Effect of oral tryptamines on the gut microbiome of rats - a preliminary study (#93535)

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Effect of oral tryptamines on the gut microbiome of rats - a preliminary study

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Background. Psilocybin and related tryptamines have come into the spotlight in recent years as potential therapeutics for depression. Research on the mechanisms of these effects has historically focused on the direct effects of these drugs on neural processes. However, in addition to such neural effects, alterations in peripheral physiology may also contribute to their therapeutic effects. In particular, substantial support exists for a gut microbiome-mediated pathway for the antidepressant efficacy of other drug classes, but no prior studies have determined the effects of tryptamines on microbiota. Methods. To address this gap, in this preliminary study, male Long Evans rats were treated with varying dosages of oral psilocybin, norbaeocystin, or vehicle and their fecal samples were collected 1 week and 3 weeks after exposure for microbiome analysis using integrated 16S ribosomal DNA sequencing to determine gut microbiome composition. Results. We found that although treatment with either psilocybin or norbaeocystin did not significantly affect overall microbiome diversity, it did cause significant dose- and time- dependent changes in bacterial abundance at the phylum level, including increases in *Verrucomicrobia* and *Actinobacteria*, and decreases in *Proteobacteria*. Conclusion and Implications: These preliminary findings support the idea that psilocybin and other tryptamines may act on the gut microbiome in a dose- and time-dependent manner, potentially identifying a novel peripheral mechanism for their antidepressant activity. The results from this preliminary study also suggest that norbaeocystin may warrant further investigation as a potential antidepressant, given the similarity of its effects to psilocybin.

Effect of Oral Tryptamines on the Gut Microbiome of Rats - A Preliminary Study

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Abstract

Background. Psilocybin and related tryptamines have come into the spotlight in recent years as potential therapeutics for depression. Research on the mechanisms of these effects has historically focused on the direct effects of these drugs on neural processes. However, in addition to such neural effects, alterations in peripheral physiology may also contribute to their therapeutic effects. In particular, substantial support exists for a gut microbiome-mediated pathway for the antidepressant efficacy of other drug classes, but no prior studies have determined the effects of tryptamines on microbiota. **Methods.** To address this gap, in this preliminary study, male Long Evans rats were treated with varying dosages of oral psilocybin, norbaeocystin, or vehicle and their fecal samples were collected 1 week and 3 weeks after exposure for microbiome analysis using integrated 16S ribosomal DNA sequencing to determine gut microbiome composition. **Results.** We found that although treatment with either psilocybin or norbaeocystin did not significantly affect overall microbiome diversity, it did cause significant dose- and time- dependent changes in bacterial abundance at the phylum level, including increases in *Verrucomicrobia* and *Actinobacteria*, and decreases in *Proteobacteria*. **Conclusion and Implications:** These preliminary findings support the idea that psilocybin and other tryptamines may act on the gut microbiome in a dose- and time-dependent manner, potentially identifying a novel peripheral mechanism for their antidepressant activity. The results from this preliminary study also suggest that norbaeocystin may warrant further investigation as a potential antidepressant, given the similarity of its effects to psilocybin.

Keywords Psilocybin; Norbaeocystin; Gut microbiome; *Proteobacteria*; *Verrucomicrobia*; *Actinobacteria*; Rat

Introduction

In addition to its hallucinogenic properties, psilocybin has gained recent interest as a potential fast-acting treatment for depression (Nichols 2004). A growing number of clinical studies have shown that when paired with talk therapy, a single dose of the tryptamine can have strong and persistent effects that are equal to or greater than traditional antidepressants (Becker et al. 2022; Carhart-Harris et al. 2021; Carhart-Harris et al. 2018; Davis et al. 2021; Goodwin et al. 2022; Goodwin et al. 2023; Griffiths et al. 2016; Gukasyan et al. 2022). The mechanisms of these effects have not yet been fully understood. While previous findings have implied that psychedelic and hallucinogenic properties may be independent to antidepressive properties and due to a mixture of actions on excitatory and inhibitory neuronal circuits, substantial evidence points to psilocybin's activation of 5-HT_{2A} receptors as a likely mechanism (De Gregorio et al. 2021; González-Maeso et al. 2007; Presti & Nichols 2004). In animal models, co-treatment with ketanserin, a 5-HT_{2A} antagonist, has been shown to block both the psychedelic effects of psilocybin and its therapeutic efficacy (Slocum et al. 2022). Additionally, animal models have shown that selective activation of 5-HT_{2A} receptors by other compounds can recapitulate many of the psychedelic effects of psilocybin (Hanks & González-Maeso 2013). This mechanism differs from conventional antidepressants such as selective serotonin reuptake inhibitors (SSRIs) that block serotonin transporters and elevate serotonin levels in neuronal synapses. Despite acute increases in serotonin levels, phenotypic depressive symptoms are usually not relieved until 4-6 weeks later (Stahl 2021).

Much of the research investigating the mechanisms of psilocybin and other antidepressants has focused on their interaction with central nervous system processes. However, a growing body of research has pointed to alternative mechanisms for antidepressant action, through peripheral processes. Specifically, substantial research has shown that modulation of gut processes such as gut motility, permeability, and gut microbiome composition may be important contributors to the antidepressant efficacy of serotonin modulating compounds. Serotonin receptors, including 5-HT_{2A}, are widely distributed throughout the gut and peripheral tissues (Mawe & Hoffman 2013) and the serotonin produced in the gut accounts for more than 60% of peripheral serotonin in mice and more than 95% in humans (Yano et al. 2015). Enteric serotonin is predominantly secreted by enterochromaffin cells that line the gut. Thus, psychedelic drugs have strong potential to influence enteric processes.

In addition to enteric factors, there is also substantial evidence that the gut microbiome has bi-directional effects on a variety of psychological disorders through a combination of neural, endocrine, and metabolic signals of the gut-brain axis (Burokas et al. 2015; Carabotti et al. 2015). The gut microbiome refers to the diverse array of microscopic organisms that exist in the gastrointestinal tract (i.e., microbiota) and their genomes. These microorganisms collectively contain a number of genes 150 times greater than that of the human genome (Weinstock 2012). Gut bacteria such as *Bacilli*, *Bifidobacterium*, *Candida*, *Enterococcus*, *Escherichia coli* (*E. coli*), *Lactobacillus*, *Streptococcus*, and *Serratia* secrete serotonin, acetylcholine, dopamine, gamma-aminobutyric acid, glycine, and catecholamine (Yano et al. 2015), which can promote serotonin production and release within the lining of the colon, affecting gut motility and permeability. Thus,

gut microbes secrete a large array of neurotransmitters, as well as other neuroactive molecules that regulate many complex cognitive processes, from mood and memory to learning and cognition (Yano et al. 2015). Both preclinical and clinical studies have observed that gut microbiota affect the symptoms of mood disorders (Cruz-Pereira et al. 2020). Most prominent are studies using germ-free rodents, which have found that, when compared with specific pathogen free (SPF) counterparts free of certain infectious pathogens but not completely free of all microbes, germ-free rats develop anxiety-like behavior and germ-free mice develop exaggerated stress responses (Crumeyrolle-Arias et al. 2014; Sudo et al. 2004). These changes have been shown to be reversible upon recolonization of the gut through dietary probiotics (Wallace & Milev 2017). These studies provide a basis for the idea that altering the gut microbiome could be an alternative therapeutic strategy to treat mood disorders. The exact relationships between bacterial populations and host remain relatively unknown due to the complexity of the gut-brain axis.

Recent work on the importance of the gut microbiome suggests a need for more understanding of how therapeutics alter these microbe populations, potentially modulating a vast array of signaling and metabolic pathways. Prior studies of treatment of various traditional antidepressants have found inconsistent changes of gut microbiota diversity, richness, and composition (Donoso et al. 2023). Additionally, a recent study on ketamine has reported dose-dependent relationships between drug treatment and shifts in gut microbe compositions in relatively short time periods (Getachew et al. 2018). Specifically, 7 days after a single ketamine treatment, some bacteria have an over 90-fold increase in abundance at the family level (Getachew et al. 2018). Combined, these studies suggest that psilocybin may also, in part, exert its antidepressant effects through similar mechanisms. This is particularly likely, since 5-HT_{2A} receptors are an essential component of the gut-brain axis (Fiorica-Howells et al. 2002). Although gut microbiome has been proposed as a potential mechanism that psychedelics act upon (Kelly et al. 2023; Kuypers 2019), no prior studies have investigated the effects of any psychedelics on gut microbe populations, however. Therefore, the primary objective of this study was to determine if psilocybin dose-dependently modulates gut bacterial composition. To accomplish this, animals were treated orally with varying dosages of psilocybin, vehicle, or norbaeocystin (a psilocybin precursor). Norbaeocystin is structurally similar to psilocybin and is also found in *Psilocybe* mushrooms. Prior studies have shown it does not cause head twitch behaviors in rats (Adams et al. 2022), a proxy for 5-HT_{2A} activation and possibly hallucinations. Thus, by comparing psilocybin's effects to those of norbaeocystin, findings could contribute to the understanding of the role of 5-HT_{2A} receptors in psilocybin's effects on the gut microbiome. Additionally, should norbaeocystin cause similar effects on the gut microbiome, this might suggest it also possesses therapeutic potential.

Materials & Methods

Production of psilocybin and precursors from E. coli

Psilocybin and norbaeocystin-containing cell broths, acquired from Dr. J. Andrew Jones' lab at Miami University, were produced using a genetically modified *E. coli* biosynthetic

production pathway (Adams et al. 2022; Adams et al. 2019). Concentrations of target metabolites in filtered cell broths were analyzed using HPLC. HPLC results indicated that the psilocybin containing broths had high levels of psilocybin (approx. 1 g/L), trace levels of norbaeocystin (<20 mg/L) and aeruginascin (<1 mg/L), and low levels of baeocystin (approx. 150 mg/L); while the norbaeocystin containing broths had high levels of norbaeocystin (approx. 1.5 g/L) only, with no baeocystin, psilocybin, or aeruginascin due to the lack of the methyltransferase responsible for the synthesis of the latter metabolites. The cell culture media broth contained none of the aforementioned metabolites.

Animals, housing condition, treatment, and fecal collection

Thirty-nine adult male Long Evans rats (90-120 days of age) were bred in-house. Rats were housed individually in standard cages with water and food provided *ad libitum* and were kept on a 12hr:12hr light/dark cycle (lights on 0700) throughout the study. All the rats were SPF animals, as the cage bedding and materials were tested periodically for certain designated pathogens to ensure safety of researchers and research quality.

Rats were randomly assigned to one of 5 groups, which received oral gavage of low (0.2 mg/kg) or high (2 mg/kg) dosages of psilocybin, low (0.25 mg/kg) or high (2.52 mg/kg) dosages of norbaeocystin, or an equivalent volumetric amount of cell culture media broth as a negative control, with the order of administration randomized. As published previously, “low” dosages show no observable head twitch responses and “high” dosages cause observable head twitches in male Long Evans rats (Adams et al. 2022).

Fresh fecal samples were collected in the morning, in the order of defecation, but was random among groups. Collections occurred from 19 rats one week after treatment (control: n = 7; low dosage psilocybin: n = 3; high dosage psilocybin: n = 2; low dosage norbaeocystin: n = 5; and high dosage norbaeocystin: n = 2) and from 20 rats 3 weeks after treatment (control: n = 8; low dosage psilocybin: n = 3; high dosage psilocybin: n = 4; low dosage norbaeocystin: n = 3; and high dosage norbaeocystin: n = 2). All the collected fecal samples were snap frozen immediately and stored at -80 ºC until processing. Each fecal sample was provided with a unique sample identification number. Thus, although researchers were aware of group allocation during drug administration and fecal sample collection, they were unaware of group allocation when samples were processed for gut microbiome analysis using 16S rDNA sequencing (see below for details).

All experimental interventions, including oral gavage and fresh fecal sample collection, were carried out in conscious rats without any anesthesia by experienced researchers. No rats showed any sign of distress throughout the study; thus, no analgesia was given. Consequently, all rats were included in this study and their results were reported. Criteria were established for euthanizing animals prior to the planned end of the experiment, but this was not needed. At the conclusion of the experiment, rats were euthanized with one IP injection of Euthasol (200 mg/kg body weight; a sodium pentobarbital-based drug). These euthanasia methods comply with AVMA standards. The research question, groups, fecal sample collection, and gut microbiome analysis using 16S rDNA sequencing were discussed before the study among involved researchers. All procedures were approved by Miami University's Institutional Animal Care and Use Committee (IACUC Project Number: 1033_2023_Apr).

16S rDNA sequencing and analysis

Genomic DNA was isolated and purified from the fecal samples via a commercialized kit (MP bio fast DNA spin kit for feces, Santa Ana, CA, USA), and underwent PCR amplification of the 16S ribosomal DNA (rDNA) V4 region using the 515f/806r primer set (Earth Microbiome Project; http://www.earthmicrobiome.org/). Gel electrophoresis was then used to check the quality and size of PCR products. Amplified 16S rDNA samples were purified by the SequalPrep Normalization Plate kit (Thermo Fisher, Waltham, MA, USA). Purified products were quantified by KAPA Library Quantification Kit Illumina Platforms (Kapa Biosystems, Wilmington, MA, USA), and used the Illumina Next Generation Sequencing MiSeq platform for amplicon sequencing at Miami University's Center for Bioinformatics and Functional Genomics for 16S rDNA sequencing based on our established protocol (Xu et al. 2020a). The project is registered with the BioProject database (BioProjectID: PRJNA1054120). Raw sequencing data are accessible via http://www.ncbi.nlm.nih.gov/bioproject/1054120. Raw sequencing data were processed and cleaned. The sequencing reads of all samples were assigned using the corresponding barcode sequences of their primers. Quality-filtering of reads was done using default parameters within QIIME to improve diversity estimates (Xu et al. 2020b).

The diversity analysis indicated by Shannon diversity index was done utilizing QIIME 2 for differentiation of bacteria at the phylum level (Bolyen et al. 2019). QIIME 2 is capable of analyzing Mi-Seq data with two or more biological replicates and tends to be conservative in revealing statistical significance (Bolyen et al. 2019), and has been used to analyze studies with sample sizes of 1 and 2 (McKenzie et al. 2017). The number and relative abundance of bacterial species were analyzed. Shannon diversity index considers the number of species indicating richness and their relative abundance indicating evenness, thus estimates the diversity of species in a community. The relative abundance refers to the percentage of one microbial phylum in relation to the total number of phyla in the community, which was analyzed to indicate the population size of specific phyla and their commonalities with other phyla in the fecal samples.

Statistical analysis

Significant differences in diversity at the phylum level and relative abundance of different bacterial populations between groups were determined by a two-way analysis of variance (ANOVA) followed by a standard two-stage linear step-up method of Benjamini, Krieger and Yekutieli post hoc multiple comparisons analysis (GraphPad™ Prism 10, San Diego, CA, USA). This statistic method assumes that test statistics are independent, and controls the false discovery rate. It first examines the distribution of P values to estimate the fraction of the null hypotheses that are actually true. It then uses this information to get more power when deciding when a P value is low enough to be called a discovery (Benjamini et al. 2006). $P < 0.05$ was considered statistically significant.

Results

Effects of psilocybin and norbaeocystin on microbial diversity

Microbial diversity measured by Shannon diversity index and analyzed by a two-way ANOVA (treatment × time) did not reveal any significant differences by psilocybin treatments at low or high dosage [$F_{(2, 21)} = 0.7496$; $p = 0.4848$], timepoints [$F_{(1, 21)} = 0.01407$; $p = 0.9067$], or interaction [$F_{(2, 21)} = 0.3673$; $p = 0.6970$] (Figure 1A). Similarly, microbial diversity was not significantly different by norbaeocystin treatment at low or high dosage [$F_{(2, 21)} = 0.3363$; $p = 0.7182$], timepoints [$F_{(1, 21)} = 0.05253$; $p = 0.8209$], or interaction [$F_{(2, 21)} = 0.5505$; $p = 0.5848$] (Figure 1B). Therefore, neither psilocybin nor norbaeocystin at either dose resulted in any significant change in microbial diversity compared to the vehicle control (Figure 1).

Effects of psilocybin and norbaeocystin on microbial abundance of dominant phyla

The populations of two major gut microbiota phyla, *Firmicutes* and *Bacteroidetes* representing ~80% of gut microbiota (Arumugam et al. 2011; Ley et al. 2008), were analyzed.

Firmicutes abundance was analyzed by a two-way ANOVA (treatment × time), revealing a significant interaction between psilocybin dose and time [($F_{(2, 21)} = 3.797$; $p = 0.0391$], but *Firmicutes* abundance was not affected by main effects of psilocybin treatment [($F_{(2, 21)} = 0.1963$; $p = 0.8233$] or time [($F_{(1, 21)} = 0.0323$; $p = 0.8591$] (Figure 2A). Post hoc multiple comparisons indicated trends of decreased *Firmicutes* abundance by the high dose of psilocybin treatment compared to the control group ($p = 0.0847$) and the low dose of psilocybin treatment ($p = 0.0512$) at the Week 1 timepoint. This trend did not persist at the Week 3 timepoint, instead the trend reversed, with trends of increased *Firmicutes* abundance after the high dose of psilocybin treatment compared to the control ($p = 0.1282$) and the low dose of psilocybin treatment ($p = 0.1451$) (Figure 2A). *Firmicutes* population was not significantly affected by norbaeocystin treatment [($F_{(2, 21)} = 0.7270$; $p = 0.4951$], time [($F_{(1, 21)} = 0.1179$; $p = 0.7347$], or their interaction [($F_{(2, 21)} = 0.8594$; $p = 0.4378$] (Figure 2B). Therefore, psilocybin or norbaeocystin at either dose did not result in any significant changes in *Firmicutes* population compared to the vehicle control (Figures 2A, 2B).

Bacteroidetes population was not significantly affected by psilocybin treatment [($F_{(2, 21)} = 2.430$; $p = 0.1124$], time [($F_{(1, 21)} = 0.1239$; $p = 0.7283$], or the interaction of treatment and time [($F_{(2, 21)} = 0.8893$; $p = 0.4259$] (Figure 2C). Similarly, *Bacteroidetes* population was not significantly affected by norbaeocystin treatment [($F_{(2, 21)} = 1.732$; $p = 0.2013$], time [($F_{(1, 21)} = 0.06842$; $p = 0.7962$], or their interaction [($F_{(2, 21)} = 1.992$; $p = 0.1613$] (Figure 2D).

Effects of psilocybin and norbaeocystin on microbial abundance of sub-dominant phyla

The populations of four minor gut microbiota phyla, *Proteobacteria*, *Tenericutes*, *Actinobacteria*, and *Verrucomicrobia* were analyzed.

Proteobacteria population at the phylum level was not affected by psilocybin treatment [($F_{(2, 21)} = 0.2846$; $p = 0.7552$], time [($F_{(1, 21)} = 0.6990$; $p = 0.4125$], or their interaction [($F_{(2, 21)} = 2.165$; $p = 0.1397$] (Figure 3A). Analysis of *Proteobacteria* population revealed a significant interaction between norbaeocystin treatment and time [($F_{(2, 21)} = 3.946$; $p = 0.0351$], but not main effects of norbaeocystin treatment [($F_{(2, 21)} = 0.7901$; $p = 0.4668$] or time [($F_{(1, 21)} = 0.7484$; $p = 0.3968$] (Figure 3B). Post hoc multiple comparisons indicated significantly decreased

Proteobacteria abundance by the low dose of norbaeocystin treatment vs. control (P = 0.0283) and vs. the high dose of norbaeocystin treatment (P = 0.0282) at Week 1 timepoint, but not at Week 3 timepoint (Figure 3B).

Actinobacteria population at the phylum level was not affected by psilocybin treatment [($F_{(2, 21)}$ = 1.399; p = 0.2701], time [($F_{(1, 21)}$ = 0.3574; p = 0.5567], or their interaction [($F_{(2, 21)}$ = 1.653; p = 0.2167] (Figure 3C). However, a significant main effect of norbaeocystin treatment [($F_{(2, 21)}$ = 3.631; p = 0.0452] and interaction between norbaeocystin treatment and time [($F_{(2, 21)}$ = 4.977; p = 0.0176] were revealed with no effect of time [($F_{(1, 21)}$ = 1.846; p = 0.1893] (Figure 3D). Post hoc comparisons indicated significantly increased *Actinobacteria* abundance at the high dose of norbaeocystin treatment vs. control (p = 0.0014) and vs. the low dose of norbaeocystin treatment (P = 0.0014) at Week 1 timepoint. This change did not persist at the Week 3 timepoint (Figure 3D).

Verrucomicrobia population was significantly affected by the interaction of psilocybin treatment and time [($F_{(2, 21)}$ = 4.027; p = 0.0331]; but there were not main effects of treatment [($F_{(2, 21)}$ = 1.646; p = 0.2167] or time [($F_{(1, 21)}$ = 0.04451; p = 0.8349] (Figure 3E). Post hoc comparisons indicated a significant increase in *Verrucomicrobia* abundance at the low dose of psilocybin treatment vs. control (p = 0.0332) and vs. the high dose of psilocybin treatment (p = 0.0355) at the Week 1 timepoint; and a significant increase in *Verrucomicrobia* abundance by the high dose of psilocybin treatment vs. control (p = 0.0288) at Week 3 timepoint (Figure 3E). *Verrucomicrobia* population at the phylum level was not changed by norbaeocystin treatment [($F_{(2, 21)}$ = 1.735; p = 0.2007], time [($F_{(1, 21)}$ = 0.07775; p = 0.7831], or their interaction [($F_{(2, 21)}$ = 0.3985; p = 0.6763] (Figure 3F).

Tenericutes population at the phylum level was not significantly affected by psilocybin treatment [($F_{(2, 21)}$ = 0.1573; p = 0.8554], time [($F_{(1, 21)}$ = 0.05945; p = 0.8097], or their interaction [($F_{(2, 21)}$ = 2.551; p = 0.1019] (Figure 3G); nor was it affected by norbaeocystin treatment [($F_{(2, 21)}$ = 0.2521; p = 0.7795], time [($F_{(1, 21)}$ = 1.386; p = 0.2523], or their interaction [($F_{(2, 21)}$ = 2.142; p = 0.1424] (Figure 3H).

Discussion

The gut-brain axis is a highly complex system, the importance of which is not yet fully understood. Although neither psilocybin nor norbaeocystin treatments significantly impacted gut microbe diversity (Figure 1), some significant changes in microbial abundance at the phylum level were observed. The human gut is predominantly composed of *Bacteroidetes* and *Firmicutes*, complemented by sub-dominant *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (Qin et al. 2010). Interestingly, neither *Firmicutes* nor *Bacteroidetes*, which together represent ~80% of gut microbes (Ley et al. 2008), was significantly impacted (Figure 2); whereas three of the four sub-dominant bacterial phyla analyzed, *Proteobacteria*, *Verrucomicrobia*, and *Actinobacteria* (Ley et al. 2008), were significantly impacted by psilocybin or norbaeocystin treatments at different timepoints. Specifically, the low dose psilocybin treatment increased *Verrucomicrobia* abundance at the Week 1 timepoint and the high dose psilocybin treatment increased *Verrucomicrobia*

abundance at the Week 3 timepoint. Additionally, while the low dose norbaeocystin decreased *Proteobacteria* abundance, the high dose norbaeocystin increased *Actinobacteria* abundance, both of which occurred at the Week 1 timepoint (Figures 3B, 3D).

Emerging evidence supports the microbiota-gut-brain axis in regulation of physiology and behavior, and suggests that disturbance of the gastrointestinal microbiota could affect the immune system and psychiatric functioning (Cruz-Pereira et al. 2020). The bidirectional communication between gastrointestinal microbiota and immune system mediates many neural processes, such as neurogenesis, neurotransmission, neuroinflammation, and neurochemical functions such as activation of stress responses, depression, and other mental health disorders (Cryan et al. 2019; Dinan & Cryan 2015; Sarkar et al. 2018). In the current study, phyla *Verrucomicrobia* and *Actinobacteria* were increased by psilocybin and norbaeocystin, respectively. Phylum *Verrucomicrobia* are mucin-degrading bacteria, constitutes 3%-5% of the bacterial community mainly residing in the intestinal mucosa that forms an interface between host and gut microbiome. Low abundance of *Verrucomicrobia* has been reported in prediabetic and type 2 diabetic patients (Zhang et al. 2013), in patients with inflammatory gut diseases such as Crohn's disease, ulcerative colitis, and inflammatory bowel disease (Papa et al. 2012; Png et al. 2010), and in populations with poorer sleep quality or disrupted sleep (Anderson et al. 2017). In contrast, abundance of *Verrucomicrobia* increases following dieting and Roux-en-Y gastric bypass in diabetic patients accompanied with many beneficial metabolic outcomes (Barlow et al. 2015). Thus, a low level of *Verrucomicrobia* has been associated with metabolic disorders and weakened immune system, while a high abundance of *Verrucomicrobia* is considered as a potential biomarker of a healthy gut status. Phylum *Actinobacteria* contributes to the maintenance of gut homeostasis and supports immune system (Binda et al. 2018). In contrast to beneficial phyla *Verrucomicrobia* and *Actinobacteria* that were increased following treatment of psilocybin and norbaeocystin, respectively, high abundance of phylum *Proteobacteria* is considered as a microbial signature of disease (Rizzatti et al. 2017) and was decreased by the low dose norbaeocystin treatment. Thus, it is possible that psilocybin and norbaeocystin could be candidates for alleviating gut dysbiosis and producing positive downstream effects.

The findings of the current study are promising, as significant alteration of the gut microbiome may provide a possible explanation as to why psilocybin users report a reduction of depressive symptoms after treatment (Grob et al. 2011). It has been proposed that psychedelics may affect gut microbiome to influence their treatment responses (Kelly et al. 2023; Kuypers 2019). To our knowledge, the current study is the first study that investigated the effects of tryptamines, psilocybin and norbaeocystin, on gut microbe populations. Unlike conventional mood modulating drugs that require chronic doses over a long timeline, it is possible that psilocybin in part works by altering microbe populations within the gut, potentially targeting a component of the disease state rather than treating the symptoms. These results also suggest that norbaeocystin, a psilocybin precursor with limited study in the peer reviewed literature (Adams et al. 2022), may warrant further investigation as a potential antidepressant.

The limitation of this study is low sample sizes analyzed for some groups. Although QIIME 2 is capable of analyzing Mi-Seq data with two or more biological replicates and tends to be conservative in revealing statistical significance (Bolyen et al. 2019), as shown in a publication with some sample sizes of 1 and 2 (McKenzie et al. 2017), we should interpret findings with caution. The lack of statistical significance in diversity and some phylum abundance, along with high variability of the relative abundance of some phyla, could be due to the small sample size of this preliminary study. Consequently, the findings from this preliminary study have limited generalizability, and would require further validation with larger sample sizes and comparing across routes of drug delivery.

One caveat to the current work is that it was conducted in normal, healthy rats. It is likely that rats modeling a disease-state, such as chronic stress, anxiety, and depression, may respond differently to psilocybin and/or norbaeocystin. Previously, we have reported the impact of chronic stress on gut microbiome diversity and composition, leading to gut dysbiosis (Xu et al. 2020a). Rats with disturbed microbiome may react very differently to these drugs. As such, findings from healthy rats in this study may not generalize to other animals or treatment conditions. Further research is needed using disease models, where multiple physiological, biochemical behavioral and microbiome outcomes are evaluated, such that biological mechanisms can be elucidated. Additionally, there is still little information on how psilocybin and norbaeocystin interact with the body, and continued study is needed in order to inform potential side effects in human trials. Altogether, psilocybin and norbaeocystin stand as strong candidates for managing gut dysbiosis.

Conclusions

The schedule I status of psilocybin has greatly hindered advancements in research to better understand the efficacy and safety of using it to treat mood disorders. Psilocybin may also be used to treat other diseases, such as those related to gut health. For example, the FDA recently approved a Phase 2A clinical trial for the treatment of irritable bowel syndrome with psilocybin. Although our study does not use a paradigm that induces stress or depression, nor does it determine psilocybin's ability to modulate mood via the gut-brain axis, it does begin to probe the mechanisms by which psilocybin affects body physiology and behavior. The observed alterations to the gut microbiome show promise for the ability of psilocybin and norbaeocystin to affect the gut microbiome in a positive manner and establish a path for future research to investigate how psilocybin, or other related tryptamines, could be used to modulate the gut microbiota to treat dysbiosis as well as other disorders. Prior to this study the potential effects of psilocybin and its biosynthetic precursor, norbaeocystin, on gut microbe populations was unknown. Further investigations building upon this work could open the door to a new potential avenue for pharmaceuticals which target the gut-brain axis.

Acknowledgements

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Protocol registration

The research question, groups, fecal sample collection, and gut microbiome analysis using 16S rDNA sequencing were discussed before the study among the researchers. The protocol was not registered.

Data access

The project is registered with the BioProject database (BioProjectID: PRJNA1054120). Raw sequencing data are accessible via http://www.ncbi.nlm.nih.gov/bioproject/1054120.

Declaration of interests

J. Andrew Jones is a significant stakeholder at PsyBio Therapeutics. PsyBio Therapeutics has licensed tryptamine biosynthesis-related technology from Miami University. J. Andrew Jones and Matthew S. McMurray are co-inventors on several patent applications related to tryptamine biosynthesis and the impacts of tryptamines on animal behavior. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. All other authors declare no conflicts of interest.

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579 10.1371/journal.pone.0071108

Figure 1(on next page)

Figure 1: Effects of psilocybin and norbaeocystin treatment on microbial diversity.

Effects of psilocybin treatment (A) and norbaeocystin treatment (B) on microbial diversity, measured by Shannon diversity index. Sample sizes for Week 1 were control (Control) n = 7; low dosage psilocybin (P-low) n = 3; high dosage psilocybin (P-high) n = 2; low dosage norbaeocystin (N-low) n = 5; and high dosage norbaeocystin (N-high) n = 2. Sample size for Week 3 were Control n = 8; P-low n = 3; P-high n = 4; N-low n = 3; and N-high n = 2. Error bars represent +/- 1 standard error of the mean of biological replicate samples.

Effect of Oral Tryptamines on the Gut Microbiome of Rats - A Preliminary Study

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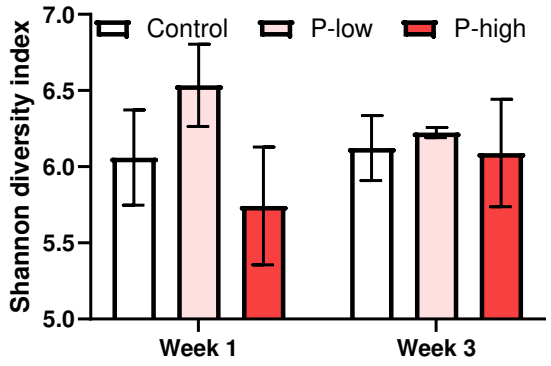
Figure 1: Effects of psilocybin and norbaeocystin treatment on microbial diversity.

A



B

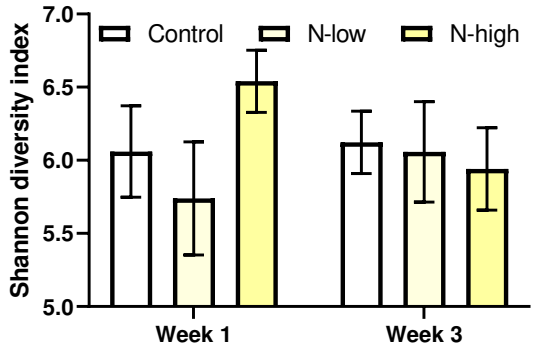


Figure 2

Figure 2: Effects of psilocybin and norbaeocystin treatment on abundance of major microbial phyla at the phylum level.

Effects of psilocybin treatment on *Firmicutes* abundance (A), norbaeocystin treatment on *Firmicutes* abundance (B), psilocybin treatment on *Bacteroidetes* abundance (C), and norbaeocystin treatment on *Bacteroidetes* abundance (D).

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Figure 2: Effects of psilocybin and norbaeocystin treatment on abundance of major microbial phyla at the phylum level.

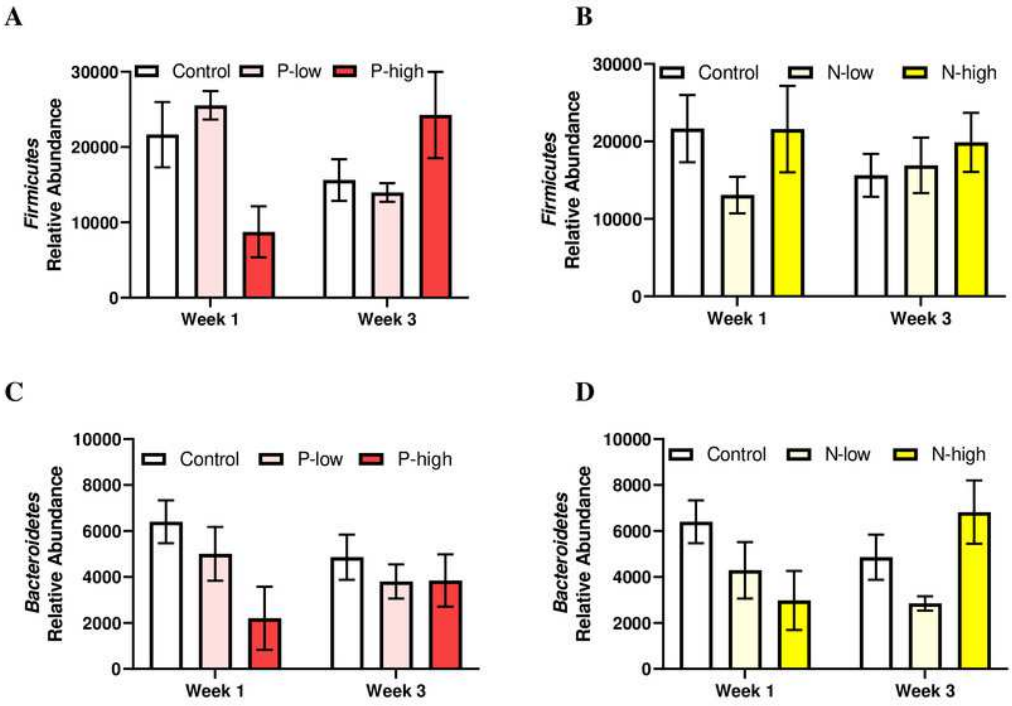


Figure 3(on next page)

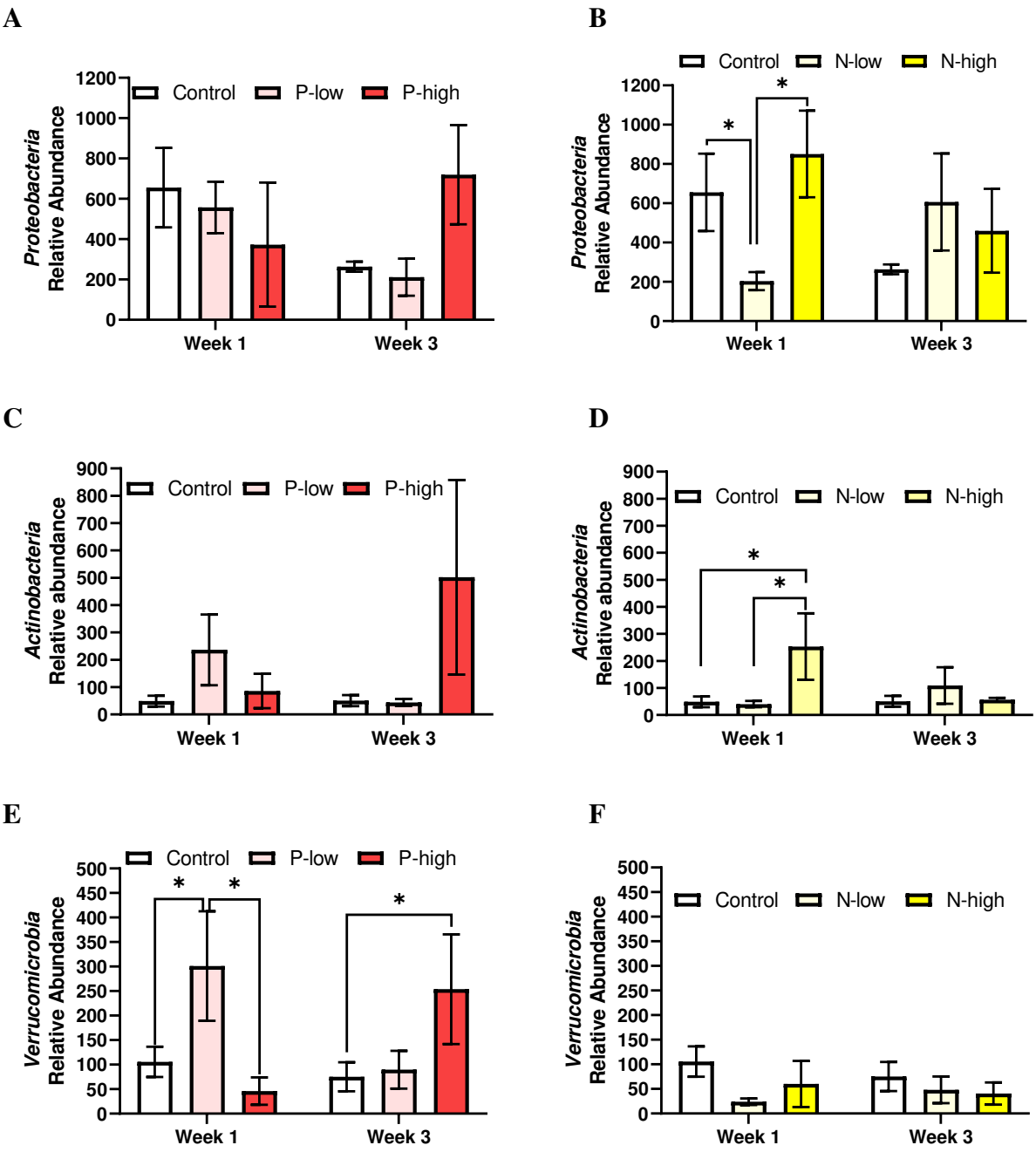
Figure 3: Effects of psilocybin and norbaeocystin treatment on abundance of minor microbial phyla at the phylum level.

Effects of psilocybin on *Proteobacteria* abundance (A), norbaeocystin on *Proteobacteria* abundance (B), psilocybin on *Actinobacteria* abundance (C), and norbaeocystin on *Actinobacteria* abundance (D), psilocybin on *Verrucomicrobia* abundance (E), and norbaeocystin on *Verrucomicrobia* abundance (F), psilocybin on *Tenericutes* abundance (G), and norbaeocystin on *Tenericutes* abundance (H). * indicated statistical significance.

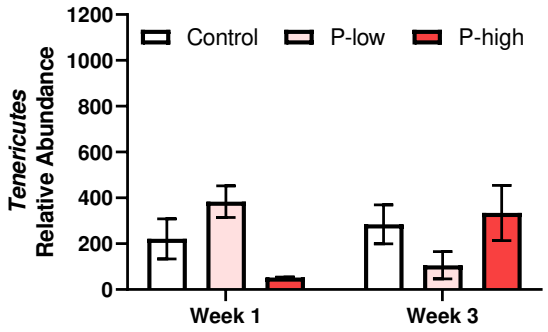
Effect of Oral Tryptamines on the Gut Microbiome of Rats - A Preliminary Study

Mengyang Xu, J. Andrew Jones, Matthew S. McMurray, and Haifei Shi

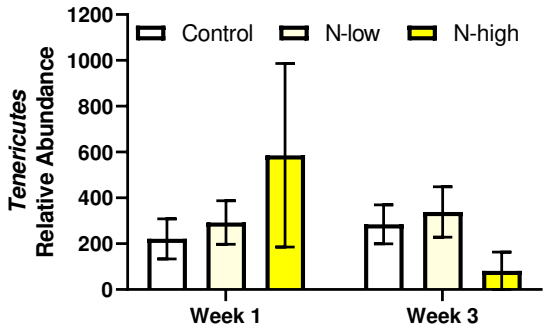
Figure 3: Effects of psilocybin and norbaeocystin treatment on abundance of minor microbial phyla at the phylum level.



G


Tenericutes
Relative Abundance
1200
1000
800
600
400
200
0
Control
P-low
P-high
Week 1
Week 3

H


Tenericutes
Relative Abundance
1200
1000
800
600
400
200
0
Control
N-low
N-high
Week 1
Week 3