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The bidirectional relationship between gut bacteria and intravenous fentanyl self-administration

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Pharmaceutical Sciences

by

Michelle Ren

Dissertation Committee:
Assistant Professor Shahrdad Lotfipour, Chair
Professor Frances M. Leslie
Professor Michael Yassa

2023

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ACKNOWLEDGMENTS

Countless people have been truly influential in my journey in graduate school and the completion of this dissertation. First, I would like to thank my advisor, Dr. Shahrdad Lotfipour, for his boundless support in my growth as a scientist and communicator. His enthusiasm and passion for research is encouraging and helped me push through many months of frustration and self-doubt. Thank you for teaching me to be simultaneously innovative and focused and for constantly reminding me to see the bigger picture.

I thank Dr. Frances Leslie, my co-advisor, who pushed me past limits that I did not believe were possible. She helped me grow in both my personal and professional life by always challenging me, offering immense guidance and support, and teaching me how to think critically, communicate effectively, set boundaries, and take risks.

Thank you to Dr. Mike Yassa for going over and beyond roles as a mentor and friend. Words cannot express my gratitude for his steadfast assistance in my academic and personal journeys.

I would also like to thank my advancement committee members, Drs. Brian Paegel, David Mobley, and Fred Ehlert, for their expertise and understanding over the years, and Dr. James Belluzzi for feedback in experimental design and statistics.

I am incredibly grateful for the camaraderie of our lab (and former Leslie Lab members), particularly Sarah Cross, Anjelica Cardenas, Emily Castro, Michelle Cano, Maricela Martinez, and Yasamin Heydary, for providing smiles and glimmers of light on particularly difficult days. Thank you to my talented undergrads, Yuexi Yin, Christie Lee, Jasmine Sran, and Michelle Nguyen, for their many, many hours of technical assistance (and youthful energy keeping me current).

Thanks to Manuella Yassa for her friendship and professional support and for introducing me to neuroscience outreach through the CNLM Ambassador Program. My time as a mentor during Brain Camp 2019 is one major highlight of graduate school.

I thank Dr. Phong Luong for his astonishing patience, kindness, and wisdom. He taught me how to stay (relatively) calm and collected through rough times and strategies to improve any situation.

I thank my friends outside of grad school for offering different perspectives and providing stress relief. Most importantly, thank you to my parents and adorable puppy for believing in me and encouraging me to never give up.

Permission was received from Wolters Kruwers Health, Inc. and MDPI to include Chapters 2, 3, and 4 of my dissertation. Financial support was provided by the University of California, Irvine T32 NIDA Training Grant (thank you to Dr. Marcelo Wood for guidance during my T32 funding period).

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Ren M, Lotfipour S, Leslie F. Unique effects of nicotine across the lifespan. Pharmacol Biochem and Behav. 2022 Mar;214:173343.

Ren M, Lotfipour S. The role of the gut microbiome in opioid use. Behav Pharmacol. 2020 Apr;31(2&3):113-121.

Ren M, Lotfipour S. Nicotine Gateway Effects on Adolescent Substance Use. West J Emerg Med. 2019 Aug;20:20(5):696-709.

Rupp AC, **Ren M**, Altimus CM, Fernandez DC, Richardson M, Turek F, Hattar S, Schmidt TM. Distinct ipRGC subpopulations mediate light's acute and circadian effects on body temperature and sleep. Elife. 2019 Jul;23:8:c44358.

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The Role of the Gut-Brain Axis in Intravenous Fentanyl Self-Administration, Jan 2023, *Winter Conference on Brain Research*.

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Gut-Brain Interactions in Intravenous Fentanyl Self-Administration, Oct 2019, Society for Neuroscience.

Animal Model of Intravenous Fentanyl Self-Administration, May 2019, *Institute for Clinical and Translational Sciences Translational Research Day*.

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ABSTRACT OF THE DISSERTATION

The bidirectional relationship between gut bacteria and intravenous fentanyl self-administration

By

Michelle Ren

Doctor of Philosophy in Pharmaceutical Sciences

University of California, Irvine, 2023

Assistant Professor Shahrdad Lotfipour, Chair

The United States is currently experiencing its worst drug crisis, which is largely driven by opioid addiction and primarily due to fentanyl. It is therefore necessary to investigate the mechanisms mediating fentanyl's rewarding and reinforcing properties to contribute to the development of successful treatment strategies. Gut bacteria communicate with the brain, and vice versa, via the gut-brain axis to regulate brain function, mood, and behavior. Addiction is a chronic brain disorder that alters circuitry involved in reward, stress, learning, and motivation, all of which have a bidirectional influence between their associated behaviors and gut bacteria. Given the associations between opioid use, gastrointestinal distress, and microbial dysbiosis in humans and rodents, I tested the hypothesis that interactions between gut bacteria and the brain mediate the reinforcing and motivational properties of fentanyl.

In this dissertation, I present my work that supports a bidirectional relationship between gut bacteria and fentanyl intravenous self-administration (IVSA) in Sprague Dawley rats. In the following studies, I implanted rats with intravenous catheters in preparation for fentanyl IVSA on an escalating schedule of reinforcement and analyzed gut microbiota by sequencing bacterial DNA from rat fecal samples. I demonstrate that based on sex and fentanyl dose, the diversity of gut bacteria is either increased or decreased following fentanyl IVSA and predicts progressive ratio breakpoint, a measure of motivation. Further, I show that depletion of gut bacteria via prolonged oral antibiotic treatment enhances fentanyl IVSA, and restoration of microbial

metabolites with short-chain fatty acid administration decreases fentanyl IVSA back to controls at higher fixed ratio schedules of reinforcement. My findings highlight an important relationship between the knockdown and rescue of gut bacterial metabolites and fentanyl self-administration in adult rats, which provides support for a relationship between gut microbiota and opioid use. Further work in this area may lead to effective, targeted treatment interventions in opioid-related disorders.

Chapter 1

Introduction

I. Background and significance

Drug overdose is one of the top leading causes of death in the United States with the majority involving opioids. Despite existing treatment options for opioid use disorder (OUD), the number of opioid-related deaths has escalated rapidly in the last decade primarily due to fentanyl use and abuse. The primary treatment for OUD is medication-assisted therapy, which assists with withdrawal symptoms and cravings. However, the underlying mechanisms driving opioid abuse need to be addressed for effective long-term treatment, as well as to prevent the development of addiction.

Addiction is a chronic brain disorder that alters circuitry involved in reward, stress, learning, memory, and motivation, all of which have a bidirectional influence between their associated behaviors and gut bacteria (Heijtz et al. 2011; Cussotto et al. 2019; Collins and Bercik 2009; Clarke et al. 2013; Selkrig et al. 2014). Opioid addiction is characterized by a compulsion to seek and voluntarily take opioids owing to their reinforcing effects and an impaired ability to control intake despite physical or psychological harm (Scholl et al. 2018). Although there are apparent associations between gut health, opioids, and addiction-related behaviors, the direct relationship between gut bacteria and opioid use is not yet understood.

Opioid crisis in the United States

The United States has been in an opioid overdose epidemic since 1999. Health and Human Services officially declared the opioid epidemic a public health emergency in 2017. There have been three distinct spikes in opioid overdose deaths, starting in the late 1990s with increased opioid prescriptions; in 2010, with rapid increases in overdose deaths involving

heroin; and most recently, beginning in 2013, a substantial rise in overdose deaths involving fentanyl (CDC 2013). Pharmaceutical fentanyl is prescribed for severe pain but is often diverted for misuse or illicitly manufactured and combined with counterfeit pills, heroin, cocaine, and methamphetamine (DEA 2019, 2021). Fentanyl is a synthetic opioid that is 50 to 100 times more potent than morphine (R. Hill et al. 2020; Schwienteck et al. 2019; Kosterlitz and Leslie 1978), so it takes very little to produce euphoric effects. The high potency of fentanyl and increased availability of fentanyl-laced drugs increase the likelihood of accidental overdose or risk of dependence and/or withdrawal in users that may or may not be aware of the presence of fentanyl (DEA 2021).

Opioid receptors and pharmacology

Opioid receptors are expressed throughout the central nervous system (CNS) (brain and spinal cord) and peripheral nervous system (nerves and ganglia outside the brain and spinal cord, including the gut). The opioid system consists of three G protein-coupled receptors: mu, delta, and kappa, which are stimulated by a family of endogenous opioid peptides (Kieffer 1995). Opioid receptors can also be activated by exogenous ligands, including heroin, morphine, oxycodone, and fentanyl, all examples that selectively activate mu opioid receptors (MORs). These opioids are highly addictive due to their activation of reward centers in the brain. The effects of MOR agonism (or activation) include euphoria, analgesia, drowsiness, nausea, tolerance, addiction, respiratory depression and/or arrest, and death. MORs are largely distributed along reward circuits where they mediate the reinforcing effects of morphine and non-opioid drugs (Contet, Kieffer, and Befort 2004).

The reason opioid overdose leads to respiratory depression is because of the abundance of opioid receptors in the brainstem, an area that controls respiration and heart rate. The widespread distribution of opioid receptors in the gut also explains why opioids can cause

gastrointestinal side effects, such as spasm and constipation (also known as opioid-induced bowel dysfunction).

II. Gut-brain communication

Enteric nervous system

The enteric nervous system (ENS) makes up a large part of the autonomic nervous system (a component of the peripheral nervous system) and comprises more than 100 million nerve cells lining the gastrointestinal tract. The intrinsic microcircuits within the ENS allow it to orchestrate gastrointestinal function independently of CNS input (Rao and Gershon 2016). However, the gut and brain do communicate with each other to regulate a variety of physiological processes via the gut-brain axis.

The gut-brain axis signifies the physical and chemical communication between the two organs. Bidirectional communication between the CNS (brain) and ENS (gut) occurs through 5 main pathways: afferent signaling via the vagus nerve, neuroendocrine signaling via the hypothalamic-pituitary-adrenal axis, enteroendocrine signaling via enteroendocrine cells, gut-derived neurotransmitters, and production of microbial metabolites (Mayer 2011). Gut-brain communication through these pathways is modulated by the trillions of bacteria (or microbiota) that inhabit the gastrointestinal system, which collectively make up one's gut microbiome (Cryan and Dinan 2012). The microbiota and the host create a mutually beneficial relationship, as a healthy microbiome prevents colonization of pathogenic bacteria, mediates the immune system, assists in fermentation of undigestible dietary fiber, and produces active peptides and proteins that regulate energy metabolism in the host (Belkaid and Hand 2014; DeGruttola et al. 2016; Bien, Palagani, and Bozko 2013; Kennedy et al. 2017; Mayer, Tillisch, and Gupta 2015; Rooks and Garrett 2016). Likewise, the host provides protection and nutrients necessary for the development of the microbial environment (Foster, Rinaman, and Cryan 2017; Mushegian and Ebert 2016).

An individual's gut microbiome is attained at birth and is influenced primarily by diet, medication, and illness, with the modern western diet, overuse of antibiotics, and drug use having detrimental impacts on gut microbiome composition (Dethlefsen et al. 2008; Spreadbury 2012; Stecher 2015; Zinöcker and Lindseth 2018). A "healthy" and balanced gut microbiome is diverse and resilient, which adapts to shifts in the community makeup and protects against an imbalance of the natural gut bacteria (Stecher, Maier, and Hardt 2013; Lozupone et al. 2012; Fassarella et al. 2021). Abnormalities in gut microbial communities, or dysbiosis, shift the microbiome balance, resulting in decreased bacterial diversity, increased abundance of potentially pathogenic bacteria, and/or decreased abundance of beneficial commensal bacteria (DeGruttola et al. 2016). An imbalanced gut microbiome leads to degradation of the gut epithelium and a translocation of microbes and metabolites that disrupts immune signaling throughout the body and brain (Levy, Blacher, and Elinav 2017).

The role of gut bacteria on the brain and behavior

A role of gut bacteria in brain function and behavior has been made evident through studies using germ-free animals, antibiotic administration, microbial supplementation, and fecal microbiota transplantation. Manipulation of the gut microbiota using these methods distinctly impacts the brain and spinal cord, gut health, and various behaviors, including those related to social interaction, sex, feeding, stress, learning and memory, mood, and addiction (Bravo et al. 2011; Bercik et al. 2011; Heijtz et al. 2011; Hegstrand and Hine 1986; K. Neufeld et al. 2011; Nobuyuki et al. 2004; Kelly et al. 2016; Desbonnet et al. 2015; McKernan et al. 2010; O'Mahony et al. 2014). Consistent with the bidirectional communication of the gut-brain axis, gut dysbiosis significantly impacts brain function and behavior and is observed in patients diagnosed with substance use disorder, depression, anxiety, Parkinson's disease, autism, and schizophrenia (Cussotto et al. 2019; Schroeder and Bäckhed 2016). Furthermore, disruption to a host's normal microbiota can lead to exaggerated stress responses and depressive symptoms (Kelly et al.

2016; Sudo et al. 2004; Ait-Belgnaoui et al. 2014; Tarr et al. 2015; Leclercq, Forsythe, and Bienenstock 2016; Rea, Dinan, and Cryan 2016).

III. The role of gut bacteria in addiction-related behavior

Few preclinical studies have directly examined the role of gut afferents and bacteria on addiction-like behavior (Kiraly et al. 2016; Han et al. 2018; Lee et al. 2018; Hofford et al. 2021). Current perspectives focus on dysfunctional reward processing, stress, and mood disorders as risk factors, characteristics, and/or co-morbidities in addiction and their implications with gut dysbiosis. Further, gut bacteria influence neural circuits and behaviors that are markedly associated with addiction, including reward, tolerance, and withdrawal (Kiraly et al. 2016; Lee et al. 2018; Hofford et al. 2021; Han et al. 2018; M. Kang et al. 2017; Wang et al. 2018; Thomaz et al. 2021).

Reward- and addiction-related behavior

Drug addiction is characterized by 1) compulsion to seek and take a drug, 2) loss of control in limiting intake, and 3) emergence of a negative emotional state when access to the drug is prevented. This chronic, relapsing disease progresses from impulsivity to compulsivity through exaggerated incentive salience, reward deficits and stress surfeits, and compromised executive function in three stages: preoccupation/anticipation, binge/intoxication, and withdrawal/negative affect (Koob and Moal 1997). The neurobiology of reward is essential to study in addiction, as drugs of abuse activate reward brain circuitry. It is also important to understand the different categories of reward, including learning, motivation, and emotion, which each include their own distinct psychological subcomponents (e.g., wanting, liking, learning) (Berridge and Robinson 2003).

Opioids and gut bacteria

A link between gut health and opioid intake is evident in the ability of opioids to significantly impact gastrointestinal function (i.e. opioid-induced bowel dysfunction). Indeed, opioid use is associated with gut dysbiosis in both humans and animals (Vincent et al. 2016; Acharya et al. 2017; Meng et al. 2013; Meng, Sindberg, and Roy 2015; Banerjee et al. 2016; M. Kang et al. 2017; Lee et al. 2018; Wang et al. 2018; Wang and Roy 2017; Zhernakova et al. 2016; Xu et al. 2017). Preclinical studies have also shown an important role of gut bacteria in drug reward (Kiraly et al. 2016; Lee et al. 2018; Hofford et al. 2021) and the development of opioid tolerance (M. Kang et al. 2017). While alterations in gut microbiota directly affect opioid-related behaviors, opioid exposure also alters the diversity and/or composition of the host gut microbiome (Wang et al. 2018; Banerjee et al. 2016; Meng et al. 2013; Ren and Lotfipour 2022; Acharya et al. 2017; Jingyuan Zhang et al. 2020; Cruz-Lebrón et al. 2021; Yafang Zhang et al. 2021; Barengolts et al. 2018). In Chapter 2, I highlight the extensive overlap in neuroadaptations between overeating and drug abuse (Volkow, Wise, and Baler 2017) and the key roles of stress and reward processing in the development of opioid addiction.

IV. Rationale for the dissertation

With the prevailing opioid crisis and escalating numbers of opioid-related deaths driven by addiction, it is critical to evaluate underlying factors that significantly influence opioid abuse. My research is focused on fentanyl due to its marked involvement in overdose deaths within the last decade. Given the associations between the gut microbiota and stress, mood, psychiatric disorders, and behavior, evaluating the role of the gut microbiota in fentanyl use is a unique approach that could lead to new paths for the treatment of addiction. Few preclinical studies have directly examined the role of gut afferents and bacteria on addiction-like behavior (Kiraly et al. 2016; Han et al. 2018; Lee et al. 2018; Hofford et al. 2021). Current perspectives focus on dysfunctional reward processing, stress, and mood disorders as risk factors, characteristics,

and/or co-morbidities in addiction and their implications with gut dysbiosis. Different pathways mediate the pain-relieving and rewarding effects of opioids, so although an apparent relationship exists between gut bacteria and analgesic tolerance to opioids, further research is needed to evaluate how the gut microbiome modulates opioid reward and reinforcement. My studies explore gut-brain interactions in fentanyl use, which has previously not been studied before in the literature. By identifying how the gut-brain axis influences fentanyl use, my research has the potential to significantly progress our understanding of the mechanisms influencing opioid addiction. Different pathways mediate the pain-relieving and rewarding effects of opioids, so although an apparent relationship exists between gut bacteria and analgesic tolerance to opioids, further research is needed to evaluate how the gut microbiome modulates opioid reward and reinforcement.

The central hypothesis of my studies is that gut bacteria are important regulators of reward, emotion, and motivation brain circuitry. In this dissertation, I review the role of the gut microbiome in various aspects of opioid addiction (Chapter 2). Next, I present an intravenous fentanyl dose-response curve in adult Sprague Dawley male and female rats, as well as the impact of fentanyl intravenous self-administration (IVSA) on gut microbiota (Chapter 3). To evaluate the bidirectional relationship between gut bacteria and behavior, I demonstrate the role of gut bacteria in fentanyl IVSA by depleting gut bacteria with oral antibiotic administration in rats (Chapter 4). Finally, I replete the animals' gut microbial metabolites via short-chain fatty acid administration to assess a potential mechanism of gut-brain communication in fentanyl IVSA (Chapter 4). Collectively, my research supports evidence of a bidirectional role of the gut-brain axis in fentanyl IVSA in adult Sprague Dawley rats, in that fentanyl use impacts gut diversity, and changes in gut diversity influence fentanyl use.

Examining the bidirectional relationship between gut microbiota and fentanyl reinforcement and reward contributes to the limited understanding of mechanisms mediating

opioid dependence and abuse. Building upon this foundation allows for the development of tractable treatment options to mitigate opioid abuse and addiction vulnerability.

Chapter 2

The Role of the Gut Microbiome in Opioid Use

Introduction

A growing field in human health is the gut-brain connection, a notion that our gut health is directly related to our emotional health. Although the gut and brain are separate organs, they are connected physically via the vagus nerve, and biochemically via neurotransmitters, gut hormones, microbial metabolites, and the immune system (Cryan and Dinan 2012). Bidirectional communication between the central nervous system (brain) and enteric nervous system (gut) occurs through the gut-brain axis, which is maintained by the trillions of intestinal bacteria that collectively make up one's gut microbiome.

An abundance of evidence in both humans and animals supports an essential role of gut microbiota in regulating brain function, mood, stress, and behavioral responses to rewards, including food and drugs of abuse (Collins and Bercik 2009; Heijtz et al. 2011; Clarke et al. 2013; Selkrig et al. 2014; Cussotto et al. 2019). Gut bacteria are heavily impacted by "diseased" states, as abnormalities in gut microbial communities, or dysbiosis, are observed in patients diagnosed with substance use disorder, depression, anxiety, Parkinson's disease, autism, and/or schizophrenia (Schroeder and Bäckhed 2016). Disruption to a host's normal microbiota can lead to exaggerated stress responses and depressive symptoms (Sudo et al. 2004; Ait-Belgnaoui et al. 2014; Tarr et al. 2015; Kelly et al. 2016; Leclercq, Forsythe, and Bienenstock 2016; Rea, Dinan, and Cryan 2016), further supporting the bidirectional relationship between the gut and brain. Given the significance of gut bacteria in obesity, stress, and motivated behaviors, as well as the extensive overlap in neuroadaptations between overeating and drug abuse (Volkow, Wise, and Baler 2017) and the key roles of stress and reward processing in the

development of addiction, we review available literature to support the hypothesis that gut-brain communication is necessary in the development and perpetuation of drug addiction.

Addiction is a chronic brain disorder that alters circuitry involved in reward, stress, learning, memory, and motivation. The development of drug addiction is driven by exaggerated incentive salience, reward deficits, stress surfeits, and compromised executive function in three distinct stages, namely binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving) (Koob and Volkow 2016). Opioid addiction is the leading cause of drug overdose in the United States and is characterized by a compulsion to seek and voluntarily take opioids owing to their reinforcing effects and an impaired ability to control intake despite physical or psychological harm (Scholl et al. 2018). Due to the rapid increase in numbers of opioid-related disabilities and deaths throughout the world (Martins et al. 2017), this review is focused on gut-brain interactions specifically in opioid use.

The endogenous opioid system regulates pain relief, reward processing, emotion, stress, and autonomic control, and consists of mu, delta, and kappa receptors (Benarroch 2012). Opioid receptors are distributed widely throughout the brain, periphery, and gut (De Schepper et al. 2004), and are activated endogenously by enkephalins, dynorphins, endorphins, and endomorphins, as well as exogenously by opioids (e.g., heroin, morphine, oxycodone, fentanyl). Opioids exert their primary clinical effects on mu opioid receptors to reduce pain perception. A link between gut health and opioid intake is evident in the ability of opioids to significantly impact gastrointestinal function (i.e. opioid-induced constipation). Indeed, opioid use is associated with gut dysbiosis in both humans (Vincent et al. 2016; Wang and Roy 2017; Zhernakova et al. 2016; Acharya et al. 2017; Xu et al. 2017; Barengolts et al. 2018). Preclinical studies have also shown an important role of the gut microbiome in drug reward (Kiraly et al. 2016; Lee et al. 2018) and the development of opioid tolerance (M. Kang et al. 2017). In this review, we present available literature assessing the bidirectional role of the gut-brain axis in addiction- and opioid-related behaviors, including stress, reward, incentive salience, mood disruption, tolerance, dependence,

withdrawal, and antinociception, as well as propose possible mechanisms of gut-brain interactions in opioid use.

With the prevailing opioid crisis and escalating numbers of opioid-related deaths worldwide driven by addiction, it is critical to evaluate mechanisms that mediate the abuse potential of opioids. By presenting a specific role of the gut-brain axis in opioid use and in factors that influence addiction, we provide a potential therapeutic target integrated with opioid regimens to mitigate abuse and addiction vulnerability. Novel interventions to limit the negative clinical outcomes of opioid use, such as tolerance, dependence, and withdrawal, may reduce the risk of addiction and opioid-related deaths. An improved understanding of how the gut is involved in addiction-related behaviors can also contribute to the development of effective treatment strategies in other disorders with shared characteristics, including depression, anxiety, and chronic pain.

Methods to study the gut microbiome

While human subjects provide valuable translational data, the majority of our understanding of gut-brain interactions in addiction-related behaviors comes from preclinical rodent studies. Animal models allow specific, targeted manipulation of gut microbiota while controlling for factors that are widely variable in humans, including disease comorbidities, opioid dose and duration of use, co-use of other opioid and/or non-opioid drugs, drug history, and genetics. Comparing human microbiomes of substance use disorder versus healthy controls may also reflect differences in lifestyles and diets and are therefore difficult to appoint solely to drug use.

The four primary ways that gut microbiota can be manipulated are with probiotics, prebiotics, antibiotics, and fecal microbiota transplantation (FMT). Probiotics are live, beneficial bacterial strains that do not repopulate on their own so need to be administered daily for benefits to the host. Prebiotics are dietary fibers that are indigestible by the host and undergo

bacterial fermentation to stimulate the growth of certain types of bacteria. An alternative to prebiotics is administration of short-chain fatty acids (SCFAs), which are bacterial fermentation byproducts. Depletion of 70-90 percent of gut bacteria in rodents can be achieved with prolonged oral treatment of non-absorbable antibiotics that do not mirror clinical doses (Bercik, Denou, et al. 2011; Reikvam et al. 2011). The goal of FMT, often known as stool transplantation, fecal transplantation, or fecal bacteriotherapy, is to restore eubiosis by transferring stool from a healthy donor into a recipient with an altered colonic microbiome (Vindigni and Surawicz 2017). FMT is an effective treatment for recurrent *Clostridium difficile* infection that has not responded to standard therapy. Potential applications of FMT for intervention in non-gastrointestinal diseases, such as obesity, ischemic stroke, Parkinson's disease, Alzheimer's disease, and depression, are also being explored (Kelly et al. 2016; Sampson et al. 2016; Chen et al. 2019; Dutta et al. 2019; Muscogiuri et al. 2019; J. Sun et al. 2019).

A unique animal model used in microbiome research is the germ-free mouse, in which mice are raised in a sterile environment from birth and remain completely absent of internal or external microbes (Faith et al. 2010). Germ-free mice are usually compared with mice containing known pathogens (conventional or specific pathogen-free) and can be colonized with microbial communities from donor animals or human subjects. Although the germ-free animal model is not entirely clinically relevant, as humans have constant exposure to environmental microbes immediately after birth, its use provides insight into early host development and function. Gut microbial depletion via prolonged oral antibiotic administration bypasses the perinatal developmental period.

As the bulk of intestinal bacteria are excreted in fecal matter, microbial analysis is most commonly achieved from fecal samples. Gut bacterial profiles are analyzed by DNA sequencing of the 16S rRNA gene found in all bacteria. Specific primers can select for the variable regions of the 16S gene to provide a profile of the different bacterial species in a given sample. DNA

sequencing of bacterial genes reveals a sample's bacterial abundance, alpha diversity (i.e. how many different species exist in the sample), and beta diversity (i.e. different species in one sample compared to another sample).

Gut microbiome role in incentive salience

Addiction is a chronic brain disease that is associated with dysregulation of reward and motivation. Incentive salience is a motivational property that when attributed to reward-predicting stimuli, or cues, triggers the approach toward and consumption of a reward (Tindell et al. 2009; Jun Zhang et al. 2009). The development of incentive salience is mediated by the mesolimbic dopamine system and promotes habits that encourage excessive cue-induced drug seeking and self-administration behaviors (Berridge 2012).

A vagal gut-to-brain circuit has been established to play a critical role in reward and motivation (Han et al., 2018), and gut bacteria influence how animals respond to various rewards, such as food and drugs (Korner and Leibel 2003; Duca et al. 2012; Alcock, Maley, and Aktipis 2014; Kiraly et al. 2016; Van de Wouw et al. 2018; Lee et al. 2018; Al-Ghezi et al. 2019). Natural rewards, such as food, sex, and nurturing, are processed by key mesocorticolimbic structures and neurotransmitters, including the ventral tegmental area (VTA), nucleus accumbens, prefrontal cortex, dopamine, serotonin, GABA, glutamate, and endogenous opioids (Russo and Nestler 2013). Drugs of abuse, including opioids, alcohol, stimulants, and cannabis, are artificial rewards that hijack this same brain system, and repeated drug use induces neurophysiological changes that contribute to addiction (Volkow and Morales 2015).

Passive exposure to or voluntary consumption of drugs of abuse can induce imbalances in gut microbiota in humans and rodents (Volpe et al. 2014; Wang and Roy 2017; Ning et al. 2017; Temko et al. 2017; Hillemacher et al. 2018; Hofford, Russo, and Kiraly 2019). In line with bidirectional gut-brain communication, these microbial imbalances influence brain function and behavior. Gut dysbiosis is associated with decreased levels of serotonin and dopamine, both

important reward-related neurotransmitters (Yano et al. 2015), and changes in the gut microbiome are correlated with altered striatal dopamine receptor expression in a model of compulsive alcohol seeking (Jadhav et al. 2018). Additionally, mice with depleted gut microbiota have abnormal behavioral responses to cocaine reward compared to controls (Kiraly et al. 2016; Lee et al. 2018), further highlighting a feedback loop between impaired reward processing and gut dysbiosis.

A growing amount of work has investigated the role of gut bacteria in obesity and overeating, which is relatable to drug addiction, as clinical and preclinical evidence uncover a significant overlap of neuroadaptations in overeating (food addiction) and drug addiction (Volkow, Wise, and Baler 2017). The rewarding properties of food and drugs are necessary for addictive potential, as foods that are highly palatable or drugs that produce significant euphoria promote repeated consumption or intake. Further, the rewarding properties of food or drugs are necessary for attributing positive motivational value to stimuli associated with reward availability and act as powerful incentives of reward-seeking behavior (Di Chiara 1999). Consistent with food and drug reward sharing similar neural mechanisms, current pharmacological and non-pharmacological (i.e. vagal nerve stimulation) treatments for obesity have also shown efficacy in reducing self-administration and/or rewarding effects of alcohol, cocaine, opiates, and nicotine in rodents (Egecioglu, Engel, and Jerlhag 2013; Graham et al. 2013; Skibicka 2013; Engel and Jerlhag 2014; H. D. Schmidt et al. 2016; Sirohi et al. 2016; Vallöf et al. 2016; Childs et al. 2017; Fortin and Roitman 2017; Tuesta et al. 2017).

As expected, the reward system is more stimulated by high energy-dense food than low-calorie food (van der Laan et al. 2014). High-fat or high-sucrose diets are associated with altered microbial diversity in mice (Daniel et al. 2014; B. Liu et al. 2018; Magnusson et al. 2015), and these diet-induced microbial changes substantially influence brain function, resulting in reduced synaptic plasticity, increased vulnerability to anxiety-like behavior, impairment in long-term and short-term memory, and disruptions in exploratory behavior (Sharma, Zhuang, and

Gomez-Pinilla 2012; Bruce-Keller et al. 2015). Bacterial byproducts that come into contact with gut epithelium stimulate production of gut hormones and neuropeptides, including peptide YY, cholecystokinin, glucagon-like peptide-1, and substance P, which mediate hunger and satiety signaling (Cani, Everard, and Duparc 2013; Cani and Knauf 2016). These findings highlight a critical function of gut bacteria in regulating appetite and feeding behaviors.

Although similar neural pathways influence food and drug reward, bariatric surgery (e.g. gastric bypass) to effectively reduce food intake, increases vulnerability and sensitivity to the reinforcing effects of opioid analgesics (Raebel et al. 2013; Biegler et al. 2016). This may be explained by the transference of one addiction (food) to another, as surgery eliminates excessive eating but does not alter individual predispositions to addictive behaviors (Niego et al. 2007; Pepino et al. 2014). In addition, altered gastrointestinal anatomy may cause changes in pharmacokinetics, as opioids are absorbed in the gastrointestinal tract (Tan et al. 1989; Lotsch et al. 1999).

Gut microbiome role in drug withdrawal/negative affect

Various biological factors likely contribute to increasing chronic opioid use, including higher pain sensitivity and lower pain detection thresholds (Dodet et al. 2013). The shift from acute drug use to addiction may be due to opposing brain circuits that mediate stress and reward, as addiction progresses from initial drug use for reward (i.e. positive reinforcement) to repeated use for distress avoidance (i.e. negative reinforcement). Once an individual adapts to regular use of opioids, a sudden decrease in intake leads to withdrawal, which interestingly yields symptoms opposite of opioid effects, as the brain tries to compensate against homeostatic disruption. The withdrawal/negative affect stage of the addiction cycle is represented by dampened reward via dopamine deficits and increased stress via activation of corticotropin-releasing factor and dynorphin (Koob et al. 2014). This recruitment of the stress system results in an emergence of negative emotional states, including dysphoria, anxiety,

irritability, and depression. Baseline stress levels are heightened and exacerbated during withdrawal or extended abstinence, which encourages a cycle of continual drug intake to avoid the dysphoric feelings associated with the negative affect.

Stress, both acute and chronic, plays a key role in mediating an animal's sensitivity to food and drug reinforcers (Sinha and Jastreboff 2013; Yau and Potenza 2013; Koob et al. 2014) and is a primary risk factor in the development of drug abuse and addiction. A substantial amount of evidence supports a bidirectional and causal relationship between gut dysbiosis and stress. Gut microbiota directly modulate general stress responses as well as drug withdrawal-induced anxiety (Xiao et al. 2018). Imbalances in gut microbial communities lead to a heightened activation of the hypothalamic-pituitary-adrenal (HPA) axis stress response (Sudo et al. 2004; Ait-Belgnaoui et al. 2014; Tarr et al. 2015; Gacias et al. 2016; Leclercq, Forsythe, and Bienenstock 2016; Rea, Dinan, and Cryan 2016), and restoring eubiosis via probiotics, SCFAs, and FMT ameliorates stress-related biomarkers and behaviors (Desbonnet et al. 2010; Ait-Belgnaoui et al. 2012; Liang, Bello, and Moran 2013; Pusceddu et al. 2015; K. Schmidt et al. 2015; Tarr et al. 2015). In addition, stress induces changes in microbiota composition and intestinal barrier function (Söderholm et al. 2002; Melanie G Gareau, Silva, and Perdue 2008). This is perhaps unsurprising given the considerable impact that stress has in aggravating gastrointestinal disorders and symptoms, such as inflammatory bowel disease, irritable bowel syndrome, gastric ulcers, and diarrhea (Klooker et al. 2009; Mayer 2011; Moloney et al. 2016).

Substance use disorders are also highly comorbid with depression and anxiety, which are both characterized as stress-related mood disorders, in which stress is a major risk factor in its onset, and affected individuals have increased stress sensitivity (Holsboer 2000; Kendler et al. 2006; Scott, Clarke, and Dinan 2013; L. Yang et al. 2015; Ramirez, Fornaguera-Trías, and Sheridan 2016). Impaired gut microbiota is reported in depression (Desbonnet et al. 2010; Jiang et al. 2015; Kelly et al. 2016; Luna and Foster 2015; Macedo et al. 2017) and anxiety (Neufeld et al. 2011; Luna and Foster 2015; Tarr et al. 2015), as well as other central nervous system

abnormalities and diseases, such as hyperactivity, cognitive deficits, Parkinson's disease, and schizophrenia (Heijtz et al. 2011; Scheperjans et al. 2015; Fröhlich et al. 2016; Severance and Yolken 2020). This link between central nervous system disorders and dysbiosis is not simply an association, as transplantation of gut bacteria from humans or animal models with obesity, chronic pain, anxiety, depression, Parkinson's disease, or schizophrenia produces matching abnormal behaviors in animals (Bravo et al. 2011; Bruce-Keller et al. 2015; Kelly et al. 2016; C. Yang et al. 2019; Zhu et al. 2020). Further, gut bacterial depletion in mice increases depressive-like behavior, alters visceral pain responses, and impairs cognition (O'Mahony et al. 2015; Fröhlich et al. 2016). Similarly in humans, a single treatment course of antibiotics is associated with an increased risk of depression and anxiety that rises with recurrent antibiotic exposure (Lurie et al. 2015). These findings collectively suggest a feedback loop exists between stress, depressive states, and gut dysbiosis, which could underlie the gut microbiome's role in drug withdrawal/negative affect.

Gut microbiome role in drug anticipation/craving

The preoccupation/anticipation stage is often linked with drug craving and hypothesized to be a key element in relapse. During this stage, the combination of excessive drug cue-induced incentive salience, diminished reward system function, and heightened stress levels promotes pathological drug seeking. Motivational withdrawal syndrome develops when access to a drug is prevented, where the primary focus is to alleviate withdrawal symptoms. According to Pavlovian conditioning analysis, drug tolerance and withdrawal symptoms are both manifestations of conditioned compensatory responses (Siegel and Ramos 2002). These drug-compensatory responses are proposed to mediate the development of tolerance by counteracting the drug effect when administered in the context of usual drug-administration cues (which may also be interpreted as "drug preparation" symptoms). In contrast, if the drug is not administered in the presence of usual cues, the conditioned compensatory responses are

not attenuated by drug effect and thus achieve full expression, increasing the risk of drug overdose. The essential role of gut microbiota in learning, memory, and stress highlights the striking impact of the gut in drug anticipation and craving (Sudo et al. 2004; Bravo et al. 2011; Heijtz et al. 2011; Desbonnet et al. 2015).

The finding that opioid tolerance to analgesia can be transferred via fecal transfer from an opioid-dependent mouse model into opioid-naive mice reveals that opioid exposure produces changes in gut bacteria that contribute to the development of tolerance to the pain-relieving effects of opioids (M. Kang et al. 2017; Lee et al. 2018; C. Yang et al. 2019). Gut microbiota diversity is in fact altered with chronic opioid use in humans and mice, and these changes alter neuronal tolerance in extrinsic sensory afferents (Akbarali and Dewey 2017). In addition, the rate of tolerance to the analgesic effects of morphine is exaggerated in the presence of colonic inflammation (Komla et al. 2019).

While tolerance to the analgesic and rewarding properties of opioids develops rapidly, the gastrointestinal-related side effects, including pain, nausea, and constipation, remain consistent and often worsen with chronic opioid exposure (Akbarali and Dewey 2017). One consideration is that gastrointestinal distress is one presentation of a conditioned compensatory response to opioid intake. As an example, diarrhea and/or vomiting are commonly observed signs of withdrawal, and administration of opioids alleviates these symptoms by inducing constipation and reduced gut motility. Gut dysbiosis may act as an interoceptive cue to opioid administration, similar to external cues, and elicit conditioned responses that mediate drug tolerance (Razran 1961). Consistent with this hypothesis, tolerance can be prevented with a peripheral mu-opioid receptor antagonist, which supports a peripheral or gut mechanism mediating opioid tolerance (Komla et al. 2019). Furthermore, the insula integrates interoceptive states into emotions and conscious feelings, and its reactivity has been suggested to serve as a biomarker to predict relapse in humans (Naqvi and Bechara 2009; Janes et al. 2010).

Possible mechanisms of the gut microbiome mediating opioid use behavior

Neuroinflammation

Gut-brain communication occurs vastly through immune pathways. Gut bacteria control the differentiation and function of immune cells in the brain, periphery, and intestines (Erny et al. 2015; Matcovitch-Natan et al. 2016; Rooks and Garrett 2016), and a healthy intestinal lining forms a tight barrier to control what gets absorbed into the bloodstream. It is therefore feasible to expect that perturbed gut bacteria and/or structure prompt immune dysfunction by triggering inflammation throughout the brain and body.

Intestinal barrier integrity can be threatened by a Western-style diet, certain medications, stress, and autoimmune conditions (Mélanie G Gareau et al. 2007; Stewart, Pratt-Phillips, and Gonzalez 2017), which cause the tight junctions in the large intestine to open up (i.e. leaky gut) and allow bacteria and their toxins to get through, eliciting a systemic inflammatory response. Neuroinflammation is characterized by increased microglial activation and/or malformed microglial morphology. Gut, brain, and systemic inflammation are seen in acute and chronic stress and mood disorders (Maes et al. 2012; Wohleb and Delpech 2017), as well as in opioid-dependent states and particularly in states of withdrawal (Taylor et al. 2016; Lee et al. 2018). Neuroinflammation disrupts the function and projections of dopaminergic neurons within VTA, leading to decreased mesolimbic dopaminergic activity and dysregulated reward, which is a shared characteristic of chronic pain, depression, and opioid addiction (Taylor et al. 2016; Cahill and Taylor 2017). Vulnerability to the negative effects of opioids may be heightened by inflammation, which can develop from chronic opioid use, creating a vicious cycle.

Microbial metabolites

Gut bacteria help break down certain nutrients, which can be further metabolized by host cells. Several of these products, short-chain fatty acids (SCFAs, e.g. butyric acid, propionic acid, and acetic acid), are associated with neural function. Gut bacteria also produce tryptophan,

serotonin, dopamine, and GABA, which play important roles in the brain as neurotransmitters or their precursors (Lyte 2011; Thomas et al. 2012; Sudo 2019).

SCFAs are considered to be beneficial to the host due to their anti-inflammatory effects and epigenetics regulation (Tsankova et al. 2007; C. H. Kim, Park, and Kim 2014; Erny et al. 2015; Stilling et al. 2016). SCFAs are able to influence memory and learning processes in the brain and alleviate stress (Chambers et al. 2015; J. Liu et al. 2015; Burokas et al. 2017; Byrne et al. 2016; Arnoldussen et al. 2017; Van de Wouw et al. 2018; Garcez et al. 2018). Additionally, administration of SCFAs normalizes microglial abnormalities in germ-free mice (Erny et al. 2015) and reverses the enhanced reward sensitivity to cocaine seen in gut bacteria-depleted mice (Kiraly et al. 2016). Opioid-related behaviors may be due in part to reduced bacterial metabolism, which can be repleted with SCFA supplementation.

Serotonin (5-HT)

Microbiota can regulate 5-hydroxytryptamine (5-HT) synthesis in the gut, which is important given that dysfunctional 5-HT signaling may underlie symptoms of gastrointestinal and mood disorders (Yano et al. 2015). Peripherally, 5-HT is involved in pain perception and regulation of gut secretion and motility, and centrally, 5-HT signaling pathways are implicated in regulating mood and cognition (Gershon and Tack 2007; O'Mahony et al. 2015). Gut bacteria may play a crucial role in tryptophan availability and metabolism to consequently impact central 5-HT concentrations (Van de Wouw et al. 2018). The relationship between gut bacteria, serotonin synthesis and signaling, and mood is imperative to note as a consideration in addiction and motivated behaviors.

Microbial diversity and presence of specific species

Dysbiosis is characterized by imbalances in bacterial species, which can be measured by total abundance, species ratios, alpha diversity, and/or the presence or absence of a specific

species. An imbalance in the body's normal gut microbiota disrupts immunity and nutrition and leads to relative overgrowth of bacteria, which can progress into a secondary infection, such as the pathogenic *Clostridium difficile*. Gut microbiomes with high diversity are posited to be more beneficial to host health than low diversity microbiomes, as many different species exist in low numbers in a high diversity environment and expend more resources competing with other bacteria rather than manipulating the host (Alcock, Maley, and Aktipis 2014).

Specific bacterial species may exert immunomodulatory effects on the central nervous system. *Lactobacillus reuteri* (*L. reuteri*) decreases anxiety-like behavior and stress-induced increase of corticosterone in mice, and alters mRNA expression of both GABA-A and GABA-B receptors in the central nervous system (Bravo et al. 2011). Vagotomy in these animals prevents the anxiolytic and antidepressant effects of *L. reuteri*, which indicates that parasympathetic innervation is necessary for *L. reuteri* to participate in the microbiota-brain interaction. Further, many species of *Lactobacillus* and *Bifidobacterium* produce GABA; *Candida, Escherichia,* and *Enterococcus* produce serotonin; and some *Bacillus* species produce dopamine (Lyte 2011; Barrett et al. 2012). Additional research on bacterial species differences in substance use disorder is necessary to understand the functions of specific species and for a precise therapeutic intervention in opioid use.

Conclusion

Few preclinical studies have directly examined the role of gut afferents and bacteria on addiction-like behavior (Kiraly et al. 2016; Han et al. 2018; Lee et al. 2018). Current perspectives focus on dysfunctional reward processing, stress, and mood disorders as risk factors, characteristics, and/or co-morbidities in addiction and their implications with gut dysbiosis. Different pathways mediate the pain-relieving and rewarding effects of opioids, so although an apparent relationship exists between gut bacteria and analgesic tolerance to

opioids, further research is needed to evaluate how the gut microbiome modulates opioid reward and reinforcement.

We highlight here the role of gut bacteria in the affected neurocircuitry and behaviors of opioid abuse and addiction, including the stress HPA axis, mesolimbic dopamine system, tolerance, withdrawal, and craving. We also provide support that the gut-brain axis and opioid use share bidirectional communication, as opioid exposure changes the gut microbiome, and manipulation of gut bacteria influences opioid-related behaviors, such as pain tolerance, withdrawal, anhedonia, and drug reward. The impact of gut dysbiosis on impaired reward, enhanced stress, and neuroinflammation, as well as the glaring feedback of these factors on gut health, strongly implicates an important role of the gut-brain axis in opioid use.

Individual differences in gut microbiomes contribute to variations in drug metabolism, which account for the disparities in therapeutic efficacy and side effects between individuals (Zimmermann et al. 2019). An interesting, targeted approach to personalized medicine would be to modulate or supplement the gut microbiota to increase the efficacy of a drug or reduce its adverse effects. Our knowledge of the gut microbiome on obesity has led to clinical trials to evaluate benefits of FMT in non-gastrointestinal disorders, including obesity (Carlucci, Petrof, and Allen-Vercoe 2016; Jayasinghe et al. 2016), autism (D. Kang et al. 2017), multiple sclerosis (Makkawi, Camara-Lemarroy, and Metz 2018), and depression (Wortelboer, Nieuwdorp, and Herrema 2019). A further understanding of the role of the gut microbiome in drug addiction and opioid use may offer a novel targeted approach in the treatment of substance use disorders and/or a combined therapy with opioid regimens.

Chapter 3

Dose- and Sex-Dependent Bidirectional Relationship Between Intravenous Fentanyl Self-Administration and Gut Microbiota

Introduction

The United States is currently in an opioid crisis: the number of drug-related deaths has been rapidly increasing every year, with most cases involving fentanyl (Rudd et al 2016). It is necessary to investigate mechanisms mediating the abuse potential of fentanyl to contribute to the development of successful treatment strategies. In this study, we evaluate the role of gut microbiota as a potential mechanism in fentanyl reward and reinforcement. A link between gut health and opioid intake is evident in the ability of opioids to significantly impact gastrointestinal function, (i.e., opioid-induced constipation). Indeed, opioid use is associated with imbalances in gut bacteria in both humans (Vincent et al. 2016; Zhernakova et al. 2016; Acharya et al. 2017; Xu et al. 2017; Barengolts et al. 2018) and animals (Meng et al. 2013; Meng, Sindberg, and Roy 2015; Banerjee et al. 2016; M. Kang et al. 2017; Lee et al. 2018; Wang et al. 2018). Preclinical studies have also shown an important role of gut microbiota in opioid-dependent behaviors, including reward, tolerance, and withdrawal (M. Kang et al. 2017; Lee et al. 2018; Hofford et al. 2021).

The gut and brain communicate via the trillions of bacteria that reside in the gastrointestinal tract, collectively known as the gut microbiome. A growing field of literature supports a bidirectional relationship between gut bacteria and host health, particularly related to emotions, behavior, and neuropsychiatric disorders (Mitrea et al. 2022; Cryan and Dinan 2012; Valdes et al. 2018). Gut microbiota provides a protective role by occupying intestinal surfaces and preventing overgrowth of pathogenic microorganisms (Karczewski et al. 2010). An unbalanced gut environment, or gut dysbiosis, is bidirectionally associated with depression,

anxiety, and learning and memory deficits (Kelly et al. 2016; Qinrui Li et al. 2017; Carabotti et al. 2015). Evidence also suggests a role of the microbiota–gut–brain axis in reward and motivation (Kiraly et al. 2016; Han et al. 2018; García-Cabrerizo et al. 2021).

The present study aims to explore gut–brain interactions in fentanyl use, which has never been studied before in the literature. Because the literature is limited on fentanyl self-administration, one primary objective of this study is to establish a dose–response relationship of fentanyl intravenous self-administration (IVSA) in adult wild-type Sprague Dawley rats. We implant rats with intravenous catheters in preparation for fentanyl IVSA on an escalating schedule of reinforcement to determine factors that influence fentanyl intake, including sex and dose. Moreover, we test the hypothesis that fentanyl IVSA decreases gut bacteria's alpha and beta diversity based on prior findings that chronic opioid treatment decreases both measures of diversity (Lee et al. 2018; Wang et al. 2018). Given the bidirectional relationship between gut bacteria and behavior, we test the hypothesis that the diversity of gut bacteria predicts fentanyl IVSA. By identifying how gut bacteria influence fentanyl use, our research has the potential to significantly progress our understanding of the mechanisms influencing opioid addiction.

Materials and Methods

Animals

Adult Sprague Dawley rats (8–9 weeks of age) were obtained from Charles River (San Diego, CA, USA) and acclimated to our vivarium at least 7 days prior to experimentation. A total of 52 animals were used in this study with 10 animals excluded from data due to technical issues or lack of catheter patency, and 1 exclusion determined as an outlier via box-and-whisker plots. The final dataset analyzed includes 23 males (n = 9, 8, and 6 at 0, 1.25, and 2.5 µg/kg/infusion, respectively), and 18 females (n = 6, 8, and 4 at 0, 1.25, and 2.5 µg/kg/infusion). Animals were pair-housed (2/cage, 26 cages in final data) in a humidity and temperature-controlled room, and the assigned drug dose for each animal was cage-matched, (i.e., all animals housed in the

same cage self-administered the same drug dose). Food and water were available ad libitum until 24-h prior to drug self-administration and onward when food was restricted to 20–25 g/animal/day (sufficient to maintain adult weight) to motivate exploration. Animals were randomly assigned to experimental groups using a random sequence generator. Animals were housed on a 12-h light-dark cycle (lights on at 07:00) and experimentation was performed during the light part of the light-dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of California Irvine and performed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care.

Catheter Implantation

All rats were anesthetized with Equithesin (0.35 mL/100 g, intraperitoneal) and catheters implanted into their right external jugular vein in preparation for intravenous (i.v.) fentanyl self-administration (implantation surgery described previously) (Belluzzi, Wang, and Leslie 2005). Animals were given carprofen (4 mg/kg, subcutaneous) (Med-Vet, Mettawa, IL, USA), a postoperative analgesic, immediately after surgery. Catheters were flushed daily with sterile heparinized saline (0.6 mL of 1000 units/mL heparin in 30 mL saline) to maintain patency. Animals were allowed at least 2 days to recover from surgery before drug self-administration.

Fentanyl Intravenous Self-Administration

Aqueous fentanyl citrate (Patterson Veterinary, Greeley, CO, USA) was mixed with saline and filtered through a 0.22 μm sterile filter (VWR, Radnor, PA, USA) prior to use. All animals were allowed to self-administer fentanyl at 0, 1.25, or 2.5 μg/kg/infusion during daily 2-h sessions for 5 days at a fixed ratio (FR) 1 schedule of reinforcement, 2 days at FR2, followed by 2 days on FR5, all with a 20-s timeout. Subsequently, animals were assessed for drug reward using a progressive ratio (PR) schedule of reinforcement, where the response requirement to earn an

injection escalates within the same session according to an approximately logarithmic series (Roberts, Loh, and Vickers 1989). Optimal fentanyl doses were selected from prior rodent studies showing acquisition of self-administration while avoiding potentially toxic effects of long-session and long-term self-administration access (van Ree, Slangen, and de Wied 1978; Morgan et al. 2002; Wade et al. 2015). Animals self-administered fentanyl in individual chambers by poking their nose into a “reinforced” nose poke hole, which signaled a cue light to turn on and delivery of one 20 µL i.v. infusion of the assigned dose of fentanyl. Non-reinforced nose pokes at a second hole were counted to control for non-specific drug effects. Data were collected by a multichannel computer system (Med Associates, St. Albans, VT, USA). After the final session on each schedule of reinforcement, propofol (0.1 mL) (Zoetis, Parsipanny, NJ, USA) was injected through the catheter to test patency as indicated by rapid (5–10 s) anesthesia. Data were discarded from animals not demonstrating rapid anesthesia.

16S Sequencing

Fecal samples were suspended in a 1.7 mL Eppendorf tube prefilled with DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and stored at −80 °C until processing. Bacterial genomic DNA from all samples was isolated using the Zymobiomics DNA Mini Kit in a 96-well format (Zymo Research, Irvine, CA, USA). The gDNA was used to target the 16S rRNA gene. A 16S rRNA amplicon PCR was performed targeting the V4-V5 region using the EMP primers (515F (barcoded) and 926R) (Caporaso et al. 2012; Walters et al., 2015). The library was sequenced at the University of California Irvine’s Genomics High Throughput Facility using a MiSeq v3 chemistry with a PE300 sequencing length. Sequencing resulted in 12M single-end reads (forward) passing filter of which 11% are PhiX with a >Q30 = 85%. The raw forward sequences were imported into QIIME2 (Bolyen et al. 2019).

Statistical Analysis

Behavioral data were analyzed with JMP (SAS Institute, Cary, NC, USA). Each self-administration schedule of reinforcement (FR1, FR2, FR5, PR) was analyzed separately with multivariate ANOVA on sex, fentanyl dose, and response, with repeated measures on response (reinforced vs. non-reinforced). Any main effects were further analyzed using Bonferroni-corrected paired (response) or unpaired (drug) t-test post hoc comparisons. One outlier determined from box-and-whisker plots on days 10–11 mean PR infusions was removed from the data. Bacterial sequence data were analyzed using QIIME2 and R Studio.

Experimental Timeline

Adult rats were implanted with an indwelling catheter in their right external jugular vein. Following 2–3 days of post-operative recovery, animals were allowed to self-administer fentanyl via reinforced nose pokes as described above. One fecal sample was collected from each animal the day before fentanyl self-administration and the last day of self-administration to assess the impact of fentanyl IVSA on gut microbiota diversity and composition (Figure 3.1).

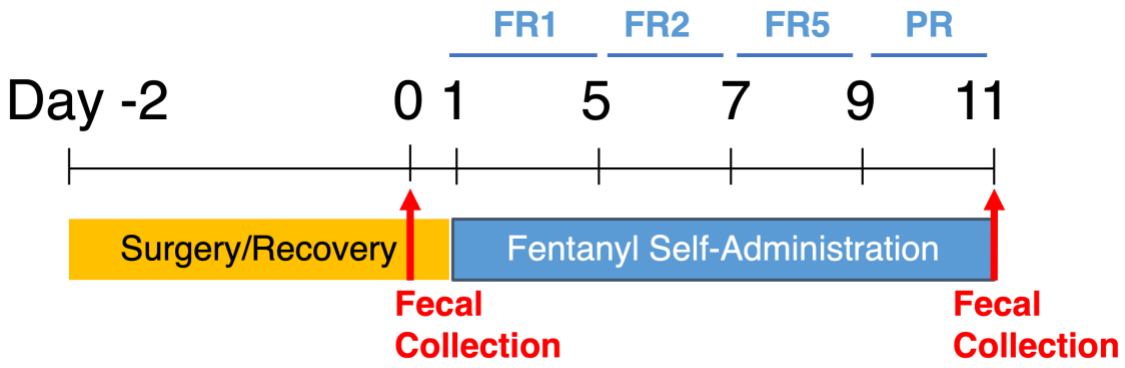

Figure 3.1. Experimental timeline for fentanyl self-administration and fecal sample collection. Animals undergo catheter implantation surgery and recover for 2 full days before starting self-administration. Animals self-administer fentanyl at fixed ratio (FR) 1 for 5 days, FR2 for 2 days, FR5 for 2 days, and progressive ratio (PR) for 2 days, for 11 days total. One fecal sample is collected from each animal the day before self-administration (day 0) and the last day of PR (day 11).

Results

Intravenous Fentanyl Self-Administration Is Dependent on Fentanyl Dose

Current data on fentanyl intravenous self-administration (IVSA) are limited, so we investigated responding for fentanyl infusions on an escalating schedule of reinforcement in wild-type Sprague Dawley rats to establish a fentanyl dose-response relationship. We analyzed fentanyl IVSA with a three-way ANOVA to investigate the roles of sex and fentanyl dose in fentanyl self-administration, in addition to discrimination between reinforced and non-reinforced nose pokes. Data were analyzed separately by schedule of reinforcement, with the mean responses of the last 2 days of each schedule, due to stabilization of learning and drug acquisition. We found a main effect of fentanyl dose across all schedules of reinforcement (fixed ratio (FR) 1: $F(5,35) = 1.69$, $p = 0.05$; FR2: $F(5,35) = 2.43$, $p = 0.01$; FR5: $F(5,35) = 2.26$, $p = 0.01$). Data are collapsed by sex due to a lack of sex differences (FR1: $p = 0.62$, FR2 and FR5: $p = 0.82$). Rats self-administered significantly more fentanyl at 1.25 vs. 0 µg/kg/infusion at FR1 ($p = 0.01$), FR2 ($p = 0.002$), and FR5 ($p = 0.003$) (Figure 3.2A, Table 3.1).

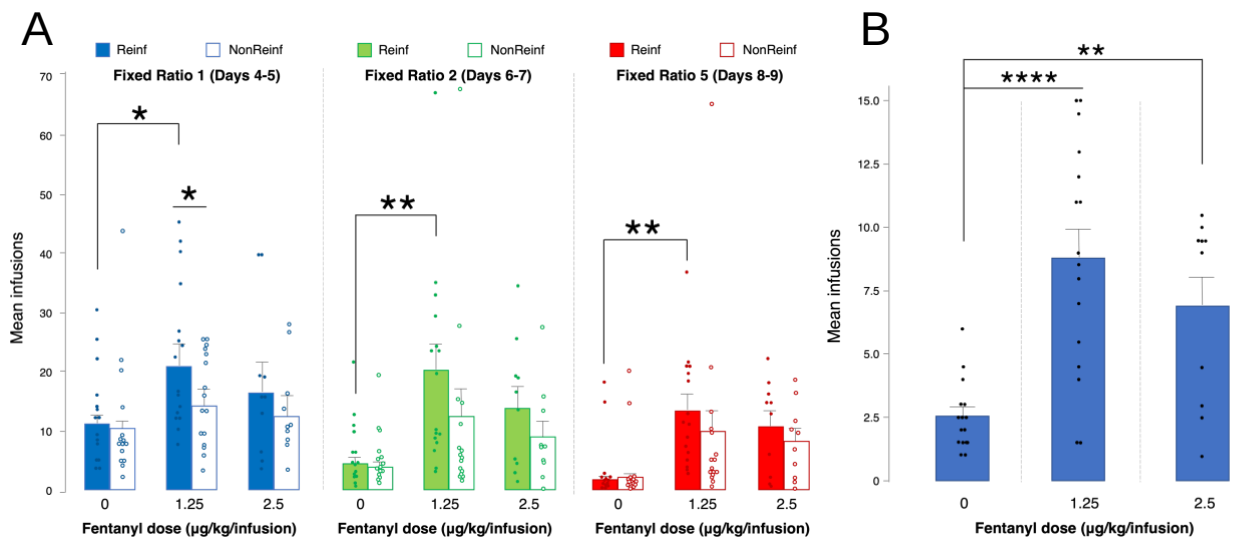

Figure 3.2. Fentanyl intravenous self-administration. (A) Mean number of infusions across 2 days at fixed ratio (FR) 1, FR2, and FR5 schedules of reinforcement, reinforced (Reinf, filled bars and circles) vs. non-reinforced (NonReinf, empty bars and circles) responses, at 0, 1.25, and 2.5 µg/kg/infusion (error bars represent S.E.M., ** $p < 0.01$, * $p < 0.05$, n = 10–16/group). (B) Mean infusion breakpoint over 2 days at a progressive ratio schedule of reinforcement at 0, 1.25, and 2.5 µg/kg/infusion (error bars represent S.E.M., **** $p < 0.0001$, ** $p < 0.01$, n = 10–16/group). Data are collapsed by sex.

Table 3.1. Fentanyl intravenous self-administration. Mean number of infusions self-administered at fixed ratio (FR) 1, FR2, FR5, and progressive ratio (PR) schedules of reinforcement at 0, 1.25, and 2.5 μg/kg/infusion.

Fentanyl Dose	Number of Animals	Results
0 μg/kg/infusion	15	Mean infusions: FR1 D4-5 Reinf: 11.80 FR1 D4-5 NonReinf: 10.60 FR2 D6-7 Reinf: 6.26 FR2 D6-7 NonReinf: 5.76 FR5 D8-9 Reinf: 4.0 FR5 D8-9 NonReinf: 4.26 PR: 2.56
1.25 μg/kg/infusion	16	Mean infusions: FR1 D4-5 Reinf: 20.90 FR1 D4-5 NonReinf: 14.65 FR2 D6-7 Reinf: 20.43 FR2 D6-7 NonReinf: 12.53 FR5 D8-9 Reinf: 13.25 FR5 D8-9 NonReinf: 9.53 PR: 8.81
2.5 μg/kg/infusion	10	Mean infusions: FR1 D4-5 Reinf: 16.45 FR1 D4-5 NonReinf: 12.40 FR2 D6-7 Reinf: 13.80 FR2 D6-7 NonReinf: 8.85 FR5 D8-9 Reinf: 10.70 FR5 D8-9 NonReinf: 8.15 PR: 6.90

Motivation for Fentanyl Infusions Is Highest at 1.25 vs. 0 or 2.5 ug/kg/Infusion

The “breakpoint” at a progressive ratio (PR) schedule of reinforcement is a measure at which an animal stops responding for a reinforcer. We define breakpoint as the last ratio of responses successfully completed by an animal [26]. As fixed ratio is a measure of reinforcement rather than reward or motivation, we measured breakpoint to determine the motivation of animals to

self-administer fentanyl infusions. A two-way ANOVA analyzing sex and fentanyl dose on mean infusions over 2 days at PR yielded a main effect of fentanyl dose ($F(5,35) = 5.08$, $p = 0.0001$). Post-hoc analysis showed significantly higher infusions self-administered at 1.25 vs. 0 µg/kg/infusion ($p < 0.0001$) and 2.5 vs. 0 µg/kg/infusion ($p = 0.008$) (Figure 3.2B, Table 3.1). The data shown are collapsed by sex, as we found no significant effect of sex ($p = 0.75$).

Fentanyl Self-Administration Changes Gut Bacteria Alpha Diversity

We isolated bacterial DNA from fecal samples collected from animals before and after self-administration to evaluate whether fentanyl IVSA alters the diversity of gut bacteria. Alpha diversity was measured using two diversity indices: Chao1, which estimates the total richness of a microbial sample based on relative abundance, and Shannon, which considers both richness and evenness of a microbial sample. We observed a main effect of sex between subjects ($F(1,35) = 6.88$, $p = 0.01$), a sex * fentanyl dose interaction ($F(2,35) = 3.52$, $p = 0.04$), and a main effect of time within subjects (before vs. after fentanyl IVSA) ($F(1,35) = 4.07$, $p = 0.05$). When evaluating post hoc comparisons, there was a significant reduction of alpha diversity in males compared to females before fentanyl IVSA (Shannon: $p = 0.02$, Chao1: $p = 0.01$). This baseline difference in alpha diversity appears to be driven by Verrumicrobia ($p = 0.01$), *Prevotella* ($p = 0.02$), and *Akkermansia* ($p = 0.01$), as relative abundance in these bacterial groups was significantly different in females than males before fentanyl IVSA (Table 3.2). When analyzing samples by sex and fentanyl dose, we found that Shannon diversity (richness and evenness) is increased after vs. before IVSA in males at 1.25 µg/kg/infusion ($p = 0.02$), and Chao1 diversity (richness) is increased after IVSA at 0 and 1.25 µg/kg/infusion ($p = 0.03$ and $p = 0.02$, respectively) (Figure 3.3A,B). Further, we observed that fentanyl IVSA at 1.25 vs. 0 µg/kg/infusion reduced alpha diversity in females ($p = 0.05$). Firmicutes/Bacteroidetes ratios remained stable before and after fentanyl IVSA (Figure 3.3C). In males that self-administered fentanyl at 1.25 µg/kg/infusion, changes in alpha diversity were seen in the bacterial phylum

Verrucomicrobia (p = 0.03) and genera *Ruminococcus* (p = 0.03) and *Akkermansia* (p = 0.03) (Table 3.2). Relative abundance of the bacterial genus *Prevotella* was greater in females that self-administered 1.25 µg/kg/infusion (Table 3.2). There were no significant differences in relative abundance or percent composition of Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes, Actinobacteria, or Lactobacillus before and after fentanyl IVSA (data not shown).

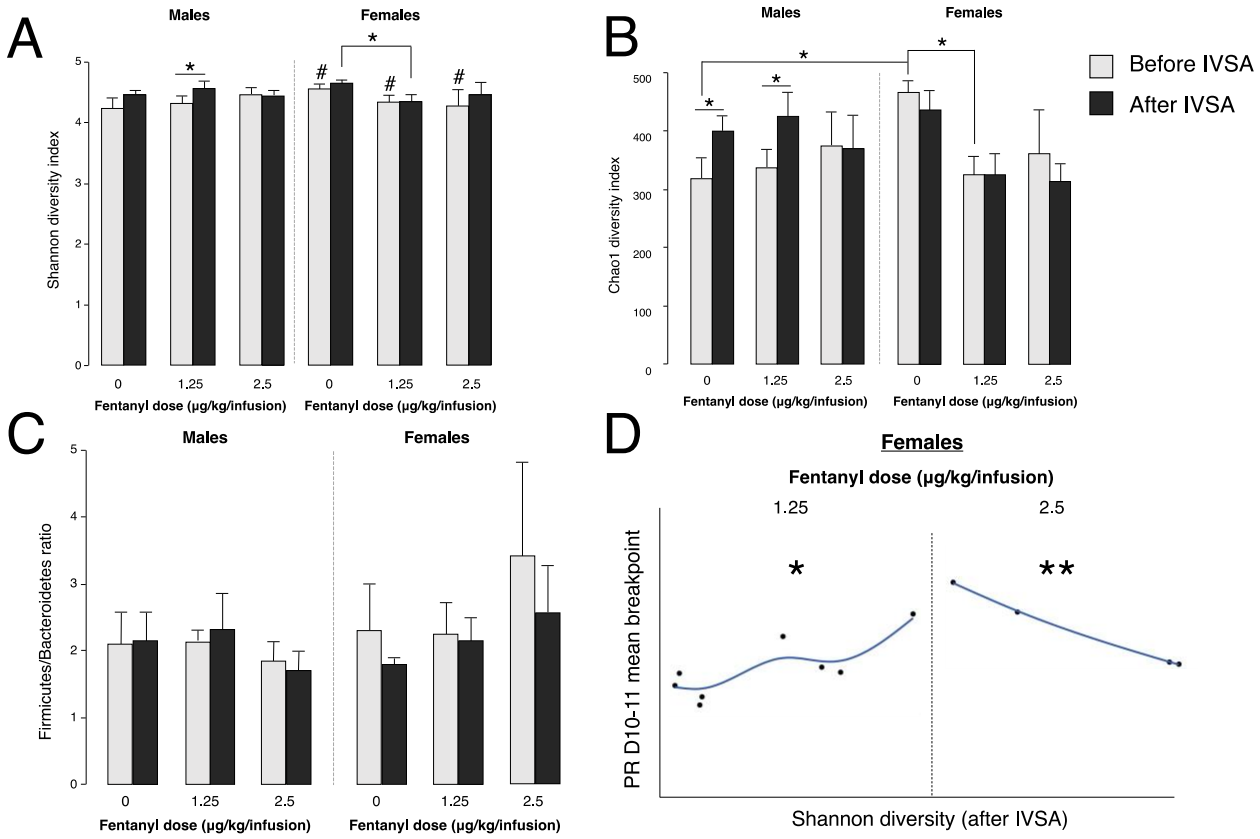

Figure 3.3. Alpha diversity of gut bacteria before and after IVSA. (A) Shannon diversity index values of gut bacteria before (gray bars) and after (black bars) IVSA in males and females (error bars represent S.E.M., * $p < 0.05$, # $p < 0.05$ vs. males, n = 4–9/group). (B) Chao1 diversity index values of gut bacteria before (gray bars) and after (black bars) in males and females (error bars represent S.E.M., * $p < 0.05$, n = 4–9/group). (C) Firmicutes/Bacteroidetes ratios before (gray bars) and after (black bars) IVSA in males and females (error bars represent S.E.M., n = 4–9/group). (D) Correlation plots of alpha diversity after IVSA vs. progressive ratio (PR) mean breakpoint on days (D) 10–11 in females at 1.25 and 2.5 µg/kg/infusion (** $p < 0.01$, * $p < 0.05$, n = 4–8/group).

Table 3.2. Bacterial groups impacted by fentanyl intravenous self-administration (IVSA).

Bacterial Phylum or Genus	Results
Verrucomicrobia	Decreased after fentanyl IVSA (males, 1.25 μg/kg/infusion; p=0.03) Increased in females vs. males before fentanyl IVSA (p=0.01)
Prevotella	Increased after fentanyl IVSA (females, 1.25 μg/kg/infusion; p=0.02) Decreased in females vs. males before fentanyl IVSA (p=0.02)
Ruminococcus	Increased after fentanyl IVSA (males, 1.25 μg/kg/infusion; p=0.03)
Akkermansia	Decreased after fentanyl IVSA (males, 1.25 μg/kg/infusion; p=0.03) Increased in females vs. males before fentanyl IVSA (p=0.01)

Baseline Gut Bacteria Alpha Diversity Does Not Predict Fentanyl Self-Administration

To investigate whether an individual animal's gut bacteria diversity impacts fentanyl self-administration behavior, we analyzed the correlation between gut bacteria alpha diversity before fentanyl IVSA and (1) mean infusions self-administered at FR1 and (2) mean breakpoint at PR. We found no significant correlations between baseline gut bacteria (alpha diversity before fentanyl IVSA) and mean infusions at FR1 or mean breakpoint at PR, suggesting that gut bacteria do not influence the motivation to self-administer fentanyl infusions.

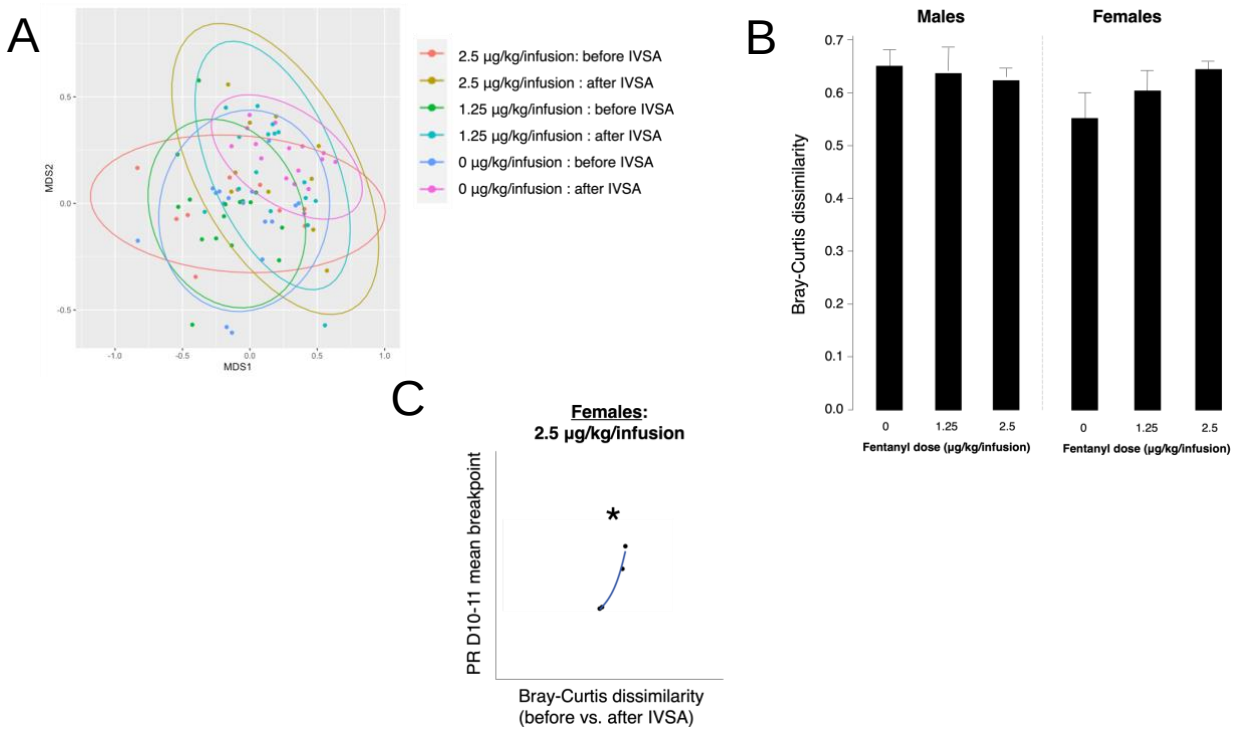

Figure 3.4. Beta diversity of gut bacteria before and after IVSA. (A) Non-metric multidimensional scaling (NMDS) ordination of gut microbiota communities before and after fentanyl IVSA at 0, 1.25, and 2.5 µg/kg/infusion. (B) Bray-Curtis dissimilarity values extracted from distance matrix comparing before and after IVSA within each animal at 0, 1.25, and 2.5 µg/kg/infusion in males and females (error bars represent S.E.M., n = 4–9/group). (C) Correlation plot of Bray–Curtis dissimilarity vs. progressive ratio (PR) mean breakpoint on days (D) 10–11 in females at 2.5 µg/kg/infusion (* $p < 0.05$, n = 4).

Gut Bacteria Alpha Diversity Predicts Progressive Ratio Responding in Females

In addition to baseline gut bacteria influencing self-administration behavior, we wanted to consider the relationship between the rewarding value of fentanyl and gut bacteria diversity. We analyzed the correlation between gut bacteria alpha diversity after fentanyl IVSA and the mean breakpoint at PR. In females only, we discovered a positive correlation between Shannon index values and mean breakpoint at 1.25 µg/kg/infusion ($p = 0.02$), and a negative correlation between these same variables at 2.5 µg/kg/infusion ($p = 0.002$). These data show that at 1.25 µg/kg/infusion, gut bacteria alpha diversity increases breakpoint, while at 2.5 µg/kg/infusion, gut bacteria alpha diversity decreases breakpoint (Figure 3.3D).

Fentanyl Self-Administration Does Not Alter Beta Diversity of Gut Bacteria

Beta diversity values were ordinated for visual representation using non-metric multidimensional scaling (NMDS) in R Studio (Figure 3.4A), as well as extracted from a distance matrix into individual Bray–Curtis dissimilarity values by comparing the distance before and after fentanyl IVSA within each animal (Figure 3.4B). A 2-way ANOVA evaluating the roles of sex and fentanyl dose on Bray–Curtis dissimilarity did not yield any significant findings ($F(5,30) = 0.54$ $p = 0.74$).

Gut Bacteria Beta Diversity Predicts Progressive Ratio Responding in Females

To evaluate the relationship between shifts in gut microbiota composition and the rewarding value of fentanyl, we tested the correlation between gut bacteria beta diversity and mean breakpoint at PR. Bray–Curtis dissimilarity measures how different two microbiota compositions are from each other. We found a positive correlation in females at 2.5 µg/kg/infusion ($p = 0.025$) (Figure 3.4C), indicating higher motivation for fentanyl as the microbiome shifts.

Discussion

The present study provides a feasible animal model of intravenous fentanyl self-administration and a bidirectional relationship between fentanyl self-administration and gut bacteria. We show here that fentanyl reinforcement and motivation are dependent on fentanyl dose, but not sex. Further, we show that both sex and dose determine the impact of fentanyl IVSA on alpha diversity of gut bacteria, as fentanyl IVSA increases diversity in males at 1.25 µg/kg/infusion and decreases alpha diversity in females at 1.25 vs. 0 µg/kg/infusion. Supporting a bidirectional relationship between fentanyl IVSA and gut bacteria, we find that bacterial diversity sex- and dose-dependently predicts responding for fentanyl infusions at a progressive ratio schedule of reinforcement.

Previous studies report sex differences in opioid abuse and reinforcement in humans and animals (Townsend et al. 2019; Lacy et al. 2016; Klein, Popke, and Grunberg 1997; Carroll,

Campbell, and Heideman 2001; Cicero, Aylward, and Meyer 2003; Kaplovitch et al. 2015). Although our results do not determine whether sex affects fentanyl self-administration at FR or PR schedules of reinforcement, we uncover an interaction between gut bacteria and sex to impact fentanyl IVSA. The discrepancy in our results compared to prior findings may be explained by methodological differences, such as species and strain used, nose poke vs. lever presses, route of drug administration, self-administration vs. experimenter-administration, and duration of drug access or exposure.

We show sex differences in baseline alpha diversity of gut microbiota, which is consistent with our hypothesis. These microbial differences are driven by Verrucomicrobia, *Prevotella*, and *Akkermansia*. The available literature on the impact of sex on gut microbiota is inconsistent, with findings either supporting or rejecting sex differences in gut microbiota diversity and composition (Y. S. Kim et al. 2019). Genotype, strain, and species appear to be stronger determinants than the sex of the rodent microbiota (Kovacs et al. 2011; Elderman et al. 2018). However, sex is an important variable in gut microbiota and behavior in both clinical and preclinical studies (Van Nas et al. 2009; Org et al. 2016). Further research is necessary to establish the role of sex on the gut microbiome, though our findings demonstrate that sex impacts baseline gut bacteria and opioid-induced changes in bacteria.

Our results support the hypothesis that opioid use affects gut bacteria. We see increased alpha diversity following fentanyl IVSA in males that self-administer 1.25 µg/kg/injection, and a reduction in alpha diversity following fentanyl IVSA in females at 1.25 vs. 0 µg/kg/injection. In addition, we find correlations between the diversity of gut bacteria and fentanyl IVSA at PR in females, but not males. The increasing breakpoint with increasing microbiome changes, both in alpha and beta diversity, is more likely that gut bacteria influence self-administration rather than responses affecting gut bacteria, as bacterial shifts do not occur that quickly (O'Toole and Ian 2015). Future studies aim to examine how direct manipulation of gut bacteria influences fentanyl self-administration.

The increase in alpha diversity after fentanyl IVSA in males is surprising and does not mimic results found in prior work focused on chronic morphine treatment in mice (Banerjee et al. 2016; Lee et al. 2018; Wang et al. 2018; Hofford et al. 2021). There are major differences in methodology from the animals examined, both in species and model, (e.g., opioid-dependent, self-administering), and drugs used. Additionally, there are arguments in favor of microbial composition rather than diversity, as high diversity is not necessarily "healthier" (Shade 2017). One possibility of increased alpha diversity in our results is an introduction of pro-inflammatory microbes (Scotti et al. 2017), although further analysis of species is essential to clarify this. We observed a difference in Verrucomicrobia (phylum), *Ruminococcus* (genus), and *Akkermansia* (genus) in males at 1.25 µg/kg/injection, and in *Prevotella* (genus) in females at 1.25 µg/kg/injection. The lack of change in the Firmicutes/Bacteroidetes ratio before and after fentanyl IVSA is expected, as these two phyla dominate a stable adult gut microbiome and are less susceptible to disruption (Paul et al. 2005) than other phyla.

The present study is limited by its relatively small sample size, which obscures the natural variability of the gut microbiome. Further, as a microbiome study, our use of a conventional environment over germ-free conditions introduces contamination as a potential factor in both natural gut bacterial shifts and in sample processing. Although previous studies have observed microbiota differences following short-term drug treatment, our 11-day paradigm may be too short to see noticeable microbial changes. Co-housing of animals also may mask potential microbial shifts. Finally, we would argue both as a limitation and a strength that fentanyl dose alone is not sufficient to reveal fentanyl-induced changes in gut microbiota, as animals self-administer varying amounts due to individual preference. Additional experimentation could examine how a fixed fentanyl intake impacts gut bacteria, although experimenter-administered vs. self-administered drug may be a stressor.

Collectively, our data show the impact of intravenous fentanyl self-administration on gut microbiota composition, as well as the role of gut microbiota on fentanyl self-administration, in

wild-type Sprague Dawley rats in a sex- and dose-dependent manner. Given the associations between the gut microbiota and stress, mood, psychiatric disorders, and behavior, evaluating the role of the gut microbiota in fentanyl use is a unique approach that could lead to new paths for the treatment of addiction. By identifying how the gut–brain axis influences fentanyl use, our research has the potential to significantly progress our understanding of the mechanisms influencing opioid addiction. The next steps are to investigate possible mechanisms underlying opioid-induced changes in gut bacteria, such as inflammation driven by decreased gut permeability (gut “leakiness”) and/or functional changes in gut peptides or bacterial metabolites, (i.e., short-chain fatty acids). Identification of direct mechanisms allows for targeted pharmacological therapeutics to address opioid abuse. Plausible clinical approaches include (1) early diagnosis and prevention by recognizing biomarkers or (2) supplementation with beneficial bacterial strains or metabolites, targeted anti-inflammatories, or microbiota transplantation.

Chapter 4

Antibiotic Knockdown of Gut Bacteria Sex-Dependently Enhances Intravenous Fentanyl Self-Administration in Adult Sprague Dawley Rats

Introduction

The number of opioid-related deaths is continuing to rise with the most recent spike in deaths driven by fentanyl (Rudd et al. 2016; SAMSA 2020). This highlights the urgency to identify underlying mechanisms contributing to fentanyl abuse and addiction. There is a clear association between opioids and gut health given the gastrointestinal (GI) side effects from opioid use (e.g., constipation, nausea) due to the widespread distribution of opioid receptors throughout the GI tract (Panchal, Müller-Schwefe, and Wurzelmann 2007; Holzer 2004). The trillions of microbes that reside in the intestines are known as gut bacteria (also called microbiota or flora) and collectively make up an organism's gut microbiome (Guarner and Malagelada 2003). In this study, we show communication between gut bacteria and the brain as a potential mechanism underlying fentanyl intravenous self-administration (IVSA) in adult Sprague Dawley rats.

Bidirectional communication through the gut-brain axis has been demonstrated to mediate neuropsychiatric disease (Iannone et al. 2019; Cryan et al. 2020). The gut and brain are physically connected via the vagus nerve (Puizillout 2005), which has been shown to mediate behavior in mouse models of anxiety and depression (Bercik, Park, et al. 2011; Ghia, Blennerhassett, and Collins 2008; Bravo et al. 2011). Gut-brain communication also occurs biochemically through hormones, neurotransmitters, immune signaling, and microbial metabolites (Berthoud 2008; Mayer 2011; Sharon et al. 2016). Evidence that the brain and gut microbiota interact with each other has been established in a variety of studies. Animals raised and maintained with no microbes (i.e., germ-free) compared to conventional animals

demonstrate differences in anxiety- and depression-like behaviors, locomotor activity, gene expression, microglia, and neurogenesis (Bercik, Denou, et al. 2011; Heijtz et al. 2011; Hegstrand and Hine 1986; K. Neufeld et al. 2011; Nobuyuki et al. 2004). Additionally, administration of specific bacterial strains, fecal microbiota transplantation, or antibiotics distinctly impacts behavior, the brain and spinal cord, and gut health (Bercik, Park, et al. 2011; Bravo et al. 2011; Kelly et al. 2016; Desbonnet et al. 2015; McKernan et al. 2010; O'Mahony et al. 2014).

Gut bacteria influence neural circuits and behaviors that are markedly associated with addiction, including reward, tolerance, and withdrawal (Kiraly et al. 2016; M. Kang et al. 2017; Wang et al. 2018; Lee et al. 2018; Hofford, Russo, and Kiraly 2019; Han et al. 2018; Thomaz et al. 2021). While alterations in gut microbiota directly affect opioid-related behaviors, opioid exposure also alters the diversity and/or composition of the host gut microbiome (Wang et al. 2018; Banerjee et al. 2016; Meng et al. 2013; Ren and Lotfipour 2022; Acharya et al. 2017; Zhang et al. 2020; Cruz-Lebrón et al. 2021; Zhang et al. 2021). We previously demonstrated the impact of fentanyl self-administration on gut microbiota (Ren and Lotfipour 2022). To highlight the bidirectional communication between the brain and gut microbiota, our present study aims to evaluate the role of gut microbiota on fentanyl self-administration. As depletion of gut bacteria via oral antibiotic treatment has been shown to enhance sensitivity to cocaine reward and disrupt opioid reward (Kiraly et al. 2016; Hofford et al. 2021), we test the hypothesis that knocking down gut bacteria will enhance fentanyl self-administration due to dysregulated reward processing. We assess alpha diversity from fecal samples of water- and antibiotic-treated animals to confirm that the selected antibiotic doses and duration of treatment significantly deplete gut bacteria. Subsequently, we administer short-chain fatty acids (SCFAs) to bacteria-depleted rats to examine the impact of gut microbial repletion on fentanyl self-administration. SCFAs are the main metabolites produced by bacterial fermentation of dietary fiber in the GI

tract, and prior work demonstrated SCFA treatment in antibiotic-treated animals restores behavioral responses akin to controls (Kiraly et al. 2016).

The lack of a normal, healthy gut microbiome is understood to contribute to depression and anxiety (Bravo et al. 2011; Heijtz et al. 2011; Kelly et al. 2016; Desbonnet et al. 2015; Burokas et al. 2017; Wong et al. 2016; K.-A. M. Neufeld et al. 2011), and accumulating evidence shows that imbalances in gut bacteria (i.e., gut dysbiosis) is also linked to opioid use disorder (Jalodia et al. 2022; Ren and Lotfipour 2020). The antibiotic treatment in this study is a means to significantly deplete gut bacteria (Kiraly et al. 2016) and does not mirror clinical doses. The cited animal studies addressing the connection between drugs of abuse and gut microbiota evaluate drug reward using conditioned place preference. We use an intravenous model of self-administration on an escalating schedule of reinforcement to assess drug reinforcement and motivation. Evaluating the bidirectional relationship between gut microbiota and fentanyl reinforcement and reward contributes to the limited understanding of mechanisms mediating opioid dependence and abuse. Building upon this foundation may allow for the development of tractable treatment options for opioid use.

Materials and Methods

Animals

Adult male and female Sprague Dawley rats (8 weeks of age) were obtained from Charles River (San Diego, CA, USA) and acclimated to our vivarium at least 7 days prior to experimentation. Animals were pair-housed in a humidity and temperature-controlled room on a 12 h light-dark cycle (lights on at 0700). Two separate experiments were run in this study, denoted as Antibiotic Treatment and Short-Chain Fatty Acid Supplementation. 37 total animals were used in the antibiotic treatment experiment (20 males and 17 females) and 5 animals were excluded due to failure of catheter patency. Fecal samples from a subset of the males were collected for analysis. 49 total animals, all males, were used in the short-chain fatty acid supplementation

experiment and 14 animals were excluded (8 due to failure of catheter patency and 6 outliers determined by box-and-whisker plots). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC protocol number AUP-21-022) at the University of California Irvine and per-formed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care.

Antibiotic and Short-Chain Fatty Acid (SCFA) Treatment

Animals were randomly assigned to treatment groups using a random sequence generator. An antibiotic cocktail (2 g/L neomycin, 0.5 g/L bacitracin, 0.2 g/L vancomycin) was mixed in drinking water and provided ad libitum. SCFAs were mixed in drinking water in the following concentrations: 67.5 mM acetate, 40 mM butyrate, and 25.9 mM propionate (Sigma Aldrich, St. Louis, MO, USA). Water bottles were changed every 2 days and weighed daily to ensure the intake of antibiotics. The selected agents, doses, and treatment duration were adapted from Kiraly et al., 2016.

Catheter Implantation and Drug Self-Administration

Methodology for catheterization surgery in preparation for fentanyl intravenous self-administration (IVSA) was described previously (Ren and Lotfipour 2022). Rats were anesthetized with Equithesin (0.35 mL/100 g, intraperitoneal) and administered carprofen (4 mg/kg, subcutaneous) (Patterson Veterinary, Greeley, CO, USA) for post-operative analgesia. Animals were given at least 2 days to recover from surgery before fentanyl self-administration (see Ren and Lotfipour, 2022 for detailed methods). Briefly, fentanyl solutions were prepared using aqueous fentanyl citrate (Patterson Veterinary, Greeley, CO, USA) and sterile saline. All animals self-administered fentanyl at 1.25 µg/kg/infusion during daily 2-hour sessions on an escalating schedule of reinforcement (i.e., 5 days at a fixed ratio (FR) 1, 2 days at FR2, 2 days at FR5, and 2 days at progressive ratio (PR)). Animals self-administered fentanyl in individual

chambers via reinforced nose pokes, with non-reinforced nose pokes at a second hole to control for non-specific drug effects. Data were collected by a multichannel computer system (Med Associates, St. Albans, VT, USA). Catheter patency was tested after the final session on each schedule of reinforcement via i.v. administration of propofol (0.1 mL) (Zoetis, Parsipanny, NJ, USA). Data were discarded from 5 out of 37 animals not demonstrating rapid anesthesia.

Experimental Timeline (Antibiotic Treatment)

Rats were treated with water or antibiotics via their home water bottles and remained on the same treatment for the duration of the experiment. One week after antibiotic treatment, rats underwent catheter implantation surgery in preparation for fentanyl IVSA and began self-administration after 2 days of recovery. One fecal sample was collected from each animal before surgery to confirm knockdown of gut bacteria prior to IVSA, and again at the end of experiment to ensure maintenance of bacterial depletion (Figure 4.1).

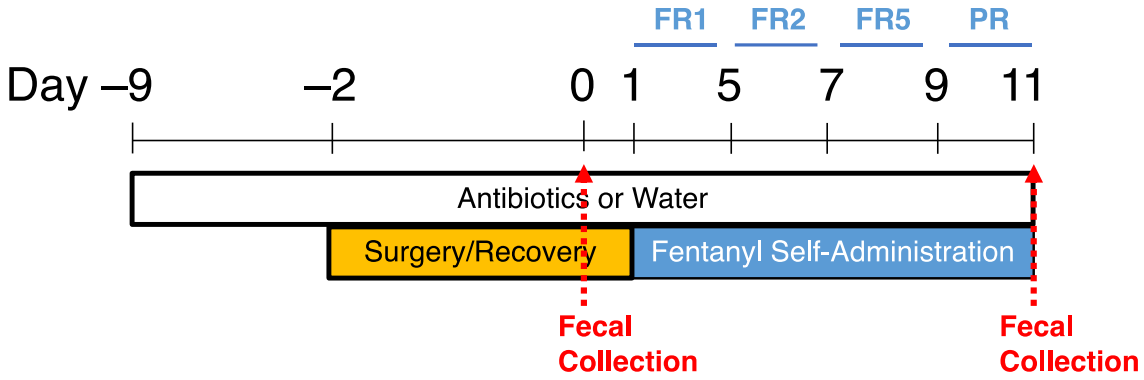

Figure 4.1. Experimental timeline for antibiotic treatment, fentanyl self-administration, and fecal sample collection. Animals were provided drinking water plus antibiotics or drinking water only (controls) and maintained their assigned treatment throughout the entire experiment. Following one week of antibiotic or water treatment, animals were implanted with intravenous catheters and given 2 full days to recover from surgery before starting self-administration. Animals self-administered fentanyl at fixed ratio (FR) 1 for 5 days, FR2 for 2 days, FR5 for 2 days, and progressive ratio (PR) for 2 days, for 11 days total. One fecal sample was collected from each animal the day before self-administration (day 0) and the last day of PR (day 11).

Experimental Timeline (SCFA Supplementation)

Animals were randomly assigned to the following experimental groups: water/water, water/SCFA, ABX/water, or ABX/SCFA. Rats were treated with water or antibiotics via their home water bottles for 3 days, and then the addition of either water or SCFA was added to the same bottle. Rats were maintained on the same treatment for the remainder of the experiment. One week after the start of antibiotic or water treatment, rats were implanted with catheters in preparation for fentanyl IVSA and began self-administration after 2 days of recovery (Figure 4.2).

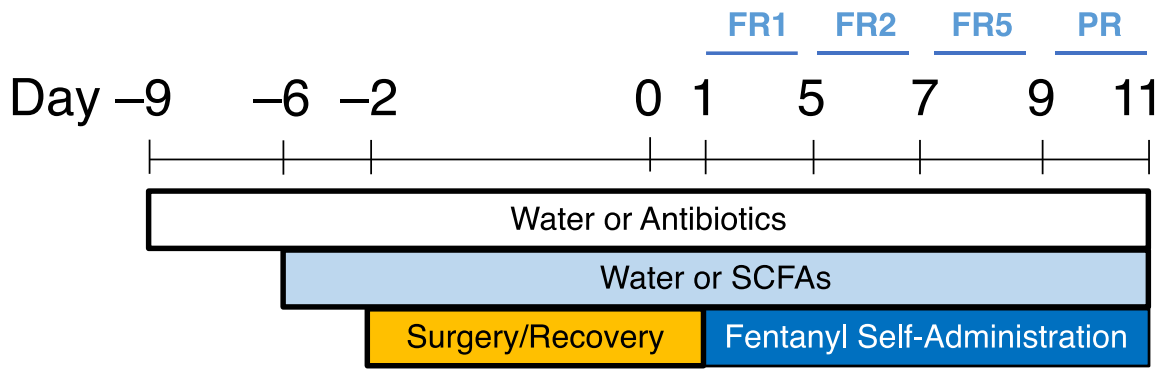

Figure 4.2. Experimental timeline for short-chain fatty acid (SCFA) supplementation and fentanyl self-administration. Animals were provided drinking water plus antibiotics or drinking water only for 3 days. SCFA or vehicle (water) was added to the treatment and rats were maintained on their assigned treatment throughout the entire experiment. Following one week of antibiotic treatment, animals were implanted with intravenous catheters and given 2 full days to recover from surgery before starting self-administration. Animals self-administered fentanyl at fixed ratio (FR) 1 for 5 days, FR2 for 2 days, FR5 for 2 days, and progressive ratio (PR) for 2 days, for 11 days total.

16S Sequencing

Fecal samples from animals were collected in individual 1.7 mL Eppendorf tubes prefilled with DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and stored at -80^{o}C until processing. Bacterial genomic DNA from all samples was isolated using the Zymobiomics DNA Mini Kit in a 96-well format (Zymo Research, Irvine, CA, USA). The genomic DNA was used to target the 16S rRNA gene. 16S rRNA amplicon PCR was performed targeting the V4-V5 region using the Earth Microbiome Project primers (515F (barcoded) and 926R) (Caporaso et al. 2012; Walters et al. 2021). The library was sequenced at the University of California Irvine's Genomics High Throughput Facility using Illumina MiSeq v3 (600 cycles) with a PE300 sequencing length.

Sequencing resulted in 12M single end reads (forward) passing filter of which 11% are PhiX with a >Q30=85%. The raw forward sequences were imported into QIIME2 (version 2020.8). After initial sample quality check and trimming (DADA2 in QIIME2), there were 9.1 M single-end non-chimeric reads, which were used for further analysis. From the sequences, the first 5 bp were trimmed and truncated at 243 bp. The sequences were assigned a taxonomic classification using the August 2013 greengenes database (greengenes.secondgenome.com), trained with the full length 16S gene region supplied by QIIME2.

Microbiome Analysis

Sequence data were exported from QIIME2 and integrated within R Studio. Within R Studio, we rarefied the feature table (organized by exact sequence variants) to the same sequencing depth (rarefaction depth = 7200, based on the lowest read distribution), plotted Shannon diversity index values, and created a distance matrix. Statistical comparison of the communities was performed using Tukey's HSD, PERMANOVA, and multivariate ANOVA.

Statistical Analysis

Behavioral data were analyzed with JMP (SAS Institute, Cary, NC, USA). Each self-administration schedule of reinforcement (FR1, FR2, FR5, PR) was analyzed separately with multivariate ANOVA on sex, treatment, and response, with repeated measures on response (reinforced vs. non-reinforced). Any main effects were further analyzed using Bonferroni-corrected paired (response) or unpaired (drug) t-test post-hoc comparisons.

Results

One-week oral antibiotic treatment significantly depletes gut bacteria (and depletion is maintained throughout experiment)

We analyzed bacteria of fecal samples from a subset of males in our self-administration study. We evaluated the diversity of gut bacteria using the Shannon diversity index (richness and evenness) and the relative abundance of specific phyla and genera. We used a 2-way, repeated measures ANOVA to analyze differences in Shannon diversity between water- and antibiotic-treated rats before and after fentanyl self-administration to ensure that the selected antibiotic cocktail knocked down gut bacteria prior to the start of self-administration and that knockdown was maintained throughout the experiment. We found a significant reduction in Shannon diversity in antibiotic-treated vs. water-drinking controls both before ($F(1,6) = 195.76$, $p < 0.0001$) and after ($F(1,6) = 116.18$, $p = 0.0001$) self-administration (Figure 4.3). In addition to a main effect of treatment, we found a significant difference in Shannon diversity before and after fentanyl self-administration in water-treated animals ($p = 0.01$) (Figure 4.3).

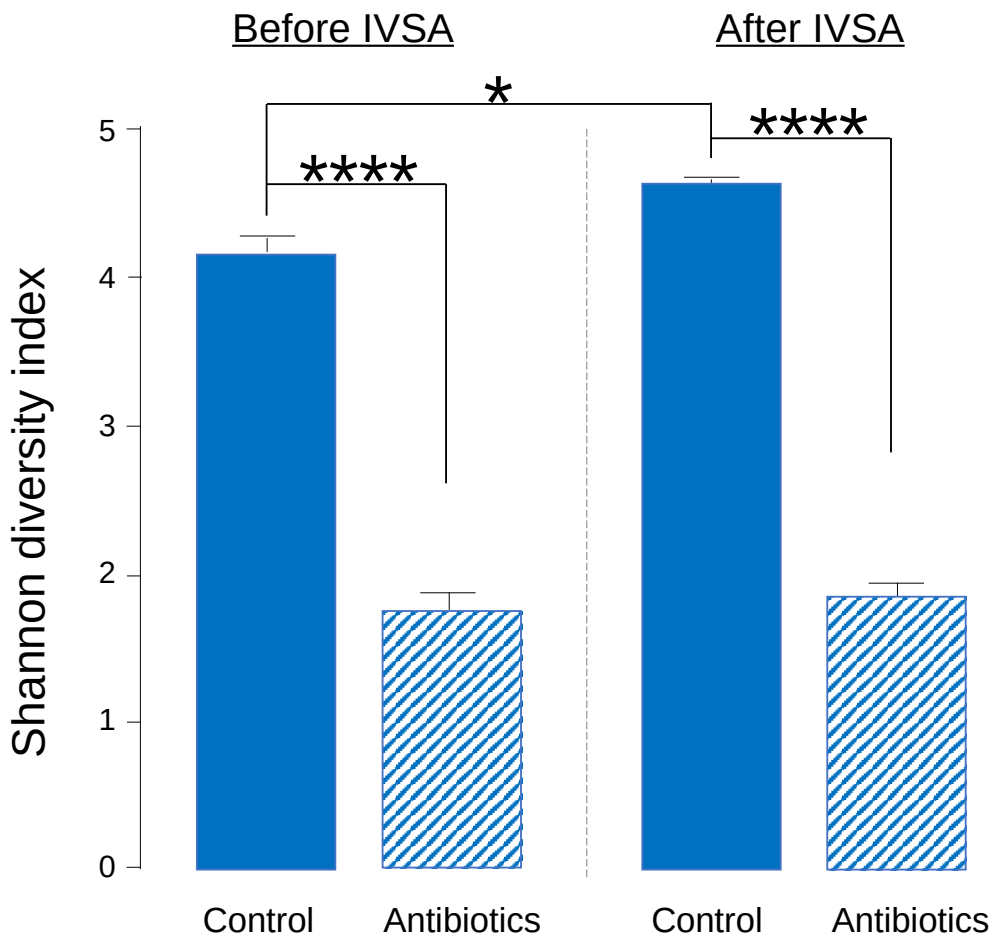

Figure 4.3. Shannon diversity index values from fecal samples before (left of dashed line) and after (right of dashed line) fentanyl intravenous self-administration (IVSA) in males treated with water only (solid bars) or water with antibiotics (dashed bars). Error bars represent S.E.M. **** $p < 0.0001$, * $p < 0.05$, n = 4–8/group.

Antibiotic treatment causes phylum- and genus-level changes in gut bacteria

To assess phylum-level changes in treatment groups before and after fentanyl self-administration, we ran a one-way, repeated measures ANOVA to identify differences in the relative abundance of Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes, Verrucomicrobia, and Actinobacteria. We observed a significant increase in Bacteroidetes ($p = 0.002$) in antibiotic-treated animals after vs. before fentanyl self-administration but saw no timepoint differences in controls (Figure 4.4). Further, we found a significant decrease in the percent

relative abundance of Firmicutes, Tenericutes, and Actinobacteria, and a significant increase in Bacteroidetes, Proteobacteria, and Verrucomicrobia in antibiotic-treated animals compared to controls independent of fecal collection timepoint (before or after self-administration) (Figure 4.4). This indicates that one week of antibiotic treatment is sufficient to change all six phyla evaluated here. Analysis at the genus level did not reveal the specific changes in Bacteroidetes in the antibiotic-treated group before vs. after fentanyl self-administration; however, we found a higher relative abundance of the genus *Prevotella* in control animals compared to those treated with antibiotics (Figure 4.5). We also found specific genus differences in the Firmicutes phylum when comparing controls and antibiotic groups (Figure 4.5). Animals drank the same amount of water and maintained the same weight regardless of the treatment group.

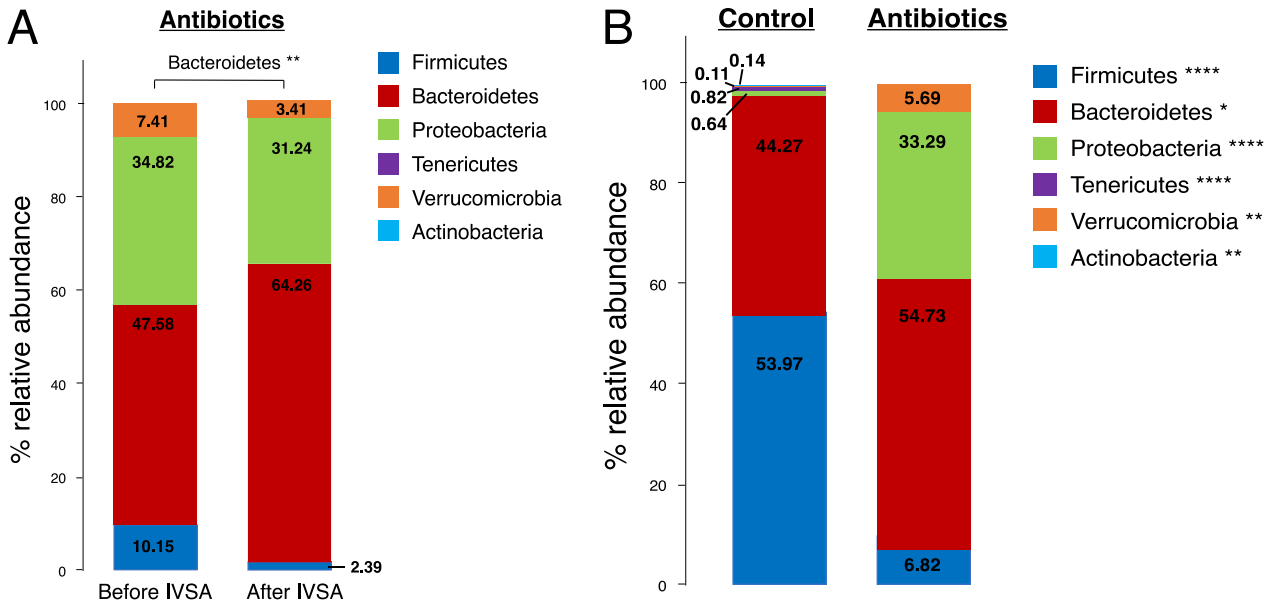

Figure 4.4. Alpha diversity at the phylum level in antibiotic-treated and control males. (A) Percent relative abundance of Firmicutes (dark blue), Bacteroidetes (red), Proteobacteria (green), Tenericutes (violet), Verrucomicrobia (orange), and Actinobacteria (light blue) in antibiotic-treated animals before and after fentanyl intravenous self-administration (IVSA). ** $p < 0.01$, n = 6–8/group. (B) Percent relative abundance of Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes, Verrucomicrobia, and Actinobacteria in controls and antibiotic-treated animals. Data are collapsed by fecal collection timepoint. Asterisks (*) next to each phylum listed in the legend represent significant differences between controls and antibiotic treatment. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, n = 9–14/group.

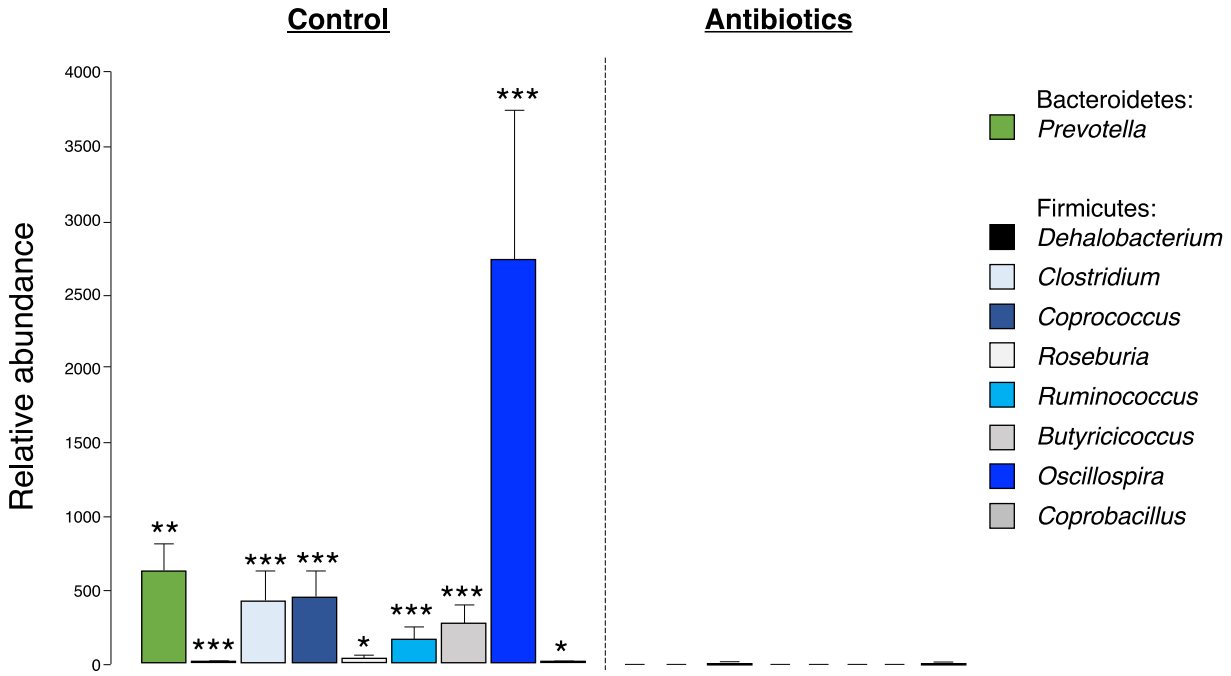

Figure 4.5. Alpha diversity at the genus level in control and antibiotic-treated males. Relative abundance of the genera *Prevotella*, *Dehalobacterium, Clostridium, Coprococcus, Roseburia, Ruminococcus, Butyricicoccus, Oscillospira,* and *Coprobaccilus* in controls and antibiotic-treated animals. Asterisks (*) above bars represent significant differences between controls and antibiotic treatment. Data are collapsed by fecal collection timepoint. Error bars represent S.E.M., *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n = 9–14/group.

Antibiotic-treated males, but not females, self-administer more fentanyl than water-treated controls at fixed ratio (FR) 1

We ran a 2-way ANOVA for each schedule of reinforcement to assess the role of treatment and sex on fentanyl self-administration. Because there was no difference in responding on any day at FR1, we analyzed a 2-day average (days 4-5). The last two days were selected, as animals may take time to acquire drug self-administration. At FR1, we found a significant interaction between treatment and sex ($F(3,31)=4.5$, $p=0.04$), thus data were analyzed separately for males and females. We observed a main effect of treatment on reinforced responding in males ($p=0.02$) but not females ($p=0.78$) (Figure 4.6A).

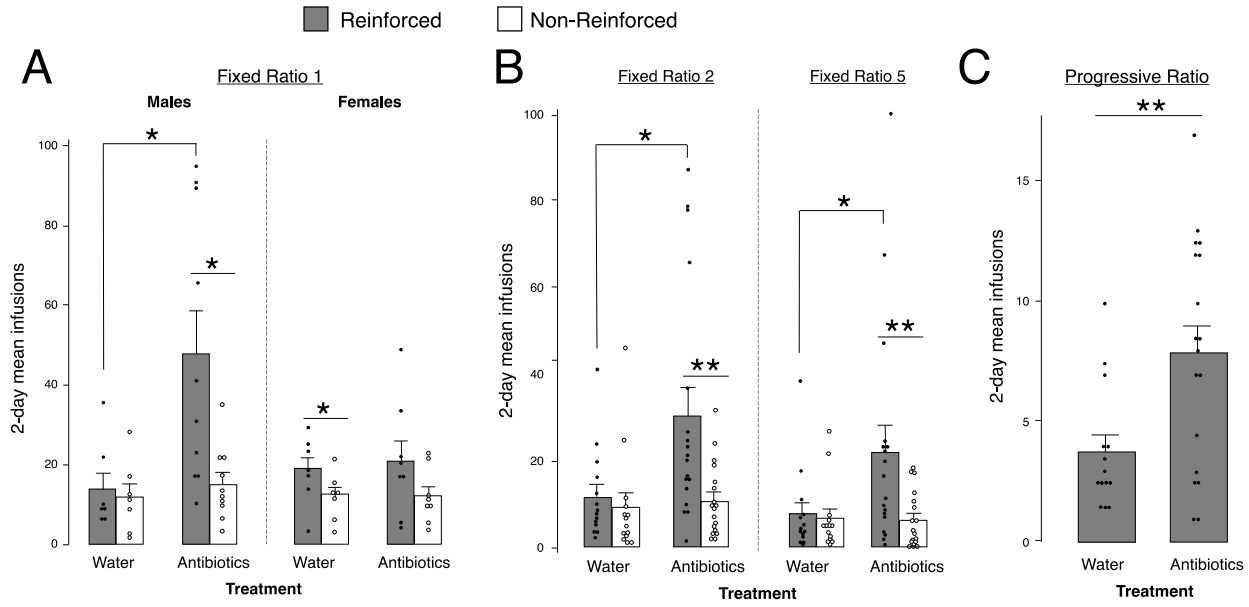

Figure 4.6. Fentanyl intravenous self-administration in controls and antibiotic-treated animals. All animals self-administered a fentanyl dose of 1.25 µg/kg/infusion. (A) Mean number of infusions across days 4–5 at fixed ratio (FR) 1 in males and females. Infusions were administered via nose pokes and are reported as reinforced (filled bars and circles) and non-reinforced (empty bars and circles) responses. Error bars represent S.E.M., * $p < 0.05$, n = 7–10/group. (B) Mean number of infusions across 2 days at FR2 and 2 days at FR5, collapsed by sex, for reinforced (filled bars and circles) and non-reinforced (empty bars and circles) nose poke responses. Error bars represent S.E.M., ** $p < 0.01$, * $p < 0.05$, n = 14–18/group. (C) Mean number of infusions across 2 days at progressive ratio, collapsed by sex. Error bars represent S.E.M., ** $p < 0.01$, n = 14–18/group.

Antibiotic-treated animals self-administer more fentanyl than water-treated controls at FR2, FR5, and progressive ratio (PR)

Subsequent 2-way ANOVAs showed a main effect of treatment at FR2 ($F(3,31)=3.1$, $p=0.02$), FR5 ($F(3,31)=3.1$, $p=0.04$), and PR ($F(3,31)=4.11$, $p=0.008$), with higher reinforced responses in antibiotic-treated animals compared to water-drinking controls (Figures 4.6B,C). Data are collapsed by sex due to a lack of sex differences at these higher schedules of reinforcement. As the responses required to earn an infusion increases logarithmically on a PR schedule of reinforcement, the number of infusions self-administered is much lower on PR than FR. Thus, the scale displaying mean infusions is smaller in PR (Figure 4.6C) compared to FR1, FR2, and FR5 (Figure 4.6A,B).

Short-chain fatty acid (SCFA) supplementation blunts fentanyl self-administration in antibiotic-treated animals at FR2 and FR5

Only males were used for the SCFA supplementation study, as the enhancement of fentanyl self-administration seen from antibiotic treatment was driven by males. A one-way ANOVA was run separately for all schedules of reinforcement to analyze differences in all 4 treatment groups on fentanyl self-administration at 1.25 μg/kg/infusion. We found a main effect of treatment at FR1 ($F(3,34) = 4.52$, $p = 0.009$), FR2 ($F(3,34) = 6.57$, $p = 0.001$), FR5 ($F(3,34) = 3.58$, $p = 0.02$), and PR ($F(3,34) = 3.41$, $p = 0.02$) (Figure 4.7). At FR1, animals treated only with antibiotics had higher reinforced responses compared to those treated with water only or water with SCFAs (Figure 4.7A). Additionally, treatment with antibiotics plus SCFAs resulted in higher reinforced responses at FR1 versus water-only controls (Figure 4.7A). At both FR2 and FR5, the same pattern of differences was observed: antibiotic treatment alone led to increased reinforced responses compared to all other treatment groups (water only, water with SCFA, and antibiotics with SCFA) (Figure 4.7A). These data show that the repletion of microbial metabolites via SCFAs restores self-administration similar to control animals at higher fixed ratio schedules of reinforcement. At PR, there was no significant difference in SCFA-supplemented animals treated with antibiotics compared to antibiotic treatment only, although this effect was trending ($p = 0.08$) (Figure 4.7B).

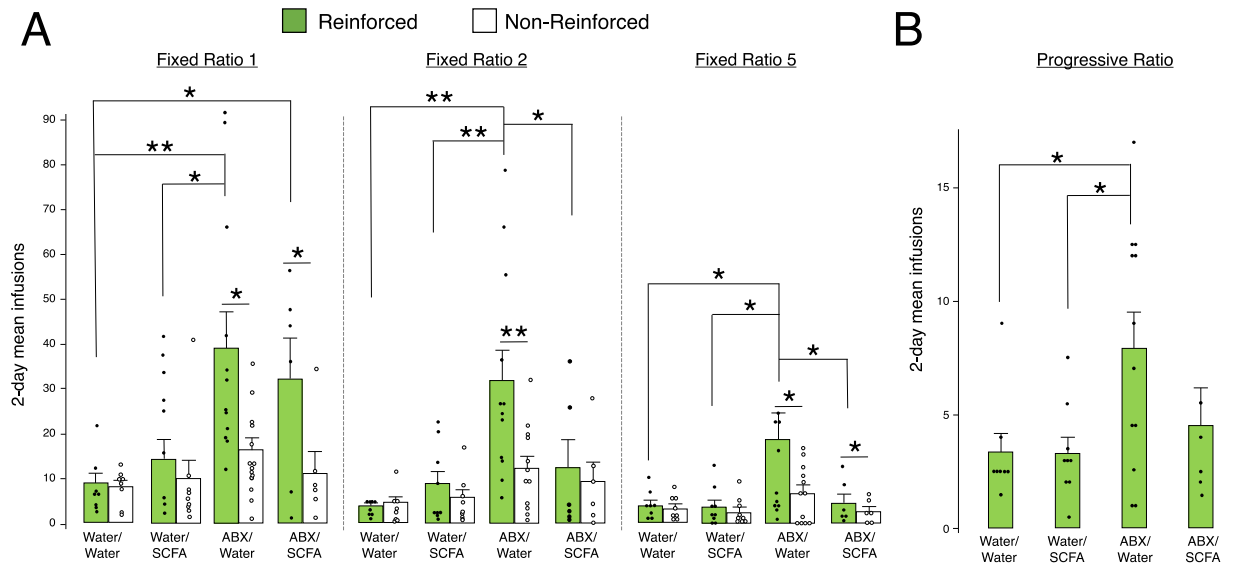

Figure 4.7. Fentanyl intravenous self-administration after short-chain fatty acid (SCFA) supplementation. All animals self-administered a fentanyl dose of 1.25 µg/kg/infusion and were treated with water, SCFA, antibiotics (ABX), or ABX plus SCFA. (A) Mean number of infusions across days 4–5 at fixed ratios 1, 2, and 5. Infusions were administered via nose pokes and are reported as rein-forced (filled bars and circles) and non-reinforced (empty bars and circles) responses. Error bars represent S.E.M., ** $p < 0.01$, * $p < 0.05$, n = 6–12/group. (B) Mean number of infusions across 2 days at progressive ratio. Error bars represent S.E.M., * $p < 0.05$, n = 6–12/group.

Discussion

Our findings highlight an important relationship between knockdown of gut bacteria and fentanyl IVSA with an enhancement of self-administration driven by males. In addition, we find that at higher schedules of reinforcement (i.e., FR2 and FR5), SCFA supplementation in antibiotic-treated males decreases fentanyl self-administration compared to males treated with antibiotics, suggesting that microbial metabolites may mediate the reinforcing efficacy of fentanyl. A previous study found a similar trend in cocaine reward in mice with knockdown and later restoration of bacterial metabolites (Kiraly et al. 2016). Our present study is consistent with prior work supporting a relationship between altered gut microbiota and opioid-related behaviors in rodents. While prior groups have reported depletion of gut bacteria to decrease opioid tolerance and preference using non-contingent drug exposure (M. Kang et al. 2017; Lee et al. 2018; Hofford et al. 2021; O'Sullivan et al. 2019), we show an increase in opioid self-

administration. Although conditioned place preference and IVSA both measure drug-related behavior and learning, they are drastically different methods and yield conclusions with subtle differences in reward, motivation, and reinforcement. These discrepancies may also be potentially explained by differences in species (rats vs. mice), the opioid used (fentanyl vs. morphine), duration of drug exposure, route of administration (intravenous vs. subcutaneous or intraperitoneal), and method of administration (self-administration vs. experimenter-administered). Additional experimentation, particularly self-administration studies, will be necessary to interpret such inconsistencies.

Our findings are also consistent with clinical studies that establish an association between opioid use and subsequent gut dysbiosis (Acharya et al. 2017; Cruz-Lebrón et al. 2021; Qiaoyan Li et al. 2020; Meng, Sindberg, and Roy 2015; Vincent et al. 2016). We observed an increase in Bacteroidetes in antibiotic-treated males before and after fentanyl IVSA. The Firmicutes/Bacteroidetes (F/B) ratio plays a role in maintaining normal intestinal homeostasis, with a higher or lower ratio observed in obesity or inflammatory bowel disease, respectively (Stojanov, Berlec, and Štrukelj 2020). However, the findings on the beneficial vs. harmful features of bacterial species within Bacteroidetes are mixed, as some studies suggest Bacteroidetes decreases inflammation and improves body composition (Trompette et al. 2014), while others highlight associations between Bacteroidetes and metabolic diseases (Johnson et al. 2017). This may be due to the various genera that make up this phylum, although we did not find specific differences at the genus level of Bacteroidetes. We observed many genera that were decreased from antibiotic treatment compared to water treatment, which is expected considering the significant depletion of bacteria from our antibiotic cocktail.

One recent national database study found that opioids prescribed in combination with antibiotics in the hospital setting are protective against the development of opioid use disorder at later time points following hospital discharge (Freedman et al. 2022). Although both short- and long-term use of antibiotics drastically disrupts the gut microbiota, the microbial community

may retain more beneficial or pathogenic bacteria (Mulder et al. 2020; Shao et al. 2020; Ianiro, Tilg, and Gasbarrini 2016). The extent of antibiotic treatment on modifying gut bacteria is dependent on the class, pharmacokinetics, pharmacodynamics, and range of action, as well as their dosage, duration, and route of administration (Ianiro, Tilg, and Gasbarrini 2016). We found that our antibiotic treatment induced a significant depletion in Firmicutes and an increase in Proteobacteria, Tenericutes, and Verrucomicrobia. Proteobacteria is presumed to be an inflammatory microbe and is elevated in diseased states (Shao et al. 2020; Shin, Whon, and Bae 2015). Prior studies also observed shifts in Firmicutes and Bacteroidetes, the gut's two dominant phyla, from antibiotic use (Shao et al. 2020; Panda et al. 2014).

The inclusion of both male and female animals in our study broadens the field of opioid research, as there is limited literature on sex differences in opioid abuse and even less when focused on gut microbial studies, which is concerning given the existing correlations between several addiction-related behaviors and the microbiome specific to sex (Peterson et al. 2020). We see that gut bacteria depletion via antibiotic treatment enhances fentanyl self-administration in males, but not females, at the lowest schedule of reinforcement (i.e., FR1), but there are no sex differences at higher order schedules of reinforcement. Prior studies report lower drug use in females versus males (United Nations Office on Drugs and Crime 2006), while others find greater self-administration and vulnerability to addiction in females (Becker and Hu 2008; B. Yang et al. 2017; Townsend et al. 2019; Cicero, Aylward, and Meyer 2003). The variation in these outcomes highlights the necessity for additional studies in gut microbiota, sex hormones, and drug-related behavior.

Our study is limited by the lack of assessment of antibiotic's impact on fentanyl metabolism or overall locomotor activity, although previous work shows no significant influence of antibiotic treatment on cocaine or morphine metabolism in mice (Kiraly et al. 2016; Hofford et al. 2021). Further, non-reinforced responses (i.e., inactive nose poke hole) control for non-specific drug effects, including locomotor activity. Our limited number of collected fecal samples

precluded an in-depth analysis of microbial interactions between fentanyl self-administration and antibiotic treatment, so future studies should evaluate these potential drug interactions. Furthermore, a causal relationship between gut microbiota and drug self-administration needs to be established. Future studies confirming gut bacterial changes with SCFA supplementation and/or evaluating microbial replacement via fecal microbiota transplantation may provide a direct connection between the gut microbiome and drug-related behavior.

Given the connections between gut microbiota and stress, mood, psychiatric dis-orders, and behavior, evaluating the role of the gut microbiota in fentanyl use is a unique approach that could lead to new paths for the treatment of addiction. By identifying the gut-brain axis role in fentanyl use, our research has the potential to significantly progress our understanding of the mechanisms influencing clinical use of opioids and addiction. One potential mechanism mediating the enhancement of fentanyl IVSA in males with depleted gut bacteria compared to control animals may be through neuroinflammation. Prior work showed that manipulation of the gut microbiota alters microglia morphology, a measure of neuroinflammation (Lee et al. 2018). Neuroinflammation disrupts the function and projections of dopaminergic neurons within reward-related regions in the brain, leading to decreased mesolimbic dopaminergic activity and dysregulated reward (Taylor et al. 2016). Although our present study is observational, future mechanistic findings may uncover direct gut-to-brain pathways or intermediate modulators, such as neuroinflammation, microbial metabolites, gut peptides, and neurotransmitters.

Chapter 5

Discussion

I. Significance

The clinical efficacy of opioids is limited by the risk of dependence and addiction. Opioid addiction is driving the escalating number of lethal drug overdoses in the United States (Rudd et al. 2016). Among the strategies to address the opioid crisis (based on the Centers for Disease Control) are to monitor trends, advance research, and increase public awareness. The cause of opioid addiction is unclear and highly necessary to delineate to develop effective treatment interventions.

Addiction is a neuropsychiatric disease that shares extensive commonalities with stress, depression, anxiety, learning and memory, reward processing, feeding, and social interaction. Emerging evidence supports a critical role of gut bacteria in these behaviors, in addition to brain function, emotion, and other neurological and psychiatric disorders. In particular, “diseased” states are associated with gut dysbiosis, and manipulation of gut bacteria impact related behaviors (Hasegawa et al. 2015; Keshavarzian et al. 2015; Li et al. 2019; Haran et al. 2019; Cosorich et al. 2017; Stasi et al. 2019; Grochowska, Laskus, and Radkowski 2019; Harach et al. 2017). Further, gut function is clearly impacted by opioid use (i.e. opioid-induced constipation). Due to the significant overlap between addiction-related behaviors, opioids, and gut health (Meckel and Kiraly 2019; Ren and Lotfipour 2020; García-Cabrerizo et al. 2021), my aim was to evaluate the role of gut bacteria in an animal model of fentanyl intravenous self-administration. As communication between the gut and brain occurs in both directions, my studies assessed both the effect of fentanyl self-administration on gut bacteria, as well as the influence of gut bacteria on fentanyl self-administration. Improving the understanding of the gut-brain axis in

opioid use can potentially contribute to the development of a tractable treatment option for opioid abuse.

II. Clinical implications

Animal model of intravenous self-administration

My studies evaluate intravenous self-administration on an escalating schedule of reinforcement, which measures reinforcement and motivation but not necessarily other aspects of drug addiction, such as drug seeking, craving, relapse, tolerance, and withdrawal. Thus, although my work assesses fentanyl intake, it is not entirely an addiction model. Other behavioral assays should be evaluated for a more comprehensive analysis, such as behavioral economics, food versus drug choice, extinction, drug tolerance, dependence, and withdrawal. Other factors can also influence these findings and should be considered, including comorbidities, pain, and social interaction. Addiction is co-morbid with mood disorders like depression and anxiety, and behavioral assays in animals may be conflicting. For example, depressive-like behavior or anhedonia may result in either increased drug intake as an attempt to self-medicate or decreased intake due to a lack of interest or motivation.

Gut-brain modulation by short-chain fatty acids (SCFAs)

SCFAs are the main metabolites produced by bacterial fermentation of non-digestible carbohydrates (i.e. dietary fiber) in the gastrointestinal tract (Miller and Wolin 1996). Circulating SCFAs can cross the blood-brain barrier and modulate neurotransmission, influence host physiology, and mediate cognition and emotion (Mitchell et al. 2011; DeCastro et al. 2005; Lewis et al. 2010; Sampson et al. 2016; Govindarajan et al. 2011; Kelly et al. 2016; Kiraly et al. 2016; Moretti et al. 2011; Resende et al. 2013). SCFAs affect the host through various mechanisms, including the regulation of histone acetylation and methylation (Krautkramer et al. 2016; Stilling et al. 2016), G-protein coupled receptors (Milligan et al. 2017), facilitation of the

secretion of hormones and neurochemicals (Chambers et al. 2015; Reigstad et al. 2015), and the induction of vagus nerve signaling (De Vadder et al. 2014; Qinrui Li et al. 2017).

Prior studies report decreased SCFA production in substance use (Touw et al. 2017; Simpson et al. 2022) and amelioration of drug-related behavior following administration of SCFAs (Kiraly et al. 2016; Hofford et al. 2021; Van de Wouw et al. 2018), which provides further support for a bidirectional, causal relationship between microbial metabolites and host behavior. One major limitation in these SCFA studies is the systemic availability is not measured (Dalile et al. 2019). Studies evaluating fecal SCFAs measure non-absorbed SCFAs but do not reflect in situ production rates, absorption, and interaction with other biologically relevant molecules or cell types. Future experimentation is critical to understand the full biological relevance of SCFAs in gut-brain communication.

Fecal microbiota transplantation (FMT)

FMT refers to the direct transfer of functional microbiota from a healthy donor to the gastrointestinal tract of a recipient with a disease that causes an imbalance in the gut microbiota (Ley, Peterson, and Gordon 2006). The goal of FMT is to treat the disease by restoring the phylogenetic diversity and bacteria of a healthy person. FMT is approved for the treatment of *Clostridium difficile* infection (Juszczuk et al. 2017) and is also increasingly being considered for treating neurological and psychiatric diseases. Studies have found that FMT in both humans and animals can be beneficial in treating symptoms of Parkinson's disease, multiple sclerosis, autism spectrum disorder, depression, and anxiety (Sun et al. 2018; Huang et al. 2019; Qiaoyan Li et al. 2020; Borody et al. 2011; Makkawi, Camara-Lemarroy, and Metz 2018; Sharon et al. 2019; D.-W. Kang et al. 2019; Yuan Zhang et al. 2019; Kelly et al. 2016). Although FMT has potential in treating a variety of diseases, one significant drawback is that transplantation of fecal microbiota does not isolate bacterial products. The microbiome also includes fungi,

viruses, and phages, making it difficult to determine how the whole community may impact the donor and match donors with patients (Sbahi and Di Palma 2016).

Probiotic and diet interventions

Administration of probiotics is a promising and valid intervention to improve neuropsychiatric disorder (Roman et al. 2018). Probiotics are live microorganisms which when administered in appreciable amounts confer a health benefit on the host (Hill et al. 2014). These bacteria are beneficial to gut health by competing for or producing growth substrates, affecting pathogens directly, improving barrier function, and regulating the immune system (Chidambaram et al. 2020; Hong et al. 2019). Specific bacterial species are associated with the clinical symptoms of depression and anxiety. There are decreased populations of *Lactobacillus* and *Bifidobacterium* in patients with depression, and administration of these species relieves depression- and anxiety-like behaviors in animal and clinical studies (Hsiao et al. 2013; Aizawa et al. 2016; Desbonnet et al. 2008; Jang et al. 2018). In addition, oral supplementation of probiotics in patients with substance use disorder reduces neuroinflammation, improves brain health, restores gut dysbiosis, reduces endotoxin, and improve tight junction and permeability (Letchumanan et al. 2022).

Although substance use is indeed associated with gut dysbiosis (Qin et al. 2021; Xu et al. 2017), it is difficult to ascertain whether gut disruption occurs as a direct result of the drug, neurobiological adaptations of disease, or even daily habits, such as diet and polydrug use, as diet is the primary contributor of changes in the gut microbiota (Power et al. 2014; Kovatcheva-Datchary and Arora 2013; Flint et al. 2012; Sandhu et al. 2017). Studies that have assessed dietary fats show a role of omega-3 polyunsaturated fatty acids in maintaining the balance between gut immunity and gut microbiota (Fu et al. 2021). Omega-3 fatty acids are essential fatty acids that humans are encouraged to obtain through their diet and have potential benefits in human health.

III. Limitations and future directions

Communication between the gut and brain has been extensively studied. It is established that their main communication pathways are the vagus nerve, immune system, hypothalamic-pituitary-adrenal axis, bacterial metabolites, and enteroendocrine cells (Mayer 2011). However, the exact mechanisms need to be identified to support a causative, direct relationship between gut bacteria and fentanyl self-administration. One potential mechanism to further evaluate is inflammation of both the gut and brain. The presence or absence of specific microbes modulates the immune system (Belkaid and Hand 2014; Zhao and Elson 2018; Lazar et al. 2018; Gensollen et al. 2016) and regulates inflammation (Lobionda et al. 2019; Blander et al. 2017; Clemente, Manasson, and Scher 2018; Tilg et al. 2020). Further, opioid use and withdrawal leads to neuroinflammation (Taylor et al. 2016; Lee et al. 2018). Other possible pathways to consider are signaling through hormones and/or gut peptides and activity in specific brain regions and pathways.

Clinical data demonstrate sex differences in opioid use, with women more likely to become dependent on opioids than men (CDC 2013). I did not find sex differences in fentanyl self-administration in my study; however, as stated earlier, the model used in my studies do not mimic opioid dependence or tolerance. Future experiments should test a variety of models of drug behavior and at various stages of addiction to assess sex differences in opioid use. Further, FMT and/or identification of specific bacterial strains affected in different addiction-related behavioral assays would provide a more comprehensive understanding of the relationship between gut bacteria and opioid use.

Another important consideration is the influence of gut bacteria on drug metabolism in these studies. Gut bacteria can affect the pharmacokinetics and bioavailability of orally administered medications by enzymatically transforming pharmaceutical compounds into active, inactive, and toxic metabolites (Flowers, Bhat, and Lee 2020; Zhang, Zhang, and Wang 2018).

Although my studies did not evaluate oral fentanyl intake, gut microbiota may have an impact on drug metabolism. Thus, future studies evaluating gut bacteria-mediated drug metabolism are critical to interpret changes in drug pharmacokinetics.

IV. Overall conclusions

Through my studies presented in this dissertation, I have discovered that 1) fentanyl intravenous self-administration (IVSA) is dependent on fentanyl dose, but not sex, at the doses evaluated in our study; 2) fentanyl IVSA changes alpha diversity, but not beta diversity, of gut bacteria in a sex-dependent manner; 3) prolonged treatment of oral antibiotics enhances fentanyl IVSA; and 4) SCFA supplementation in antibiotic-treated males reverses the enhancement of fentanyl IVSA at higher-order schedules of reinforcement. Collectively, these findings strongly suggest a bidirectional relationship between gut bacteria and intravenous fentanyl self-administration.

REFERENCES

Acharya, C, NS Betrapally, PM Gillevet, RK Sterling, H Akbarali, MB White, D Ganapathy, A Fagan, M Sikaroodi, and JS27868217 Bajaj. 2017. “Chronic Opioid Use Is Associated with Altered Gut Microbiota and Predicts Readmissions in Patients with Cirrhosis.” *Alimentary Pharmacology & Therapeutics* 45 (2): 319–31.

Ait-Belgnaoui, Afifa, André Colom, Viorica Braniste, Ludovic Ramalho, Alain Marrot, Christel Cartier, Eric Houdeau, Vassilia Theodorou, and Thomas Tompkins. 2014. “Probiotic Gut Effect Prevents the Chronic Psychological Stress-induced Brain Activity Abnormality in Mice.” *Neurogastroenterology & Motility* 26 (4): 510–20.

Ait-Belgnaoui, Afifa, Henri Durand, Christel Cartier, Gilles Chaumaz, Hélène Eutamene, Laurent Ferrier, Eric Houdeau, Jean Fioramonti, Lionel Bueno, and Vassilia Theodorou. 2012. “Prevention of Gut Leakiness by a Probiotic Treatment Leads to Attenuated HPA Response to an Acute Psychological Stress in Rats.” *Psychoneuroendocrinology* 37 (11): 1885–95.

Aizawa, Emiko, Hirokazu Tsuji, Takashi Asahara, Takuya Takahashi, Toshiya Teraishi, Sumiko Yoshida, Miho Ota, Norie Koga, Kotaro Hattori, and Hiroshi Kunugi. 2016. “Possible Association of Bifidobacterium and Lactobacillus in the Gut Microbiota of Patients with Major Depressive Disorder.” *Journal of Affective Disorders* 202 (September): 254–57. https://doi.org/10.1016/j.jad.2016.05.038.

Akbarali, Hamid I, and William L Dewey. 2017. “The Gut–Brain Interaction in Opioid Tolerance.” *Current Opinion in Pharmacology* 37: 126–30.

Alcock, Joe, Carlo C Maley, and C Athena Aktipis. 2014. “Is Eating Behavior Manipulated by the Gastrointestinal Microbiota? Evolutionary Pressures and Potential Mechanisms.” *Bioessays* 36 (10): 940–49.

Al-Ghezi, Zinah Zamil, Philip Brandon Busbee, Hasan Alghetaa, Prakash S Nagarkatti, and Mitzi Nagarkatti. 2019. “Combination of Cannabinoids, Delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD), Mitigates Experimental Autoimmune Encephalomyelitis (EAE) by Altering the Gut Microbiome.” *Brain, Behavior, and Immunity* 82: 25–35.

Arnoldussen, IAC, M Wiesmann, CE Pelgrim, EM Wielemaker, W Van Duyvenvoorde, PL Amaral-Santos, L Verschuren, BJF Keijser, A Heerschap, and R Kleemann. 2017. “Butyrate Restores HFD-Induced Adaptations in Brain Function and Metabolism in Mid-Adult Obese Mice.” *International Journal of Obesity* 41 (6): 935–44.

Banerjee, S., G. Sindberg, F. Wang, J. Meng, U. Sharma, L. Zhang, P. Dauer, et al. 2016. “Opioid-Induced Gut Microbial Disruption and Bile Dysregulation Leads to Gut Barrier Compromise and Sustained Systemic Inflammation.” *Mucosal Immunology* 9 (6): 1418–28. https://doi.org/10.1038/mi.2016.9.

Barengolts, Elena, Stefan J Green, Yuval Eisenberg, Arfana Akbar, Bharathi Reddivari, Brian T Layden, Lara Dugas, and George Chlipala. 2018. “Gut Microbiota Varies by Opioid Use, Circulating Leptin and Oxytocin in African American Men with Diabetes and High Burden of Chronic Disease.” *PLoS One* 13 (3): e0194171.

Barrett, Eoin, RP Ross, Paul W O’Toole, Gerald F Fitzgerald, and Catherine Stanton. 2012. “Γ-Aminobutyric Acid Production by Culturable Bacteria from the Human Intestine.” *Journal of Applied Microbiology* 113 (2): 411–17.

Becker, Jill B, and Ming Hu. 2008. “Sex Differences in Drug Abuse.” *Frontiers in Neuroendocrinology* 29 (1): 36–47.

Belkaid, Yasmine, and Timothy W Hand. 2014. “Role of the Microbiota in Immunity and Inflammation.” *Cell* 157 (1): 121–41.

Belluzzi, James D, Ruihua Wang, and Frances M Leslie. 2005. “Acetaldehyde Enhances Acquisition of Nicotine Self-Administration in Adolescent Rats.” *Neuropsychopharmacology* 30 (4): 705–12. https://doi.org/10.1038/sj.npp.1300586.

Benarroch, Eduardo E. 2012. “Endogenous Opioid Systems: Current Concepts and Clinical Correlations.” *Neurology* 79 (8): 807–14.
Bercik, Premysl, Emmanuel Denou, Josh Collins, Wendy Jackson, Jun Lu, Jennifer Jury, Yikang Deng, Patricia Blennerhassett, Joseph Macri, and Kathy D McCoy. 2011. “The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice.” *Gastroenterology* 141 (2): 599–609.
Bercik, Premysl, AJ Park, D Sinclair, A Khoshdel, J Lu, X Huang, Y Deng, PA Blennerhassett, M Fahnestock, and D Moine. 2011. “The Anxiolytic Effect of Bifidobacterium Longum NCC3001 Involves Vagal Pathways for Gut–Brain Communication.” *Neurogastroenterology & Motility* 23 (12): 1132–39.
Berridge, Kent C. 2012. “From Prediction Error to Incentive Salience: Mesolimbic Computation of Reward Motivation.” *European Journal of Neuroscience* 35 (7): 1124–43.
Berridge, Kent C., and Terry E. Robinson. 2003. “Parsing Reward.” *Trends in Neurosciences* 26 (9): 507–13. https://doi.org/10.1016/S0166-2236(03)00233-9.
Berthoud, H-r. 2008. “Vagal and Hormonal Gut–Brain Communication: From Satiation to Satisfaction.” *Neurogastroenterology & Motility* 20: 64–72.
Biegler, Jessica M, Christopher S Freet, Nelli Horvath, Ann M Rogers, and Andras Hajnal. 2016. “Increased Intravenous Morphine Self-Administration Following Roux-En-Y Gastric Bypass in Dietary Obese Rats.” *Brain Research Bulletin* 123: 47–52.
Bien, Justyna, Vindhya Palagani, and Przemyslaw Bozko. 2013. “The Intestinal Microbiota Dysbiosis and Clostridium Difficile Infection: Is There a Relationship with Inflammatory Bowel Disease?” *Therapeutic Advances in Gastroenterology* 6 (1): 53–68.
Blander, J Magarian, Randy S Longman, Iliyan D Iliev, Gregory F Sonnenberg, and David Artis. 2017. “Regulation of Inflammation by Microbiota Interactions with the Host.” *Nature Immunology* 18 (8): 851–60.
Bolyen, Evan, Jai Ram Rideout, Matthew R Dillon, Nicholas A Bokulich, Christian C Abnet, Gabriel A Al-Ghalith, Harriet Alexander, Eric J Alm, Manimozhiyan Arumugam, and Francesco Asnicar. 2019. “Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2.” *Nature Biotechnology* 37 (8): 852–57.
Borody, Thomas, Sharyn Leis, Jordana Campbell, Margaux Torres, and Anna Nowak. 2011. “Fecal Microbiota Transplantation (FMT) in Multiple Sclerosis (MS): 942.” *Official Journal of the American College of Gastroenterology| ACG* 106: S352.
Bravo, Javier A, Paul Forsythe, Marianne V Chew, Emily Escaravage, Hélène M Savignac, Timothy G Dinan, John Bienenstock, and John F Cryan. 2011. “Ingestion of Lactobacillus Strain Regulates Emotional Behavior and Central GABA Receptor Expression in a Mouse via the Vagus Nerve.” *Proceedings of the National Academy of Sciences* 108 (38): 16050–55.
Bruce-Keller, Annadora J, J Michael Salbaum, Meng Luo, Eugene Blanchard IV, Christopher M Taylor, David A Welsh, and Hans-Rudolf Berthoud. 2015. “Obese-Type Gut Microbiota Induce Neurobehavioral Changes in the Absence of Obesity.” *Biological Psychiatry* 77 (7): 607–15.
Burokas, Aurelijus, Silvia Arboleya, Rachel D Moloney, Veronica L Peterson, Kiera Murphy, Gerard Clarke, Catherine Stanton, Timothy G Dinan, and John F Cryan. 2017. “Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice.” *Biological Psychiatry* 82 (7): 472–87.
Byrne, Claire S, Edward S Chambers, Habeeb Alhabeeb, Navpreet Chhina, Douglas J Morrison, Tom Preston, Catriona Tedford, Julie Fitzpatrick, Cherag Irani, and Albert Busza. 2016. “Increased Colonic Propionate Reduces Anticipatory Reward Responses in the Human Striatum to High-Energy Foods.” *The American Journal of Clinical Nutrition* 104 (1): 5–14.

Cahill, Catherine M, and Anna MW Taylor. 2017. “Neuroinflammation—a Co-Occurring Phenomenon Linking Chronic Pain and Opioid Dependence.” *Current Opinion in Behavioral Sciences* 13: 171–77.
Cani, Patrice D, Amandine Everard, and Thibaut Duparc. 2013. “Gut Microbiota, Enteroendocrine Functions and Metabolism.” *Current Opinion in Pharmacology* 13 (6): 935–40.
Cani, Patrice D, and Claude Knauf. 2016. “How Gut Microbes Talk to Organs: The Role of Endocrine and Nervous Routes.” *Molecular Metabolism* 5 (9): 743–52.
Caporaso, J Gregory, Christian L Lauber, William A Walters, Donna Berg-Lyons, James Huntley, Noah Fierer, Sarah M Owens, et al. 2012. “Ultra-High-Throughput Microbial Community Analysis on the Illumina HiSeq and MiSeq Platforms.” *The ISME Journal* 6 (8): 1621–24. https://doi.org/10.1038/ismej.2012.8.
Carabotti, Marilia, Annunziata Scirocco, Maria Antonietta Maselli, and Carola Severi. 2015. “The Gut-Brain Axis: Interactions between Enteric Microbiota, Central and Enteric Nervous Systems.” *Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology* 28 (2): 203.
Carlucci, Christian, Elaine O Petrof, and Emma Allen-Vercoe. 2016. “Fecal Microbiota-Based Therapeutics for Recurrent Clostridium Difficile Infection, Ulcerative Colitis and Obesity.” *EBioMedicine* 13: 37–45.
Carroll, Marilyn E, Una C Campbell, and Paul Heideman. 2001. “Ketoconazole Suppresses Food Restriction–Induced Increases in Heroin Self-Administration in Rats: Sex Differences.” *Experimental and Clinical Psychopharmacology* 9 (3): 307.
Centers for Disease Control and Prevention (CDC). Vital signs: overdoses of prescription opioid pain relievers—United States, 1999–2008. MMWR MorbMortal Wkly Rep. 2011 Nov 4; 60(43):1487-1492.
Center for Disease Control and Prevention. (2013). Prescription Painkiller Overdoses: A Growing Epidemic, Especially Among Women. Atlanta, GA: Centers for Disease Control and Prevention. Available at http://www.cdc.gov/vitalsigns/prescriptionpainkilleroverdoses/index.html.
Chambers, Edward S, Alexander Viardot, Arianna Psichas, Douglas J Morrison, Kevin G Murphy, Sagen EK Zac-Varghese, Kenneth MacDougall, Tom Preston, Catriona Tedford, and Graham S Finlayson. 2015. “Effects of Targeted Delivery of Propionate to the Human Colon on Appetite Regulation, Body Weight Maintenance and Adiposity in Overweight Adults.” *Gut* 64 (11): 1744–54.
Chen, Zigui, Pak Chun Hui, Mamie Hui, Yun Kit Yeoh, Po Yee Wong, Martin CW Chan, Martin CS Wong, Siew C Ng, Francis KL Chan, and Paul KS Chan. 2019. “Impact of Preservation Method and 16S RRNA Hypervariable Region on Gut Microbiota Profiling.” *Msystems* 4 (1): e00271-18.
Chidambaram, Saravana Babu, Sunanda Tuladhar, Abid Bhat, Arehally Marappa Mahalakshmi, Bipul Ray, Musthafa Mohamed Essa, Muhammed Bishir, Srinivasa Rao Bolla, Nandakumar Dalavaikodihalli Nanjaiah, and Gilles J Guillemin. 2020. “Autism and Gut–Brain Axis: Role of Probiotics.” *Personalized Food Intervention and Therapy for Autism Spectrum Disorder Management*, 587–600.
Childs, Jessica E, Jaime DeLeon, Emily Nickel, and Sven Kroener. 2017. “Vagus Nerve Stimulation Reduces Cocaine Seeking and Alters Plasticity in the Extinction Network.” *Learning & Memory* 24 (1): 35–42.
Cicero, Theodore J, Shawn C Aylward, and Edward R Meyer. 2003. “Gender Differences in the Intravenous Self-Administration of Mu Opiate Agonists.” *Pharmacology Biochemistry and Behavior* 74 (3): 541–49.
Clarke, Gerard, S Grenham, P Scully, P Fitzgerald, RD Moloney, F Shanahan, TG Dinan, and JF22688187 Cryan. 2013. “The Microbiome-Gut-Brain Axis during Early Life Regulates

the Hippocampal Serotonergic System in a Sex-Dependent Manner." *Molecular Psychiatry* 18 (6): 666–73.
Clemente, Jose C, Julia Manasson, and Jose U Scher. 2018. "The Role of the Gut Microbiome in Systemic Inflammatory Disease." *Bmj* 360.
Collins, Stephen M, and Premsyl Bercik. 2009. "The Relationship between Intestinal Microbiota and the Central Nervous System in Normal Gastrointestinal Function and Disease." *Gastroenterology* 136 (6): 2003–14.
Contet, Candice, Brigitte L Kieffer, and Katia Befort. 2004. "Mu Opioid Receptor: A Gateway to Drug Addiction." *Current Opinion in Neurobiology* 14 (3): 370–78. https://doi.org/10.1016/j.conb.2004.05.005.
Cosorich, Ilaria, Gloria Dalla-Costa, Chiara Sorini, Roberto Ferrarese, Maria Josè Messina, Jayashree Dolpady, Elisa Radice, Alberto Mariani, Pier Alberto Testoni, and Filippo Canducci. 2017. "High Frequency of Intestinal TH17 Cells Correlates with Microbiota Alterations and Disease Activity in Multiple Sclerosis." *Science Advances* 3 (7): e1700492.
Cruz-Lebrón, Angélica, Ramona Johnson, Claire Mazahery, Zach Troyer, Samira Joussef-Piña, Miguel E. Quiñones-Mateu, Christopher M Strauch, Stanley L. Hazen, and Alan D. Levine. 2021. "Chronic Opioid Use Modulates Human Enteric Microbiota and Intestinal Barrier Integrity." *Gut Microbes* 13 (1): 1946368. https://doi.org/10.1080/19490976.2021.1946368.
Cryan, John F, and Timothy G Dinan. 2012. "Mind-Altering Microorganisms: The Impact of the Gut Microbiota on Brain and Behaviour." *Nature Reviews Neuroscience* 13 (10): 701–12.
Cryan, John F, Kenneth J O'Riordan, Kiran Sandhu, Veronica Peterson, and Timothy G Dinan. 2020. "The Gut Microbiome in Neurological Disorders." *The Lancet Neurology* 19 (2): 179–94.
Cussotto, Sofia, Gerard Clarke, Timothy G Dinan, and John F Cryan. 2019. "Psychotropics and the Microbiome: A Chamber of Secrets…." *Psychopharmacology* 236 (5): 1411–32. https://doi.org/10.1007/s00213-019-5185-8.
Dalile, Boushra, Lukas Van Oudenhove, Bram Vervliet, and Kristin Verbeke. 2019. "The Role of Short-Chain Fatty Acids in Microbiota–Gut–Brain Communication." *Nature Reviews Gastroenterology & Hepatology* 16 (8): 461–78. https://doi.org/10.1038/s41575-019-0157-3.
Daniel, Hannelore, Amin Moghaddas Gholami, David Berry, Charles Desmarchelier, Hannes Hahne, Gunnar Loh, Stanislas Mondot, Patricia Lepage, Michael Rothballer, and Alesia Walker. 2014. "High-Fat Diet Alters Gut Microbiota Physiology in Mice." *The ISME Journal* 8 (2): 295–308.
De Schepper, HU, F Cremonini, M-I Park, and M Camilleri. 2004. "Opioids and the Gut: Pharmacology and Current Clinical Experience." *Neurogastroenterology & Motility* 16 (4): 383–94.
De Vadder, Filipe, Petia Kovatcheva-Datchary, Daisy Goncalves, Jennifer Vinera, Carine Zitoun, Adeline Duchampt, Fredrik Bäckhed, and Gilles Mithieux. 2014. "Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits." *Cell* 156 (1–2): 84–96.
DeCastro, Manuel, Bistra B. Nankova, Parul Shah, Pranav Patel, Pradeep V. Mally, Ravi Mishra, and Edmund F. La Gamma. 2005. "Short Chain Fatty Acids Regulate Tyrosine Hydroxylase Gene Expression through a CAMP-Dependent Signaling Pathway." *Molecular Brain Research* 142 (1): 28–38. https://doi.org/10.1016/j.molbrainres.2005.09.002.
DeGruttola, Arianna K, Daren Low, Atsushi Mizoguchi, and Emiko Mizoguchi. 2016. "Current Understanding of Dysbiosis in Disease in Human and Animal Models." *Inflammatory Bowel Diseases* 22 (5): 1137–50.

Desbonnet, Lieve, Gerard Clarke, Alexander Traplin, Orla O'Sullivan, Fiona Crispie, Rachel D Moloney, Paul D Cotter, Timothy G Dinan, and John F Cryan. 2015. "Gut Microbiota Depletion from Early Adolescence in Mice: Implications for Brain and Behaviour." *Brain, Behavior, and Immunity* 48: 165–73.

Desbonnet, Lieve, Lillian Garrett, Gerard Clarke, John Bienenstock, and Timothy G Dinan. 2008. "The Probiotic Bifidobacteria Infantis: An Assessment of Potential Antidepressant Properties in the Rat." *Journal of Psychiatric Research* 43 (2): 164–74.

Desbonnet, Lieve, Lillian Garrett, Gerard Clarke, Barry Kiely, John F Cryan, and TG20696216 Dinan. 2010. "Effects of the Probiotic Bifidobacterium Infantis in the Maternal Separation Model of Depression." *Neuroscience* 170 (4): 1179–88.

Dethlefsen, Les, Sue Huse, Mitchell L Sogin, and David A Relman. 2008. "The Pervasive Effects of an Antibiotic on the Human Gut Microbiota, as Revealed by Deep 16S RRNA Sequencing." *PLoS Biology* 6 (11): e280.

Di Chiara, Gaetano. 1999. "Drug Addiction as Dopamine-Dependent Associative Learning Disorder." *European Journal of Pharmacology* 375 (1–3): 13–30.

Dodet, Pauline, Serge Perrot, Lauriane Auvergne, Aline Hajj, Guy Simoneau, Xavier Declèves, Christine Poitou, Jean-Michel Oppert, Katel Peoc'h, and Stéphane Mouly. 2013. "Sensory Impairment in Obese Patients? Sensitivity and Pain Detection Thresholds for Electrical Stimulation after Surgery-Induced Weight Loss, and Comparison with a Nonobese Population." *The Clinical Journal of Pain* 29 (1): 43–49.

Drug Enforcement Administration. DEA warning to police and public: fentanyl exposure kills. June 10, 2016

Drug Enforcement Administration. March 2021. 2020 National Drug Threat Assessment. https://www.dea.gov/sites/default/files/2021-02/DIR-00821%202020%20National%20Drug%20Threat%20Assessment_WEB.pdf

Duca, Frank A, Timothy D Swartz, Yassine Sakar, and Mihai Covasa. 2012. "Increased Oral Detection, but Decreased Intestinal Signaling for Fats in Mice Lacking Gut Microbiota." *PLoS One* 7 (6): e39748.

Dutta, Sudhir K, Sandeep Verma, Vardhmaan Jain, Balarama K Surapaneni, Rakesh Vinayek, Laila Phillips, and Padmanabhan P Nair. 2019. "Parkinson's Disease: The Emerging Role of Gut Dysbiosis, Antibiotics, Probiotics, and Fecal Microbiota Transplantation." *Journal of Neurogastroenterology and Motility* 25 (3): 363.

Eckburg Paul B., Bik Elisabeth M., Bernstein Charles N., Purdom Elizabeth, Dethlefsen Les, Sargent Michael, Gill Steven R., Nelson Karen E., and Relman David A. 2005. "Diversity of the Human Intestinal Microbial Flora." *Science* 308 (5728): 1635–38. https://doi.org/10.1126/science.1110591.

Egecioglu, Emil, Jörgen A Engel, and Elisabet Jerlhag. 2013. "The Glucagon-like Peptide 1 Analogue, Exendin-4, Attenuates the Rewarding Properties of Psychostimulant Drugs in Mice." *PloS One* 8 (7): e69010.

Elderman, Marlies, Floor Hugenholtz, Clara Belzer, Mark Boekschoten, Adriaan van Beek, Bart de Haan, Huub Savelkoul, Paul de Vos, and Marijke Faas. 2018. "Sex and Strain Dependent Differences in Mucosal Immunology and Microbiota Composition in Mice." *Biology of Sex Differences* 9 (1): 26. https://doi.org/10.1186/s13293-018-0186-6.

Engel, Jörgen A, and Elisabet Jerlhag. 2014. "Role of Appetite-Regulating Peptides in the Pathophysiology of Addiction: Implications for Pharmacotherapy." *CNS Drugs* 28 (10): 875–86.

Erny, Daniel, Anna Lena Hrabě de Angelis, Diego Jaitin, Peter Wieghofer, Ori Staszewski, Eyal David, Hadas Keren-Shaul, Tanel Mahlakoiv, Kristin Jakobshagen, and Thorsten Buch. 2015. "Host Microbiota Constantly Control Maturation and Function of Microglia in the CNS." *Nature Neuroscience* 18 (7): 965–77.

Faith, Jeremiah J, Federico E Rey, David O'donnell, Maria Karlsson, Nathan P McNulty, George Kallstrom, Andrew L Goodman, and Jeffrey I Gordon. 2010. "Creating and Characterizing Communities of Human Gut Microbes in Gnotobiotic Mice." *The ISME Journal* 4 (9): 1094–98.

Fassarella, Marina, Ellen E Blaak, John Penders, Arjen Nauta, Hauke Smidt, and Erwin G Zoetendal. 2021. "Gut Microbiome Stability and Resilience: Elucidating the Response to Perturbations in Order to Modulate Gut Health." *Gut* 70 (3): 595–605.

Flint, Harry J, Karen P Scott, Petra Louis, and Sylvia H Duncan. 2012. "The Role of the Gut Microbiota in Nutrition and Health." *Nature Reviews Gastroenterology & Hepatology* 9 (10): 577–89.

Flowers, Stephanie A., Shubha Bhat, and James C. Lee. 2020. "Potential Implications of Gut Microbiota in Drug Pharmacokinetics and Bioavailability." *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 40 (7): 704–12. https://doi.org/10.1002/phar.2428.

Fortin, Samantha M, and Mitchell F Roitman. 2017. "Central GLP-1 Receptor Activation Modulates Cocaine-Evoked Phasic Dopamine Signaling in the Nucleus Accumbens Core." *Physiology & Behavior* 176: 17–25.

Foster, Jane A, Linda Rinaman, and John F Cryan. 2017. "Stress & the Gut-Brain Axis: Regulation by the Microbiome." *Neurobiology of Stress* 7: 124–36.

Freedman, Zachary G., Jennifer A. Kane, Tonya S. King, and Nicholas M. Graziane. 2022. "The Effect of Prescribing Antibiotics with Opioids on the Development of Opioid Use Disorder: A National Database Study." *Journal of Addictive Diseases* 40 (1): 62–70. https://doi.org/10.1080/10550887.2021.1926889.

Fröhlich, Esther E, Aitak Farzi, Raphaela Mayerhofer, Florian Reichmann, Angela Jačan, Bernhard Wagner, Erwin Zinser, Natalie Bordag, Christoph Magnes, and Eleonore Fröhlich. 2016. "Cognitive Impairment by Antibiotic-Induced Gut Dysbiosis: Analysis of Gut Microbiota-Brain Communication." *Brain, Behavior, and Immunity* 56: 140–55.

Fu, Yawei, Yadong Wang, Hu Gao, DongHua Li, RuiRui Jiang, Lingrui Ge, Chao Tong, and Kang Xu. 2021. "Associations among Dietary Omega-3 Polyunsaturated Fatty Acids, the Gut Microbiota, and Intestinal Immunity." Edited by Miaomiao Wu. *Mediators of Inflammation* 2021 (January): 8879227. https://doi.org/10.1155/2021/8879227.

Gacias, Mar, Sevasti Gaspari, Patricia-Mae G Santos, Sabrina Tamburini, Monica Andrade, Fan Zhang, Nan Shen, Vladimir Tolstikov, Michael A Kiebish, and Jeffrey L Dupree. 2016. "Microbiota-Driven Transcriptional Changes in Prefrontal Cortex Override Genetic Differences in Social Behavior." *Elife* 5: e13442.

Garcez, Michelle Lima, Carlos Alberto de Carvalho, Francielle Mina, Tatiani Bellettini-Santos, Gustavo Luis Schiavo, Sabrina da Silva, Ana Carolina Brunatto Falchetti Campos, Roger Bitencourt Varela, Samira S Valvassori, and Adriani Paganini Damiani. 2018. "Sodium Butyrate Improves Memory and Modulates the Activity of Histone Deacetylases in Aged Rats after the Administration of D-Galactose." *Experimental Gerontology* 113: 209–17.

García-Cabrerizo, Rubén, Carina Carbia, Kenneth J. O´Riordan, Harriet Schellekens, and John F. Cryan. 2021. "Microbiota-Gut-Brain Axis as a Regulator of Reward Processes." *Journal of Neurochemistry* 157 (5): 1495–1524. https://doi.org/10.1111/jnc.15284.

Gareau, Mélanie G, Jennifer Jury, Glenda MacQueen, Philip M Sherman, and Mary H Perdue. 2007. "Probiotic Treatment of Rat Pups Normalises Corticosterone Release and Ameliorates Colonic Dysfunction Induced by Maternal Separation." *Gut* 56 (11): 1522–28.

Gareau, Melanie G, Manuel A Silva, and Mary H Perdue. 2008. "Pathophysiological Mechanisms of Stress-Induced Intestina Damage." *Current Molecular Medicine* 8 (4): 274–81.

Gensollen, Thomas, Shankar S Iyer, Dennis L Kasper, and Richard S Blumberg. 2016. “How Colonization by Microbiota in Early Life Shapes the Immune System.” *Science* 352 (6285): 539–44.
Gershon, Michael D, and Jan Tack. 2007. “The Serotonin Signaling System: From Basic Understanding to Drug Development for Functional GI Disorders.” *Gastroenterology* 132 (1): 397–414.
Ghia, Jean-Eric, Patricia Blennerhassett, and Stephen M. Collins. 2008. “Impaired Parasympathetic Function Increases Susceptibility to Inflammatory Bowel Disease in a Mouse Model of Depression.” *The Journal of Clinical Investigation* 118 (6): 2209–18. https://doi.org/10.1172/JCI32849.
Govindarajan, Nambirajan, Roberto Carlos Agis-Balboa, Jonas Walter, Farahnaz Sananbenesi, and André Fischer. 2011. “Sodium Butyrate Improves Memory Function in an Alzheimer’s Disease Mouse Model When Administered at an Advanced Stage of Disease Progression.” *Journal of Alzheimer’s Disease* 26 (1): 187–97.
Graham, Devon L, Kevin Erreger, Aurelio Galli, and Gregg D Stanwood. 2013. “GLP-1 Analog Attenuates Cocaine Reward.” *Molecular Psychiatry* 18 (9): 961–62.
Grochowska, Marta, Tomasz Laskus, and Marek Radkowski. 2019. “Gut Microbiota in Neurological Disorders.” *Archivum Immunologiae et Therapiae Experimentalis* 67 (6): 375–83.
Guarner, Francisco, and Juan-R Malagelada. 2003. “Gut Flora in Health and Disease.” *The Lancet* 361 (9356): 512–19.
Han, Wenfei, Luis A Tellez, Matthew H Perkins, Isaac O Perez, Taoran Qu, Jozelia Ferreira, Tatiana L Ferreira, Daniele Quinn, Zhong-Wu Liu, and Xiao-Bing Gao. 2018. “A Neural Circuit for Gut-Induced Reward.” *Cell* 175 (3): 665–78.
Harach, Taoufiq, Nittaya Marungruang, Nicolas Duthilleul, Victoria Cheatham, KD Mc Coy, Giovanni Frisoni, JJ Neher, F Fåk, Mathias Jucker, and Theo Lasser. 2017. “Reduction of Abeta Amyloid Pathology in APPPS1 Transgenic Mice in the Absence of Gut Microbiota.” *Scientific Reports* 7 (1): 1–15.
Haran, John P, Shakti K Bhattarai, Sage E Foley, Protiva Dutta, Doyle V Ward, Vanni Bucci, and Beth A McCormick. 2019. “Alzheimer’s Disease Microbiome Is Associated with Dysregulation of the Anti-Inflammatory P-Glycoprotein Pathway.” *MBio* 10 (3): e00632-19.
Hasegawa, Satoru, Sae Goto, Hirokazu Tsuji, Tatsuya Okuno, Takashi Asahara, Koji Nomoto, Akihide Shibata, Yoshiro Fujisawa, Tomomi Minato, and Akira Okamoto. 2015. “Intestinal Dysbiosis and Lowered Serum Lipopolysaccharide-Binding Protein in Parkinson’s Disease.” *PloS One* 10 (11): e0142164.
Hegstrand, Linda R, and R Jean Hine. 1986. “Variations of Brain Histamine Levels in Germ-Free and Nephrectomized Rats.” *Neurochemical Research* 11 (2): 185–91.
Heijtz, Rochellys Diaz, Shugui Wang, Farhana Anuar, Yu Qian, Britta Björkholm, Annika Samuelsson, Martin L Hibberd, Hans Forssberg, and Sven Pettersson. 2011. “Normal Gut Microbiota Modulates Brain Development and Behavior.” *Proceedings of the National Academy of Sciences* 108 (7): 3047–52.
Hill, Colin, Francisco Guarner, Gregor Reid, Glenn R Gibson, Daniel J Merenstein, Bruno Pot, Lorenzo Morelli, Roberto Berni Canani, Harry J Flint, and Seppo Salminen. 2014. “Expert Consensus Document: The International Scientific Association for Probiotics and Prebiotics Consensus Statement on the Scope and Appropriate Use of the Term Probiotic.” *Nature Reviews Gastroenterology & Hepatology*.
Hill, Rob, Rakulan Santhakumar, William Dewey, Eamonn Kelly, and Graeme Henderson. 2020. “Fentanyl Depression of Respiration: Comparison with Heroin and Morphine.” *British Journal of Pharmacology* 177 (2): 254–65.

Hillemacher, Thomas, Oliver Bachmann, Kai G Kahl, and Helge Frieling. 2018. “Alcohol, Microbiome, and Their Effect on Psychiatric Disorders.” *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 85: 105–15.

Hofford, Rebecca S., Nicholas L. Mervosh, Tanner J. Euston, Katherine R. Meckel, Amon T. Orr, and Drew D. Kiraly. 2021. “Alterations in Microbiome Composition and Metabolic Byproducts Drive Behavioral and Transcriptional Responses to Morphine.” *Neuropsychopharmacology*, June. https://doi.org/10.1038/s41386-021-01043-0.

Hofford, Rebecca S, Scott J Russo, and Drew D Kiraly. 2019. “Neuroimmune Mechanisms of Psychostimulant and Opioid Use Disorders.” *European Journal of Neuroscience* 50 (3): 2562–73.

Holsboer, Florian. 2000. “The Corticosteroid Receptor Hypothesis of Depression.” *Neuropsychopharmacology* 23 (5): 477–501.

Holzer, Peter. 2004. “Opioids and Opioid Receptors in the Enteric Nervous System: From a Problem in Opioid Analgesia to a Possible New Prokinetic Therapy in Humans.” *Neuroscience Letters* 361 (1–3): 192–95.

Hong, Liang, Whee-Soo Kim, Sang-Mok Lee, Sang-Kee Kang, Yun-Jaie Choi, and Chong-Su Cho. 2019. “Pullulan Nanoparticles as Prebiotics Enhance the Antibacterial Properties of Lactobacillus Plantarum through the Induction of Mild Stress in Probiotics.” *Frontiers in Microbiology* 10: 142.

Hsiao, Elaine Y., Sara W. McBride, Sophia Hsien, Gil Sharon, Embriette R. Hyde, Tyler McCue, Julian A. Codelli, et al. 2013. “Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders.” *Cell* 155 (7): 1451–63. https://doi.org/10.1016/j.cell.2013.11.024.

Huang, Hongli, Haoming Xu, Qingling Luo, Jie He, Mengyan Li, Huiting Chen, Wenjuan Tang, Yuqiang Nie, and Yongjian Zhou. 2019. “Fecal Microbiota Transplantation to Treat Parkinson’s Disease with Constipation: A Case Report.” *Medicine* 98 (26).

Ianiro, Gianluca, Herbert Tilg, and Antonio Gasbarrini. 2016. “Antibiotics as Deep Modulators of Gut Microbiota: Between Good and Evil.” *Gut* 65 (11): 1906. https://doi.org/10.1136/gutjnl-2016-312297.

Iannone, Luigi Francesco, Alberto Preda, Hervé M. Blottière, Gerard Clarke, Diego Albani, Vincenzo Belcastro, Marco Carotenuto, et al. 2019. “Microbiota-Gut Brain Axis Involvement in Neuropsychiatric Disorders.” *Expert Review of Neurotherapeutics* 19 (10): 1037–50. https://doi.org/10.1080/14737175.2019.1638763.

Jadhav, Kshitij S, Veronica L Peterson, Olivier Halfon, Grace Ahern, Fiona Fouhy, Catherine Stanton, Timothy G Dinan, John F Cryan, and Benjamin Boutrel. 2018. “Gut Microbiome Correlates with Altered Striatal Dopamine Receptor Expression in a Model of Compulsive Alcohol Seeking.” *Neuropharmacology* 141: 249–59.

Jalodia, Richa, Yaa Fosuah Abu, Mark Ryan Oppenheimer, Bridget Herlihy, Jingjing Meng, Irina Chupikova, Junyi Tao, et al. 2022. “Opioid Use, Gut Dysbiosis, Inflammation, and the Nervous System.” *Journal of Neuroimmune Pharmacology*, January. https://doi.org/10.1007/s11481-021-10046-z.

Janes, Amy C, Diego A Pizzagalli, Sarah Richardt, Blaise deB Frederick, Sarah Chuzi, Gladys Pachas, Melissa A Culhane, Avram J Holmes, Maurizio Fava, and A Eden Evins. 2010. “Brain Reactivity to Smoking Cues Prior to Smoking Cessation Predicts Ability to Maintain Tobacco Abstinence.” *Biological Psychiatry* 67 (8): 722–29.

Jang, Se-Eun, Jin-Ju Jeong, Jeon-Kyung Kim, Myung Joo Han, and Dong-Hyun Kim. 2018. “Simultaneous Amelioratation of Colitis and Liver Injury in Mice by Bifidobacterium Longum LC67 and Lactobacillus Plantarum LC27.” *Scientific Reports* 8 (1): 1–14.

Jayasinghe, Thilini N, Valentina Chiavaroli, David J Holland, Wayne S Cutfield, and Justin M O’Sullivan. 2016. “The New Era of Treatment for Obesity and Metabolic Disorders:

Evidence and Expectations for Gut Microbiome Transplantation." *Frontiers in Cellular and Infection Microbiology* 6: 15.
Jiang, Haiyin, Zongxin Ling, Yonghua Zhang, Hongjin Mao, Zhanping Ma, Yan Yin, Weihong Wang, Wenxin Tang, Zhonglin Tan, and Jianfei Shi. 2015. "Altered Fecal Microbiota Composition in Patients with Major Depressive Disorder." *Brain, Behavior, and Immunity* 48: 186–94.
Johnson, Elizabeth L., Stacey L. Heaver, William A. Walters, and Ruth E. Ley. 2017. "Microbiome and Metabolic Disease: Revisiting the Bacterial Phylum Bacteroidetes." *Journal of Molecular Medicine* 95 (1): 1–8. https://doi.org/10.1007/s00109-016-1492-2.
Juszczuk, Klaudia, Katarzyna Grudlewska, Agnieszka Mikucka, and Eugenia Gospodarek. 2017. "Fecal Microbiota Transplantation-Methods of Treatment of Recurrent Clostridium Difficile Infections and Other Diseases." *Postepy Higieny i Medycyny Doswiadczalnej (Online)* 71: 220–26.
Kang, Dae-Wook, James B Adams, Devon M Coleman, Elena L Pollard, Juan Maldonado, Sharon McDonough-Means, J Gregory Caporaso, and Rosa Krajmalnik-Brown. 2019. "Long-Term Benefit of Microbiota Transfer Therapy on Autism Symptoms and Gut Microbiota." *Scientific Reports* 9 (1): 1–9.
Kang, DW, JB Adams, AC Gregory, T Borody, L Chittick, A Fasano, A Khoruts, E Geis, J Maldonado, and S McDonough-Means. 2017. "Microbiota Transfer Therapy Alters Gut Ecosystem and Improves Gastrointestinal and Autism Symptoms: An Open-Label Study. Microbiome."
Kang, Minho, Ryan A Mischel, Sukhada Bhave, Essie Komla, Alvin Cho, Charity Huang, William L Dewey, and Hamid I Akbarali. 2017. "The Effect of Gut Microbiome on Tolerance to Morphine Mediated Antinociception in Mice." *Scientific Reports* 7 (1): 1–17.
Kaplovitch, Eric, Tara Gomes, Ximena Camacho, Irfan A Dhalla, Muhammad M Mamdani, and David N Juurlink. 2015. "Sex Differences in Dose Escalation and Overdose Death during Chronic Opioid Therapy: A Population-Based Cohort Study." *PloS One* 10 (8): e0134550.
Karczewski, Jurgen, Freddy J Troost, Irene Konings, Jan Dekker, Michiel Kleerebezem, Robert-Jan M Brummer, and Jerry M Wells. 2010. "Regulation of Human Epithelial Tight Junction Proteins by Lactobacillus Plantarum in Vivo and Protective Effects on the Epithelial Barrier." *American Journal of Physiology-Gastrointestinal and Liver Physiology* 298 (6): G851–59.
Kelly, John R, Yuliya Borre, Ciaran O'Brien, Elaine Patterson, Sahar El Aidy, Jennifer Deane, Paul J Kennedy, Sasja Beers, Karen Scott, and Gerard Moloney. 2016. "Transferring the Blues: Depression-Associated Gut Microbiota Induces Neurobehavioural Changes in the Rat." *Journal of Psychiatric Research* 82: 109–18.
Kendler, Kenneth S, Margaret Gatz, Charles O Gardner, and Nancy L Pedersen. 2006. "A Swedish National Twin Study of Lifetime Major Depression." *American Journal of Psychiatry* 163 (1): 109–14.
Kennedy, Paul J, John F Cryan, Timothy G Dinan, and Gerard Clarke. 2017. "Kynurenine Pathway Metabolism and the Microbiota-Gut-Brain Axis." *Neuropharmacology* 112: 399–412.
Keshavarzian, Ali, Stefan J Green, Phillip A Engen, Robin M Voigt, Ankur Naqib, Christopher B Forsyth, Ece Mutlu, and Kathleen M Shannon. 2015. "Colonic Bacterial Composition in Parkinson's Disease." *Movement Disorders* 30 (10): 1351–60.
Kieffer, Brigitte L. 1995. "Recent Advances in Molecular Recognition and Signal Transduction of Active Peptides: Receptors for Opioid Peptides." *Cellular and Molecular Neurobiology* 15 (6): 615–35.
Kim, Chang H, Jeongho Park, and Myunghoo Kim. 2014. "Gut Microbiota-Derived Short-Chain Fatty Acids, T Cells, and Inflammation." *Immune Network* 14 (6): 277–88.

Kim, Yong Sung, Tatsuya Unno, Byung-Yong Kim, and Mi-Sung Park. 2019. “Sex Differences in Gut Microbiota.” *Wjmh* 38 (1): 48–60. https://doi.org/10.5534/wjmh.190009.
Kiraly, Drew D, Deena M Walker, Erin S Calipari, Benoit Labonte, Orna Issler, Catherine J Pena, Efrain A Ribeiro, Scott J Russo, and Eric J Nestler. 2016. “Alterations of the Host Microbiome Affect Behavioral Responses to Cocaine.” *Scientific Reports* 6 (1): 1–12.
Klein, Laura Cousino, E Jon Popke, and Neil E Grunberg. 1997. “Sex Differences in Effects of Predictable and Unpredictable Footshock on Fentanyl Self-Administration in Rats.” *Experimental and Clinical Psychopharmacology* 5 (2): 99.
Klooker, Tamira K, Breg Braak, Rebecca C Painter, Susanne R De Rooij, Ruurd M Van Elburg, Rene M Van Den Wijngaard, Tessa J Roseboom, and Guy E Boeckxstaens. 2009. “Exposure to Severe Wartime Conditions in Early Life Is Associated with an Increased Risk of Irritable Bowel Syndrome: A Population-Based Cohort Study.” *Official Journal of the American College of Gastroenterology| ACG* 104 (9): 2250–56.
Komla, Essie, David L Stevens, Yi Zheng, Yan Zhang, William L Dewey, and Hamid I Akbarali. 2019. “Experimental Colitis Enhances the Rate of Antinociceptive Tolerance to Morphine via Peripheral Opioid Receptors.” *Journal of Pharmacology and Experimental Therapeutics* 370 (3): 504–13.
Koob, George F, Cara L Buck, Ami Cohen, Scott Edwards, Paula E Park, Joel E Schlosburg, Brooke Schmeichel, Leandro F Vendruscolo, Carrie L Wade, and Timothy W Whitfield Jr. 2014. “Addiction as a Stress Surfeit Disorder.” *Neuropharmacology* 76: 370–82.
Koob, George F, and Michel Le Moal. 1997. “Drug Abuse: Hedonic Homeostatic Dysregulation.” *Science* 278 (5335): 52–58.
Koob, George F, and Nora D Volkow. 2016. “Neurobiology of Addiction: A Neurocircuitry Analysis.” *The Lancet Psychiatry* 3 (8): 760–73.
Korner, Judith, and Rudolph L Leibel. 2003. “To Eat or Not to Eat-How the Gut Talks to the Brain.” *New England Journal of Medicine* 349 (10): 926–27.
Kosterlitz, HW, and FRANCES M Leslie. 1978. “Comparison of the Receptor Binding Characteristics of Opiate Agonists Interacting with Mu-or Kappa-Receptors.” *British Journal of Pharmacology* 64 (4): 607.
Kovacs, Amir, Noa Ben-Jacob, Hanna Tayem, Eran Halperin, Fuad A. Iraqi, and Uri Gophna. 2011. “Genotype Is a Stronger Determinant than Sex of the Mouse Gut Microbiota.” *Microbial Ecology* 61 (2): 423–28. https://doi.org/10.1007/s00248-010-9787-2.
Kovatcheva-Datchary, Petia, and Tulika Arora. 2013. “Nutrition, the Gut Microbiome and the Metabolic Syndrome.” *Best Practice & Research Clinical Gastroenterology* 27 (1): 59–72.
Krautkramer, Kimberly A, Julia H Kreznar, Kymberleigh A Romano, Eugenio I Vivas, Gregory A Barrett-Wilt, Mary E Rabaglia, Mark P Keller, Alan D Attie, Federico E Rey, and John M Denu. 2016. “Diet-Microbiota Interactions Mediate Global Epigenetic Programming in Multiple Host Tissues.” *Molecular Cell* 64 (5): 982–92.
Laan, Laura Nynke van der, Denise TD de Ridder, Max A Viergever, and Paul AM Smeets. 2014. “Activation in Inhibitory Brain Regions during Food Choice Correlates with Temptation Strength and Self-Regulatory Success in Weight-Concerned Women.” *Frontiers in Neuroscience* 8: 308.
Lacy, Ryan T, Justin C Strickland, Max A Feinstein, Andrea M Robinson, and Mark A Smith. 2016. “The Effects of Sex, Estrous Cycle, and Social Contact on Cocaine and Heroin Self-Administration in Rats.” *Psychopharmacology* 233 (17): 3201–10.
Lazar, Veronica, Lia-Mara Ditu, Gratiela Gradisteanu Pircalabioru, Irina Gheorghe, Carmen Curutiu, Alina Maria Holban, Ariana Picu, Laura Petcu, and Mariana Carmen Chifiriuc. 2018. “Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer.” *Frontiers in Immunology* 9: 1830.

Leclercq, Sophie, Paul Forsythe, and John Bienenstock. 2016. “Posttraumatic Stress Disorder: Does the Gut Microbiome Hold the Key?” *The Canadian Journal of Psychiatry* 61 (4): 204–13.

Lee, Kevin, Helen E Vuong, David J Nusbaum, Elaine Y Hsiao, Christopher J Evans, and Anna MW Taylor. 2018. “The Gut Microbiota Mediates Reward and Sensory Responses Associated with Regimen-Selective Morphine Dependence.” *Neuropsychopharmacology* 43 (13): 2606–14.

Letchumanan, Vengadesh, See-Nee Low, Loh Teng-Hern Tan, Jodi Woan-Fei Law, Vanassa Ratnasingam, Sivakumar Thurairajasingam, and Learn-Han Lee. 2022. “IDDF2022-ABS-0212 Exploring the Potential Role of Probiotics in Substance Use Disorders.” *Gut* 71 (Suppl 2): A58. https://doi.org/10.1136/gutjnl-2022-IDDF.66.

Levy, Maayan, Eran Blacher, and Eran Elinav. 2017. “Microbiome, Metabolites and Host Immunity.” *Current Opinion in Microbiology* 35: 8–15.

Lewis, Kimberley, Femke Lutgendorff, Van Phan, Johan D Söderholm, Philip M Sherman, and Derek M McKay. 2010. “Enhanced Translocation of Bacteria across Metabolically Stressed Epithelia Is Reduced by Butyrate.” *Inflammatory Bowel Diseases* 16 (7): 1138–48.

Ley, Ruth E, Daniel A Peterson, and Jeffrey I Gordon. 2006. “Ecological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine.” *Cell* 124 (4): 837–48.

Li, Binyin, Yixi He, Jianfang Ma, Pei Huang, Juanjuan Du, Li Cao, Yan Wang, Qin Xiao, Huidong Tang, and Shengdi Chen. 2019. “Mild Cognitive Impairment Has Similar Alterations as Alzheimer’s Disease in Gut Microbiota.” *Alzheimer’s & Dementia* 15 (10): 1357–66.

Li, Qiaoyan, Siqi Chen, Ke Liu, Danfeng Long, Diru Liu, Zhengchao Jing, and Xiaodan Huang. 2020. “Differences in Gut Microbial Diversity Are Driven by Drug Use and Drug Cessation by Either Compulsory Detention or Methadone Maintenance Treatment.” *Microorganisms* 8 (3). https://doi.org/10.3390/microorganisms8030411.

Li, Qinrui, Ying Han, Angel Belle C Dy, and Randi J Hagerman. 2017. “The Gut Microbiota and Autism Spectrum Disorders.” *Frontiers in Cellular Neuroscience*, 120.

Liang, NC, NT Bello, and TH Moran. 2013. “Additive Feeding Inhibitory and Aversive Effects of Naltrexone and Exendin-4 Combinations.” *International Journal of Obesity* 37 (2): 272–78.

Liu, Baoning, Yali Zhang, Rong Wang, Yingfeng An, Weiman Gao, Liang Bai, Yandong Li, Sihai Zhao, Jianglin Fan, and Enqi Liu. 2018. “Western Diet Feeding Influences Gut Microbiota Profiles in ApoE Knockout Mice.” *Lipids in Health and Disease* 17 (1): 1–8.

Liu, Jiaming, Jing Sun, Fangyan Wang, Xichong Yu, Zongxin Ling, Haixiao Li, Huiqing Zhang, Jiangtao Jin, Wenqian Chen, and Mengqi Pang. 2015. “Neuroprotective Effects of Clostridium Butyricum against Vascular Dementia in Mice via Metabolic Butyrate.” *BioMed Research International* 2015.

Lobionda, Stefani, Panida Sittipo, Hyog Young Kwon, and Yun Kyung Lee. 2019. “The Role of Gut Microbiota in Intestinal Inflammation with Respect to Diet and Extrinsic Stressors.” *Microorganisms* 7 (8): 271.

Lotsch, Jorn, Michel Weiss, Gabi Ahne, Gerd Kobal, and Gerd Geisslinger. 1999. “Pharmacokinetic Modeling of M6G Formation after Oral Administration of Morphine in Healthy Volunteers.” *The Journal of the American Society of Anesthesiologists* 90 (4): 1026–38.

Lozupone, Catherine A, Jesse I Stombaugh, Jeffrey I Gordon, Janet K Jansson, and Rob Knight. 2012. “Diversity, Stability and Resilience of the Human Gut Microbiota.” *Nature* 489 (7415): 220–30.

Luna, Ruth Ann, and Jane A Foster. 2015. “Gut Brain Axis: Diet Microbiota Interactions and Implications for Modulation of Anxiety and Depression.” *Current Opinion in Biotechnology* 32: 35–41.

Lurie, Ido, Yu-Xiao Yang, Kevin Haynes, Ronac Mamtani, and Ben Boursi. 2015. “Antibiotic Exposure and the Risk for Depression, Anxiety, or Psychosis: A Nested Case-Control Study.” *The Journal of Clinical Psychiatry* 76 (11): 825.
Lyte, Mark. 2011. “Probiotics Function Mechanistically as Delivery Vehicles for Neuroactive Compounds: Microbial Endocrinology in the Design and Use of Probiotics.” *Bioessays* 33 (8): 574–81.
Macedo, Danielle, Adriano José Maia Chaves Filho, Caren Nádia Soares de Sousa, João Quevedo, Tatiana Barichello, Hélio Vitoriano Nobre Júnior, and David Freitas de Lucena. 2017. “Antidepressants, Antimicrobials or Both? Gut Microbiota Dysbiosis in Depression and Possible Implications of the Antimicrobial Effects of Antidepressant Drugs for Antidepressant Effectiveness.” *Journal of Affective Disorders* 208: 22–32.
Maes, Michael, Michael Berk, Lisa Goehler, Cai Song, George Anderson, Piotr Gałecki, and Brian Leonard. 2012. “Depression and Sickness Behavior Are Janus-Faced Responses to Shared Inflammatory Pathways.” *BMC Medicine* 10 (1): 1–19.
Magnusson, KR, Laura Hauck, BM Jeffrey, V Elias, A Humphrey, R Nath, A Perrone, and LE Bermudez. 2015. “Relationships between Diet-Related Changes in the Gut Microbiome and Cognitive Flexibility.” *Neuroscience* 300: 128–40.
Makkawi, Seraj, Carlos Camara-Lemarroy, and Luanne Metz. 2018. “Fecal Microbiota Transplantation Associated with 10 Years of Stability in a Patient with SPMS.” *Neurology-Neuroimmunology Neuroinflammation* 5 (4).
Martins, Silvia S, Luis E Segura, Julian Santaella-Tenorio, Alexander Perlmutter, Miriam C Fenton, Magdalena Cerdá, Katherine M Keyes, Lilian A Ghandour, Carla L Storr, and Deborah S Hasin. 2017. “Prescription Opioid Use Disorder and Heroin Use among 12-34 Year-Olds in the United States from 2002 to 2014.” *Addictive Behaviors* 65: 236–41.
Matcovitch-Natan, Orit, Deborah R Winter, Amir Giladi, Stephanie Vargas Aguilar, Amit Spinrad, Sandrine Sarrazin, Hila Ben-Yehuda, Eyal David, Fabiola Zelada González, and Pierre Perrin. 2016. “Microglia Development Follows a Stepwise Program to Regulate Brain Homeostasis.” *Science* 353 (6301): aad8670.
Mayer, Emeran A. 2011. “Gut Feelings: The Emerging Biology of Gut–Brain Communication.” *Nature Reviews Neuroscience* 12 (8): 453–66. https://doi.org/10.1038/nrn3071.
Mayer, Emeran A, Kirsten Tillisch, and Arpana Gupta. 2015. “Gut/Brain Axis and the Microbiota.” *The Journal of Clinical Investigation* 125 (3): 926–38.
McKernan, DP, Patrick Fitzgerald, TG Dinan, and JF Cryan. 2010. “The Probiotic Bifidobacterium Infantis 35624 Displays Visceral Antinociceptive Effects in the Rat.” *Neurogastroenterology & Motility* 22 (9): 1029-e268.
Meckel, Katherine R, and Drew D Kiraly. 2019. “A Potential Role for the Gut Microbiome in Substance Use Disorders.” *Psychopharmacology* 236 (5): 1513–30.
Meng, Jingjing, Gregory M Sindberg, and Sabita Roy. 2015. “Disruption of Gut Homeostasis by Opioids Accelerates HIV Disease Progression.” *Frontiers in Microbiology* 6: 643.
Meng, Jingjing, Haidong Yu, Jing Ma, Jinghua Wang, Santanu Banerjee, Rick Charboneau, Roderick A Barke, and Sabita Roy. 2013. “Morphine Induces Bacterial Translocation in Mice by Compromising Intestinal Barrier Function in a TLR-Dependent Manner.” *PloS One* 8 (1): e54040.
Miller, Terry L, and Meyer J Wolin. 1996. “Pathways of Acetate, Propionate, and Butyrate Formation by the Human Fecal Microbial Flora.” *Applied and Environmental Microbiology* 62 (5): 1589–92.
Milligan, Graeme, Bharat Shimpukade, Trond Ulven, and Brian D Hudson. 2017. “Complex Pharmacology of Free Fatty Acid Receptors.” *Chemical Reviews* 117 (1): 67–110.
Mitchell, Ryan W, Ngoc H On, Marc R Del Bigio, Donald W Miller, and Grant M Hatch. 2011. “Fatty Acid Transport Protein Expression in Human Brain and Potential Role in Fatty

Acid Transport across Human Brain Microvessel Endothelial Cells." *Journal of Neurochemistry* 117 (4): 735–46.
Mitrea, Laura, Silvia-Amalia Nemeş, Katalin Szabo, Bernadette-Emőke Teleky, and Dan-Cristian Vodnar. 2022. "Guts Imbalance Imbalances the Brain: A Review of Gut Microbiota Association With Neurological and Psychiatric Disorders." *Frontiers in Medicine* 9 (March): 813204–813204. https://doi.org/10.3389/fmed.2022.813204.
Moloney, Rachel D, Anthony C Johnson, Siobhain M O'mahony, Timothy G Dinan, Beverley Greenwood-Van Meerveld, and John F Cryan. 2016. "Stress and the Microbiota–Gut–Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome." *CNS Neuroscience & Therapeutics* 22 (2): 102–17.
Moretti, Morgana, Samira S Valvassori, Roger B Varela, Camila L Ferreira, Natalia Rochi, Joana Benedet, Giselli Scaini, Flavio Kapczinski, Emilio L Streck, and Alexandra I Zugno. 2011. "Behavioral and Neurochemical Effects of Sodium Butyrate in an Animal Model of Mania." *Behavioural Pharmacology* 22 (8): 766–72.
Morgan, Andrew D., Una C. Campbell, Ryen D. Fons, and Marilyn E. Carroll. 2002. "Effects of Agmatine on the Escalation of Intravenous Cocaine and Fentanyl Self-Administration in Rats." *Pharmacology Biochemistry and Behavior* 72 (4): 873–80. https://doi.org/10.1016/S0091-3057(02)00774-8.
Mulder, M., D. Radjabzadeh, J. C. Kiefte-de Jong, A. G. Uitterlinden, R. Kraaij, B. H. Stricker, and A. Verbon. 2020. "Long-Term Effects of Antimicrobial Drugs on the Composition of the Human Gut Microbiota." *Gut Microbes* 12 (1): 1791677. https://doi.org/10.1080/19490976.2020.1791677.
Muscogiuri, Giovanna, Elena Cantone, Sara Cassarano, Dario Tuccinardi, Luigi Barrea, Silvia Savastano, and Annamaria Colao. 2019. "Gut Microbiota: A New Path to Treat Obesity." *International Journal of Obesity Supplements* 9 (1): 10–19.
Mushegian, Alexandra A, and Dieter Ebert. 2016. "Rethinking 'Mutualism' in Diverse Host-symbiont Communities." *BioEssays* 38 (1): 100–108.
Naqvi, Nasir H, and Antoine Bechara. 2009. "The Hidden Island of Addiction: The Insula." *Trends in Neurosciences* 32 (1): 56–67.
Neufeld, Karen-Anne M, Nancy Kang, John Bienenstock, and Jane A Foster. 2011. "Effects of Intestinal Microbiota on Anxiety-like Behavior." *Communicative & Integrative Biology* 4 (4): 492–94.
Neufeld, KM, Nancy Kang, John Bienenstock, and Jane A Foster. 2011. "Reduced Anxiety-like Behavior and Central Neurochemical Change in Germ-free Mice." *Neurogastroenterology & Motility* 23 (3): 255-e119.
Niego, Sara H, Michele D Kofman, Jeffrey J Weiss, and Allan Geliebter. 2007. "Binge Eating in the Bariatric Surgery Population: A Review of the Literature." *International Journal of Eating Disorders* 40 (4): 349–59.
Ning, Tingting, Xiaokang Gong, Lingling Xie, and Baomiao Ma. 2017. "Gut Microbiota Analysis in Rats with Methamphetamine-Induced Conditioned Place Preference." *Frontiers in Microbiology* 8: 1620.
Nobuyuki, Sudo, Chida Yoichi, Aiba Yuji, Sonoda Junko, Oyama Naomi, Yu Xiao-Nian, Kubo Chiharu, and Koga Yasuhiro. 2004. "Postnatal Microbial Colonization Programs the Hypothalamic-Pituitary-Adrenal System for Stress Response in Mice." *J Physiol* 558 (1): 263–75.
O'Mahony, Siobhain M, Gerard Clarke, YE Borre, Tig G Dinan, and JF Cryan. 2015. "Serotonin, Tryptophan Metabolism and the Brain-Gut-Microbiome Axis." *Behavioural Brain Research* 277: 32–48.
O'mahony, SM, VD Felice, K Nally, HM Savignac, MJ Claesson, P Scully, J Woznicki, NP Hyland, F Shanahan, and Eamonn Martin Quigley. 2014. "Disturbance of the Gut

Microbiota in Early-Life Selectively Affects Visceral Pain in Adulthood without Impacting Cognitive or Anxiety-Related Behaviors in Male Rats." *Neuroscience* 277: 885–901.
Org, Elin, Margarete Mehrabian, Brian W. Parks, Petia Shipkova, Xiaoqin Liu, Thomas A. Drake, and Aldons J. Lusis. 2016. "Sex Differences and Hormonal Effects on Gut Microbiota Composition in Mice." *Gut Microbes* 7 (4): 313–22. https://doi.org/10.1080/19490976.2016.1203502.
O'Sullivan, Sean J, Evangelia Malahias, James Park, Ankita Srivastava, Beverly AS Reyes, Jon Gorky, Rajanikanth Vadigepalli, Elisabeth J Van Bockstaele, and James S Schwaber. 2019. "Single-Cell Glia and Neuron Gene Expression in the Central Amygdala in Opioid Withdrawal Suggests Inflammation with Correlated Gut Dysbiosis." *Frontiers in Neuroscience* 13: 665.
O'Toole Paul W. and Jeffery Ian B. 2015. "Gut Microbiota and Aging." *Science* 350 (6265): 1214–15. https://doi.org/10.1126/science.aac8469.
Panchal, SJ, PJIW Müller-Schwefe, and JI Wurzelmann. 2007. "Opioid-induced Bowel Dysfunction: Prevalence, Pathophysiology and Burden." *International Journal of Clinical Practice* 61 (7): 1181–87.
Panda, Suchita, Ismail El Khader, Francesc Casellas, Josefa Lopez Vivancos, Montserrat Garcia Cors, Alba Santiago, Silvia Cuenca, Francisco Guarner, and Chaysavanh Manichanh. 2014. "Short-Term Effect of Antibiotics on Human Gut Microbiota." *PloS One* 9 (4): e95476.
Pepino, Marta Yanina, Richard I Stein, J Christopher Eagon, and Samuel Klein. 2014. "Bariatric Surgery-induced Weight Loss Causes Remission of Food Addiction in Extreme Obesity." *Obesity* 22 (8): 1792–98.
Peterson, Veronica L., Jerry B. Richards, Paul J. Meyer, Raul Cabrera-Rubio, Jordan A. Tripi, Christopher P. King, Oksana Polesskaya, et al. 2020. "Sex-Dependent Associations between Addiction-Related Behaviors and the Microbiome in Outbred Rats." *EBioMedicine* 55 (May): 102769. https://doi.org/10.1016/j.ebiom.2020.102769.
Power, Susan E, Paul W O'Toole, Catherine Stanton, R Paul Ross, and Gerald F Fitzgerald. 2014. "Intestinal Microbiota, Diet and Health." *British Journal of Nutrition* 111 (3): 387–402.
Puizillout, Jean-Jacques. 2005. *Central Projections of Vagal Afferents*. Editions Publibook.
Pusceddu, Matteo M, Sahar El Aidy, Fiona Crispie, Orla O'Sullivan, Paul Cotter, Catherine Stanton, Philip Kelly, John F Cryan, and Timothy G Dinan. 2015. "N-3 Polyunsaturated Fatty Acids (PUFAs) Reverse the Impact of Early-Life Stress on the Gut Microbiota." *PloS One* 10 (10): e0139721.
Qin, Chenyuan, Jiawei Hu, Yiming Wan, Mengyao Cai, Zhenting Wang, Zhao Peng, Yuxiao Liao, et al. 2021. "Narrative Review on Potential Role of Gut Microbiota in Certain Substance Addiction." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 106 (March): 110093. https://doi.org/10.1016/j.pnpbp.2020.110093.
Raebel, Marsha A, Sophia R Newcomer, Liza M Reifler, Denise Boudreau, Thomas E Elliott, Lynn DeBar, Ameena Ahmed, Pamala A Pawloski, David Fisher, and W Troy Donahoo. 2013. "Chronic Use of Opioid Medications before and after Bariatric Surgery." *Jama* 310 (13): 1369–76.
Ramirez, Karol, Jaime Fornaguera-Trías, and John F Sheridan. 2016. "Stress-Induced Microglia Activation and Monocyte Trafficking to the Brain Underlie the Development of Anxiety and Depression." *Inflammation-Associated Depression: Evidence, Mechanisms and Implications*, 155–72.
Rao, Meenakshi, and Michael D Gershon. 2016. "The Bowel and beyond: The Enteric Nervous System in Neurological Disorders." *Nature Reviews Gastroenterology & Hepatology* 13 (9): 517–28.
Razran, G. 1961. "The Observable Unconscious." *Psych. Rev*.

Rea, Kieran, Timothy G Dinan, and John F Cryan. 2016. “The Microbiome: A Key Regulator of Stress and Neuroinflammation.” *Neurobiology of Stress* 4: 23–33.
Ree, J M van, J L Slangen, and D de Wied. 1978. “Intravenous Self-Administration of Drugs in Rats.” *Journal of Pharmacology and Experimental Therapeutics* 204 (3): 547.
Reigstad, Christopher S, Charles E Salmonson, John F Rainey III, Joseph H Szurszewski, David R Linden, Justin L Sonnenburg, Gianrico Farrugia, and Purna C Kashyap. 2015. “Gut Microbes Promote Colonic Serotonin Production through an Effect of Short-chain Fatty Acids on Enterochromaffin Cells.” *The FASEB Journal* 29 (4): 1395–1403.
Reikvam, Dag Henrik, Alexander Erofeev, Anders Sandvik, Vedrana Grcic, Frode Lars Jahnsen, Peter Gaustad, Kathy D McCoy, Andrew J Macpherson, Leonardo A Meza-Zepeda, and Finn-Eirik Johansen. 2011. “Depletion of Murine Intestinal Microbiota: Effects on Gut Mucosa and Epithelial Gene Expression.” *PloS One* 6 (3): e17996.
Ren, Michelle, and Shahrdad Lotfipour. 2020. “The Role of the Gut Microbiome in Opioid Use.” *Behavioural Pharmacology* 31 (2-# x000263): 113.
———. 2022. “Dose- and Sex-Dependent Bidirectional Relationship between Intravenous Fentanyl Self-Administration and Gut Microbiota.” *Microorganisms* 10 (6). https://doi.org/10.3390/microorganisms10061127.
Resende, Wilson R, Samira S Valvassori, Gislaine Z Reus, Roger B Varela, Camila O Arent, Karine F Ribeiro, Daniela V Bavaresco, Monica L Andersen, Alexandra I Zugno, and Joao Quevedo. 2013. “Effects of Sodium Butyrate in Animal Models of Mania and Depression: Implications as a New Mood Stabilizer.” *Behavioural Pharmacology* 24 (7): 569–79.
Roberts, David, Elliot A Loh, and Gary Vickers. 1989. “Self-Administration of Cocaine on a Progressive Ratio Schedule in Rats: Dose-Response Relationship and Effect of Haloperidol Pretreatment.” *Psychopharmacology* 97 (4): 535–38.
Roman, Pablo, Raquel Abalo, Eva M Marco, and Diana Cardona. 2018. “Probiotics in Digestive, Emotional, and Pain-Related Disorders.” *Behavioural Pharmacology* 29 (2): 103–19.
Rooks, Michelle G, and Wendy S Garrett. 2016. “Gut Microbiota, Metabolites and Host Immunity.” *Nature Reviews Immunology* 16 (6): 341–52.
Rudd, Rose A, Puja Seth, Felicita David, and Lawrence Scholl. 2016. “Increases in Drug and Opioid-Involved Overdose Deaths—United States, 2010–2015.” *Morbidity and Mortality Weekly Report* 65 (50 & 51): 1445–52.
Russo, Scott J, and Eric J Nestler. 2013. “The Brain Reward Circuitry in Mood Disorders.” *Nature Reviews Neuroscience* 14 (9): 609–25.
Sampson, Timothy R, Justine W Debelius, Taren Thron, Stefan Janssen, Gauri G Shastri, Zehra Esra Ilhan, Collin Challis, Catherine E Schretter, Sandra Rocha, and Viviana Gradinaru. 2016. “Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson’s Disease.” *Cell* 167 (6): 1469–80.
Sandhu, Kiran V., Eoin Sherwin, Harriët Schellekens, Catherine Stanton, Timothy G. Dinan, and John F. Cryan. 2017. “Feeding the Microbiota-Gut-Brain Axis: Diet, Microbiome, and Neuropsychiatry.” *Microbiome and Human Disease Pathogenesis* 179 (January): 223–44. https://doi.org/10.1016/j.trsl.2016.10.002.
Sbahi, Hani, and Jack A Di Palma. 2016. “Faecal Microbiota Transplantation: Applications and Limitations in Treating Gastrointestinal Disorders.” *BMJ Open Gastroenterology* 3 (1): e000087.
Scheperjans, Filip, Velma Aho, Pedro AB Pereira, Kaisa Koskinen, Lars Paulin, Eero Pekkonen, Elena Haapaniemi, Seppo Kaakkola, Johanna Eerola-Rautio, and Marjatta Pohja. 2015. “Gut Microbiota Are Related to Parkinson’s Disease and Clinical Phenotype.” *Movement Disorders* 30 (3): 350–58.
Schmidt, Heath D, Elizabeth G Mietlicki-Baase, Kelsey Y Ige, John J Maurer, David J Reiner, Derek J Zimmer, Duncan S Van Nest, Leonardo A Guercio, Mathieu E Wimmer, and

Diana R Olivos. 2016. “Glucagon-like Peptide-1 Receptor Activation in the Ventral Tegmental Area Decreases the Reinforcing Efficacy of Cocaine.” *Neuropsychopharmacology* 41 (7): 1917–28.
Schmidt, Kristin, Philip J Cowen, Catherine J Harmer, George Tzortzis, Steven Errington, and Philip WJ Burnet. 2015. “Prebiotic Intake Reduces the Waking Cortisol Response and Alters Emotional Bias in Healthy Volunteers.” *Psychopharmacology* 232 (10): 1793–1801.
Scholl, Isabelle, Allison LaRussa, Pola Hahlweg, Sarah Kobrin, and Glyn Elwyn. 2018. “Organizational-and System-Level Characteristics That Influence Implementation of Shared Decision-Making and Strategies to Address Them—a Scoping Review.” *Implementation Science* 13 (1): 1–22.
Schroeder, Bjoern O, and Fredrik Bäckhed. 2016. “Signals from the Gut Microbiota to Distant Organs in Physiology and Disease.” *Nature Medicine* 22 (10): 1079–89.
Schwienteck, Kathryn L, Kaycee E Faunce, Kenner C Rice, Samuel Obeng, Yan Zhang, Bruce E Blough, Travis W Grim, S Stevens Negus, and Matthew L Banks. 2019. “Effectiveness Comparisons of G-Protein Biased and Unbiased Mu Opioid Receptor Ligands in Warm Water Tail-Withdrawal and Drug Discrimination in Male and Female Rats.” *Neuropharmacology* 150: 200–209.
Scott, Lucinda V, Gerard Clarke, and Timothy G Dinan. 2013. “The Brain-Gut Axis: A Target for Treating Stress-Related Disorders.” *Inflammation in Psychiatry* 28: 90–99.
Scotti, Elena, Stéphanie Boué, Giuseppe Lo Sasso, Filippo Zanetti, Vincenzo Belcastro, Carine Poussin, Nicolas Sierro, James Battey, Anne Gimalac, and Nikolai V Ivanov. 2017. “Exploring the Microbiome in Health and Disease: Implications for Toxicology.” *Toxicology Research and Application* 1: 2397847317741884.
Selkrig, Joel, Peiyan Wong, Xiaodong Zhang, and Sven Pettersson. 2014. “Metabolic Tinkering by the Gut Microbiome: Implications for Brain Development and Function.” *Gut Microbes* 5 (3): 369–80.
Severance, Emily G, and Robert H Yolken. 2020. “Deciphering Microbiome and Neuroactive Immune Gene Interactions in Schizophrenia.” *Neurobiology of Disease* 135: 104331.
Shade, Ashley. 2017. “Diversity Is the Question, Not the Answer.” *The ISME Journal* 11 (1): 1–6. https://doi.org/10.1038/ismej.2016.118.
Shao, Haoqing, Chenyang Zhang, Nenqun Xiao, and Zhoujin Tan. 2020. “Gut Microbiota Characteristics in Mice with Antibiotic-Associated Diarrhea.” *BMC Microbiology* 20 (1): 313. https://doi.org/10.1186/s12866-020-01999-x.
Sharma, Sandeep, Yumei Zhuang, and Fernando Gomez-Pinilla. 2012. “High-Fat Diet Transition Reduces Brain DHA Levels Associated with Altered Brain Plasticity and Behaviour.” *Scientific Reports* 2 (1): 1–8.
Sharon, Gil, Nikki Jamie Cruz, Dae-Wook Kang, Michael J Gandal, Bo Wang, Young-Mo Kim, Erika M Zink, Cameron P Casey, Bryn C Taylor, and Christianne J Lane. 2019. “Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice.” *Cell* 177 (6): 1600–1618.
Sharon, Gil, Timothy R Sampson, Daniel H Geschwind, and Sarkis K Mazmanian. 2016. “The Central Nervous System and the Gut Microbiome.” *Cell* 167 (4): 915–32.
Shin, Na-Ri, Tae Woong Whon, and Jin-Woo Bae. 2015. “Proteobacteria: Microbial Signature of Dysbiosis in Gut Microbiota.” *Trends in Biotechnology* 33 (9): 496–503. https://doi.org/10.1016/j.tibtech.2015.06.011.
Siegel, Shepard, and Barbara Ramos. 2002. “Applying Laboratory Research: Drug Anticipation and the Treatment of Drug Addiction.” *Experimental and Clinical Psychopharmacology* 10 (3): 162.
Simpson, Sierra, Rio Mclellan, Emma Wellmeyer, Frederic Matalon, and Olivier George. 2022. “Drugs and Bugs: The Gut-Brain Axis and Substance Use Disorders.” *Journal of*

Neuroimmune Pharmacology 17 (1): 33–61. https://doi.org/10.1007/s11481-021-10022-7.
Sinha, Rajita, and Ania M Jastreboff. 2013. “Stress as a Common Risk Factor for Obesity and Addiction.” *Biological Psychiatry* 73 (9): 827–35.
Sirohi, Sunil, Jennifer D Schurdak, Randy J Seeley, Stephen C Benoit, and Jon F Davis. 2016. “Central & Peripheral Glucagon-like Peptide-1 Receptor Signaling Differentially Regulate Addictive Behaviors.” *Physiology & Behavior* 161: 140–44.
Skibicka, Karolina P. 2013. “The Central GLP-1: Implications for Food and Drug Reward.” *Frontiers in Neuroscience* 7: 181.
Söderholm, Johan D, Ping–Chang Yang, Peter Ceponis, Angeli Vohra, Robert Riddell, Philip M Sherman, and Mary H Perdue. 2002. “Chronic Stress Induces Mast Cell–Dependent Bacterial Adherence and Initiates Mucosal Inflammation in Rat Intestine.” *Gastroenterology* 123 (4): 1099–1108.
Spreadbury, Ian. 2012. “Comparison with Ancestral Diets Suggests Dense Acellular Carbohydrates Promote an Inflammatory Microbiota, and May Be the Primary Dietary Cause of Leptin Resistance and Obesity.” *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 5: 175.
Stasi, Cristina, Anna Caserta, Cristiana Nisita, Sonia Cortopassi, Bernardo Fani, Stefano Salvadori, Andrea Pancetti, Lorenzo Bertani, Dario Gambaccini, and Nicola de Bortoli. 2019. “The Complex Interplay between Gastrointestinal and Psychiatric Symptoms in Irritable Bowel Syndrome: A Longitudinal Assessment.” *Journal of Gastroenterology and Hepatology* 34 (4): 713–19.
Stecher, Bärbel. 2015. “The Roles of Inflammation, Nutrient Availability and the Commensal Microbiota in Enteric Pathogen Infection.” *Metabolism and Bacterial Pathogenesis*, 297–320.
Stecher, Bärbel, Lisa Maier, and Wolf-Dietrich Hardt. 2013. “’Blooming’in the Gut: How Dysbiosis Might Contribute to Pathogen Evolution.” *Nature Reviews Microbiology* 11 (4): 277–84.
Stewart, Amy Stieler, Shannon Pratt-Phillips, and Liara M Gonzalez. 2017. “Alterations in Intestinal Permeability: The Role of the ‘Leaky Gut’ in Health and Disease.” *Journal of Equine Veterinary Science* 52: 10–22.
Stilling, Roman M, Marcel van de Wouw, Gerard Clarke, Catherine Stanton, Timothy G Dinan, and John F Cryan. 2016. “The Neuropharmacology of Butyrate: The Bread and Butter of the Microbiota-Gut-Brain Axis?” *Neurochemistry International* 99: 110–32.
Stojanov, Spase, Aleš Berlec, and Borut Štrukelj. 2020. “The Influence of Probiotics on the Firmicutes/Bacteroidetes Ratio in the Treatment of Obesity and Inflammatory Bowel Disease.” *Microorganisms* 8 (11). https://doi.org/10.3390/microorganisms8111715.
Substance Abuse and Mental Health Services Administration. Key Substance Use and Mental Health Indicators in the United States: Results from the 2019 National Survey on Drug Use and Health. HHS Publication; Rockville, MD, USA: 2020.
Sudo, Nobuyuki. 2019. “Biogenic Amines: Signals between Commensal Microbiota and Gut Physiology.” *Frontiers in Endocrinology*, 504.
Sudo, Nobuyuki, Yoichi Chida, Yuji Aiba, Junko Sonoda, Naomi Oyama, Xiao-Nian Yu, Chiharu Kubo, and Yasuhiro Koga. 2004. “Postnatal Microbial Colonization Programs the Hypothalamic–Pituitary–Adrenal System for Stress Response in Mice.” *The Journal of Physiology* 558 (1): 263–75.
Sun, Jing, Jingxuan Xu, Yi Ling, Fangyan Wang, Tianyu Gong, Changwei Yang, Shiqing Ye, Keyue Ye, Dianhui Wei, and Ziqing Song. 2019. “Fecal Microbiota Transplantation Alleviated Alzheimer’s Disease-like Pathogenesis in APP/PS1 Transgenic Mice.” *Translational Psychiatry* 9 (1): 1–13.

Sun, Meng-Fei, Ying-Li Zhu, Zhi-Lan Zhou, Xue-Bing Jia, Yi-Da Xu, Qin Yang, Chun Cui, and Yan-Qin Shen. 2018. “Neuroprotective Effects of Fecal Microbiota Transplantation on MPTP-Induced Parkinson’s Disease Mice: Gut Microbiota, Glial Reaction and TLR4/TNF-α Signaling Pathway.” *Brain, Behavior, and Immunity* 70: 48–60.

TAN, Takashi, Masashi Kuramoto, Terutaka TAKAHASHI, Hideo NAKAMURA, Yutaka NAKANISHI, Yu IMASATO, and Hidetoshi YOSHIMURA. 1989. “Characteristics of the Gastrointestinal Absorption of Morphine in Rats.” *Chemical and Pharmaceutical Bulletin* 37 (1): 168–73.

Tarr, Andrew J, Jeffrey D Galley, Sydney E Fisher, Maciej Chichlowski, Brian M Berg, and Michael T Bailey. 2015. “The Prebiotics 3′ Sialyllactose and 6′ Sialyllactose Diminish Stressor-Induced Anxiety-like Behavior and Colonic Microbiota Alterations: Evidence for Effects on the Gut–Brain Axis.” *Brain, Behavior, and Immunity* 50: 166–77.

Taylor, Anna MW, Annie Castonguay, Atefeh Ghogha, Pia Vayssiere, Amynah AA Pradhan, Lihua Xue, Sadaf Mehrabani, Juli Wu, Pat Levitt, and Mary C Olmstead. 2016. “Neuroimmune Regulation of GABAergic Neurons within the Ventral Tegmental Area during Withdrawal from Chronic Morphine.” *Neuropsychopharmacology* 41 (4): 949–59.

Temko, Jamie E, Sofia Bouhlal, Mehdi Farokhnia, Mary R Lee, John F Cryan, and Lorenzo Leggio. 2017. “The Microbiota, the Gut and the Brain in Eating and Alcohol Use Disorders: A ‘Ménage à Trois’?” *Alcohol and Alcoholism* 52 (4): 403–13.

Thomas, Carissa M, Teresa Hong, Jan Peter Van Pijkeren, Peera Hemarajata, Dan V Trinh, Weidong Hu, Robert A Britton, Markus Kalkum, and James Versalovic. 2012. “Histamine Derived from Probiotic Lactobacillus Reuteri Suppresses TNF via Modulation of PKA and ERK Signaling.” *PloS One* 7 (2): e31951.

Thomaz, Ana C., Vishakh Iyer, Taylor J. Woodward, and Andrea G. Hohmann. 2021. “Fecal Microbiota Transplantation and Antibiotic Treatment Attenuate Naloxone-Precipitated Opioid Withdrawal in Morphine-Dependent Mice.” *Experimental Neurology* 343 (September): 113787. https://doi.org/10.1016/j.expneurol.2021.113787.

Tilg, Herbert, Niv Zmora, Timon E Adolph, and Eran Elinav. 2020. “The Intestinal Microbiota Fuelling Metabolic Inflammation.” *Nature Reviews Immunology* 20 (1): 40–54.

Tindell, Amy J, Kyle S Smith, Kent C Berridge, and J Wayne Aldridge. 2009. “Dynamic Computation of Incentive Salience:‘Wanting’ What Was Never ‘Liked.’” *Journal of Neuroscience* 29 (39): 12220–28.

Touw, Ketrija, Daina L. Ringus, Nathaniel Hubert, Yunwei Wang, Vanessa A. Leone, Anuradha Nadimpalli, Betty R. Theriault, et al. 2017. “Mutual Reinforcement of Pathophysiological Host-Microbe Interactions in Intestinal Stasis Models.” *Physiological Reports* 5 (6): e13182. https://doi.org/10.14814/phy2.13182.

Townsend, E Andrew, S Stevens Negus, S Barak Caine, Morgane Thomsen, and Matthew L Banks. 2019. “Sex Differences in Opioid Reinforcement under a Fentanyl vs. Food Choice Procedure in Rats.” *Neuropsychopharmacology* 44 (12): 2022–29.

Trompette, Aurélien, Eva S Gollwitzer, Koshika Yadava, Anke K Sichelstiel, Norbert Sprenger, Catherine Ngom-Bru, Carine Blanchard, et al. 2014. “Gut Microbiota Metabolism of Dietary Fiber Influences Allergic Airway Disease and Hematopoiesis.” *Nature Medicine* 20 (2): 159–66. https://doi.org/10.1038/nm.3444.

Tsankova, Nadia, William Renthal, Arvind Kumar, and Eric J Nestler. 2007. “Epigenetic Regulation in Psychiatric Disorders.” *Nature Reviews Neuroscience* 8 (5): 355–67.

Tuesta, Luis M, Zuxin Chen, Alexander Duncan, Christie D Fowler, Masago Ishikawa, Brian R Lee, Xin-An Liu, Qun Lu, Michael Cameron, and Matthew R Hayes. 2017. “GLP-1 Acts on Habenular Avoidance Circuits to Control Nicotine Intake.” *Nature Neuroscience* 20 (5): 708–16.

United Nations Office on Drugs and Crime. 2006. *World Drug Report*. Vol. 1. Boom Koninklijke Uitgevers.

Valdes, Ana M, Jens Walter, Eran Segal, and Tim D Spector. 2018. “Role of the Gut Microbiota in Nutrition and Health.” *Bmj* 361.
Vallöf, Daniel, Paola Maccioni, Giancarlo Colombo, Minja Mandrapa, Julia Winsa Jörnulf, Emil Egecioglu, Jörgen A Engel, and Elisabet Jerlhag. 2016. “The Glucagon-like Peptide 1 Receptor Agonist Liraglutide Attenuates the Reinforcing Properties of Alcohol in Rodents.” *Addiction Biology* 21 (2): 422–37.
Van de Wouw, Marcel, Marcus Boehme, Joshua M Lyte, Niamh Wiley, Conall Strain, Orla O’Sullivan, Gerard Clarke, Catherine Stanton, Timothy G Dinan, and John F Cryan. 2018. “Short-chain Fatty Acids: Microbial Metabolites That Alleviate Stress-induced Brain–Gut Axis Alterations.” *The Journal of Physiology* 596 (20): 4923–44.
Van Nas, Atila, Debraj GuhaThakurta, Susanna S Wang, Nadir Yehya, Steve Horvath, Bin Zhang, Leslie Ingram-Drake, Gautam Chaudhuri, Eric E Schadt, and Thomas A Drake. 2009. “Elucidating the Role of Gonadal Hormones in Sexually Dimorphic Gene Coexpression Networks.” *Endocrinology* 150 (3): 1235–49.
Vincent, Caroline, Mark A Miller, Thaddeus J Edens, Sudeep Mehrotra, Ken Dewar, and Amee R Manges. 2016. “Bloom and Bust: Intestinal Microbiota Dynamics in Response to Hospital Exposures and Clostridium Difficile Colonization or Infection.” *Microbiome* 4 (1): 1–11.
Vindigni, Stephen M, and Christina M Surawicz. 2017. “Fecal Microbiota Transplantation.” *Gastroenterology Clinics* 46 (1): 171–85.
Volkow, Nora D, and Marisela Morales. 2015. “The Brain on Drugs: From Reward to Addiction.” *Cell* 162 (4): 712–25.
Volkow, Nora D, Roy A Wise, and Ruben Baler. 2017. “The Dopamine Motive System: Implications for Drug and Food Addiction.” *Nature Reviews Neuroscience* 18 (12): 741–52.
Volpe, Gretchen E, Honorine Ward, Mkaya Mwamburi, Duy Dinh, Seema Bhalchandra, Christine Wanke, and Anne V Kane. 2014. “Associations of Cocaine Use and HIV Infection with the Intestinal Microbiota, Microbial Translocation, and Inflammation.” *Journal of Studies on Alcohol and Drugs* 75 (2): 347–57.
Wade, Carrie L, Leandro F Vendruscolo, Joel E Schlosburg, Daniel O Hernandez, and George F Koob. 2015. “Compulsive-like Responding for Opioid Analgesics in Rats with Extended Access.” *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 40 (2): 421–28. https://doi.org/10.1038/npp.2014.188.
Walters William, Hyde Embriette R., Berg-Lyons Donna, Ackermann Gail, Humphrey Greg, Parada Alma, Gilbert Jack A., et al. n.d. “Improved Bacterial 16S RRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys.” *MSystems* 1 (1): e00009-15. https://doi.org/10.1128/mSystems.00009-15.
Wang, Fuyuan, Jingjing Meng, Li Zhang, Timothy Johnson, Chi Chen, and Sabita Roy. 2018. “Morphine Induces Changes in the Gut Microbiome and Metabolome in a Morphine Dependence Model.” *Scientific Reports* 8 (1): 3596–3596. https://doi.org/10.1038/s41598-018-21915-8.
Wang, Fuyuan, and Sabita Roy. 2017. “Gut Homeostasis, Microbial Dysbiosis, and Opioids.” *Toxicologic Pathology* 45 (1): 150–56.
Wohleb, Eric S, and Jean-Christophe Delpech. 2017. “Dynamic Cross-Talk between Microglia and Peripheral Monocytes Underlies Stress-Induced Neuroinflammation and Behavioral Consequences.” *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 79: 40–48.
Wong, Ma-Li, Antonio Inserra, MD Lewis, Claudio A Mastronardi, LEX Leong, Jocelyn Choo, Sandra Kentish, P Xie, M Morrison, and SL Wesselingh. 2016. “Inflammasome Signaling

Affects Anxiety-and Depressive-like Behavior and Gut Microbiome Composition." *Molecular Psychiatry* 21 (6): 797–805.
Wortelboer, Koen, Max Nieuwdorp, and Hilde Herrema. 2019. "Fecal Microbiota Transplantation beyond Clostridioides Difficile Infections." *EBioMedicine* 44: 716–29.
Xiao, Hui-wen, Chang Ge, Guo-xing Feng, Yuan Li, Dan Luo, Jia-li Dong, Hang Li, Haichao Wang, Ming Cui, and Sai-jun Fan. 2018. "Gut Microbiota Modulates Alcohol Withdrawal-Induced Anxiety in Mice." *Toxicology Letters* 287: 23–30.
Xu, Yu, Zhenrong Xie, Huawei Wang, Zongwen Shen, Youbing Guo, Yunhong Gao, Xin Chen, Qiang Wu, Xuejun Li, and Kunhua Wang. 2017. "Bacterial Diversity of Intestinal Microbiota in Patients with Substance Use Disorders Revealed by 16S RRNA Gene Deep Sequencing." *Scientific Reports* 7 (1): 1–9.
Yang, Bao-Zhu, Shizhong Han, Henry R Kranzler, Abraham A Palmer, and Joel Gelernter. 2017. "Sex-specific Linkage Scans in Opioid Dependence." *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 174 (3): 261–68.
Yang, Chun, Xi Fang, Gaofeng Zhan, Niannian Huang, Shan Li, Jiangjiang Bi, Riyue Jiang, Ling Yang, Liying Miao, and Bin Zhu. 2019. "Key Role of Gut Microbiota in Anhedonia-like Phenotype in Rodents with Neuropathic Pain." *Translational Psychiatry* 9 (1): 1–11.
Yang, Longfei, Yinghao Zhao, Yicun Wang, Lei Liu, Xingyi Zhang, Bingjin Li, and Ranji Cui. 2015. "The Effects of Psychological Stress on Depression." *Current Neuropharmacology* 13 (4): 494–504.
Yano, Jessica M, Kristie Yu, Gregory P Donaldson, Gauri G Shastri, Phoebe Ann, Liang Ma, Cathryn R Nagler, Rustem F Ismagilov, Sarkis K Mazmanian, and Elaine Y Hsiao. 2015. "Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis." *Cell* 161 (2): 264–76.
Yau, Yvonne HC, and Marc N Potenza. 2013. "Stress and Eating Behaviors." *Minerva Endocrinologica* 38 (3): 255.
Zhang, Jingyuan, Jun Yang, Cheng Yang, Ti Chen, Ziwei Wang, Junyi Li, Fanglin Qin, Qijian Deng, and Xiaojie Zhang. 2020. "Sensitivity to Morphine Reward Associates with Gut Dysbiosis in Rats with Morphine-Induced Conditioned Place Preference." *Frontiers in Psychiatry* 11: 631.
Zhang, Juanhong, Junmin Zhang, and Rong Wang. 2018. "Gut Microbiota Modulates Drug Pharmacokinetics." *Drug Metabolism Reviews* 50 (3): 357–68. https://doi.org/10.1080/03602532.2018.1497647.
Zhang, Jun, Kent C Berridge, Amy J Tindell, Kyle S Smith, and J Wayne Aldridge. 2009. "A Neural Computational Model of Incentive Salience." *PLoS Computational Biology* 5 (7): e1000437.
Zhang, Yafang, Suditi Rahematpura, Kael H. Ragnini, Amanda Moreno, Kamryn S. Stecyk, Michelle W. Kahng, Brandon T. Milliken, Matthew R. Hayes, Robert P. Doyle, and Heath D. Schmidt. 2021. "A Novel Dual Agonist of Glucagon-like Peptide-1 Receptors and Neuropeptide Y2 Receptors Attenuates Fentanyl Taking and Seeking in Male Rats." *Neuropharmacology* 192 (July): 108599. https://doi.org/10.1016/j.neuropharm.2021.108599.
Zhang, Yuan, Rongrong Huang, Mengjing Cheng, Lirui Wang, Jie Chao, Junxu Li, Peng Zheng, Peng Xie, Zhijun Zhang, and Honghong Yao. 2019. "Gut Microbiota from NLRP3-Deficient Mice Ameliorates Depressive-like Behaviors by Regulating Astrocyte Dysfunction via CircHIPK2." *Microbiome* 7 (1): 1–16.
Zhao, Qing, and Charles O Elson. 2018. "Adaptive Immune Education by Gut Microbiota Antigens." *Immunology* 154 (1): 28–37.
Zhernakova, Alexandra, Alexander Kurilshikov, Marc Jan Bonder, Ettje F Tigchelaar, Melanie Schirmer, Tommi Vatanen, Zlatan Mujagic, Arnau Vich Vila, Gwen Falony, and Sara

Vieira-Silva. 2016. “Population-Based Metagenomics Analysis Reveals Markers for Gut Microbiome Composition and Diversity.” *Science* 352 (6285): 565–69.
Zhu, Feng, Ruijin Guo, Wei Wang, Yanmei Ju, Qi Wang, Qingyan Ma, Qiang Sun, Yajuan Fan, Yuying Xie, and Zai Yang. 2020. “Transplantation of Microbiota from Drug-Free Patients with Schizophrenia Causes Schizophrenia-like Abnormal Behaviors and Dysregulated Kynurenine Metabolism in Mice.” *Molecular Psychiatry* 25 (11): 2905–18.
Zimmermann, Michael, Maria Zimmermann-Kogadeeva, Rebekka Wegmann, and Andrew L Goodman. 2019. “Mapping Human Microbiome Drug Metabolism by Gut Bacteria and Their Genes.” *Nature* 570 (7762): 462–67.
Zinöcker, Marit K, and Inge A Lindseth. 2018. “The Western Diet–Microbiome-Host Interaction and Its Role in Metabolic Disease.” *Nutrients* 10 (3): 365.