



Research Article

<https://doi.org/10.1631/jzus.B2500219>



IL-1 β pathway-dependent regulation of glutamate receptor activity by gut microbiota in bipolar depression

Anying TANG^{1,2,3,4*}, Yi CHEN^{1*}, Kaijing DING^{5*}, Jinyu ZHANG^{1,6}, Le XU¹, Wenhao CHEN¹,
Shaohua HU^{1,2,3,4,7,8,9,10}✉, Jianbo LAI^{1,2,8,9}

¹Department of Psychiatry, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China

²Zhejiang Key Laboratory of Precision Psychiatry, Hangzhou 310003, China

³Nanhu Brain-computer Interface Institute, Hangzhou 311100, China

⁴Liangzhu Laboratory, Zhejiang University School of Medicine, Hangzhou 311121, China

⁵Affiliated Mental Health Center & Hangzhou Seventh People's Hospital, Zhejiang University School of Medicine, Hangzhou 310013, China

⁶Wenzhou Medical University, Wenzhou 325035, China

⁷Brain Research Institute of Zhejiang University, Hangzhou 310058, China

⁸Zhejiang Engineering Center for Mathematical Mental Health, Hangzhou 310003, China

⁹The MOE Frontier Science Center for Brain Science and Brain-machine Integration, Zhejiang University School of Medicine, Hangzhou 310058, China

¹⁰Department of Psychology and Behavioral Sciences, Zhejiang University, Hangzhou 310058, China

Abstract: Objective: Neuroinflammation may disrupt neurotransmitter signaling. This study investigated whether gut microbiota-induced neuroinflammation can regulate glutamate pathways in bipolar disorder (BD). Methods: Fecal microbiota transplantation (FMT) was performed to observe behavioral changes in the antibiotic-treated C57BL/6J male mouse model of bipolar depression. Gut microbial structure, circulating, and prefrontal levels of inflammatory factors, microglial activation, and transcription levels of *N*-methyl-D-aspartate receptor (*NMDAR*) and α -amino-3-hydroxy-5-methyl-4 isoxazole receptor (*AMPA*) genes were measured in the “BD” and control mice. Furthermore, the effects of interleukin-1 (IL-1) receptor antagonist (IL-1RA) on the glutamate pathways were assessed. Results: Compared with the control mice, “BD” mice displayed depression-like behaviors, with a lower diversity of gut bacteria and a decreased abundance of certain species. In addition, “BD” mice showed increased levels of inflammatory factors (e.g., IL-1 β) in the serum and prefrontal cortex, microglial activation, and changes in the messenger RNA (mRNA) levels of *NMDAR* and *AMPA*. Treatment with IL-1RA partially reversed the behavioral patterns, neuroinflammation, and transcription levels of glutamate receptors. Conclusions: The findings suggest that gut microbiota may influence glutamate receptor gene expression via an IL-1 β -dependent pathway in a mouse model of BD, potentially contributing to neuroinflammatory mechanisms relevant to this disorder.

Key words: Bipolar disorder; Gut microbiota; Neuroinflammation; Glutamate receptor; Interleukin-1 (IL-1)

1 Introduction

Bipolar disorder (BD) is a severe and debilitating disease, characterized by alternating episodes of (hypo-)mania and depression (Anderson et al., 2012). Since the underlying etiology remains largely unclear, the clinical management of BD poses consistent challenges (Bauer et al., 2018). Thus, more efforts are needed to elucidate the etiology of BD. Genetic background, environmental factors, and their interplay have been considered to contribute to the development of BD (Anderson et al., 2012). The gut microbiota as part of the gut-brain axis has been recognized as an

✉ Shaohua HU, dorhushaohua@zju.edu.cn

Jianbo LAI, laijianbo@zju.edu.cn

* The three authors contributed equally to this work

Shaohua HU, <https://orcid.org/0000-0003-0570-670X>

Jianbo LAI, <https://orcid.org/0000-0002-8137-7701>

Anying TANG, <https://orcid.org/0009-0001-0171-4959>

Yi CHEN, <https://orcid.org/0000-0002-4923-3716>

Kaijing DING, <https://orcid.org/0000-0001-6491-9305>

Jinyu ZHANG, <https://orcid.org/0009-0006-8123-5136>

Le XU, <https://orcid.org/0000-0002-9801-8764>

Wenhao CHEN, <https://orcid.org/0000-0002-1715-2698>

Received May 7, 2025; Revision accepted Aug. 25, 2025;
Crosschecked June 23, 2026; Published online July 1, 2026

© Zhejiang University Press 2026

essential factor affecting human health (Sharon et al., 2016) and plays an important role in emotional regulation (Liu et al., 2025). To date, accumulating studies have revealed that the gut microbiota in individuals with BD is dysregulated. In most cases, the alpha diversity of gut microbiota was decreased in patients with bipolar depression (Sublette et al., 2021). In addition, specific gut microbiota species were associated with the severity of BD symptoms. Notably, the abundance of butyrate-producing bacteria decreased significantly, while the proportion of pro-inflammatory bacteria increased in patients with bipolar depression (Nikolova et al., 2021). These evidences indicate that gut microbial dysbiosis may disrupt the inflammatory balance in BD patients.

Studies have also shown that gut-brain regulation in various psychiatric disorders is closely related to neuroinflammatory pathways (Goldsmith et al., 2023). In terms of mood disorders such as BD, the inflammasome is considered to be a key mediator of the response to physiological and psychological stressors. Inflammasome activation induces the maturation of caspase-1 and the secretion of interleukin-1 β (IL-1 β) and IL-18, two pro-inflammatory cytokines involved in neuroimmunomodulation, neuroinflammation, and neurodegeneration. The genetic deficiency of caspase-1 was associated with decreased depression- and anxiety-like behaviors and increased spontaneous locomotion and locomotory skills, as well as altered compositions of fecal microbiota, including *Akkermansia*, *Blautia*, and *Lachnospiraceae* (Wong et al., 2016). Depression-like behaviors could be observed in healthy mice when transplanted with gut microbiota derived from depressed mice, which might be mediated by elevated serum inflammatory factors and neuroinflammatory responses (e.g., activation of nucleotide-binding domain (NOD)-like receptor thermal protein domain-associated protein 3 (NLRP3) inflammasome) in the brain (Yao et al., 2023). Changes in the gut microbiota in BD are also related to pathophysiological processes such as systemic inflammation. For example, the relative abundance of fecal *Lactobacillaceae* and *Streptococcaceae* in BD patients was positively correlated with elevated serum inflammatory factors such as IL-6 (Hu et al., 2019). Therefore, it is plausible that the inflammatory pathway may play an important role in the gut-brain communication associated with BD. While previous clinical research has indicated potential links between gut microbiota and inflammatory dysregulation in BD,

there is an urgent need to deepen the mechanistic exploration of neuroinflammation-dependent gut-brain communication in this disease.

Glutamate receptors are classified into ionotropic and metabotropic receptors. The former mainly include *N*-methyl-D-aspartate receptor (NMDAR), kainate receptor (KAR), and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), which couple with ion channels to form receptor-channel complexes that mediate interneuronal signaling in the brain. Meanwhile, metabotropic receptors mainly regulate intracellular second messengers and produce slower physiological responses (Traynelis et al., 2010). At present, it has been widely recognized that the glutamate system plays an important role in the antidepressant effects of ketamine (Ma et al., 2023). In addition, the glutamate system may also be involved in synaptic plasticity (Newpher and Ehlers, 2008), memory, and learning, as well as in the pathogenesis of psychiatric disorders such as schizophrenia (Hardingham and Do, 2016) and neurocognitive disorders (Li et al., 2022). In response to environmental stimuli, activated inflammatory cells in the brain (e.g., microglia) can release inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and IL-1 β , which lead to an increase in synaptic glutamate in neurons and in turn mediate oxidative stress, thus affecting the function of glutamate receptors on glial cells and neurons (Haroon et al., 2017). Although neuroinflammation and glutamate receptor dysfunction are both implicated in BD, their causal relationship remains poorly defined. To fill this gap, we created a fecal microbiota transplantation (FMT) mouse model that allows the direct measurement of brain inflammatory markers and glutamate receptor expression, providing mechanistic insights that cannot be obtained from human clinical studies.

The present study proposes a hypothesis that gut dysbiosis may modulate glutamate receptors via IL-1 β -dependent pathways, which contribute to the neuroinflammatory processes underlying the BD phenotype. To test this hypothesis, we established a mouse model of BD by transplanting fecal microbiota from patients with bipolar depression into antibiotic-treated mice. We then examined the behavioral patterns of mice in response to treatment, as well as the peripheral and cerebral inflammation responses, the changes in glutamate receptor activity, and their relationship with the inflammatory process. The findings provide new perspectives on gut-brain communication in BD by

regulating the activity of glutamate receptors in an IL-1 β -dependent manner.

2 Materials and methods

2.1 Animals

The study experiments involving animals were approved by the Animal Experimental Ethical Inspection Protocol of The First Affiliated Hospital, Zhejiang University School of Medicine (No. 2024-376).

Seventy-two male C57BL/6J specific pathogen free (SPF) mice aged 6–8 weeks were selected for the animal experiments, with an average weight of (20 $\pm$ 2) g. Animals were housed in groups of 5–6 individuals per cage using sterilized polypropylene containers. The feeding environment included a stable room temperature ((20 $\pm$ 1) °C) and automated 12-h light/dark alternation. All mice received a continuous supply of standard laboratory feed and drinking water for 7 d before the formal experiment. Since the FMT method in this study was exploratory, we used the resource equation approach (Arifin and Zahiruddin, 2017) for sample size calculation in animal experiments, which recommended 5–11 animals per group. Considering model failures and animal deaths, 14–15 mice per group were finally included.

2.2 Fecal sample collection and treatment

The diagnosis of BD patients ($n=4$) with a current depressive episode was made according to the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). All fecal donors were screened according to the following inclusion criteria: (1) the first episode or psychotropic drug-free for at least three months; (2) no use of antibiotics, probiotics, or prebiotics for at least one month before sample collection; and (3) 17-item Hamilton Depression Rating Scale (HDRS-17) score $\geq$ 14. Healthy participants ($n=4$) with strictly matched age, gender, and body mass index were also recruited. Written consent forms were obtained from all participants. Fecal samples from all BD patients and healthy controls were collected and immediately preserved in labeled tubes at -80 °C. The demographic profiles of BD and healthy donors are provided in Table S1.

For fecal suspension preparation, 2–3 g of fecal samples were weighed under anaerobic conditions and

homogenized with a 15–30 mL phosphate-buffered saline (PBS)-glycerol mixture (20%, volume fraction). Then, the mixture was passed through 0.60 mm sterile filters and centrifuged at 2500 r/min and 4 °C for 60 s. The supernatant was dispensed into cryotubes and stored at -80 °C. The above procedures were performed on ice. Before gavaging the mice, frozen fecal suspensions were thawed in ice-water mixtures for 2–4 h.

2.3 Antibiotic treatment and fecal microbiota transplantation

Considering that germ-free mice have defects in metabolism and immunity (Bhattarai et al., 2018), as well as in the absorption function of intestinal epithelial cells (Martinez-Guryn et al., 2018), we used antibiotic treatment to eliminate the intestinal microorganisms of mice. After one week of adaptation, all mice were given a cocktail comprising vancomycin, neomycin, metronidazole, and ampicillin (1 mg/mL for all reagents) in their drinking water for seven consecutive days. At the same time, 200 μ L of the antibiotic mixture was administered daily by oral gavage to deplete intestinal microorganisms in each mouse. Subsequently, after a one-day antibiotic washout phase, mice were randomly divided into two groups, one treated with suspension from BD individuals (BD mice) and another treated with suspension from healthy controls (HC mice). Groups of 3–4 mice received bacterial suspensions derived from a single donor source. Fecal microbiota suspension (10 μ L/g body weight) was gavaged on Days 1, 3, 5, 8, 10, and 12, to reconstruct the gut microbiome in each mouse.

2.4 Treatment with IL-1 receptor antagonist

At the end of modeling, BD mice were randomly divided into two groups: one group was intraperitoneally injected with IL-1 receptor antagonist (IL-1RA) (BBI Life Sciences, Shanghai, China) at 50 pg/kg for seven consecutive days, and the other group was intraperitoneally injected with the same amount of saline. The control mice were intraperitoneally injected with the same amount of saline.

2.5 Behavioral tests

2.5.1 Open field test (OFT)

Before the formal experiment, all experimental mice underwent 2 h environmental adaptation in a room

with dim light. During the formal experiment, each mouse was placed in the center of open field (50 cm×50 cm×50 cm) for 5 min in the same darkened room. The movement of each mouse was tracked by a video camera and recorded by the ANY-maze system. For each mouse, the parameters measured included the total distance and the number of crossings in the total area, the number of crossings in the central area (25 cm×25 cm), as well as distance and time in the central area.

2.5.2 Forced swim test (FST)

Each mouse individually was placed gently in a transparent plastic cylinder (12 cm in diameter, 25 cm in height) of water at (25±1) °C. The heads of the mice were allowed to be submerged under the water during this step. Six-minute tests were digitally recorded, and the immobile time for the last four minutes was finally counted. The immobility criteria were defined as the absence of all movement except for small motions required to remain afloat. Quantitative analysis was performed by independent investigators under blinded experimental conditions.

2.5.3 Tail suspension test (TST)

All mice were individually suspended approximately 40 cm above the room floor using medical adhesive tape applied to the tails. All trials were conducted in a dim light environment with continuous video recording for six minutes. Immobility duration during the final four minutes was calculated by blinded investigators. The immobility time was defined as the complete absence of limb movement.

2.6 Gut microbiota 16S rRNA sequencing of mice after FMT

To confirm the success of FMT and its effects on the gut microecology, fecal samples from mice before and after antibiotic treatment and the those after FMT were collected for 16S ribosomal RNA (rRNA) detection.

Fecal genomic DNA was isolated using a DNA extraction kit (QIAamp 96 PowerFecal QIAcube HT Kit, QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA purity and concentrations were tested by the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and preserved at -20 °C. Bacterial strains were investigated using 16S rRNA gene sequencing. The hypervariable V3-V4

regions of bacterial 16S rRNA were amplified using high-fidelity polymerase with universal primers (forward primer: 5'-TACGGRAGGCAGCAG-3'; reverse primer: 5'-AGGGTATCTAATCCT-3'). The purified amplicons obtained by the QIAquick Gel Extraction Kit (QIAGEN) were subjected to library preparation and quantification via NanoDrop and Qubit validation. According to the data volume of 50 000 reads for each sample, an appropriate library volume was added. Illumina was used for PE250 sequencing.

Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity threshold using hierarchical clustering Alg (HCA). The SILVA (<https://www.arb-silva.de>) database was used for species annotation and species classification analysis, the OTUs were classified into various taxonomic ranks. The alpha diversity and beta diversity of OTUs in different groups were analyzed, and the differences in community structure and species composition among samples could be mined through a variety of statistical comparisons.

2.7 Real-time polymerase chain reaction (PCR)

Within 24 h of the behavioral test, all mice were sacrificed after anesthesia, and then the prefrontal lobe tissues were dissected on ice and preserved at -80 °C until testing. Total RNA from prefrontal lobe tissues was extracted using the TRIzol reagent according to the manufacturer's instructions (Accurate Biology, China), and total RNA purity and concentration were detected using Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Reverse transcription reactions (20 µL total volume) were performed with the ABScript II cDNA First Strand Synthesis Kit (ABclonal, China), maintaining the thermal cycling parameters as the manufacturer's recommendations. Target genes were detected by QuantStudio 5DX Real-Time PCR System with SYBR Green Fast qPCR Mix (ABclonal, China). β -Actin was used as an internal control and each sample was tested three times. The primers for β -actin, glutamate ionotropic receptor NMDA type subunit 1 (*Grin1*), *Grin2A*, *Grin2B*, *Grin2C*, *Grin2D*, glutamate ionotropic receptor AMPA type subunit 1 (*Gria1*), *Gria2*, *Gria3*, and *Gria4* are given in Table S2.

2.8 Enzyme-linked immunosorbent assay (ELISA)

The prefrontal lobe tissues were homogenized in ice-cooled radio immunoprecipitation assay (RIPA)

lysis buffer containing protease inhibitors, homogenized for 2 min, and then lysed for 30 min on ice. This was followed by centrifugation at 13 000 r/min for 15 min at 4 °C, and the supernatant was removed to obtain protein samples. Protein concentrations were determined through bicinchoninic acid assay (BCA Kit P0010, Beyotime, Shanghai, China) using bovine serum albumin standards. Absorbance measurements were performed at 562 nm wavelength.

TNF- α , IL-6, brain-derived neurotrophic factor (BDNF), interferon- γ (IFN- γ), IL-10, and IL-1 β protein concentrations in the prefrontal lobe tissues and IL-6, IL-1 β , and IFN- γ in serum were assessed using ELISA kits according to the manufacturer's protocols. All ELISA kits were obtained from ABclonal (RK00008, RK00016, RK00027, RK00019, and RK00006).

2.9 Immunofluorescence analysis

Following transcardial perfusion with 4% (0.04 g/mL) paraformaldehyde (PFA), the whole brain tissues were harvested and immersed in PFA at 4 °C for 24 h. Subsequently, the brain tissues were removed into 0.3 g/mL sucrose (in PBS) until complete tissue equilibrium, followed by optimal cutting temperature (OCT) compound embedding (Sakura Finetek, Japan).

Next, sequential 25 μ m coronal sections were prepared using a cryostat microtome (Leica, Wetzlar, Germany) at -20 °C chamber temperature. Sections were blocked with 5% (0.05 g/mL) bovine serum albumin (BSA/PBS) and permeabilized with 0.5% (volume fraction) Triton X-100 in blocking solution for 60 min. Anti-ionized calcium-binding adaptor molecule 1 (anti-Iba1) rabbit antibody (1:200 (volume ratio, the same below), Cell Signaling Technology, USA) was applied and maintained at 4 °C for 16 h. Alexa Fluor 488-conjugated goat anti-rabbit immunoglobulin G (IgG) (1:500) was incubated for 1 h at room temperature. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). Images were acquired using an Olympus FV3000 confocal laser scanning microscope at 40 $\times$ magnification. All images were processed with FIJI software (National Institutes of Health, Bethesda, MD, USA).

2.10 Statistics and reproducibility

The results were summarized and presented as mean $\pm$ standard error of mean (SEM). To ensure unbiased group distribution, subject allocation followed

the complete randomization protocols. The sample sizes for each experiment are indicated in the respective figure legends. Normality test was conducted for all datasets. Data that did not exhibit normal distribution were analyzed by the Shapiro-Wilk test. The specific statistical methods were detailed in the figure legends of each experiment. All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software Inc., CA, USA). The statistical significance threshold was established at $P < 0.05$ when compared to control groups.

3 Results

3.1 BD symptoms partially recapitulated by FMT

We reconstructed the gut microbiota of C57BL/6 male mice by applying antibiotics for seven consecutive days. After a one-day antibiotic washout period, the mice were gavaged with feces from either BD patients or healthy controls according to the grouping, followed by behavioral tests (OFT, FST, and TST) (Fig. 1a).

Behavioral tests demonstrated that mice transplanted with BD feces developed a depression-like phenotype. In the OFT, the total distance of the BD group was significantly less than that of the control mice (Fig. 1b) and the number of accesses in the central area was significantly decreased (Fig. 1d), whereas the locomotor distance in the central area and the locomotor time in central area were unchanged (Figs. 1c and 1e). In the FST, the BD mice also developed a depression-like phenotype, mainly manifested by a significant increase in immobility time in the water (Fig. 1f). Nonetheless, the immobility time was not significantly changed in the TST (Fig. 1g).

To determine the colonization efficiency of FMT, we compared the fecal microbiota of BD donors, healthy controls, and the two groups of mice after FMT. The results showed that 1465 bacterial species were present in the fecal microbiota of both BD patients and BD mice, and 1745 bacterial species were present in both HC donors and control mice (Fig. 1h). We also compared the gut microbiota of BD patients and HC donors and their "humanized" mice at the genus level. The results showed that in both BD and HC groups, except for *Muribaculaceae*, a bacterium that is widely present in the mouse microbiome (Smith

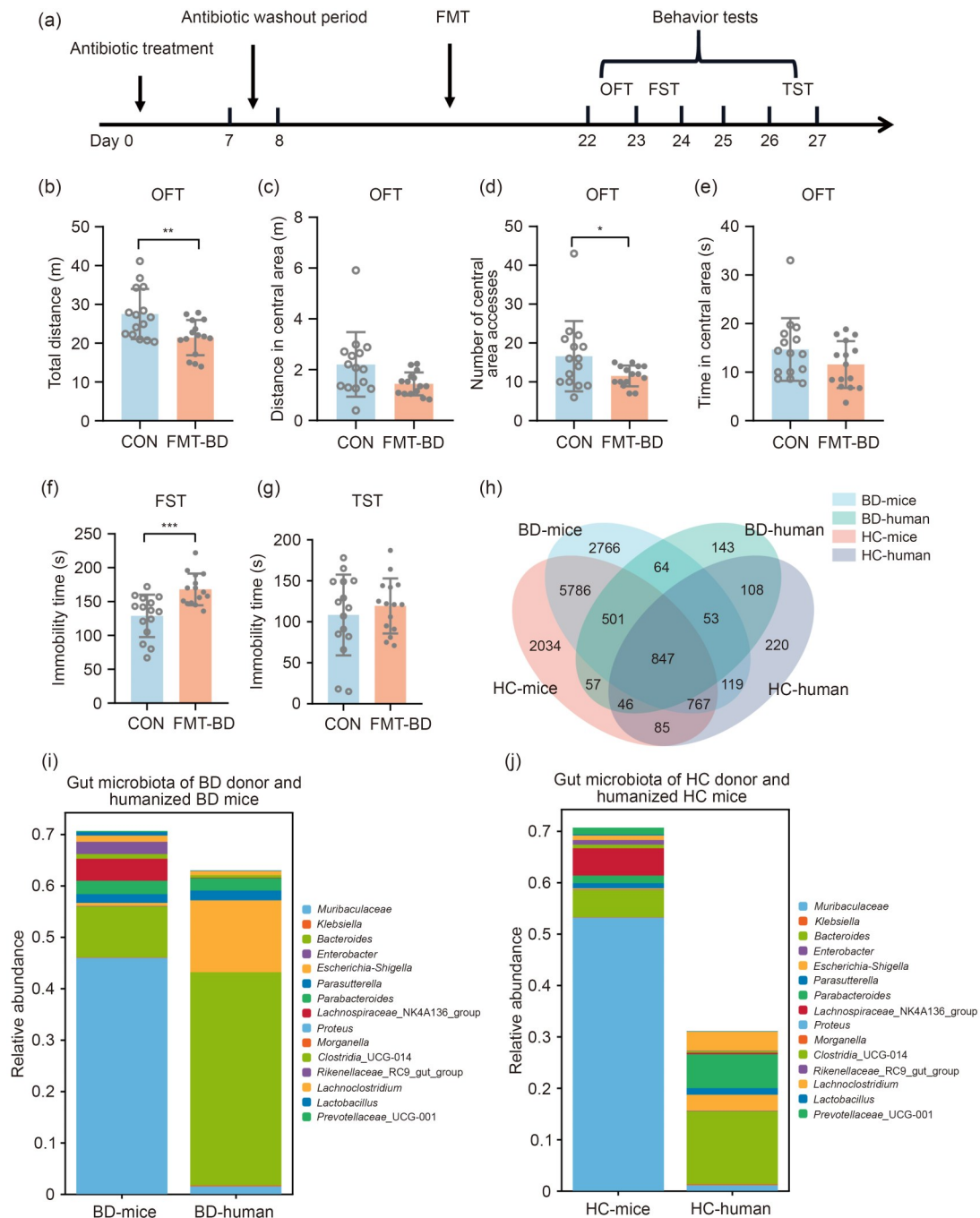


Fig. 1 Depression-like behaviors and gut microbiota humanization in mice induced by FMT from BD patients. (a) Mouse experimental schedule. (b–e) In the OFT, BD mice traveled shorter total distances and accessed the central area frequently, although there were no differences in traveled distance or time in the central area. (f, g) In the FST, BD mice showed increased immobility time in the water, whereas the TST revealed no difference between the two groups. (h) Venn diagrams of the number of bacterial species in the feces of human donors and humanized mice. (i) The gut microbiota of BD donors and humanized BD mice were analyzed and compared at the genus level. (j) The gut microbiota of healthy donors and humanized control mice were analyzed and compared at the genus level. In the OFT, FST, and TST, CON ($n=15$), FMT-BD ($n=15$); in the 16S ribosomal RNA (rRNA) analysis of gut microbiota, HC-mice ($n=17$), BD-mice ($n=19$), HC-human ($n=4$), BD-human ($n=4$). (b–g) Data are presented as mean $\pm$ SEM. Significant differences were measured by independent-samples t -test (b, f, g) or Kruskal-Wallis test (c, d, e) ($P<0.05$, $**P<0.01$, $***P<0.001$). FMT: fecal microbiota transplantation; OFT: open field test; FST: forced swimming test; TST: tail suspension test; BD: bipolar disorder; HC: healthy control; SEM: standard error of the mean; CON: control.

et al., 2021), the proportions of bacteria in the humanized mice group were similar to those of their donors (Figs. 1i and 1j). In addition, we also detected the abundance of fecal microbiota in the mice before and after antibiotic treatment. Alpha diversity analysis showed that this was significantly decreased after antibiotic treatment (Figs. S1a–S1d).

In order to verify whether the change in behavioral patterns was related to the FMT procedure in mice, we analyzed the fecal microbiota of mice after FMT by sequencing the 16S rRNA gene amplicon. Differences in bacterial community structure between the two groups were evident in the principal coordinate analysis (PCoA) of the beta-diversity Euclidean distance algorithm, which revealed a clear clustering of microbial compositions (Fig. 2a). In terms of alpha

diversity, the BD mice group harbored a significantly lower level of number of OTUs actually observed (observed species: control, 1985.00 ± 67.05 ; FMT-BD, 1671.00 ± 104.00 ; $P=0.0187$) and species richness (Chao1 index: control, 3240.00 ± 109.50 ; FMT-BD, 2880.00 ± 154.20 ; $P=0.0196$) when compared to the control mice (Figs. 2c and 2d). No significant differences were observed between the two groups in Simpson's index (control: 0.9753 ± 0.0032 ; FMT-BD: 0.9767 ± 0.0041 ; $P=0.8214$) or Shannon's index (control: 7.7540 ± 0.1051 ; FMT-BD: 7.5190 ± 0.1968 ; $P=0.2784$) (Figs. 2b and 2e). In addition, the bar plot and heatmap revealed a significant change in the relative abundance of microbial communities at the genus level in both groups of mice (Figs. 2f and S1h), as evidenced by a significant increase in the relative abundance of microbial

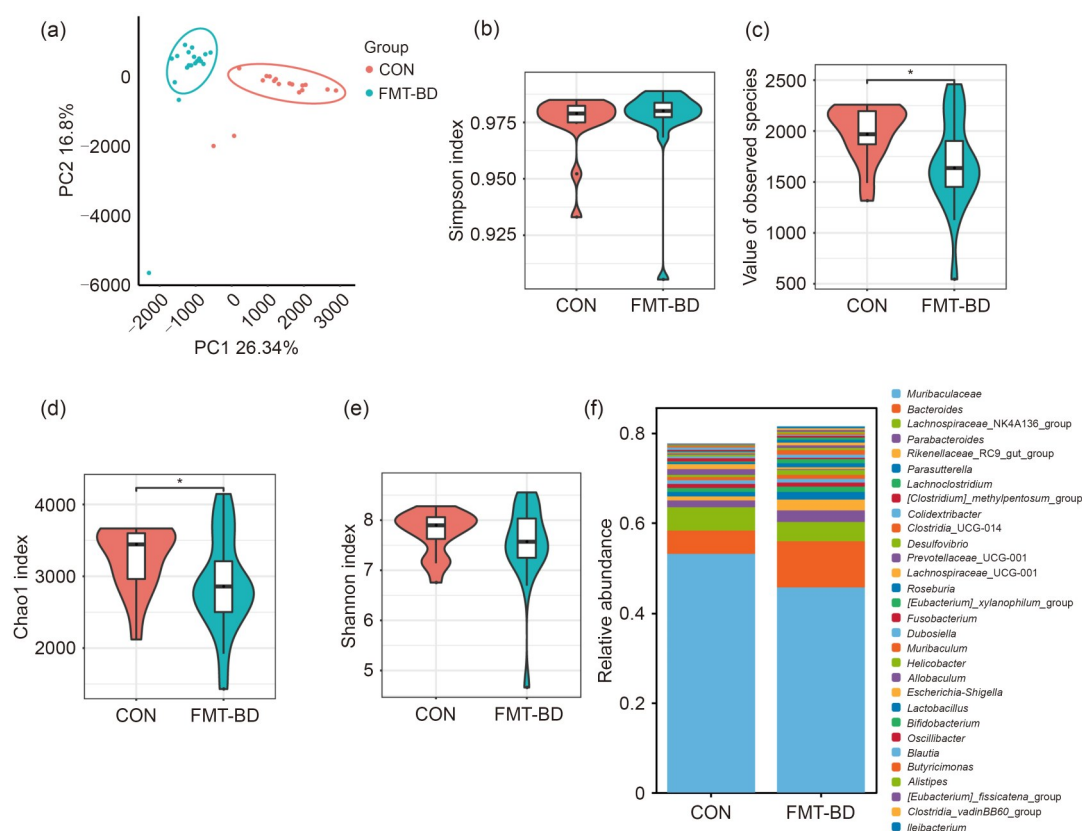


Fig. 2 Gut microbial features in mice with fecal bacteria transplant based on 16S rRNA sequencing. (a) Principal coordinate analysis (PCoA) of the beta-diversity Euclidean distance algorithm. (b) Alpha diversity measured by Simpson's index showed no difference between the FMT-BD group and the control group. (c) The number of OTUs by observed species in the FMT-BD group was lower than that in the control group. (d) The Chao1 index in the FMT-BD group was lower than that in the control group. (e) There was no difference in Shannon index between the FMT-BD group and the control group. (f) Bar plot showed the relative abundance of gut microbiota in the two groups at the genus level. CON ($n=17$), FMT-BD ($n=19$). Data are presented as mean $\pm$ SEM. Significant differences were measured by the Wilcoxon rank-sum test (b–e) (* $P<0.05$). rRNA: ribosomal RNA; PC: principal component; FMT: fecal microbiota transplantation; BD: bipolar disorder; OTUs: operational taxonomic units; CON: control; SEM: standard error of the mean.

communities in the BD mice group, including *Roseburia*, *Eubacterium_xylanophilum_group*, *Alistipes*, *Lactobacillus*, *Clostridia_vadinBB60_group*, and *Muribaculum*, and decreased OTU abundance of *Prevotellaceae_UCG-001* (Figs. 3a–3g). Significant differences in microbial communities were observed not only at the genus level but also at the class, order, and family levels (Figs. S1e–S1g).

3.2 FMT of BD microbiota elevated the expression of IL-1 β in the periphery and brain

After 27 d of experimental and behavioral testing, serum samples were taken from mice after anesthesia and prefrontal lobe tissues were collected after euthanasia. In order to assess the level of inflammation induced by FMT in mice, we examined the levels of various inflammatory factors in the serum and prefrontal tissues of mice by ELISA. To comprehensively evaluate the gut microbiota-induced inflammatory response spectrum, we simultaneously examined multiple

core inflammatory factors, including pro-inflammatory cytokines IL-1 β , TNF- α , IL-6, IFN- γ , and anti-inflammatory cytokine IL-10, which could help to distinguish broad-spectrum inflammatory activation from the role of specific factors. In the serum, the IL-1 β level in the BD mice group was significantly higher than that of the control group (Fig. 4c), while IFN- γ and IL-6 levels were not significantly different (Figs. 4a and 4b). In the prefrontal lobe tissues, the IL-1 β level in the BD mice group was also significantly higher than that in the control group (Fig. 4g), whereas the levels of IL-10, IFN- γ , IL-6, and TNF- α were not (Figs. 4d–4f and 4h), indicating that disordered microbiota did not cause extensive inflammatory activation.

3.3 FMT of BD microbiota activated microglia in the brain

To determine whether gut microbiota is associated with neuroinflammation in the brain, we used immunofluorescence to detect the main innate immune cells

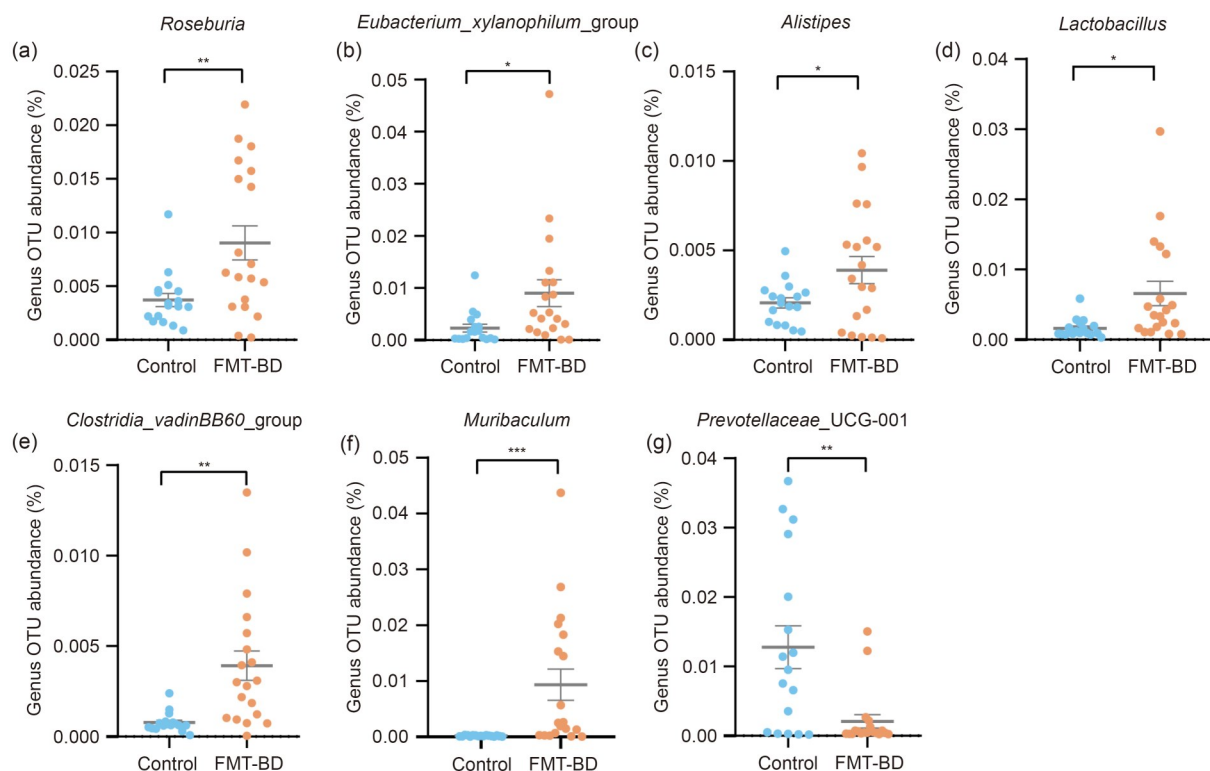


Fig. 3 Significant changes in the relative abundance of gut microbes at the genus level based on 16S rRNA sequencing. The abundance of *Roseburia* ($P=0.0063$) (a), *Eubacterium_xylanophilum_group* ($P=0.0486$) (b), *Alistipes* ($P=0.0365$) (c), *Lactobacillus* ($P=0.0156$) (d), *Clostridia_vadinBB60_group* ($P=0.001$) (e), and *Muribaculum* ($P=0.0001$) (f) in FMT-BD was increased significantly, while that of *Prevotellaceae_UCG-001* ($P=0.0031$) (g) was decreased. Control ($n=17$), FMT-BD ($n=19$). Data are presented as mean $\pm$ SEM. Significant differences were measured by an independent samples t -test (c) or Kruskal-Wallis test (a, b, d, e, f, g) (* $P<0.05$, ** $P<0.01$, *** $P<0.001$). rRNA: ribosomal RNA; FMT: fecal microbiota transplantation; BD: bipolar disorder; OTU: operational taxonomic unit; SEM: standard error of the mean.

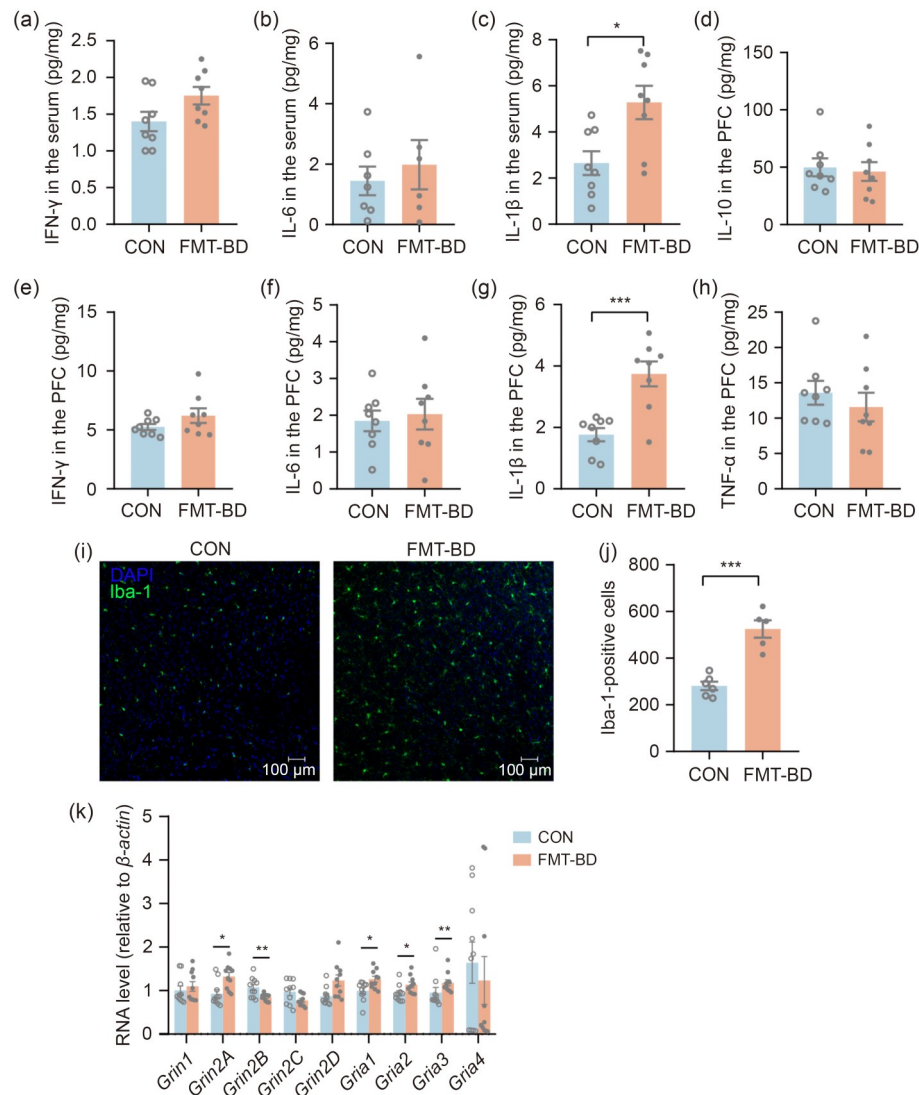


Fig. 4 Changes in inflammatory factor levels and glutamate receptor gene expression caused by FMT. (a–c) ELISA analysis revealed the serum levels of inflammatory factors, including IFN- γ , IL-6, and IL-1 β . The serum IL-1 β level was increased significantly in the FMT-BD group. (d–h) The levels of inflammatory factors in the prefrontal cortex (PFC), including IL-10, IFN- γ , IL-6, IL-1 β , and TNF- α . The PFC IL-1 β level was increased significantly in the FMT-BD group. (i) Immunofluorescence staining for Iba-1 (green) and DAPI (blue) in the prefrontal tissues. (j) Analysis of Iba-1-positive cells. The number of Iba-1-positive cells was increased significantly in the FMT-BD group compared to the control group. (k) Gene expression of *NMDAR* and *AMPA* in the prefrontal tissues in the FMT-BD and control groups. The levels of *Grin2A*, *Gria1*, *Gria2*, and *Gria3* were increased significantly in BD mice, while the level of *Grin2B* was decreased significantly. In the serum ELISA analysis, CON ($n=7$ or 8), FMT-BD ($n=6$ – 8); in the prefrontal ELISA analysis, CON ($n=8$), FMT-BD ($n=8$); in the immunofluorescence staining, CON ($n=6$), FMT-BD ($n=5$); in the RT-PCR test, CON ($n=10$), FMT-BD ($n=10$). Data are presented as mean $\pm$ standard error of the mean (SEM). Significant differences were measured by an independent samples *t*-test (a–h, j) or Kruskal-Wallis test (k) (* $P<0.05$, ** $P<0.01$, *** $P<0.001$). FMT: fecal microbiota transplantation; IFN: interferon; IL: interleukin; BD: bipolar disorder; Iba-1: ionized calcium-binding adaptor molecule 1; DAPI: 4',6-diamidino-2-phenylindole; *NMDAR*: *N*-methyl-D-aspartate receptor; *AMPA*: α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; ELISA: enzyme-linked immunosorbent assay; CON: control; RT-PCR: reverse transcription-polymerase chain reaction; *Grin1*: glutamate ionotropic receptor NMDA type subunit 1; *Gria1*: glutamate ionotropic receptor AMPA type subunit 1.

in the brain, microglia (Nakagawa and Chiba, 2015). We calculated the number of positive ionized calcium-binding adaptor molecule 1 (Iba-1 $^{+}$) (a microglia marker)

cells in the prefrontal lobe region and found it to be increased in the brains of the BD mice group (Figs. 4i and 4j).

3.4 FMT of BD microbiota modulated the glutamate receptor levels in the brain

To demonstrate the effect of neuroinflammation induced by FMT on the prefrontal lobe function in mice, we examined the levels of glutamate-related receptors in the prefrontal lobe. We found that the levels of *Grin2A* (CON: 0.92780 ± 0.08991 ; FMT-BD: 1.32500 ± 0.09202 ; $P=0.0149$), *Gria1* (CON: 0.99280 ± 0.07008 ; FMT-BD: 1.26000 ± 0.06757 ; $P=0.0133$), *Gria2* (CON: 0.93780 ± 0.05979 ; FMT-BD: 1.13300 ± 0.06337 ; $P=0.0376$), and *Gria3* (CON: 0.95300 ± 0.12120 ; FMT-BD: 1.17800 ± 0.07187 ; $P=0.0033$) were increased in BD mice, while the level of *Grin2B* (CON: 1.07300 ± 0.07268 ; FMT-BD: 0.84220 ± 0.02567 ; $P=0.0078$) was decreased. Otherwise, there was no difference in the levels of *Grin1* (CON: 1.0070 ± 0.1001 ; FMT-BD: 1.0960 ± 0.1098 ; $P=0.9883$), *Grin2C* (CON: 0.97170 ± 0.08701 ; FMT-BD: 0.77360 ± 0.04389 ; $P=0.0571$), *Grin2D* (CON: 0.86770 ± 0.06592 ; FMT-BD: 1.2330 ± 0.1282 ; $P=0.1641$), or *Gria4* (CON: 1.6410 ± 0.4742 ; FMT-BD: 1.2320 ± 0.5492 ; $P=0.7591$) between the two groups (Fig. 4k).

3.5 IL-1RA partially reversed the behavioral phenotypes and neuroinflammation in BD fecal microbiota-recipient mice

The above findings suggested that the gut microbiota from BD patients might mediate neuroinflammation in the prefrontal lobe through the IL-1 β pathway, which ultimately causes changes in the levels of glutamate receptors. Therefore, we speculated that IL-1RA administration to mice after fecal transplantation could reverse the behavioral phenotype due to FMT. After the FMT period, we administered IL-1RA intraperitoneally (50 pg/kg) to some of the BD-transplanted mice, while saline was administered intraperitoneally to the rest of these mice and control mice. Behavioral tests were performed one week after administration (Fig. 5a).

Behavioral tests showed that IL-1RA administration caused partial reversal of depression-like behavior in BD mice. In the TST, the immobility time of mice in the saline-treated BD group was significantly higher than that of the control group, whereas the immobility time of IL-1RA-treated BD mice was significantly shorter than that of the saline-treated BD group (Fig. 5g), suggesting that IL-1RA treatment may improve the depression-like behavior in the mice. In the FST, the

immobility time of the mice in the IL-1RA-treated BD group was similar to that of the control mice, while the immobility time of the saline-treated BD mice was significantly higher than that of the control mice (Fig. 5f). In the OFT, however, in terms of the total distance of movement and the distance of movement in the central area, as well as the number of accesses in the central area, the mice in the IL-1RA-treated BD group and in the saline-treated BD group differed significantly from the mice in the control group (Figs. 5b–5d), indicating that IL-1RA treatment was unable to improve the anxiety-like behavior of mice.

The transcription levels of the *NMDAR* and *AMPA* genes were also examined after IL-1RA treatment. From the RT-PCR results, we found that the levels of *Grin2A*, *Gria1*, *Gria2*, and *Gria3* in the saline-treated BD mice were increased compared with those in the control group, which was consistent with the aforementioned findings, while there was no change in *Grin2B*. IL-1RA treatment significantly decreased the levels of *Gria1* and *Gria3*, and partially decreased the *Grin2A* level, which remained higher than that of the control group. IL-1RA treatment had no obvious effect on *Gria2* (Fig. 5h). Immunofluorescence analysis of prefrontal lobe tissues from IL-1RA-treated BD mice showed that IL-1RA also partially reversed the activation of microglia (Figs. 5i and 5j).

4 Discussion

In the current study, we found that FMT of BD microbiota in mice not only induced behavioral changes but also elicited IL-1 β -dependent neuroinflammation that eventually impacted the activity of glutamate receptors in the prefrontal lobe (Fig. 6). To date, numerous clinical studies have investigated the characteristics of the gut microbiota in different mental disorders, including BD (McGuinness et al., 2022). Meanwhile, FMT has also emerged as a potential approach to establish disease-related animal models. However, few studies utilizing animal models have been conducted to investigate the gut–brain axis in BD due to its complex phenotype of recurrent manic or depressive episodes. A preliminary study has demonstrated that the gut microecology is different during the manic and depressive phases of BD (Lucidi et al., 2021). In the current study, we attempted to establish

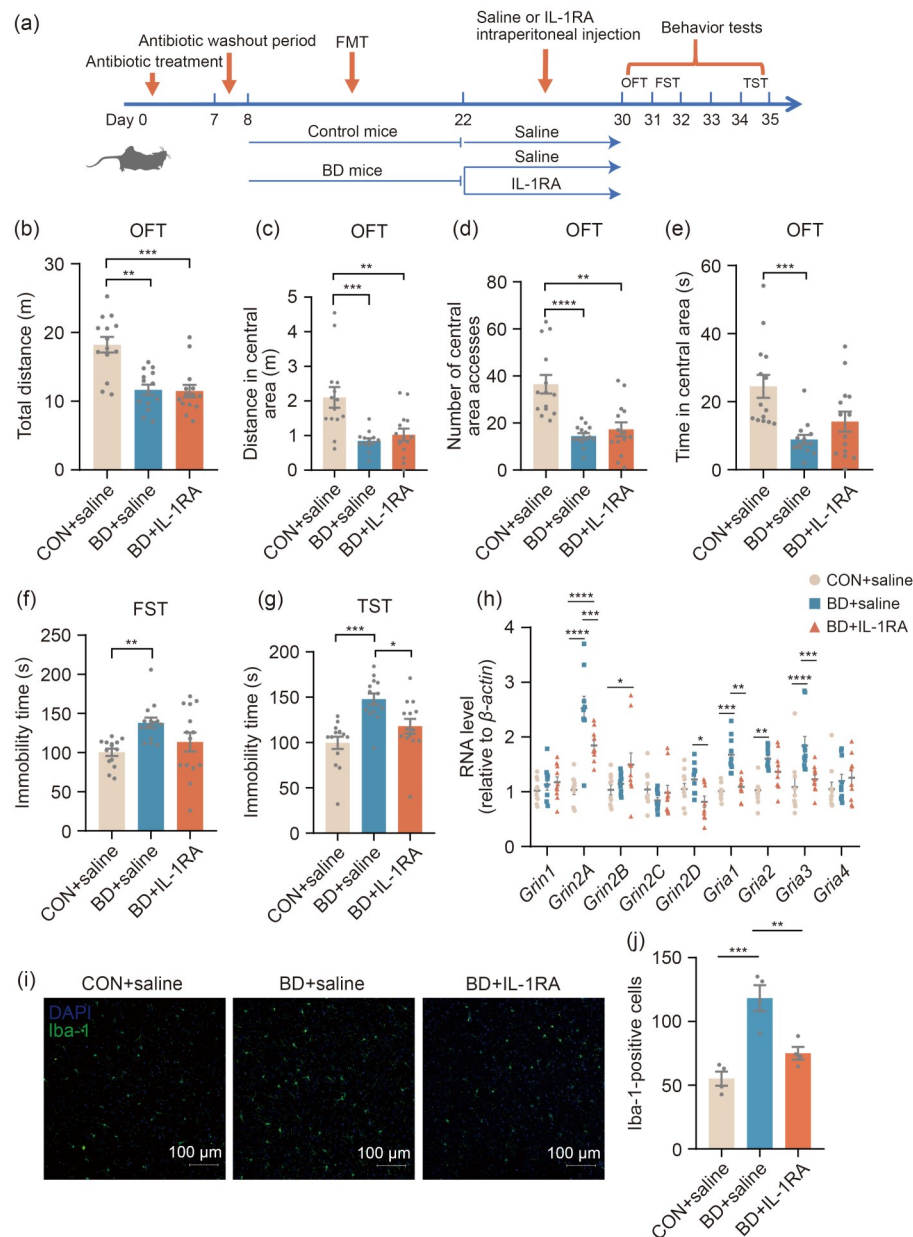


Fig. 5 Partial reversal of the FMT-induced behavioral phenotype in mice by intraperitoneal injection of IL-1RA. (a) Experimental schedule for IL-1RA injection in mice. (b–e) The OFT examined the total movement distance and movement distance, time, and number of accesses in the central area of mice. (f) The FST showed the immobility time of mice. The IL-1RA reduced the immobility time of FMT-BD mice in the FST, but did not restore it to normal levels. (g) The TST showed the immobility time of mice. The IL-1RA reduced the immobility time of FMT-BD mice in the TST. (h) The gene expression of *NMDAR* and *AMPA* in the prefrontal tissues in the three groups. (i, j) Immunofluorescence staining for Iba-1 (green) and DAPI (blue) in the prefrontal tissues and quantitative analysis of Iba-1-positive cells of three groups. In behavioral tests, CON ($n=14$), BD+saline ($n=14$), BD+IL-1RA ($n=14$); in the RT-PCR test, CON ($n=8$), BD+saline ($n=8$), BD+IL-1RA ($n=8$); in the immunofluorescence staining, CON ($n=4$), BD+saline ($n=4$), BD+IL-1RA ($n=4$). Data are presented as mean $\pm$ standard error of the mean (SEM). Significant differences were measured by one-way ANOVA (c, h, j) or Kruskal-Wallis test (b, d, e, f, g) (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$). IL-1RA: IL-1 receptor antagonist; FMT: fecal microbiota transplantation; OFT: open field test; FST: forced swimming test; TST: tail suspension test; BD: bipolar disorder; *NMDAR*: *N*-methyl-D-aspartate receptor; *AMPA*: α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; Iba-1: ionized calcium-binding adaptor molecule 1; DAPI: 4',6-diamidino-2-phenylindole; RT-PCR: reverse transcription-polymerase chain reaction; CON: control; ANOVA: analysis of variance; *Grin1*: glutamate ionotropic receptor NMDA type subunit 1; *Gria1*: glutamate ionotropic receptor AMPA type subunit 1.

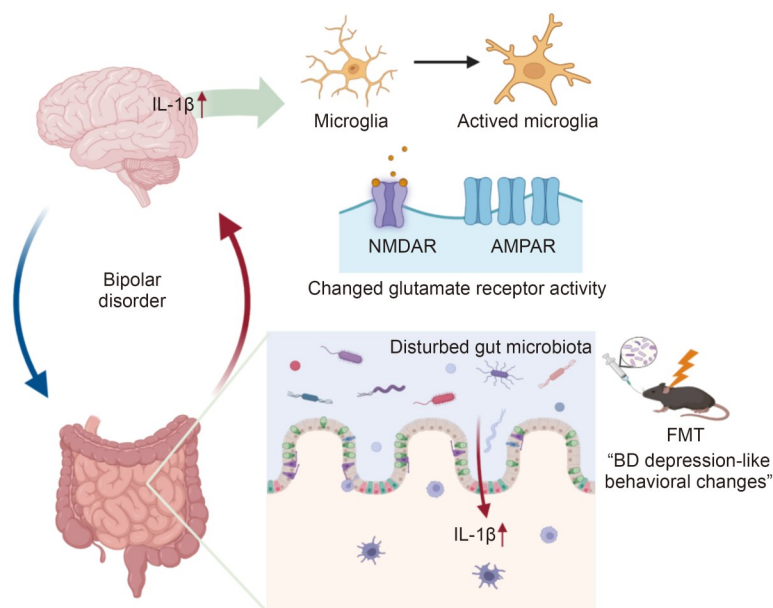


Fig. 6 Crosstalk between gut microbiota and glutamate receptors via an IL-1 β -dependent manner. In bipolar disorder, the disordered gut microbiota triggers the release of systemic IL-1 β . The circulating IL-1 β passes through the BBB and induces neuroinflammation, including the activity of microglia, ultimately mediating changes in glutamate receptor activity and bipolar depression-like behaviors. NMDAR: *N*-methyl-D-aspartate receptor; AMPAR: α -amino-3-hydroxy-5-methyl-4 isoxazole receptor; IL-1 β : interleukin-1 β ; BBB: blood–brain barrier; FMT: fecal microbiota transplantation; BD: bipolar disorder. Image created with BioRender.com, with permission.

a mouse model of bipolar depression via FMT. As observed in Fig. 1, in the OFT and FST, the BD mice exhibited depression- and anxiety-like behaviors, which was consistent with previously reported animal models of BD. For example, *syt7*-knockout (*syt7*-KO) mice, that is, BD risk gene knockout mice that can mimic the behavioral abnormalities of BD, also showed significantly longer immobility in the FST during the daytime depressive phase (Shen et al., 2020; Wang QW et al., 2021). However, the inter-group behavioral outcomes were not significantly different in TST, which may reflect intrinsic variations in the test sensitivity, stress paradigms, and neurobiological mechanisms induced by gut microbiota. While both FST and TST ostensibly measure behavioral despair, their distinct stressors engage partially divergent neural circuits, neurotransmitter systems, and coping strategies. Prior studies indicate that FST exhibits greater sensitivity to serotonergic modulators, whereas TST may be more responsive to noradrenergic agents (Cryan et al., 2005). This could explain why gut microbiota transfer from BD patients, which may predominantly dysregulate serotonergic pathways, had measurable effects in FST but not TST. The absence of TST differences does not invalidate the overall depressive phenotype. Future

work should delineate whether this divergence stems from test duration, stressor intensity, or distinct circuit modulation by donor microbiota.

Previous studies have reported overall differences in the composition of gut microbiota in patients with BD compared with healthy controls, whereas the findings were inconsistent across different studies (Spulber et al., 2009; McGuinness et al., 2022; Obi-Azuike et al., 2023). In most studies, changes in the abundance of *Lactobacillus*, *Faecalibacterium*, and *Ruminococcus* were consistent in BD patients compared with controls (Obi-Azuike et al., 2023). Meanwhile, in another study, *Actinobacteria* and *Coriobacteria* were significantly more abundant in BD patients, and *Rumencoccaceae* and *Faecalibacterium* were significantly more abundant in healthy controls (Painold et al., 2019). In our sequencing results for gut microbiota, a lower alpha diversity of gut microbiota was found in BD when compared to control mice, as shown in Fig. 2. In addition, Fig. 3 illustrates that six genera of bacteria were found to be significantly increased in BD mice, including *Roseburia*, *Eubacterium_xylanophilum_group*, *Alistipes*, *Lactobacillus*, *Clostridia_vadinBB60_group*, and *Muribaculum*, while the abundance of *Prevotellaceae_UCG-001* was reduced.

These taxa altered in FMT-BD mice exhibit functional profiles with significant implications for BD pathophysiology. *Alistipes* may exert detrimental effects through neurotransmitter disruptions. On the one hand, reduced tryptophan availability in the intestine could impair serotonergic signaling (Jiang et al., 2015), while its glutamate decarboxylase activity may dysregulate γ -aminobutyric acid (GABAergic) transmission (Polansky et al., 2016), collectively contributing to mood dysregulation in psychiatric disorders (Duman et al., 2019). *Prevotellaceae*_UCG-001 can secrete short-chain fatty acids, which are related to reducing intestinal inflammation (Zhu et al., 2019). Conversely, *Roseburia* and *Clostridia_vadinBB60_group* demonstrate anti-inflammatory potential via butyrate production, which is typically associated with gut barrier integrity and protection against inflammatory pathology (Machiels et al., 2014; Shen et al., 2022; Kang et al., 2023; Song et al., 2023). Their elevation in BD mice may represent a compensatory response counteracting FMT-induced gut inflammation, despite established reductions in *Roseburia* in depressive states correlating with neuroinflammation (Knuesel and Mohajeri, 2021; Eicher and Mohajeri, 2022). The role of *Lactobacillus* is notably complex: while its lactate production confers benefits including metabolic regulation, pathogen elimination, and immunomodulation (George et al., 2018; Kleerebezem et al., 2010), its consistent elevation in depression, schizophrenia, and BD (McGuinness et al., 2022) suggests potential neurotoxicity. Excessive lactate may disrupt neuronal energy metabolism and contribute to neuropsychiatric symptoms, as evidenced by elevated CSF lactate in depression (Ernst et al., 2017). Few functional data are available on *Eubacterium_xylanophilum_group* and *Muribaculum*, which may be associated with lipid metabolism (Lozano et al., 2022; Xu et al., 2023; Zhang et al., 2023; Zhao et al., 2023), but lack clear mechanistic links to BD. Therefore, the above findings not only advocate that the gut microecology in bipolar depression is disturbed but also indicate that the metabolic and inflammation pathways are involved in the gut-brain communication of bipolar depression. Inflammatory dysregulation may play an important role in the pathogenesis of the brain-gut axis in mental disorders.

Neuroinflammation represents a critical pathophysiological mechanism implicated in the development of numerous psychiatric disorders, including

BD. This inflammatory cascade has been increasingly linked to gut-brain axis dysregulation. For instance, in Alzheimer's disease (AD), compositional shifts in gut microbiota correlate with elevated phenylalanine and isoleucine levels, which promote M1 microglial activation and contribute to AD neuroinflammation (Wang et al., 2019). Furthermore, studies in sleep-deprived mouse models demonstrated that gut microbiota alterations drive inflammatory responses leading to cognitive impairment, while these effects were absent in germ-free mice, and microbiota depletion reversed both inflammation and cognitive dysfunction (Wang Z et al., 2021). Previous studies confirmed that the serum IL-1 β level was significantly higher in BD patients during the remission phase than in controls (Goldsmith et al., 2016), which was associated with neurocognitive deficits in BD (Poletti et al., 2021). In addition, IL-1 β single-nucleotide polymorphism was associated with an increased putamen volume in the left hemisphere in bipolar patients (Strenn et al., 2021). It has been reported that the chronic systemic elevation of IL-1 β can affect the permeability of the blood-brain barrier (Argaw et al., 2006; Versele et al., 2022) and exacerbate the level of central inflammation (Murta et al., 2015). In the present study, a higher IL-1 β level in the serum and prefrontal lobe tissues was also observed in the BD mice group in Fig. 4, while IL-6, IFN- γ , TNF- α , and IL-10 levels remained unchanged. This pattern suggests that IL-1 β has a specific role in driving neuroinflammation in our model, rather than being part of a general inflammatory response. The stable levels of these other cytokines provide strong evidence that IL-1 β acts as a key driver in this context. Besides, immunofluorescence staining of microglia in the prefrontal lobe further confirmed the presence of neuroinflammation in the brain. The above results suggest that the gut microbiota may participate in regulating the brain function of bipolar depression via an IL-1 β -mediated inflammatory pathway.

Postmortem evidence indicated the presence of excitotoxicity and neuroinflammation in the prefrontal cortex of BD individuals, particularly with the activation of inflammatory factors. In our study, the messenger RNA (mRNA) levels of *Grin2A*, *Gria1*, *Gria2*, and *Gria3* were increased and the mRNA level of *Grin2B* was decreased (Fig. 4), indicating that IL-1 β activation may induce prefrontal NMDAR- and AMPAR-related neurotoxicity. Increased *Grin2A* may enhance

calcium influx through NMDARs, promoting excitotoxicity and dendritic pruning (Yamada-Fowler et al., 2014). This is further compounded by reduced *Grin2B*, which typically mediates neuroprotective signaling (Bell et al., 2018). The concurrent upregulation of AMPAR subunits suggests heightened neuronal excitability and susceptibility to excitotoxic injury (Wu et al., 2025; Xu et al., 2025), mirroring the elevated glutamate levels and cortical hyperexcitability in bipolar depression patients. However, at present, in the postmortem brains of patients with BD, there is still a lack of research on the encoding of *N*-methyl-D-aspartate receptor subunit 2 (NR2) and AMPAR subunits. Nevertheless, researchers discovered that NMDAR regulatory subunit GluN3A (*NR3A*) mRNA was significantly decreased in the dorsolateral prefrontal cortex (DLPFC) region of BD patients (Mueller and Meador-Woodruff, 2004), while the expression of NR1 was decreased (Beneyto and Meador-Woodruff, 2008), which was related to the levels of IL-1 β , IL-1 receptor, astrocyte- and microglia-related markers, as well as several oxidative stress-related proteins and mRNAs (Rao et al., 2010). The effect of intestinal bacteria on NMDAR and AMPAR may affect neuronal firing (van Vugt et al., 2020), synaptic signal transduction (Chipman et al., 2022), and synaptic plasticity (Renteria et al., 2017), thus influencing the development of BD.

We further used IL-1RA in mice transplanted with BD feces. As shown in Fig. 5, we observed that desperation-like behaviors of the mice were alleviated in the FST, but the behavioral patterns in the OFT were not altered, indicating that IL-1RA treatment did not improve the motor ability and anxiety-like behavior of animals. Meanwhile, treatment with IL-1RA reversed the changes in NMDAR and AMPAR levels and the microglial activation in the prefrontal lobe. As a key mediator of the inflammatory response, IL-1 β exists in balance with its endogenous receptor antagonist (IL-1RA) and is widely expressed in the brain (Spulber et al., 2009). It has been reported that plasma levels of IL-1 β and IL-1RA, and IL-1 β :IL-1RA ratio are higher in patients with bipolar depression than in healthy individuals before treatment: after one week of antidepressant treatment, IL-1 β was significantly decreased and IL-1RA was significantly increased, and the ratio of IL-1 β to IL-1RA was significantly decreased (Benedetti et al., 2021). In addition, it has

been reported that there is an imbalance between IL-1RA and IL-1 β in the hippocampus of a rat model with chronic social defeat stress (CSDS)-induced depression (Li et al., 2023). Chronic intraventricular injection of IL-1RA blocked the depression-like behaviors induced by CSDS and alleviated the reduction in dendritic spine density and impairments in AMPAR-mediated neurotransmission (Li et al., 2023). Interestingly, intraperitoneal injection of IL-1RA could alleviate lipopolysaccharide (LPS)-induced depression-like behaviors (Bluthé et al., 1992). Current treatment algorithms for BD, particularly bipolar depression, remain inadequately defined. The existing pharmacopeia are limited and supported by insufficient evidence. Some agents carry risks, such as treatment-emergent affective switching and adverse metabolic effects (Dai et al., 2025). Our findings put forward new approaches to treating BD depression by targeting the IL-1 β pathway.

Some limitations of our study must be mentioned. First of all, it should be noted that the observed changes in glutamate receptor gene expression require validation at the protein level to confirm functional relevance rather than being based solely on mRNA data. In future studies, incorporating proteomic and electrophysiological analyses will be essential to substantiate these findings. While donor microbiota profiles reflect established BD dysbiosis patterns, our small cohort size may not capture the full heterogeneity of human BD. Multi-center studies with larger donor pools are warranted to enhance the translational potential. Finally, our mouse model only mimics gut-brain communication in bipolar depression and does not fully replicate the complexity of BD symptoms. Whether a similar mechanism exists in the manic episode of BD has not been explored in the current study because of the lack of stool samples from BD patients with mania. In future studies, we will focus on this issue to decipher the exact mechanism underlying gut-brain communication in BD.

5 Conclusions

In summary, our findings suggest that during depressive episodes in BD, alterations in intestinal flora may be associated with neuroinflammation and changes in the expression of glutamate receptor (*NMDAR* and *AMPA*) genes in the prefrontal lobe, potentially

involving an IL-1 β -dependent pathway, which proves our hypothesis.

Data availability statement

All datasets analyzed in this study are available from the corresponding author upon request.

Acknowledgments

This study was supported by the National Key Research and Development Program of China (Nos. 2023YFC2506200 and 2023YFC2506203), the National Natural Science Foundation of China (Nos. 82571735 and 82471542), the Key R&D Program of Zhejiang Province (Nos. 2024C03098 and 2025C02109), the Research Project of Jinan Microecological Biomedicine Shandong Laboratory (No. JNL-2023001B), the Fundamental Research Funds for the Central Universities (Nos. 2023ZFJH01-01 and 2024ZFJH01-01), and the Major Science and Technology Plan Guidance Project of Xiaoshan District (No. 2023316), China.

Author contributions

Anying TANG, Shaohua HU, and Jianbo LAI were responsible for the overall experimental design. Anying TANG and Jinyu ZHANG performed the behavioral tests. Yi CHEN and Kaijing DING performed the immunofluorescence staining, RT-PCR, and ELISA. Wenhao CHEN analyzed the microbiome profile within the fecal samples. Le XU and Jianbo LAI performed the data analysis. Anying TANG, Yi CHEN, and Kaijing DING outlined and wrote the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Anying TANG, Yi CHEN, Kaijing DING, Jinyu ZHANG, Le XU, Wenhao CHEN, Shaohua HU, and Jianbo LAI declare that they have no conflicts of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (No. 2017-397, Institutional Review Board of The First Affiliated Hospital, School of Medicine of Zhejiang University, China) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

All institutional and national guidelines for the care and use of laboratory animals were followed. This study was approved by the Animal Experimental Ethical Inspection Committee of The First Affiliated Hospital, Zhejiang University School of Medicine (No. 2024-376).

Declaration on the use of generative AI tools

No generative AI tools were used in the preparation of this manuscript.

References

- Anderson IM, Haddad PM, Scott J, 2012. Bipolar disorder. *BMJ*, 345:e8508.
<https://doi.org/10.1136/bmj.e8508>
- Argaw AT, Zhang YT, Snyder BJ, et al., 2006. IL-1 β regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. *J Immunol*, 177(8):5574-5584.
<https://doi.org/10.4049/jimmunol.177.8.5574>
- Arifin WN, Zahiruddin WM, 2017. Sample size calculation in animal studies using resource equation approach. *Malays J Med Sci*, 24(5):101-105.
<https://doi.org/10.21315/mjms2017.24.5.11>
- Bauer M, Andreassen OA, Geddes JR, et al., 2018. Areas of uncertainties and unmet needs in bipolar disorders: clinical and research perspectives. *Lancet Psychiatry*, 5(11):930-939.
[https://doi.org/10.1016/S2215-0366\(18\)30253-0](https://doi.org/10.1016/S2215-0366(18)30253-0)
- Bell S, Maussion G, Jefri M, et al., 2018. Disruption of *GRIN2B* impairs differentiation in human neurons. *Stem Cell Rep*, 11(1):183-196.
<https://doi.org/10.1016/j.stemcr.2018.05.018>
- Benedetti F, Dallaspesza S, Melloni EMT, et al., 2021. Effective antidepressant chronotherapeutics (sleep deprivation and light therapy) normalize the IL-1 β :IL-1RA ratio in bipolar depression. *Front Physiol*, 12:740686.
<https://doi.org/10.3389/fphys.2021.740686>
- Beneyto M, Meador-Woodruff JH, 2008. Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. *Neuropsychopharmacology*, 33(9):2175-2186.
<https://doi.org/10.1038/sj.npp.1301604>
- Bhattarai Y, Williams BB, Battaglioli EJ, et al., 2018. Gut microbiota-produced tryptamine activates an epithelial G-protein-coupled receptor to increase colonic secretion. *Cell Host Microbe*, 23(6):775-785.e5.
<https://doi.org/10.1016/j.chom.2018.05.004>
- Bluthé RM, Dantzer R, Kelley KW, 1992. Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. *Brain Res*, 573(2):318-320.
[https://doi.org/10.1016/0006-8993\(92\)90779-9](https://doi.org/10.1016/0006-8993(92)90779-9)
- Chipman PH, Fetter RD, Panzera LC, et al., 2022. NMDAR-dependent presynaptic homeostasis in adult hippocampus: synapse growth and cross-modal inhibitory plasticity. *Neuron*, 110(20):3302-3317.e7.
<https://doi.org/10.1016/j.neuron.2022.08.014>
- Cryan JF, Valentino RJ, Lucki I, 2005. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev*, 29(4-5):547-569.
<https://doi.org/10.1016/j.neubiorev.2005.03.008>
- Dai CX, Fu YY, Li XW, et al., 2025. Clinical efficacy and safety of vortioxetine as an adjuvant drug for patients with bipolar depression. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 26(1):26-38.
<https://doi.org/10.1631/jzus.B2400470>
- Duman RS, Sanacora G, Krystal JH, 2019. Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. *Neuron*, 102(1):

- 75-90.
<https://doi.org/10.1016/j.neuron.2019.03.013>
- Eicher TP, Mohajeri MH, 2022. Overlapping mechanisms of action of brain-active bacteria and bacterial metabolites in the pathogenesis of common brain diseases. *Nutrients*, 14(13):2661.
<https://doi.org/10.3390/nu14132661>
- Ernst J, Hock A, Henning A, et al., 2017. Increased pregenual anterior cingulate glucose and lactate concentrations in major depressive disorder. *Mol Psychiatry*, 22(1):113-119.
<https://doi.org/10.1038/mp.2016.73>
- George F, Daniel C, Thomas M, et al., 2018. Occurrence and dynamism of lactic acid bacteria in distinct ecological niches: a multifaceted functional health perspective. *Front Microbiol*, 9:2899.
<https://doi.org/10.3389/fmicb.2018.02899>
- Goldsmith DR, Rapaport MH, Miller BJ, 2016. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry*, 21(12):1696-1709.
<https://doi.org/10.1038/mp.2016.3>
- Goldsmith DR, Bekhbat M, Mehta ND, et al., 2023. Inflammation-related functional and structural dysconnectivity as a pathway to psychopathology. *Biol Psychiatry*, 93(5):405-418.
<https://doi.org/10.1016/j.biopsych.2022.11.003>
- Hardingham GE, Do KQ, 2016. Linking early-life NMDAR hypofunction and oxidative stress in schizophrenia pathogenesis. *Nat Rev Neurosci*, 17(2):125-134.
<https://doi.org/10.1038/nrn.2015.19>
- Haroon E, Miller AH, Sanacora G, 2017. Inflammation, glutamate, and glia: a trio of trouble in mood disorders. *Neuropsychopharmacology*, 42(1):193-215.
<https://doi.org/10.1038/npp.2016.199>
- Hu SH, Li A, Huang TT, et al., 2019. Gut microbiota changes in patients with bipolar depression. *Adv Sci (Weinh)*, 6(14):1900752.
<https://doi.org/10.1002/advs.201900752>
- Jiang HY, Ling ZX, Zhang YH, et al., 2015. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun*, 48:186-194.
<https://doi.org/10.1016/j.bbi.2015.03.016>
- Kang X, Liu CA, Ding YQ, et al., 2023. *Roseburia intestinalis* generated butyrate boosts anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8⁺ T cells. *Gut*, 72(11):2112-2122.
<https://doi.org/10.1136/gutjnl-2023-330291>
- Kleerebezem M, Hols P, Bernard E, et al., 2010. The extracellular biology of the lactobacilli. *FEMS Microbiol Rev*, 34(2):199-230.
<https://doi.org/10.1111/j.1574-6976.2009.00208.x>
- Knuesel T, Mohajeri MH, 2021. The role of the gut microbiota in the development and progression of major depressive and bipolar disorder. *Nutrients*, 14(1):37.
<https://doi.org/10.3390/nu14010037>
- Li MX, Han L, Xiao JL, et al., 2023. IL-1RA treatment prevents chronic social defeat stress-induced depression-like behaviors and glutamatergic dysfunction via the upregulation of CREB-BDNF. *J Affect Disord*, 335:358-370.
<https://doi.org/10.1016/j.jad.2023.05.049>
- Li QQ, Chen J, Hu P, et al., 2022. Enhancing GluN2A-type NMDA receptors impairs long-term synaptic plasticity and learning and memory. *Mol Psychiatry*, 27(8):3468-3478.
<https://doi.org/10.1038/s41380-022-01579-7>
- Liu ML, Fan GH, Meng LK, et al., 2025. New perspectives on microbiome-dependent gut-brain pathways for the treatment of depression with gastrointestinal symptoms: from bench to bedside. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 26(1):1-25.
<https://doi.org/10.1631/jzus.B2300343>
- Lozano CP, Wilkens LR, Shvetsov YB, et al., 2022. Associations of the dietary inflammatory index with total adiposity and ectopic fat through the gut microbiota, LPS, and C-reactive protein in the Multiethnic Cohort-Adiposity Phenotype Study. *Am J Clin Nutr*, 115(5):1344-1356.
<https://doi.org/10.1093/ajcn/nqab398>
- Lucidi L, Pettorruso M, Vellante F, et al., 2021. Gut microbiota and bipolar disorder: an overview on a novel biomarker for diagnosis and treatment. *Int J Mol Sci*, 22(7):3723.
<https://doi.org/10.3390/ijms22073723>
- Ma SS, Chen M, Jiang YH, et al., 2023. Sustained antidepressant effect of ketamine through NMDAR trapping in the LHb. *Nature*, 622(7984):802-809.
<https://doi.org/10.1038/s41586-023-06624-1>
- Machiels K, Joossens M, Sabino J, et al., 2014. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut*, 63(8):1275-1283.
<https://doi.org/10.1136/gutjnl-2013-304833>
- Martinez-Guryn K, Hubert N, Frazier K, et al., 2018. Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. *Cell Host Microbe*, 23(4):458-469.e5.
<https://doi.org/10.1016/j.chom.2018.03.011>
- McGuinness AJ, Davis JA, Dawson SL, et al., 2022. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Mol Psychiatry*, 27(4):1920-1935.
<https://doi.org/10.1038/s41380-022-01456-3>
- Mueller HT, Meador-Woodruff JH, 2004. NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. *Schizophr Res*, 71(2-3):361-370.
<https://doi.org/10.1016/j.schres.2004.02.016>
- Murta V, Fariás MI, Pitossi FJ, et al., 2015. Chronic systemic IL-1 β exacerbates central neuroinflammation independently of the blood-brain barrier integrity. *J Neuroimmunol*, 278:30-43.
<https://doi.org/10.1016/j.jneuroim.2014.11.023>
- Nakagawa Y, Chiba K, 2015. Diversity and plasticity of microglial cells in psychiatric and neurological disorders. *Pharmacol Ther*, 154:21-35.
<https://doi.org/10.1016/j.pharmthera.2015.06.010>
- Newpher TM, Ehlers MD, 2008. Glutamate receptor dynamics in dendritic microdomains. *Neuron*, 58(4):472-497.
<https://doi.org/10.1016/j.neuron.2008.04.030>
- Nikolova VL, Smith MRB, Hall LJ, et al., 2021. Perturbations

- in gut microbiota composition in psychiatric disorders: a review and meta-analysis. *JAMA Psychiatry*, 78(12):1343-1354.
<https://doi.org/10.1001/jamapsychiatry.2021.2573>
- Obi-Azuike C, Ebai R, Gibson T, et al., 2023. A systematic review on gut-brain axis aberrations in bipolar disorder and methods of balancing the gut microbiota. *Brain Behav*, 13(6):e3037.
<https://doi.org/10.1002/brb3.3037>
- Painold A, Mörk S, Kashofer K, et al., 2019. A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disord*, 21(1):40-49.
<https://doi.org/10.1111/bdi.12682>
- Polansky O, Sekelova Z, Faldynova M, et al., 2016. Important metabolic pathways and biological processes expressed by chicken cecal microbiota. *Appl Environ Microbiol*, 82(5):1569-1576.
<https://doi.org/10.1128/aem.03473-15>
- Poletti S, Mazza MG, Calesella F, et al., 2021. Circulating inflammatory markers impact cognitive functions in bipolar depression. *J Psychiatr Res*, 140:110-116.
<https://doi.org/10.1016/j.jpsychires.2021.05.071>
- Rao JS, Harry GJ, Rapoport SI, et al., 2010. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. *Mol Psychiatry*, 15(4):384-392.
<https://doi.org/10.1038/mp.2009.47>
- Renteria R, Maier EY, Buske TR, et al., 2017. Selective alterations of NMDAR function and plasticity in D1 and D2 medium spiny neurons in the nucleus accumbens shell following chronic intermittent ethanol exposure. *Neuropharmacology*, 112:164-171.
<https://doi.org/10.1016/j.neuropharm.2016.03.004>
- Sharon G, Sampson TR, Geschwind DH, et al., 2016. The central nervous system and the gut microbiome. *Cell*, 167(4):915-932.
<https://doi.org/10.1016/j.cell.2016.10.027>
- Shen W, Wang QW, Liu YN, et al., 2020. Synaptotagmin-7 is a key factor for bipolar-like behavioral abnormalities in mice. *Proc Natl Acad Sci USA*, 117(8):4392-4399.
<https://doi.org/10.1073/pnas.1918165117>
- Shen ZH, Luo WW, Tan B, et al., 2022. *Roseburia intestinalis* stimulates TLR5-dependent intestinal immunity against Crohn's disease. *eBioMedicine*, 85:104285.
<https://doi.org/10.1016/j.ebiom.2022.104285>
- Smith BJ, Miller RA, Schmidt TM, 2021. *Muribaculaceae* genomes assembled from metagenomes suggest genetic drivers of differential response to acarbose treatment in mice. *mSphere*, 6(6):e00851-21.
<https://doi.org/10.1128/msphere.00851-21>
- Song WD, Wang YY, Li GC, et al., 2023. Modulating the gut microbiota is involved in the effect of low-molecular-weight *Glycyrrhiza* polysaccharide on immune function. *Gut Microbes*, 15(2):2276814.
<https://doi.org/10.1080/19490976.2023.2276814>
- Spulber S, Bartfai T, Schultzberg M, 2009. IL-1/IL-1ra balance in the brain revisited – evidence from transgenic mouse models. *Brain Behav Immun*, 23(5):573-579.
<https://doi.org/10.1016/j.bbi.2009.02.015>
- Strenn N, Pålsson E, Liberg B, et al., 2021. Influence of genetic variations in *IL1B* on brain region volumes in bipolar patients and controls. *Psychiatry Res*, 296:113606.
<https://doi.org/10.1016/j.psychres.2020.113606>
- Sublette ME, Cheung S, Lieberman E, et al., 2021. Bipolar disorder and the gut microbiome: a systematic review. *Bipolar Disord*, 23(6):544-564.
<https://doi.org/10.1111/bdi.13049>
- Traynelis SF, Wollmuth LP, McBain CJ, et al., 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev*, 62(3):405-496.
<https://doi.org/10.1124/pr.109.002451>
- van Vugt B, van Kerkoele T, Vartak D, et al., 2020. The contribution of AMPA and NMDA receptors to persistent firing in the dorsolateral prefrontal cortex in working memory. *J Neurosci*, 40(12):2458-2470.
<https://doi.org/10.1523/jneurosci.2121-19.2020>
- Versele R, Sevin E, Gosselet F, et al., 2022. TNF- α and IL-1 β modulate blood-brain barrier permeability and decrease amyloid- β peptide efflux in a human blood-brain barrier model. *Int J Mol Sci*, 23(18):10235.
<https://doi.org/10.3390/ijms231810235>
- Wang QW, Wang YH, Wang B, et al., 2021. Synaptotagmin-7-mediated activation of spontaneous NMDAR currents is disrupted in bipolar disorder susceptibility variants. *PLoS Biol*, 19(7):e3001323.
<https://doi.org/10.1371/journal.pbio.3001323>
- Wang XY, Sun GQ, Feng T, et al., 2019. Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. *Cell Res*, 29(10):787-803.
<https://doi.org/10.1038/s41422-019-0216-x>
- Wang Z, Chen WH, Li SX, et al., 2021. Gut microbiota modulates the inflammatory response and cognitive impairment induced by sleep deprivation. *Mol Psychiatry*, 26(11):6277-6292.
<https://doi.org/10.1038/s41380-021-01113-1>
- Wong ML, Inserra A, Lewis MD, et al., 2016. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol Psychiatry*, 21(6):797-805.
<https://doi.org/10.1038/mp.2016.46>
- Wu WL, Gong XX, Qin ZH, et al., 2025. Molecular mechanisms of excitotoxicity and their relevance to the pathogenesis of neurodegenerative diseases—an update. *Acta Pharmacol Sin*, 46:3129-3142.
<https://doi.org/10.1038/s41401-025-01576-w>
- Xu HT, Fang F, Wu KZ, et al., 2023. Gut microbiota-bile acid crosstalk regulates murine lipid metabolism via the intestinal FXR-FGF19 axis in diet-induced humanized dyslipidemia. *Microbiome*, 11:262.
<https://doi.org/10.1186/s40168-023-01709-5>
- Xu YW, Ye LY, Li ZS, et al., 2025. Microglial transient receptor potential melastatin 2 deficiency accelerates seizure development via increasing AMPAR-mediated neuronal excitability. *MedComm*, 6(8):e70271.
<https://doi.org/10.1002/mco2.70271>

- Yamada-Fowler N, Fredrikson M, Söderkvist P, 2014. Caffeine interaction with glutamate receptor gene *GRIN2A*: Parkinson's disease in swedish population. *PLoS ONE*, 9(6):e99294.
<https://doi.org/10.1371/journal.pone.0099294>
- Yao H, Zhang DL, Yu H, et al., 2023. Gut microbiota regulates chronic ethanol exposure-induced depressive-like behavior through hippocampal NLRP3-mediated neuroinflammation. *Mol Psychiatry*, 28(2):919-930.
<https://doi.org/10.1038/s41380-022-01841-y>
- Zhang GX, Ma FM, Zhang ZW, et al., 2023. Associated long-term effects of decabromodiphenyl ethane on the gut microbial profiles and metabolic homeostasis in sprague-dawley rat offspring. *Environ Int*, 172:107802.
<https://doi.org/10.1016/j.envint.2023.107802>
- Zhao Q, Hao Y, Yang XQ, et al., 2023. Mitigation of maternal fecal microbiota transplantation on neurobehavioral deficits of offspring rats prenatally exposed to arsenic: role of microbiota-gut-brain axis. *J Hazard Mater*, 457:131816.
<https://doi.org/10.1016/j.jhazmat.2023.131816>
- Zhu HZ, Liang YD, Ma QY, et al., 2019. Xiaoyaosan improves depressive-like behavior in rats with chronic immobilization stress through modulation of the gut microbiota. *Biomed Pharmacother*, 112:108621.
<https://doi.org/10.1016/j.biopha.2019.108621>

Supplementary information

Tables S1 and S2; Fig. S1