consumption contribute to the development of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). The brain is a major organ of alcohol accumulation, and this is linked to brain damage. Long-term alcohol abuse increases glutamate excitotoxicity and oxidative stress, resulting in neuronal damage (5). Besides alcohol, psychostimulants like cocaine, amphetamines, and nicotine have also been implicated in disruption of blood-brain-barrier (BBB), neural plasticity, and neuroinflammation (6, 7). There are case reports suggesting the association of cocaine overuse with accelerated neurodegeneration exhibiting symptoms similar to that found in Parkinson's disease (8). It has also been shown that iron metabolism regulation and storage lead to dopamine accumulation in cocaine-abusing individuals, resulting in neuroadaptive changes in the basal ganglia (9). Other than genetic events, epigenetic events also play a major role in neurodegeneration mediated by abuse of substances such as cocaine and Meth, as well as opioids. Epigenetic changes are established by classical pathways, including the class III histone deacetylase-sirtuin family modifications by the stimulatory effects of drugs in the form of psychostimulants (10). Drugs of abuse have also been extensively reported to cause dysbiosis of the gut microbiome and, there is significant amount of evidence that links the dysbiotic gut microbiome to mental health and neurodegeneration (11-14). In this review, we summarize existing research, including preclinical and clinical studies about correlation between HIV-1-linked drug abuse and the intestinal microbiota, and the potential role of the resultant dysbiotic gut microbiome in the pathogenesis of neurodegenerative disorders (Figure 1).

Gut microbiome and the gut-brain axis

The gut microbiome comprises a highly diverse repertoire of trillions of microbes that dwell in the gut linings and has been identified as a marker associated with various disease conditions. There is a standard composition of microbes in the gut for the metabolism and energy assimilation of the body. Several environmental, nutritional and genetic factors influence the multiplication of gut microorganisms and their compositional modifications (15). The human gut microbiome consists of various microbes that are beneficial to the body for metabolism and involved in a communication pathway to the CNS *via* the bidirectional "microbiota-gut-brain axis," which was initially termed the "gut-brain axis." In the 1960s, the brain was thought to control gut function which served as the basis for coining the term "gut-brain axis." Later, bidirectional interactions between the gut microbiota and the CNS was discovered and was defined as the "microbiota-gut-brain axis (16-19). Recent findings implicate the role of the gut-brain axis in regulating behavior and responses to drugs and, thus, underpinning its role in reward and satiety (20-22). The vagus nerve physically connects the gut and the brain through an interplay of neurotransmitters and metabolites (23-26). The existence of specific microbes in the gut is known to regulate both the immune system (27-30) and inflammation (31-34). Both preclinical and clinical studies have demonstrated a pivotal role of the gut microbiota in brain functioning (35), mood (36), and behavior (37, 38). Gut microbiota regulates the differentiation and function of immune cells of the intestine, periphery, and brain (39-41). Growing evidence also points to the critical role of the gut microbiota and the immune system in regulating the pathogenesis of neurodevelopmental and neurodegenerative diseases (12).

There is an altered influx and efflux of microbial metabolites and immune mediators between the gut and brain, leading to impaired neurotransmission and the advent of many neuropsychiatric and neurological disorders (42). Microbiome changes, termed dysbiosis, result in acute and chronic stages of several diseases, such as depression (43). Further, dysfunctional glutamate neurotransmission is involved in the D-glutamate signaling pathway observed in AD models in which the gut microbiome metabolized D-glutamate influences the glutamate N-methyl-D-aspartate receptors and cognitive function in dementia patients (44). The gut microbiota has also been linked to the development of schizophrenia (42). The "gut-brain axis" encompasses several key signaling pathways. The immune system, the vagus nerve, or microbiota-modulating neuroactive compounds may drive these pathways. Existing literature also points toward the fact that bacteria present in the gut microbiome are responsible for the production and consumption of several mammalian neurotransmitters, such as dopamine, serotonin, norepinephrine, or gamma-aminobutyric acid (GABA). Reports suggest that, on the one hand, any change in the levels of these neurotransmitters by bacteria could impact host physiology, while on the other hand, any form of microbiota-based interventions could also alter neurotransmitter levels (45). A prime example of this regulation is the impact of the gut microbiome on tryptophan metabolism and the serotonergic system (46). In such a scenario, the interaction between gut microbes with drugs of abuse is complex since gut microbes can directly impact the response of an individual to a specific drug by enzymatically modifying the structure of the drug and, in turn, affecting its availability, activity, or toxicity in the system. The drugs of abuse can also influence the microbiome composition (47-51).

Drug abuse and gut microbiome

Recent evidence implicates the gut-brain axis in the regulation of not only behavior but also a response to drugs in terms of reward and satiety. The vagus nerve connects the gut and brain, but several metabolites, hormones, and neurotransmitters regulate this connection. Such an influence of gut microbes on brain functions has been supported by studies in both preclinical and clinical models (52). During drug abuse, the gut-brain axis is disrupted, leading to modifications in the normal microbiota composition and dysregulated expression of neurotransmitters, bile acids, and metabolites, such as short-chain fatty acids (SCFA). Alterations in SCFA levels mediate tight junction dysfunction resulting in aberrant permeability of the gut epithelium, which can activate a wide range of proinflammatory signaling pathways (53). The hypothalamic-pituitary axis is linked to this inflammation in the gut, which subsequently sends feedback to the CNS, resulting in pain, stress, and anxiety (52). Herein, we discuss the role of the microbiota-gut-brain communication in the context of drug abuse in people living with HIV-1 (PLWH).

Opioids and HIV

Opioids comprise a large class of compounds with different mechanisms of action and include heroin, morphine, oxycodone, fentanyl, methadone, buprenorphine, and nalorphine, among several others (54). Opioid receptors are widely distributed in the central and peripheral nervous systems and the digestive tract (55, 56). Prescription opioid drugs are used to treat moderate to severe chronic pain. Recently, the use of various opioid drugs and their abuse, which can lead to tolerance and dependence, has become a severe public health issue (57). According to the Centers for Disease Control and Prevention, out of the 92000 people who died from a drug overdose in 2020, 75% were due to prescription or illicit opioid use (58). Most studies on the gut-brain axis and opioid abuse are based on the exogenous

opioid compound morphine. Severe constipation is a primary physiological manifestation of chronic morphine use and has been linked to disruption of the gut epithelium and microbial dysbiosis (59). Animal models commonly used to study the pathways involved in the interaction between the host-gut microbiome and opioid drugs involve rodents, particularly mice and rats, primarily for economic reasons. However, recent studies have also focused on non-human primates (NHPs) as they are both physiologically and genetically closer to humans (60). The major outcome of these studies is a gut microbial imbalance or dysbiosis due to opioid use. Preclinical animal studies show that morphine exposure increases the abundance of pathogenic bacteria (*Flavobacterium, Enterococcus, Fusobacterium, Sutterella, Clostridium, Rikenellaceae,* and *Ruminococcus*). Once tolerance is developed, it causes a significant decrease in the quantities of beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) (59, 61). It is difficult to extrapolate the data from the rodent preclinical models to the effect of morphine on human microbiota due to several factors such as genetic background, geographical setting, and lifestyle (60, 62). Human clinical studies also display variations in the presence of Bacteroidetes, Firmicutes, and Actinobacteria phylum of microbiota, consistent with rodent studies. However, there are only a limited number of studies utilizing NHPs to comment on any close association between the effect of opioids on gut microbiota in humans and that of NHPs.

It has been reported that opioid-induced gut dysbiosis, which causes structural changes in the gut epithelium, is responsible for tolerance and withdrawal behaviors. Disruption of the gut epithelium, in turn, allows bacteria and their toxic products to enter the host circulatory system, subsequently activating several inflammatory pathways and neuroinflammation. Withdrawal and tolerance linked to chronic opioid use have been related to this neuroinflammation (61, 63). The integrity of the gut epithelium depends on several factors, like the disruption of tigh-junction (TJ) organization and the restoration of the depleted epithelial layer by intestinal stem cells. The toll-like receptor (TLR) signaling is responsible for regulating intestinal TJ protein (TJP) organization. It has also been reported that morphine can disrupt the arrangement of the TJPs *via* modulation of myosin light chain kinase signaling (MLCK) in a TLR-dependent manner (64).

Opioid-induced microbial dysbiosis is responsible for continuous immune activation leading to HIV-1 disease progression. Several studies report that opioid addicts are at a greater risk of HIV-1 infection (65). Several factors, including the usage of contaminated needles and the nutritional status of the infected individual, could likely play a role in the heightened susceptibility of opioid abusers to HIV-1 infection. However, reports indicate that opioid use alone can also increase the risk of HIV-1 infection (66). There is ample evidence suggesting that HIV-1 infection disrupts the structure and function of the gut epithelium, leading to AIDS progression. Reports suggest that HIV-1 modulates tight junctions by disrupting $CD4^+$ T cells, which are responsible for maintaining tight junctions (67). HIV-1 proteins such as Tat (transactivator of transcription) and gp120 have also been reported to disrupt tight junctions on epithelial cells in culture (68). Studies also report that simian immunodeficiency virus (SIV) infection results in early upregulation of proinflammatory cytokine IL-1β in the colon of the rhesus macaques (69) as well as in the intestine of HIV-1-infected patients (70), which, in turn, could activate the MLCK, resulting in mucosal damage. SIV-infected African green monkeys exhibit an accelerated depletion of $CD4^+$ T cells in the intestine (71). An identical phenomenon is found in HIV-1-infected humans and SIV-infected rhesus macaques, suggesting that microbial translocation through the disrupted gut epithelium affects SIV disease progression.

Opioid users have been reported to display rapid HIV-1 disease progression while demonstrating severe long-term effects such as neurocognitive disorders (72). Certain opioid abusers infected with HIV-1 show elevated levels of lipopolysaccharide (LPS) in their serum compared to non-users, thus underscoring that disruption of the gut epithelium is more acute in HIV-1 patients who use opioid drugs (73). Preclinical and clinical studies done in HIV-1-infected patients indicate that morphine-mediated disruption of intestinal tight junctions involves activation of MLCK. This has also been validated in rodent models where combination of opioids and HIV-1 infection either synergistically and/or additively activate MLCK, leading to increased gut epithelium permeability, which is observed in HIV-1-infected patients misusing opioids (74). Opioids have also been reported to promote HIV-1 disease progression by disrupting the intestinal epithelial self-repair mechanism and reducing epithelial proliferation in bone marrow-liver-thymus humanized mice and in opioid-using HIV-1+ patients (75). Cumulatively these studies underscore the pivotal role of gut microbiota in the disease progression of HIV-1 infection while also demonstrating that opioid abuse by HIV-1 patients can lead to severe disruption of gut homeostasis, resulting in an accelerated progression of the disease in comparison to drug naïve, infected individuals.

Cannabis and HIV

Despite controlling the HIV-1 viral load with combined antiretroviral therapy (cART), gut epithelium defects and intestinal $CD4^+$ cell depletion continue to persist. In HIV-1 infected patients compromised gut barrier function is aided by the increase in apoptosis, and chronic inflammatory signals on the one hand and the decrease in proliferation and repair of epithelial cells, on the other hand. Alterations in tryptophan metabolism leading to defects in microbes that produce butyrate in PLWH and likely contribute to increased gut permeability have been reported (76-78). A dysfunctional gut epithelium

allows inflammatory microbial products such as LPS in the periphery to be translocated (79-82). In particular, defects in the gut epithelium make HIV-1+ individuals vulnerable to increased exposure to proinflammatory ligands produced by gut microbiota (78, 83, 84). These alterations lead to poor HIV-1 disease outcomes, including associated neurocognitive disorders (77).

Cannabis effectively alleviates symptoms associated with HIV-1 disease and other conditions such as cancer and neuropathic pain (85). Cannabinoids act on inflammatory pathways through mechanisms distinct from agents such as non-steroidal anti-inflammatory drugs (NSAIDs) (86). Naturally occurring endocannabinoids, including cannabis, have antioxidative and anti-inflammatory characteristics that help in healing and restoration and thus can be used as adjunctive therapy. As a class, cannabinoids are generally free from the adverse effects of NSAIDs. A concise survey of the anti-inflammatory actions of the phytocannabinoids Δ^9-tetrahydrocannabinol (THC), cannabidiol, cannabichromene, and cannabinol has been reported (85-90). Meta-analyses of several clinical trials have established the efficacy of cannabis in HIV-1-related neuropathic pain and nausea (85-92), although dosing and administration routes varied widely. Some studies suggest that titrating dosing to effectiveness and side effects is a valuable strategy for dose selection. While acute cannabis exposure disturbs cognition, how its long-term use affects brain function in the context of HIV-1 is yet to be elucidated clearly (93, 94). Medicinal use of cannabis is becoming rapidly accepted, and a state-level authorized disease management strategy (95, 96). Healthcare providers identify the potential benefits of cannabis by understanding the potential benefits of symptom management. However, a few clinical studies on patients using cannabis as therapy showed potential dependence or possible adverse effects (93, 97). A better understanding of the strategic use of cannabis could aid clinicians in better treatment and therapeutic options with their patients. Since not much research has been done to assess the effects of cannabis in PLWH, there is a dearth of reliable data for cannabis use recommendations in the clinical field.

The endocannabinoid system is a complex network of receptors and enzymes involved in synthesizing and detecting endogenous lipid ligands (98-100). Most human tissues express cannabinoid (type-1 and -2) receptors (98, 99). Cannabinoid receptors type-2 are densely expressed in diverse immune cell types, including macrophages, microglia, splenocytes, monocytes, and T-cells resident in the thymus, spleen, and bone marrow tonsils (98-100). Endocannabinoid system signaling pathways are essential in HIV-1 infection for several reasons and has been pursued as a target for future pharmacotherapy to reduce inflammation (98-100). In HIV-1 infection, cannabis use has been shown to reduce systemic inflammation and activate the immune system (101). Furthermore, HIV-1 DNA is reported to decline more rapidly in individuals taking antiretroviral therapy and using cannabis than those not using cannabis (102). Cannabis use in PLWH leads to aggravated dysbiosis and epithelial barrier dysfunction of the gut, along with chronic inflammation and consequential ill effect on overall health (79, 81, 82, 103). Chronic cannabis use is reported to lower the abundance of *Prevotella* and increase the abundance of *Bacteriodes* bacteria in the gut microbiome. Lower abundance of *Prevotella* leads to systemic mitochondrial dysfunction and reduction of gut SCFA production in cannabis users which is linked to impairment in cognitive function (104). It is also reported that administration of cannabidiol-rich cannabis extract resulted in increased abundance of A. *muciniphila* and significant decrease in *Alistipes finegoldii*, *Lachnoclostridium sp. YL32*, and *Ruminiclostridium sp. KB18* alongwith remarkable downregulation of mucin-2 which is associated with maintenance of gut integrity. The study also found upregulation of inflammatory markers IL-1β, CXCL1, and CXCL2 which points towards the disruptive effect of long-term cannabis use (105).

Cocaine and HIV

Cocaine is one of the most commonly abused drugs among PLWH, and it has been suggested that it accelerates AIDS progression. Based on the evidence that the limbic system of the brain, comprising a set of interconnected regions regulating pleasure and motivation, is the primary site of action for cocaine helps explain its high potential for addiction and relapse. Cocaine, a commonly used psychostimulant among PLWH, is a cofactor for HIV-1 infection and progression to AIDS. Globally almost 22.5 million people worldwide are affected by cocaine use disorder, thus making it a significant public health crisis with a high socioeconomic burden (106). Although cocaine is known to have immunomodulatory functions (107-109), the underlying mechanism(s) by which cocaine accentuates HIV-1 replication remains unclear. There are reports that cocaine increases HIV-1 infection/replication by inhibiting HIV-1 protective chemokines and/or upregulating the HIV-1 entry co-receptor (110, 111). Cocaine is a potent vasoconstrictor and brain stimulant. Its abuse leads to severe neurological (fainting attacks, hemorrhagic brain strokes, CNS vasculitis, and encephalopathies), cardiovascular (cardiac arrhythmia and heart attacks), and gastrointestinal complications (112-117).

Cocaine abuse has been reported to alter the gut microbiota composition which in turn affects the uptake and clearence of neurotransmitters. One particular study reports higher accumulation of norepinephrine in intestines of cocaine-administred mice helped the resident *Citrobacter rodentium* to flourish which resulted in depletion of the intestinal neurotransmitter glycine. This also resulted in glycine

depletion in circulation and cerebrospinal fluid of cocaine-administered mice, which was in correlation with increased hyperlocomotion and escalation of drug-seeking behavior (118). The authors also reported alteration of synaptic plasticity pathways at the transcriptome-level in the nucleus accumbens of the cocaine-administered mice, and also that the behavioral changes were reversed with dietary supplementation of glycine or sarcosine (118). Another study reports that cocaine administration in mice reduces the abundance of *Mucispirillum, Butrycicoccus, Ruminococcaceae, Pseudoflavonifractor, and Lachnospiracea* species of bacteria in the gut microbiota which are the involved in the synthesis of SCFAs involved in maintaining mucosal epithelium integrity. Cocaine administration resulted in alteration of TJPs of the gut membrane, upregulated expression of proinflammatory markers NF-κB and IL-1β, and also disruption of the mucosal permeability *via* MAPK/ERK1/2 signaling pathway (106). Also, in case of mice with reduced gut-bacteria, cocaine admistration resulted in increased sensitivity towards drug reward as well as increased locomotor-sensitivity (119). These studies reveal the critical role of gut microbiome in the behavioral effects of cocaine addiction. The research on the gut microbiome and its relationship with drug abuse is currently in its infancy with a bright future, and still a long way to go.

Methamphetamine and HIV

Similar to other drugs of abuse, several preclinical and clinical studies have demonstrated that Meth induces alterations in the gut microbiome (49,120-123). However, there is a lack of evidence directly linking the gut microbiota with Meth-induced brain dysfunction (124). Meth has been reported to promote the release of norepinephrine and dopamine, leading to a markedly decreased intestinal contractility and motor capacity (125). This decrease in intestinal muscle tone is associated with oxidative and nitrosative stress, which, in turn, can cause neuronal injury and death in the intestine and disrupt intestinal barrier functioning (126). Disruption of the intestinal mucosal barrier increases the permeability of the gut epithelium and plays an essential role in contributing to anxiogenic behavior (127), stress (128), depression (129), cognitive decline (130), and eating and sleep disorders (131). Disruption of the intestinal barrier also leads to the leakage of several inflammatory factors (like TNF-α, interferon-γ, IL-6), microbes, and metabolites from the gut epithelium to the circulatory and lymphatic systems (132). It has been reported that Meth use can increase the permeability of the blood-brain barrier (133), thereby facilitating the entry of microbial communities and metabolites to enter the brain (134). In mouse models, Meth-exposure has been reported to increase the abundance of pathogenic bacteria in the fecal microbiota (120), with increased inflammation, reduced TJP expression in the intestine, and decreased relative quantity of probiotics and fecal metabolites. Further, Meth exposure was also shown to enhance the intestinal autophagy-associated flora, concomitantly leading to the induction of autophagy in the CNS (123). Intestinal inflammatory biomarkers, including the proinflammatory cytokines, are upregulated in Meth abusers and have been reported to infiltrate the brain regions related to depression (135), causing alterations in neurotransmitter metabolism, neuroendocrine function, and neuroplasticity. A recent study has also shown that gene sequencing of the 16S rRNA of the rectal swab samples collected from individuals using Meth, showed increased presence of bacterial species such as *Finegoldia, Peptoniphilus*, *Parvimonas,* and *Porphyromonas* and depletion of species like *Faecalibacterium* and *Butyricicoccus* (122). In line with this study, other studies have also shown that there were alterations in the composition of microbes present in the gut of Meth users with decrease in quantity of *Bacteroidaceae* and *Deltaproteobacteria,* and increased abundance of *Sphingomonadales*, *Xanthomonadales, Romboutsia* and *Lachnospiraceae* (49). Interestingly, these alterations have been reported in those bacterial species which had previously been demonstrated to be altered in individuals with psychotic syndromes, thus pointing towards a potential link between Meth abuse and psychotic disorders (49). Forouzan et al. showed that Meth exposure and withdrawal in rats resulted in gut dysbiosis, which was linked to depression-like behavior as evidenced by the forced swim test. However, the authors reported no alterations in anxiety-like behaviors which was assessed by either the elevated plus maze or the open field test (136).

HIV-1 has been reported to alter the human intestinal microbiome. An exciting study showed significant changes in the microbiome in the context of drug abuse and sexual behavior during HIV-1 infection. Rectal swab samples, urine drug test results, along with responses to substance use and sex behavior questionnaires were collected from 37 HIV-1-positive individuals at two-time points, in a 6-month gap period, in a group that was being evaluated for the effects of drug abuse in men who have sex with men. The samples were subjected to 16S ribosomal RNA gene sequencing, and the association of the data with behavioral factors was examined using 0-inflated negative binomial regression. Further analyses demonstrated that abuse of Meth and marijuana exhibit unique associations. Meth use was linked with increased *Granulicatella* and *Porphyromonas* organisms in HIV-1 patients and a decrease in abundance of *Collinsella*, *Ruminococcus*, and Parabacteroides organisms. In contrast, marijuana use was associated with an increased abundance of *Clostridium* cluster IV, Ruminococcus, *Fusobacterium*, and Solobacterium organisms and decreased *Acidaminococcus*, *Dialister*, *Prevotella*, *Anaerostipes*, and *Dorea* organisms. From this study, it can be concluded that drug use and sexual behavior are important factors associated with intestinal dysbiosis during chronic HIV-1 infection among young men

who have sex with men (137). Further, studies are warranted in the field, specifically in association with HIV-1 infection and drug abuse-related disorders.

Nicotine and HIV

Several reports have, on the other hand, demonstrated an association between nicotine and microbiome dysregulation (138-142). In one study aimed at assessing the link between the smoking status of an individual and their intensity of smoking with the relative abundance of gut microbial species in 249 Bangladesh participants, it was reported that there was an increase in the relative abundance of *Erysipelotrichi* and *Catenibacterium* in current smokers in comparison to those who had never smoked (139). Another interesting study showed that long-term nicotine administration in rats resulted in alterations of gut microbiota, which was more prominent in rodents fed a high-fat diet than a regular chow diet, thus indicating diet-dependent changes (142). In line with this study, another study showed that cigarette smoke altered gut microbiota composition, which was linked to modifications in the distribution of primary bile acids and cholesterol homeostasis (138). Another study also showed that oral administration of nicotine in mice differentially reorganized the gut microbiome in a gender-specific manner and, furthermore, modified the levels of metabolites such as GABA and glutamate, which are involved in gut-brain communication (142). A recent study has also demonstrated that nicotine altered the gut microbiome and metabolites involved in the gut-brain axis in a sex-specific manner. This study employed high-throughput sequencing and gas chromatography-mass spectrometry to evaluate the effect of nicotine exposure on the gut microbiome and its metabolism in C57BL/6J mice in a sex-dependent manner, with special emphasis on the signaling pathways involved in the gut-brain axis. The 16S sequencing results from this study indicated that the composition of the gut microbiome was differentially altered by nicotine in both females and males. Also, the differential changes in the bacterial carbohydrate metabolic pathways were consistent with lower body weight gain in nicotine-administered males. Genes related to oxidative stress response and DNA repair were also explicitly upregulated in the gut microbiome of the nicotine-treated male mice. Analysis of the fecal metabolome demonstrated that several neurotransmitters, such as glutamate, GABA, and glycine, and neuroactive metabolites-leucine and uric acid, were also differentially altered in female *versus* male mice. This study showed a sex-dependent effect of nicotine on gut microbiome composition, functional bacterial genes, and the fecal metabolome (141). However, studies are lacking on gut-brain axis in the context of nicotine and HIV.

Conclusion and future perspectives

Understanding the impact of the gut microbiome on gut-brain axis communication has been the topic of momentous research over the past few years. There is a mounting effort to delineate the mechanism(s) of this communication at all axis nodes. It has been now well-established that gut microbiota is crucial for the proper development and maintenance of brain functions. Additionally, as discussed above, there is accumulating evidence from preclinical and clinical studies that implicate the role of microbial dysbiosis in various psychiatric, neurological, and neurodegenerative diseases in the context of HIV-1 and drug abuse. However, it is still a very nascent field of research, and caution must be exerted in over-interpreting these studies. Many unanswered questions remain regarding the beneficial effects of probiotics, with extensive work required to test optimal dosing, strain, and timing in therapeutic applications. The emphasis in the field must shift from correlative analyses to prospective longitudinal study design, causative and mechanistic investigations, and larger-scale trials of potential therapeutic approaches, especially in the case of HIV-1 and drug abuse comorbidity. One big conundrum in microbiota-based research is the ideal definition of healthy microbiota. Inter-individual differences in the gut microbiota composition can be very critical, making it challenging to apply a "one size fits all" approach to target the microbiota. However, this also provides future opportunities for practical personalized medicine approaches. We have also moved from focusing on single bacterial strains as pathogens to an emphasis on nurturing an entire community of microbes, lest they become pathological entities. There are many challenges to conventional wisdom at play, with the possibility that the alterations in the gut microbiota noted in many CNS disorders may have a causal role in symptomatology and that many of the drugs used to treat those disorders could be toxic to or support the diversity of our gut microbes.

Author contributions

Study conception and design: SR, PP, and SB; Draft manuscript preparation: SR, SS, MK, and PP. All authors reviewed the contents and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Nass SR, Ohene-Nyako M, Hahn YK, Knapp PE, Hauser KF. Neurodegeneration within the amygdala is differentially induced by opioid and HIV-1 Tat exposure. *Front Neurosci* (2022) 16:804774. doi:10.3389/fnins.2022.804774

2. Arzua T, Yan Y, Jiang C, Logan S, Allison RL, Wells C, et al. Modeling alcohol-induced neurotoxicity using human induced pluripotent stem cell-derived three-dimensional cerebral organoids. *Transl Psychiatry* (2020) 10(1):347. doi:10.1038/s41398-020-01029-4

3. de la Monte SM, Kril JJ. Human alcohol-related neuropathology. *Acta Neuropathol* (2014) 127(1):71–90. doi:10.1007/s00401-013-1233-3

4. Araujo I, Henriksen A, Gamsby J, Gulick D. Impact of alcohol abuse on susceptibility to rare neurodegenerative diseases. *Front Mol Biosci* (2021) 8:643273. doi:10.3389/fmolb.2021.643273

5. Peng B, Yang Q, B Joshi R, Liu Y, Akbar M, Song BJ, et al. Role of alcohol drinking in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Int J Mol Sci*, 2020. 21, 2316. doi:10.3390/ijms21072316

6. Clark KH, Wiley CA, Bradberry CW. Psychostimulant abuse and neuroinflammation: Emerging evidence of their interconnection. *Neurotox Res* (2013) 23(2):174–88. doi:10.1007/s12640-012-9334-7

7. Sajja RK, Rahman S, Cucullo L. Drugs of abuse and blood-brain barrier endothelial dysfunction: A focus on the role of oxidative stress. *J Cereb Blood Flow Metab* (2016) 36(3):539–54. doi:10.1177/0271678X15616978

8. Illes A, Balicza P, Molnar V, Bencsik R, Szilvasi I, Molnar MJ. Dynamic interaction of genetic risk factors and cocaine abuse in the background of Parkinsonism - a case report. *BMC Neurol* (2019) 19(1):260. doi:10.1186/s12883-019-1496-y

9. Ersche KD, Acosta-Cabronero J, Jones PS, Ziauddeen H, van Swelm RPL, Laarakkers CMM, et al. Disrupted iron regulation in the brain and periphery in cocaine addiction. *Transl Psychiatry* (2017) 7(2):e1040. doi:10.1038/tp.2016.271

10. Sivalingam K, Doke M, Khan MA, Samikkannu T. Influence of psychostimulants and opioids on epigenetic modification of class III histone deacetylase (HDAC)-sirtuins in glial cells. *Sci Rep* (2021) 11(1):21335. doi:10.1038/s41598-021-00836-z

11. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin Gastroenterol* (2015) 31(1):69–75. doi:10.1097/MOG.0000000000000139

12. Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous systems in health and disease. *Nat Neurosci* (2017) 20(2):145–55. doi:10.1038/nn.4476

13. Capuco A, Urits I, Hasoon J, Chun R, Gerald B, Wang JK, et al. Gut microbiome dysbiosis and depression: A comprehensive review. *Curr Pain Headache Rep* (2020) 24(7):36. doi:10.1007/s11916-020-00871-x

14. Mossad O, Erny D. The microbiota-microglia axis in central nervous system disorders. *Brain Pathol* (2020) 30(6):1159–77. doi:10.1111/bpa.12908

15. Gomaa EZ. Human gut microbiota/microbiome in health and diseases: A review. *Antonie Van Leeuwenhoek* (2020) 113(12):2019–40. doi:10.1007/s10482-020-01474-7

16. Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat Rev Gastroenterol Hepatol* (2009) 6(5):306–14. doi:10.1038/nrgastro.2009.35

17. Holzer P, Farzi A. Neuropeptides and the microbiota-gut-brain axis. *Adv Exp Med Biol* (2014) 817:195–219. doi:10.1007/978-1-4939-0897-4_9

18. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* (2015) 28(2):203–9.

19. Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic signaling along the microbiota-gut-brain Axis. *Int J Mol Sci* (2019) 20(6):1482. doi:10.3390/ijms20061482

20. Bliss ES, Whiteside E. The gut-brain Axis, the human gut microbiota and their integration in the development of obesity. *Front Physiol* (2018) 9:900. doi:10.3389/fphys.2018.00900

21. Han W, Tellez LA, Perkins MH, Perez IO, Qu T, Ferreira J, et al. A neural circuit for gut-induced reward. *Cell* (2018) 175(3):887–8. doi:10.1016/j.cell.2018.10.018

22. Van Oudenhove L. Does the gut-brain axis control anticipatory food reward? Novel insights from bariatric surgery. *Gut* (2014) 63(6):868–9. doi:10.1136/gutjnl-2013-305488

23. Brookes SJ, Spencer NJ, Costa M, Zagorodnyuk VP. Extrinsic primary afferent signalling in the gut. *Nat Rev Gastroenterol Hepatol* (2013) 10(5):286–96. doi:10.1038/nrgastro.2013.29

24. Cryan JF, Dinan TG. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* (2012) 13(10):701–12. doi:10.1038/nrn3346

25. Forsythe P, Kunze W, Bienenstock J. Moody microbes or fecal phrenology: What do we know about the microbiota-gut-brain axis? *BMC Med* (2016) 14:58. doi:10.1186/s12916-016-0604-8

26. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the manipulation of bacteria-gut-brain signals. *Trends Neurosci* (2016) 39(11):763–81. doi:10.1016/j.tins.2016.09.002

27. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* (2014) 157(1):121–41. doi:10.1016/j.cell.2014.03.011

28. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science* (2016) 352(6285):539–44. doi:10.1126/science.aad9378

29. Lazar V, Ditu LM, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, et al. Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. *Front Immunol* (2018) 9:1830. doi:10.3389/fimmu.2018.01830

30. Zhao Q, Elson CO. Adaptive immune education by gut microbiota antigens. *Immunology* (2018) 154(1):28–37. doi:10.1111/imm.12896

31. Blander JM, Longman RS, Iliev ID, Sonnenberg GF, Artis D. Regulation of inflammation by microbiota interactions with the host. *Nat Immunol* (2017) 18(8):851–60. doi:10.1038/ni.3780

32. Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. *BMJ* (2018) 360:j5145. doi:10.1136/bmj.j5145

33. Lobionda S, Sittipo P, Kwon HY, Lee YK. The role of gut microbiota in intestinal inflammation with respect to diet and extrinsic stressors. *Microorganisms* (2019) 7(8):271. doi:10.3390/microorganisms7080271

34. Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* (2020) 20(1):40–54. doi:10.1038/s41577-019-0198-4

35. Mohajeri MH, La Fata G, Steinert RE, Weber P. Relationship between the gut microbiome and brain function. *Nutr Rev* (2018) 76(7):481–96. doi:10.1093/nutrit/nuy009

36. Huang TT, Lai JB, Du YL, Xu Y, Ruan LM, Hu SH. Current understanding of gut microbiota in mood disorders: An update of human studies. *Front Genet* (2019) 10:98. doi:10.3389/fgene.2019.00098

37. Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, et al. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci U S A* (2008) 105(6):2117–22. doi:10.1073/pnas.0712038105

38. Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold G, et al. The gut microbiota and host health: A new clinical frontier. *Gut* (2016) 65(2):330–9. doi:10.1136/gutjnl-2015-309990

39. Microbiology: Gut bacteria linked to Parkinson's. *Nature* (2016) 540(7632):172–3. doi:10.1038/540172d

40. Erny D, Prinz M. Microbiology: Gut microbes augment neurodegeneration. *Nature* (2017) 544(7650):304–5. doi:10.1038/nature21910

41. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* (2016) 167(6):1469–80. doi:10.1016/j.cell.2016.11.018

42. From the American Association of Neurological Surgeons (AANS); American Society of Neuroradiology (ASNR); Cardiovascular and Interventional Radiology Society of Europe (CIRSE); Canadian Interventional Radiology Association (CIRA); Congress of Neurological Surgeons (CNS); European Society of Minimally Invasive Neurological Therapy (ESMINT) et al. Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. *Int J Stroke* (2018) 13(6):612–32. doi:10.1177/1747493018778713

43. Capuco A, Urits I, Hasoon J, Chun R, Gerald B, Wang JK, et al. Current perspectives on gut microbiome dysbiosis and depression. *Adv Ther* (2020) 37(4):1328–46. doi:10.1007/s12325-020-01272-7

44. Chang CH, Lin CH, Lane HY. D-Glutamate and gut microbiota in Alzheimer's disease. *Int J Mol Sci* (2020) 21(8):2676. doi:10.3390/ijms21082676

45. Strandwitz P. Neurotransmitter modulation by the gut microbiota. *Brain Res* (2018) 1693:128–33. doi:10.1016/j.brainres.2018.03.015

46. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res* (2015) 277:32–48. doi:10.1016/j.bbr.2014.07.027

47. Wang F, Roy S. Gut homeostasis, microbial dysbiosis, and opioids. *Toxicol Pathol* (2017) 45(1):150–6. doi:10.1177/0192623316679898

48. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* (2012) 302(9):G966–78. doi:10.1152/ajpgi.00380.2011

49. Yang Y, Yu X, Liu X, Liu G, Zeng K, Wang G. Altered fecal microbiota composition in individuals who abuse methamphetamine. *Sci Rep* (2021) 11(1): 18178. doi:10.1038/s41598-021-97548-1

50. Salavrakos M, Leclercq S, De Timary P, Dom G. Microbiome and substances of abuse. *Prog Neuropsychopharmacol Biol Psychiatry* (2021) 105:110113. doi:10.1016/j.pnpbp.2020.110113

51. Weersma RK, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. *Gut* (2020) 69(8):1510–9. doi:10.1136/gutjnl-2019-320204

52. Simpson S, Mclellan R, Wellmeyer E, Matalon F, George O. Drugs and bugs: The gut-brain Axis and substance use disorders. *J Neuroimmune Pharmacol* (2021) 17:33–61. doi:10.1007/s11481-021-10022-7

53. Meng J, Sindberg GM, Roy S. Disruption of gut homeostasis by opioids accelerates HIV disease progression. *Front Microbiol* (2015) 6:643. doi:10.3389/fmicb.2015.00643

54. Ren M, Lotfipour S. The role of the gut microbiome in opioid use. *Behav Pharmacol* (2020) 31(2&3):113–21. doi:10.1097/FBP.0000000000000538

55. Cussotto S, Clarke G, Dinan TG, Cryan JF. Psychotropics and the microbiome: A chamber of secrets. *Psychopharmacology (Berl)* (2019) 236(5): 1411–32. doi:10.1007/s00213-019-5185-8

56. Trang T, Al-Hasani R, Salvemini D, Salter MW, Gutstein H, Cahill CM. Pain and poppies: The good, the bad, and the ugly of opioid analgesics. *J Neurosci* (2015) 35(41):13879–88. doi:10.1523/JNEUROSCI.2711-15.2015

57. Kalkman GA, Kramers C, van Dongen RT, van den Brink W, Schellekens A. Trends in use and misuse of opioids in The Netherlands: A retrospective, multi-source database study. *Lancet Public Health* (2019) 4(10):e498–e505. doi:10.1016/S2468-2667(19)30128-8

58. Hedegaard H, Minino AM, Spencer MR, Warner M. Drug overdose deaths in the United States, 1999-2020. *NCHS Data Brief* (2021) 2021(426):1–8.

59. Banerjee S, SindberG G, Wang F, Meng J, Sharma U, Zhang L, et al. Opioid-induced gut microbial disruption and bile dysregulation leads to gut barrier compromise and sustained systemic inflammation. *Mucosal Immunol* (2016) 9(6):1418–28. doi:10.1038/mi.2016.9

60. Nagpal R, Wang S, Solberg Woods LC, Seshie O, Chung ST, Shively CA, et al. Comparative microbiome signatures and short-chain fatty acids in mouse, rat, non-human primate, and human feces. *Front Microbiol* (2018) 9:2897. doi:10.3389/fmicb.2018.02897

61. Zhang L, Meng J, Ban Y, Jalodia R, Chupikova I, Fernandez I, et al. Morphine tolerance is attenuated in germfree mice and reversed by probiotics, implicating the role of gut microbiome. *Proc Natl Acad Sci U S A* (2019) 116(27):13523–32. doi:10.1073/pnas.1901182116

62. Park JC, Im SH. Of men in mice: The development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. *Exp Mol Med* (2020) 52(9):1383–96. doi:10.1038/s12276-020-0473-2

63. Lee K, Vuong HE, Nusbaum DJ, Hsiao EY, Evans CJ, Taylor AMW. The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence. *Neuropsychopharmacology* (2018) 43(13): 2606–14. doi:10.1038/s41386-018-0211-9

64. Meng J, Yu H, Ma J, Wang J, Banerjee S, Charboneau R, et al. Morphine induces bacterial translocation in mice by compromising intestinal barrier function in a TLR-dependent manner. *PLoS One* (2013) 8(1):e54040. doi:10.1371/journal.pone.0054040

65. Nath A, Hauser KF, Wojna V, Booze RM, Maragos W, Prendergast M, et al. Molecular basis for interactions of HIV and drugs of abuse. *J Acquir Immune Defic Syndr* (2002) 31(2):S62–9. doi:10.1097/00126334-200210012-00006

66. Roy S, Ninkovic J, Banerjee S, Charboneau RG, Das S, Dutta R, et al. Opioid drug abuse and modulation of immune function: Consequences in the susceptibility to opportunistic infections. *J Neuroimmune Pharmacol* (2011) 6(4):442–65. doi:10.1007/s11481-011-9292-5

67. Shanahan F. Intestinal lymphoepithelial communication. *Adv Exp Med Biol* (1999) 473:1–9. doi:10.1007/978-1-4615-4143-1_1

68. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, Gray-Owen SD, et al. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. *Plos Pathog* (2010) 6(4):e1000852. doi:10.1371/journal.ppat.1000852

69. Hirao LA, Grishina I, Bourry O, Hu WK, Somrit M, Sankaran-Walters S, et al. Early mucosal sensing of SIV infection by paneth cells induces IL-1β production and initiates gut epithelial disruption. *Plos Pathog* (2014) 10(8):e1004311. doi:10.1371/journal.ppat.1004311

70. McGowan I, Elliott J, Fuerst M, Taing P, Boscardin J, Poles M, et al. Increased HIV-1 mucosal replication is associated with generalized mucosal cytokine activation. *J Acquir Immune Defic Syndr* (2004) 37(2):1228–36. doi:10.1097/01.qai.0000131846.12453.29

71. Pandrea IV, Gautam R, Ribeiro RM, Brenchley JM, Butler IF, Pattison M, et al. Acute loss of intestinal CD4+ T cells is not predictive of simian immunodeficiency virus virulence. *J Immunol* (2007) 179(5):3035–46. doi:10.4049/jimmunol.179.5.3035

72. Byrd DA, Fellows RP, Morgello S, Franklin D, Heaton RK, Deutsch R, et al. Neurocognitive impact of substance use in HIV infection. *J Acquir Immune Defic Syndr* (2011) 58(2):154–62. doi:10.1097/QAI.0b013e318229ba41

73. Ancuta P, Kamat A, Kunstman KJ, Kim EY, Autissier P, Wurcel A, et al. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One* (2008) 3(6):e2516. doi:10.1371/journal.pone.0002516

74. Sindberg GM, Sharma U, Banerjee S, Anand V, Dutta R, Gu CJ, et al. An infectious murine model for studying the systemic effects of opioids on early HIV pathogenesis in the gut. *J Neuroimmune Pharmacol* (2015) 10(1):74–87. doi:10.1007/s11481-014-9574-9

75. Meng J, Banerjee S, Zhang L, Sindberg G, Moidunny S, Li B, et al. Opioids impair intestinal epithelial repair in HIV-infected humanized mice. *Front Immunol* (2019) 10:2999. doi:10.3389/fimmu.2019.02999

76. Dillon SM, Kibbie J, Lee EJ, Guo K, Santiago ML, Austin GL, et al. Low abundance of colonic butyrate-producing bacteria in HIV infection is associated with microbial translocation and immune activation. *AIDS* (2017) 31(4):511–21. doi:10.1097/QAD.0000000000001366

77. Dillon SM, Lee EJ, Kotter CV, Austin GL, Dong Z, Hecht DK, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunol* (2014) 7(4): 983–94. doi:10.1038/mi.2013.116

78. Jenabian MA, El-Far M, Vyboh K, Kema I, Costiniuk CT, Thomas R, et al. Immunosuppressive tryptophan catabolism and gut mucosal dysfunction following early HIV infection. *J Infect Dis* (2015) 212(3):355–66. doi:10.1093/infdis/jiv037

79. Brenchley JM, Douek DC. The mucosal barrier and immune activation in HIV pathogenesis. *Curr Opin HIV AIDS* (2008) 3(3):356–61. doi:10.1097/COH.0b013e3282f9ae9c

80. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* (2006) 12(12):1365–71. doi:10.1038/nm1511

81. Somsouk M, Estes JD, Deleage C, Dunham RM, Albright R, Inadomi JM, et al. Gut epithelial barrier and systemic inflammation during chronic HIV infection. *AIDS* (2015) 29(1):43–51. doi:10.1097/QAD.0000000000000511

82. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med* (2013) 5(193):193ra91. doi:10.1126/scitranslmed.3006438

83. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, et al. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. *Microbiome* (2013) 1(1):26. doi:10.1186/2049-2618-1-26

84. Sandler NG, Douek DC. Microbial translocation in HIV infection: Causes, consequences and treatment opportunities. *Nat Rev Microbiol* (2012) 10(9):655–66. doi:10.1038/nrmicro2848

85. Abrams DI, Jay CA, Shade SB, Vizoso H, Reda H, PreSS S, et al. Cannabis in painful HIV-associated sensory neuropathy: A randomized placebo-controlled trial. *Neurology* (2007) 68(7):515–21. doi:10.1212/01.wnl.0000253187.66183.9c

86. Zurier RB, Burstein SH. Cannabinoids, inflammation, and fibrosis. *FASEB J* (2016) 30(11):3682–9. doi:10.1096/fj.201600646R

87. Whiting PF, Wolff RF, Deshpande S, Di Nisio M, Duffy S, Hernandez AV, et al. Cannabinoids for medical use: A systematic review and meta-analysis. *JAMA* (2015) 313(24):2456–73. doi:10.1001/jama.2015.6358

88. Abrams DI, Hilton JF, Leiser RJ, Shade SB, Elbeik TA, Aweeka FT, et al. Short-term effects of cannabinoids in patients with HIV-1 infection: A randomized, placebo-controlled clinical trial. *Ann Intern Med* (2003) 139(4):258–66. doi:10.7326/0003-4819-139-4-200308190-00008

89. Ellis RJ, Toperoff W, Vaida F, van den Brande G, Gonzales J, Gouaux B, et al. Smoked medicinal cannabis for neuropathic pain in HIV: A randomized, crossover clinical trial. *Neuropsychopharmacology* (2009) 34(3):672–80. doi:10.1038/npp.2008.120

90. Lutge EE, Gray A, Siegfried N. The medical use of cannabis for reducing morbidity and mortality in patients with HIV/AIDS. *Cochrane Database Syst Rev* (2013)(4) CD005175. doi:10.1002/14651858.CD005175.pub3

91. Andreae MH, Carter GM, Shaparin N, Suslov K, Ellis RJ, Ware MA, et al. Inhaled cannabis for chronic neuropathic pain: A meta-analysis of individual patient data. *J Pain* (2015) 16(12):1221–32. doi:10.1016/j.jpain.2015.07.009

92. Prospero-Garcia O, Amancio-Belmont O, Becerril Melendez AL, Ruiz-Contreras AE, Mendez-Diaz M. Endocannabinoids and sleep. *Neurosci Biobehav Rev* (2016) 71:671–9. doi:10.1016/j.neubiorev.2016.10.005

93. Brooks E, Gundersen DC, Flynn E, Brooks-Russell A, Bull S. The clinical implications of legalizing marijuana: Are physician and non-physician providers prepared? *Addict Behav* (2017) 72:1–7. doi:10.1016/j.addbeh.2017.03.007

94. Carlini BH, Garrett SB, Carter GT. Medicinal cannabis: A survey among health care providers in Washington state. *Am J Hosp Palliat Care* (2017) 34(1):85–91. doi:10.1177/1049909115604669

95. Azofeifa A, Mattson ME, Schauer G, McAfee T, Grant A, Lyerla R. National estimates of marijuana use and related indicators - national survey on drug use and health, United States, 2002-2014. *MMWR Surveill Summ* (2016) 65(11):1–28. doi:10.15585/mmwr.ss6511a1

96. Bonn-Miller MO, Oser ML, Bucossi MM, Trafton JA. Cannabis use and HIV antiretroviral therapy adherence and HIV-related symptoms. *J Behav Med* (2014) 37(1):1–10. doi:10.1007/s10865-012-9458-5

97. Wilson NL, Azuero A, Vance DE, Richman JS, Moneyham LD, Raper JL, et al. Identifying symptom patterns in people living with HIV disease. *J Assoc Nurses AIDS Care* (2016) 27(2):121–32. doi:10.1016/j.jana.2015.11.009

98. Acharya N, Penukonda S, Shcheglova T, Hagymasi AT, Basu S, Srivastava PK. Endocannabinoid system acts as a regulator of immune homeostasis in the gut. *Proc Natl Acad Sci U S A* (2017) 114(19):5005–10. doi:10.1073/pnas.1612177114

99. Li C, Jones PM, Persaud SJ. Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacol Ther* (2011) 129(3):307–20. doi:10.1016/j.pharmthera.2010.10.006

100. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* (2006) 58(3):389–462. doi:10.1124/pr.58.3.2

101. Manuzak JA, Gott TM, Kirkwood JS, Coronado E, Hensley-McBain T, Miller C, et al. Heavy cannabis use associated with reduction in activated and inflammatory immune cell frequencies in antiretroviral therapy-treated human immunodeficiency virus-infected individuals. *Clin Infect Dis* (2018) 66(12):1872–82. doi:10.1093/cid/cix1116

102. Chaillon A, Nakazawa M, Anderson C, Christensen-Quick A, Ellis RJ, Franklin D, et al. Effect of cannabis use on human immunodeficiency virus DNA during suppressive antiretroviral therapy. *Clin Infect Dis* (2020) 70(1):140–3. doi:10.1093/cid/ciz387

103. Klatt NR, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease. *Trends Microbiol* (2013) 21(1):6–13. doi:10.1016/j.tim.2012.09.001

104. Panee J, Gerschenson M, Chang L. Associations between microbiota, mitochondrial function, and cognition in chronic marijuana users. *J Neuroimmune Pharmacol* (2018) 13(1):113–22. doi:10.1007/s11481-017-9767-0

105. Skinner CM, Nookaew I, Ewing LE, Wongsurawat T, Jenjaroenpun P, Quick CM, et al. Potential probiotic or trigger of gut inflammation - the janus-faced nature of cannabidiol-rich cannabis extract. *J Diet Suppl* (2020) 17(5):543–60. doi:10.1080/19390211.2020.1761506

106. Chivero ET, Ahmad R, Thangaraj A, Periyasamy P, Kumar B, Kroeger E, et al. Cocaine induces inflammatory gut milieu by compromising the mucosal barrier integrity and altering the gut microbiota colonization. *Sci Rep* (2019) 9(1):12187. doi:10.1038/s41598-019-48428-2

107. Rofael HZ, Turkall RM, Abdel-Rahman MS. Immunomodulation by cocaine and ketamine in postnatal rats. *Toxicology* (2003) 188(1):101–14. doi:10.1016/s0300-483x(03)00081-7

108. Watzl B, Watson RR. Immunomodulation by cocaine--a neuroendocrine mediated response. *Life Sci* (1990) 46(19):1319–29. doi:10.1016/0024-3205(90)90331-k

109. Halpern JH, Sholar MB, Glowacki J, Mello NK, Mendelson JH, Siegel AJ. Diminished interleukin-6 response to proinflammatory challenge in men and women after intravenous cocaine administration. *J Clin Endocrinol Metab* (2003) 88(3):1188–93. doi:10.1210/jc.2002-020804

110. Nair MP, Mahajan S, Chadha KC, Nair NM, Hewitt RG, Pillai SK, et al. Effect of cocaine on chemokine and CCR-5 gene expression by mononuclear cells from normal donors and HIV-1 infected patients. *Adv Exp Med Biol* (2001) 493:235–40. doi:10.1007/0-306-47611-8_28

111. Nair MP, Chadha KC, Hewitt RG, Mahajan S, Sweet A, Schwartz SA. Cocaine differentially modulates chemokine production by mononuclear cells from normal donors and human immunodeficiency virus type 1-infected patients. *Clin Diagn Lab Immunol* (2000) 7(1):96–100. doi:10.1128/cdli.7.1.96-100.2000

112. Dash S, Balasubramaniam M, Villalta F, Dash C, Pandhare J. Impact of cocaine abuse on HIV pathogenesis. *Front Microbiol* (2015) 6:1111. doi:10.3389/fmicb.2015.01111

113. Daras M. Neurologic complications of cocaine. *NIDA Res Monogr* (1996) 163:43–65.

114. Riezzo I, Fiore C, De Carlo D, Pascale N, Neri M, Turillazzi E, et al. Side effects of cocaine abuse: Multiorgan toxicity and pathological consequences. *Curr Med Chem* (2012) 19(33):5624–46. doi:10.2174/092986712803988893

115. Marasco CC, Goodwin CR, Winder DG, Schramm-Sapyta NL, McLean JA, Wikswo JP. Systems-level view of cocaine addiction: The interconnection of the immune and nervous systems. *Exp Biol Med (Maywood)* (2014) 239(11):1433–42. doi:10.1177/1535370214537747

116. Gawin FH. Cocaine addiction: Psychology and neurophysiology. *Science* (1991) 251(5001):1580–6. doi:10.1126/science.2011738

117. Soder HE, Berumen AM, Gomez KE, Green CE, Suchting R, Wardle MC, et al. Elevated neutrophil to lymphocyte ratio in older adults with cocaine use disorder as a marker of chronic inflammation. *Clin Psychopharmacol Neurosci* (2020) 18(1):32–40. doi:10.9758/cpn.2020.18.1.32

118. Cuesta S, Burdisso P, Segev A, Kourrich S, Sperandio V. Gut colonization by Proteobacteria alters host metabolism and modulates cocaine neurobehavioral responses. *Cell Host Microbe* (2022) 30(11):1615–29 e5. doi:10.1016/j.chom.2022.09.014

119. Kiraly DD, Walker DM, Calipari ES, Labonte B, Issler O, Pena CJ, et al. Alterations of the host microbiome affect behavioral responses to cocaine. *Sci Rep* (2016) 6:35455. doi:10.1038/srep35455

120. Ning T, Gong X, Xie L, Ma B. Gut microbiota analysis in rats with methamphetamine-induced conditioned place preference. *Front Microbiol* (2017) 8:1620. doi:10.3389/fmicb.2017.01620

121. Xu Y, Xie Z, Wang H, Shen Z, Guo Y, Gao Y, et al. Bacterial diversity of intestinal microbiota in patients with substance use disorders revealed by 16S rRNA gene deep sequencing. *Sci Rep* (2017) 7(1):3628. doi:10.1038/s41598-017-03706-9

122. Cook RR, Fulcher JA, Tobin NH, Li F, Lee DJ, Woodward C, et al. Alterations to the gastrointestinal microbiome associated with methamphetamine use among young men who have sex with men. *Sci Rep* (2019) 9(1):14840. doi:10.1038/s41598-019-51142-8

123. Chen LJ, Zhi X, Zhang KK, Wang LB, Li JH, Liu JL, et al. Escalating dose-multiple binge methamphetamine treatment elicits neurotoxicity, altering gut microbiota and fecal metabolites in mice. *Food Chem Toxicol* (2021) 148:111946. doi:10.1016/j.fct.2020.111946

124. Deng D, Su H, Song Y, Chen T, Sun Q, Jiang H, et al. Altered fecal microbiota correlated with systemic inflammation in male subjects with methamphetamine use disorder. *Front Cel Infect Microbiol* (2021) 11:783917. doi:10.3389/fcimb.2021.783917

125. Li Y, Kong D, Bi K, Luo H. Related effects of methamphetamine on the intestinal barrier via cytokines, and potential mechanisms by which methamphetamine may occur on the brain-gut Axis. *Front Med (Lausanne)* (2022) 9:783121. doi:10.3389/fmed.2022.783121

126. Boschetti E, Accarino A, Malagelada C, Malagelada JR, Cogliandro RF, Gori A, et al. Gut epithelial and vascular barrier abnormalities in patients with chronic intestinal pseudo-obstruction. *Neurogastroenterol Motil* (2019) 31(8):e13652. doi:10.1111/nmo.13652

127. Stevens BR, Goel R, Seungbum K, Richards EM, Holbert RC, Pepine CJ, et al. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. *Gut* (2018) 67(8):1555–7. doi:10.1136/gutjnl-2017-314759

128. Camilleri M. Leaky gut: Mechanisms, measurement and clinical implications in humans. *Gut* (2019) 68(8):1516–26. doi:10.1136/gutjnl-2019-318427

129. Trzeciak P, Herbet M. Role of the intestinal microbiome, intestinal barrier and psychobiotics in depression. *Nutrients* (2021) 13(3):927. doi:10.3390/nu13030927

130. Wang Y, An Y, Ma W, Yu H, Lu Y, Zhang X, et al. 27-Hydroxycholesterol contributes to cognitive deficits in APP/PS1 transgenic mice through microbiota dysbiosis and intestinal barrier dysfunction. *J Neuroinflammation* (2020) 17(1):199. doi:10.1186/s12974-020-01873-7

131. Gao T, Wang Z, Dong Y, Cao J, Lin R, Wang X, et al. Role of melatonin in sleep deprivation-induced intestinal barrier dysfunction in mice. *J Pineal Res* (2019) 67(1):e12574. doi:10.1111/jpi.12574

132. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci* (2013) 70(4):631–59. doi:10.1007/s00018-012-1070-x

133. Coelho-Santos V, Leitao RA, Cardoso FL, Palmela I, Rito M, Barbosa M, et al. The TNF-α/NF-κB signaling pathway has a key role in methamphetamine-induced blood-brain barrier dysfunction. *J Cereb Blood Flow Metab* (2015) 35(8):1260–71. doi:10.1038/jcbfm.2015.59

134. Northrop NA, Yamamoto BK. Methamphetamine effects on blood-brain barrier structure and function. *Front Neurosci* (2015) 9:69. doi:10.3389/fnins.2015.00069

135. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* (2009) 65(9):732–41. doi:10.1016/j.biopsych.2008.11.029

136. Forouzan S, Hoffman KL, Kosten TA. Methamphetamine exposure and its cessation alter gut microbiota and induce depressive-like behavioral effects on rats. *Psychopharmacology (Berl)* (2021) 238(1):281–92. doi:10.1007/s00213-020-05681-y

137. Fulcher JA, Hussain SK, Cook R, Li F, Tobin NH, Ragsdale A, et al. Effects of substance use and sex practices on the intestinal microbiome during HIV-1 infection. *J Infect Dis* (2018) 218(10):1560–70. doi:10.1093/infdis/jiy349

138. Yang Y, Yang C, Lei Z, Rong H, Yu S, Wu H, et al. Cigarette smoking exposure breaks the homeostasis of cholesterol and bile acid metabolism and induces gut microbiota dysbiosis in mice with different diets. *Toxicology* (2021) 450:152678. doi:10.1016/j.tox.2021.152678

139. Nolan-Kenney R, Wu F, Hu J, Yang L, Kelly D, Li H, et al. The association between smoking and gut microbiome in Bangladesh. *Nicotine Tob Res* (2020) 22(8):1339–46. doi:10.1093/ntr/ntz220

140. Kobayashi T, Fujiwara K. Identification of heavy smokers through their intestinal microbiota by data mining analysis. *Biosci Microbiota Food Health* (2013) 32(2):77–80. doi:10.12938/bmfh.32.77

141. Chi L, Mahbub R, Gao B, Bian X, Tu P, Ru H, et al. Nicotine alters the gut microbiome and metabolites of gut-brain interactions in a sex-specific manner. *Chem Res Toxicol* (2017) 30(12):2110–9. doi:10.1021/acs.chemrestox.7b00162

142. Wang R, Li S, Jin L, Zhang W, Liu N, Wang H, et al. Four-week administration of nicotine moderately impacts blood metabolic profile and gut microbiota in a diet-dependent manner. *Biomed Pharmacother* (2019) 115:108945. doi:10.1016/j.biopha.2019.108945

ADAR

MINI REVIEW
published: 15 March 2022
doi: 10.3389/adar.2022.10311

Gut-Microbiome Implications in Opioid Use Disorder and Related Behaviors

Bridget Herlihy[1,2] *and Sabita Roy*[1]*

[1]*Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, United States,* [2]*Department of Neuroscience, University of Miami Miller School of Medicine, Miami, FL, United States*

Substance use disorder (SUD) is a prevalent disease that has caused hundreds of thousands of deaths and affected the lives of even more. Despite its global impact, there is still no known cure for SUD, or the psychological symptoms associated with drug use. Many of the behavioral consequences of drug use prevent people from breaking the cycle of addiction or cause them to relapse back into the cycle due to the physical and psychological consequences of withdrawal. Current research is aimed at understanding the cause of these drug related behaviors and therapeutically targeting them as a mechanism to break the addiction cycle. Research on opioids suggests that the changes in the microbiome during drug use modulated drug related behaviors and preventing these microbial changes could attenuate behavioral symptoms. This review aims to highlight the relationship between the changes in the microbiome and behavior during opioid treatment, as well as highlight the additional research needed to understand the mechanism in which the microbiome modulates behavior to determine the best therapeutic course of action.

Keywords: opioid use disorder, substance use disorder, microbiome, behavior, gut-brain, withdrawal

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Edited by:
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Berhanu Geresu Kibret,
Addis Ababa University, Ethiopia
Declan Ali,
University of Alberta, Canada

***Correspondence:**
Sabita Roy
sabita.roy@miami.edu

Received: *15 December 2021*
Accepted: *25 February 2022*
Published: *15 March 2022*

Citation:
Herlihy B and Roy S (2022) Gut-Microbiome Implications in Opioid Use Disorder and Related Behaviors. Adv. Drug. Alco. Res. 2:10311. doi: 10.3389/adar.2022.10311

THE MICROBIOME AND BEHAVIOR

There is a well-studied relationship between the gut microbiome and mental health disorders such as anxiety and depression (1–3). Both anxiety and depression induce changes to the composition and proper functioning of the gut microbiota (4–6). Stress disorders, like anxiety, cause disruptions to the integrity of the gut barrier, which can allow translocation of gut bacterial products, commonly referred to as "leaky gut" (7,8). This likely results in a microbiota-driven proinflammatory response. Animal studies also show an increase of *Bacteroidetes* and a decrease of *Firmicutes* in mice expressing depressive like behavior, indicating a state of microbial dysbiosis (9–11). Interestingly antidepressant medications such as monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs) have antimicrobial properties, suggesting gut modulation as a potential therapeutic target (12,13). Additionally, specific antibiotics show antidepressant properties in both human and rodent studies (14,15).

Animal studies using germ free mice further investigate the role of the microbiome in anxiety and depression. Numerous studies show that germ free mice display lower levels of anxiety-like behavior across multiple models of anxiety: Elevated Plus Maze, Open Field Test, and Light Dark test (16–18). Upon reconstitution of the microbiome in these germ-free mice, anxiety like behavior was only normalized if the reconstitution happened in early life (16,17,19). Reconstitution during adulthood resulted in the persistence of lower anxiety-like behavior. This indicates specific developmental

windows in which the microbiome alters neuronal circuitry important for anxiety-like behavior. However, alterations to a healthy microbiome in adulthood can still show alterations to anxiety-like behavior (20). Antibiotic depletion of the microbiome using a broad spectrum antibiotics causes reduced levels of anxiety-like behavior, and once antibiotic administration ceases, the anxiety-like response normalizes as the microbiome replenishes (20). Antibiotic treatment does not affect anxiety-like behavior in germ-free mice, confirming behavioral changes are due to changes in the microbiome. While eliminating harmful bacteria with antibiotics can reduce anxiety-like behavior, it has also been shown that supplementing the microbiome with beneficial bacteria via probiotic treatment can also reduce anxiety-like and depressive-like behavior. Probiotic treatment causes a reduction of anxiety-like and depressive-like behaviors, even in rats experiencing maternal separation during neonatal development, an event known to increase depressive like behavior in adulthood (21,22). The probiotic effects are comparable to results from antidepressant treatment. Clinically, studies show similar antidepressant effects of probiotics with healthy participants displaying less psychological distress when treated with probiotics, and subjects initially scoring highest in depression, showing significant improvement in symptoms (23). Additionally, patients with chronic fatigue syndrome, which has a high comorbidity with anxiety, show a disrupted microbiome composition, and also see improvement of anxiety severity with the treatment of probiotics (24).

Other disruptions to the gut-homeostasis, such as infection and inflammation, are also seen to change anxiety-like behavior. Infections with *C. rodentium* and *C. jejuni* increased anxiety-like behaviors as early as 8 h post infection, lasting up to 2 days (25,26). Interestingly this behavioral change is seen even without a periphery immune response, suggesting that pathogenic bacteria in the gut can produce behavioral changes independently from an immune response (27). Increased anxiety-like behavior is also seen when there is an increase in GI inflammation. This behavioral change was reversed with probiotic treatment.

This relationship between the microbiome and anxiety and depression has great interest in opioid research, as anxiety and depression both have high comorbidity with opioid use disorder, and elevated levels of anxiety and depression are common throughout the stages of opioid use (28). Initial drug is often used as a form of self-medication to alleviate stress and anxieties (29). However, these effects of opioids are short lived, and consumption of drugs can lead to increased depression symptoms instead (30). Once opioid dependence is formed, the abstinence of opioids in the body induces a withdrawal response, which can result in elevated anxiety and depression symptoms, even after the physical symptoms of withdrawal have passed (31–34). For many the severity of anxiety and depression during withdrawal drives continued drug use to alleviate the symptoms (33). The implication of microbiome in these behaviors are especially interesting considering the impact that opioid use has on the gut microbiome.

MORPHINE INDUCED CHANGES TO THE MICROBIOME

Opioid use has been shown to disrupt multiple areas of gut homeostasis. Animal studies show that morphine treatment results in specific changes to the relative abundance of bacteria (35). Morphine exposure, even in the short-term, causes an increased abundance of pathogenic bacteria (*Flavobacterium*, *Enterococcus*, *Fusobacterium*, *Sutterella*, *Clostridium*, *Rikenellaceae*, and *Ruminococcus*). Once tolerance is developed a significant decrease in the abundance of beneficial bacteria (*Lactobacillus* and *Bifidobacterium*) is observed as well (36). This pattern of microbial change is an indication of microbial dysbiosis (37,38). Additional changes in abundance of individual bacteria are seen across morphine exposure time and doses, all indicating the same pattern of increasing of harmful bacteria and decreasing of beneficial bacteria (36,38–46). The functional consequences of the dysbiosis of the microbiome include a decrease in gut motility and an increase in gut-barrier permeability, creating a risk of bacterial translocation and proinflammatory signaling (47).

The diversity of the gut microbiota is also greatly impacted by morphine treatment. Functional and taxonomic diversity of the microbiota are very important for maintaining gut-homeostasis, and a non-diverse microbiota is associated with inflammatory bowel disease and obesity (48,49). Opioid treatment causes a decrease in the alpha diversity of the gut-microbiota, which signifies diminished species richness and a less diverse array of present bacteria within the microbiome (35). Additionally, the beta diversity measuring the similarities or dissimilarities of microbiome composition between groups shows distinct clustering in morphine treated animals as compared to placebo treated controls. This is additional confirmation of a dysbiosis of the gut microbiome caused by morphine treatment.

Studies have been targeting these opioid induced changes in the gut to understand their relevance to the behavioral consequences of opioid use. Tolerance development is the most well studied relationship between the morphine induced dysbiosis and drug related behavior. Germ free mice showed an attenuation of morphine tolerance and the reconstitution of the microbiome *via* a fecal matter transplant (FMT) of a healthy microbiome reinstated the tolerance development, indicating the microbiome is necessary to the development of morphine tolerance (36). Antibiotic depletion of the microbiome in specific pathogen-free (SPF) mice also shows an attenuation of morphine tolerance, however a FMT of a healthy microbiome does not recover the tolerance development. Instead, the FMT of a morphine treated microbiome is needed for the proper development of morphine tolerance. Additionally, treatment with a probiotic cocktail of the bacteria that showed significantly reduced abundance during morphine exposure, both prevents the dysbiosis effects of morphine as well as attenuates morphine tolerance (36). This suggests the state of the microbiome during morphine induced dysbiosis is a requirement for tolerance development. Similar relationships have been seen in other stages of drug use as well. Research shows that antibiotic depletion of the microbiome causes impaired cocaine reward processing, suggesting a need for the

microbiome in the rewarding pathways involved in addiction, though no studies have examined the impact of the microbiome in any addiction paradigms for morphine specifically (44). Studies examining addiction paradigms such as Conditioned Place Preference (CPP) show that mice that display higher CPP scores have a unique microbial composition compared to mice that display lower CPP scores (50). A new area of research is the relationship of the morphine induced gut changes on the withdrawal response. However, the limited research shows conflicting results on antibiotics effects on the withdrawal severity. Withdrawal symptoms are seen to both decrease and remain the same depending on the morphine treatment regimen and the specifics of antibiotic treatment (46,51). Though the withdrawal state of morphine use does still show changes to the microbiome as well as neuronal changes that may be linked to gut dysbiosis (52).

The well-studied relationship of the microbiome's influence on morphine tolerance, as well as emerging evidence of its roles in addition and withdrawal behavior, show that the dysbiosis of the microbiome caused by opioids has an impact on drug related behaviors (36,38,46,51). While researchers are still investigating the extent to which this opioid induced dysbiosis contributes to the severity and development of these behaviors, the importance of this relationship between drug use, microbial changes, and behavior is paramount. Understanding this relationship can lead to the development of gut targeted therapeutic strategies to treat the behavioral symptoms of drug use, an area that is lacking in therapeutic intervention. However, additional research on the mechanisms in which the microbiome is modulating behavior during opioid use is needed. Potential mechanisms are understudied in opioid research, however research with other drugs of misuse has discovered some potential links between microbial and behavioral changes.

POSSIBLE LINKS BETWEEN MICROBIAL AND BEHAVIORAL CHANGES

The exact mechanism in which the microbiome influences behavioral changes is unknown, though there are many potential links currently being researched (53–59). One possible connection is the inflammatory response that results from drug induced disruption to the epithelial barrier. The microbial changes that occur during use of opioids, and other drugs of misuse, cause damage to the tight junction proteins (47,60–62). This leads to a compromised integrity of the epithelial lining, allowing for translocation of bacteria. Additionally, there is a higher risk of the translocation of pathogenic bacteria, due to the microbial dysbiosis caused by drug exposure. Host epithelial cells recognize the bacteria and initiate a toll-like receptor (TLR) modulated immune response, resulting in the release of proinflammatory cytokines (63). Studies suggest that these cytokines can cross the blood brain barrier and modulate behavior to contribute to the behavioral consequences of drug use (53,54). However, not all proinflammatory cytokines produce the same behavioral responses. Activation of the TLR4 signaling driven by interleukin 1 beta (IL-1b) results in an increase of CPP and self-administration to cocaine. This indicates that proinflammatory activation drives the addiction process, but conflicting results are found for proinflammatory TNF-a. An increase of TNF-a levels results in a decrease of CPP response to morphine, as well as a decrease of behavioral drug response to both morphine and cocaine. These inconsistent findings suggest the role of inflammation in drug related behavior could depend on the specific cytokine or drug being studied (64–66).

Another potential link is the vagus nerve, as it provides direct communication between the brain and gut. Even though the vagus nerve does not cross the gut epithelial layer and have direct contact with the microbiome, studies have shown that the vagus nerve may be sensitive to signals from the microbiome (55,56). Antimicrobial treatment of the microbiome, resulting in an increase of *Lactobacilli*, modified GABA expression in numerous brain areas and decreased anxiety-like behavior. These findings are thought to be a result of vagal signaling, and additional studies have shown that vagal nerve integrity is crucial to the successful attenuation of anxiety-like behavior by probiotic treatment (21,57). There is also preliminary evidence that shows vagal nerve stimulation facilitates the extinction of drug seeking behavior during the withdrawal process of cocaine treatment (67). While research shows the vagus nerve may be important to the behavioral responses of drug use, there is limited evidence to prove microbiome is relying on the vagus nerve to modulate behavior, or if the microbiome alone can stimulate the vagus nerve enough to produce behavioral changes. Others believe the microbiome modulates behavior *via* hippocampal brain-derived neurotropic factor (BDNF), independent of vagal nerve stimulation (20). Cocaine studies show an epigenetic regulation of BDNF levels during drug use, and BDNF has been shown to mediate cocaine self-administration, and drug seeking (68). Expression of BDNF also changes in response to changes in the microbiome, and these changes are associated with altered behavioral responses to alcohol and cocaine (69–71). A FMT of a microbiome samples of alcohol exposed donors to healthy recipients, resulted in a decrease of BDNF levels in the hippocampus, as well as an increase of anxiety and depressive-like withdrawal behaviors (70). There are many correlations of drug exposure and microbial changes with changes in BDNF expression levels, however causal studies to determine a mechanism in which the microbiome is influencing the BDNF expression have not been done (71).

There is a wealth of data implementing microglial activation as a mechanism that drives microbiome modulated drug related behaviors. It is well documented that the microbiome is crucial for the proper development of microglia (72,73). In fact, germ free mice display deformed microglial cells as well as slight behavioral differences that may be a result of the lack of properly matured microglia (72). On a less severe model, microglial defects can be seen with prolonged antibiotic treatment, and microglial function can be restored with probiotic treatment (72). Also, microglia become significantly more activated during drug exposure (52,58,59,74). Elevated microglial activation occurs as a result of chronic ethanol treatment, and microglia remain overactive throughout long-term ethanol withdrawal (74).

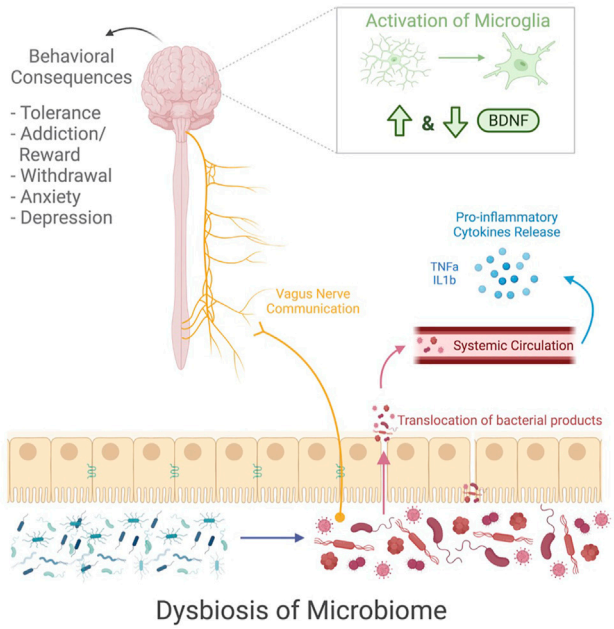

FIGURE 1 | This schematic shows the microbial and neuronal changes that occur during drug use. Exposure to drugs cause a dysbiosis of the microbiome and impaired gut barrier function which leads to the translocation of pathogenic bacteria, resulting in a release of pro-inflammatory cytokines. The vagus nerve sends communications from the gut microbiome to the brain. Additionally, within the brain there is an increased activation of microglia and alterations to BDNF signaling. All of these factors seem to play in a role in drug related behavior, leading to tolerance development, addiction and reward signaling, withdrawal symptoms, anxiety, and depression. Image created with BioRender.com.

Methamphetamine treatment also results in microglia activation, and upon inhibition of microglia cells, locomotor sensitization to methamphetamine attenuated (58). Additionally, high levels of microglial activation in the nucleus accumbens are observed during cocaine treatment (59). This change in microglia activation could be a response to the drug presence itself or a consequence of drug induced microbial changes. The elevation of microglial activation is seen in many brain areas crucial to addiction and reward pathways, and inhibition of microglia has attenuated some behaviors related to drug use. Further research is needed to understand the implications of the gut-microbiome in microglial modulation of drug related behaviors.

SUMMARY

Drug use causes dysregulation from the gut to the brain (**Figure 1**). Research shows a drug induced dysbiosis of the gut microbiome, causing the diverse microbial environment to become overpopulated with pathogenic bacteria (35,36). The consequence of the microbial shift leads to a compromised gut barrier, resulting in a translocation of bacteria that trigger a proinflammatory cytokine release (47). While the microbiome is activating an inflammatory response, it also communicates with the vagus nerve to send signals to the brain (55,56). Additionally, the microbiome may be responsible for the increase in microglial activation as well as dysregulation of BDNF signaling during drug use (52,68,74). All of these factors affected by drug use also have behavioral implications that are relevant to behavioral consequences of drug use. Thus, the dysregulation of these factors during drug use may be during the behavioral consequences of drug use.

In conclusion, there is a lot of evidence showing a relationship between the microbiome and behavior, and the microbiome undergoes substantial changes when exposed to opioids that may further modulate drug related behaviors, such as reward processing, tolerance, withdrawal, anxiety, and depression. However, the exact mechanism between microbial changes and behavior is still not understood, especially for opioids specifically. The collective literature for drugs of misuse, provide potential links between the microbiome and the brain: inflammation, the vagus nerve, BDNF, and microglia. These are areas that need additional research for opioids, as well as every drug individually, to determine how the gut and brain are communicating and regulating drug related behaviors. Understanding this relationship could lead to potential treatment options for the psychiatric symptoms of SUD and provide an easier path out of the cycle of addition.

AUTHOR CONTRIBUTIONS

BH researched the literature in the field, wrote the manuscript, and created the figure. SR provided edits and collaborative ideas for the paper.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Nardone G, Compare D. The Psyche and Gastric Functions. *Dig Dis* (2014) 32(3):206–12. doi:10.1159/000357851
2. Vuong HE, Yano JM, Fung TC, Hsiao EY. The Microbiome and Host Behavior. *Annu Rev Neurosci* (2017) 40:21–49. doi:10.1146/annurev-neuro-072116-031347
3. Lach G, Schellekens H, Dinan TG, Cryan JF. Anxiety, Depression, and the Microbiome: A Role for Gut Peptides. *Neurotherapeutics* (2018) 15(1):36–59. doi:10.1007/s13311-017-0585-0
4. Bailey MT, Coe CL. Maternal Separation Disrupts the Integrity of the Intestinal Microflora in Infant Rhesus Monkeys. *Dev Psychobiol* (1999) 35(2):146–55. doi:10.1002/(sici)1098-2302(199909)35:2<146::aid-dev7>3.0.co;2-g
5. Park AJ, Collins J, Blennerhassett PA, Ghia JE, Verdu EF, Bercik P, et al. Altered Colonic Function and Microbiota Profile in a Mouse Model of Chronic

Depression. *Neurogastroenterol Motil* (2013) 25(9):733–e575. doi:10.1111/nmo.12153
6. O'Malley D, Julio-Pieper M, Gibney SM, Dinan TG, Cryan JF. Distinct Alterations in Colonic Morphology and Physiology in Two Rat Models of Enhanced Stress-Induced Anxiety and Depression-like Behaviour. *Stress* (2010) 13(2):114–22. doi:10.3109/10253890903067418
7. Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking Down the Barriers: The Gut Microbiome, Intestinal Permeability and Stress-Related Psychiatric Disorders. *Front Cel Neurosci.* (2015) 9:392. doi:10.3389/fncel.2015.00392
8. Maes M, Kubera M, Leunis JC. The Gut-Brain Barrier in Major Depression: Intestinal Mucosal Dysfunction with an Increased Translocation of LPS from Gram Negative Enterobacteria (Leaky Gut) Plays a Role in the Inflammatory Pathophysiology of Depression. *Neuro Endocrinol Lett* (2008) 29(1):117–24.
9. Labus JS, Hollister EB, Jacobs J, Kirbach K, Oezguen N, Gupta A, et al. Differences in Gut Microbial Composition Correlate with Regional Brain Volumes in Irritable Bowel Syndrome. *Microbiome* (2017) 5(1):49. doi:10.1186/s40168-017-0260-z
10. Yu M, Jia H, Zhou C, Yang Y, Zhao Y, Yang M, et al. Variations in Gut Microbiota and Fecal Metabolic Phenotype Associated with Depression by 16S rRNA Gene Sequencing and LC/MS-based Metabolomics. *J Pharm Biomed Anal* (2017) 138:231–9. doi:10.1016/j.jpba.2017.02.008
11. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a Social Stressor Alters the Structure of the Intestinal Microbiota: Implications for Stressor-Induced Immunomodulation. *Brain Behav Immun* (2011) 25(3):397–407. doi:10.1016/j.bbi.2010.10.023
12. Lieb J. The Immunostimulating and Antimicrobial Properties of Lithium and Antidepressants. *J Infect* (2004) 49(2):88–93. doi:10.1016/j.jinf.2004.03.006
13. Munoz-Bellido JL, Munoz-Criado S, Garcìa-Rodrìguez JA. Antimicrobial Activity of Psychotropic Drugs. *Int J Antimicrob Agents* (2000) 14(3):177–80. doi:10.1016/s0924-8579(99)00154-5
14. Ferreira Mello BS, Monte AS, McIntyre RS, Soczynska JK, Custódio CS, Cordeiro RC, et al. Effects of Doxycycline on Depressive-like Behavior in Mice after Lipopolysaccharide (LPS) Administration. *J Psychiatr Res* (2013) 47(10):1521–9. doi:10.1016/j.jpsychires.2013.06.008
15. Miyaoka T, Wake R, Furuya M, Liaury K, Ieda M, Kawakami K, et al. Minocycline as Adjunctive Therapy for Patients with Unipolar Psychotic Depression: An Open-Label Study. *Prog Neuro-Psychopharmacology Biol Psychiatry* (2012) 37(2):222–6. doi:10.1016/j.pnpbp.2012.02.002
16. Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal Gut Microbiota Modulates Brain Development and Behavior. *Proc Natl Acad Sci* (2011) 108(7):3047–52. doi:10.1073/pnas.1010529108
17. Neufeld K-AM, Kang N, Bienenstock J, Foster JA. Effects of Intestinal Microbiota on Anxiety-like Behavior. *Communicative Integr Biol* (2011) 4(4):492–4. doi:10.4161/cib.15702
18. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced Anxiety-like Behavior and central Neurochemical Change in Germ-free Mice. *Neurogastroenterol Motil* (2011) 23(3):255–e119. doi:10.1111/j.1365-2982.2010.01620.x
19. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, et al. The Microbiome-Gut-Brain axis during Early Life Regulates the Hippocampal Serotonergic System in a Sex-dependent Manner. *Mol Psychiatry* (2013) 18(6):666–73. doi:10.1038/mp.2012.77
20. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The Intestinal Microbiota Affect central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. *Gastroenterology* (2011) 141(2):599–609. doi:10.1053/j.gastro.2011.04.052
21. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus Strain Regulates Emotional Behavior and central GABA Receptor Expression in a Mouse via the Vagus Nerve. *Proc Natl Acad Sci* (2011) 108(38):16050–5. doi:10.1073/pnas.1102999108
22. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the Probiotic Bifidobacterium Infantis in the Maternal Separation Model of Depression. *Neuroscience* (2010) 170(4):1179–88. doi:10.1016/j.neuroscience.2010.08.005
23. Messaoudi M, Violle N, Bisson J-F, Desor D, Javelot H, Rougeot C. Beneficial Psychological Effects of a Probiotic Formulation (Lactobacillus helveticusR0052 andBifidobacterium longumR0175) in Healthy Human Volunteers. *Gut Microbes* (2011) 2(4):256–61. doi:10.4161/gmic.2.4.16108
24. Benton D, Williams C, Brown A. Impact of Consuming a Milk Drink Containing a Probiotic on Mood and Cognition. *Eur J Clin Nutr* (2007) 61(3):355–61. doi:10.1038/sj.ejcn.1602546
25. Lyte M, Varcoe JJ, Bailey MT. Anxiogenic Effect of Subclinical Bacterial Infection in Mice in the Absence of Overt Immune Activation. *Physiol Behav* (1998) 65(1):63–8. doi:10.1016/s0031-9384(98)00145-0
26. Lyte M, Li W, Opitz N, Gaykema R, Goehler L. Induction of Anxiety-like Behavior in Mice during the Initial Stages of Infection with the Agent of Murine Colonic Hyperplasia Citrobacter Rodentium. *Physiol Behav* (2006) 89(3):350–7. doi:10.1016/j.physbeh.2006.06.019
27. Bercik P, Verdu EF, Foster JA, MacRi J, Potter M, Huang X, et al. Chronic Gastrointestinal Inflammation Induces Anxiety-like Behavior and Alters central Nervous System Biochemistry in Mice. *Gastroenterology* (2010) 139(6):2102–12. doi:10.1053/j.gastro.2010.06.063
28. Hasin DS, Stinson FS, Ogburn E, Grant BF. Prevalence, Correlates, Disability, and Comorbidity of DSM-IV Alcohol Abuse and Dependence in the United States. *Arch Gen Psychiatry* (2007) 64(7):830–42. doi:10.1001/archpsyc.64.7.830
29. Labouvie E. Maturing Out of Substance Use: Selection and Self-Correction. *J Drug Issues* (1996) 26(2):457–76. doi:10.1177/002204269602600208
30. Bleichmar H. Droga Y Depresión. Un camino a Doble Vía. *Proy Hombre* (1994) 10:11–4.
31. Powell JE, Taylor D. Anger, Depression, and Anxiety Following Heroin Withdrawal. *Int J Addict* (1991) 27(1):25–35. doi:10.3109/10826089109063460
32. Sullivan RM, Duchesne A, Hussain D, Waldron J, Laplante F. Effects of Unilateral Amygdala Dopamine Depletion on Behaviour in the Elevated Plus Maze: Role of Sex, Hemisphere and Retesting. *Behav Brain Res* (2009) 205(1):115–22. doi:10.1016/j.bbr.2009.07.023
33. Tian M, Mao R-R, Wang L-P, Zhou Q-X, Cao J, Xu L. Interaction between Behavioral Despair and Addictive Behaviors in Rats. *Physiol Behav* (2011) 102(1):7–12. doi:10.1016/j.physbeh.2010.10.002
34. Anraku T, Ikegaya Y, Matsuki N, Nishiyama N. Withdrawal from Chronic Morphine Administration Causes Prolonged Enhancement of Immobility in Rat Forced Swimming Test. *Psychopharmacology* (2001) 157(2):217–20. doi:10.1007/s002130100793
35. Wang F, Meng J, Zhang L, Johnson T, Chen C, Roy S. Morphine Induces Changes in the Gut Microbiome and Metabolome in a Morphine Dependence Model. *Sci Rep* (2018) 8(1):3596–15. doi:10.1038/s41598-018-21915-8
36. Zhang L, Meng J, Ban Y, Jalodia R, Chupikova I, Fernandez I, et al. Morphine Tolerance Is Attenuated in Germfree Mice and Reversed by Probiotics, Implicating the Role of Gut Microbiome. *Proc Natl Acad Sci USA* (2019) 116(27):13523–32. doi:10.1073/pnas.1901182116
37. Sommer F, Bäckhed F. The Gut Microbiota - Masters of Host Development and Physiology. *Nat Rev Microbiol* (2013) 11(4):227–38. doi:10.1038/nrmicro2974
38. Banerjee S, Sindberg G, Wang F, Meng J, Sharma U, Zhang L, et al. Opioid-induced Gut Microbial Disruption and Bile Dysregulation Leads to Gut Barrier Compromise and Sustained Systemic Inflammation. *Mucosal Immunol* (2016) 9(612):1418–28. doi:10.1038/mi.2016.9
39. Acharya C, Betrapally NS, Gillevet PM, Sterling RK, Akbarali H, White MB, et al. Chronic Opioid Use Is Associated with Altered Gut Microbiota and Predicts Readmissions in Patients with Cirrhosis. *Aliment Pharmacol Ther* (2017) 45(2):319–31. doi:10.1111/apt.13858
40. Barengolts E, Green SJ, Eisenberg Y, Akbar A, Reddivari B, Layden BT, et al. Gut Microbiota Varies by Opioid Use, Circulating Leptin and Oxytocin in African American Men with Diabetes and High burden of Chronic Disease. *PLoS One* (2018) 13(3):e0194171. doi:10.1371/journal.pone.0194171
41. Xu Y, Xie Z, Wang H, Shen Z, Guo Y, Gao Y, et al. Bacterial Diversity of Intestinal Microbiota in Patients with Substance Use Disorders Revealed by 16S rRNA Gene Deep Sequencing. *Sci Rep* (2017) 7(1):3628. doi:10.1038/s41598-017-03706-9
42. Sindberg GM, Callen SE, Banerjee S, Meng J, Hale VL, Hegde R, et al. Morphine Potentiates Dysbiotic Microbial and Metabolic Shifts in Acute SIV Infection. *J Neuroimmune Pharmacol* (2019) 14(2):200–14. doi:10.1007/s11481-018-9805-6
43. Meng J, Banerjee S, Li D, Sindberg GM, Wang F, Ma J, et al. Opioid Exacerbation of Gram-Positive Sepsis, Induced by Gut Microbial

Modulation, Is Rescued by IL-17A Neutralization. *Sci Rep* (2015) 5:10918. doi:10.1038/srep10918
44. Lee K, Vuong HE, Nusbaum DJ, Hsiao EY, Evans CJ, Taylor AMW. The Gut Microbiota Mediates Reward and Sensory Responses Associated with Regimen-Selective Morphine Dependence. *Neuropsychopharmacol* (2018) 43(13):2606–14. doi:10.1038/s41386-018-0211-9
45. Sharma U, Olson RK, Erhart FN, Zhang L, Meng J, Segura B, et al. Prescription Opioids Induce Gut Dysbiosis and Exacerbate Colitis in a Murine Model of Inflammatory Bowel Disease. *J Crohn's Colitis* (2020) 14(6):801–17. doi:10.1093/ecco-jcc/jjz188
46. Simpson S, Kimbrough A, Boomhower B, McLellan R, Hughes M, Shankar K, et al. Depletion of the Microbiome Alters the Recruitment of Neuronal Ensembles of Oxycodone Intoxication and Withdrawal. *eNeuro* (2020) 7, ENEURO.0312-19.2020. doi:10.1523/ENEURO.0312-19.2020
47. Meng J, Yu H, Ma J, Wang J, Banerjee S, Charboneau R, et al. Morphine Induces Bacterial Translocation in Mice by Compromising Intestinal Barrier Function in a TLR-dependent Manner. *PLoS One* (2013) 8(1):e54040. doi:10.1371/journal.pone.0054040
48. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human Gut Microbiome Viewed across Age and Geography. *Nature* (2012) 486(7402):222–7. doi:10.1038/nature11053
49. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human Gut Microbes Associated with Obesity. *Nature* (2006) 444(7122):1022–3. doi:10.1038/4441022a
50. Zhang J, Yang J, Yang C, Chen T, Wang Z, Li J, et al. Sensitivity to Morphine Reward Associates with Gut Dysbiosis in Rats with Morphine-Induced Conditioned Place Preference. *Front Psychiatry* (2020) 11:631. doi:10.3389/fpsyt.2020.00631
51. Rock EM, Ayoub SM, Limebeer CL, Gene A, Wills KL, DeVuono MV, et al. Acute Naloxone-Precipitated Morphine Withdrawal Elicits Nausea-like Somatic Behaviors in Rats in a Manner Suppressed by N-Oleoylglycine. *Psychopharmacology* (2020) 237(2):375–84. doi:10.1007/s00213-019-05373-2
52. O'Sullivan SJ, Malahias E, Park J, Srivastava A, Reyes BAS, Gorky J, et al. Single-Cell Glia and Neuron Gene Expression in the Central Amygdala in Opioid Withdrawal Suggests Inflammation with Correlated Gut Dysbiosis. *Front Neurosci* (2019) 13:665–14. doi:10.3389/fnins.2019.00665
53. Pan W, P. Stone K, Hsuchou H, K. Manda V, Zhang Y, J. Kastin A. Cytokine Signaling Modulates Blood-Brain Barrier Function. *Cpd* (2011) 17(33):3729–40. doi:10.2174/138161211798220918
54. Yarlagadda A, Alfson E, Clayton AH. The Blood Brain Barrier and the Role of Cytokines in Neuropsychiatry. *Psychiatry (Edgmont)* (2009) 6(11):18–22.
55. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals. *Trends Neurosciences* (2016) 39(11):763–81. doi:10.1016/j.tins.2016.09.002
56. Bonaz B, Bazin T, Pellissier S. The Vagus Nerve at the Interface of the Microbiota-Gut-Brain axis. *Front Neurosci* (2018) 12:49. doi:10.3389/fnins.2018.00049
57. Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, et al. The Anxiolytic Effect of Bifidobacterium Longum NCC3001 Involves Vagal Pathways for Gut-Brain Communication. *Neurogastroenterol Motil* (2011) 23(12):1132–9. doi:10.1111/j.1365-2982.2011.01796.x
58. Thomas DM, Walker PD, Benjamins JA, Geddes TJ, Kuhn DM. Methamphetamine Neurotoxicity in Dopamine Nerve Endings of the Striatum Is Associated with Microglial Activation. *J Pharmacol Exp Ther* (2004) 311(1):1–7. doi:10.1124/jpet.104.070961
59. Miguel-Hidalgo JJ. The Role of Glial Cells in Drug Abuse. *Curr Drug Abuse Rev* (2009) 2(1):72–82. doi:10.2174/1874473710902010076
60. Rao RK, Seth A, Sheth P. Recent Advances in Alcoholic Liver Disease I. Role of Intestinal Permeability and Endotoxemia in Alcoholic Liver Disease. *Am J Physiology-Gastrointestinal Liver Physiol* (2004) 286(6):G881–G884. doi:10.1152/ajpgi.00006.2004
61. Keshavarzian A, Farhadi A, Forsyth CB, Rangan J, Jakate S, Shaikh M, et al. Evidence that Chronic Alcohol Exposure Promotes Intestinal Oxidative Stress, Intestinal Hyperpermeability and Endotoxemia Prior to Development of Alcoholic Steatohepatitis in Rats. *J Hepatol* (2009) 50(3):538–47. doi:10.1016/j.jhep.2008.10.028
62. Feng P, Truant AL, Meissler JJ, Gaughan JP, Adler MW, Eisenstein TK. Morphine Withdrawal Lowers Host Defense to Enteric Bacteria: Spontaneous Sepsis and Increased Sensitivity to Oral Salmonella enterica Serovar Typhimurium Infection. *Infect Immun* (2006) 74(9):5221–6. doi:10.1128/iai.00208-06
63. Kelly D, Conway S, Aminov R. Commensal Gut Bacteria: Mechanisms of Immune Modulation. *Trends Immunol* (2005) 26(6):326–33. doi:10.1016/j.it.2005.04.008
64. Northcutt AL, Hutchinson MR, Wang X, Baratta MV, Hiranita T, Cochran TA, et al. DAT Isn't All that: Cocaine Reward and Reinforcement Require Toll-like Receptor 4 Signaling. *Mol Psychiatry* (2015) 20(12):1525–37. doi:10.1038/mp.2014.177
65. Niwa M, Nitta A, Yamada Y, Nakajima A, Saito K, Seishima M, et al. Tumor Necrosis Factor-α and its Inducer Inhibit Morphine-Induced Rewarding Effects and Sensitization. *Biol Psychiatry* (2007) 62(6):658–68. doi:10.1016/j.biopsych.2006.10.009
66. Lewitus GM, Konefal SC, Greenhalgh AD, Pribiag H, Augereau K, Stellwagen D. Microglial TNF-α Suppresses Cocaine-Induced Plasticity and Behavioral Sensitization. *Neuron* (2016) 90(3):483–91. doi:10.1016/j.neuron.2016.03.030
67. Han W, Tellez LA, Perkins MH, Perez IO, Qu T, Ferreira J, et al. A Neural Circuit for Gut-Induced Reward. *Cell* (2018) 175(3):665–78. doi:10.1016/j.cell.2018.08.049
68. Li X, Wolf ME. Multiple Faces of BDNF in Cocaine Addiction. *Behav Brain Res* (2015) 279:240–54. doi:10.1016/j.bbr.2014.11.018
69. Kiraly DD, Walker DM, Calipari ES, Labonte B, Issler O, Pena CJ, et al. Alterations of the Host Microbiome Affect Behavioral Responses to Cocaine. *Sci Rep* (2016) 6:35455. doi:10.1038/srep35455
70. Xiaowen H-w., Ge C, Feng G-x., Li Y, Luo D, Dongli J-l., et al. Gut Microbiota Modulates Alcohol Withdrawal-Induced Anxiety in Mice. *Toxicol Lett* (2018) 287:23–30. doi:10.1016/j.toxlet.2018.01.021
71. Xu Z, Wang C, Dong X, Hu T, Wang L, Zhao W, et al. Chronic Alcohol Exposure Induced Gut Microbiota Dysbiosis and its Correlations with Neuropsychic Behaviors and Brain BDNF/Gabra1 Changes in Mice. *BioFactors* (2019) 45(2):187–99. doi:10.1002/biof.1469
72. Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host Microbiota Constantly Control Maturation and Function of Microglia in the CNS. *Nat Neurosci* (2015) 18(7):965–77. doi:10.1038/nn.4030
73. Thion MS, Low D, Silvin A, Chen J, Grisel P, Schulte-Schrepping J, et al. Microbiome Influences Prenatal and Adult Microglia in a Sex-specific Manner. *Cell* (2018) 172(3):500–16. doi:10.1016/j.cell.2017.11.042
74. Cruz C, Meireles M, Silva SM. Chronic Ethanol Intake Induces Partial Microglial Activation that Is Not Reversed by Long-Term Ethanol Withdrawal in the Rat Hippocampal Formation. *Neurotoxicology* (2017) 60:107–15. doi:10.1016/j.neuro.2017.04.005

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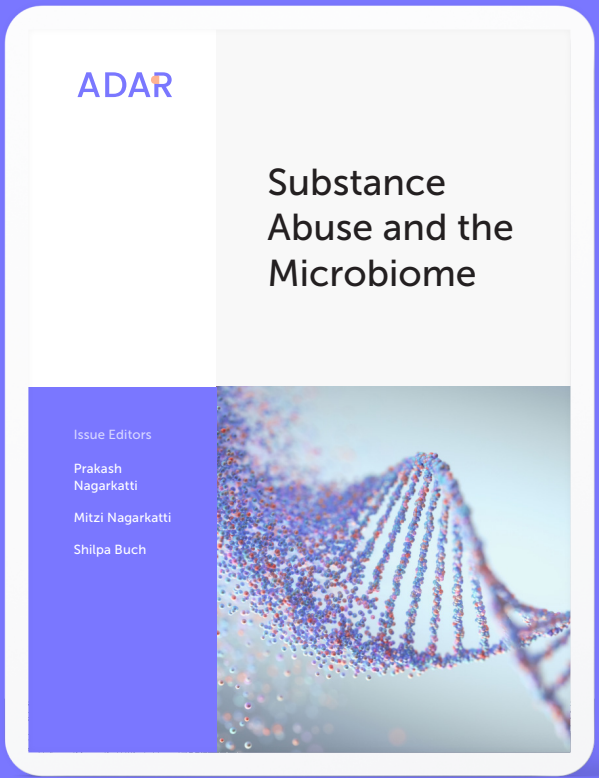

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+41 (0) 21 510 17 40
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