# Pakistan Veterinary Journal

**RESEARCH ARTICLE**

# Gut-Derived Indole-3-Propionic Acid Protects Against Neuroinflammation After Traumatic Brain Injury Through the NLRP3 Signaling Pathway

Yue Zhao[1], Lu Zhou[1], Xiuxiu Xu[1], Jiexin Zou[2], Kai Hu[2] and Lijuan Quan*[1]

[1]Department of Rehabilitation, The 1st affiliated hospital, Jiangxi Medical College, Nanchang University, Nanchang, China; [2]Animal Center of Core Facility for Biomedical Science, Nanchang University
*Corresponding author: ndyfy01461@ncu.edu.cn

**A B S T R A C T**

Traumatic brain injury (TBI) poses a significant threat to human and veterinary health, with secondary neuroinflammation driven by the gut-brain axis exacerbating brain damage. This study aimed to investigate the role of gut microbiota-derived indole-3-propionic acid (IPA) in mitigating neuroinflammation following traumatic brain injury (TBI) and to elucidate its mechanism. TBI models were established using controlled rotational acceleration. Gut microbiota composition and serum metabolites were analyzed via 16S rRNA sequencing and untargeted metabolomics. Fecal microbiota transplantation (FMT) from TBI rats to antibiotic-treated healthy rats was performed to validate microbiota-dependent effects. IPA was orally administered post-TBI and neuroinflammation was assessed by immunofluorescence, qRT-PCR and proteomics. Our results demonstrate that TBI induces dysbiosis of gut commensals (e.g., Alistipes, Allobaculum) and reduces serum IPA levels. Supplementation with IPA suppressed microglial activation and NLRP3 inflammasome signaling, highlighting its therapeutic potential for neuroinflammatory disorders. These findings reveal a novel gut-microbiota-neuroinflammation axis, supporting IPA-based interventions for TBI management in both humans and companion animals.

**To Cite This Article:** Zhao Y, Zhou L, Xu X, Zou J, Hu K and Quan L, 2025. Gut-Derived Indole-3-Propionic Acid Protects Against Neuroinflammation After Traumatic Brain Injury Through the NLRP3 Signaling Pathway. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.173

## INTRODUCTION

Traumatic brain injury (TBI) is a destructive damage to the brain structure and function resulting from external physical trauma and has emerged as a leading threat to human and companion animal health and life (Finnie, 2012; Finnie, 2014; Kuo et al., 2018; Brett et al., 2022; Wart et al., 2024). The pathological mechanisms underlying TBI encompass intricate abnormalities within cortical networks, characterized by the degeneration of functional neurons and disruption of synaptic circuits which involves both primary and secondary injuries (Orive et al., 2009; Wang et al., 2020). Secondary brain injury is an important component of chronic neurodegeneration and neurological dysfunction post-TBI, and the mechanisms include inflammatory response, oxidative stress, apoptosis, mitochondrial dysfunction, and calcium overload (Schweitzer et al., 2019). Neuroinflammation is a crucial mechanism of secondary injury post-TBI. Neuroinflammation results in the release of harmful substances and activation of the innate immune system cause neuronal apoptosis, leading to blood-brain barrier damage, worsening cerebral edema, and increasing brain tissue damage (Sulimai and Lominadze, 2020; Drieu et al., 2022; Liu et al., 2022). Neuroinflammation is a complex response in the central nervous system to harmful stimuli like TBI, infections, and other conditions, primarily driven by the activation of the innate immune system. When the brain experiences an injury, as in TBI, a complex network of signaling pathways activates the innate immune response(Kalra et al., 2022). This triggers the release mediators that help recruit immune cells, clear debris and start repair processes (Amanollahi et al., 2023). While the initial inflammatory response is crucial for brain healing, prolonged or dysregulated inflammation can be harmful. Chronic neuroinflammation may disrupt the blood-brain barrier (BBB), creating a cycle of inflammation and cell death. This maladaptive response is linked to various neurological conditions (Kalra et al., 2022). Thus, inhibiting neuroinflammation has become a key therapeutic goal for TBI in human beings and animals.

Current clinical and veterinary treatments for TBI primarily focus on symptomatic management, with

limited options to modulate neuroinflammation (Verdoodt *et al.*, 2022; Elfers *et al.*, 2024; Blanquet *et al.*, 2025). In recent years, the interplay between neurological disorders and the gut microbiota, along with its metabolites, has been increasingly elucidated (Morais *et al.*, 2021; Socala *et al.*, 2021). TBI has already reported to induce modifications in the gut microbiota, and these alterations may subsequently affect the extent of acute pathological damage following TBI (Hanscom *et al.*, 2021). The gut microbiota and relative metabolites can regulate neuroinflammation through the microbiome-gut-brain axis, while gut microbiota transplantation can effectively alleviate inflammatory responses, thus improving neurological dysfunction (Hu *et al.*, 2023).

Studies have increasingly focused on the anti-inflammatory effects of metabolites derived from gut microbiota. Research showed that there are significant abnormalities in tryptophan metabolism post-TBI, particularly in indole and its derivatives (Sun *et al.*, 2023). Indoles were found to decrease proinflammatory cytokine production in astrocytes and reduce central nervous system inflammation in antibiotic-treated mice (Rothhammer *et al.*, 2016). IPA is one of the tryptophan derivatives produced by gut bacterium. Past few years, there has been a significant increase in understanding the biological actions of IPA, which providing new evidence of the biological function of this metabolite (Konopelski *et al.*, 2019; Fang *et al.*, 2022). IPA reduces the release of pro-inflammatory cytokines in mammalian disease models (Zhao *et al.*, 2019; Garcez *et al.*, 2020; Du *et al.*, 2021; Li *et al.*, 2021; Konopelski and Mogilnicka, 2022). While IPA exhibits anti-inflammatory properties across various organs, its impact on neuroinflammation following TBI remains uncertain.

The neuroinflammation induced by the NLRP3 inflammasome is emerging as a significant area of research, particularly in the context of TBI. NLRP3 inflammasome stimulation sets off a series of inflammatory events that may critically influence neurological outcomes after TBI (Tan *et al.*, 2021). The NLRP3 inflammasome is pivotal in brain injury post-TBI(Xu *et al.*, 2018). Recent research has confirmed that the expression of NLRP3 inflammasome significantly increases post-TBI, while NLRP3 inflammasome inhibitors can notably reduce neuroinflammation in TBI rats. The NLRP3 signaling axis emerges as a key therapeutic target to mitigate microglial overactivation and secondary brain damage in TBI (Chakraborty *et al.*, 2023). But it remains unclear whether IPA improves the neuroinflammatory responses to TBI by regulating the NLRP3 pathway.

In this study, we evaluated the gut microbiota and serum metabolites altered in TBI rats and analyzed the correlation between gut microbiota and circulating metabolites. In addition, we examined the mechanism by which the tryptophan metabolite IPA attenuates hippocampal neuroinflammation and ameliorates TBI via modulation of the NLRP3 inflammasome pathway.

## MATERIALS AND METHODS

**TBI rat model:** Experimental procedures involving animals were authorized by the Nanchang University Institutional Animal Care and Use Committee (IACUC),

ensuring compliance with national regulatory requirements. This approval reflects our commitment to uphold the highest ethical standards in research involving animal subjects. Prior to the initiation of the study, a comprehensive proposal detailing the experimental design, methodology, and anticipated impacts on animal welfare was submitted and thoroughly reviewed by the committee.

SPF Sprague-Dawley (SD) male rats (250-300g) were obtained from Laboratory Animal Center (LAC) of Nanchang University. Rats were housed at a constant temperature and humidity room (temperature: 22-24°C; humidity: 45-60%). The experiment commenced after a one-week acclimation period. Brain injury model was established as previously described (Quan *et al.*, 2023). All surgical procedures adhered to institutional ethical guidelines for vertebrate research. Rats were anesthetized via isoflurane inhalation and immobilized in a stereotactic frame to maintain a 90° torso-to-horizontal angle. A custom-built lateral cranial rotation device was applied to induce controlled rotational acceleration of the cranium, mimicking diffuse axonal injury (DAI) pathophysiology. Post-injury, animals were transferred to a temperature-controlled recovery chamber for continuous monitoring of core temperature, respiratory rate, and motor function until stabilization (≥2 hours). Following stabilization, rats were singly housed in pathogen-free conditions with *ad libitum* access to food/water. Full-thickness skin incisions were closed using absorbable sutures, and neurological recovery was assessed daily. At 48 hours post-TBI, rats were humanely euthanized via pentobarbital overdose followed by transcardial perfusion fixation for histological and molecular analyses.

**Fecal microbiome transplantation (FMT):** To investigate the role of gut microbiota in the neuroinflammation after TBI, freshly prepared antibiotic cocktail mix (Abx) was administered to the daily drinking water of rats to facilitate the establishment of a suitable environment for FMT. The specific concentrations of antibiotics in the cocktail included neomycin at 100mg/L, metronidazole at 100mg/L, vancomycin at 50mg/L, penicillin at 100mg/L, and streptomycin at 50mg/L. This combination was designed to effectively suppress the gut microbiota and disrupt the existing microbial community, thereby allowing for a more controlled reintroduction of microbiota through FMT. FMT was performed as previously described (Wang *et al.*, 2022). Fecal specimens were aseptically procured from TBI rat models and subjected to mechanical disintegration using phosphate-buffered saline (PBS) at a 1:10 (weight/volume) dilution. Centrifugal separation was performed at 500×g for 5 minutes under refrigerated conditions (4°C), yielding a supernatant fraction enriched with viable microbial biomass and bioactive metabolites critical for effective FMT. Following a week of daily high-dose antibiotics, the normal rats received 150μL of the supernatant orally each day for 4 weeks (C-TBI).

**IPA supplementation experiment:** Previous studies suggest that the oral administration of IPA at a dosage of 20mg/kg/day in rats does not lead to significant toxicity or adverse effects. Furthermore, there even some evidence

indicating its potential neuroprotective properties(Li *et al.*, 2021; Socala *et al.*, 2021). Thus, in our experiment involving IPA supplementation, the TBI rats were administered a daily oral gavage of 20mg/kg IPA for a duration of two weeks, whereas both the TBI and sham groups were subjected to an equivalent dosage of standard saline treatment.

**Gut microbiota profiling:** Fecal microbial DNA was extracted from the collected fecal samples. Following the extraction of DNA, the next step involved amplifying the V3-V4 hypervariable regions of the bacterial 16S ribosomal RNA (rRNA) gene. These regions are of particular interest due to their variability among different bacterial taxa, making them ideal targets for studying microbial diversity and composition. The amplification process was conducted using specific primers, 515F and 806R. Further sequence analysis was conducted using the QIIIME2 platform (version 2019.10). Gut microbiota bioinformatics, encompassing diversity analysis, species compositions, and LEfSe analysis across groups, was evaluated using the R package microeco (version 0.12.0).

**Serum metabolomic analysis:** Sample extraction and instrumental analysis followed rigorously validated protocols to ensure reliable and reproducible results(Agirman *et al.*, 2021). Specifically, 100µL serum and 400µL methanol were mixed in a 1mL centrifuge tube and sonicated for 5 minutes to ensure thorough homogenization and disruption of cellular debris. The sonication-treated suspension underwent differential centrifugation at 15,000×g (equivalent to 15,000rpm under specified conditions) for 10 minutes at 4°C. The resulting clarified fraction was carefully decanted into sterile filtration systems equipped with 0.22µm membrane filters to eliminate microbial contaminants and host-derived debris. Subsequent recentrifugation under identical parameters further purified the supernatant, which was then aliquoted (0.4mL) into LC-MS vials for untargeted metabolomics profiling using a Triple TOF 5600+ mass spectrometer coupled with a Waters Acquity UPLC HSS T3 C18 column (2.1×100mm, 1.8µm). Chromatographic separation was achieved with a binary mobile phase system (0.1% formic acid in water/acetonitrile) at 0.35mL/min flow rate and 40°C column temperature, with metabolite detection optimized by electrospray ionization (ESI) in positive/negative switching mode. Data processing incorporated HMDB-based metabolite annotation and differential abundance analysis through DESeq2 (v1.34.0), while multivariate statistical evaluation was performed using MetaboAnalyst (v5.0) with false discovery rate (FDR) correction.

**Brain amino acid metabolomic analysis:** Amino acids in hippocampus region tissue were analyzed using LC-MS/MS. the LC-MS/MS method provides accurate identification and quantification of amino acids, making it an ideal choice for examining metabolic alterations in hippocampus region tissue. Specifically, a total of 800µL of ice-cold methanol-acetonitrile (1:1, v/v) was added to 60mg of hippocampal tissue and grinded with a tissue homogenizer at 4°C, then vortexed for 30 seconds and sonicated for 30 minutes in an ice-water bath. The mixture

was then incubated at -20°C for 60 minutes to precipitate proteins. After centrifugation at 14,000rpm for 20 minutes at 4°C, the supernatant was promptly transferred. To prepare an additional supernatant, combine 100µL of acetonitrile-water (v/v=1:1) solution was used to dissolve the extract. The supernatant was centrifuged at 14,000rpm for 15 minutes at 4°C, and filtered through 0.22µm microporous filter membrane. Based on the LC-MS detection results, the standard curve was applied to perform quantitative analysis on all samples.

**Proteomic Analysis:** Sample extraction and instrumental analysis were conducted in accordance with established protocols as referenced reported (Opeyemi *et al.*, 2021). 20mg of rat hippocampal tissue were carefully lysed in 200µL of SDT lysis buffer (Solarbio, Beijing, China) at a controlled temperature of 4°C. Following in-solution digestion with sequencing-grade trypsin (37°C, 16h), peptide mixtures were acidified using 0.1% formic acid (FA) and purified via C18 solid-phase extraction (SPE) cartridges (Roager and Licht, 2018). The desalted peptides were lyophilized to dryness and reconstituted in 0.1% FA prior to LC-MS/MS analysis using a Thermo Scientific Q Exactive Plus mass spectrometer equipped with a nanoLC system. Data processing incorporated missing value imputation through the k-nearest neighbors algorithm (k=10) using the lowest intensity value as reference. Differentially expressed proteins (DEPs) were identified with |log2FC| >1 and adjusted p-value (FDR <0.05) from three biological replicates. Systems biology platforms including KEGG Mapper and Gene Ontology (GO) enrichment analysis were implemented using DAVID Bioinformatics Resources (v6.8), while pathway overrepresentation was further validated through GSEA v4.2.3 with MSigDB c5.bp.v7.5.1 gene sets.

**Immunofluorescence:** To evaluate the proliferation of cells, rats were administered bromodeoxyuridine (BrdU, Sigma-Aldrich) solution (dissolved in PBS, 100mg/kg) twice per day intraperitoneally. BrdU was injected for a total of 6 days, starting from 2 days before cortical impact (or only craniotomy) and continuing until 3 days post-injury. BrdU-positive (BrdU+) Iba1+ cells colabeled with Iba1 were then quantified. The hippocampus was removed, stored in 4% paraformaldehyde overnight. Coronal sections (50µm) of fixed hippocampus were cut using a vibratome (LT-1000S, Leica). Tissue sections underwent dual immunolabeling with rabbit-derived primary antibodies against ionized calcium-binding adapter molecule 1 (Iba1, 1:500 dilution) and bromodeoxyuridine (BrdU, 1:200 dilution) under refrigerated conditions (4°C, 16h). Following sequential PBS washing steps (3×5min), specimens were exposed to Alexa Fluor 488-conjugated goat anti-rabbit IgG secondary antibody (1:500 dilution) at ambient temperature for 2h under controlled humidity to minimize photobleaching. The nuclei of cells were counterstained with DAPI (Sigma) for 20 minutes. Following washing with PBS, sections were sealed with Dako (Glostrup, Denmark).

**Quantitative Real-Time PCR (qRT-PCR) for RNA Expression Analysis:** Total RNA was extracted from rat

hippocampal tissue using TRIzol reagent (Tiangen Biotech, Beijing, China) following manufacturer specifications. Subsequent reverse transcription was performed utilizing the FastKing RT kit (Tiangen) to synthesize complementary DNA (cDNA). Quantitative transcriptomic profiling was conducted on a QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific) employing SYBR® Green Master Mix (Bio-Rad) optimized for high fidelity amplification. Primer sequences (Table S1) were designed using Primer-BLAST with amplicon lengths between 80-200bp and GC content of 50±5%. Gene expression normalization was achieved through the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH), validated by geNorm analysis. Relative quantification was performed using the comparative threshold cycle ($2-\Delta\Delta Ct$) method, incorporating amplification efficiency corrections derived from standard curves.

**Statistical analysis:** The experimental data was analyzed with SPSS 25.0 and GraphPad Prism 10.4.0. To express the data effectively, results were reported as Mean ± Standard Deviation (SD). For multi-group comparisons, we utilized one-way analysis of variance (ANOVA). For comparisons between two samples, Student's t-tests were employed. In addition to the statistical software mentioned, all statistical analyses were conducted using R (version 4.0.3).

## RESULTS

**TBI disturbed the serum tryptophan metabolism:** To elucidate the effects of TBI on systemic metabolic homeostasis, we performed the widely-targeted metabolomics analysis on serum samples collected from both the sham-operated group and the TBI model group of SD rats two days post-surgery (Fig. 1A). From the ion feature matrix, 165 significant features were identified for further analysis (Wilcox test, P<0.05). The differential substance classification was conducted to classify metabolites altered by TBI. Three chemical categories were clustered, indicating that TBI predominantly impacted amino acids and fatty acids (Fig. 1B). We conducted a comprehensive metabolite analysis utilizing individual principal component analysis (PCA) to estimate and elucidate the structural changes in metabolites affected by TBI. The results showed that the TBI presented an altered metabolites structure (Fig. 1C). To further identify the biological pathways affected by TBI-altered metabolites, we performed the quantitative metabolite set enrichment analysis (qMSEA). Our findings indicate that TBI significantly alters the systemic metabolism of phenylalanine, tyrosine and tryptophan (Fig.1D). The TBI significantly downregulated the serum

IPA pathway (Fig. 1E), as evidenced by reduced IPA concentrations and in serum (Fig. 1F). Above results suggested that TBI leads to serum tryptophan metabolism disorder, which is characterized by downregulation of the IPA pathway.

**Gut bacteria dysregulation linked to serum IPA concentration:** To investigate whether gut microbiota changes in TBI rats, 16S rRNA sequencing of colonic contents were conducted and the data revealed that TBI group rats showed a significantly decreased gut bacterial richness (Fig. 2A) and a distinct bacterial structure compared to sham group rats, as determined by PERMANOVA using the Adonis method (P=0.001) (Fig. 2B). The linear discriminant analysis (LDA) effect size (LEfSe) was used to estimate TBI-affected bacterial taxa, the results showed an richness in the genera *Bacteroides, Colidextribacter*, *Escherichia_Shigella*, and *Muribaculum*, while a decrease in the genera *Alistipes*, *Allobaculum*, *Candidatus_Saccharimonas*, *Desulfovibrio*, among others in rats with TBI (Fig. 2C, D). To investigate association between the gut microbiota and serum metabolites, we performed the Mantel analysis and found a strong correlation between TBI-altered bacterial taxa and disturbed serum metabolites in TBI rats, indicating that changes in bacterial populations were closely linked to alterations in the serum metabolic profile (Fig.2E). Furthermore, a linear -regression analysis was conducted and the results showed that the abundance of *Alistipes, Allobaculum, Candidatus_Saccharimonas, Desulfovibrio, Dubosiella* and *Ileibacterium,* which decreased in TBI rats were positively associated with IPA level in the serum (Fig. 2F). These findings indicate that TBI-induced gut dysbiosis, marked by reduced levels of *Alistipes, Allobaculum, Candidatus_Saccharimonas, Desulfovibrio, Dubosiella* and *Ileibacterium* are strongly linked to decreased serum IPA levels.

**TBI affected the IPA pathway by gut microbiota:** To further investigate the causal relationship between TBI-induced gut bacterial dysbiosis and peripheral IPA pathway dysfunction, the fecal microbiota of TBI rats was transplanted into normal rats (C-TBI) and detected changes in gut bacterial and serum metabolites in rats. Rats were given antibiotic cocktail mix (Abx) for three days before fecal microbiota transplantation (FMT) in order to eliminate the gut bacteria and increase the efficacy of FMT. 4 weeks later, the microbial composition of C-TBI group rats was closely to TBI group rats (P=0.001) (Fig. 3A). The bacterial composition at the genus level was also more similar to that of TBI group rats (Fig. 3B). Furthermore, those bacteria that strongly associated with IPA level in C-TBI group were found to have similar tendency with TBI group rats (Fig. 3C).

**Table 1:** Sequences of primers used for qRT-PCR

| Gene | ID | Forward Primer (5'–3') | Reverse Primer (5'–3') | T(℃) | Size(bp) |
|------|----|-----------------------|------------------------|------|----------|
| iNOS | NM_010927.3 | TGGAACAGTATAAGGCAAACACC | TTCTGGTCGATGTCATGAGCAAAGG | 61.5 | 497 |
| TNF-α | NM_013693.3 | GCCTCTTCTCATTCCTGCTT | TGGGAACTTCTCATCCCTTTG | 54.8 | 329 |
| IL-6 | NM_031168.2 | GCCCTTCAGGAACAGCTATGA | TGTCAACAACATCAGTCCCAAGA | 61.2 | 227 |
| NLRP3 | NM_145827.4 | TGTTGTCAGGATCTCGCA | AGTGAAGTAAGGCCGGAAT | 62.7 | 200 |
| IL-1β | NM_008361.4 | AACCTGCTGGTGTGTGACGTTC | CAGCACGAGGCTTTTTTGTTGT | 60.6 | 323 |
| IL-18 | NM_008360.2 | AACGAATCCCAGACCAGAC | AGAGGGTAGACATCCTTCCAT | 58.2 | 431 |
| GAPDH | NM_008084.2 | CATCACCATCTTCCAGGAGCGAGA | TGCAGGAGGCATTGCTGATGATCT | 60.0 | 318 |
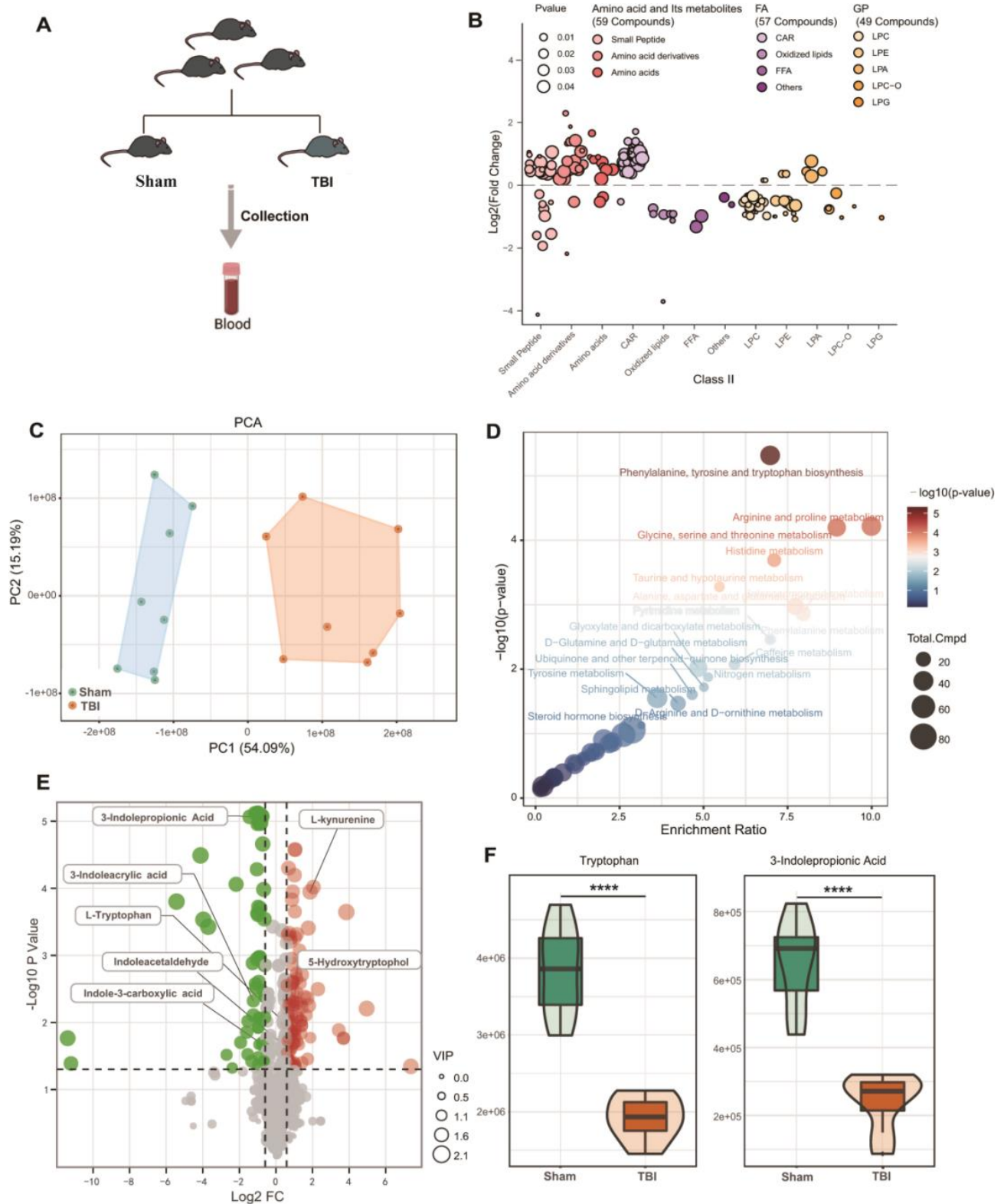
**Fig.1:** TBI affects tryptophan metabolism. A Flow chart of animal grouping and sample collection (n=8/group). B Cluster analysis of 165 serum metabolites with statistically altered TBI was conducted using differential substance classification methods; Node size indicated the total number of compounds per cluster, while node color differentiated the compounds. C PCA score to evaluate serum metabolic profile of TBI model group and sham group rats. D qMSEA identified the top 20 significantly disturbed serum metabolic biological pathways induced by TBI (P<0.05). E The TBI model group rats exhibited significant down regulation of the phenylalanine, tyrosine, and tryptophan metabolic pathways. F TBI model group rats significantly down-regulated serum tryptophan and IPA metabolism. Data are shown as Mean ± SD. ****P≤0.0001.

These findings suggest that healthy rats subjected to FMT from TBI rats exhibited significant alterations in their gut microbiota, resulting in a bacterial composition more closely resembling that of the TBI rats.

We then isolated the serum of C-TBI group and used metabolomics to detect changes in serum metabolites of C-TBI group rats. Our findings indicate that the C-TBI group rats induced significant alterations in the serum metabolite profile of normal rats, making it more

similar to that of TBI rats (P=0.001) (Fig. 3D). Serum IPA levels were further examined, revealing significantly reduced serum tryptophan concentrations in both the C-TBI and TBI group rats (Fig. 3E). Our findings indicated a significant reduction in serum IPA concentration in rats from the C-TBI and TBI groups compared to the sham group (Fig. 3F). These results suggest that gut microbiota dysbiosis of genus *Alistipes*, *Allobaculum*, *Candidatus_Saccharimonas*, *Desulfovibrio* and *Ileibacterium* played a decisive role in peripheral IPA metabolism dysregulation induced by TBI.

**TBI-disturbed IPA level in brain:** IPA can cross the blood-brain barrier via peripheral circulation, enabling it to exert neuroprotective effects (Xie *et al.*, 2022). We evaluated amino acid metabolic alterations in the brain tissue of TBI rats to determine if IPA can cross from peripheral circulation into the brain. Significant differences in amino acid metabolites were observed between Sham and TBI rat brain tissues (PERMANOVA by Adonis, P=0.031) (Fig. 4A). Metabolomic profiling revealed a significant reduction of IPA in the brain tissue

of TBI rats (Fig. 4B, C). Collectively, these results highlighted that TBI disturbed IPA level.

**IPA supplementation decreased TBI-induced neuroinflammation:** Microglia, key mediators of neuroinflammation following central nervous system injury (Donat *et al.*, 2017). Following TBI, microglia change from a steady-state rod-like phenotype to an enlarged cell body with contractile cell protrusions and eventually to an amoeboid shapeor rod shape, then produce cytokines, which result in local neuroinflammation(Kaur and Sharma, 2018). To examine the impact of IPA on microglial proliferation in the hippocampus of TBI rats, BrdU was administered intraperitoneally for 6 days. We quantified the number of BrdU⁺Iba1⁺ positive cells and microglial density in the hippocampus tissue to assess the effect of IPA supplementation on neuroinflammation following TBI (Fig. 5A). Our findings showed that the expression of inflammatory factors iNOS, TNF-a, and IL-6 was significantly upregulated in the TBI group rats, while supplementation with IPA markedly reduced the expression of these inflammatory factors (Fig. 5B-D). We
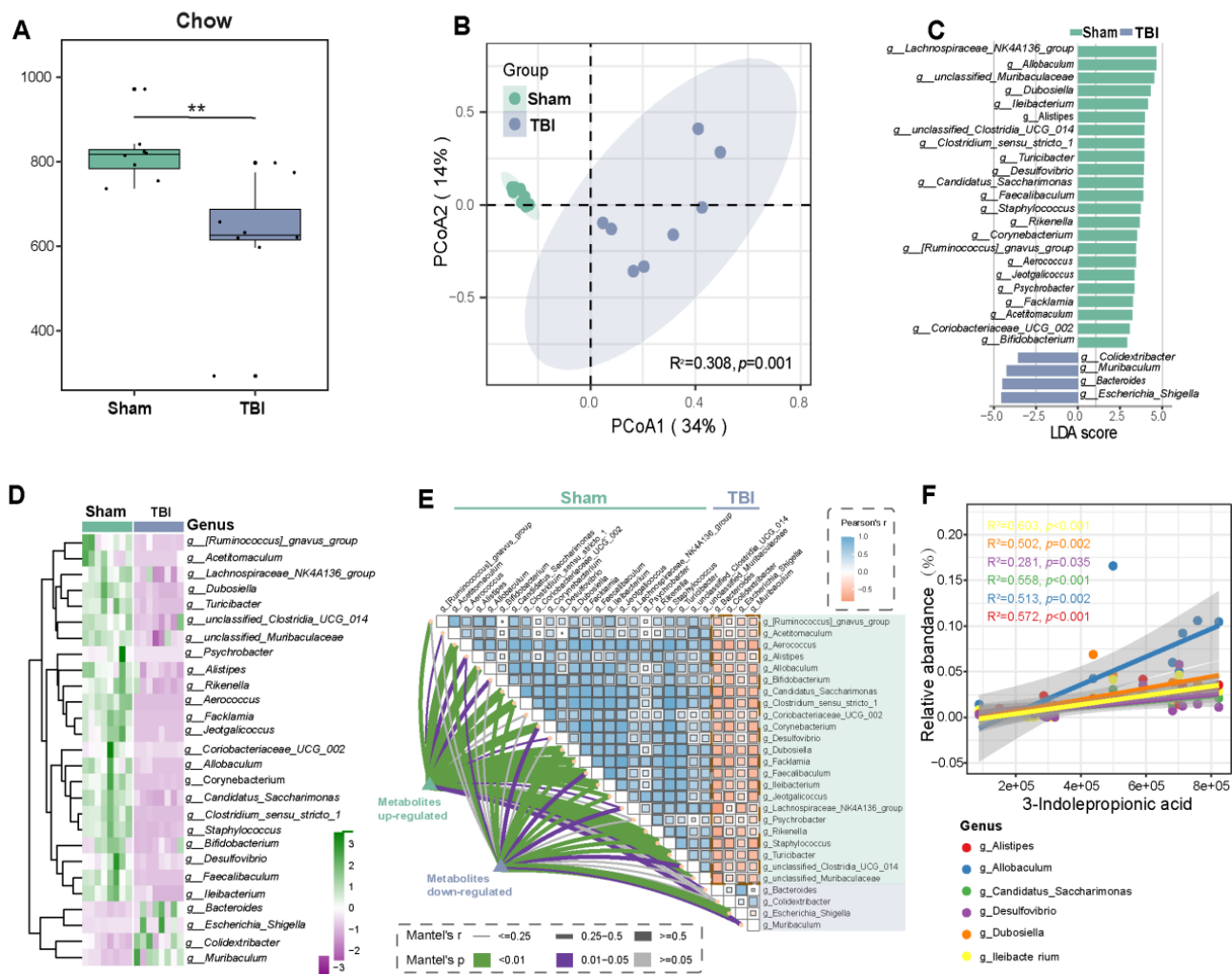


**Fig. 2**: TBI-induced gut bacteria dysregulation was highly linked to serum IPA concentration. A Chow index was used to analyze the alpha diversity of gut bacteria (n=8/group). B PCoA plot showing gut bacteria compositional differences of Sham group and TBI group rats. (PERMANOVA). C LEfSe identified discriminative taxa between TBI and sham group rats (log₁₀LDA>2.5; P<0.05). D Heatmap of bacterial abundance estimated by LEfSe analysis. E Mantel tests reveal correlations between serum differential metabolites and microbial diversity in rats, depicted using a color gradient for Pearson's correlation coefficient (based on 999 permutations). F Linear regression analysis of *Alistipes, Allobaculum, Candidatus_Saccharimonas, Desulfovibrio, Dubosiella and Ileibacterium* abundance and serum IPA concentration. Data are shown as Mean ± SD. In A, **P≤0.01.
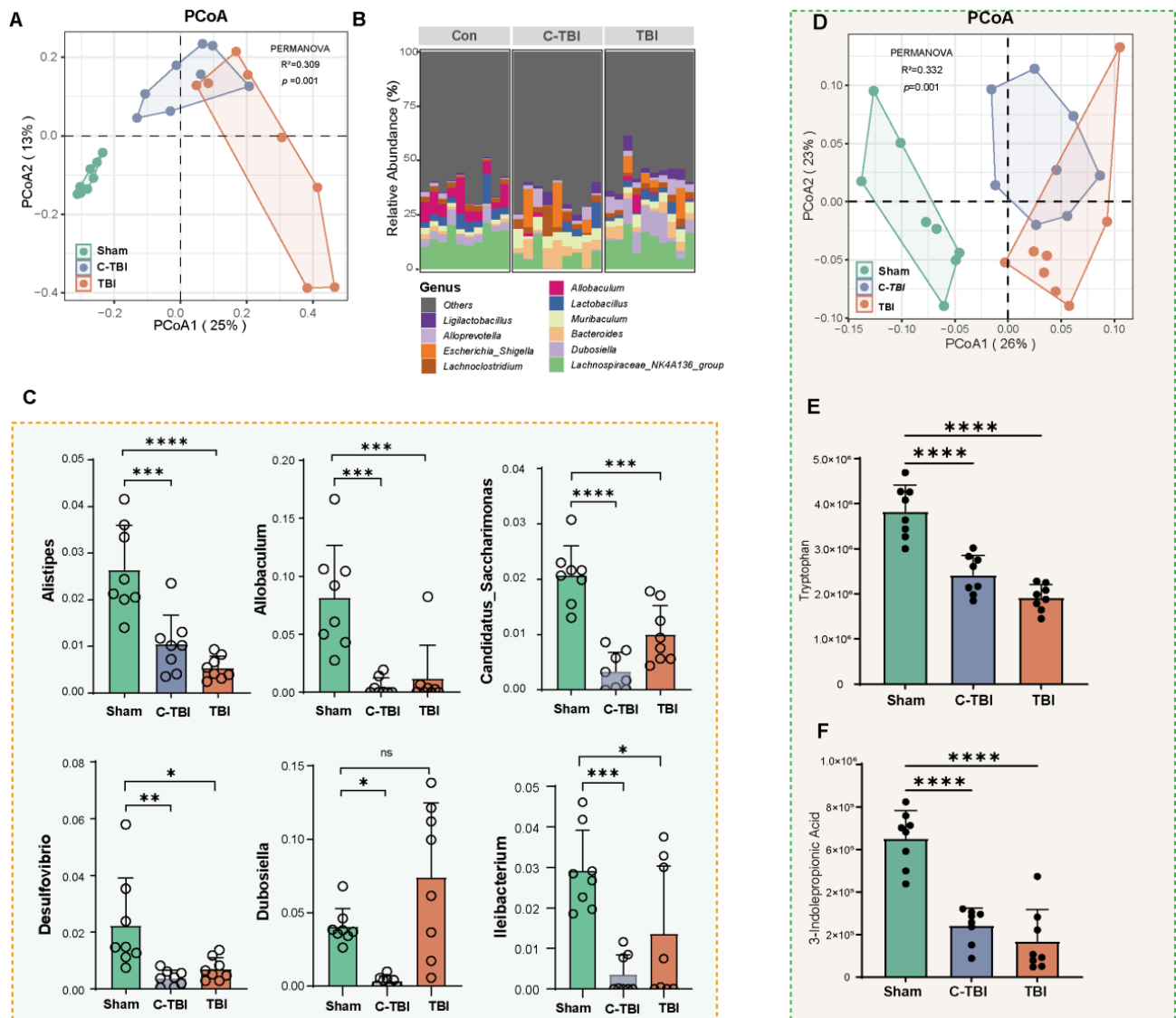
**Fig. 3:** TBI disrupted the IPA pathway in a gut microbiota-dependent manner. A Bray-Curtis distance-based PCoA illustrates the microbiota composition similarity between rats post-FMT and their donors (n=8/group, PERMANOVA by Adonis, R²=0.309, P=0.001). B Bar graph depicting genus-level bacterial abundance. C The abundance of the genera *Alistipes, Allobaculum, Candidatus_Saccharimonas, Desulfovibrio, Dubosiella* and *Ileibacterium* comparison in the Sham group, C-TBI group and TBI group rats. D PCoA presented the similarity of serum metabolite composition in rats post-FMT from TBI rats. E Serum tryptophan levels significantly decreased in both C-TBI and TBI rat groups. F The serum IPA concentration significantly decreased in both C-TBI and TBI groups. Data are shown as Mean ± SD. In C, E, and F, ****P≤0.0001.
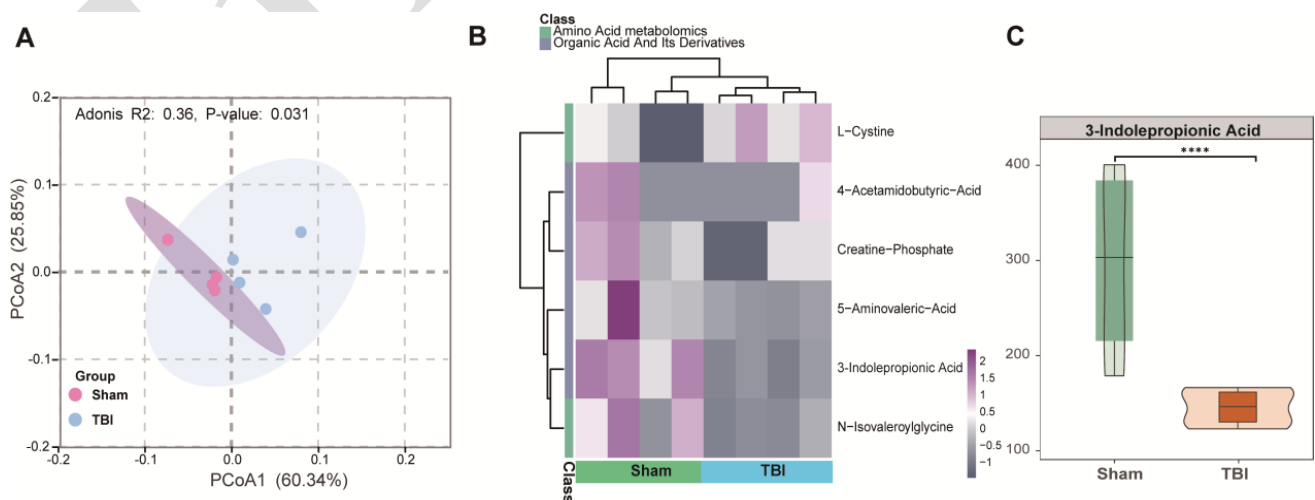


**Fig. 4:** TBI disturbed IPA level in brain. A PCoA using Bray–Curtis distance was conducted to evaluate the amino acid metabolomic data in brain tissue from TBI model group and sham group rats (n=4/group). B Heatmap showing the TBI-altered amino acid metabolism in brain tissues. C The IPA level in the brain tissue of TBI model group rats was significantly downregulated. Data are shown as Mean ± SD. In C, ****P≤0.0001.
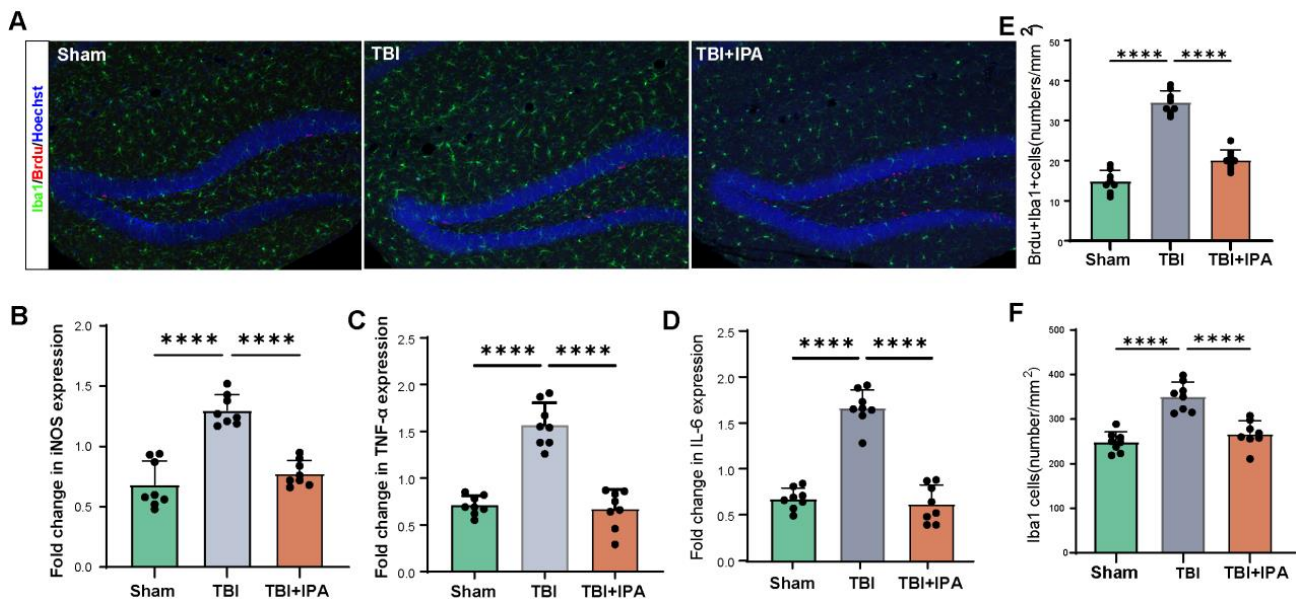
**Fig.5:** IPA supplementation decreased TBI-induced neuroinflammation. A Confocal images showing BrdU (red) and Iba1 (green) double labeling in the hippocampus of TBI rats. B-D qRT-PCR analysis revealed that IPA supplementation significantly reduced the mRNA levels of iNOS, TNF-a, and IL-6 in the hippocampus of TBI rats (n=3/group). E Supplementation with IPA significantly decreased the number of BrdU$^+$Iba1$^+$ in TBI rats. F IPA supplementation markedly decreased the density of microglia in TBI rats. Data are represented as mean ± SD. In B-F, ****P≤0.0001.

also found that TBI led to an increase in the number of BrdU+Iba1+ and the density of microglia, whereas supplementation with IPA significantly reduced the number of BrdU+Iba1+ and the density of microglia (Fig. 5E, F). These results show that IPA reduced TBI-induced neuroinflammation by inhibiting microglial activation and inflammatory factor release.

**IPA inhibited the upregulation of NLRP3 signaling pathway:** To investigate the mechanism by which IPA inhibited TBI-induced neuroinflammation, we used proteomics to analyze the effect of IPA on protein expression in brain tissue of TBI model rats. We identified 211 differentially expressed proteins (DEPs, absolute $log_2$-fold change ($log_2FC$)>1, P<0.05), of which 127 were upregulated and 84 were downregulated (Fig. 6A). We further conducted KEGG pathway enrichment analysis on DEPs, accumulating a total of 20 pathways, mainly involving Herpes simplex virus 1 infection, NLRP3 signaling pathway, and Epstein Barr virus infection (Fig. 6B). Gene set enrichment analysis (GSEA) is conducted to estimate the differential protein enrichment without applying a threshold. Our findings indicate a significant upregulation of the NLRP3 signaling pathway in the brain tissue of TBI rats compared to Sham rats. However, IPA supplementation markedly inhibited this upregulation (P<0.001) (Fig. 6C). We observed no significant differences (P>0.05) in the NF-KB, Toll-Like Receptor, and TGF-β signaling pathways (P>0.05) (Fig. 6D-F). To further confirm the effect of IPA on NLRP3 signaling pathway in the hippocampal tissue, we performed qRT-PCR analysis revealed a significant increase in NLRP3, IL-1β, and IL-18 expression in the hippocampal tissue of TBI rats, which was notably reduced by IPA supplementation (Fig. 6G-I). These data indicate that IPA supplementation mitigated TBI-induced neuroinflammation by inhibiting NLRP3 signaling pathway activation.

## DISCUSSION

TBI is now acknowledged as a chronic, progressive condition with lasting effects(Brett *et al.*, 2022). In recent years, the gut microbiome has been proved crucial to the pathophysiology of acute CNS injuries like TBI. The protection of gut function is important for curbing the body excessive inflammatory response and alleviating secondary neurological damage (Brenner *et al.*, 2017; Armstrong *et al.*, 2023). Current evidence suggests that while specific gut microbial consortia exhibit modulatory potential in neuroinflammatory regulation, the precise mechanistic pathways and contextual determinants underlying these interactions remain incompletely characterized, necessitating multidimensional investigative frameworks.

The gut microbiota interacts with CNS mainly through producing gut microbiota metabolites and influencing the neural, immune and endocrine systems(Rooks and Garrett, 2016; Taraskina *et al.*, 2022; Zheng *et al.*, 2022). In this process, gut microbiota metabolites traverse the gut barrier to enter the peripheral circulation system and then cross the blood-brain barrier to directly regulate neuronal cell function, or influence neuronal cell function by regulating the immune system (El Aidy *et al.*, 2014; Agirman *et al.*, 2021). The gut microbiota derived metabolites mainly play the role of regulating lipid metabolism and glucose homeostasis. A reduction in bile acids, particularly secondary bile acids, was observed in both fecal and plasma samples following TBI. This alteration was correlated with specific bacterial taxa (Xu *et al.*, 2018). Furthermore, the administration of SCFAs was found to enhance spatial learning post TBI (Opeyemi *et al.*, 2021).

TBI was further correlated with alterations in tryptophan metabolism. Tryptophan, an essential amino acid, plays a critical role in various physiological processes within the body and must be obtained through

dietary sources, as it cannot be synthesized endogenously. The gut microbiota contributes significantly to the metabolism of tryptophan. Various gut microbial species possess the enzymatic capabilities to convert tryptophan into several other metabolites, including indole, which is associated with anti-inflammatory and immunomodulatory properties. Since tryptophan metabolism is affected in pathological conditions, tryptophan and its metabolites are very attractive as biomarkers to aid in diagnosis, prognosis, and selection of therapies of human beings and animals (Roager and Licht, 2018; Li *et al.*, 2021). Gut microbiota compounds contribute to host health through various beneficial effects. The beneficial effects of certain compounds can include the promotion of immune system function, modulation of inflammatory responses, enhancement of gut barrier integrity, and support for metabolic processes (Roager and Licht, 2018). Biological effects of tryptophan metabolites and their alterations in disease suggest that tryptophan metabolites or their receptors may be targets for disease treatment for human beings and animals (Roager and Licht, 2018; Laursen *et al.*, 2021; Tintelnot *et al.*, 2023). IPA circulates within the mammalian bloodstream, and its synthesis is dependent on the presence of gut microbiota (Wikoff *et al.*, 2009). As one of the key metabolites of tryptophan, IPA demonstrates notable radical scavenging activity and has gained considerable attention for its neuroprotective properties(Venkatesh *et al.*, 2014; Rothhammer *et al.*, 2016). Besides, IPA has been reported to regulate gut barrier function effectively. The modulation of this barrier function by IPA may involve various mechanisms, including the enhancement of epithelial cell tight junctions and the balance of gut microbiota, contributing to overall digestive health (Chyan *et al.*, 1999; Xue *et al.*, 2022; Zhang *et al.*, 2022).

Our study revealed that serum IPA level is positively associated with the abundance of genera *Alistipes, Allobaculum, Candidatus_Saccharimonas, Desulfovibrio, Dubosiella,* and *Ileibacterium*, significantly suppressed inflammation and NLRP3 pathway activation induced by TBI. These findings suggest that gut-derived metabolites influence neuroinflammation following TBI.

In summary, our findings indicate that gut microbiota-derived IPA reduces TBI-related neuroinflammation via the NLRP3 pathway, suggesting a potential treatment strategy for TBI-induced neuroinflammation in human beings and animals.

**Author Contributions:** Yue Zhao, Kai Hu and Jiexin Zou performed the experiments; Lu Zhou and Xiu-Xiu Xu analyzed the data; Li-Juan Quan designed the study; Li-Juan Quan wrote the paper.

**Institutional Review Board Statement:** All processes involved in this research were rigorously approved by the Institutional Animal Care and Ethics Committee of the College of Veterinary Medicine at Nanchang University. This approval process is critical to ensure that the study adheres to ethical guidelines and regulatory standards concerning the treatment and welfare of animals used in research. The procedures followed the guidelines of the Care and Use of Laboratory Animals.

**Data Availability Statement:** Data presented in this study is contained in the article and reasonable requirement can be available to obtain additional information from the corresponding author.

**Conflicts of Interest:** All the authors have declared that they have no conflict of interest regarding the publication of this work.

## REFERENCES

Agirman G, Yu KB, Hsiao EY, 2021. Signaling inflammation across the gut-brain axis. Science 374:1087-92.

Amanollahi M, Jameie M, Heidari A, *et al.*, 2023. The dialogue between neuroinflammation and adult neurogenesis: Mechanisms involved and alterations in neurological diseases. Mol Neurobiol 60:923-59.

Armstrong PA, Venugopal N, Wright TJ, *et al.*, 2023. Traumatic brain injury, abnormal growth hormone secretion, and gut dysbiosis. Best Pract Res Clin Endocrinol Metab 37:101841.

Blanquet L, Serra D, Marrinhas C, *et al.*, 2025. Exploring gut microbiota-targeted therapies for canine idiopathic epilepsy. Int J Mol Sci 26.

Brenner LA, Stearns-Yoder KA, Hoffberg AS, *et al.*, 2017. Growing literature but limited evidence: A systematic review regarding prebiotic and probiotic interventions for those with traumatic brain injury and/or posttraumatic stress disorder. Brain Behav Immun 65:57-67.

Brett BL, Gardner RC, Godbout J, *et al.*, 2022. Traumatic brain injury and risk of neurodegenerative disorder. Biol Psychiatry 91:498-507.

Chakraborty R, Tabassum H, Parvez S, 2023. Nlrp3 inflammasome in traumatic brain injury: Its implication in the disease pathophysiology and potential as a therapeutic target. Life Sci 314:121352.

Chyan YJ, Poeggeler B, Omar RA, *et al.*, 1999. Potent neuroprotective properties against the alzheimer beta-amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. J Biol Chem 274:21937-42.

Donat CK, Scott G, Gentleman SM, *et al.*, 2017. Microglial activation in traumatic brain injury. Front Aging Neurosci 9:208.

Drieu A, Lanquetin A, Prunotto P, *et al.*, 2022. Persistent neuroinflammation and behavioural deficits after single mild traumatic brain injury. J Cereb Blood Flow Metab 42:2216-29.

Du L, Qi R, Wang J, *et al.*, 2021. Indole-3-propionic acid, a functional metabolite of clostridium sporogenes, promotes muscle tissue development and reduces muscle cell inflammation. Int J Mol Sci 22.

El Aidy S, Dinan TG, Cryan JF, 2014. Immune modulation of the brain-gut-microbe axis. Front Microbiol 5:146.

Elfers K, Watanangura A, Hoffmann P, *et al.*, 2024. Fecal supernatants from dogs with idiopathic epilepsy activate enteric neurons. Front Neurosci 18:1281840.

Fang Z, Pan T, Wang H, *et al.*, 2022. Limosilactobacillus reuteri attenuates atopic dermatitis via changes in gut bacteria and indole derivatives from tryptophan metabolism. Int J Mol Sci 23.

Finnie JW, 2012. Comparative approach to understanding traumatic injury in the immature, postnatal brain of domestic animals. Aust Vet J 90:301-7.

Finnie JW, 2014. Pathology of traumatic brain injury. Vet Res Commun 38:297-305.

Garcez ML, Tan VX, Heng B, *et al.*, 2020. Sodium butyrate and indole-3-propionic acid prevent the increase of cytokines and kynurenine levels in lps-induced human primary astrocytes. Int J Tryptophan Res 13:1178646920978404.

Hanscom M, Loane DJ, Shea-Donohue T, 2021. Brain-gut axis dysfunction in the pathogenesis of traumatic brain injury. J Clin Invest 131.

Hu X, Jin H, Yuan S, *et al.*, 2023. Fecal microbiota transplantation inhibited neuroinflammation of traumatic brain injury in mice via regulating the gut-brain axis. Front Cell Infect Microbiol 13:1254610.

Kalra S, Malik R, Singh G, *et al.*, 2022. Pathogenesis and management of traumatic brain injury (tbi): Role of neuroinflammation and anti-inflammatory drugs. Inflammopharmacology 30:1153-66.

Kaur P and Sharma S, 2018. Recent advances in pathophysiology of traumatic brain injury. Curr Neuropharmacol 16:1224-38.

Konopelski P, Konop M, Gawrys-Kopczynska M, *et al.*, 2019. Indole-3-propionic acid, a tryptophan-derived bacterial metabolite, reduces weight gain in rats. Nutrients 11.

Konopelski P and Mogilnicka I, 2022. Biological effects of indole-3-propionic acid, a gut microbiota-derived metabolite, and its precursor tryptophan in mammals' health and disease. Int J Mol Sci 23.

Kuo KW, Bacek LM, Taylor AR, 2018. Head trauma. Vet Clin North Am Small Anim Pract 48:111-28.

Laursen MF, Sakanaka M, Von Burg N, *et al.*, 2021. Bifidobacterium species associated with breastfeeding produce aromatic lactic acids in the infant gut. Nat Microbiol 6:1367-82.

Li K, Hao Z, Du J, *et al.*, 2021. Bacteroides thetaiotaomicron relieves colon inflammation by activating aryl hydrocarbon receptor and modulating cd4(+)t cell homeostasis. Int Immunopharmacol 90:107183.

Li X, Zhang B, Hu Y, *et al.*, 2021. New insights into gut-bacteria-derived indole and its derivatives in intestinal and liver diseases. Front Pharmacol 12:769501.

Liu X, Zhang L, Cao Y, *et al.*, 2022. Neuroinflammation of traumatic brain injury: Roles of extracellular vesicles. Front Immunol 13:1088827.

Morais LH, Schreiber HLT, Mazmanian SK, 2021. The gut microbiota-brain axis in behaviour and brain disorders. Nat Rev Microbiol 19:241-55.

Opeyemi OM, Rogers MB, Firek BA, *et al.*, 2021. Sustained dysbiosis and decreased fecal short-chain fatty acids after traumatic brain injury and impact on neurologic outcome. J Neurotrauma 38:2610-21.

Orive G, Anitua E, Pedraz JL, *et al.*, 2009. Biomaterials for promoting brain protection, repair and regeneration. Nat Rev Neurosci 10:682-92.

Quan L, Zhao Y, Ouyang C, *et al.*, 2023. The mechanism of chemokine receptor ackr2 in cognitive impairment in young rats with traumatic brain injury. Am J Transl Res 15:1654-66.

Roager HM and Licht TR, 2018. Microbial tryptophan catabolites in health and disease. Nat Commun 9:3294.

Rooks MG and Garrett WS, 2016. Gut microbiota, metabolites and host immunity. Nat Rev Immunol 16:341-52.

Rothhammer V, Mascanfroni ID, Bunse L, *et al.*, 2016. Type i interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. Nat Med 22:586-97.

Schweitzer AD, Niogi SN, Whitlow CT, *et al.*, 2019. Traumatic brain injury: Imaging patterns and complications. Radiographics 39:1571-95.

Socala K, Doboszewska U, Szopa A, *et al.*, 2021. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacol Res 172:105840.

Sulimai N and Lominadze D, 2020. Fibrinogen and neuroinflammation during traumatic brain injury. Mol Neurobiol 57:4692-703.

Sun Y, Wang S, Liu B, *et al.*, 2023. Host-microbiome interactions: Tryptophan metabolism and aromatic hydrocarbon receptors after traumatic brain injury. Int J Mol Sci 24.

Tan SW, Zhao Y, Li P, *et al.*, 2021. Hmgb1 mediates cognitive impairment caused by the nlrp3 inflammasome in the late stage of traumatic brain injury. J Neuroinflammation 18:241.

Taraskina A, Ignatyeva O, Lisovaya D, *et al.*, 2022. Effects of traumatic brain injury on the gut microbiota composition and serum amino acid profile in rats. Cells 11.

Tintelnot J, Xu Y, Lesker TR, *et al.*, 2023. Microbiota-derived 3-iaa influences chemotherapy efficacy in pancreatic cancer. Nature 615:168-74.

Venkatesh M, Mukherjee S, Wang H, *et al.*, 2014. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor pxr and toll-like receptor 4. Immunity 41:296-310.

Verdoodt F, Watanangura A, Bhatti SFM, *et al.*, 2022. The role of nutrition in canine idiopathic epilepsy management: Fact or fiction? Vet J 290:105917.

Wang H, Zhou XM, Wu LY, *et al.*, 2020. Aucubin alleviates oxidative stress and inflammation via nrf2-mediated signaling activity in experimental traumatic brain injury. J Neuroinflammation 17:188.

Wang Y, Tang J, Lv Q, *et al.*, 2022. Establishment and resilience of transplanted gut microbiota in aged mice. iScience 25:103654.

Wart M, Edwards TH, Rizzo JA, *et al.*, 2024. Traumatic brain injury in companion animals: Pathophysiology and treatment. Top Companion Anim Med 63:100927.

Wikoff WR, Anfora AT, Liu J, *et al.*, 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A 106:3698-703.

Xie Y, Zou X, Han J, *et al.*, 2022. Indole-3-propionic acid alleviates ischemic brain injury in a mouse middle cerebral artery occlusion model. Exp Neurol 353:114081.

Xu X, Yin D, Ren H, *et al.*, 2018. Selective nlrp3 inflammasome inhibitor reduces neuroinflammation and improves long-term neurological outcomes in a murine model of traumatic brain injury. Neurobiol Dis 117:15-27.

Xue H, Chen X, Yu C, *et al.*, 2022. Gut microbially produced indole-3-propionic acid inhibits atherosclerosis by promoting reverse cholesterol transport and its deficiency is causally related to atherosclerotic cardiovascular disease. Circ Res 131:404-20.

Zhang B, Jiang M, Zhao J, *et al.*, 2022. The mechanism underlying the influence of indole-3-propionic acid: A relevance to metabolic disorders. Front Endocrinol (Lausanne) 13:841703.

Zhao ZH, Xin FZ, Xue Y, *et al.*, 2019. Indole-3-propionic acid inhibits gut dysbiosis and endotoxin leakage to attenuate steatohepatitis in rats. Exp Mol Med 51:1-14.

Zheng Z, Wang S, Wu C, *et al.*, 2022. Gut microbiota dysbiosis after traumatic brain injury contributes to persistent microglial activation associated with upregulated lyz2 and shifted tryptophan metabolic phenotype. Nutrients 14.