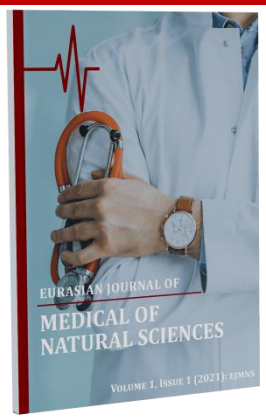
EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
IF = 7.921 www.in-academy.uz



EFFECTS OF ADRENALECTOMY ON THE GUT MICROBIOME AND HYPERTHERMIA INDUCED BY MDMA

Usanova Saodat Turayevna
Senior lecturer at the Medical faculty of the Alfraganus University
Email: usanovasadi1974@gmail.com
https://doi.org/10.5281/zenodo.14642828

ARTICLE INFO

Received: 08th January 2025
Accepted: 13th January 2025
Online: 14th January 2025

KEYWORDS

Adrenalectomy, gut microbiome, MDMA, hyperthermia, sympatho-adrenal axis, corticosterone, norepinephrine, phenylethylamine, microbiome diversity, gut-brain axis, thermoregulation, microbiota modulation, 16S rRNA sequencing.

ABSTRACT

The widespread use of the stimulant drug 3,4-methylenedioxymethamphetamine (MDMA), commonly referred to as Ecstasy, Molly, or X, has been associated with life-threatening hyperthermia in both humans and animal models. This study aimed to explore the role of the gut-adrenal axis in MDMA-induced hyperthermia by examining the effects of acute exogenous supplementation with norepinephrine (NE) or corticosterone (CORT) in adrenalectomized (ADX) rats following MDMA administration. MDMA (10 mg/kg, subcutaneous) significantly elevated body temperature in SHAM animals compared to ADX animals at 30-, 60-, and 90-minute intervals post-treatment. The attenuated hyperthermic response observed in ADX animals was partially restored with exogenous administration of NE (3 mg/kg, intraperitoneal) or CORT (3 mg/kg, intraperitoneal) 30 minutes after MDMA treatment. Additionally, 16S rRNA analysis revealed notable differences in gut microbiome composition and diversity, including a higher abundance of minor phyla Actinobacteria, Verrucomicrobia, and Proteobacteria in ADX rats compared to control and SHAM rats. MDMA administration further induced significant changes in the dominant phyla Firmicutes and Bacteroidetes, as well as minor phyla Actinobacteria, Verrucomicrobia, and Proteobacteria in ADX animals. Exogenous CORT treatment increased Bacteroidetes and decreased Firmicutes, while NE treatment increased Firmicutes and decreased Bacteroidetes and Proteobacteria. These findings highlight a potential relationship between the sympathoadrenal axis, gut microbiome composition, and MDMA-induced hyperthermia.

1. Introduction

The use of the substituted phenylethylamine 3,4-methylenedioxymethamphetamine (MDMA) continues to rise across the world (Garc\u00eda-Montes et al., 2021). According to the 2016 National Survey on Drug Use and Health, over 18 million individuals reported having used MDMA at least once in their lifetime (2016 National Survey on Drug Use and Health). While MDMA is known for its euphoric and entactogenic effects (Garc\u00eda-Montes et al., 2021), its use is associated with several severe adverse effects, including hypertension, psychosis, seizures, and hyperthermia, which can escalate to rhabdomyolysis, multi-organ failure, and even death (Colado et al., 1995; Mas et al., 1999; Sprague et al., 2003a).

Phenylethylamines, including MDMA, have been linked to elevated corticosterone (CORT) levels in both rodents and humans (Downey et al., 2015; Fernandez et al., 2002; Herring et al., 2010; Makisumi et al., 1998; McNamara et al., 1995; Parrott et al., 2014; Wolff et al., 2012). The activation of the hypothalamic-pituitary-adrenal (HPA) axis by phenylethylamines has been implicated in phenylethylamine-induced hyperthermia (Fernandez et al., 2002; Makisumi et al., 1998). Nash et al. (1988) demonstrated that MDMA administration triggered a hyperthermic response and increased CORT levels within 30 minutes post-administration. Similarly, Makisumi et al. (1998) reported that adrenalectomized (ADX) rats exhibited a blunted hyperthermic response to methamphetamine (MA), which was restored with the administration of dexamethasone. In human studies, MDMA was shown to acutely increase plasma cortisol levels (Seibert et al., 2014), and regular MDMA users displayed nearly four times higher hair cortisol levels compared to non-users (Parrott et al., 2014).

Norepinephrine (NE) has also been identified as a significant contributor to MDMA-induced hyperthermia (Sprague et al., 2007). Administration of the phenylethanolamine N-methyltransferase inhibitor \u00b1 2,3-dichloro-\u03b1-methylbenzylamine prior to MDMA treatment not only increased plasma NE levels but also amplified the hyperthermic response (Sprague et al., 2007). Additionally, prazosin, an \u03b11-adrenoreceptor antagonist, has been shown to attenuate MDMA-induced hyperthermia in rats (Sprague et al., 2003a; Sprague et al., 2003b). Consistent with these findings, \u03b11-antagonism (Hysek et al., 2013a) or combined \u03b1-\u03b2 adrenoceptor blockade (Hysek et al., 2013b) also reduced the thermogenic response to MDMA in humans.

Changes in CORT levels have been associated with modulations in gut microbiome composition and diversity. Sudo et al. (2004) demonstrated that germ-free (GF) mice exhibited higher plasma adrenocorticotropic hormone (ACTH) and CORT levels under stress compared to specific pathogen-free (SPF) mice. These effects were reversed by recolonizing the gut with *Bifidobacterium infantis* (Sudo et al., 2004). Similarly, Crumeyrolle-Arias et al. (2014) found that GF rats showed higher CORT levels compared to SPF rats. Although these studies suggest a link between CORT levels and gut microbiome composition, the direct correlation between phenylethylamine-induced hyperthermia, changes in CORT levels, and gut microbiome alterations remains unreported.

In this study, we investigated the correlation between gut microbiome composition and diversity, NE and CORT levels, and MDMA-induced hyperthermia in SHAM and ADX male rats.

2. Materials and Methods

2.1. Animals

Male SHAM and adrenalectomized (ADX) Sprague-Dawley rats (*Rattus norvegicus domesticus*), initially weighing between 300–324 g, were acquired from Envigo (Indianapolis, IN). In line with previous studies following adrenalectomy (Makisumi et al., 1998; Terada et al., 2021), rats were housed individually in cages measuring 21.0 × 41.9 × 20.3 cm under a 12:12 h light/dark cycle. Animals had ad libitum access to food and water, were maintained at an ambient temperature of 25–28 °C, and were provided with a diet containing a minimum of 10% fat to optimize thermogenic responses (Dafters, 1995; Mills et al., 2007).

Animal care and research procedures adhered to the eighth edition of the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health) and were approved by the Animal Care and Use Committee at Bowling Green State University (protocol #1751804). All animals were acclimated to the facility for one week before beginning treatments.

2.2. Drugs and Chemicals

Racemic MDMA, obtained as a hydrochloride salt from Cayman Chemicals (Ann Arbor, MI), was prepared fresh on the day of the study at a concentration of 10 mg/ml in 0.9% normal saline. This dose of MDMA (10 mg/kg) was selected based on prior studies demonstrating its significant hyperthermic effects in rats (Colado et al., 1995; Mills et al., 2007).

Norepinephrine hydrochloride (NE) and corticosterone (CORT) HBC complex were sourced from Sigma Chemical (St. Louis, MO). Both compounds were prepared fresh on the day of treatment at a concentration of 3 mg/ml in 0.9% normal saline. The 3 mg/kg intraperitoneal (ip) dose of NE was chosen based on evidence of its adrenergic effects in rats (Neri et al., 2007). Similarly, a 3 mg/kg ip dose of CORT was selected based on its ability to increase norepinephrine levels in the amygdala following avoidance training in rats (McReynolds et al., 2010). Given the role of the sympathetic nervous system in MDMA-induced hyperthermia, these doses were employed in the present study.

2.3. Study Design

2.3.1. Fecal Droppings Collection

Rats were randomly assigned to one of five treatment groups: saline, SHAM-MDMA, ADX-MDMA, ADX-MDMA-CORT, or ADX-MDMA-NE (Fig. 1). Twenty-four hours before MDMA treatment, rats were placed in cages with sterile bedding to allow for the accumulation of fresh fecal droppings, which were collected and flash-frozen using liquid nitrogen. Samples were then transferred to a -80 °C freezer for storage until analysis (pre-treatment samples).

On the treatment day, fecal samples were collected again from each animal following the same procedure. Twenty-four hours post-treatment, fecal droppings were collected once more using the same process and stored for later analysis (post-treatment samples).

EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
IF = 7.921 www.in-academy.uz

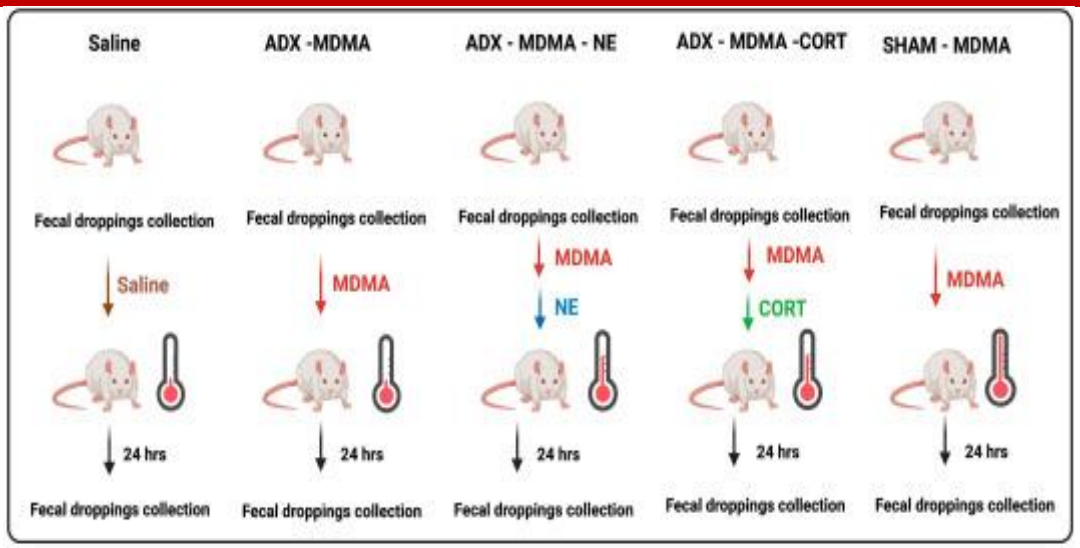


Fig. 1. Experimental study design. Male Sprague-Dawley rats were randomly divided into five groups according to the type of treatment. On treatment day [Day 1], right before the treatments fecal samples were collected and core body temperature (basal temperature) recorded for all rats in each group. Treatment with saline or MDMA (10 mg/kg sc.) followed immediately after. For the groups treated with either corticosterone (CORT) or norepinephrine (NE), freshly prepared solutions (3 mg/kg ip.) were administered 30 min after MDMA treatment. The core body temperature was recorded in intervals of 30 min after MDMA or saline challenge for 90 min 24 h post-treatment [Day 2], fecal samples were collected similarly for all rats in each group. Rats were sacrificed shortly after.

2.3.2. Drug Treatment

On the treatment day, animals were weighed, and their baseline core body temperatures were recorded. Following the baseline temperature measurements, treatment commenced as follows: saline (n = 6), SHAM-MDMA (10 mg/kg, subcutaneous, n = 6), ADX-MDMA (10 mg/kg, subcutaneous, n = 5), ADX-MDMA-CORT (CORT 3 mg/kg intraperitoneal, administered 30 minutes after MDMA 10 mg/kg subcutaneous, n = 5), and ADX-MDMA-NE (NE 3 mg/kg intraperitoneal, administered 30 minutes after MDMA 10 mg/kg subcutaneous, n = 5). The saline control group did not undergo surgery.

Temperature measurements continued at 30-minute intervals for 90 minutes after the initial MDMA or saline administration. Core body temperature was measured using a rectal probe thermometer. The probe was lubricated with white petroleum jelly and gently inserted into the rectum, reaching a steady reading within 10 seconds. All drug dosages were selected based on previously published studies (Fernandez et al., 2002; McReynolds et al., 2010; Neri et al., 2007).

2.4. Microbial Community Analysis

DNA extraction was performed using fecal samples pooled by treatment groups. Approximately 250 mg of fecal material per group was processed using the DNeasy Powersoil Pro Kit (Qiagen Inc., CA), following the manufacturer\u2019s instructions. Baseline samples were pooled from all animals within each group, including ADX-MDMA and saline control groups. Post-treatment samples varied due to animal mortality from MDMA-induced hyperthermia: ADX-MDMA-CORT (n = 4), ADX-MDMA-NE (n = 1), and SHAM-MDMA (n = 0).

DNA quality was assessed using 0.8% agarose gel electrophoresis, and concentrations were measured with a NanoDrop Spectrophotometer (Thermo, MI). Library preparation and sequencing of the 16S rRNA genes were performed by Laragen Sequencing and Genotyping (Culver City, CA) using primers BAC357TS-for (CCTACGGGNGGCWGCAG) and BAC806TS-rev (GACTACHVGGGTATCTAATCC). The resulting ~450-bp amplicons spanned the V3 and V4 regions of the 16S rRNA gene.

Amplicon libraries were sequenced on the Illumina MiSeq platform as paired-end reads (2x250 bp). Raw sequence reads were trimmed to remove primers and low-quality ends, error-corrected, and dereplicated using the DADA2 package (v1.18.0) (Callahan et al., 2016) in R (v4.0.5). This process generated a total of 172,014 amplicon sequence variants (ASVs) across all samples. Taxonomic assignments were made using the Ribosomal DNA Project (RDP) (v16) database, and analyses were performed on a rarefied sample size of 9,000 reads per sample.

2.5. Statistical Analysis

All statistical analyses were performed using GraphPad InStat v6.0, and figures were generated using GraphPad Prism v6.0. Results are expressed as mean ± SEM. Statistical significance within treatment groups over time was determined using one-way ANOVA with a Student-Newman-Keuls post hoc test for comparisons between groups at each time point. To calculate the maximum change in temperature (maximum Δ °C), the highest recorded core body temperature was compared to the animal's baseline temperature. A significance threshold of $p < 0.05$ was set a priori.

3. Results

3.1. Effect of Adrenalectomy on MDMA-Induced Hyperthermia

Figure 2A illustrates the time-course of temperature changes following acute administration of MDMA or saline in male Sprague-Dawley rats. Temperature measurements were taken at baseline (time 0) and at 30, 60, and 90 minutes post-treatment. Adrenalectomy significantly suppressed the hyperthermic response to MDMA compared to SHAM animals treated under identical conditions (Fig. 2A).

In SHAM animals, a noticeable increase in body temperature began 30 minutes after MDMA administration, with a continued rise reaching a peak temperature change of 3.9 °C at the 90-minute time point (Fig. 2B). In contrast, ADX animals exhibited no significant change in body temperature throughout the monitoring period, demonstrating the absence of a hyperthermic response.

Acute supplementation of NE or CORT in ADX animals partially restored the increase in body temperature, as evidenced by a significant rise in maximum temperature change. However, the maximum temperature change in these groups remained significantly lower compared to the SHAM-MDMA treatment group (Fig. 2B).

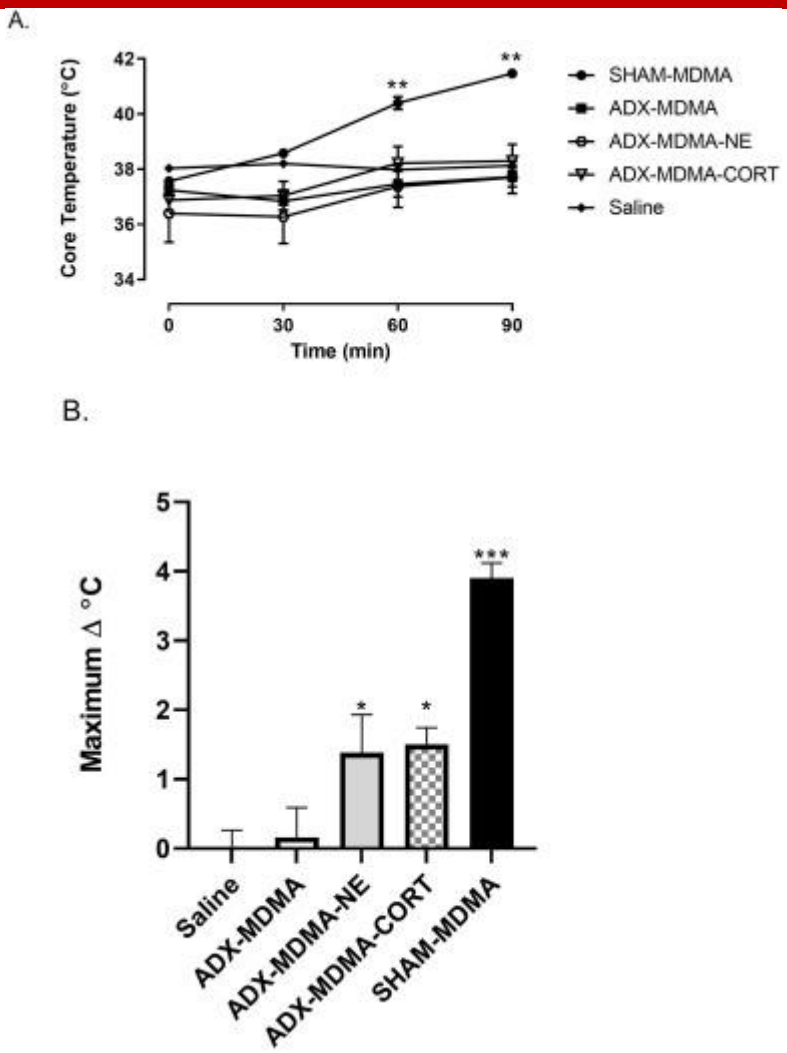


Fig. 2. The Impact of Adrenalectomy and External Corticosterone or NE Supplementation on MDMA-Induced Hyperthermia. (A) Adrenalectomized (ADX) rats (n = 5) showed no significant change in body temperature following MDMA administration (10 mg/kg, subcutaneous) compared to SHAM animals (n = 6). However, supplementation with norepinephrine (NE) or corticosterone (CORT) (3 mg/kg, intraperitoneal) partially restored the hyperthermic response in ADX rats. (B) The effect of NE and corticosterone supplementation on the maximum temperature change induced by MDMA was also examined. No significant difference was observed in the maximal temperature change between ADX animals treated with corticosterone and those treated with NE. *P < 0.05, **P < 0.01, and ***P < 0.001 indicate significant differences in the effect of MDMA between ADX and SHAM rats.

3.2. Effect of Adrenalectomy and Drug Challenge on Gut Microbiome Diversity and Composition

3.2.1. Impact of Adrenalectomy on Gut Microbiome

To investigate the effects of adrenalectomy on the gut microbiome, pre-treatment (Day 1) fecal samples from three ADX rat groups were compared to those from the saline control and SHAM groups. Diversity and relative abundance were analyzed across various taxonomic levels (Fig. 3, Fig. 4).

The beta diversity plot (PCoA - Bray-Curtis based, Fig. 3) for Day 1 (closed structures) indicated that the SHAM group clustered more closely with the control group, whereas the

ADX groups formed distinct clusters, separate from both the control and SHAM groups. This observation suggests that adrenalectomy significantly alters the gut microbiome composition.

The Bray-Curtis dissimilarity index between the control and SHAM groups was 0.37, while the dissimilarity between the control and ADX groups ranged from 0.51 to 0.58, further highlighting the impact of adrenalectomy on microbiome diversity.

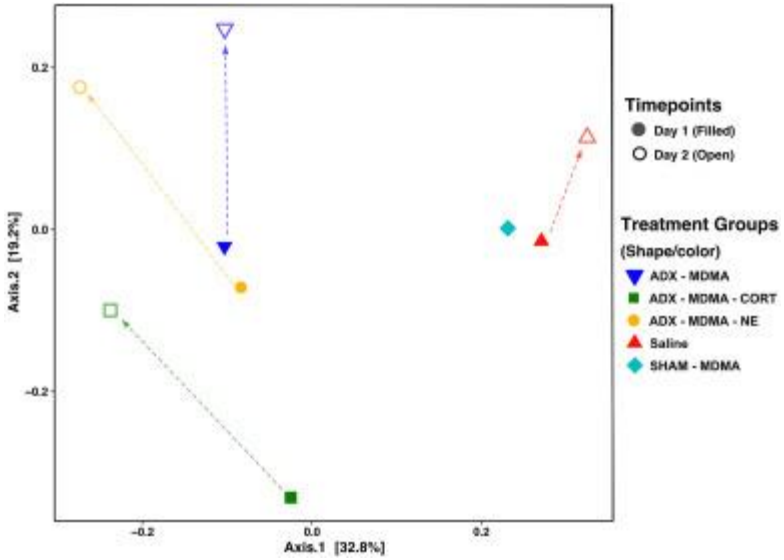


Fig. 3. Principal Coordinates Analysis (PCoA) plot showing the clustering of all treatment groups (represented by different shapes and colors) at various time points. Filled symbols represent pre-treatment groups ([Day 1]), while open symbols represent post-treatment groups ([Day 2]).

At the phylum level (Fig. 4), **Firmicutes** were the most abundant across all groups, with relative abundances of 94.3% in the Control group, 89.2 ± 4.9% in the ADX group, and 88.9% in the SHAM group. Conversely, **Bacteroidetes** showed comparatively higher levels in the ADX (6.9 ± 3.5%) and SHAM groups (9.1%) compared to the Control group (4.6%). Interestingly, the ADX groups were notably enriched with minor phyla, including **Actinobacteria** (ADX: 1.4 ± 0.2%, Control: 0.49%, SHAM: 0.59%), **Verrucomicrobia** (ADX: 0.60 ± 0.40%, Control: undetected, SHAM: 0.01%), and **Proteobacteria** (ADX: 1.8 ± 1.7%, Control: 0.61%, SHAM: 1.5%).

At the class level, the differences between groups became more pronounced (Fig. 4). In the Control group, **Clostridia** dominated with 82% relative abundance, followed by **Bacilli** (8.3%) and **Erysipelotrichia** (3.8%; all within Firmicutes). Similarly, the ADX groups were also dominated by Clostridia (69.1 ± 3.5%) but exhibited a higher abundance of Bacilli (18.3 ± 5.1%) and a lower abundance of Erysipelotrichia (1.8 ± 0.6%) compared to the Control group.

The SHAM group exhibited a composition similar to the Control group for the dominant classes, with Clostridia comprising 74.1% and Bacilli 9.2%. However, SHAM rats showed the highest relative abundance of **Erysipelotrichia** (5.5%), **Bacteroidia** (9.1%, compared to ADX: 6.8 ± 3.5% and Control: 4.5%), and the minor class **Betaproteobacteria** (SHAM: 0.97%, ADX: 0.22 ± 0.11%, Control: 0.22%). Other Proteobacteria classes (each with relative abundance < 1%) were more prevalent in ADX rats compared to the Control and SHAM groups.

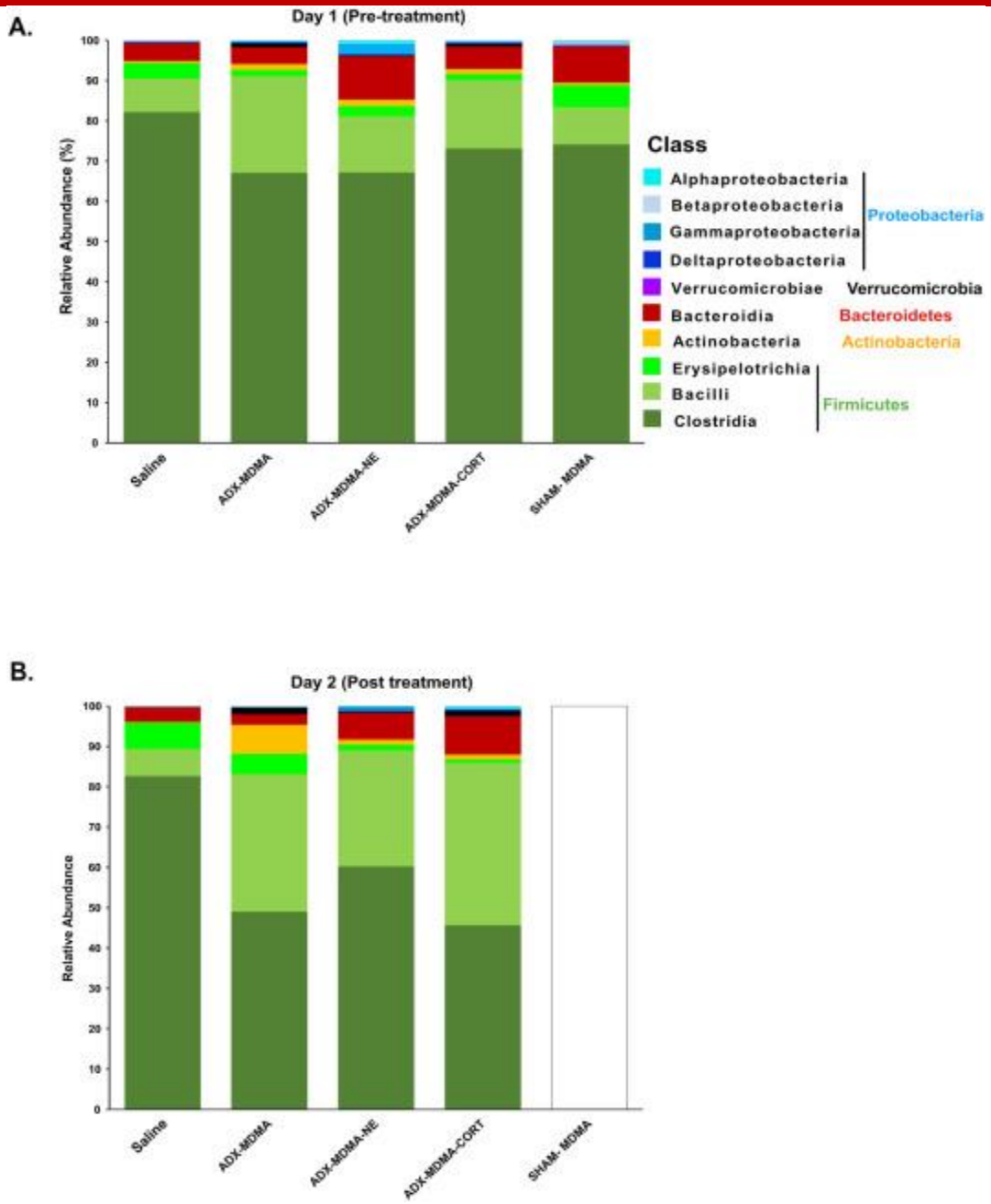


Fig. 4. This figure illustrates the relative abundance of various classes (>0.1%) within the dominant phyla across different groups on Day 1 (pre-treatment) and Day 2 (post-treatment). Baseline samples were pooled from all animals assigned to each group, including the ADX-MDMA and saline control groups post-treatment (n = 5 or 6). The sample size for post-treatment samples from the remaining groups varied due to MDMA-induced hyperthermia-related mortality: ADX-MDMA-CORT (n = 4), ADX-MDMA-NE (n = 1), and SHAM-MDMA (n = 0).

3.2.2. Effect of MDMA Treatment on Gut Microbiome in ADX Rats

To assess the effects of MDMA on the gut microbiome in ADX rats, pre-treatment (Day 1) and post-treatment (Day 2) fecal samples from all three ADX groups were compared. Since all ADX groups received MDMA (prior to CORT/NE supplementation in two groups), common changes were analyzed across these groups. Compared to saline-treated controls, MDMA induced notable changes in the gut microbiome composition in the ADX-MDMA, ADX-MDMA-

CORT, and ADX-MDMA-NE groups, as indicated by the PCoA analysis (Fig. 3). The Bray-Curtis (BC) dissimilarity value was lower in the saline group (0.33) and higher in the ADX-MDMA (0.45), ADX-MDMA-CORT (0.45), and ADX-MDMA-NE (0.45) groups following treatment.

Post-MDMA treatment (Day 2), all three ADX groups exhibited a further shift in beta diversity compared to their respective pre-treatment (Day 1) counterparts. This shift was indicated by an increase in BC dissimilarity values: from 0.51 (Day 1) to 0.54 (Day 2) for ADX-MDMA, from 0.52 (Day 1) to 0.65 (Day 2) for ADX-MDMA-CORT, and from 0.58 (Day 1) to 0.66 (Day 2) for ADX-MDMA-NE groups.

Taxonomic analysis of the saline-treated control group revealed minimal changes in major phyla, with Firmicutes remaining relatively stable (94.3% pre-treatment vs. 95.7% post-treatment) and Clostridia (the dominant class within Firmicutes) maintaining ~82% abundance. Minor phyla showed some changes, but overall, the microbial composition remained consistent (Fig. 4). In contrast, common post-MDMA changes were observed across all ADX groups, including an increase in **Verrucomicrobia** (from 0.6 ± 0.4% to 1 ± 0.7%) and significant alterations in Firmicutes classes. Clostridia abundance decreased (69.1 ± 3.5% to 51.6 ± 7.7%), while Bacilli abundance increased (18.3 ± 5.1% to 34.3 ± 5.8%). In the control group, these classes remained stable (Clostridia: 82.2% to 82.5%, Bacilli: 8.3% to 6.7%), suggesting that MDMA uniquely affects the gut microbiome in ADX rats.

3.2.3. Effect of CORT/NE Supplementation on Gut Microbiome

In the ADX-MDMA group, distinct post-treatment changes were observed in **Actinobacteria** (phylum Actinobacteria) and **Erysipelotrichia** (phylum Firmicutes), which increased from 1.5% to 7.8% and 1.5% to 5%, respectively, between Day 1 (pre-treatment) and Day 2 (post-treatment). In contrast, these classes remained unchanged or decreased in the other ADX groups. For example, Actinobacteria abundance ranged between 1.2% and 1.6% across ADX-MDMA-CORT and ADX-MDMA-NE groups, while Erysipelotrichia decreased from 1.5% (Day 1) to 0.86% (Day 2) in ADX-MDMA-CORT and from 2.5% (Day 1) to 1.4% (Day 2) in ADX-MDMA-NE.

Compared to ADX-MDMA, the ADX-MDMA-CORT group exhibited a higher post-treatment abundance of **Bacteroidetes** (class Bacteroidia; 5.5% pre-treatment to 9.5% post-treatment) and **Proteobacteria** (class Alphaproteobacteria; 0.8% to 1.2%). In contrast, Bacteroidetes and Proteobacteria decreased in the ADX-MDMA group (Bacteroidetes: 4.2% to 2.6%, Proteobacteria: 0.73% to 0.40%) and in the NE-supplemented group (Bacteroidetes: 10.8% to 6.7%, Proteobacteria: 3.7% to 1.4%). These findings suggest a unique modulatory effect of CORT on the gut microbiome in ADX-MDMA rats.

In the ADX-MDMA-NE group, notable changes were observed in **Firmicutes**, **Bacteroidetes**, and **Proteobacteria**. Firmicutes increased from 83.3% to 90.3%, while Bacteroidetes decreased from 10.8% to 6.6% (class Bacteroidia). Proteobacteria (all classes) also significantly declined from 3.7% to 1.4%. In comparison, the ADX-MDMA group showed decreased Firmicutes (92.5% to 88%) and Bacteroidetes (4.2% to 2.6%), whereas the ADX-MDMA-CORT group exhibited decreased Firmicutes (91.6% to 86.7%) and increased Bacteroidetes (5.5% to 9.5%). These findings highlight distinct gut microbiome alterations following NE supplementation compared to other treatments.

4. Discussion

This study explored the relationship between the gut microbiome, the sympatho-adrenal axis, and their role in MDMA-induced hyperthermia. The complete suppression of the hyperthermic response in adrenalectomized (ADX) male Sprague-Dawley rats compared to SHAM-operated rats after MDMA treatment aligns with previously reported findings (Fernandez et al., 2002; Makisumi et al., 1998). These studies suggested that corticosterone (CORT) plays a critical role in phenylethylamine-induced hyperthermia, primarily through its indirect regulation of the sympathetic nervous system (SNS) (Fernandez et al., 2002; Makisumi et al., 1998).

In earlier work, acute dexamethasone supplementation (0.5 mg/kg subcutaneous) (Makisumi et al., 1998) or chronic CORT administration (50 mg via subcutaneous implant for two weeks) (Fernandez et al., 2002) nearly restored phenylethylamine-induced hyperthermia. In this study, acute CORT supplementation (3 mg/kg intraperitoneal) 30 minutes post-MDMA treatment partially restored the hyperthermic response in ADX rats compared to those treated with MDMA alone, though this effect was still significantly lower than in SHAM rats under the same conditions. Similarly, norepinephrine (NE, 3 mg/kg intraperitoneal) supplementation 30 minutes after MDMA treatment elevated core body temperature in ADX rats, further supporting the involvement of the SNS in phenylethylamine-induced hyperthermia, consistent with prior research (Makisumi et al., 1998; Sprague et al., 2007).

Gut microbiome alterations have been linked to NE and CORT activity (Aburahma et al., 2020; Desbonnet et al., 2015). The changes in microbial diversity and taxa observed after NE and CORT supplementation in this study highlight the potential role of the gut microbiome in MDMA-induced hyperthermia. Previous work from our laboratory supports this, demonstrating that gut microbiome attenuation via antibiotics diminished MDMA-induced hyperthermia (Ridge et al., 2019). Additionally, Goldsmith et al. (2022) showed that repeated methylone exposure reduced hyperthermic responses, and fecal microbiota transplantation (FMT) between methylone-tolerant and methylone-naïve groups restored hyperthermic responses in the former.

Consistent with prior findings in animal and human studies (Angoa-Pérez et al., 2020; Yang et al., 2021), MDMA treatment in ADX rats resulted in distinct shifts in the dominant phyla Firmicutes and Bacteroidetes, as well as minor phyla Actinobacteria, Verrucomicrobia, and Proteobacteria. In particular, Actinobacteria were highly enriched in the ADX-MDMA group. Common post-MDMA changes across all ADX groups included decreased Clostridia and increased Bacilli (both within Firmicutes), indicating a shared effect of MDMA. Yang et al. (2021) also reported variations in Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria between chronic methamphetamine users and controls, while Angoa-Pérez et al. (2021) highlighted significant changes in Firmicutes and Bacteroidetes with phenylethylamine derivatives.

Desbonnet et al. (2015) reported that antibiotic-induced gut microbiome depletion altered behavior and reduced CORT-induced microbiome changes, such as elevated Bacteroidetes levels. In this study, acute CORT supplementation increased Bacteroidetes and decreased Firmicutes, consistent with stress-related changes observed in previous studies.

Liu et al. (2020), however, reported contrasting results, with stress-induced increases in Firmicutes and decreases in Bacteroidetes, Actinobacteria, and Proteobacteria in mice.

NE supplementation in this study predominantly increased Firmicutes and decreased Bacteroidetes and Proteobacteria, supporting prior findings on NE's role in microbial modulation (Bailey et al., 1999; Lyte et al., 1996). Lyte and Bailey (1997) showed that sympathectomy led to increased gram-negative bacteria, particularly *Escherichia coli,* though gram-positive bacteria changes were not examined. Variations in results across studies may stem from differences in animal models, environmental conditions, and treatment protocols.

5. Conclusion

This study examined the influence of the gut microbiome and the sympatho-adrenal axis on MDMA-induced hyperthermia by assessing the effects of acute CORT or NE supplementation in ADX rats. While adrenalectomy suppressed MDMA-induced hyperthermia, CORT and NE supplementation partially restored the hyperthermic response. Furthermore, these treatments were associated with distinct changes in gut microbiome composition and diversity, as evidenced by 16S rRNA analysis. These findings highlight the interplay between the adrenal-gut axis and MDMA-induced hyperthermia and suggest the potential for microbiome-targeted interventions, such as probiotics, to modulate phenylethylamine-induced hyperthermia. This work provides a foundation for future research into microbiome-based therapeutic strategies targeting the adrenal-gut axis.

References:

1. A. Aburahma, S. Pachhain, S.R. Choudhury, S. Rana, V. Phuntumart, R. Larsen, J.E. Sprague. Potential contribution of the intestinal microbiome to phenthylamine-induced hyperthermia. *Brain Behav. Evol.*, 95 (2020), pp. 256-271. https://doi.org/10.1159/000512098

2. M. Angoa-Pérez, B. Zagorac, A.D. Winters, J.M. Greenberg, M. Ahmad, K.R. Theis, D.M. Kuhn. Differential effects of synthetic psychoactive cathinones and amphetamine stimulants on the gut microbiome in mice. *PLoS One*, 15 (2020), Article e0227774. https://doi.org/10.1371/journal.pone.0227774

3. M. Angoa-Pérez, B. Zagorac, D.M. Francescutti, K.R. Theis, D.M. Kuhn. Responses to chronic corticosterone on brain glucocorticoid receptors, adrenal gland, and gut microbiota in mice lacking neuronal serotonin. *Brain Res.*, 1751 (2021), Article 147190. https://doi.org/10.1016/j.brainres.2020.147190

4. M.T. Bailey, J.W. Karaszewski, G.R. Lubach, C.L. Coe, M. Lyte. In vivo adaptation of attenuated *Salmonella typhimurium* results in increased growth upon exposure to norepinephrine. *Physiol. Behav.*, 67 (1999), pp. 359-364. https://doi.org/10.1016/S0031-9384(99)00087-6

5. B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods*, 13 (2016), pp. 581-583. https://doi.org/10.1038/nmeth.3869

6. M.I. Colado, J.L. Williams, A.R. Green. The hyperthermic and neurotoxic effects of “Ecstasy” (MDMA) and 3,4 methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a

model of the CYP2D6 poor metabolizer phenotype. *Br. J. Pharmacol.*, 115 (1995), pp. 1281-1289.

7. M. Crumeyrolle-Arias, M. Jaglin, A. Bruneau, S. Vancassel, A. Cardona, V. Daugé, L. Naudon, S. Rabot. Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, 42 (2014), pp. 207-217. https://doi.org/10.1016/j.psyneuen.2014.01.014

8. R.I. Dafters. Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. *Physiol. Behav.*, 58 (1995), pp. 877-882. https://doi.org/10.1016/0031-9384(95)00136-7

9. L. Desbonnet, G. Clarke, A. Traplin, O. O'Sullivan, F. Crispie, R.D. Moloney, P.D. Cotter, T.G. Dinan, J.F. Cryan. Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav. Immun.*, 48 (2015), pp. 165-173. https://doi.org/10.1016/j.bbi.2015.04.004

10. L.A. Downey, H. Sands, L. Jones, A. Clow, P. Evans, T. Stalder, A.C. Parrott. Reduced memory skills and increased hair cortisol levels in recent Ecstasy/MDMA users: significant but independent neurocognitive and neurohormonal deficits. *Hum. Psychopharmacol. Clin. Exp.*, 30 (2015), pp. 199-207. https://doi.org/10.1002/hup.2474

11. F. Fernandez, S. Aguerre, P. Mormède, F. Chaouloff. Influences of the corticotropic axis and sympathetic activity on neurochemical consequences of 3,4-methylenedioxymethamphetamine (MDMA) administration in Fischer 344 rats. *Eur. J. Neurosci.*, 16 (2002), pp. 607-618. https://doi.org/10.1046/j.1460-9568.2002.02110.x

12. J.M. García-Montes, M. Pérez-Álvarez, M.Á. Sánchez-Moya, J.A.C. Torres, D.F. Carreno, S.P. Garcelán, L. del C. Sánchez-Sánchez. Ecstasy (MDMA): a rebellion coherent with the system. *Nordic Stud. Alcohol Drugs*, 38 (2021), pp. 89-102. https://doi.org/10.1177/1455072520954329

13. R. Goldsmith, A. Aburahma, S. Pachhain, S.R. Choudhury, V. Phuntumart, R. Larsen, C.S. Ward, J.E. Sprague. Reversal of temperature responses to methylone mediated through bi-directional fecal microbiota transplantation between hyperthermic tolerant and naïve rats. *Temperature*, 9 (2022), pp. 318-330. https://doi.org/10.1080/23328940.2022.2069965

14. N.R. Herring, G.A. Gudelsky, C.V. Vorhees, M.T. Williams. (+)-Methamphetamine-induced monoamine reductions and impaired egocentric learning in adrenalectomized rats is independent of hyperthermia. *Synapse*, 64 (2010), pp. 773-785. https://doi.org/10.1002/syn.20784

15. C.M. Hysek, A.E. Fink, L.D. Simmler, M. Donzelli, E. Grouzmann, M.E. Liechti. Alpha-adrenergic receptors contribute to the acute effects of MDMA in humans. *J. Clin. Psychopharmacol.*, 33 (2013), pp. 658-666. https://doi.org/10.1097/JCP.0b013e3182979d32

16. C.M. Hysek, Y. Schmid, A. Rickli, M.E. Liechti. Carvedilol inhibits the cardiostimulant and thermogenic effects of MDMA in humans: lost in translation. *Br. J. Pharmacol.*, 170 (2013), pp. 1273-1275. https://doi.org/10.1111/bph.12398

17. Q.F. Liu, H.-M. Kim, S. Lim, M.-J. Chung, C.-Y. Lim, B.-S. Koo, S.-S. Kang. Effect of probiotic administration on gut microbiota and depressive behaviors in mice. *DARU J. Pharm. Sci.*, 28 (2020), pp. 181-189. https://doi.org/10.1007/s40199-020-00329-w

18. M. Lyte, B.P. Arulanandam, C.D. Frank. Production of Shiga-like toxins by *Escherichia coli* O157:H7 can be influenced by the neuroendocrine hormone norepinephrine. *J. Lab. Clin. Med.*, 128 (1996), pp. 392-398. https://doi.org/10.1016/s0022-2143(96)80011-4

19. M. Lyte, M.T. Bailey. Neuroendocrine-bacterial interactions in a neurotoxin-induced model of trauma. *J. Surg. Res.*, 70 (1997), pp. 195-201. https://doi.org/10.1006/jsre.1997.5130

20. T. Makisumi, K. Yoshida, T. Watanabe, N. Tan, N. Murakami, A. Morimoto. Sympatho-adrenal involvement in methamphetamine-induced hyperthermia through skeletal muscle hypermetabolism. *Eur. J. Pharmacol.*, 363 (1998), pp. 107-112. https://doi.org/10.1016/S0014-2999(98)00758-4