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Evaluation of Dietary Probiotic Supplements on Methamphetamine-Induced Impulsive Action in Rats Maintained on a Differential Reinforcement of Low Rate Schedule

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EVALUATION OF DIETARY PROBIOTIC SUPPLEMENTS ON METHAMPHETAMINE-INDUCED IMPULSIVE ACTION IN RATS MAINTAINED ON A DIFFERENTIAL REINFORCEMENT OF LOW RATE SCHEDULE

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Substance Use Disorder (SUD) is a chronic, debilitating condition often comorbid with anxiety and depression. Both SUD and affective disorders are characterized by cognitive dysfunction, including impaired decision-making and impulsivity. A mounting body of research implicates gut microbiome alterations as a contributing factor to the pathophysiology of affective disorders. No published studies have assessed behavioral effects of dietary interventions targeting psychostimulant-induced gut microbiome changes. The present study utilized a differential reinforcement of low rate responding schedule (DRL 18 s) as a behavioral index of drug-induced impulsive action to determine if a dietary probiotic supplement alters the behavioral effects of (+)-methamphetamine in rats. Thirty-two adult male Sprague-Dawley rats were trained to lever press for food reinforcement under a DRL 18 s schedule. Rats were then assigned to two dietary treatment groups, matched on reinforcement rate; the supplement group received continuous access to Bio-Kult Advance® in their drinking water and the control group received standard drinking water. Half the rats in each diet group received intraperitoneal injections of 1 mg/kg (+)-methamphetamine and the remaining rats received saline injections for eight consecutive days. DRL 18 s test sessions were conducted on day 1 and day 8, and subsequently 24 h, 48 h, 96 h, after the last injection. Statistically significant increases in response rate and corresponding decreases in reinforcement rate were observed in (+) methamphetamine-treated animals compared to saline-treated animals. The probiotic-treated rats displayed a higher drug-induced increase in response rate, but reinforcement rates were comparable between diet groups, and the main effect of diet was not statistically significant.

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by

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INTRODUCTION

Substance use disorders (SUD) are characterized by patterns of chronic or frequent use of alcohol or other drugs resulting in health problems, disabilities, and failure to perform at work, school, or home (SAMHSA, 2021). As of 2020, 40.3 million people in the United States aged 12 or older had a SUD within the past year. Federal Drug Administration (FDA) approved medications are available for alcohol and opioid use disorders. Unlike opioid or alcohol withdrawal syndrome, psychostimulant withdrawal is characterized by psychological symptoms similar to anxiety and/or depression, and minimal clear overt physical signs. Overlap between anxiety, depression, and psychostimulant withdrawal symptoms are evident, and these negative affective states are associated with relapse to drug use (Forouzan et al., 2021b). Currently, no FDA-approved pharmacotherapies are available for psychostimulant use disorders, despite decades of medication-development research.

Recent evidence implicates psychostimulant-induced gut dysbiosis and negative affect in the sequelae of psychostimulant use disorder (Forouzan et al., 2021b). The gut microbiome is the community of organisms residing in the gut. Dysbiosis refers to changes in the composition of the microbiome that negatively impacts gut health. Forouzan et al. (2021b) describe experimental evidence for stress-induced alterations in the gut microbiome that coincide with behavioral indices of negative affect in rodents.

The nervous system and gut microbiome interact through several channels, including the immune response system, metabolic pathways, neuroendocrine pathways and the vagus nerve (Salavrakos et al., 2021). Communication through immune response is a result of bacterial products within the gut lumen and their passage into the bloodstream. Metabolic pathways allow communication between the gut and brain through production of short-chain fatty acids (SCFAs)

and tryptophan. SCFAs have been shown to increase intestinal barrier integrity and diffuse across the blood-brain barrier, which may impact brain functioning and influence behavior (Salavrakos et al., 2021). Lastly, the gut and brain communicate through neuroendocrine pathways and the vagus nerve. The hypothalamic-pituitary-adrenal axis may be influenced, through afferents of the vagus nerve, by bacterial products that activate brain microglia (Salavrakos et al., 2021).

Salavrakos et al. (2021) examined various psychoactive drugs and their effects on gut-brain communication through the gut-brain axis. The bulk of the scientific literature reviewed by Salavrakos et al. (2021) was based on research on alcohol-induced changes to the gut microbiome and their correlation with neurocognitive functioning. Given the correlational nature of much of the published research, the direction of causality between gut microbiome changes and brain function changes are currently unknown. Preclinical studies that allow for rigorous experimental control are essential to advancing scientific knowledge and its relevance to gut microbiome/brain interactions in substance use disorders.

A few preclinical studies have evaluated psychostimulant effects on various indices of gut microbiome function. Yang et al. (2020) evaluated methamphetamine-induced conditioned place preference (CPP) in male rats and assessed the gut microbiota before and after methamphetamine exposure. Based on CPP scores, methamphetamine-treated rats were characterized as high CPP responders or low CPP responders. The gut microbiota composition differed between the high CPP and low CPP groups as evidenced by greater levels of the bacteria, *Akkermansia,* in the high CPP animals, which was positively correlated with CPP scores. Moreover, the gut microbiome composition of the animals prior to methamphetamine differed between the high CPP and low CPP animals. The high CPP animals had higher levels of the bacteria *Ruminococcus*. Additionally, this study found that pre-treatment with antibiotics enhanced methamphetamine-

induced CPP. These results indicate the gut microbiota may modulate drug-induced conditioned reward.

Forouzan et al. (2021a) assessed chronic methamphetamine administration and cessation on the gut microbiome and changes to depressive- or anxiety-like effects in rodents. Microbial DNA was isolated from rodent fecal samples and analyzed using 16s rRNA sequencing to determine changes to the gut microbiome. The administration of methamphetamine did not alter the abundance of bacteria but did change the composition. These changes were normalized after seven days of methamphetamine cessation (Forouzan et al., 2021a). The researchers conducted three behavioral tests to assess methamphetamine-induced changes in affect. The open field test and elevated plus maze test were conducted as indices of anxiety and the forced swim test was conducted as an index of depression. Methamphetamine cessation did not alter anxiety-like behavior in the open field test or elevated plus maze, but it did alter depressive-like behavior with increased immobility in the forced swim test (Forouzan et al., 2021a). Based on these results, Forouzan et al. (2021a) suggest gut dysbiosis following methamphetamine cessation is related to depressive-like effects in rats.

Zhang et al. (2022) conducted a similar study in mice to assess the impact of methamphetamine on gut homeostasis and brain changes. The researchers examined locomotor sensitization, light-dark activity test, tail suspension test, forced swim test, and open field test. Results of the behavioral tests indicate an increase in locomotor sensitization, depression-, and anxiety-like behavior. The 16S rRNA sequencing of the fecal microbiome revealed methamphetamine decreased microbial diversity and altered the microbiota (Zhang et al., 2022). Furthermore, the gut microbiome changes were correlated with behavioral changes. The authors

concluded that methamphetamine alters gut homeostasis and maybe be related to methamphetamine-induced neurotoxicity and changes to behavioral outcomes.

Considering these pre-clinical findings that methamphetamine alters the gut microbiome, treatments that target the gut microbiome may be worth exploring as a complimentary treatment for psychostimulant use disorder. A review of the scientific literature revealed no preclinical studies evaluating the effects of probiotics on drug-induced microbiome changes. However, a few studies have evaluated the effects of probiotics in rodent models predictive of depression, anxiety and stress-induced behaviors. For example, Desbonnet et al. (2008) assessed the probiotic *Bifidobacteria infantis* for its antidepressant-like properties in rats. The probiotic was administered orally by dissolving prepared powder in the animal's drinking water. The authors evaluated treatment effects in the forced swim test, biomarkers of immune function, neuroendocrine function, and central monoaminergic activity. No changes in swimming, climbing, or immobility were observed in probiotic-treated animals compared to controls. However, an attenuation of pro-inflammatory immune responses and elevation of tryptophan in the probiotic-treated animals were noted (Desbonnet et al., 2008).

In a more recent study, Haas et al. (2020) assessed the effects of chronic administration of the probiotic *Bifidobacterium longum* on male and female rats under pharmacologically induced stress or control conditions. This single species probiotic was administered orally by mixing the probiotic powder with Nutella®. Animals received daily corticosterone injections to mimic the physiological features of stress, and sesame oil injections served as the control treatment. The open field test was conducted as a measure of anxiety and the forced swim test was conducted as a measure of depression. Corticosterone injections produced predicted effects consistent with stress-induced increases in anxiety and depression, and these effects were not altered by the

probiotic treatment (Haas et al., 2020). Blood samples were collected to assess basal and stress-induced corticosterone levels and brain tissue was analyzed to assess for evidence of neurogenesis. Probiotics did not induce changes in neurogenesis but did impact hypothalamic-pituitary-adrenal axis functioning within the male rats. Unfortunately, this study did not assess gut microbiome composition following experimental treatments.

Li et al. (2018) investigated the effects of a multi species probiotic, *L. helveticus R0052, L. plantarum R1012, and B. longum R0175*, on chronic mild stress induced by a number of stressors including animal cage tilted at 45° as well as food and water deprivation. Behavioral testing included the sucrose preference test to assess anhedonia, the elevated plus maze to assess anxiety-like behavior, and the forced swim test to assess depressive-like behavior. Probiotics and a control antidepressant, fluoxetine, were administered via oral gavage. Fluoxetine significantly improved depressive- and anxiety-like behaviors in the sucrose preference test, the elevated plus maze, and the forced swim test. Probiotics, however, only improved behavior in the elevated plus maze and the forced swim test but did not improve anhedonia-like behavior in the sucrose preference test (Li et al., 2018). The authors noted chronic mild stress depleted *Lactobacillus* in the mice and administration of the probiotic reversed these changes.

As summarized above, a few preclinical studies have examined the effects of probiotics in behavioral assays indicative of depression and anxiety (Desbonnet et al., 2008; Hass et al., 2020; Li et al., 2018). In considering the potential for dietary interventions targeting the gut microbiome as a complementary treatment for SUD, behavioral indices of proimpulsive behavior may be a relevant outcome measure to consider. Impulsive behaviors are strongly associated with psychological disorders including SUD and may reflect reward seeking or salient outcomes (Jentsch et al., 2014). Impulsivity may be involved in all stages of substance abuse. Jentsch et al.

(2014) describe the relationship to these stages as an increased likelihood of initial drug use, the rapid escalation of drug use, and an inability to decrease drug use and abstain from further use. Impulsivity is often regarded as suppression of bottom-up control mechanisms and diminished cognitive control (Kozak et al., 2019). According to Kozak et al. (2019), numerous studies assessing a variety of behavioral measures of impulsivity, such as delayed discounting and behavioral inhibition, reveal SUD alter human performance. These results indicate drug use may increase impulsivity. Impulsive action may also be assessed in behavioral paradigms in which low rates of responding are established by a differential reinforcement of low rate responding (DRL) schedule, also known as interresponse time greater than t (IRT > t). DRL schedules require the animal to withhold responding for the duration of the IRT to receive reinforcement. If a response is made prior to the IRT elapsing, the IRT is reset, and reinforcement is delayed. Impulsive action is characterized by the inability to withhold inappropriate or premature responses. Under a DRL 20 sec schedule (e.g., Hyatt et al., 2020) impulsive action is determined by an increase in response rate, accompanied by a decreased reinforcement rate.

Training operant responding on a DRL schedule produces organized patterns of responding that provide a baseline for assessing the effects of various drugs on behavior. Sabol et al. (1995) evaluated amphetamine and several structurally-related compounds for changes in response rate, reinforcement rate, and shape of the interresponse time (IRT) distribution in rats maintained on a DRL 36 s schedule. Psychostimulants characterized as dopamine releasers, amphetamine and methamphetamine, produced large increases in response rate, whereas substituted amphetamines that increase serotonin and dopamine, methylenedioxymethamphetamine (MDMA) and para-chloroamphetamine (PCA) produced small increases in response rate, and the selective serotonin releaser, fenfluramine had no effect on

response rate. Amphetamine, methamphetamine, MDMA and PCA produced dose-dependent decreases in reinforcement rate. Fenfluramine did not affect reinforcement rate. Similarly, the former four drugs produced a leftward shift in the IRT distribution and fenfluramine had no effect.

Hyatt et al. (2020) examined the effects of the psychostimulants cocaine and 3,4-methylenedioxypyrovalerone (MDPV) on impulsive action in rats trained on a DRL 20 s schedule. The results of the study indicate proimpulsive effects of the assessed psychostimulant drugs. The authors determined the psychostimulant drugs resulted in an increase in premature responding (0-15s) and a decrease in timing error responding (15-20s) and reinforcers earned (Hyatt et al., 2020). These results suggest the assessed drugs increased impulsive action.

Peterson et al. (2003) evaluated amphetamine withdrawal in rats trained under a DRL 30 s schedule. Once animals were trained on a DRL 30 s schedule, the animals underwent five days of no treatment, saline, or 5 mg/kg d-amphetamine injections. No behavioral tests were conducted on the first two days of withdrawal. Performance on a DRL 30 s schedule was assessed three days after amphetamine withdrawal and then daily for the next eight days. On withdrawal day 3, the amphetamine treated animals responding was significantly different from the no treatment and saline-treated animals. On each test day, the amphetamine-treated animals had the greatest number of 1 s IRTs responses compared to the saline treated animals as well as the greatest number of 28 s IRTs on testing days 6, 8, 9, and 11. These findings suggest a decrease in training efficacy in the amphetamine treated animals. The authors suggest these behavioral findings are the result of increased impulsivity associated with amphetamine withdrawal due to disrupted dopamine in the prefrontal cortex (Peterson et al., 2003).

Previous studies have established that psychostimulant drugs alter the gut microbiome and produce changes to the gut microbiome (Yang et al., 2020; Forouzan et al., 2021a; Zang et al., 2022). Additionally, several studies examining the effects of probiotics on the gut and behavior revealed the probiotic diet produced changes which may be beneficial to the gut-brain axis (Desbonnet et al., 2008; Hass et al., 2020). In consideration of evidence that psychostimulants alter gut microbiome composition, targeting the gut microbiome through dietary interventions could improve neurocognitive deficits associated with SUD. A DRL paradigm provides insight into drug-induced impulsivity and is appropriate to assess the effects of a dietary intervention on drug-induced impulsive action (Hyatt et al., 2020; Peterson et al., 2003). The present study aimed to assess the effects of a dietary probiotic supplement on (+) methamphetamine-induced changes on impulsive action in rats trained on a DRL 18 s schedule. In addition to assessing behavioral changes, fecal samples were collected weekly, and analysis is currently ongoing to assess the effects of the probiotic supplement and (+) methamphetamine on the gut microbiome.

METHODS

Subjects

Thirty-two drug naïve adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used. Animals were single housed in polycarbonate cages with corncob bedding and maintained on a 12:12 hour light-dark cycle (lights on at 0700). Rats were fed daily rations of rodent diet (LabDiet®, PMI® Nutrition International, LLC, Brentwood, MO, USA or Purina®, Richmond, IN, USA) and maintained at 80-90% of their free feeding weight. Animals were approximately six months old at the beginning of the study and were previously

used in an undergraduate operant conditioning laboratory course. This study was approved by the Institutional Animal Care Use Committee at Western Michigan University.

Apparatus

Sixteen standard operant chambers (ENV-001, Med Associates, Inc., St. Albans, VT, USA) were used for all training and testing sessions. Chambers were equipped with a food pellet dispenser and fan on the front panel, a 28-V house light on the back panel, housed within sound-attenuating shells, and one retractable lever was used. Dustless Precision Pellets (45 mg; Product# F0021; BioServ, Flemington, NJ) were used to reinforce lever pressing.

DRL Training Procedures

Preliminary training consisted of two sessions to acclimate the animals to the operant chamber and food pellet delivery. During these sessions, no levers were present and food pellets were delivered on a fixed time 60-second schedule. Preliminary training next consisted of four 60 min sessions in which lever pressing was established under a continuous reinforcement schedule. Once lever pressing was established in each rat, training commenced with a DRL schedule, in which the programmed interval was gradually increased (2.25, 4.5, 9 sec.) over the course of six sessions. Once the rats were trained on a DRL 18 s schedule, they continued training three days per week for 11 weeks, until animals reached stability as determined by visual analysis, before dietary interventions commenced.

Probiotic Treatment and Fecal Sample Collection

Once response rate and reinforcement rate were stable, with no visually discernable trends, the 32 rats were divided into four treatment groups (N=8), counterbalanced across training cohorts. For each rat, an average response rate and average reinforcement rate were calculated

from the last three training sessions during week 11, and group means were calculated to ascertain that these measures were comparable across the four treatment groups. Beginning in week 12, two groups were randomly assigned to receive continuous access to a probiotic supplement containing 14 probiotic strains (Bio-Kult ®) in their drinking water and the other two groups received unaltered drinking water. Prior to the onset of probiotic treatment, fecal samples were collected from each individual rat, once per week, over three weeks. From there, fecal samples were collected once per week and continued once every other week for four weeks after cessation of treatment. Fecal samples were collected 24 hours following home cage changes and stored in a -80º C freezer for later analysis. Behavioral testing continued during this time.

Drug Testing Phase

Five weeks after initiating the dietary intervention, the drug testing phase commenced. A 2 x 2 treatment design was implemented, such that one of each of the aforementioned dietary treatment groups received (+) methamphetamine injections and the remaining two groups received saline injections. Animals were injected once per day for eight days. DRL 18 test sessions were conducted on injection day 1 and injection day 8, and subsequently 24 h, 48 h, and 96 h after drug withdrawal.

Bio-Kult ® Preparation

Bio-Kult® was prepared in sterile water and diluted with deionized drinking water. Each capsule containing approximately 800 mg Bio-Kult ® was added to 10 mL of sterile water. Each 10 ml solution was vortexed for 30 seconds and left for five minutes to allow for separation. The supernatant (ranging 6 to 8 mL), which contained the live bacteria, was aspirated, and deposited into a graduated cylinder and deionized water was added to a total volume of 100 mL. A 2-liter

batch was prepared every two days and each rat received a fresh batch of 125 ml of the Bio-Kult® solution every two days.

Drug Preparation

(+) Methamphetamine was provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD). (+) Methamphetamine (1 mg/kg) was dissolved in 0.9% saline and was administered by intraperitoneal injection at a volume of 1.0 ml/kg.

Data Analysis

Response rate (responses/minute), reinforcement rate (reinforcers/minute), and IRT distributions were graphed for visual analysis during the preliminary training phase to determine stability and during the treatment phase to assess drug-induced changes in these measures. The primary dependent variables analyzed were response rate and reinforcement rate during test sessions compared to each group's baseline rates. Baseline rates were calculated for individual rats by averaging the rates obtained during the six training sessions preceding the test phase. These training sessions occurred during the third and fourth week of the dietary intervention. Response rates during the drug treatment phase were calculated and plotted as a percentage of baseline values. A two-way ANOVA was conducted on Days One and Eight and 24, 48, and 96 hrs following the last injection. Additionally, the relative frequency of interresponse times (IRT) was calculated for each rat, and IRT distributions were compared for the last baseline session, and each test day during the drug treatment and the withdrawal phase using visual analysis.

RESULTS

Response rate and reinforcement rate were the primary dependent variables assessed to address the research hypothesis that a dietary probiotic intervention would alter

methamphetamine-induced impulsive action. Interresponse time distributions were also compared among treatment groups during baseline and on each test session. Figure 1 displays the response and reinforcement rates during baseline, drug treatment days one and eight, and withdrawal days as percent baseline. Visual analysis indicated comparable response rates among treatment groups during baseline training sessions, and increased response rates following acute and sub-chronic (+)-methamphetamine treatment. Figure 2 displays response rate expressed as a percent baseline for each treatment group on each test session. In both the control diet and probiotic diet groups, response rates increased following a single acute injection of 1 mg/kg (+)-methamphetamine. A two-way ANOVA indicated a statistically significant drug effect [$F(1, 28) = 36.19$, $P<0.0001$] and a statistically significant diet x drug interaction [$F(1, 28) = 5.380$, $P=0.028$]. However, the main effect of diet on response rate on Day 1 was not statistically significant [$F(1, 28) = 1.967$, $P=0.17$]. For the reinforcement rate on injection day one, only the drug treatment was statistically significant [$F(1, 28) = 30.55$, $P<0.0001$]. Diet [$F(1, 28) = 0.6691$, $P=0.4203$] and the interaction between diet and drug [$F(1, 28) = 1.083$, $P=0.31$] were not statistically significant..

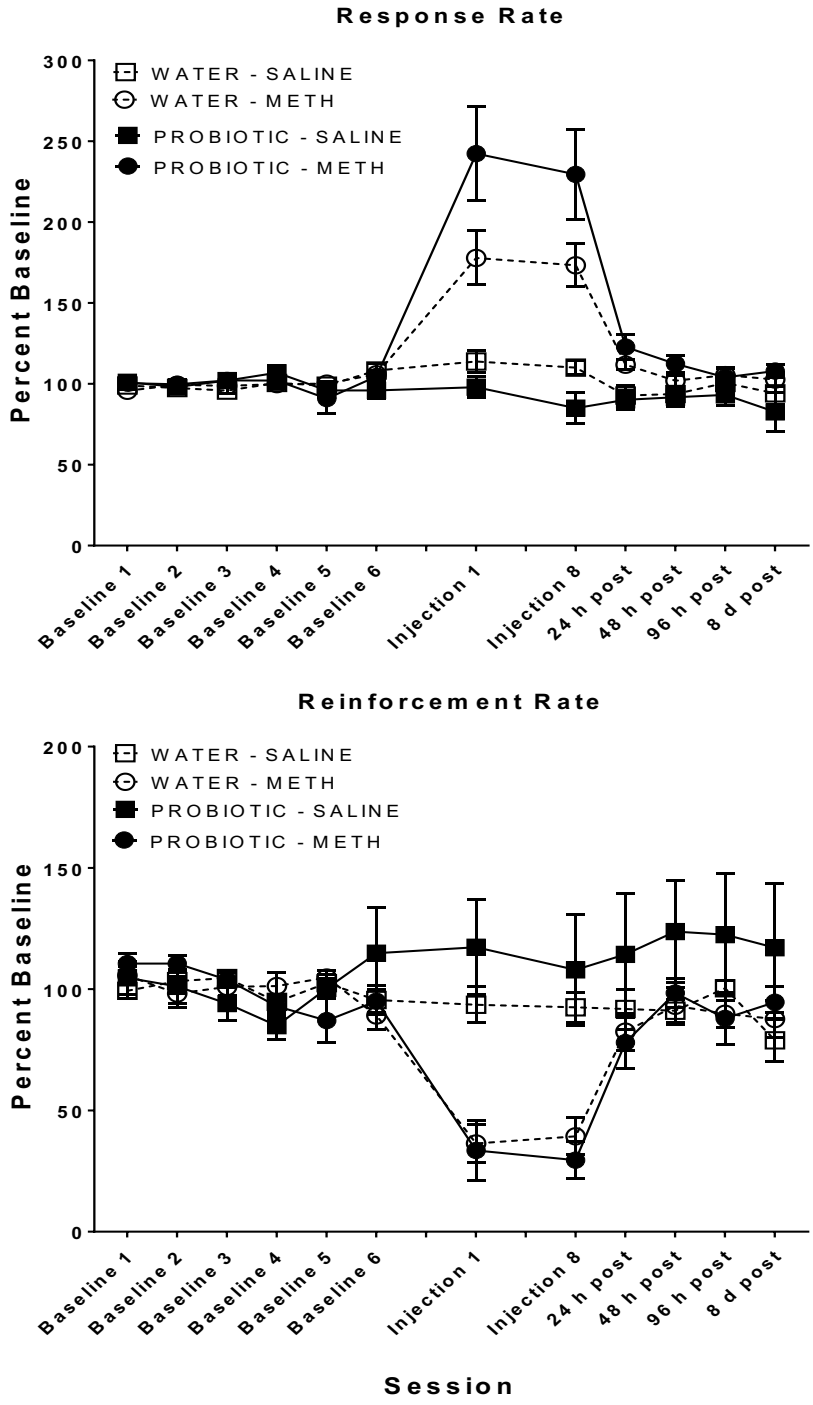

Figure 1. Response and reinforcement rates expressed as percent baseline on the last six baseline training sessions, injection days one and eight, and 24, 48 and 96 h after the last injection.

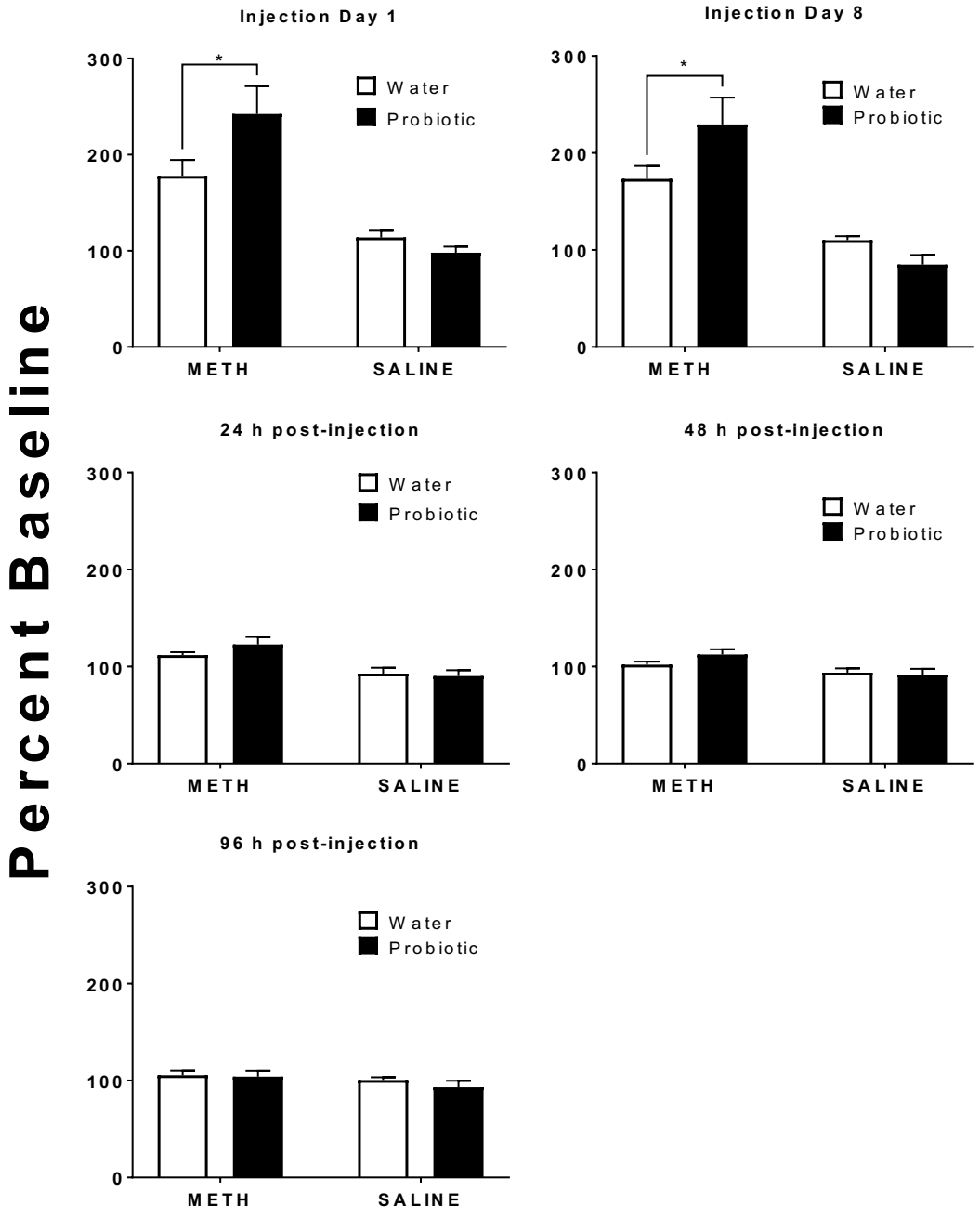

Figure 2. Response rates expressed as percent baseline in probiotic- and water-treated animals on injection days one and eight and 24, 48, and 96 h after the last injection.

Figure 3 depicts the reinforcement rates expressed as a percent of baseline on each test day. On injection day eight, a two-way ANOVA on response rates revealed a statistically

significant drug effect [$F(1, 28) = 41.06$, $P<0.0001$] and diet x drug interaction [$F(1, 28) = 6.287$, $P=0.0182$]. Diet was not statistically significant [$F(1, 28) = 0.9239$, $P=0.3447$]. Similarly to injection day one, for the reinforcement rate only the drug treatment was statistically significant [$F(1, 28) = 25.63$, $P<0.0001$]. Diet [$F(1, 28) = 0.04646$, $P=0.8309$] and the interaction [$F(1, 28) = 0.9432$, $P=0.3398$] were not statistically significant.

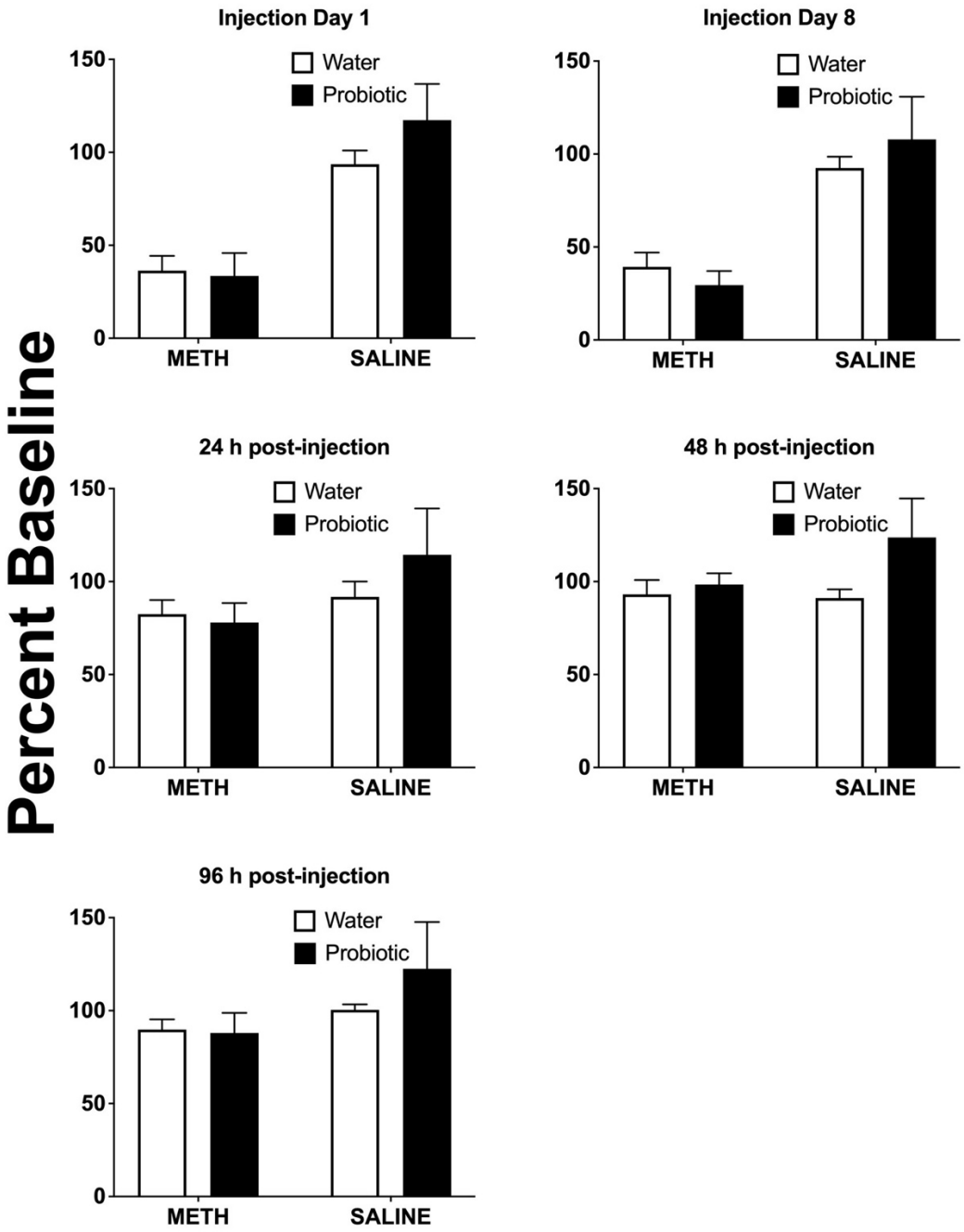

Figure 3. Reinforcement rate (SR) of probiotic and water treated animals on injection days one and eight and withdrawal 24, 48, and 96 h following drug administration.

A two-way ANOVA on response rates 24 hours following the last injection, indicated a statistically significant main effect of drug [F (1, 28) = 19.00, P=0.0002]. Neither the diet [F (1,

28) = 0.4943, P=0.4878] nor the diet x drug interaction [F (1, 28) = 1.309, P=0.2622] was statistically significant. There was no statistically significant drug effect [F (1, 28) = 2.433, P=0.1300], diet [F (1, 28) = 0.3792, P=0.5430], nor a significant drug x diet interaction [F (1, 28) = 0.8514, P=0.3640] on the reinforcement rate 24 hours after the last injection.

Forty-eight hours following the last injection, the effect of drug on response rate was statistically significant [F (1, 28) = 8.697, P=0.0064]. The diet [F (1, 28) = 0.7625, P=0.3900] and drug x diet interaction [F (1, 28) = 1.624, P=0.2130] were not statistically significant. Reinforcement rate on this test day showed no statistically significant effect of drug [F (1, 28) = 0.9662, P=0.3340], diet [F (1, 28) = 2.568, P=0.1203], or drug x diet interaction [F (1, 28) = 1.333, P=0.2580].

Ninety-six hours after the last injection, there was no statistically significant effect of drug [F (1, 28) = 2.399, P=0.1327], diet [F (1, 28) = 0.7303, P=0.4000], or drug x diet interaction [F (1, 28) = 0.3589, P=0.5540] on response rate. Additionally, there was no statistically significant effect of drug [F (1, 28) = 2.574, P=0.1199], diet [F (1, 28) = 0.5172, P=0.4780], or drug x diet interaction [F (1, 28) = 0.7260, P=0.4014] on reinforcement rate.

During baseline training sessions, all four treatment groups' peak IRT distributions, the highest frequency of responses that occur at a given time, were approximately 18 s. On the first day of acute (+) methamphetamine assessment, the saline-treated animals' peak IRT remained around 18 s. The control diet animals displayed a leftward shift in the IRT distribution following (+) methamphetamine treatment the peak IRT was between 7-9 s. The probiotic-diet animals also showed a leftward shift in the IRT distribution following (+) methamphetamine treatment, with a peak IRT between 7-9 s. Following the repeated daily administration of drug or saline for eight days, there was no change to the control diet or probiotic diet animals' IRT distribution when

assessed after an acute injection on day 8. Both the probiotic and control diet groups displayed peak IRTs around 7-9 s following the last (+) methamphetamine injection. All four treatment groups displayed comparable IRT distributions when assessed 24 h, 48 h, and 96 h after the last injection. Figure four depicts the IRT distributions on injection days 1 and eight and 24 h withdrawal.

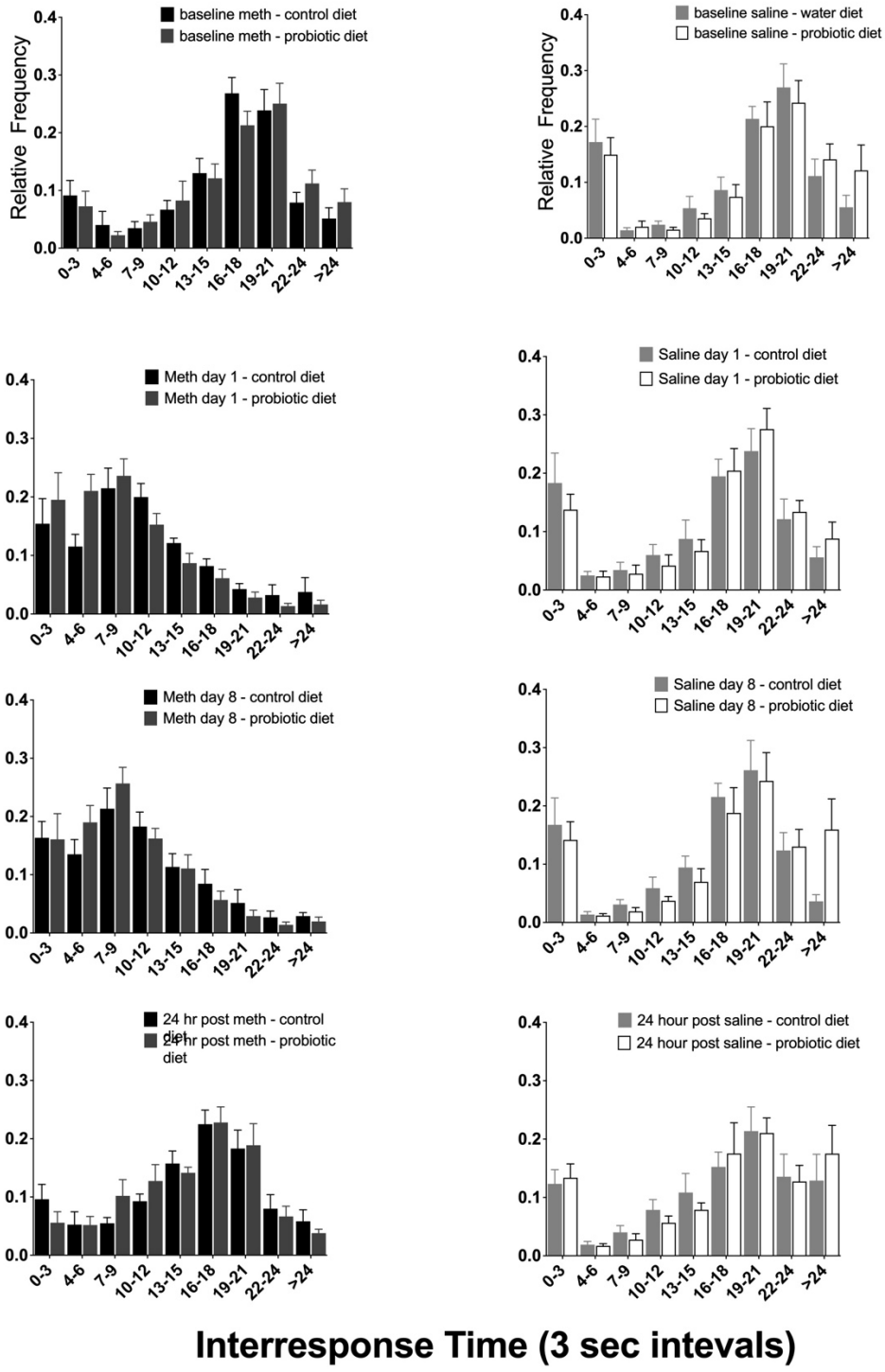

Figure 4. IRT relative frequency distributions during baseline, injection days one and eight, and 24 h withdrawal in probiotic and control diet groups. The left set of graphs depict the drug treated animals and the right set of graphs depict the saline-treated animals.

DISCUSSION

Evidence-based treatment options for psychostimulant disorder are limited. Behavioral therapies utilizing contingency management are highly effective in reducing relapse, but they can be costly and they are not readily accessible to all populations. Medication-assisted therapies are not currently available for psychostimulant use disorder. Complementary and alternative treatments targeting both physical and psychological health may improve treatment outcomes. Therapeutic interventions targeting gut health may be worth exploring as complimentary treatment approach, in light of recent evidence of psychostimulant-induced gut dysbiosis and negative affect associated with relapse to drug use (Forouzan et al., 2021b). A wide variety of probiotic supplements are commercially available and offer a convenient way to target gut health. Recent preclinical findings are mixed regarding the effects of probiotics in rodent behavioral assays predictive of depression and anxiety (Desbonnet et al., 2008; Hass et al., 2020; Li et al., 2018). To date, no studies have evaluated probiotics as a potential protective factor for psychostimulant-induced gut dysbiosis. The current study evaluated the effects of a dietary probiotic supplement on methamphetamine-induced impulsive behaviors in rats. The probiotic-treated rats displayed a higher drug-induced increase in response rate, but reinforcement rates were comparable between diet groups, and the main effect of diet was not statistically significant.

Impulsive behavior is a component of SUD relevant to the assessment of potential therapeutic interventions (Jentsch et al., 2014). Operant responding under a DRL schedule is amenable to the assessment of impulsive behavior because it requires an organism to withhold responding to maximize reinforcement. The current study results failed to support the research hypothesis that a multispecies probiotic supplement would attenuate methamphetamine-induced

increases in impulsive behavior in rats as measured changes in responding maintained by a DRL 18 schedule. Statistically significant increases in response rate and corresponding decreases in reinforcement rate were observed in (+) methamphetamine-treated animals compared to saline-treated animals. Several potential limitations of this study are worth noting.

One limitation of the current study was the administration of the probiotic supplement via the animal's drinking water. Animals had free access to water in their home cages and individual animals consumed varying amounts from day to day. Although water bottles were weighed each day to obtain estimates of consumption, the specific amount of probiotic supplement was not precisely controlled to be identical across rats. Future studies should utilize a different approach to administer oral probiotics, such as mixing the probiotic supplement with daily food rations to assure animals consume the complete amount. Although oral gavage has been used in previous studies to administer probiotics (Li et al., 2018), this route was not used in the current study because it is technically more challenging and potentially stressful to the animal. Another limitation of this study was that the animals were not experimentally naïve. Prior to the present study, the rats were used in an undergraduate operant conditioning laboratory course. Due to this, the animals were approximately six-months old at the start of the experiment. Future studies should consider using experimentally naive rats and younger rats due to developmental variations.

The present study was also limited to the investigation of alterations to the gut microbiome in male rats. Thus, no female rats were assessed in the present study. Previous research on alterations to the gut microbiome has revealed sex differences between dietary intervention and behavioral outcomes (Hass et al., 2020). The findings of Haas et al. (2020) are consistent with previous literature indicating sex differences and the outcomes of dietary

interventions on behavior. The authors note possible behavioral differences may be due to sex steroid hormones, such as estrogen, and bacterial interactions (Haas et al., 2020). Further research should be conducted to assess sex differences associated with dietary interventions and subsequent behavioral outcomes.

The present study findings are consistent with previous reports regarding psychostimulant drug effects on rodent behavior maintained by DRL schedules. For example, Sabol et al. (1995) assessed several psychoactive compounds on behavior maintained under a DRL 36 s schedule. Differential results were produced by prototypical psychostimulant drugs that act primarily as dopamine releasers (amphetamine and methamphetamine) and drugs that act as serotonin releasers (MDMA, fenfluramine). The psychostimulants produced the largest increase in response rate, dose-dependent decrease in reinforcement rate, and a leftward shift in the peak IRT distribution. Additionally, Hyatt et al. (2020) assessed the effects of MDPV and cocaine on rodent behavior on a DRL 20 s schedule. The results suggest an increase in impulsive action as assessed by an increase in premature responses, a decrease in timing error, and reinforcers earned. These results coincide with the present study in which (+) methamphetamine increased response rate, decreased in reinforcement rate, and produced an overall leftward shift in the IRT distribution. Collectively, these results are consistent that psychostimulants produce an increase in impulsive action, as indexed by changes in low-rate behaviors maintained on a DRL schedule.

Previous preclinical research has assessed the effects of psychostimulant drugs, such as methamphetamine, on gut homeostasis and behavioral changes. Zang et al. (2022) examined methamphetamine-induced changes to the gut microbiome and behavior using a variety of behavioral assays. The authors noted a correlation between gut microbiome changes and an increase in locomotor sensitization and depression-like behavior as indexed by the open field

test, forced swim test, and tail suspension test. Anxiety-like behaviors were also noted as indexed by the light-dark activity (Zang et al., 2022). Together, the findings of Zang et al. (2020) indicate methamphetamine induces gut-dysbiosis and correlated behavioral changes which are relevant to understanding methamphetamine-induced toxicity. A study assessing CPP in male rats before and after exposure to methamphetamine also revealed behavioral differences associated with gut dysbiosis and methamphetamine administration (Yang et al., 2020). Lastly, Forouzan et al. (2021a) assessed the gut microbiome and methamphetamine-induced changes to behavior in the open field test, the elevated plus maze, and the forced swim test. The authors suggest methamphetamine and its cessation is associated with dysbiosis of the gut and depressive-like behaviors in rodents. In the present study, analysis of microbial DNA is ongoing. Therefore, the dietary intervention and drug treatment effects on gut microbiome composition are yet to be determined.

Previous preclinical studies have assessed the effects of probiotics in rodent behavioral assays predictive of depression and anxiety. Desbonnet et al. (2008) evaluated *Bifidobacteria infantis* in behavioral assays predictive of antidepressant-like effects. No behavioral differences were obtained between the probiotic-treated and control animals. Similarly, Haas et al. (2020) assessed *Bifidobacterium longum* in the open field test and the forced swim test to assess anxiety and depression. In addition to the probiotic intervention, animals were treated with corticosterone or control injections to mimic stress. The behavioral effects produced by the corticosterone were not altered by the probiotic treatment. The present study results are consistent with these findings as the probiotic intervention did not produce statistically significant behavioral differences. The present study assessed impulsive action of animals in a DRL 18 s paradigm whereas previous literature has investigated anxiety- and depression-like behavior in paradigms such as the forced

swim test and the open field test (Desbonnet et al., 2008; Haas et al., 2020). Additionally, the current study evaluated a multispecies probiotic supplement, Bio-Kult ®, whereas many previous studies have evaluated single strains of probiotic. The present findings are consistent with previous literature despite differences in the behavioral assays and probiotic supplement assessed.

In summary, the current study is the first to examine the effects of an oral probiotic supplement targeting gut health on (+) methamphetamine-induced behavioral changes indicative of impulsive action. The study was conducted to assess the hypothesis that dietary interventions could improve neurocognitive deficits associated with SUD. This hypothesis was based on previous reports implicating a relationship between gut microbiome changes and overall brain function. The results obtained in the present study failed to indicate any protective effects of a commercially available multispecies probiotic supplement on methamphetamine-induced impulsive action in adult male rats. Despite these negative findings, additional investigations are warranted to further address this research hypothesis. Future studies could implement a behavioral assay of impulsive choice, such as delayed discounting. Delayed discounting utilizes operant conditioning to assess variables associated with impulsive choice. Choice is assessed by presenting concurrent schedules of reinforcement with the chance to earn either a small immediate reward or a larger delayed reward. Other animal behavior assays relevant to SUD could also be explored, such as conditioned drug reward and drug self-administration. Future studies could also examine the wide variety of commercially available probiotic supplements for efficacy in altering gut microbiome composition. The present study utilized Bio-Kult® which is a 14-strain probiotic supplement. Investigation of various probiotic strains, both in combination

and isolation, is warranted to assess the potential changes to the gut microbiome and its impact on drug-induced behavioral changes.

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WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

Date: November 11, 2020

To: Lisa Baker, Principal Investigator

From: Kathryn Eckler, Vice Chair Kathryn A Eckler, PhD

Re: IACUC Protocol Number 20-11-02

This letter will serve as confirmation that your research project "Behavioral Assessments of Probiotic Treatments in Rats" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: **November 10, 2021**

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