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A Potential Role for the Gut Microbiome in Substance Use Disorders

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Abstract

Pathological substance use disorders represent a major public health crisis with limited effective treatment options. While much work has been done to understand the neuronal signaling networks and intracellular signaling cascades associated with prolonged drug use, these studies have yielded few successful treatment options for substance use disorders. In recent years, there has been a growing interest to explore interactions between the peripheral immune system, the gut microbiome and the CNS. In this review we will present a summary of existing evidence, suggesting a potential role for gut dysbiosis in the pathogenesis of substance use disorders. Clinical evidence of gut dysbiosis in human subjects with substance use disorder as well as preclinical evidence of gut dysbiosis in animal models of drug addiction are discussed in detail. Additionally, we examine how changes in the gut microbiome and its metabolites may not only be a consequence of substance use disorders but may in fact play a role in mediating behavioral response to drugs of abuse. While much work still needs to be done, understanding the interplay of gut microbiome in substance use disorders may offers a promising avenue for future therapeutic development.

Keywords

Addiction; Microbiome; Microbiota; Dysbiosis; Metabolites; Opioid; Cocaine; Alcohol; Gut-Brain

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Conflicts of Interest:

The authors have no potential conflicts of interest to disclose.

I. Introduction

A. Drug Addiction

1. Social Costs of Drug Addiction—Pathological substance use disorders represent a major public health crisis resulting in profound personal, economic, and social cost (Murray et al. 2012; Degenhardt et al. 2014; Substance Abuse and Mental Health Services Administration 2016). Recent reports from the Office of the US Surgeon General estimate that substance use disorders cost \$442 billion annually in healthcare expenses, lost productivity, and criminal justice enforcement (Substance Abuse and Mental Health Services Administration 2016). Furthermore, substance use disorders are a leading cause of disability in the USA (Substance Abuse and Mental Health Services Administration 2016). Despite the profound burden of these disorders, the available pharmacotherapy options for afflicted patients are limited. While there are FDA-approved pharmacotherapies for alcohol and opioid use disorders, there are currently no approved medications for the treatment of psychostimulant use disorder (Shorter et al. 2015; Soyka and Müller 2017; Hofford et al. 2018).

While much work has been done to understand the neuronal signaling networks and intracellular signaling cascades associated with prolonged drug use, the biological basis of the transition from “use” to “disorder” has still not been elucidated. While the majority of this work has focused primarily on mesolimbic cells and circuits in recent years, there has been a growing understanding that there are extensive connections between the periphery and the CNS that may be playing a role in the pathogenesis of psychiatric disorders, including substance use disorders. A growing body literature has identified roles for the immune system as well as the gut microbiome in these conditions. Here, we will present a brief overview of clinical and preclinical data suggesting how gut-brain connections may be affecting the pathogenesis of substance use disorders. This will include a discussion of neuroactive metabolites, literature examining the effects of drugs of abuse on gut microbial communities, the effects of the gut microbiome on behavioral response to drugs of abuse, as well as potential mechanisms of microbiome-brain communication.

B. The Gut Microbiome

1. Characteristics of the Gut Microbiome—Interest in the impact of the gut microbiome on human health and disease has grown rapidly, with many looking to the microbiome as a target for novel therapeutics (Franzosa et al. 2014; Sun and Chang 2014; Kelly et al. 2015; Sarkar et al. 2016). The gut microbiome together contains a genome of approximately 10 million genes (approximately 150-fold larger than the human genome) (Kelly et al. 2015; Skosnik and Cortes-Briones 2016). The intestinal tract is the most heavily colonized area of the human body, with bacterial concentrations in the gut ranging from 10^2 – 10^4 cells per gram in the duodenum to 10^{11} – 10^{12} cells per gram of bowel content in the large intestine (O’Hara and Shanahan 2006; Faith et al. 2010; Kelly et al. 2015; Derrien and van Hylckama Vlieg 2015). Within each individual’s gut, a unique microbial signature of 100–1000 species is seen (Aziz et al. 2013). Although most of these species belong to the phyla Bacteroidetes and Firmicutes, other major phyla including Actinobacteria, Proteobacteria, Fusobacteria, Verrucomicrobia, and Cyanobacteria can also be found within

the gut (Sun and Chang 2014; Kelly et al. 2015). While the gut microbiome can remain relatively stable throughout one's lifetime, it can be altered through changes in diet, medications, and stress (Turnbaugh et al. 2009; Aziz et al. 2013; Faith et al. 2013; Kelly et al. 2015; Sandhu et al. 2017; Wu et al. 2017; Singh et al. 2017; Xu et al. 2017).

Throughout the host's lifetime, the microbiome promotes intestinal homeostasis, producing beneficial, bioactive metabolites and protecting the host from colonization by exogenous, pathogenic bacteria (Belkaid and Hand 2014; DeGruttola et al. 2016). Dysbiosis occurs when this homeostatic balance of the microbiome is disrupted (Cryan and Dinan 2012; Belkaid and Hand 2014). Dysbiosis can have several effects, which are often seen simultaneously, including a reduction in overall bacterial diversity as well as a relative increase in the presence of potentially pathogenic bacteria and a relative decrease in the presence of beneficial commensal bacteria (DeGruttola et al. 2016). Such changes are also often associated with altered microbial metabolism (DeGruttola et al. 2016).

Recent studies have demonstrated that alterations to the gut microbiome can have profound impacts on the brain and behavior (Sudo et al. 2004; Diaz Heijtz et al. 2011; Bercik et al. 2011; Gacias et al. 2016; Wong et al. 2016; Zheng et al. 2016; Kiraly et al. 2016). Although the exact mechanism by which the microbiome influences brain function and behavior are unknown, microbial products such as short-chain fatty acids (SCFAs) and bile acids are suspected to play a role in this process by influencing gene expression, host immune activation, blood-brain barrier (BBB) permeability, and synaptic signaling (Kelly et al. 2015; Sarkar et al. 2016).

II. Bacterial Metabolites: Potential Mechanisms of Gut-Brain Communication

A. Short Chain Fatty Acids

SCFAs are a primary byproduct of the fermentation of dietary fibers by microbes in the colon; when fermentable fibers are limited, these microbes can also metabolize dietary fats as well as amino acids from endogenous and dietary proteins (Sun and Chang 2014; Koh et al. 2016). Although colonocytes utilize most of the SCFA metabolites as an energy source, some SCFAs are transported to the liver via the hepatic portal vein; from there, they are ultimately released into systemic circulation (Sun and Chang 2014; Joseph et al. 2017). Local SCFAs promote enhanced gut epithelial barrier function as well as immune homeostasis (Smith et al. 2013; Morrison and Preston 2016). Circulating SCFAs, especially butyrate and to a lesser extent propionate and acetate, are known to cross the BBB, allowing them to exert their effects directly on neurons and glial cells (Joseph et al. 2017). Microbiome-derived SCFAs have been shown to have widespread effects on brain function, altering the maturation and function of microglia as well as the permeability of the BBB (Braniste et al. 2014; Erny et al. 2015). Systemic administration of sodium butyrate has also been shown to influence behavior, producing antidepressant-like effects in rats (Sun et al. 2016). Although the exact mechanisms by which SCFAs influence the brain and behavior is unknown, multiple reports point to a potential role for SCFAs as histone deacetylase (HDAC) inhibitors (Stilling et al. 2014; Koh et al. 2016). SCFAs remain an important target

for future studies examining the role of the gut microbiome in substance abuse disorder and other psychiatric diseases (Kiraly et al. 2016).

B. Bile Acids

Bile acids are the key metabolites of cholesterol catabolism (Chiang 2013). They serve as important signaling molecules, influencing nutrient absorption and metabolism as well as gut immune homeostasis (Chiang 2013; van de Wouw et al. 2017; Li et al. 2018). Primary bile acids—specifically cholic acid (CA) and chenodeoxycholic acid (CDA) in humans—are produced by the liver, stored in the gallbladder, and secreted into the duodenum following food consumption (Chiang 2013; van de Wouw et al. 2017). Gut microbes within the small intestine deconjugate bile acids, preventing their reuptake by the small intestine and allowing their entry into the colon, where they are metabolized into secondary bile acids, such as deoxycholic and lithocholic acids (Lefebvre et al. 2009; Staels and Fonseca 2009). Bile acids and the gut microbiome influence each other in a bidirectional manner (Wahlström et al. 2016; van de Wouw et al. 2017). Microbial composition and metabolites can impact the synthesis of primary bile acids in the liver, the reabsorption of primary bile acids in the ileum, and the conversion into secondary bile acids in the colon (Riottot and Sacquet 1985; Sayin et al. 2013; van de Wouw et al. 2017). In turn, bile acid signaling can influence microbial composition (Ursell et al. 2014; Wang and Roy 2017; Li et al. 2018).

C. Tryptophan Metabolites

Tryptophan is an essential amino acid that is primarily derived from dietary protein (O'Mahony et al. 2015; Agus et al. 2018; Roager and Licht 2018). Following absorption by the gut, tryptophan enters circulation and is able to cross the BBB where it can serve as a key precursor for serotonin synthesis (O'Mahony et al. 2015; Waclawiková et al. 2018). Tryptophan metabolism proceeds along three main metabolic pathways within the gut (Agus et al. 2018), and the tight balance of these pathways governs the bioavailability of both circulating tryptophan and its metabolites, many of which regulate immune homeostasis and inflammatory response within the gut and the CNS (Waclawiková et al. 2018).

1. The Kynurenine Pathway—Host immune and epithelial cells within the gut metabolize approximately 90–95% of ingested tryptophan along the kynurenine pathway via indoleamine 2,3-dioxygenase 1 (IDO1), the first and rate-limiting enzyme within this pathway (Agus et al. 2018; Gao et al. 2018; Sofia et al. 2018). Within the gastrointestinal tract, IDO1 acts as brake to host inflammatory response, and it is upregulated to counteract excessive tissue damage in subjects with active inflammatory bowel disease (IBD) (Gupta et al. 2012; Sofia et al. 2018). Subjects with active IBD also display increased circulating kynurenine and its metabolite kynurenic acid relative to circulating tryptophan (Gupta et al. 2012; Sofia et al. 2018). Notably, peripheral kynurenine is able to cross the BBB (O'Mahony et al. 2015). Within the gut and the CNS, kynurenine is further metabolized into two main metabolites: kynurenic acid, which has neuroprotective effects, and quinolinic acid, which has excitotoxic effects (Vécsei et al. 2013; O'Mahony et al. 2015).

2. Aryl Hydrocarbon Receptor Ligands—Approximately 4–6% of the ingested tryptophan is metabolized by the gut microbiome into several molecules, including

tryptamine and indole, which serve as ligands for the aryl hydrocarbon receptor (AhR) (Levy et al. 2016; Agus et al. 2018; Gao et al. 2018; Roager and Licht 2018). AhR ligands are associated with decreased hepatic and intestinal inflammation as well as improved intestinal barrier function in rodent models (Levy et al. 2016; Gao et al. 2018; Krishnan et al. 2018; Roager and Licht 2018). Within the CNS, microbially-derived tryptophan metabolites activate AhR signaling in astrocytes, resulting in suppressed inflammation in a mouse model of autoimmune encephalitis (Rothhammer et al. 2016).

3. Serotonin—Third, host enterochromaffin cells convert approximately 1–3% of the ingested tryptophan into serotonin (Agus et al. 2018; Gao et al. 2018; Waclawiková et al. 2018). In response to stimulation of the GI tract, enterochromaffin cells release serotonin onto the 5-HT₃ receptor terminals of vagal afferents, modulating vagal signaling (Breit et al. 2018). Serotonin synthesis is further modulated by the gut microbiome via signaling from its metabolites, bile acids and SCFAs, on host enterochromaffin cells (Reigstad et al. 2015; Yano et al. 2015). Notably, competing metabolism of tryptophan into AhR ligands, kynurenine and its metabolites, limits the bioavailability of circulating tryptophan for serotonin synthesis (O'Mahony et al. 2015). Some groups have suggested this relative tryptophan depletion may contribute to increased risk of depression in vulnerable populations (Maes et al. 2009; O'Mahony et al. 2015).

III. The Gut Microbiome in Models of Psychiatric Diseases

While data from human subjects is critical for translational research into gut-brain connections, much of our current mechanistic understanding of gut-brain signaling derives from the use of rodent models. Rodent models offer excellent tools to interrogate the role of the gut microbiome in addiction and other psychiatric diseases, as they allow for specific and targeted manipulations of microbial content overlaid with translationally-relevant behavioral models. Below is a brief primer on the most commonly used methods of microbiome manipulation in rodent models of psychiatric disease.

For investigation of the effects of the gut microbiome on behavior, one of the more common paradigms is to use germ-free animals that are raised in a sterile environment from birth and lack any internal or external microbiome (Faith et al. 2010). Germ-free animals are often compared to specific pathogen-free (SPF) or conventional animals, both of which possess a normal, complex microbiome (Umesaki 2014). Germ-free animals can be colonized with both simple and complex microbial communities from donor animals or human subjects in a process known as conventionalization; this allows researchers to interrogate the contribution of a defined donor microbiome to a phenotype of interest (Faith et al. 2010). Seminal work showed that germ-free mice exhibited increased corticosterone levels in response to restraint stress compared to controls, and that these effects could be reversed through gut bacterial colonization via the probiotic *Bifidobacterium infantis* (Sudo et al. 2004; Sarkar et al. 2016). Further studies demonstrated that germ-free mice exhibit reduced anxiety-like behavior compared to controls, and that fecal microbial transplants between mouse strains could transpose strain-specific anxiety-like behaviors from one strain to another (Diaz Heijtz et al. 2011; Bercik et al. 2011a). Similar effects were seen in a mouse model of depression-like behavior with germ-free mice demonstrating reduced immobility in the forced swim test

compared to conventionally raised control mice; when those germ-free mice were colonized with fecal microbiome samples from patients with Major Depressive Disorder, they demonstrated increased depression-like behaviors compared to germ-free mice that were colonized with fecal microbiome samples from healthy controls (Zheng et al. 2016).

Despite their benefits, germ-free animals also have significant limitations. The gut microbiome has profound effects on the developing immune system and gastrointestinal tract, as well as additional effects on microglia and oligodendrocyte functioning, making assessments complicated in adult animals (Mortha et al. 2014; Erny et al. 2015; Zhang et al. 2015; Hoban et al. 2016). Moreover, studies in germ-free animals are costly and technically challenging due to the need to maintain animals in a completely sterile environment. Antibiotic-induced depletion of the gut microbiome allows for bacterial community perturbations without the confounding developmental issues of germ-free mice. Studies examining the role of the microbiome in antibiotic-treated mice recapitulate earlier findings that germ-free animals exhibit decreased depression- and anxiety-like behavior in models such as forced swim, step down, and social interaction tests (Bercik et al. 2011a; Gacias et al. 2016; Wong et al. 2016). Overall, antibiotic and germ-free studies are often best performed in tandem in a complimentary manner. Antibiotic-treatment studies are useful to assess the broad effects of microbiome depletion and microbial metabolite restoration on a phenotype of interest. However, antibiotic treatment studies are limited in the degree of mechanistic insight they can provide. Subsequent studies in conventionalized germ-free animals are best used to assess mechanistic contributions of particular microbial strains.

IV. The Gut Microbiome in Addiction

Although research suggests a role of the gut microbiome in animal models of depression and anxiety disorders, mechanistic studies on the impact of the gut microbiome on behavioral response to drugs of abuse remain quite limited (Kiraly et al. 2016; Kang et al. 2017; Lee et al. 2018). Nonetheless, there is growing clinical and preclinical evidence of bacterial dysbiosis in response to drugs of abuse, which should be discussed and investigated further (Volpe et al. 2014; Temko et al. 2017; Wang and Roy 2017; Hillemacher et al. 2018; Hofford et al. 2018). Future studies on the impact of the microbiome in substance abuse disorders may help provide insight into shared molecular mechanisms that accelerate disease pathogenesis in comorbid mood and anxiety disorders as well.

A. Alcohol

1. Clinical Literature—Some preliminary studies have demonstrated examples of gut dysbiosis in human patients with substance use disorders, though most have focused on alcohol users. A summary of currently published findings of changes in the composition of the gut microbiome from human subjects with substance use disorders and in animal models of substance use disorders is presented in Table 1. Findings by Kirpich et al. (2008) demonstrate decreased Firmicutes (specifically *Lactobacilli* and *Enterococci*) and Actinobacteria (specifically *Bifidobacteria*) in stool cultures from alcoholics. Volpe et al. (2014) examined the effects of alcohol in cocaine and non-cocaine users with and without a history of HIV; after controlling for the effects of HIV infection and cocaine use, they

observed decreased relative abundance of Firmicutes as well as increased relative abundance of Bacteroidetes in the stool of alcohol users compared to healthy controls. These findings are consistent with rodent studies by Yan et al. (2011). One conflicting study demonstrated lower relative abundance of Bacteroidetes as well as increased relative abundance of Proteobacteria in alcoholic subjects, but this study analyzed the mucosa-associated microbiome rather than stool (Mutlu et al. 2012). The mucosa-associated microbiome is considerably different from that of the gut lumen (Sun and Chang 2014). Importantly, polyphenol-containing alcoholic beverages such as red wine appear to have differing effects on the microbiome diversity compared to other alcoholic beverages (Gibson et al. 1995; Engen et al. 2015). Thus, variability in the types of alcoholic beverages consumed, the amount and frequency of alcohol intake as well as the presence or absence of concomitant alcoholic liver disease may contribute to differences in the microbiome profiles of these cohorts. Interactions between the gut microbiome and liver diseases are well-examined and reviewed elsewhere (Schnabl and Brenner 2014; Betrapally et al. 2017; Acharya et al. 2017; Zuo et al. 2017; Adolph et al. 2018; Tripathi et al. 2018).

Multu et al. (2012) estimate that approximately 31% of alcoholics are dysbiotic, with Leclercq et al. (2014b) showing that the overall gut bacterial load was decreased only in alcohol-dependent subjects with increased intestinal permeability compared to alcohol-dependent subjects without increased intestinal permeability or age- and BMI-matched social drinker controls (<20g alcohol/day). Notably, the alcohol-dependent patients with altered intestinal permeability exhibited persistent symptoms of increased anxiety, depression, and alcohol craving—even following a short-term detoxification program (Leclercq et al. 2014b). These findings are consistent with additional studies, which demonstrated associations between increased intestinal permeability and inflammation to depression and alcohol-craving in non-cirrhotic alcohol-dependent patients (Leclercq et al. 2012, 2014a). Further studies demonstrated that gut-derived microbial products, such as lipopolysaccharide (LPS) and peptidoglycan (PGN), are elevated in the blood of alcohol-dependent subjects, and this elevation is associated with increased expression of their respective receptors, Toll-like receptor 4 (TLR4) and TLR2, as well as increased expression of proinflammatory cytokines (Leclercq et al. 2014a). This elevation in LPS and PGN may be due to altered gut permeability in response to alcohol use. Interestingly, binge drinking in healthy adults with no prior history of alcohol abuse disorder is also associated with a modest increase in serum LPS and 16S rDNA as well as elevated levels of inflammatory cytokines, indicating that acute alcohol exposure may also alter gut permeability and facilitate bacterial translocation into the circulatory system (Bala et al. 2014). Rao (2009) suggests that acute alcohol exposure results in transient effects to gut barrier function, while chronic alcohol exposure results in persistent epithelial barrier dysfunction.

2. Preclinical Literature—Consistent with the clinical literature, preclinical studies in rodent models of alcohol abuse also indicate that alcohol induces gut dysbiosis, though the exact microbial communities that are altered varies from study to study—likely due to differences in alcohol administration regimen as well as concomitant liver disease (Mutlu et al. 2009; Yan et al. 2011; Bull-Ottersen et al. 2013; Zhang et al. 2015; Peterson et al. 2017; Samuelson et al. 2017; Xiao et al. 2018; Xu et al. 2018; Zallar et al. 2019). For instance, one

group found that chronic alcohol feeding in mice resulted in decreased relative abundance of both Bacteroidetes and Firmicutes as well as an increased relative abundance of Proteobacteria and Actinobacteria in stool samples (Bull-Otterson et al. 2013). Fan et al. (2018) found that chronic alcohol treatment resulted in a significant increase in both the Bacteroidetes and Proteobacteria phyla as well as a significant decrease in the Firmicutes phyla in the colonic contents of alcohol-dependent rats compared to control groups. Differences in these studies may be attributable differences in sample type and location (stool versus colon contents) (Bull-Otterson et al. 2013; Fan et al. 2018). Furthermore, work by Wang et al. (2018b) suggests that alcohol use not only affects the composition of the gut microbiome, but also its production of bioactive metabolites such as serotonin and bile acids.

To control for confounding effects of inconsistent alcohol dose resulting of variable food consumption amongst subjects, Peterson et al. (2017) instead investigated the effects of chronic intermittent vaporized alcohol (CIE) exposure on the composition of the gut microbiome in C57BL/6J mice. They found an overall decrease in alpha diversity in the CIE mice. Relative abundance of the *Alistipes* genus was increased, while relative abundance of *Clostridium* IV and *Clostridium* XIVb, *Dorea*, and *Coproccoccus* genera were decreased in the CIE mice compared to controls (Peterson et al. 2017). Interestingly, supplementation with either *Lactobacillus GG*, a probiotic, or oats, a prebiotic, prevented alcohol-induced changes in bacterial diversity in a rat model of alcoholic steatohepatitis (Mutlu et al. 2009).

Animal models indicate that alcohol disrupts the epithelial barrier by increasing oxidative stress in the intestine, resulting in damage to the tight junction proteins of the gut (Rao et al. 2004; Engen et al. 2015; Bishehsari et al. 2017). Gut bacteria are then able to translocate to the circulatory system, resulting in elevated levels of toxic bacterial byproducts such as LPS and PGN (Hillemacher et al. 2018). Blednov et al. (2011) suggested that LPS may play a role in addiction-like behavior in a continuous two-bottle free choice test and that this effect may be mediated through TLR4 signaling, but follow up studies using the continuous two-bottle free choice test as well as operant self-administration and a limited-access drinking in the dark (DID) binge drinking model have not reproduced these findings (Harris et al. 2017; Lainiola and Linden 2017). Additionally, Jadhav et al. (2018) trained rats to self-administer alcohol and stratified them as vulnerable or resilient to alcohol use disorder (AUD). Changes in the gut flora were significantly correlated with increased impulsivity, vulnerability to AUD, and striatal dopamine 1 receptor expression as well as decreased striatal dopamine receptor 2 expression (Jadhav et al. 2018). Further research is needed to understand whether gut dysbiosis and elevated inflammation in alcohol-dependent subjects is a consequence of increased alcohol craving and consumption or whether the changes to the gut microbiome and its immunomodulatory metabolites may drive alcohol-seeking behavior.

B. Psychostimulants

1. Clinical Literature—Human studies of dysbiosis in cocaine users are sparse, but one group compared microbial diversity in stool samples from patients with cocaine use disorder to those of healthy controls, examining patients with and without HIV (Volpe et al. 2014). Compared to healthy controls, cocaine use disorder was associated with decreased relative abundance of Firmicutes phylum and increased relative abundance of Bacteroidetes phylum

in stool samples of patients with and without HIV (Volpe et al. 2014). Cocaine use disorder independently predicted gut dysbiosis with increased relative abundance of the Bacteroidetes phylum (Volpe et al. 2014).

2. Preclinical Literature—Because cocaine is frequently smoked, Scorza et al. (2019) examined the effects of volatilized cocaine as well as caffeine and phenacetin, two common adulterants in cocaine, by exposing rats to the fume of these compounds for 14 days. Significant shifts in fecal microbial communities were noted in cocaine and phenacetin-treated rats compared to controls, and stool samples from cocaine-treated rats exhibited elevated microbial expression of genes involved in the synthesis of the SCFA butyrate. Caffeine-treatment alone, however, had little effect on the composition of the fecal microbiome. Further research should examine whether these changes in gut microbial composition are associated with altered behavioral response to cocaine or phenacetin (Scorza et al. 2019).

A recent study by our lab interrogated the role of the gut microbiome in a translational C57BL/6J mouse model of drug addiction. We found that a cocktail of Bacitracin, Neomycin, Vancomycin, and Pimaricin in the drinking water successfully knocked down gut bacterial content without altering cocaine metabolism or serum corticosterone levels (Kiraly et al. 2016). Antibiotic-treated mice demonstrated increased sensitivity to injection of intraperitoneal cocaine in both conditioned place preference (CPP) and locomotor sensitization tasks. While both control and antibiotic-treated mice formed a place preference at a high dose of 10 mg/kg cocaine, only antibiotic-treated mice forming a robust place preference to a low dose of cocaine (5mg/kg) (Kiraly et al. 2016). Likewise, while both control and antibiotic-treated mice exhibited locomotor sensitization following five days of high dose cocaine, only the antibiotic-treated mice demonstrated a sensitized locomotor response following five days of the low dose of cocaine (Kiraly et al. 2016). Importantly, antibiotic treatment in the absence of cocaine had no effect on CPP or locomotor sensitization (Kiraly et al. 2016). When their antibiotic drink was supplemented with SCFAs, microbial metabolites known to act as HDAC inhibitors, antibiotic-treated mice demonstrated a CPP response to low dose cocaine similar to that of control mice, indicating that SCFAs are crucial for the behavioral effects of the microbiome (Kiraly et al. 2016). Interestingly, Lee et al. (2018) found pretreatment with antibiotics prior to cocaine CPP resulted in decreased formation of cocaine CPP to a dose of 10mg/kg cocaine. Interestingly, these two papers used different antibiotic regimens, and different antibiotic pretreatment intervals and found disparate effects. This discrepancy suggests that the duration and extent of microbiome depletion is likely important for interpretation of behavior and highlights how far we have to go to fully understand the complexities of gut-brain interactions in influencing behavior. A summary of currently published results examining the effects of microbiome manipulations on behavioral response to drugs of abuse is presented as Table 2.

Ning et al. (2017) examined the gut microbiome of rats that were subjected to methamphetamine CPP compared to controls, observing increased relative abundance of several taxa from Proteobacteria and Fusobacteria phyla in methamphetamine-treated animals compared to saline-treated controls. Within the Firmicutes phylum, Bacillaceae and Ruminococcaceae were more abundant in the methamphetamine-treated animals, and

Acidaminococcaceae was more abundant in the saline-treated animals (Ning et al. 2017). Levels of the SCFA propionate were also found to be significantly decreased in the caecal contents of methamphetamine-treated rats compared to controls. Propionate, along with SCFAs butyrate and acetate, was found to be a key mediator of the behavioral effects of the microbiome on response to cocaine in our earlier work (Kiraly et al. 2016). Although this study demonstrates dysbiosis and altered metabolism in methamphetamine-treated rats compared to controls, more mechanistic research utilizing antibiotics and/or fecal microbial transplants is needed to determine the effects of the microbiome on behavioral response to methamphetamine.

C. Opioids

1. Clinical Literature—Emerging evidence points to an association between gut dysbiosis and opioid use in human subjects, but such studies have often been limited by small sample size as well as potentially confounding factors such as differing patient comorbidities, inconsistencies in opioid dose and duration of use, as well as concomitant non-opioid drug use (Vincent et al. 2016; Zhernakova et al. 2016; Acharya et al. 2017; Xu et al. 2017; Barengolts et al. 2018). A recent study by Xu et al. (2017) compared the gut flora of 48 healthy controls (HCs) to those of 45 patients with a history of substance use disorders (SUDs). The SUD group was heterogeneous with users of the opioid heroin as well as psychostimulants including methamphetamine (also known as ice) and ephedrine; some SUDs used heroin + ephedrine or heroin + ethamphetamine, and all SUDs also partook in daily smoking and alcohol consumption. Although significant differences were seen in the intestinal microbiome of SUDs compared to HCs, these changes were not associated specifically with use of heroin, methamphetamine, or ephedrine. It is possible that these findings instead reflect the effects of alcohol and tobacco use (seen in all SUDs) on the gut microbiome, or perhaps changes in the lifestyles and diets of SUDs compared to controls. (Xu et al. 2017) The functional diversity of the putative metagenomes from the microbiome of SUDs and HCs was also analyzed; SUDs demonstrated enrichment of pathways involved in translation, cell growth and death, and DNA replication and repair, as well as a decline in pathways involved in metabolism, cellular processes, and signaling. Thus, SUD is associated not only with changes in gut microbial communities, but also in the functional repertoire of those communities (Xu et al. 2017).

Although additional studies have reported associations between gut dysbiosis and opioid use, these studies did not examine patients with a history of SUD. In a cohort of 98 hospitalized patients, opioid use was associated with an increase in alpha diversity, possibly due to opioid-induced delays in colon transit time facilitating increased bacterial growth within the gut (Vincent et al. 2016). An association between opioid use and altered beta diversity was also recently noted in a study of 1135 subjects (Zhernakova et al. 2016). Two other studies examining distinct patient populations (MELD-matched cirrhotic subjects and diabetic African American males, respectively) also demonstrated differences in the gut microbial communities of opioid users compared to non-opioid users (Acharya et al. 2017; Barengolts et al. 2018).

Clinical literature suggests that opioid use is associated with worsening patient outcomes and increased risk of sepsis (Glattard et al. 2010; Meng et al. 2015a; Acharya et al. 2017; Zhang et al. 2018). Further research is needed to understand how gut dysbiosis and altered microbial metabolism may impact response to the antinociceptive and rewarding properties of opioid medications in human subjects.

2. Preclinical Literature—Animal models provide further evidence that opioids induce significant alterations in gut microbial communities and their metabolites, pushing the gut microbiome towards a more dysbiotic phenotype. Dysbiosis in response to opioid treatment can occur rapidly; within 1 day of morphine pellet implantation there is a dramatic shift in the gut microbiome of mice compared to placebo-treated animals (Wang et al. 2018a). Non-human primate studies suggest that the resultant dysbiosis is maintained with long-term morphine treatment, despite the development of increasing tolerance to the drug (Sindberg et al. 2018). At the phylum level, morphine treatment results in a relative reduction of Gram-negative Bacteroidetes and a relative expansion of Gram-positive Firmicutes, particularly potentially pathogenic bacterial families such as Enterococcaceae and Staphylococcaceae (Meng et al. 2013, 2015a; Banerjee et al. 2016; Kang et al. 2017; Wang et al. 2018a). Of note, such reductions in the Bacteroidetes/Firmicutes ratio are correlated with increased systemic inflammatory biomarkers in obese patients (Verdam et al. 2013). Animal models strongly suggest that opioid use is associated with impaired gut permeability, resulting in bacterial translocation and ultimately sepsis (Hilburger et al. 1997; Feng et al. 2006; Breslow et al. 2011; Meng et al. 2015a; Banerjee et al. 2016; Wang and Roy 2017).

Emerging evidence suggests that the gut microbiome may modulate the behavioral effects of opioids, though such studies have been limited. Wang et al. (2018) found that inoculation of *E. faecalis* via oral gavage was sufficient to augment analgesic tolerance to morphine in a tail flick assay. Kang et al. (2017) treated mice with an oral gavage of a broad-spectrum antibiotic cocktail vs saline control and implanted either a morphine pellet or a placebo pellet. Tail-immersion and acetic acid stretch assays were performed to measure nociceptive response to morphine (Kang et al. 2017). Broad spectrum antibiotic treatment prevented the development of antinociceptive tolerance to morphine, a major limiting factor in clinical use of opioid medications for pain management (Kang et al. 2017). Oral vancomycin, which has low bioavailability and is not absorbed from the intestine, was sufficient to recapitulate the effects of broad-spectrum antibiotics, indicating that Gram-positive bacteria may be mediating nociceptive response to morphine (Kang et al. 2017). Notably, morphine metabolism did not appear to be altered in this study, as there was no difference in the brain morphine concentrations of antibiotic and placebo-treated mice (Kang et al. 2017).

Conflicting results were seen from Lee et al. (2018), however. They also treated mice with antibiotics and measured nociceptive response to morphine in the tail immersion assay, finding that antibiotic-treated animals did develop tolerance to the antinociceptive properties of morphine (Lee et al. 2018). These conflicting results may be due to differences in antibiotic cocktail and duration of use, as well as differences in the regimen of morphine administration. Kang et al. (2017) used a continual release 75mg morphine pellet for their study. Lee et al. (2018) examined both a 25 mg pellet as well as intermittent, escalating intraperitoneal injections of morphine administered twice daily. Intermittent morphine

injections are associated with a withdrawal period, which can have unique immunomodulatory effects (Eisenstein et al. 2006). Examining both intermittent morphine injections as well as continual release pellets allowed Lee et al. (2018) to separate the effects of morphine withdrawal from those of the drug itself. They found similar results in tolerance for mice treated with continual morphine release via a 25 mg pellet compared to mice treated with intermittent morphine injections (Lee et al. 2018). However, they only saw changes in microglial activation in mice that experienced a morphine withdrawal period, either due to intermittent morphine administration or morphine pellet removal (Lee et al. 2018). Future studies that incorporate morphine withdrawal may provide a more translational model, as withdrawal periods are common in drug users (Eisenstein et al. 2006).

D. Microbiome and Addiction: Possible Links

1. Short-Chain Fatty Acids—Emerging evidence suggests that SCFAs may exert their effects through epigenetic alterations to chromatin structure (Stilling et al. 2014; Koh et al. 2016). Butyrate, in particular, is known to be a potent HDAC inhibitor, and other SCFAs such as propionate have also demonstrated HDAC inhibition (Stilling et al. 2014; Koh et al. 2016). The role of SCFAs as HDAC inhibitors is especially critical, because histone acetylation serves as an essential switch, mediating the transition between heterochromatin (condensed, inaccessible, deacetylated) and euchromatin (decondensed, accessible, acetylated); by preventing histone deacetylation, HDAC inhibitors such as SCFAs potentially facilitate increased transcription of repressed genes (Bagot et al. 2014; Peña et al. 2014; Koh et al. 2016). Notably, Braniste et al. (2014) found that 3 days of oral gavage of the SCFA sodium butyrate was associated with increased expression of the tight junction protein occludin as well as increased histone acetylation in brain tissues. These findings were later confirmed by Sun et al. (2016), who likewise found that two weeks of intragastric administration of sodium butyrate was associated with increased expression of tight junction proteins.

Although prior studies have demonstrated that SCFAs alter behavioral response to cocaine, further studies are needed to determine if SCFAs are exerting their effects through HDAC inhibition and, if so, what gene targets they may be acting upon (Kiraly et al. 2016). The role of SCFAs as HDAC inhibitors in animal models of drug addiction represents a promising area of research, as prior studies have demonstrated that changes in histone acetylation at specific gene loci can drive molecular and ultimately behavioral responses to multiple drugs of abuse (Romieu et al. 2008, 2011; Malvaez et al. 2010; Rogge and Wood 2013; Kennedy et al. 2013; Kyzar and Pandey 2015; Heller et al. 2016). Further studies should examine whether antibiotic-induced knockdown of SCFA-producing bacteria results in reduced HDAC inhibitor activity at critical gene loci that mediate behavioral effects of drugs of abuse.

2. Bile Acids and Other Metabolites—Energy sources from the host's diet are fermented by the gut microbiome to support their growth (Ursell et al. 2014; Li et al. 2018). These microbial metabolites not only provide nutrients for the host; they can also influence gut immune homeostasis, host metabolism, and the composition of the gut microbiome itself

(Ursell et al. 2014; Wang and Roy 2017; Li et al. 2018). Compared to placebo treatment, morphine treatment induces a gradual but distinct shift in microbial metabolome of mice in a time-dependent manner, with a decrease seen in bile acid metabolites and an increase seen in phosphatidylethanolamine and saturated fatty acid metabolites in response to morphine treatment (Wang et al. 2018a). These effects were antagonized by naltrexone (Wang et al. 2018a). Additional studies have also demonstrated that morphine treatment results in a decrease in primary and secondary bile acids (Banerjee et al. 2016; Sindberg et al. 2018). These findings are especially important, because bile acids can prevent the overgrowth and translocation of gut bacteria. Bile acids can also exert effects on the gut microbiome by altering gut motility (Kang et al. 2017).

Strict anaerobes such as *Bacteroides* and *Bifidobacteria* produce β -glucuronidase (GUS) enzymes, which play an essential role in bile acid metabolism (Stain-Textier et al. 1998). Recent evidence from Wang et al. (2018) suggests that they impact morphine pharmacokinetics as well. Bacterial β -glucuronidase hydrolyzes morphine metabolites M6G and M3G, allowing them to be reabsorbed as morphine. Morphine treatment is associated with decreased abundance of Bacteroidales communities as well as decreased levels of deconjugated M3G. These changes result in reduced enterohepatic recirculation of morphine into the circulatory system, reduced bioavailability of morphine, and ultimately reduced antinociceptive efficacy of morphine (Wang et al. 2018a).

Research by Reddy et al. (2018) in a mouse model of bariatric surgery indicates that bile acids can also impact behavioral response to cocaine. GB-IL (gallbladder to ileum) mice underwent ligation of the common bile duct as well as anastomosis of the gallbladder to the ileum in order to chronically elevate circulating levels of bile acids, while GB-D (gallbladder to duodenum) control mice underwent anastomosis of the gallbladder to the duodenum. GB-IL mice exhibited reduced CPP and locomotor sensitization to cocaine. There were no significant differences in the microbiome composition of the two surgical groups, nor were there significant differences in cocaine bioavailability in the striatum. The behavioral effects seen in the GB-IL mice appeared to be mediated by signaling of the bile acid receptor TGR5 (Reddy et al. 2018).

3. Epithelial Barrier Dysfunction, Bacterial Translocation, and Inflammation

—Both clinical and preclinical literature suggest that alcohol disrupts the epithelial barrier by increasing oxidative stress in the intestine, resulting in damage to the tight junction proteins of the gut (Rao et al. 2004; Keshavarzian et al. 2009; Engen et al. 2015; Bishehsari et al. 2017). Bacteria are able to translocate in to the circulating blood supply, resulting in a systemic immune response (Leclercq et al. 2012, 2014a, b; Bala et al. 2014; Hillemecher et al. 2018). Similar to the alcohol literature, animal models suggest that chronic opioid use results in impaired intestinal epithelial barrier function that leaves the gut susceptible to bacterial translocation and ultimately sepsis (Hilburger et al. 1997; Ocasio et al. 2004; Feng et al. 2006; Breslow et al. 2011; Babrowski et al. 2012; Meng et al. 2013). Commensal microbes can sense morphine in the environment and develop a virulent phenotype in response to morphine treatment. *P. aeruginosa* is able to sense the presence of morphine in the local environment and respond by expressing mucus-suppressing PA-I lectin proteins, which are known to impair gut barrier function and cause gut-derived sepsis (Laughlin et al.

2000; Babrowski et al. 2012). Furthermore, opioids directly act on enteric neurons to slow colonic transit, which can in turn alter the gut microbiome due to changes in gut motility (Rhee et al. 2009; Smith et al. 2012; Zhu et al. 2014; Meng et al. 2015b; Parthasarathy et al. 2016). Impaired gut motility increases the risk of bacterial translocation across the gut epithelial barrier, which can induce inflammation and ultimately gut-derived sepsis (Balzan et al. 2007; Meng et al. 2015a; Banerjee et al. 2016). When bacteria come in contact with host epithelial cells, TLRs trigger immune responses, including release of proinflammatory cytokines (Kelly et al. 2005).

Microbial metabolites such as SCFAs and tryptophol, an indole derivative, also modulate host cytokine production (Schirmer et al. 2016). Circulating cytokines are able to cross the blood brain barrier and then act on the brain to modulate behavioral response to drugs of abuse (Yarlagadda et al. 2009; Pan et al. 2011). Indeed, emerging evidence suggests that changes in immune activation may not only be a consequence of substance use disorders, but potentially a driving force in the development and persistence of substance use disorders (Hofford et al. 2018). Intraperitoneal injection of proinflammatory TNF- α inhibited the rewarding and sensitizing effects of morphine (Niwa et al. 2007). Likewise, release of TNF- α by microglia was found to inhibit behavioral sensitization to cocaine (Lewitus et al. 2016). However, TLR4 signaling through the proinflammatory cytokine interleukin 1 beta (IL-1 β) was found to drive behavioral response to cocaine (Northcutt et al. 2015). Treatment with different anti-inflammatory cytokines can also result in either enhanced or reduced response to drugs of abuse. Intraperitoneal injection of granulocyte colony stimulating factor (G-CSF), which is primarily anti-inflammatory, resulted in enhanced locomotor sensitization to cocaine in mice as well as increased motivation to self-administer cocaine in rats (Calipari et al. 2018). In contrast, plasmid delivery of anti-inflammatory interleukin 10 (IL-10) into the NAc attenuated self-administration of the opioid remifentanyl in rats (Lacagnina et al. 2017). Overall, it appears that cytokines can modulate behavior in animal models of drug addiction, but the effects are specific to individual cytokines; not all proinflammatory cytokines produce consistent behavioral effects, nor do all anti-inflammatory cytokines (Hofford et al. 2018).

4. Vagus Nerve—The vagus nerve is a key branch of the parasympathetic nervous system, providing a means of bidirectional communication between the brain and the gut as recent evidence suggests that the vagus nerve is sensitive to signals from the microbiome (Sarkar et al. 2016; Bonaz et al. 2018). Because the vagal afferent fibers do not cross the gut epithelial layer, the vagus nerve does not come into direct contact with the gut microbiome (Wang and Powley 2007; Bonaz et al. 2018). However, the vagus nerve does receive and respond to signals from bacterial metabolites such as SCFAs (Lal et al. 2001). Additionally, enteroendocrine cells of the gut epithelium make direct contact with the bacteria within the gut lumen and relay signals to the vagus nerve through the release of serotonin, cholecystokinin, peptide YY, and glucagon-like peptide-1 (Raybould 2010; Bonaz et al. 2018). Some evidence suggests that vagal nerve integrity is necessary to mediate the behavioral effects of probiotic treatment in animal models of anxiety-like behavior (Bravo et al. 2011; Bercik et al. 2011b). However, other studies suggest that the gut microbiome can influence behavior and hippocampal BDNF expression independent of the vagus nerve

(Bercik et al. 2011a). No studies have yet examined whether vagal nerve integrity is necessary for mediating the effects of the gut microbiome on animal models of drug addiction.

Some preliminary evidence suggests that the vagus nerve signaling may be an interesting target for future studies on substance use disorders. In a study by Childs et al. (2017), rats self-administered cocaine and were then treated with vagus nerve stimulation (VNS) or sham stimulation during extinction training. VNS-treatment facilitated extinction training and reduced cocaine-seeking behavior in a cue-induced reinstatement paradigm (Childs et al. 2017). More recent work by Han et al. (2018) proposes a role for the vagus nerve in brain reward circuitry. They found that optogenetic stimulation of the right vagal sensory ganglion induced dopamine release from the substantia nigra into the dorsal striatum of mice. Additional experiments demonstrated that this stimulation was rewarding in both flavor and place preference tasks and that mice would nose-poke for self-stimulation of the vagus nerve (Han et al. 2018). Importantly, these studies rely on electrical and optogenetic stimulation of the vagus nerve. Further work is needed to understand whether the gut microbiome may be able to exert similar effects on vagus nerve activity.

5. Microglia—Microglia, the resident myeloid cells of the brain, use their motile, stellate processes to survey the local microenvironment (Aguzzi et al. 2013; Ayata et al. 2018). In response to CNS infection or injury, activated microglia release cytokines and phagocytize cellular debris, promoting a return to tissue homeostasis (Miguel-Hidalgo 2009; De Biase et al. 2017). During both postnatal development and adulthood, phagocytotic microglia also perform synaptic pruning and circuit remodeling (Schafer and Stevens 2013, 2015). The gut microbiome plays a critical role in shaping microglial function and promoting microglial homeostasis (Erny et al. 2015; Thion et al. 2018). Microglia from germ-free mice exhibit altered morphology and an immature phenotype that results in impaired innate immune response (Erny et al. 2015). Microglial deficits were also observed in antibiotic-treated mice as well as mice colonized with a non-complex Schaedler flora consisting of three bacterial strains (Erny et al. 2015). Microglia function was restored through further colonization with a complex microbiome or supplementation of the microbial metabolites SCFAs (Erny et al. 2015). Recent work by Thion et al. (2018), demonstrates that the gut microbiome contributes to microglial development and function in a sex-specific manner. Germ-free males exhibit greater differences in microglial transcriptomic signature during embryonic development, while germ-free females exhibit greater transcriptomic differences compared to SPF controls during adulthood (Thion et al. 2018).

Evidence indicates that microglia are altered in response to drugs of abuse (Thomas et al. 2004b; Miguel-Hidalgo 2009; Cruz et al. 2017). For instance, chronic ethanol administration results in increased microglial activation, which was not reversed by long-term withdrawal (Cruz et al. 2017). Additionally, administration of methamphetamine results in microglial activation within the striatum, and methamphetamine-induced neurotoxicity is thought to be mediated, at least in part, by microglia (Thomas et al. 2004a, b; Thomas and Kuhn 2005). Recent studies have sought to understand the contribution of microglia to addictive behaviors (Zhang et al. 2006; Lewitus et al. 2016). Zhang et al. (2006) found that treatment with minocycline, a microglial inhibitor, attenuates locomotor sensitization to

methamphetamine. Moreover, microglia within the NAc respond to repeated intraperitoneal injections of cocaine by releasing tumor necrosis factor alpha (TNF- α), resulting in depressed glutamatergic synaptic strength which then antagonizes cocaine-induced synaptic plasticity and locomotor sensitization. Further research is needed to understand what role the gut microbiome may play in modulating microglial response to drugs of abuse.

7. Brain-Derived Neurotrophic Factor—Brain-derived neurotrophic factor (BDNF) may be an especially interesting target for interrogating the role of the gut microbiome on drug addiction. BDNF is synthesized by both neuronal and glial cell populations within the brain as well as peripheral immune cells and the vascular endothelium (Subedi et al. 2017). Through its effects on synaptic plasticity, BDNF plays a critical role in mediating behavioral responses to cocaine in tasks such as CPP and locomotor sensitization, as well as self-administration tasks, including but not limited to post-extinction reinstatement and drug-seeking activity (Li and Wolf 2015). BDNF is known to be epigenetically regulated by cocaine via alterations in histone acetylation, and it has also been shown to mediate behavioral effects of alcohol and opioids (Rogge and Wood 2013; Barker et al. 2015; Pandey 2016; Felipe Palma-Álvarez et al. 2017). Future studies should seek to examine if microbiome derived metabolites such as SCFAs or others affect the epigenetic regulation of BDNF (Rogge and Wood 2013; Kiraly et al. 2016).

Although there have been no causal studies examining the exact mechanisms by which the gut microbiome may influence BDNF expression, studies have demonstrated that BDNF expression is dysregulated in multiple brain regions in response to changes in the gut microbiome; these changes are associated with altered behavioral response to cocaine and alcohol (Kiraly et al. 2016; Xiao et al. 2018; Xu et al. 2018). Our group found that treatment with antibiotics and cocaine (but not cocaine alone) was associated with increased expression of BDNF as well as decreased expression of its receptor Ntrk2 (Tyrosine receptor kinase B) within the nucleus accumbens (NAc) as measured by qPCR (Kiraly et al. 2016). Increased levels of BDNF are associated with increased locomotor activity in response to cocaine, which may explain why the mice treated with antibiotics and cocaine exhibited increased locomotor activity compared to saline controls or mice treated with cocaine alone. Xiao et al. (2018) performed fecal microbial transplantation (FMT) via oral gavage of enteric microbial samples from alcohol-exposed donors to healthy recipients. Compared to saline-gavage controls, FMT mice exhibited increased immobility time in the forced swim and tail suspension, suggesting an increase in alcohol withdrawal-induced anxiety-like behaviors. These changes were also associated with decreased hippocampal BDNF in FMT mice compared to saline-gavaged animals (Xiao et al. 2018). Likewise, Xu et al. (2018) found that chronic alcohol exposure was associated with decreased BDNF in the prefrontal cortex (PFC) of mice as well as increased anxiety and depression-like behaviors. These changes were correlated with specific changes in the gut bacterial populations (Xu et al. 2018). Notably, decreased levels of BDNF in the mPFC, striatum and the hippocampus are all associated with increased alcohol consumption (Pandey 2016).

V. Conclusions

A. Therapeutic Goals

At this time, there are no FDA-approved medications for the treatment of cocaine use disorder (Shorter et al. 2015). Dopaminergic, noradrenergic, and GABAergic agents have been previously studied as potential therapeutics for cocaine addiction, but these compounds have failed in early-phase human clinical trials (Shorter et al. 2015). Pharmacological treatments for alcohol and opioid use disorders are available, but are not effective for all patients (Soyka and Müller 2017; Hofford et al. 2018). It is therefore important that we improve our understanding of the role of the microbiome in drug addiction, as new discoveries may elucidate novel targets for future therapeutics. These therapies could target pathways involved in microbial signaling within the brain-gut axis either by manipulating the composition of the gut microbiome or by manipulating its products via drugs designed to alter and exploit microbial metabolism. While these potential therapeutics may not prove a panacea, they have significant potential to be developed as therapies.

Sarkar et al. (2016) recently proposed an expanded definition of psychobiotics to include both probiotics and prebiotics that influence microbial-neural signaling. Although no clinical trials have examined the effects of psychobiotics on drug addiction at this time, there have been proposals to investigate the effects of psychobiotics on alcohol addiction (de Timary et al. 2017). Several studies have demonstrated modest positive effects of psychobiotics—primarily probiotics—on comorbid mood disorders in human subjects (Benton et al. 2007; Messaoudi et al. 2011a, b; Steenbergen et al. 2015; Kato-Kataoka et al. 2016; Slykerman et al. 2017). Given the comorbidity of substance use disorders with mood disorders, as well as the high overlap of the neural circuitry and molecular machinery involved in these conditions, there is a strong possibility that psychobiotics may be affecting mesolimbic circuits that are affected in both conditions (Bagot et al. 2014; Peña et al. 2014). This overlap of mesolimbic circuitry increases the pre-test probability that psychobiotics may be effective for the treatment of substance use disorders as well, but much work needs to be done to test this hypothesis.

B. Final Remarks

Preliminary studies by our group and others indicate that the gut microbiome is dysregulated in response to psychostimulants, alcohol and opioids (de Timary et al. 2015; Kiraly et al. 2016; Ning et al. 2017; Peterson et al. 2017; Wang and Roy 2017; Lee et al. 2018). These shifts in the gut microbiome influence behavioral response to drugs of abuse, possibly through changes in gut microbial metabolites such as SCFAs and bile acids (Kiraly et al. 2016; Wang et al. 2018a; Lee et al. 2018). While there is significant promise for the microbiome as a translational research target for substance use disorders, there is still much work that has to be done. As shown in Table 1, there is no clear signature of changes induced by all classes of drugs of abuse, and the changes found within classes of drugs are also inconsistent. Currently published studies of microbiome manipulations, shown in Table 2, have primarily relied on broad spectrum antibiotics. Going forward it will be necessary to expand our database of how different classes of drugs of abuse lead to shifts in the microbiome and develop a more nuanced understanding of which bacterial populations and

metabolites affect the behavioral response to drugs of abuse. Better understanding of these mechanisms of microbiome-brain crosstalk may provide insight into new therapies for substance abuse disorders.

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**Table 1 -
Summary of effects of drugs of abuse on microbiome contents.**

This table outlines currently published findings in which the effects of alcohol, stimulants, or opioids on the composition of the microbiome and its metabolites were assessed.

Species	Drug	Administration	Effect on Microbiome	Effect on Microbial Metabolites	Citation
Human	Alcohol	Active Users	↓ <i>Bifidobacteria</i> ↓ <i>Enterococci</i> ↓ <i>Lactobacilli</i>	Not examined	Kirpich et al. 2008
Human	Alcohol	Active Users	↑ Bacteroidetes ↓ Firmicutes	Not examined	Volpe et al. 2014
Human	Alcohol	Active Users	↓ Bacteroidetes ↑ Proteobacteria	↑ Blood endotoxin	Mutlu et al. 2012
Human	Alcohol	Active Users	↓ Overall bacterial load ↑ Lachnospiraceae ↓ Ruminococcaceae Only in subjects with increased intestinal permeability	Not examined	Leclercq et al. 2014
Human	Alcohol	Active Users	Not examined	↑ Blood LPS	Leclercq et al. 2012
Human	Alcohol	Active Users	Not examined	↑ Blood peptidoglycan	Leclercq et al. 2014
Mouse	Alcohol	Oral	↑ Actinobacteria ↓ Bacteroidetes ↓ Firmicutes ↑ Proteobacteria	↑ Blood endotoxin	Bull-Otterson et al. 2013
Rat	Alcohol	Oral	↑ Bacteroidetes ↓ Firmicutes ↑ Proteobacteria	↑ Amino acid metabolism	Fan et al. 2018
Mouse	Alcohol	Oral	↑ Firmicutes	↑ Serotonin	Wang et al. 2018b
Mouse	Alcohol	Oral	↑ Bacteroidetes ↑ Verrucomicrobia	Not examined	Yan et al. 2011
Mouse	Alcohol	Vaporized	↑ <i>Alistipes</i> ↓ <i>Clostridium</i> IV ↓ <i>Clostridium</i> XIVb ↓ <i>Coprococcus</i> ↓ <i>Dorea</i>	Not examined	Peterson et al. 2017
Human	Cocaine	Active Users	↑ Bacteroidetes ↓ Firmicutes	No significant effect on blood LPS	Volpe et al. 2014
Rat	Cocaine	Volatilized	↓ Alpha diversity ↓ Beta diversity	Not examined	Scorza et al. 2019
Rat	Methamphetamine	Intraperitoneal	↓ <i>Acidaminococcaceae</i> ↑ Bacillaceae and ↑ Ruminococcaceae	↓ Propionate	Ning et al. 2017
Human	Heroin Methamphetamine Ephedrine	Active Users	No changes specific to heroin, methamphetamine or ephedrine	Not examined	Xu et al. 2017
Human	Opioids	Active Users Inpatient	↑ Alpha diversity	Not examined	Vincent et al. 2016
Human	Opioids	Active Users	↓ <i>Bacteroidacea</i> ↓ <i>Clostridiales</i> XI ↓ <i>Ruminococcaceae</i>	↑ Metabolism of aromatic amino acids	Acharya et al. 2017

Species	Drug	Administration	Effect on Microbiome	Effect on Microbial Metabolites	Citation
				↑ Degradation of branched-chain amino acids	
Human	Opioids	Active Users	↑ <i>Bifidobacterium</i>	Not examined	Barengolts et al. 2018
Mouse	Morphine	Implanted Pellet	↑ <i>Enterococcus faecalis</i>	↓ Bile acids ↑ Saturated fatty acids ↑ Phosphatidylethanolamines	Wang et al. 2018a
Nonhuman Primate	Morphine	Intramuscular	↑ <i>Methanobacteriaceae</i> ↓ <i>Streptococcaceae</i> <i>Streptococcus</i> ↓ <i>Pasteurellaceae</i> <i>Aggregatibacter</i>	↓ Primary bile acids ↑ Secondary bile acids	Sindberg et al. 2018
Mouse	Morphine	Implanted Pellet	↓ Bacteroidetes ↑ Firmicutes	↓ Primary bile acids ↓ Secondary bile acids	Banerjee et al. 2016

**Table 2 -
Effect of microbiome manipulations on behavioral responses to drugs of abuse.**

This table summarizes the currently published preclinical literature of studies in which the composition of the gut microbiome was manipulated prior to behavioral testing. The column “Administration” refers to the route of administration of cocaine or morphine. All antibiotic solutions were delivered orally.

Species	Drug	Administration	Microbiome or Metabolite manipulation	Behavioral Effect	Citation
Mouse	Cocaine	Intraperitoneal	Antibiotics SCFA + Antibiotics	↑ Locomotor sensitization ↑ Conditioned place preference No difference from controls for locomotor sensitization or conditioned place preference	Kiraly et al. 2016
Mouse	Cocaine	Intraperitoneal	Surgical intervention to increase bile acids	↓ Locomotor sensitization ↓ Conditioned place preference	Reddy et al. 2018
Mouse	Cocaine Morphine	Intraperitoneal Implanted Pellet + Intraperitoneal	Antibiotics Antibiotics	↓ Conditioned place preference ↓ Latency in tail immersion test	Lee et al. 2018
Mouse	Morphine	Implanted Pellet	Antibiotics	Prevented antinociceptive tolerance in tail immersion test and acetic acid stretch assay	Kang et al. 2017