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A Review of Pre-Clinical Studies on Meth-Induced Gut Microbiome Alterations

Payton Brabant
Western Michigan University

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Payton Brabant

Department of Psychology, Western Michigan University

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Dr. Lisa Baker

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` Substance use disorder (SUD) is a chronic, pervasive disease that affects approximately 40.3 million people aged 12 and over within the United States (SAMHSA, 2021). Recent investigations have emphasized preventive measures targeting the development of SUDs and the occurrence of relapse. However, to date, FDA-approved medications are only available for those with alcohol (AUD) and opioid use disorders (OUD) (Forouzan et al., 2021). Given that about 964,000 people have methamphetamine use disorder (MUD) within the United States, investigation of novel treatment approaches for psychostimulant abuse is warranted (Forouzan, Hoffman, and Kosten, 2020). Research investigations have increased over the past decade regarding the involvement of the gut-brain axis and the gut microbiota in central nervous system disorders, including SUD (Salavrakos et al., 2020). The gut microbiota houses the ensemble of microorganisms such as bacteria, viruses, fungi, and yeasts that live within the gastrointestinal tract (Salavrakos et al., 2020). The gut microbiome refers to the total genetic material of bacteria in the digestive tract. The gut microbiome has between 100-1,000 different identified species (Salavrakos et al., 2020). It is difficult to determine what is a “normal” gut microbiome composition since it can be impacted by genetics, mode of delivery at birth, race, diet, and environment (Salavrakos et al., 2020). Alterations in the gut microbiome have been reported in those with AUD and OUD (Salavrakos et al., 2020). This is a cause of concern given that several channels in the gut microbiome communicate with the brain. The most recognized gut-brain interactions are the immune response, metabolic pathway, neuroendocrine pathway, and vagus nerve which can all be affected by alterations within the gut microbiome (Salavrakos et al., 2020). Several studies have demonstrated evidence for gut dysbiosis in AUD and OUD

populations. Fewer studies have examined the microbiome alterations associated with methamphetamine use.

MUD and Affective Disorders

MUD withdrawal is characterized by drug-cravings, physical discomfort, anxiety, stress, and depression (Forouzan et al., 2021). Depression and anxiety disorders are reported in about 30.2% of people with MUD (Forouzan et al., 2021). Since the withdrawal phase includes symptoms that increase the desire to relapse, treatments that attenuate these symptoms are necessary (Forouzan et al., 2021). Preclinical studies utilizing objective behavioral measures are essential to research and discovery of novel treatments for relapse prevention. Furthermore, animal models are invaluable to the assessment of therapies to attenuate withdrawal symptoms. Methamphetamine withdrawal symptoms such as anxiety, stress, and depression are also present within affective disorders. Additionally, both SUD and affective disorders can include cognitive dysfunctions, such problems with decision-making and impulsivity (Forouzan et al., 2021). Given these similarities in symptoms and the comorbidity of affective disorders and SUD, Forouzan et al. (2021) proposed that they share commonalities within the gut-microbiome. Alterations in gut microbiome composition have been observed in depressed patients (Salavrakos et al., 2020; Zhang et al., 2022). Moreover, this behavioral phenotype has been transferred to mice through a fecal transplant (Zhang et al., 2022). This suggests that alterations in the gut microbiome can influence cognition, emotions, and behaviors. These findings have allowed researchers to examine the relationship between SUD and the gut microbiome as well as propose treatments to combat relapse by targeting the gut microbiome with probiotic interventions (Zhang et al., 2022).

Gut Microbiota Dysbiosis and Probiotics

Dysbiosis is described as disturbances in homeostasis of the microbiota and has been implicated in Parkinson's disease, Alzheimer's disease, autism, anxiety, depression, and SUDs (Forouzan, Hoffman, and Kosten, 2020). The research literature has described a relationship between intestinal inflammation and alcohol-induced gut dysbiosis (Forouzan, Hoffman, and Kosten, 2020). Therefore, it has been hypothesized that probiotics may attenuate gut dysbiosis, as some probiotic products have been used to treat irritable bowel syndrome (IBS) and have reduced both inflammation and depression/anxiety symptoms (Wang et al., 2016). Probiotics also have been reported to affect the HPA-axis stress responses by decreasing corticosteroid levels (Wang et al., 2016). Additionally, probiotics have been shown to affect levels of neurotransmitters such as serotonin, aminobutyric acid (GABA), and dopamine thus influencing cognition and behavior (Wang et al., 2016). In a literature review by Wang et al. (2016), several animal studies reported that probiotic formulations improved behavioral indices of anxiety and depression. These findings suggest that the cognitive and behavioral symptoms of methamphetamine-induced gut dysbiosis may also be attenuated by probiotic formulations.

Methods

The studies included within this review were obtained through Western Michigan University's library databases. This review contains five preclinical studies and three literature reviews. The inclusion criteria were that the preclinical studies utilized behavioral tests predictive of depression and anxiety. Additionally, the studies reviewed addressed gut microbiota dysbiosis and examined the effects of methamphetamine on gut microbiome composition.

Results

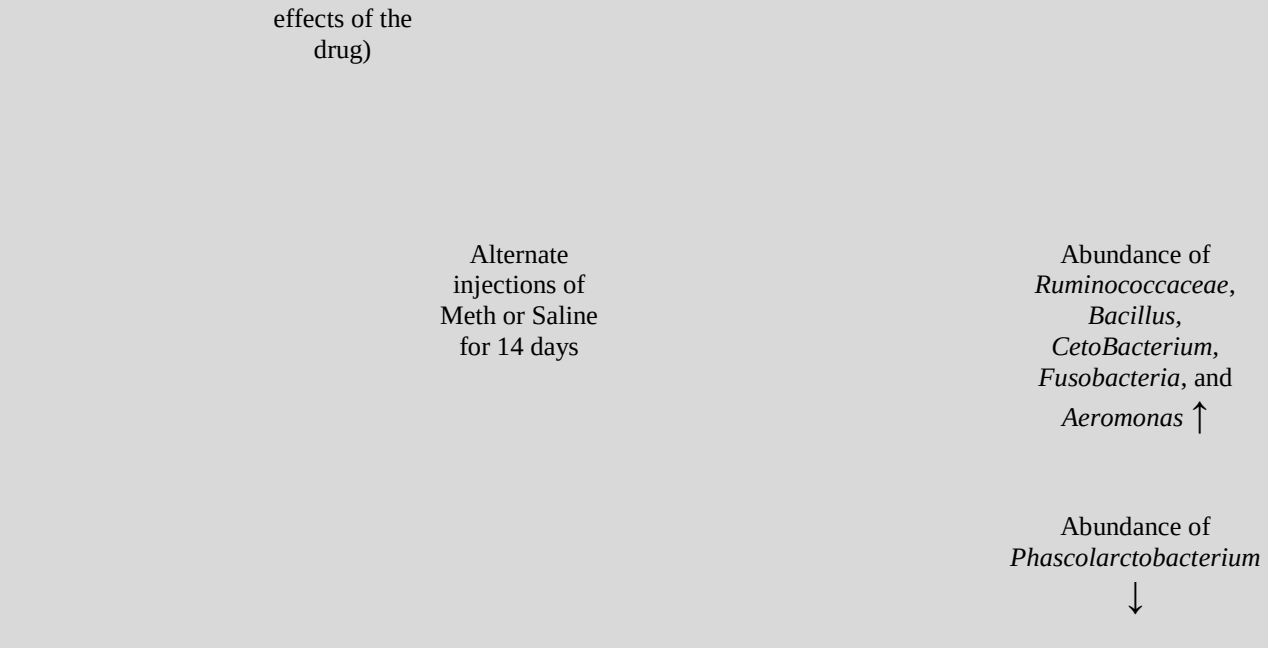
All five studies included in this review were conducted with rodents. Three studies used male Sprague-Dawley rats (Forouzan, Hoffman, and Kosten, 2020; Ning et al., 2017; Yang et al., 2020) whereas the other two used different types of mice (Chen et al., 2021; Zhang et al. 2022). All of these studies assessed the effects of methamphetamine on gut dysbiosis but varied in dose and dosing regimen. Chen et al. (2021) treated the methamphetamine group with one injection per day for 6 days as follows 1.5, 4.5, and 7.5mg/kg. The last two days of the study they exposed the mice to a binge dose involving four successive sessions of 10mg/kg at two-hour intervals. Conversely, Forouzan, Hoffman, and Kosten (2020) treated the methamphetamine group with 2mg/kg injections for 14 days then conducted behavioral interventions post-cessation of methamphetamine. Zang et al. (2022) also treated the methamphetamine group with 2mg/kg injections, but for only 6 days. Finally, Yang et al. (2020) treated the methamphetamine group with alternative injections of methamphetamine (2mg/kg), or saline for 9 days as did Ning et al. (2017) but with injections of 1mg/kg and for 14 days. Since these studies have similar methods, conclusions on methamphetamine-induced gut microbiome alterations may be made. A summary of these outcomes can be found in Table 1.

Table 1: *Study Outcomes*

Study	Animal	Behavioral Assays	Meth (dosage, duration)	Behavioral Outcomes	Neurotoxicity	Gut Microbiome Alterations
Zhang et al. (2022)	Wild Type C57BL/6 male mice (8-10 weeks)	Locomotor Sensitization (open field test)	2mg/kg - 24hr Intervals	Locomotor ↑ Sensitization	DAT, TH, and MAOA ↑	Microbial Diversity ↓

		Light-Dark Test (measuring anxiety)	6-days	Anxiety-Like Behaviors ↑	Beclin1, Atg5, and LC3-II proteins ↑	*Bacteroidetes*, A*ctinobacteria, and unclassified K_norank* ↑
		Tail Suspension Test (measuring depression)		Immobility ↑	Apoptosis related proteins (p53, Bax, caspase 3, and cleaved caspase 3) ↑	*Firmicutes, Proteobacteria, Tenericutes, and Deferribacteres* ↓
		Forced Swim Test (measuring depression)		Immobility ↑		Expression of TLR4, MyDD88, NF-kB, and NLRP3 ↑
Forouzan, Hoffman, and Kosten (2020)	16 Sprague-Dawley male rats (60-90 days old)	Open Field Test (measuring anxiety)	2mg/kg (twice daily)	Anxiety-like Behaviors ↑	N/a	*Actinobacteria* on meth day 7, 14, and 24h post cessation ↑
		Elevated Plus Maze (measuring anxiety)	14 days of meth treatment	No significant difference between groups was found		*Allobaculum*, B*ifidobacterium*, and L*actobacillus* ↑ (abundance of these genera returned to baseline after 7 days of cessation)
		Forced Swim Test (measuring depression)	Behavioral Assays conducted initially with Vehicle and then during cessation from meth	Immobility ↑		Significant microbial community differences were observed on meth day 7, 15, and 24h post cessation of meth. These differences dissipated by 96h post Meth cessation
Chen et al. (2021)	14 BALB/c male mice (6-8 weeks old)	N/a	One injection per day for 6 days as follows; 1.5, 4.5, and 7.5	N/a	MAOA ↑	Inflammation factors in the colonic mucosa (overexpression of TLR4, MyDD88 and Nf-kB proteins) ↑

			On days 7-8, mice exposed to binge dose involving four successive injections of 10.0mg/kg at two-hour intervals for two successive days		TH ↓	Microbial Diversity ↑
					Beclin1, Agt5, and LC3II proteins ↑	*Bacteroidetes* and F*irmicutes* ↓
						Proteobacteria and A*ctinobacteria* ↑
						Relative abundance of F*usobacteriaceae*, L*actobacillacea*, and P*revotellaceae* ↓
Yang et al. (2020)	22 Sprague-Dawley male rats	Conditioned Place Preference Assessment (measuring the rewarding effects of the drug)	2mg/kg (one injection per day)	Preference for Meth- paired chamber ↑	N/a	Gut dysbiosis in rats pre- treated with antibiotics ↑
			Alternate injections of Meth or Saline for 9 days			Abundance of *Akkermansia, Allobaculum, and Olsenella* ↑
			Pre-treated with antibiotics			Abundance of *Acetivibrio* ↓
						Abundance of *Butyricimonas* ↑
Ning et al. (2017)	16 Sprague-Dawley male rats	Conditioned Place Preference Assessment (measuring the rewarding	1mg/kg (one injection per day)	Preference for Meth- paired chamber ↑	N/a	Microbial Diversity ↑



Methods of Analysis

Each study utilized similar methods of data analysis. All five studies collected fecal samples and conducted 16s rRNA sequencing to determine the fecal microbiome composition. Additionally, they all reported Simpson and Shannon indices to draw conclusions about microbial diversity (Chen et al., 2021; Forouzan, Hoffman, and Kosten, 2020; Ning et al., 2017; Yang et al., 2020; Zhang et al., 2022). For the behavioral assays, the investigators performed ANOVAs and applicable t-tests to compare experimental and control groups (Chen et al., 2021; Forouzan, Hoffman, and Kosten, 2020; Ning et al., 2017; Yang et al., 2020; Zhang et al., 2022). Chen et al. (2021). Only two studies measured neurotoxicity which was achieved by Western Blot Analysis of enzymes and transporter proteins in the striatum (Chen et al., 2021; Zhang et al., 2022). The proteins measured included monoamine oxidase (MAOA) and tyrosine hydroxylase (TH). Due to the similarities in analysis, these studies show some replication.

Behavioral Assays and Outcomes

Of the five studies reviewed, only one did not conduct behavioral assays. However, the others conducted similar behavioral assays. For example, both Yang et al. (2020) and Ning et al. (2017) used conditioned place preference assessments (CPP) to measure the conditioned rewarding effects of methamphetamine. After seven sessions of methamphetamine treatment, significant preference for the methamphetamine-paired chamber was established. The rats spent about six out of 15 minutes of their time in the methamphetamine-paired chamber (Ning et al., 2017). Yang et al. (2020) found similar results after only five sessions of methamphetamine treatment. After five sessions of methamphetamine treatment, the methamphetamine group showed a four-fold higher preference for the methamphetamine-paired chamber compared with the saline group. Eight out of the 17 methamphetamine treatment rats were labeled as high-CPP group and the other eight were labeled as the low-CPP group (median rat excluded). The CPP scores for the low group showed no significant difference when compared to the saline group. Additionally, some of the rats were pre-treated with antibiotics and methamphetamine to determine whether antibiotics further induced dysbiosis. These rats presented significantly higher CPP than those pre-treated with water. The established preference for the methamphetamine-paired chamber suggests that methamphetamine induces drug-seeking behaviors due to the drug's actions on brain reward pathways. Thus, it is important to understand how to attenuate these drug-seeking behaviors.

The other two studies in this review focused on methamphetamine-induced anxiety and depression-like behaviors as well as locomotor sensitization (Forouzan, Hoffman, and Kosten, 2020; Zhang et al., 2022). Forouzan, Hoffman, and Kosten (2020) used an open-field test and an elevated plus maze to measure anxiety, and the forced swim test to measure depression. In the open-field test, a decrease in total distance traveled and time spent in the open chamber are

measures of anxiety-like behavior. The results showed that the methamphetamine-treated group exhibited a significant decrease in distance traveled. The methamphetamine-treated group spent greater time in the open chamber on saline treatment day 6 than they did 48 h after cessation from methamphetamine (Forouzan, Hoffman, and Kosten, 2020). The elevated plus maze was used to determine whether anxiety-like behaviors were induced from methamphetamine cessation. Anxiety-like behaviors within this assay are characterized by greater time spent in closed arms. In this assay, no differences were found between the methamphetamine and saline-treated groups. In the forced swim test, increased immobility is an indicator of depression-like behaviors. Forouzan, Hoffman, and Kosten (2020) found an increase in immobility after methamphetamine cessation. Similarly, Zhang et al., (2022) used the forced swim test to measure depression as well as the tail suspension test. They also used the light-dark test to measure anxiety and the open field test to measure locomotor sensitization. In the forced swim test and tail suspension test, the methamphetamine group had a significantly higher immobility time than the saline group suggesting methamphetamine-induced depression-like behavior. In the open field test, they found an increase in locomotor sensitization. Finally, in the light-dark test, the methamphetamine-treated mice spent less time in the light compartment suggesting induced anxiety (Zhang et al., 2022). These findings support the theory that methamphetamine induces anxiety and depression-like behaviors as seen in affective disorders.

Neurotoxicity

Only two out of the five studies examined methamphetamine-induced neurotoxicity along with gut dysbiosis. Chen et al. (2020) found through the Western Blot Analysis that methamphetamine significantly elevated MAOA expression and decreased the expression of the enzyme (TH), which reflects dopamine terminal toxicity. They also observed up-regulated

autophagy related proteins such as Beclin1, Agt5, and LC3II in comparison to the saline group. Autophagy related proteins are essential to maintaining cellular homeostasis and growth. They also play a key role in cell death/survival (Chen et al., 2020). These upregulated autophagy proteins caused by methamphetamine treatment can alter the dopaminergic system in a negative way. Zhang et al. (2022) found very similar results with the same mode of analysis. They found the methamphetamine group had an overexpression of DAT, TH, and MAOA. Additionally, they also found an overexpression of Beclin1, Atg5, and LC3II proteins and apoptosis related proteins. Apoptosis is a form of programmed cell death, so it is concerning that methamphetamine induces an overregulation of these proteins. While both studies had similar outcomes, Chen et al. (2020) found that TH was downregulated. Therefore, there is a need for further studies to examine methamphetamine-induced neurotoxicity to better understand the role of the gut-brain axis.

Gut Microbiome Alterations

All five of the studies examined methamphetamine-induced gut microbiome alterations. Ning et al. (2017) reported a greater microbial diversity in the methamphetamine-treated group than the control group. This result was surprising as greater diversity is assumed to be beneficial. However, the influence of gut bacterial diversity on the central nervous system is not yet fully understood. They also found a higher abundance of *Ruminococcacea* (related to anxiety), *Bacillus*, *CetoBacterium, Fusobacteria,* and *Aeromonas* in the methamphetamine-treated group compared to the control group (Ning et al., 2017). Moreover, the abundance of *Phascolarctobacterium,* which has been characterized by producing propionate, was lower in the methamphetamine group than the saline group. These results are consistent with the idea that methamphetamine induces dysbiosis as propionate has been assumed to have health-promoting

benefits (Ning et al., 2017). In the Yang et al. (2020) study, some rats were pre-treated with antibiotics to see if that would further affect methamphetamine-induced dysbiosis. They also conducted correlational statistics to determine the relationship between abundance of phyla in the gut bacteria and CPP scores. The rats that received the antibiotics and methamphetamine had higher gut dysbiosis than the rats that were only treated with methamphetamine (Yang et al., 2020). Additionally, the methamphetamine-treated group's CPP scores correlated with the increase in abundance of bacteria in the gut (Yang et al., 2020). This suggests that pro-biotics could help attenuate these effects. The abundance of *Akkermansia*, *Allobaculum*, *Osenella*, and *Butyricimonas* were higher in the methamphetamine group whereas *Acetivibrio* had higher abundance in the saline group (Yang et al., 2020). Higher levels of *Akkermansia* and *Allobaculum* have also been observed in alcohol-treated mice indicating substances do alter the gut microbiome (Yang et al., 2020). Forouzan, Hoffman, and Kosten (2020) described similar results, however, these changes in the microbial composition returned to baseline after 7 days of cessation for methamphetamine. *Actinobacteria* had higher levels of abundance on methamphetamine treatment day 7, 14, and 24h post cessation. *Allobaculum*, *Bifidobacterium*, and *Lactobacillus* were also discovered to be higher in abundance. The microbial community differences observed on methamphetamine treatment day 7 dissipated by 96 h post cessation (Forouzan, Hoffman, and Kosten, 2020). The temporary changes in microbial composition may imply a window of time that methamphetamine-induced gut dysbiosis can be reversed (Forouzan, Hoffman, and Kosten 2020). Chen et al. (2021) found multiple alterations within the gut microbiome due to the escalating doses that were higher than those used in the other studies. Like Ning et al. (2017), they discovered that microbial diversity increased following methamphetamine treatment. They also saw an overexpression of MyDD88 and Nf-kB proteins

involved in inflammation. Proteobacteria and actinobacteria were higher in abundance whereas *Bacteriodetes, Firmicutes, Fusobacteriaceae, Lactobacillacea,* and *Prevotellaceae* were lower in abundance (Chen et al., 2021). *Fusobacteriaceae, Lactobacillacea, Prevotellaceae,* and *Alloprevotella* have been known to produce short-chain fatty acids which are important for maintaining the balance in the gastrointestinal tract (Chen et al., 2021). Thus, a decrease in these bacteria is consistent with the idea that methamphetamine-exposure induces dysbiosis. Finally, Zhang et al. (2022) produced similar results. The overexpression of inflammatory proteins was found along with a higher abundance of *Bacteriodetes*, *Actinobacteria*, and *unclassified K_norank*. *Firmicutes*, *Proteobacteria*, *Tenericutes*, and *Deferribacteres* were lower in abundance (Zhang et al. 2022). They also found that microbial diversity was decreased which shows the differences in results and the need for further replication of these studies.

Discussion

Given the results of the studies addressed within this review, methamphetamine can produce gut dysbiosis and subsequent neurotoxicity that may be associated with cognitive and behavioral symptoms related to relapse risk. All of the preclinical studies reported increases in anxiety and depressive-like behavior following methamphetamine treatment. Moreover, they all reported methamphetamine-induced changes in the gut microbial composition. These findings implicate the attenuation of gut dysbiosis as a possible treatment strategy for MUD. Future studies are warranted to identify a form of treatment to attenuate behavioral symptoms such as impulsivity, anxiety, and depression as well as inflammation in the gut. Since probiotics have been shown to decrease inflammation and anxiety/depression in patients with IBS, probiotics could curb the negative effects of MUD such as anxiety, depression, impulsivity (Wang et. Al, 2016). Impulsivity contributes to relapse which suggests that strategies to reduce impulsivity

may assist with relapse prevention. Future studies should evaluate the effects of methamphetamine on impulsivity as well as examine whether probiotics can attenuate these effects. Moreover, more research should be conducted on the health-related effects of microbial diversity. Despite the need for further preclinical and clinical research in this area, it can be difficult to translate animal research to humans. Several ethical concerns may contribute to the difficulty in translating the research such as inducing anxiety and depression for behavioral tests. Additionally, there have been debates over whether the behavioral assays truly measure anxiety and depression-like behaviors (Wang et al., 2016). The use of an accurate assessment of relevant behavioral outcomes is essential to measure the effects of methamphetamine-induced gut dysbiosis and the possible effects of probiotics on dysbiosis.

Conclusion

Overall, the studies reviewed have shown a significant relationship between exposure to methamphetamine and gut dysbiosis as well as anxiety and depression-like behaviors in preclinical behavioral assessments. Based on these preclinical findings, future research is warranted to evaluate probiotic treatments with human SUD populations. The research methods must be translated to human populations such as developing behavioral assays that give a more accurate view of one's emotional symptoms than self-report questionaries. Additionally, more preclinical studies should be conducted to determine the functions of the gut-brain axis to further understand the role of probiotics. Finally, longer treatment periods should be considered to determine the window of time that gut dysbiosis can be attenuated by probiotics.

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