

## Potential of *Limosilactobacillus*-Linked 3-OMDP as a Treatment Target in Depression

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### Abstract

Although the gut microbiota has been strongly implicated in the development of depression, its precise molecular mechanisms are still poorly understood. This study aimed to clarify the relationship between gut microbial alterations, fluctuations in neurotransmitters, and inflammatory responses in a murine model of depression. A chronic social defeat stress (CSDS) paradigm was used to induce depressive-like states. Fecal samples were analyzed for microbial composition and neurotransmitter levels, while neurotransmitters were also assessed in colon tissue, blood, and hippocampus. Inflammatory mediators were quantified in the hippocampus. After identifying a key neurotransmitter of interest, an intervention study was conducted to investigate its therapeutic potential in mitigating depressive symptoms. The analysis revealed that six gut microbial genera differed between groups, fourteen neurotransmitters altered along the gut-brain axis, and two hippocampal cytokines—interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6)—with significant changes in the depressed mice. Strong correlations emerged between altered microbial taxa, neurotransmitter profiles, and the expression of IL-1 $\beta$  and IL-6. Notably, 3-O-Methyldopa (3-OMDP) showed consistent decreases in feces, colon, circulation, and hippocampus, and was closely associated with *Limosilactobacillus* abundance and IL-1 $\beta$  levels. 3-OMDP administration alleviated depressive-like behaviors and normalized hippocampal IL-1 $\beta$  and IL-6 levels. The findings suggest that gut microbes may influence neuroinflammation through neurotransmitter modulation, thereby contributing to depressive pathophysiology. A potential mechanistic route, the *Limosilactobacillus*–3-OMDP–IL-1 $\beta$ /IL-6 axis, was identified, with 3-OMDP showing therapeutic promise for the treatment of depression.

**Keywords:** Depression, Inflammation, Gut microbiota, Neurotransmitters

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### Introduction

Depression is a prevalent mood disorder that significantly impacts both mental and physical well-being [1, 2]. It is

characterized by persistent sadness, lack of interest, sleep disturbances, and, in severe cases, suicidal thoughts [3]. Globally, depression ranks as the fourth most common disorder, affecting over 350 million individuals, with the incidence steadily rising [4]. Despite notable

advancements in understanding the causes and treatment options for depression over recent decades, the precise mechanisms underlying the disorder remain largely elusive [5, 6]. This knowledge gap poses substantial obstacles for effective prevention and therapeutic interventions, highlighting the urgent need to uncover novel pathological mechanisms to inform better treatment strategies.

The human gut hosts nearly 100 trillion microorganisms that play crucial roles in host physiology, including digestion, immune regulation, and neurotransmitter production [7–9]. Increasing evidence points to gut microbiota as a critical contributor to depression [10–12]. Our prior studies demonstrated that individuals with depression exhibit distinct gut microbial profiles compared to healthy controls [13–16], which aligns with findings from other research groups [17, 18]. Typically, individuals with depression exhibit reduced microbial diversity and an increased abundance of pathogenic bacteria [19, 20]. Dysbiosis can compromise intestinal barrier integrity, trigger inflammatory responses, and potentially contribute to depression onset and progression [21, 22]. Furthermore, recent studies in mice suggest that gut-derived metabolites can influence brain activity and anxiety-like behaviors [23, 24]. Nevertheless, the precise mechanisms by which gut microbiota interacts with depressive pathology remain to be elucidated.

Gut microbes produce a variety of metabolites, including short-chain fatty acids, neurotransmitters, and inflammatory mediators [25, 26], all of which are implicated in neuropsychiatric disorders such as depression [13, 27]. Neurotransmitters, in particular, are essential for maintaining cerebral chemical balance [28, 29]. Previous work by our group has found altered levels of specific neurotransmitters, such as tryptophan and tyrosine, in depressed mice [23, 30, 31]. Morais *et al.* [32] have reported that gut microbiota communicates with the brain via neurotransmitters and inflammatory molecules through the so-called “gut-brain axis”. Moreover, gut microbial composition can influence central nervous system (CNS) physiology and neuroinflammation [33, 34], suggesting a complex interplay between microbiota, neurotransmission, inflammation, and the development of depression.

In this study, we further investigated the interplay between gut microbiota and depression using a chronic social defeat stress (CSDS) mouse model. Neurotransmitter levels were assessed along the gut-brain axis, including feces, colon, blood, and hippocampus, while four hippocampal inflammatory factors were also measured. Our focus was on neurotransmitters involved in GABAergic and catecholaminergic pathways. By integrating microbial, neurotransmitter, and inflammatory data, we aimed to elucidate how gut microbiota may

contribute to depressive pathology. Furthermore, upon identifying a critical neurotransmitter, we conducted an intervention study to assess its potential to mitigate depressive-like behaviors, providing new insights into depression pathogenesis and therapeutic strategies.

## Methods and Materials

### *Ethics statement*

This study was designed, executed, and reported in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. All experimental protocols were reviewed and approved by the Ethics Committee of Chongqing Medical University (Chongqing, China; approval number: 2017013). Male C57BL/6J mice, aged 7–8 weeks, were sourced from ENSIWEIER Laboratory Animal Co., Ltd. (Chongqing, China). Retired CD1 breeders (7 months old, 30–35 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animals were maintained following the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals: Eighth Edition. At the study’s conclusion, mice were anesthetized and cardiac-perfused according to the American Veterinary Medical Association guidelines.

### *CSDS depression model construction*

To establish a chronic social defeat stress (CSDS) model of depression, mice were randomly allocated into either the control (CON) group or the CSDS group, ensuring comparable body weights and baseline sucrose preference (SP) between groups. Each group consisted of eight mice, consistent with our prior studies [23, 30, 31]. Mice in the CSDS group were exposed daily to different aggressive CD1 mice for 5–10 minutes, followed by 24 hours of sensory contact, over a period of 10 consecutive days. Control mice were individually housed under standard laboratory conditions. All animals were maintained in a controlled environment with a 12-hour light/dark cycle (lights on at 8:00 am) and had unrestricted access to food and water.

Following CSDS exposure, behavioral assessments were performed to validate the depression model. The tests included: the sucrose preference test (SPT) to measure anhedonia; the open field test (OFT) to evaluate anxiety-like and exploratory behaviors, with center distance (CD%) or center time as indicators; the forced swim test (FST) and tail suspension test (TST) to assess behavioral despair, using immobility time as the metric. All procedures followed the protocols previously described in our studies [20].

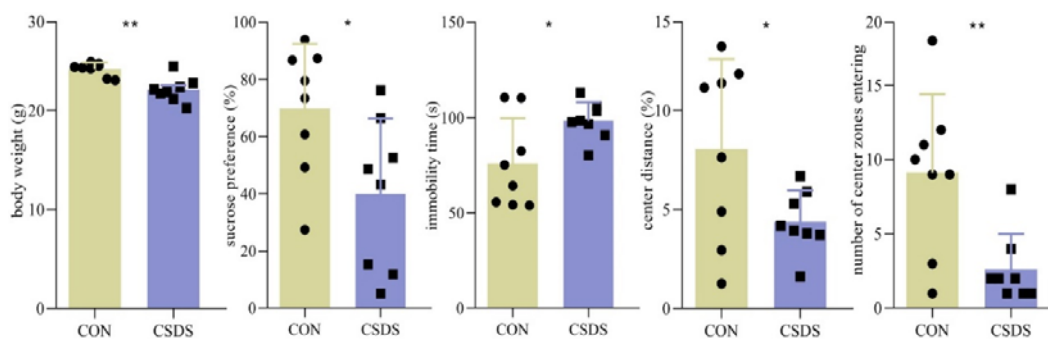
### *Gut microbiota, neurotransmitters, and inflammatory factor assessment*

Samples, including feces, colon, blood, and hippocampus, were collected promptly and stored at  $-80^{\circ}\text{C}$ . Fecal samples were analyzed to assess gut microbiota composition, while neurotransmitters in all sample types were quantified using liquid chromatography–mass spectrometry (LC-MS), following previously established protocols [23, 30, 35]. Hippocampal levels of alpha 1-antitrypsin (AAT), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were measured using ELISA kits (Jiangsu Meimian Industrial Co., Ltd). Tissue homogenates were prepared in sterile phosphate-buffered saline (PBS) at a 1:9 ratio, then centrifuged at 12,000 rpm for 15 minutes. The resulting supernatant was collected for analysis, following the manufacturer's instructions.

### 3-O-Methyldopa (3-OMDP) intervention

3-OMDP (MedChemExpress, HY-113468A) was dissolved in sterile PBS and administered at a dose of 3 mg/kg/day, based on prior literature [36]. Following the establishment of the CSDS model, mice in the CSDS group were divided into two subgroups, matched for body weight and SP: one received intraperitoneal PBS ( $n = 8$ ), and the other received intraperitoneal 3-OMDP ( $n = 8$ ) daily for 10 days. Similarly, CON mice were assigned to PBS or 3-OMDP treatment subgroups ( $n = 8$  each). After the intervention, behavioral tests were repeated to evaluate antidepressant effects, and hippocampal inflammatory markers were measured.

### Statistical analysis



**Figure 1.** Depressive-like behaviors in CSDS mice. Mice subjected to chronic social defeat stress (CSDS) displayed pronounced depressive-like phenotypes compared to control (CON) mice. Specifically, CSDS mice exhibited reduced body weight, lower sucrose consumption, decreased time spent in the center zone, and increased immobility duration. Each group included eight mice

### Differential gut microbiota compositions in CSDS mice

No significant differences in alpha diversity metrics were observed between CSDS and CON mice (ACE,  $P = 0.87$ ; Chao,  $P = 0.75$ ; Simpson,  $P = 0.07$ ; Shannon,  $P = 0.11$ ;  $n = 8$  per group; **Figure 2(A)**). At the phylum level, Firmicutes and Bacteroidetes dominated the fecal

Group allocation was blinded to all researchers except the two corresponding authors. Data were analyzed using SPSS 20.0, Cytoscape 3.10.0, and R 4.0.5. Appropriate statistical tests included Student's t-test, non-parametric tests, and Spearman's correlation. Multiple testing corrections were applied using the Benjamini–Hochberg method. Alpha diversity was evaluated using ACE, Chao, Simpson, and Shannon indices, while beta diversity was assessed via principal coordinate analysis (PCoA). Differential microbial genera were identified using Linear Discriminant Analysis Effect Size (LEfSe;  $\text{LDA} > 2.0$ ,  $P < 0.05$ ). Correlations among behavioral outcomes, microbial genera, neurotransmitters, and inflammatory factors were analyzed to explore gut-brain interactions.

## Results

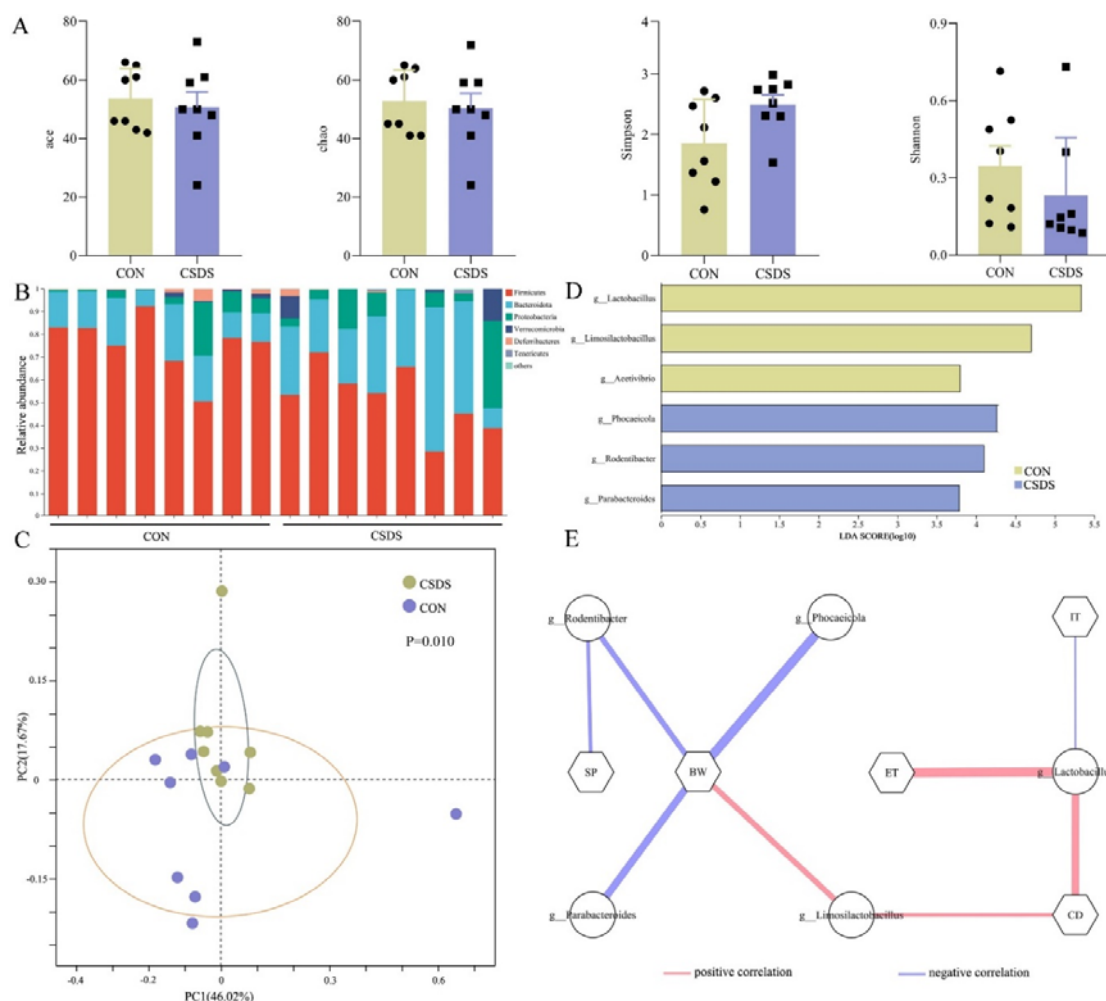
### Behavioral characteristics in CSDS mice

At baseline, no significant differences were observed in body weight or SP between CON and CSDS groups. After 10 days of CSDS exposure, mice in the stress group exhibited clear depressive-like behaviors. Specifically, CSDS mice had significantly lower body weight and SP compared to controls (**Figure 1**). In the FST, CSDS mice displayed longer immobility times, indicative of behavioral despair (**Figure 1**). In the OFT, both the percentage of center distance (CD%) and the number of entries into center zones (ET) were reduced in CSDS mice relative to controls (**Figure 1**). These findings confirm that the CSDS procedure effectively induced depressive-like phenotypes in mice.

microbiota in both groups (**Figure 2(B)**). Despite similar alpha diversity, overall microbial composition differed markedly, as revealed by principal coordinate analysis (PCoA) (**Figure 2(C)**). Linear discriminant analysis (LDA) identified taxa driving these differences: *Lactobacillus*, *Limosilactobacillus*, and *Acetivibrio* were enriched in CON mice, whereas CSDS mice showed higher abundances of *Phocaecicola*, *Rodentibacter*, and

*Parabacteroides* (Figure 2(D)). Correlation analysis further linked specific bacterial genera to depressive-like phenotypes: body weight was associated with *Rodentibacter*, *Phocaeicola*, *Parabacteroides*, and *Limosilactobacillus*; center zone activity correlated with

*Lactobacillus* and *Limosilactobacillus*; and sucrose preference, exploration time, and immobility time were connected to *Rodentibacter* and *Lactobacillus*, respectively (Figure 2(E)).

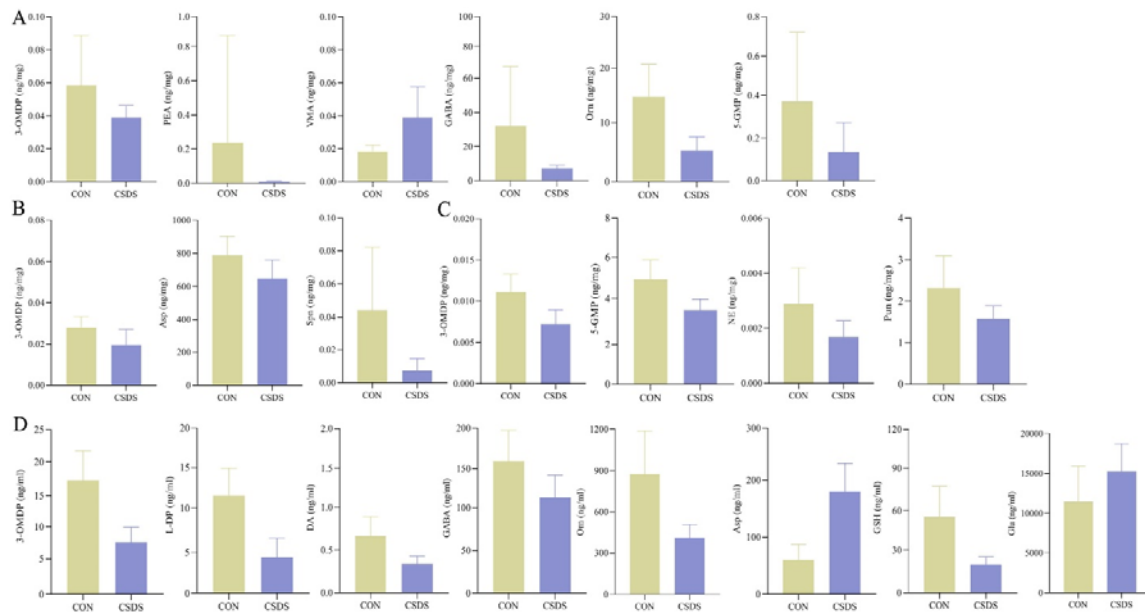


**Figure 2.** Alterations in gut microbiota between CON and CSDS mice. Analysis of alpha diversity showed no significant variation between CON and CSDS groups (Figure 2A). At the phylum level, Firmicutes and Bacteroidetes dominated the microbial communities in both sets of mice (Figure 2B). Despite similar overall diversity, principal coordinate analysis (PCoA) revealed clear differences in microbial composition between the groups (Figure 2C). Using LEfSe (LDA > 2.0,  $P < 0.05$ ), six bacterial taxa were identified as characteristic of either CON or CSDS mice (Figure 2D). Further correlation analysis demonstrated strong links between these specific microbes and depressive-like behaviors (Figure 2E). Each group consisted of eight mice

### Neurochemical changes in CSDS mice

Chronic social defeat stress induced significant changes in neurotransmitter levels across multiple biological compartments. In fecal samples, CSDS mice exhibited elevated 3-OMDP, phenylethylamine (PEA), and GABA, while Ornithine (Orn) and 5-Guanylic acid (5-GMP) decreased, and 4-Hydroxy-3-methoxymandelic acid (VMA) was increased (Figure 3A). Colonic analysis showed lower levels of 3-OMDP, L-aspartic acid (Asp), and spermine (Spn) in stressed mice compared with

controls (Figure 3B). Within the hippocampus, reductions were observed for 3-OMDP, 5-GMP, norepinephrine (NE), and 1,4-diaminobutane (Pun) (Figure 3C). Blood analysis revealed that CSDS mice had decreased concentrations of 3-OMDP, levodopa (L-DP), dopamine (DA), GABA, Orn, and glutathione (GSH), accompanied by increased Asp and L-glutamic acid (Glu) (Figure 3D). Functionally, 3-OMDP, PEA, VMA, NE, L-DP, and DA are components of the catecholaminergic pathway, whereas GABA, Orn, 5-GMP, Asp, Spn, Pun, GSH, and Glu are associated with GABAergic signaling.

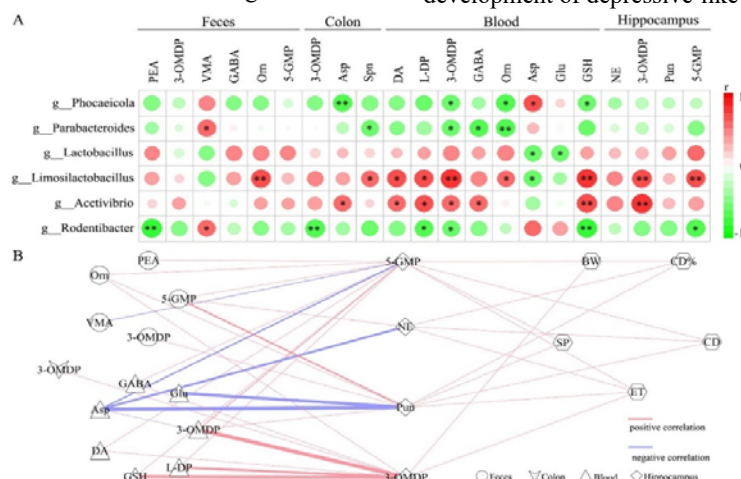


**Figure 3.** Alterations in peripheral and central neurotransmitters in CSDS mice: (A) Six neurotransmitters in fecal samples were identified as significantly altered in CSDS mice, (B) Three neurotransmitters showed differential levels in the colon, (C) Four neurotransmitters were altered in the hippocampus, and (D) Eight neurotransmitters in the blood exhibited significant differences compared with controls. Among these changes, 3-OMDP, PEA, VMA, NE, L-DP, and DA are components of the catecholaminergic pathway, while GABA, Orn, 5-GMP, Asp, Spn, Pun, GSH, and Glu are linked to GABAergic signaling. Each group consisted of eight mice

#### Associations between altered gut microbiota and neurotransmitters

Correlation analyses revealed strong relationships between six differentially abundant bacterial genera and 14 neurotransmitters across the gut-brain axis, including feces, colon, blood, and hippocampus ( $n = 8$  per group). Among the genera, *Limosilactobacillus* appeared to exert the strongest influence on these neurotransmitters (**Figure 4A**). Specifically, blood 3-OMDP levels were significantly associated with five of the six differential genera, while *Limosilactobacillus* showed significant

correlations with 3-OMDP in both blood and hippocampus (**Figure 4A**). Further investigation indicated that peripheral neurotransmitter alterations were closely linked with central neurotransmitter changes: central 5-GMP, NE, Pun, and 3-OMDP were significantly associated with 10, 2, 6, and 8 peripheral neurotransmitters, respectively (**Figure 4B**). Moreover, all central neurotransmitters examined showed significant correlations with at least one measure of depressive-like behavior (**Figure 4B**). These findings suggest that interactions between peripheral and central neurotransmitter systems may play a key role in the development of depressive-like states.



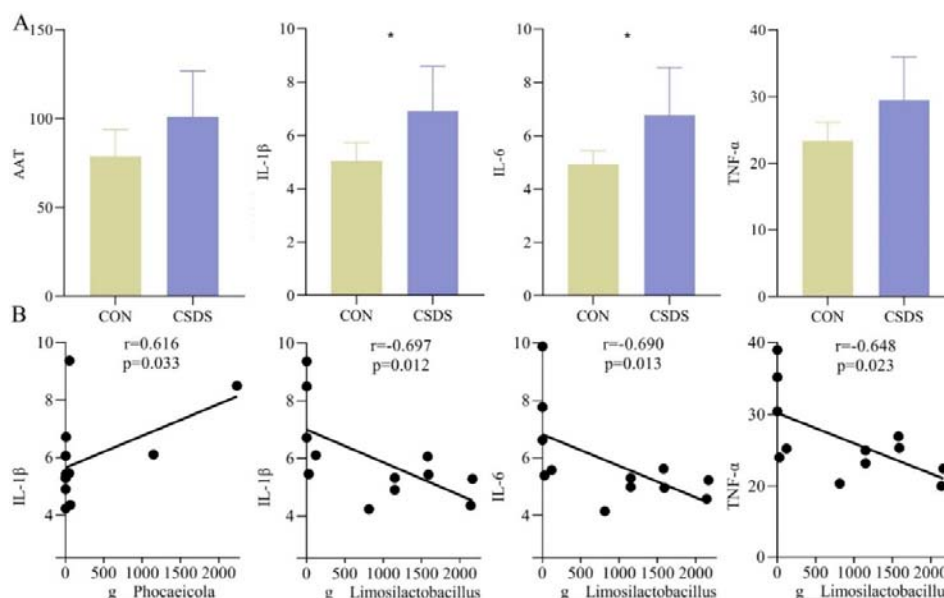
**Figure 4.** Relationships between altered gut microbiota and neurotransmitters. (A) Strong associations were observed between the differentially abundant bacterial genera and the altered neurotransmitters. *Limosilactobacillus* exhibited the

broadest influence, showing correlations with the most significant number of neurotransmitters. Blood levels of 3-OMDP were significantly associated with five out of the six differential genera. (B) Correlation analysis further demonstrated that changes in peripheral neurotransmitters could substantially impact central neurotransmitters, particularly 5-GMP and 3-OMDP. Each group contained eight mice

### Altered inflammatory markers in CSDS mice

Four inflammation-related cytokines—AAT, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6—were measured in the hippocampus. CSDS mice displayed significantly elevated IL-1 $\beta$  and IL-6 levels compared to CON mice ( $n = 8$  per group; **Figure 5A**). Although AAT and TNF- $\alpha$  tended to increase, these changes were not statistically significant. These findings indicate that CSDS-induced depression is associated with heightened neuroinflammation. Correlation analysis revealed negative relationships between *Limosilactobacillus* and IL-6, IL-1 $\beta$ , and TNF- $\alpha$ , while IL-

1 $\beta$  showed a positive correlation with *Phocaeicola* (**Figure 5B**). Further investigation into the links between cytokines and neurotransmitters demonstrated that IL-6 was positively correlated with Glu ( $r = 0.609$ ,  $P = 0.047$ ) and VMA ( $r = 0.657$ ,  $P = 0.020$ ), and negatively with GSH ( $r = -0.636$ ,  $P = 0.035$ ). IL-1 $\beta$  was positively associated with VMA ( $r = 0.72$ ,  $P = 0.018$ ) and negatively with 3-OMDP ( $r = -0.601$ ,  $P = 0.039$ ). Collectively, these results suggest that disruptions in gut microbiota may contribute to elevated neuroinflammation through modulation of both peripheral and central neurotransmitter systems.

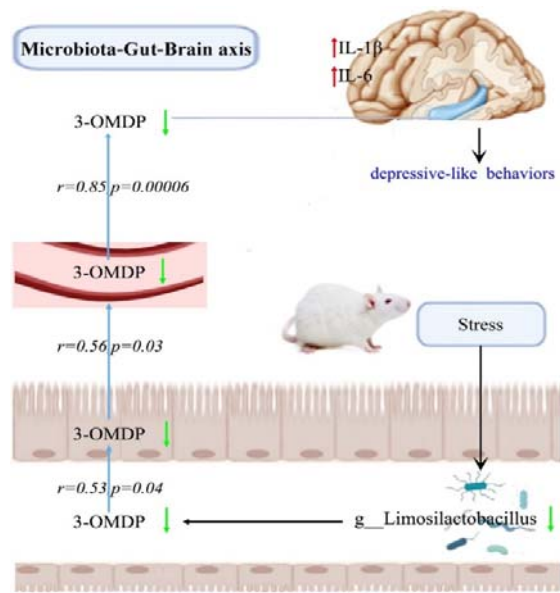


**Figure 5.** Links between gut microbiota and brain inflammation. CSDS mice exhibited significantly higher hippocampal levels of IL-1 $\beta$  and IL-6 compared to controls, whereas AAT and TNF- $\alpha$  levels were elevated but not statistically significant (Figure 5A). Correlation analysis revealed that *Phocaeicola* was positively associated with IL-1 $\beta$ , while *Limosilactobacillus* showed inverse correlations with IL-1 $\beta$ , IL-6, and TNF- $\alpha$  (Figure 5B). Each experimental group included eight mice

### Insights into the gut-brain axis

Throughout the study, 3-OMDP was consistently reduced in CSDS mice across fecal, colonic, blood, and hippocampal samples ( $n = 8$  per group; **Figure 3**). Sequential correlations suggested a directional relationship along the gut-brain axis: fecal 3-OMDP correlated with colonic levels ( $r = 0.53$ ,  $P = 0.04$ ), colonic levels correlated with blood ( $r = 0.56$ ,  $P = 0.03$ ), and blood 3-OMDP strongly correlated with hippocampal concentrations ( $r = 0.85$ ,  $P = 0.00006$ ). This pattern indicates that gut 3-OMDP may influence central neurotransmitter levels, positioning it as a critical

molecule in depressive pathology. *Limosilactobacillus* abundance was closely linked with 3-OMDP concentrations in both peripheral and central compartments (**Figure 4A**). Notably, reductions in 3-OMDP in the hippocampus were accompanied by elevated IL-6 and IL-1 $\beta$ , suggesting that decreased *Limosilactobacillus* may disrupt 3-OMDP homeostasis, thereby promoting neuroinflammation and contributing to depressive-like behavior (**Figure 6**).

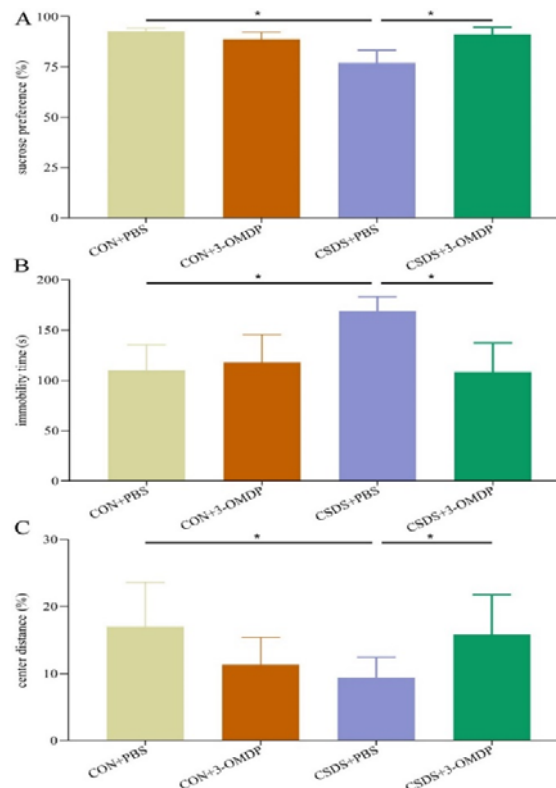


**Figure 6.** Proposed *Limosilactobacillus*–3-OMDP–IL-1 $\beta$ /IL-6 pathway in the gut-brain axis. In CSDS mice, 3-OMDP levels were consistently reduced across feces, colon, blood, and hippocampus. Correlation analyses revealed sequential associations: fecal 3-OMDP correlated with colonic 3-OMDP, colonic 3-OMDP correlated with blood 3-OMDP, and blood 3-OMDP correlated with hippocampal 3-OMDP. *Limosilactobacillus* abundance was closely linked with both peripheral and central 3-OMDP, and 3-OMDP levels were associated with IL-1 $\beta$ . These findings suggest that *Limosilactobacillus* may modulate brain IL-6 and IL-1 $\beta$  levels via effects on peripheral and central 3-OMDP, ultimately contributing to the development of depressive-like behaviors

#### Antidepressant potential of 3-OMDP

To evaluate the therapeutic effects of 3-OMDP, CSDS mice ( $n = 8$  per group) received intraperitoneal injections of 3-OMDP, followed by behavioral assessments including sucrose preference test (SPT), open field test (OFT), and tail suspension test (TST). After ten days of treatment, CSDS mice administered 3-OMDP exhibited significant improvements in depressive-like behaviors compared with CSDS mice given PBS: sucrose preference (SP) increased ( $P = 0.015$ , **Figure 7A**), immobility time (IT) decreased ( $P = 0.008$ , **Figure 7B**), and center zone activity (CD%) increased ( $P = 0.043$ , **Figure 7C**). Notably, these measures were comparable to those in control mice receiving PBS, indicating near-complete behavioral restoration. Additionally, no significant differences were observed between CON mice treated with PBS and CON mice treated with 3-OMDP, whereas both groups differed significantly from CSDS + PBS mice (SP:  $P = 0.004$ ; IT:  $P = 0.012$ ; CD%:  $P = 0.014$ , **Figure 7**). Together, these results demonstrate that 3-OMDP

administration can effectively ameliorate depressive-like behaviors in CSDS mice.

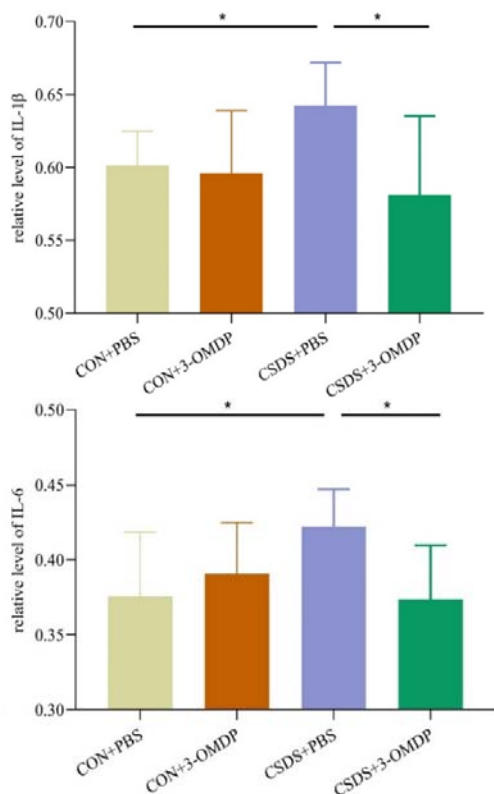


**Figure 7.** Impact of 3-OMDP on depressive-like behaviors in CSDS mice. Administration of 3-OMDP significantly alleviated depressive-like behaviors in CSDS mice. Specifically, sucrose preference (%) was markedly increased in CSDS mice treated with 3-OMDP compared with CSDS mice receiving PBS (Figure 7A). Immobility time was reduced following 3-OMDP treatment (Figure 7B), and center zone activity (%) was elevated (Figure 7C). Behavioral measures in the CON + PBS group were comparable to those in both the CON + 3-OMDP and CSDS + 3-OMDP groups. Still, they differed significantly from the CSDS + PBS group, demonstrating that 3-OMDP effectively restored depressive-like behaviors in stressed mice. Each group included eight mice

#### Effects of 3-OMDP on hippocampal inflammatory markers

We further examined the influence of 3-OMDP on hippocampal levels of IL-1 $\beta$  and IL-6 in CSDS mice ( $n = 8$  per group). Following treatment, CSDS + 3-OMDP mice exhibited significantly reduced IL-1 $\beta$  ( $P = 0.035$ ) and IL-6 ( $P = 0.021$ ) compared with CSDS + PBS mice, while their cytokine levels were comparable to CON + PBS mice (**Figure 8**). Similarly, IL-1 $\beta$  and IL-6 concentrations in the CON + PBS group were similar to those in the CON + 3-OMDP group but remained significantly lower than in the CSDS + PBS mice ( $P = 0.031$  and  $P = 0.049$ , respectively;

**Figure 8).** These findings indicate that 3-OMDP effectively attenuates elevated hippocampal IL-1 $\beta$  and IL-6 in CSDS mice.



**Figure 8.** Effects of 3-OMDP on hippocampal IL-1 $\beta$  and IL-6 in CSDS mice. Following administration of 3-OMDP, hippocampal IL-1 $\beta$  and IL-6 levels were significantly reduced in CSDS mice compared to the CSDS + PBS group. The CON + PBS group exhibited cytokine levels similar to both CON + 3-OMDP and CSDS + 3-OMDP groups. These findings demonstrate that 3-OMDP can effectively reverse the elevated IL-1 $\beta$  and IL-6 observed in the hippocampus of CSDS mice. Each group consisted of eight mice

## Discussion

This study identified six differential bacterial genera in feces and fourteen neurotransmitters across the gut-brain axis that differed between CSDS and control mice. Additionally, two inflammatory markers, IL-6 and IL-1 $\beta$ , were significantly increased in the hippocampus of CSDS mice. Notably, 3-OMDP levels were consistently decreased in both peripheral and central tissues. Correlation analyses revealed that *Limosilactobacillus* was strongly associated with 3-OMDP as well as IL-6 and IL-1 $\beta$  levels. Previous studies have suggested that chronic stress-induced alterations in neurotransmitter systems and inflammatory responses contribute to depression [37, 38]. To further investigate the role of 3-OMDP in this context, we conducted intervention experiments, which

demonstrated that 3-OMDP administration improved depressive-like behaviors and normalized elevated hippocampal levels of IL-6 and IL-1 $\beta$  in CSDS mice. Collectively, these findings suggest that gut microbiota may influence depression through modulation of neurotransmitter-mediated inflammation, and that a “*Limosilactobacillus*–3-OMDP–IL-1 $\beta$ /IL-6” axis could represent a critical pathway in gut-brain communication in depression.

Neurotransmitters are central regulators of mental functions, including emotion, cognition, and mood [39, 40]. Catecholamines such as dopamine (DA) and norepinephrine (NE) are essential monoamine neurotransmitters, and their depletion is associated with depression risk [41]. In this study, CSDS mice exhibited reduced DA and NE levels. Catecholamine intermediates, including phenylethylamine (PEA) and levodopa (L-DP), were also decreased. PEA acts as a neuromodulator of aminergic synapses, enhancing energy and mood [42], while L-DP is clinically used to supplement DA in Parkinson’s disease [43]. These observations suggest that dysregulation of catecholaminergic neurotransmission may contribute to the development of depressive-like behaviors.

GABA, the primary inhibitory neurotransmitter in the central nervous system, is generated via glutamate metabolism. Evidence indicates that GABA levels are significantly altered in both depressed patients and animal models [44, 45], with magnetic resonance spectroscopy studies reporting generally reduced GABA in individuals with depression [46]. Consistent with these findings, GABA was decreased in the feces and blood of CSDS mice in our study. Furthermore, previous studies have shown that guanosine monophosphate (5-GMP) exhibits antidepressant-like effects, potentially by indirectly activating serotonin through NMDA receptor blockade [47]. Here, 5-GMP levels were significantly decreased in both feces and hippocampus of CSDS mice, reinforcing the notion that disruptions in GABAergic neurotransmission play a pivotal role in depression pathogenesis.

As a metabolic product of L-DP, 3-OMDP is formed via the action of catechol-O-methyltransferase (COMT) [48]. Previous research indicated that 3-OMDP could influence brain dopamine (DA) levels by inhibiting dopamine transporter (DAT) function and DA uptake in both striatal membranes and PC12 cells [49]. DA and its metabolites are critical components of the brain’s reward circuitry. Moriya *et al.* [50] observed that both DAT binding and dopamine concentrations were markedly reduced in patients experiencing depression and anhedonia. Moreover, as a substrate of COMT, 3-OMDP may exert feedback inhibition on COMT itself. In essence, elevated 3-OMDP levels could restrict catechol conversion of L-

DP, thereby favoring its transformation into DA and helping to maintain CNS DA levels. Considering the central role of the reward system in depression, we hypothesized that 3-OMDP may contribute to depressive pathology by modulating DA levels. Notably, our study revealed significant reductions in DA in blood and 3-OMDP in the serum, hippocampus, colon, and feces of CSDS mice. Furthermore, administration of 3-OMDP alleviated depression-like behaviors in these mice, highlighting its potential as a therapeutic target for depression.

Depression is closely linked with inflammation, and individuals with chronic inflammatory disorders are more susceptible to depression [51–53]. Previous studies reported elevated levels of inflammatory mediators, including IL-6, IL-1 $\beta$ , and TNF- $\alpha$ , in the serum of patients with depression [54, 55]. Genetic analyses have also demonstrated strong associations between IL-6/IL-1 $\beta$  gene variants and depression risk [56, 57]. IL-6 and IL-1 $\beta$  are prominent pro-inflammatory cytokines that induce systemic inflammatory responses. In this study, CSDS mice exhibited significantly increased levels of IL-6 and IL-1 $\beta$ . Furthermore, gut microbiota dysbiosis is known to activate the immune system, triggering the release of inflammatory factors [58, 59]. Here, we found significant correlations between *Limosilactobacillus/Phocaeicola* and IL-6/IL-1 $\beta$  in CSDS mice, suggesting that the gut microbiota may influence depression through modulation of inflammatory processes.

Growing evidence indicates that gut microbiota dysregulation is associated with various diseases, and the microbiome includes both beneficial and potentially harmful microbes [60–63]. *Limosilactobacillus* is recognized as a beneficial genus in humans [64]. Tyagi *et al.* [65] reported that fermented products of *Limosilactobacillus reuteri* can enhance bioactive compounds and antioxidants, which may contribute to antidepressant effects by mitigating oxidative stress. Additionally, Bron *et al.* [66] highlighted that folic acid and vitamin B12 deficiencies elevate homocysteine levels, which are linked to depression onset, and *Limosilactobacillus* can promote the synthesis of these vitamins. These findings suggest a potential antidepressant role for *Limosilactobacillus*. Consistent with this, our study showed that reductions in *Limosilactobacillus* had the most significant impact on gut-brain axis neurotransmitters in CSDS mice, particularly 3-OMDP, and that 3-OMDP intervention mitigated elevated IL-6 and IL-1 $\beta$  levels. Therefore, *Limosilactobacillus* may play a key role in depression onset, with the ‘*Limosilactobacillus*-3-OMDP-IL-1 $\beta$ /IL-6’ axis representing a potential gut-brain pathway in depressive pathology.

Several limitations should be acknowledged. First, only four inflammatory mediators were measured; future work should explore additional inflammatory factors and their interactions with gut microbiota. Second, the mechanism by which *Limosilactobacillus* contributes to 3-OMDP production—directly or indirectly—was not investigated. Future studies using microbiota transplantation could clarify this relationship. Third, each experimental group included only eight mice, necessitating further validation. Fourth, this study focused solely on intraperitoneal 3-OMDP administration; other delivery routes and the potential for metabolite formation should be explored. Finally, only a single 3-OMDP dose was tested; dose-response studies are needed to establish optimal therapeutic levels.

In summary, we identified six differential bacterial genera, 14 neurotransmitters across central and peripheral tissues, and two pro-inflammatory cytokines (IL-6 and IL-1 $\beta$ ) in the hippocampus of CSDS mice. Strong associations were observed among these microbial, neurotransmitter, and inflammatory variables. Notably, *Limosilactobacillus* was closely linked with both central and peripheral 3-OMDP and with IL-6/IL-1 $\beta$ . Intervention experiments demonstrated that 3-OMDP significantly reduced elevated IL-6/IL-1 $\beta$  levels and alleviated depression-like behaviors. These findings suggest that gut microbiota disturbances may contribute to depression by modulating neurotransmitters and influencing inflammation, with the ‘*Limosilactobacillus*-3-OMDP-IL-1 $\beta$ /IL-6’ axis representing a potential gut-brain mechanism. This study provides insights into the pathogenesis of depression and highlights potential therapeutic targets.

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