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Research Article

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

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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 was performed to observe behavioral changes in the antibiotic-treated C57 BL/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 (AMPAR) genes were measured in the "BD" and control mice. Furthermore, the effects of IL-1 receptor antagonist on the glutamate pathways was 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-1β) in the serum and prefrontal cortex, microglial activation, and changes in the mRNA levels of NMDAR and AMPAR. Treatment with IL-1 receptor antagonist partially reversed the behavioral patterns, neuroinflammation, and transcription levels of glutamate receptors. Conclusion: The findings suggest that gut microbiota may influence glutamate receptor gene expression via an IL-1β-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 receptors; Interleukin 1

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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). Gut microbiota as part of the gut-brain axis has been recognized as an essential factor affecting human health (Sharon et al., 2016) and play an important role in emotional regulation (Liu et al., 2025). To date, accumulating studies have revealed that 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 the 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 IL-1β 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 *Lachnospiracea* (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 response (e.g., activation of 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.

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Glutamate receptors are classified into ionotropic and metabotropic receptors. The former mainly include N-methyl-D-aspartate receptors (NMDAR), kainate receptors (KAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), 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 (TNF-α) and IL-1β, which lead to an increase in synaptic glutamate in neurons and in turn mediates oxidative stress, thus affecting the function of glutamate receptors on glial cells and neurons (Haroon et al., 2016). 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-1β-dependent pathways, which contributes 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 antibiotics-treated mice. We then examined the behavioral patterns of mice in response to treatment, as well as the peripheral and cerebral inflammation response, the changes in glutamate receptor activity and their relationship with the inflammatory process. The findings provide new perspectives on the gut-brain communication in BD by regulating the activity of glutamate receptors in an IL-1β-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 (Reference No. 2024-376).

Male C57BL/6J SPF mice aged 6-8 weeks were selected for the animal experiments, with an average weight of (20±2) g. Animals were housed in groups of 5-6 individuals per cage using sterilized polypropylene containers. The feeding environment included stable room temperature ((20±1) °C) and automated 12-hour 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.

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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 Diagnostic and Statistical Manual of Mental Disorders (DSM-5). All fecal donors were screened according to the following inclusion criteria: (1) First episode or psychotropic drug-free for at least 3 months; (2) No use of antibiotics, probiotics, or prebiotics for at least one month before sample collection; (3) 17-item Hamilton Depression Rating Scale (HDRS-17) score ≥ 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℃. 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 15-30 mL PBS-glycerol mixture (20%, volume fraction). Then, the mixture was passed through 0.60 mm sterile filters and centrifuged at 2,500 r/min, 4 °C, 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 suspension was thawed in ice-water mixtures for 2-4 h.

2.3 Antibiotic treatment and fecal microbiota transplantation (FMT)

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 7 consecutive days. At the same time, 200 μL 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 (control mice). Groups of 3-4 mice received bacterial suspension derived from a single donor source. Fecal microbiota suspension (10 μL per gram body weight) was gavaged on Days 1, 3, 5, 8, 10, and 12, respectively, 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-1RA (BBI life sciences) at 50 pg/kg for 7 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

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.

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 (25±1°C). The heads of 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. 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.

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 complete absence of limb movement.

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2.6 Gut microbiota 16S rRNA sequencing of mice after FMT

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

Fecal genomic DNA was isolated using a DNA Extraction Kit (QIAamp 96 PowerFecal QIAcube HT kit, QIAGEN) following the manufacturer's instructions. DNA purity and concentration 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 primers: 5'- TACGGRAGGCAGCAG -3'; reverse primers: 5'- AGGGTATCTAATCCT-3'). The purified amplicons (QIAquick Gel Extraction Kit) 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 database was used for species annotation and species classification analysis, and 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 PCR

Within 24 hours of the behavioral test, all mice were sacrificed after anesthesia, then the prefrontal lobe tissues were dissected on ice and preserved at -80°C until testing. Total RNAs from prefrontal lobe tissues were 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 ABScript II cDNA First Strand Synthesis Kit (Abclonal, China), maintaining the thermal cycling parameters as per the manufacturer's recommendations. Target genes were detected by QuantStudio 5DX Real-Time PCR System with SYBR Green Fast qPCR Mix (ABclonal, China). Beta-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*), glutamate ionotropic receptor NMDA type subunit 2A (*grin2A*), glutamate ionotropic receptor NMDA type subunit 2B (*grin2B*), glutamate ionotropic receptor NMDA type subunit 2C (*grin2C*), glutamate ionotropic receptor NMDA type subunit 2D (*grin2D*), glutamate ionotropic receptor AMPA type subunit 1 (*gria1*), glutamate ionotropic receptor AMPA type subunit 2 (*gria2*), glutamate ionotropic receptor AMPA type subunit 3 (*gria3*), glutamate ionotropic receptor AMPA type subunit 4 (*gira4*) are given in **Table S2**.

2.8 Enzyme-linked immunosorbent assay (ELISA) analysis

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 minutes on ice. This was followed by centrifugation at 13,000 rpm for 15 min at 4℃, and the supernatant was removed to obtain protein samples. Protein concentrations were determined through bicinchoninic acid assay (BCA Kit P0010, Beyotime) using bovine serum albumin standards. Absorbance measurements were performed at 562 nm wavelength.

TNF-α, IL-6, BDNF, IFN-γ, IL-10 and IL-1β protein concentrations in the prefrontal lobe tissues and IL-6, IL-1β, and IFN-γ in serum were assessed using enzyme-linked immunosorbent assay kits according to the manufacturer's protocols. All ELISA kits were obtained from Abclonal (RK00008, RK00016, RK00027, RK00019, RK00006).

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2.9 Immunofluorescence analysis

Following transcardial perfusion with 4% paraformaldehyde (PFA/PBS), the whole brain tissues were harvested and immersed in PFA at 4℃ for 24 hours. Subsequently, the brain tissues were removed into 30% sucrose (in PBS) until complete tissue equilibrium, followed by Optimal Cutting Temperature compound (OCT) embedding (Sakura Finetek, Japan).

Next, sequential 25 μm coronal sections were prepared using a cryostat microtome (Leica, Wetzlar, Germany) at -20℃ chamber temperature. Sections were blocked with 5% bovine serum albumin (BSA/PBS) and permeabilized with 0.5% Triton X-100 in blocking solution for 60 minutes. Anti-Ionized Calcium Binding Adaptor Molecule 1 (Anti-Iba1) rabbit antibody (1:200, Cell Signaling technology, USA) was applied and maintained at 4℃ for 16 hours. Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:500) was incubated for 1 hour 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× 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 ± SEM (standard error of mean). To ensure unbiased group distribution, subject allocation followed the complete randomization protocols. The sample sizes (*n*) 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 7 consecutive days. After a one-day antibiotic washout period, the mice were gavaged with feces either from 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 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 1,465 bacterial species were present in the fecal microbiota of both BD patients and BD mice, and 1,745 bacterial species were present in both HC donors and control mice (**Fig. 1h**). We also compared the gut microbiota of BD patients and healthy controls 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 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)**.

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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 of 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 species richness (Chao1 index: CON: 3240.00±109.50; FMT-BD: 2880.00±154.20; *P*=0.0196) and the number of OTUs actually observed (observed species: CON: 1985.00±67.05; FMT-BD: 1671.00±104.00; *P*=0.0187) when compared to the control mice **(Figs. 2c and 2d)**. No significant differences were observed between the two groups in Simpson's (CON: 0.9753±0.0032; FMT-BD: 0.9767±0.0041; *P*=0.8214) or Shannon's index (CON: 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 communities in the BD mice group, including *Roseburia*, *[Eubacterium]_xylanophilum_group*, *Alistipes*, *Lactobacillus*, *Clostridia_vadinBB60_group*, *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)**.

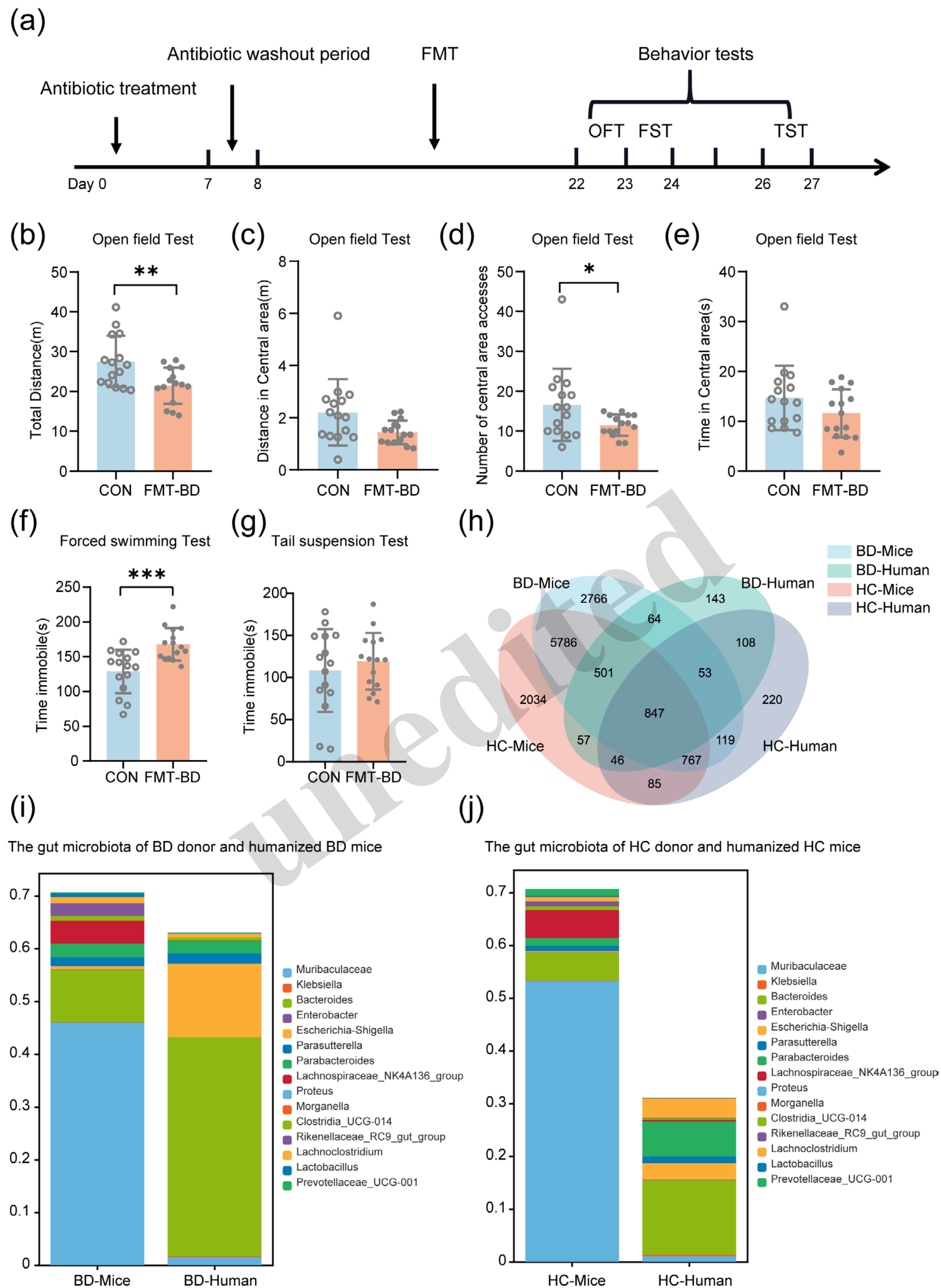

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Fig. 1 The depression-like behaviors and gut microbiota humanization in mice induced by fecal microbiota transplantation from BD patients.

(a) Mouse experimental schedule. (b–e) In the OFT, BD mice traveled shorter total distances and accessed the central area frequently, though 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 plots 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 rRNA analysis of gut microbiota, HC-Mice (n=17), BD-Mice (n=19), HC-Human (n=4), BD-Human (n=4). Data are presented as mean ± standard error of the mean (± 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). Abbreviations: Fecal microbiota transplantation (FMT), Open field test (OFT), Forced swimming test (FST), Tail suspension test (TST), Bipolar disorder (BD).

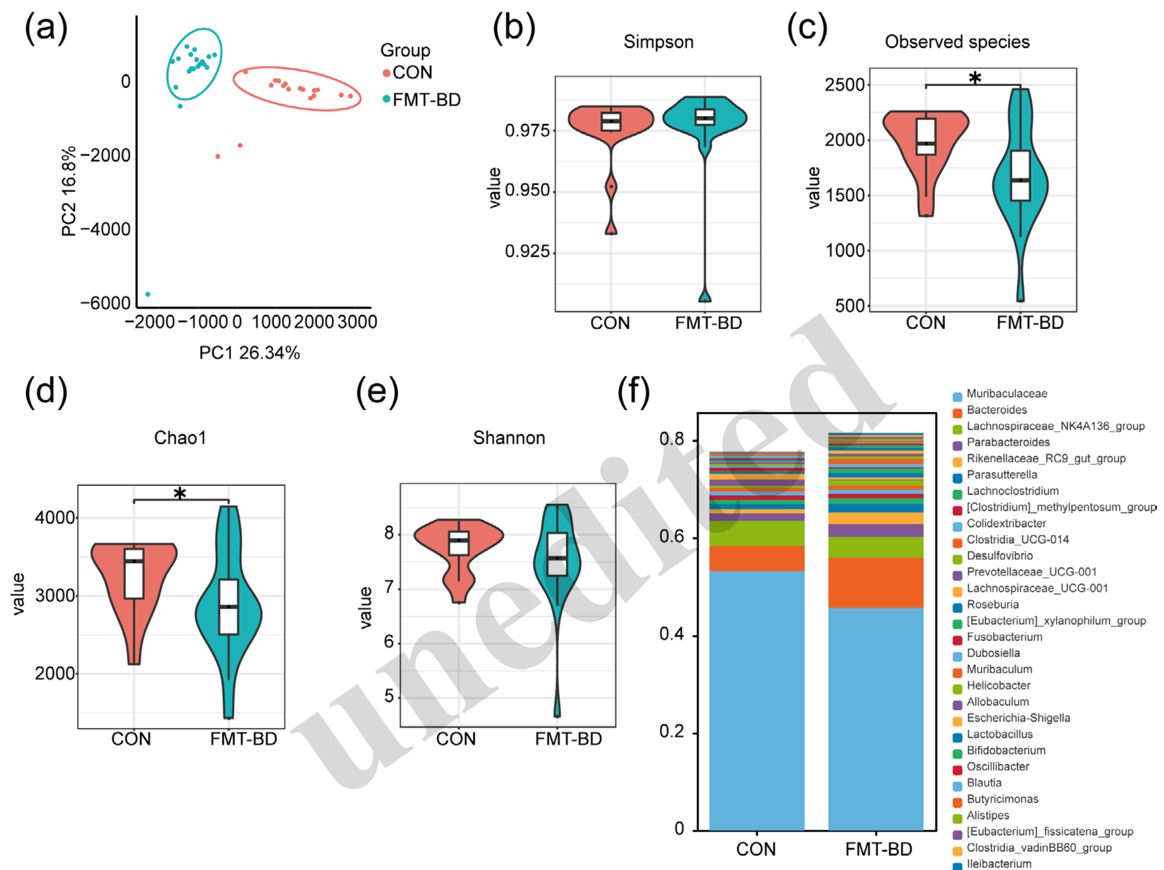

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Fig 2. Gut microbial features in mice with fecal bacteria transplant based on 16S rRNA sequencing.
(a) Principal coordinate analysis (PCoA) of the beta diversity Euclidean distance algorithm. (b) Alpha diversity measured by Simpson's index showed no difference between the FMT-BD group and the control group. (c) The number of OTUs by observed species in 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 between the FMT-BD group and the control group. (f) Bar plot showing 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 ± standard error of the mean (± SEM). Significant differences were measured by the Wilcoxon rank-sum test (b, c, d, e) ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

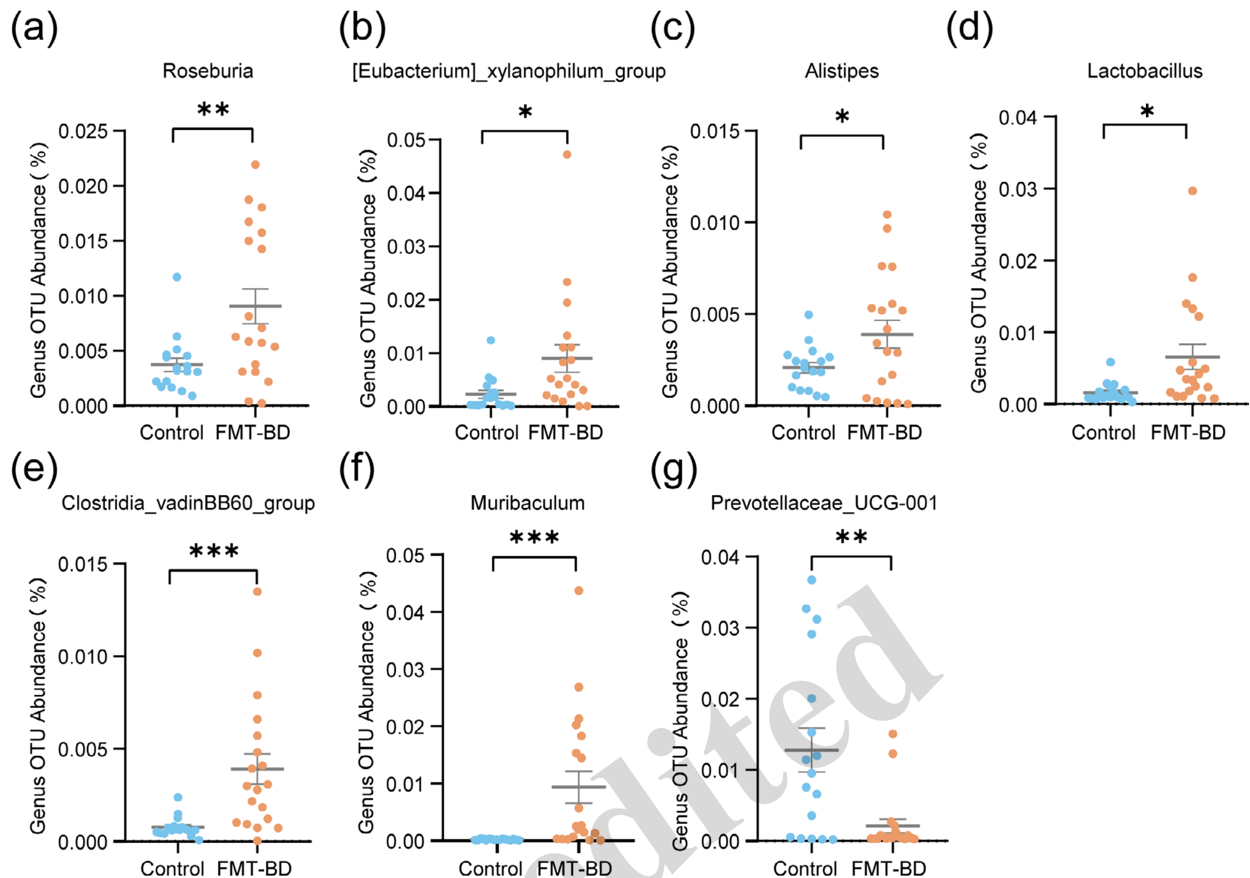

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Fig 3. Significant changes in the relative abundance of gut microbes at the genus level based on 16S rRNA sequencing. (a-g) The abundance of *Roseburia* (P=0.0063), *[Eubacterium]_xylanophilum_group* (P=0.0486), *Alistipes* (P=0.0365), *Lactobacillus* (P=0.0156), *Clostridia_vadinBB60_group* (P=0.001), and *Muribaculum* (P=0.0001) in FMT-BD was increased significantly, while that of *Prevotellaceae_UCG-001* (P=0.0031) was decreased. CON (n=17), FMT-BD (n=19). Data are presented as mean ± standard error of the mean (± SEM). Significant differences were measured by an independent samples t-test (c) or Kruskal-wallis test (a, b, d, e, f, g) (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

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

After 27 days 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-1β, 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-1β levels in the BD mice group were significantly higher than that of the control group **(Fig. 4c)**, while IFN-γ and IL-6 levels were not different **(Figs. 4a and 4b)**. In the prefrontal lobe tissues, the IL-1β level in the BD mice group was also significantly higher than that in the control group **(Fig. 4g)**, whereas the levels of IL-6, IL-10, IFN-γ, and TNF-α were not **(Figs. 4d, 4e, 4f, 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 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), Gria3 (CON: 0.9530±0.1212; 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)**.

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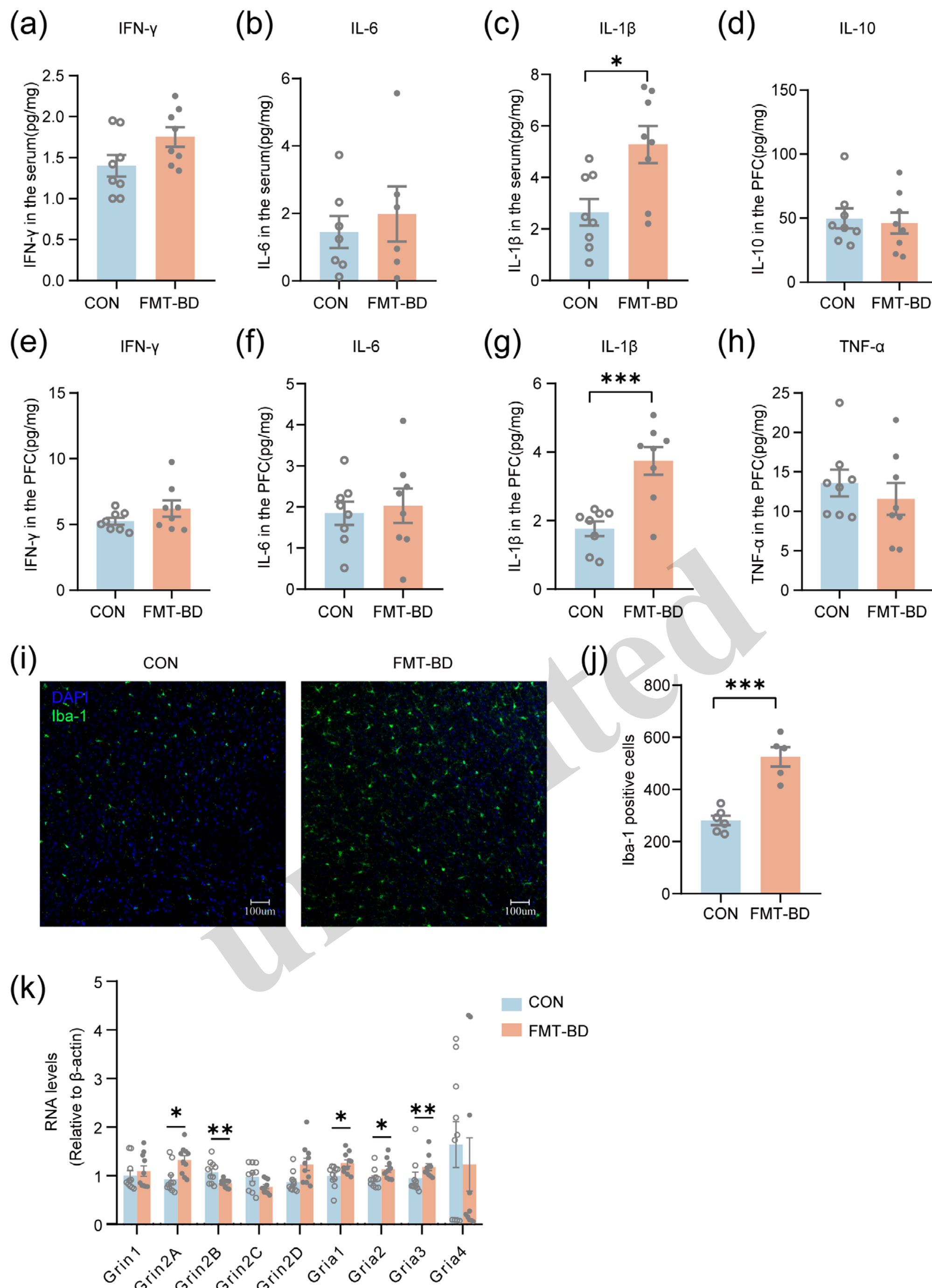

Fig 4. Changes in inflammatory factor levels and glutamate receptor gene expression caused by fecal microbiota transplantation.

(a-c) Enzyme-linked immunosorbent assay (ELISA) analysis revealed the serum levels of inflammatory factors, including IFN-γ, IL-6 and IL-1β. The serum IL-1β level was increased significantly in the FMT-BD group. (d-h) The levels of inflammatory factors in the prefrontal cortex, including IL-10, IFN-γ, IL-6, IL-1β, and TNF-α. The prefrontal cortex IL-1β level was increased significantly in the FMT-BD group. (i) Immunofluorescence staining for Iba-1 (green) and DAPI (blue) in the prefrontal tissues. The number of Iba-1 positive cells in the FMT-BD group was increased compared to the control group. Scale bar = 100 μm. (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 AMPAR in the prefrontal tissues in FMT-BD and control groups. The levels of *Grin2A*, *Gria1*, *Gria2* and *Gria3* were increased in BD mice, while the level of *Grin2B* was decreased. In the serum ELISA analysis, CON (n=7-8), 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 group (*n*=5). In the RT-PCR test, CON (*n*=10), FMT-BD (*n*=10). Data are presented as mean ± standard error of the mean (± SEM). Significant differences were measured by an independent samples t-test (a, b, c, d, e, h, j) or Kruskal-wallis test (k) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Abbreviations: Glutamate ionotropic receptor NMDA type subunit 1 (*Grin1*), Glutamate ionotropic receptor NMDA type subunit 2A (*Grin2A*), Glutamate ionotropic receptor NMDA type subunit 2B (*Grin2B*), Glutamate ionotropic receptor NMDA type subunit 2C (*Grin2C*), Glutamate ionotropic receptor NMDA type subunit 2D (*Grin2D*), Glutamate ionotropic receptor AMPA type subunit 1 (*Gria1*), Glutamate ionotropic receptor AMPA type subunit 2 (*Gria2*), Glutamate ionotropic receptor AMPA type subunit 3 (*Gria3*), Glutamate ionotropic receptor AMPA type subunit 4 (*Gira4*).

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

The above findings suggested that gut microbiota from BD patients might mediate neuroinflammation in the prefrontal lobe through the IL-1β 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 (50pg kg^{-1}) 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 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 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 crossing and time of movement in the central area, mice both 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-5e)**, indicating that IL-1RA treatment was unable to improve the anxiety-like behavior of mice.

Transcription levels of NMDAR and AMPAR genes were also examined after IL-1RA treatment. From the RT-qPCR results, we found that the levels of *Grin2A*, *Gria1*, *Gria2* and *Gria3* in the saline-treated BD mice were increased compared with the control group, which was consistent with aforementioned findings, while there was no change in *Grin2B*. IL-1RA treatment significantly decreased the levels of *Gria1* and *Gria3*, and partially decreased *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)**.

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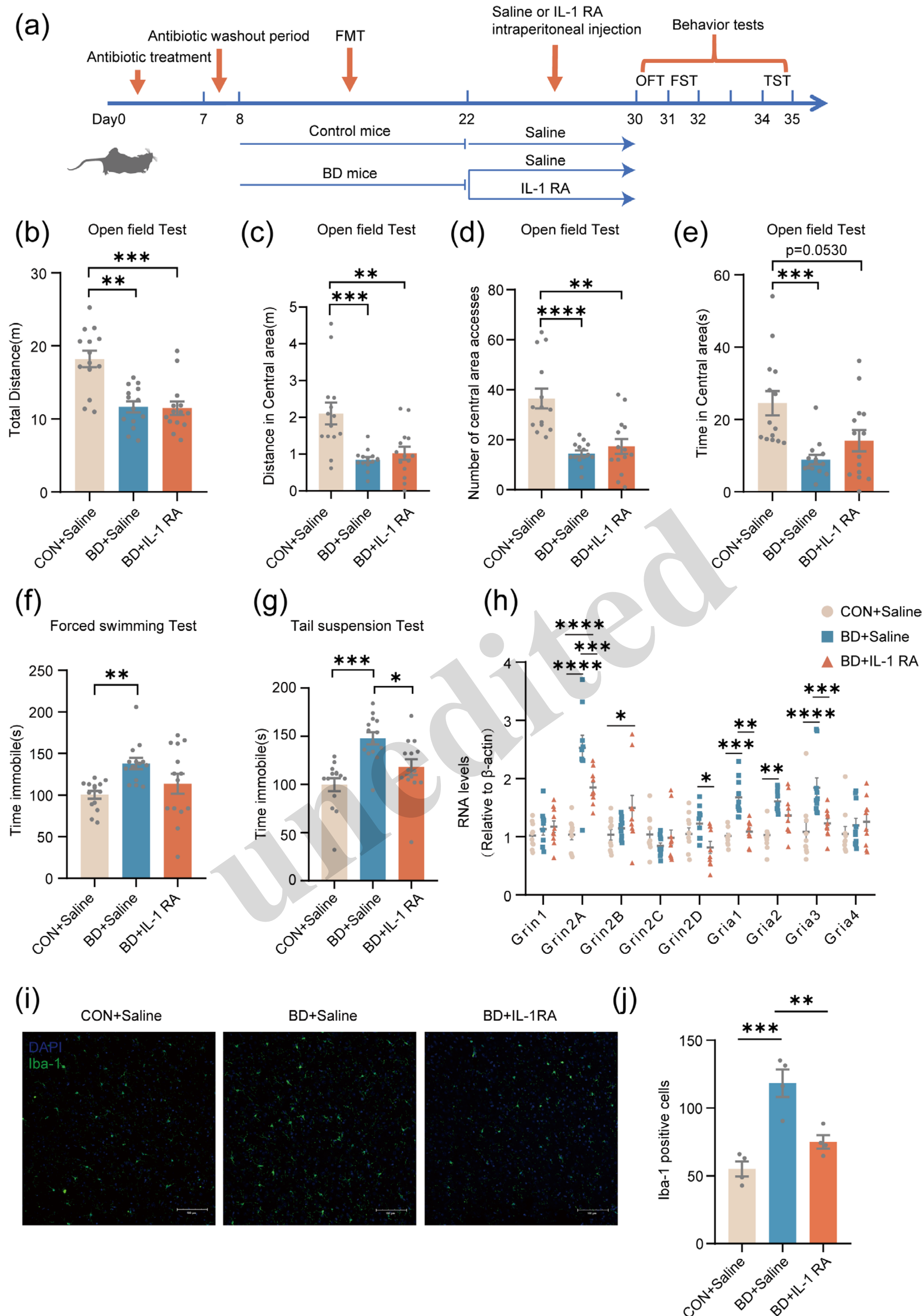

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Fig 5. Partial reversal of the FMT-induced behavioral phenotype in mice by intraperitoneal injection of IL-1RA. (a) Experimental schedule for IL-1 RA injection in mice. (b-e) The OFT examined the total movement distance, movement distance, time, and 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 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 TST. (h) The gene expression of NMDAR and AMPAR in the prefrontal tissues in three groups. (i-j) Immunofluorescence staining for Iba-1 (green) and DAPI

(blue) in the prefrontal tissues and 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 ± 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) (*$P<0.05$, **$P<0.01$, ***$P<0.001$). Abbreviations: Fecal microbiota transplantation (FMT), Open field test (OFT), Forced swimming test (FST), Tail suspension test (TST), Bipolar disorder (BD), Glutamate ionotropic receptor NMDA type subunit 1 (*Grin1*), Glutamate ionotropic receptor NMDA type subunit 2A (*Grin2A*), Glutamate ionotropic receptor NMDA type subunit 2B (*Grin2B*), Glutamate ionotropic receptor NMDA type subunit 2C (*Grin2C*), Glutamate ionotropic receptor NMDA type subunit 2D (*Grin2D*), Glutamate ionotropic receptor AMPA type subunit 1 (*Gria1*), Glutamate ionotropic receptor AMPA type subunit 2 (*Gria2*), Glutamate ionotropic receptor AMPA type subunit 3 (*Gria3*), Glutamate ionotropic receptor AMPA type subunit 4 (*Gira4*).

4 Discussion

In the current study, we found that FMT of BD microbiota in mice not only induced behavioral changes but also elicited IL-1β-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 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 a mouse model of bipolar depression via FMT. As observed in Fig 1, in the OFT and FST, the BD mice exhibited depression-like and anxiety-like behavior, which was consistent with previously reported animal models of BD. For example, 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; Hughson 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.

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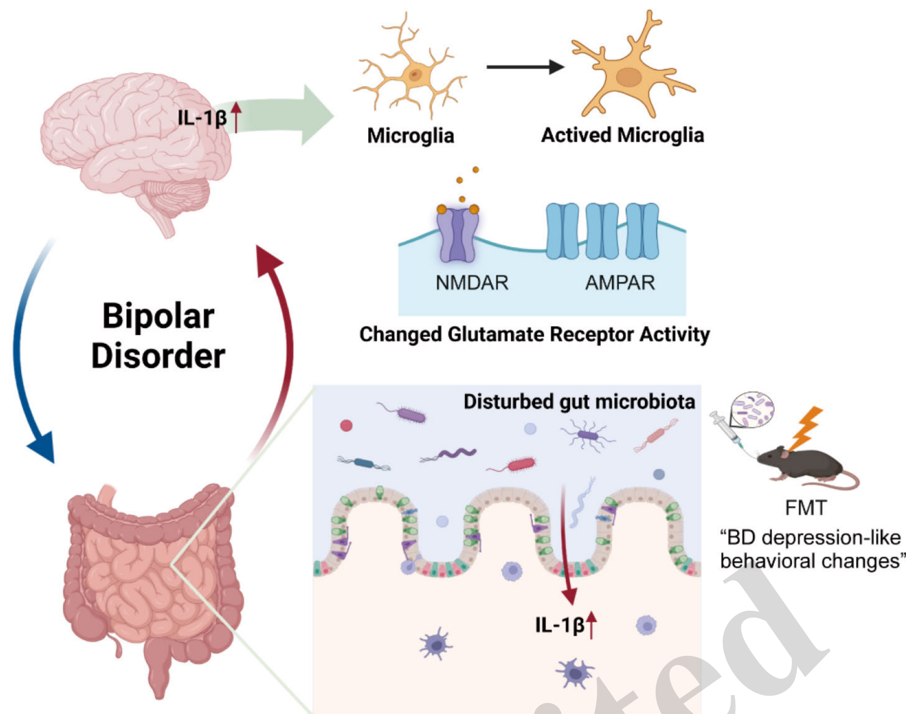

Fig. 6 Crosstalk between gut microbiota and glutamate receptors via an IL-1β-dependent manner.
In bipolar disorder, the disordered gut microbiota triggers the release of systemic IL-1β. The circulating IL-1β passes through the blood-brain barrier (BBB) and induces neuroinflammation including the activity of microglia, ultimately mediating changes in glutamate receptor activity and mediating bipolar depression-like behaviors. Image created with BioRender.com, with permission. Abbreviations: Fecal microbiota transplantation (FMT), Bipolar disorder (BD).

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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*, *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 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, typically associated with gut barrier integrity and protection against inflammatory pathology (Kang et al., 2023) (Shen et al., 2022) (Machiels et al., 2014) (Song et al., 2023). Their elevation in BD mice may

represent a compensatory response counteracting FMT-induced gut inflammation, despite established reductions of *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 elmination, 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 et al., 2021). Previous studies confirmed that the serum IL-1β level was significantly higher in BD patients during remission phase compared with controls (Goldsmith et al., 2016), which was associated with neurocognitive deficits in BD (Poletti et al., 2021). In addition, IL-1β 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-1β 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-1β 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-1β 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-1β 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-1β-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 mRNA levels of *Grin2A*, *Gria1*, *Gria2* and *Gria3* were increased and the mRNA level of *Grin2B* was decreased (see Fig 4), indicating that IL-1β activation may induce prefrontal NMDAR and AMPAR-related neurotoxicity. Increased Grin2A may enhance calcium influx through NMDA receptors, 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 NR2 and AMPAR subunits. Nevertheless, researchers discovered that NR3A mRNA was significantly decreased in the 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 levels of IL-1β, IL-1 receptor, astrocyte and microglia-related markers, as well as several oxidative stress-related proteins and mRNAs (Rao et al., 2010).

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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 of NMDAR and AMPAR levels and the microglial activation in the prefrontal lobe. As a key mediator of inflammatory response, IL-1β 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-1β, IL-1RA, and the IL-1β: IL-1RA ratio are higher in patients with bipolar depression than healthy individuals before treatment: after one week of antidepressant treatment, IL-1β was significantly decreased and IL-1RA was significantly increased, and the ratio of IL-1β 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-1β 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 of dendritic spine density and impairments in AMPARs-mediated neurotransmission (Li, et al., 2023). Interestingly, intraperitoneal injection of IL-1RA could alleviate LPS-induced depression-like behaviors (Bluthé et al., 1992). Current treatment algorithms for BD, particularly bipolar depression, remain inadequately defined. The existing pharmacopeia is limited and supported by insufficient evidence. Some agents carry risks, such as treatment-emergent affective switch and metabolic adverse effects (Dai et al., 2025). Our findings put forward new approaches to treating BD depression by targeting the IL-1β 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 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.

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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 AMPAR) genes in the prefrontal lobe, potentially involving an IL-1β-dependent pathway, which proves our hypothesis.

Data availability statement

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

Acknowledgments

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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. Kaijing DING and Yi CHEN performed the immunofluorescence staining and RT-PCR and ELISA analysis. 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, which was reviewed by all authors.

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 conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Institutional Review Board of the First Affiliated Hospital, School of Medicine of Zhejiang University, China) and with the Helsinki Declaration of 1975, as revised in 2008. This study was approved by the Institutional Review Board of the First Affiliated Hospital, School of Medicine of Zhejiang University (Reference No. 2017-397). 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 (Reference No. 2024-376).

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Supplementary information
Tables S1 and S2; Fig. S1

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