

Research report

Esketamine relieves depressive-like behaviors in MPTP-induced Parkinson disease mice via GPR109A-dependent reduction of neuroinflammation

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ABSTRACT

Background: Depression is a common complication of Parkinson disease (PD). This study investigated whether a single dose of esketamine could alleviate depressive symptoms in an MPTP mouse model by activating G protein-coupled receptor 109 A (GPR109A) and reducing neuroinflammation.

Methods: Adult male C57BL/6 J mice (8 weeks, 25–30 g) were allocated into three groups: saline, Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and MPTP plus esketamine. To elucidate the specific functional contribution of GPR109A signaling to esketamine's effects, the selective GPR109A antagonist mepenzolate bromide (MPN) was administered. Depressive-like behaviors were evaluated using the tail suspension test (TST) and forced swim test (FST) for assessing behavioral despair, and the sucrose preference test (SPT) for measuring anhedonia. Western blot analysis was employed to quantify protein expression levels of GPR109A, Tumor Necrosis Factor- α (TNF- α), Interleukin-1 beta (IL-1 β), and Interleukin-6 (IL-6). The presence of GPR109A in microglia was also evaluated using immunofluorescence and flow cytometry.

Results: A single injection of esketamine elicited a rapid improvement in the behavior of MPTP-treated mice, accompanied by increased GPR109A expression in the medial prefrontal cortex (mPFC) and reduced pro-inflammatory markers. However, co-administration of MPN negated these benefits, suggesting that intact GPR109A signalling is involved in the antidepressant-like and anti-inflammatory effects of esketamine.

Conclusions: Esketamine alleviates PD-related depressive-like behavior by suppressing microglial inflammation in the mPFC via GPR109A. These findings emphasise the importance of GPR109A in linking immune modulation to the rapid behavioral effects of esketamine, and indicate the need for further research into GPR109A-targeted treatments for PD-related depression.

1. Introduction

Parkinson's disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). This leads to bradykinesia, resting tremor, and muscular rigidity (Zamanian et al., 2025). In addition to motor impairments, depressive symptoms affect 40 %–50 % of PD patients and are associated with neuroinflammation, imbalances in neurotransmitters and abnormalities in the circuits that regulate mood. These abnormalities are particularly prevalent in the mPFC. (Rashid et al., 2023). Studies have reported prefrontal cortical thinning and reduced volume in patients with PD and

depression (Jellinger, 2022).

Neuroinflammation is a central pathological process in PD, characterised by the activation of microglia and astrocytes, the release of pro-inflammatory cytokines and the infiltration of peripheral immune cells. Triggers include infection, traumatic brain injury, neurodegenerative disease, and autoimmunity. While acute responses may facilitate repair, persistent activation can damage synapses and neurons, accelerating progression (Ivraghi et al., 2024; Zamanian et al., 2022). Inflammation within the mPFC, which is a key area for regulating mood, is closely linked to depressive behaviors (Liu et al., 2025). This highlights the importance of identifying mPFC-specific molecular mechanisms to

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improve the treatment of PD-related depression.

GPR109A, also known as the niacin receptor or hydroxycarboxylic acid receptor 2 (HCA2), is a G protein-coupled receptor which has recently emerged as a promising target for modulating neuroinflammation (Wei et al., 2023). It is predominantly expressed in immune-competent cells such as microglia, and activating the receptor has been shown to suppress neuroinflammatory responses in various neurodegeneration models (Viatchenko-Karpinski et al., 2022). Therefore, targeting GPR109A could be a viable therapeutic strategy for reducing neuroinflammation and alleviating symptoms in PD. However, its specific role in PD and PD-associated depression remains unclear.

Esketamine, the S-enantiomer of ketamine, has attracted considerable attention as a rapid-acting antidepressant (Smith-Apeldoorn et al., 2022). Although both (R)- and (S)-ketamine attenuate MPTP-induced striatal dopamine transporter loss in mice, (R)-ketamine demonstrates more extensive neuroprotective effects, including enhanced preservation of tyrosine hydroxylase (TH) in nigrostriatal pathways. These effects are mediated primarily through BDNF–TrkB signaling rather than NMDA receptor antagonism, indicating enantiomer-specific mechanisms (Fujita et al., 2020). Unlike conventional antidepressants, esketamine modulates the glutamatergic system, representing a novel therapeutic approach (Moore et al., 2022). Recent preclinical studies suggest that esketamine may also possess immunomodulatory properties (Chen et al., 2024), though the molecular basis for these effects—particularly in the context of PD-related depressive behaviors—is not fully elucidated. Although NMDA receptor inhibition is considered central to its antidepressant action, esketamine's anti-inflammatory effects imply the involvement of alternative pathways. We hypothesize that esketamine acts through GPR109A-mediated mechanisms. Support for this idea comes from a study by Ibrahim et al. (2022), which showed that the benefits of niacin in a ketamine-induced psychosis model were diminished by the GPR109A antagonist MPN, highlighting the functional importance of this receptor (Ibrahim et al., 2022; Song et al., 2025). Given the structural and metabolic similarities between esketamine and ketamine, it is plausible that esketamine engages the GPR109A pathway to exert anti-inflammatory and neuroprotective effects (Wadie et al., 2023). This hypothesis is further strengthened by the overlap between niacin/GPR109A signaling pathways and the known therapeutic effects of esketamine.

This study investigates whether GPR109A-expressing microglia are responsible for the antidepressant effects of esketamine in a mouse model of PD. We hypothesize that GPR109A activation in microglial attenuates neuroinflammation in the mPFC and alleviates depressive-like behaviors in PD mice. Additionally, we will examine whether esketamine's antidepressant properties depend on GPR109A receptor signaling and modulation of neuroinflammatory processes. Elucidating these mechanisms may address critical gaps in the treatment of PD-related depression and identify new therapeutic strategies targeting GPR109A. This research aims to clarify the molecular basis of depressive symptoms in PD and uncover novel pharmacologic targets. By exploring the interaction between esketamine and GPR109A within the mPFC, this study may contribute to the development of innovative therapies for depression in PD.

2. Materials and methods

2.1. Animals and treatments

A cohort of 130 male C57BL/6 J mice (28–32 g; Cavens, Jiangsu, China) was acclimated for one week. They were housed in groups of four or five in an environment with a temperature of $24 \pm 2^\circ\text{C}$, humidity of $55 \pm 10\%$, and a 12/12-hour light/dark cycle. Chow and filtered water were provided ad libitum. The total sample size included a 10% buffer to accommodate anticipated attrition from MPTP-induced mortality or technical procedures. Following acclimation, all animals were randomly assigned to experimental groups using a computer-generated

random number table (GraphPad Prism 9.0) to minimize selection bias. Group sizes were determined in strict adherence to the 3Rs principle (Replacement, Reduction, and Refinement), utilizing the minimum number of animals necessary for statistically valid results: $n = 6$ per group were used for Western blot and immunofluorescence analyses, while $n = 12$ per group were allocated for behavioral tests to account for higher individual variability in functional assessments. All procedures complied with international animal care standards and were approved by Jiangsu Vocational College of Medicine's IACUC (XMLL-2023-753).

2.2. Treatments

A schematic overview of the experimental workflow is presented in Fig. 1A. A subacute PD model was established through administration of MPTP (MCE HY-15608) as described previously. This model is featuring progressive depletion of nigrostriatal dopaminergic neurons, decreased tyrosine hydroxylase immunoreactivity and a marked decline in dopamine levels in the substantia nigra (Jackson-Lewis and Przedborski, 2007; Özkan et al., 2021). A randomization procedure was employed to allocate the mice between groups (all drugs were diluted to a volume of 200 μL): the MPTP group, saline control group, monomethyl fumarate (MMF, MCE HY-103252)-treated MPTP group (45 mg/kg/d) (Yao et al., 2016), esketamine-treated MPTP group (10 mg/kg) (Ma et al., 2025), and esketamine combined with MPN (MCE, HY-17585)-treated MPTP group (5 mg/kg/d) (Ibrahim et al., 2022). MPTP-HCl (30 mg/kg dissolved in saline) was injected intraperitoneally once daily at 09:00 for five consecutive days. After successful induction of parkinsonian phenotypes was confirmed, mice received subsequent intraperitoneal treatments with MMF, MPN for 2 weeks. Esketamine or saline were administered one day prior to behavioral testing.

2.3. Behavior test

2.3.1. Pole test

Bradykinesia was assessed using the pole test (Sun et al., 2021). The mice underwent three training sessions, after which they descended a 75 cm, 1 cm-diameter pole while their descent time was recorded. Each animal performed three trials, with a 30-minute rest period between each one. The mean time per mouse was then used for analysis.

2.3.2. Rotarod test

Motor coordination and balance were assessed using an automated rotarod apparatus following established methods (Haque et al., 2021). A three-day pretest training period was conducted to allow the animals to familiarize themselves with the equipment. Rotarod assessment began at 4 rpm with gradual acceleration to 40 rpm over 5 min. Time spent on the rotating rod before falling was measured as the primary outcome.

2.3.3. Sucrose preference test, SPT

SPT was conducted on days 15–17. Following a 72-h acclimation period with alternating 1% sucrose and water bottles (positions switched every 12 h), mice were food/water-deprived for 22 h. During testing, individually housed animals had 2-h access to both solutions, with consumption measured.

2.3.4. Tail suspension test, TST

The mice were suspended by their tails (1 cm from tip) 65 cm above a surface for six minutes, with immobility time recorded by blinded observers as a measure of behavioral despair (Meng et al., 2024).

2.3.5. Forced swim test, FST

The forced swim test was conducted to evaluate depressive-like behavior according to established protocols. Mice were individually placed in transparent cylinders (10 cm diameter, 25 cm height) filled with water ($23\text{--}25^\circ\text{C}$, depth 10 cm) for a 6-minute session. The onset of immobility (time to first immobility episode) and the total immobility

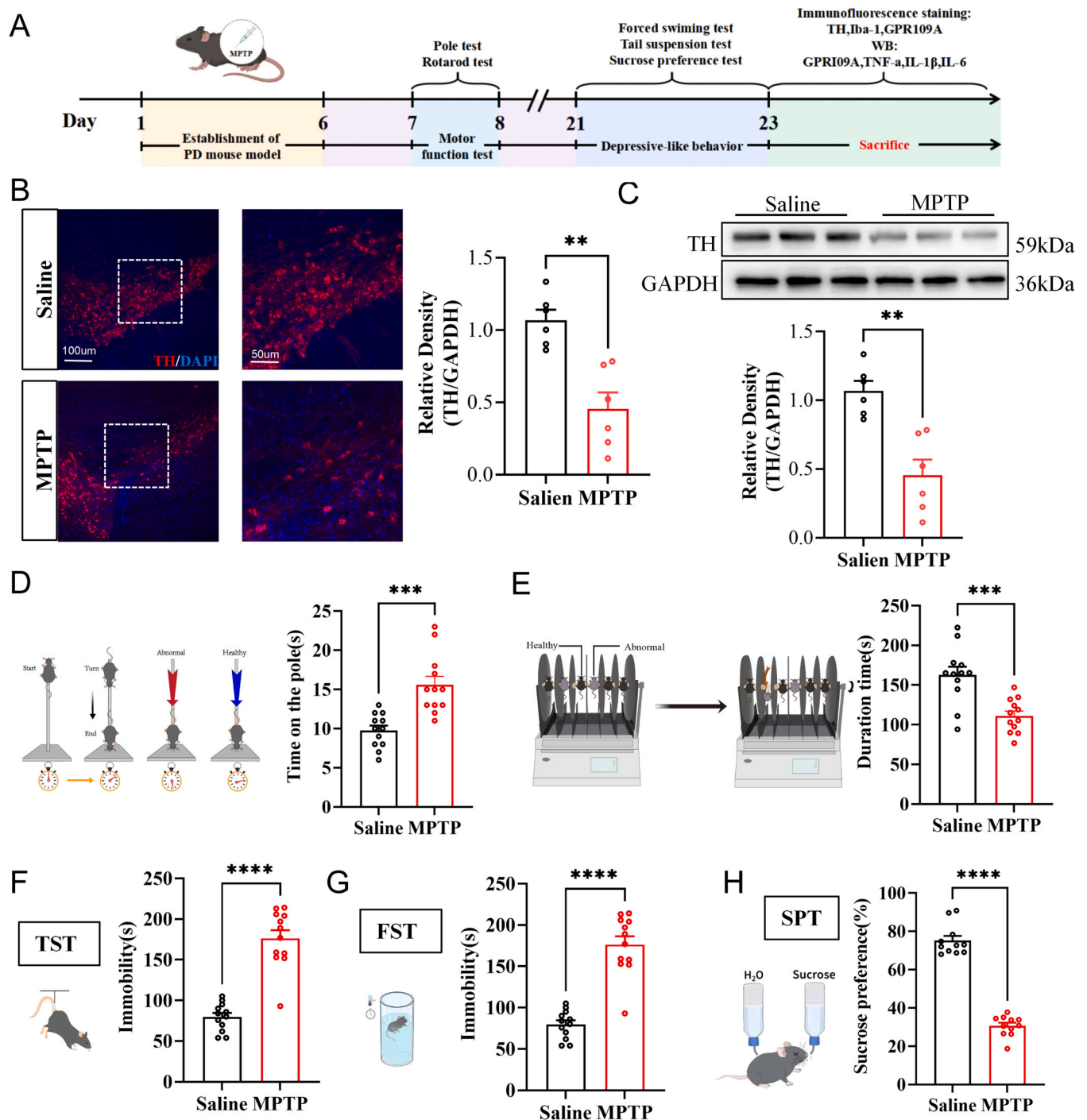


Fig. 1. Evaluation of dopaminergic neuron loss, motor function deficits and depressive-like behaviors. (A) Timeline for MPTP induction and behavioral testing. (B) Representative SNpc immunofluorescence for TH (red) and DAPI (blue) in the saline and MPTP groups (scale bars: first row, 100 μ m; second row 50 μ m) and the number of TH⁺ dopaminergic neurons in the SNpc (n = 6). (C) Western blot of TH in the SNpc (n = 6). (D) Time to descend on the pole (n = 12). (E) Time to fall off the rod on the rotarod (n = 12). (F) Immobility time in the tail suspension test (TST) of MPTP-treated mice (n = 12). (G) Immobility time in the forced swim test (FST) of MPTP-treated mice (n = 12). (H) Preference of the MPTP mice for sucrose solution (n = 12). Data are shown as means \pm SEM. Group differences were analysed using an independent t test (significance: * P < 0.05, ** P < 0.01, *** P < 0.001).

time during the final 4 min of the test were recorded. Immobility was defined as the cessation of active movement while maintaining minimal effort to keep the head above water (Trunnell et al., 2024).

2.4. Immunofluorescence staining

Following behavioral testing, mice were transcardially perfused with 4 % paraformaldehyde. Whole brains were post-fixed for 24 h and

cryoprotected in a 30 % sucrose solution for 48 h at 4 °C. Coronal sections (25 μ m) through mPFC and SNpc regions were prepared with a Leica CM1800 cryostat (Leica, Germany). After permeabilization with 0.1 % Triton X-100/PBS and blocking with 10 % goat serum for 1 h, sections were exposed to primary antibodies against TH (HUABIO ET1612-65, 1:200), GPR109A (Abclonal, A15611, 1:200), or Iba-1 (Invitrogen MA5-38266, 1:500) at 4°C overnight. Secondary antibody incubation (anti-rabbit/rat IgG, 1:400, HUABIO HA1121/HA1133)

preceded fluorescence detection. and subsequently subjected to fluorescence detection (the details of the secondary antibody and detection system can be added as needed). To assess dopaminergic neuron loss, the mean number of TH-positive neurons was quantified in three consecutive sections from the SNpc of each mouse ($n = 9\text{--}12$ sections per group, 3–4 mice per group). TH-positive neurons were counted manually using fluorescence microscopy (Olympus, Tokyo, Japan). Additionally, the number of Iba-1 and GPR109A positive cells was quantified using Image-Pro Plus 6.0 software (Media Cybernetics, USA), with five representative micrographs analyzed per mouse. The total SNpc area was measured using ImageJ software. TH-positive neuron density was determined by counting immunoreactive cells per mm^2 across multiple fields within each tissue section. Quantitative analyses were conducted in a double-blinded manner by two independent evaluators, and statistical processing was performed on the aggregated results.

2.5. Western blot (WB)

Dissected SNpc and mPFC tissues were homogenized in ice-cold RIPA buffer with complete protease inhibitors. Post-centrifugation ($12,000 \times g$, 15 min, 4°C), the protein-containing supernatants were aliquoted for BCA-based protein quantification. SDS-PAGE separation of equalized protein loads (3–6/group) preceded PVDF membrane transfer and 5 % non-fat milk/TBST blocking. Overnight membrane probing at 4°C employed primary antibodies (TH, HUABIO, 1:3000), GPR109A (Abclonal, 1:400), interleukin-1 β (IL-1 β , BOSTER, PB0055, 1:1000), interleukin-6 (IL-6, Invitrogen, 701028, 1:2000), and tumor necrosis factor- α (TNF- α , BOSTER, BA0131 1:1000), followed by HRP-conjugated secondary antibody (Boster, BA1061, 1:2000) incubation. Protein expression was detected by ECL, and all subsequent quantification of band intensities was performed using ImageJ software by an investigator blinded to the experimental group assignments.

2.6. Flow cytometry and cell sorting

The mPFC tissues were dissected and enzymatically digested in RPMI 1640 medium containing collagenase II (1 mg/mL, VIC080, Vicmed) and DNase I (0.1 mg/mL, VIC115, Vicmed) at 37°C with 200 rpm shaking for 60 min to obtain single-cell suspensions. Following 100- μm nylon mesh filtration, the tissue digest was resuspended in PBS supplemented with 2 % (w/v) FBS. After extensive washes, cell suspensions were stained with fluorophore-conjugated antibodies targeting surface epitopes (30 min, 4°C , light-protected). Samples were acquired on a BD FACSCanto II instrument, applying standard viability gating (FSC-A/SSC-A) and single-cell discrimination (FSC-H/FSC-A). Data analysis was conducted using FlowJo X software (TreeStar), with cell population frequencies quantified by percentage and antigen density measured by median fluorescence intensity (MFI). In the brain, microglia were identified as CD11b $^+$ CD45 $^{\text{low}}$ (CD45 Biolegend 103112 1:200; CD11b Biolegend 101206 1:200).

2.7. Statistical analysis

Statistical evaluations employed GraphPad Prism 9.0 software. Parametric assumptions were assessed through Shapiro-Wilk normality testing and Brown-Forsythe variance analysis. Comparisons between two groups were performed using an unpaired Student's t -test. Multi-group comparisons utilized post-hoc Tukey's test via one-way ANOVA adjusted multiple comparisons. Correlations between variables were determined using Pearson correlation coefficient. The data are expressed as the mean \pm standard error of the mean (SEM). Statistical significance thresholds were defined as follows: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; non-significant differences are indicated as n.s.

3. Results

3.1. Evaluation of dopaminergic neuron loss, motor function deficits and depressive-like behaviors in MPTP-induced PD mice

A subacute PD model was generated through daily intraperitoneal MPTP hydrochloride administration (30 mg/kg) over five consecutive days. (Fig. 1A). Dopaminergic neurons were characterized by TH expression. We first assessed TH $^+$ neuron counts in the SNpc using immunofluorescence staining. TH $^+$ cell numbers were significantly lower in MPTP-treated animals versus saline-treated groups ($t = 5.46$, $P = 0.0003$, Fig. 1B). Western blot detection revealed significantly reduced TH levels in the MPTP group ($t = 4.521$, $P = 0.0011$, Fig. 1C). MPTP group exhibited significant motor impairments at day 7, with worsened rotarod performance and delayed pole test completion compared to saline-treated mice. ($t = 4.612$, $P = 0.0001$; $t = 4.295$, $P = 0.0003$, Fig. 1D, E). Beginning on the twenty-first day, we evaluated depressive like behaviors. The MPTP group exhibited substantially increased immobility periods during TST compared to control animals ($t = 8.534$, $P < 0.0001$, Fig. 1F). Similarly, the FST revealed a significant increase in immobility time in MPTP-treated mice ($t = 10.60$, $P < 0.0001$, Fig. 1G). Additionally, the SPT indicated a marked reduction in sucrose preference in the MPTP group versus control, with the difference was significant ($t = 15.31$, $P < 0.0001$, Fig. 1H). These findings collectively suggest that MPTP-induced PD mice exhibit depressive-like behaviors.

3.2. MPTP-treated mice exhibit significant neuroinflammation and greater GPR109A expression in the mPFC correlates with depressive-like behaviors

Western blot quantification revealed significantly higher mPFC levels of IL-1 β , IL-6, and TNF- α ($t = 2.895$, $P = 0.016$; $t = 8.451$, $P < 0.0001$; $t = 5.275$, $P = 0.0004$, Fig. 2A–C) and enhanced GPR109A expression ($t = 4.717$, $P = 0.0008$; $t = 4.16$, $P = 0.002$, Fig. 2D, E) in MPTP-treated mice versus saline controls. Quantitative immunofluorescence analysis demonstrated significantly increased GPR109A-immunopositive cell density in the mPFC compared to saline-treated controls. Furthermore, Pearson correlation analysis revealed positive associations between the levels of pro-inflammatory cytokines and immobility times in depressive-like behavioral tests. Specifically: IL-1 β levels showed a strong correlation with both FST ($R^2 = 0.856$, $P = 0.008$, Fig. 2F) and TST ($R^2 = 0.907$, $P = 0.003$, Fig. 2F); IL-6 levels were highly correlated with FST ($R^2 = 0.908$, $P = 0.003$, Fig. 2G) and TST ($R^2 = 0.769$, $P = 0.022$, Fig. 2G); Similarly, TNF- α levels were significantly correlated with performance in both FST ($R^2 = 0.874$, $P = 0.006$, Fig. 2H) and TST ($R^2 = 0.882$, $P = 0.006$, Fig. 2H). The elevated mPFC proinflammatory cytokine levels were significantly associated with the manifestation of depressive-like behaviors in PD mice.

3.3. GPR109A expression is significantly upregulated in microglia within the mPFC of PD model mice

To investigate the relationship between GPR109A and microglia in the mPFC of PD mice, we used immunofluorescence confocal microscopy, Western blotting and flow cytometry to analyze changes in GPR109A expression in mPFC microglia. Compared with saline control-treated mice, MPTP-treated mice presented increased microglial activation and increased colocalization of microglia with GPR109A in the mPFC ($t = 4.795$, $P = 0.0007$, Fig. 3A, B). Compared with saline control treatment, Western blot analysis revealed significantly higher expression of microglial markers in the mPFC of MPTP-treated mice ($t = 4.262$, $P = 0.0017$, Fig. 3C). Flow cytometry revealed a marked increase in the GPR109A MFI in microglia in the mPFC of PD model mice ($t = 5.908$, $P = 0.0001$, Fig. 3D, E). These convergent findings

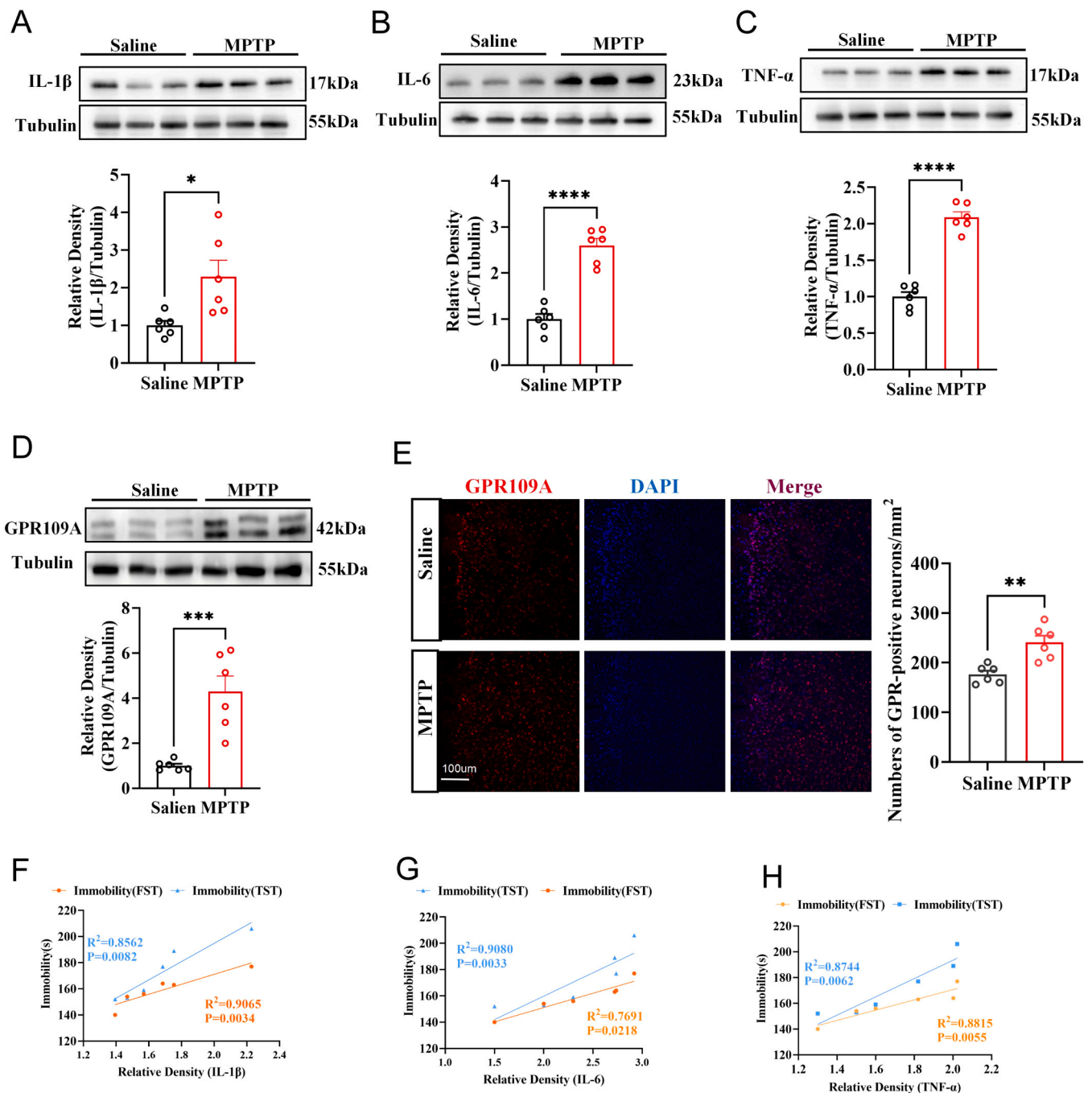


Fig. 2. MPTP-treated mice present elevated levels of IL-1 β , IL-6, and TNF- α and a strong correlation with depressive-like behaviors and upregulated GPR109A expression in the mPFC. Western blot (A-C) analysis of IL-1 β , IL-6 and TNF- α in the mPFC of the control and MPTP groups ($n = 6$). (D) shows GPR109A expression in the mPFC of the saline and MPTP groups ($n = 6$). (E) Immunofluorescence for GPR109A, with quantification of GPR109A $^{+}$ neurons in the mPFC ($n = 9-12$ sections/3 mice per group). Scale bars: 100 μ m (overview). (F-H) Correlation analysis between proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) and depressive-like behaviors. Immobility time in the TST and FST and levels of IL-1 β , IL-6, and TNF- α in the mPFC ($n = 6$). Data are shown as means \pm SEM. Significant differences between groups were assessed using independent t test, pearson correlation analysis to assess the strength and direction of linear relationships between continuous variables. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

demonstrate that GPR109A overexpression in activated mPFC microglia is a hallmark of PD-associated neuroinflammation, suggesting a critical role for GPR109A in microglia-mediated pathological responses.

3.4. Esketamine exerts antidepressant effects in PD mice correlate with GPR109A and inflammatory mediator regulation in the mPFC

To investigate the antidepressant potential of esketamine in PD, a series of behavioral tests were performed 24 h post-final drug

administration (Fig. 4A). In the TST, a significant intergroup difference in immobility time was observed. Compared with the saline controls, MPTP-exposed mice exhibited prolonged immobility, whereas ESK treatment significantly reduced immobility time in the ESK+MPTP group ($F(2, 33) = 68.01$, $P < 0.0001$, Fig. 4B). The FST also revealed that MPTP administration markedly increased immobility time relative to that of the control group. Esketamine treatment reversed this effect, significantly decreasing immobility ($F(2, 33) = 10.45$, $P = 0.0003$, Fig. 4C). MPTP-induced sucrose preference deficit was reversed by

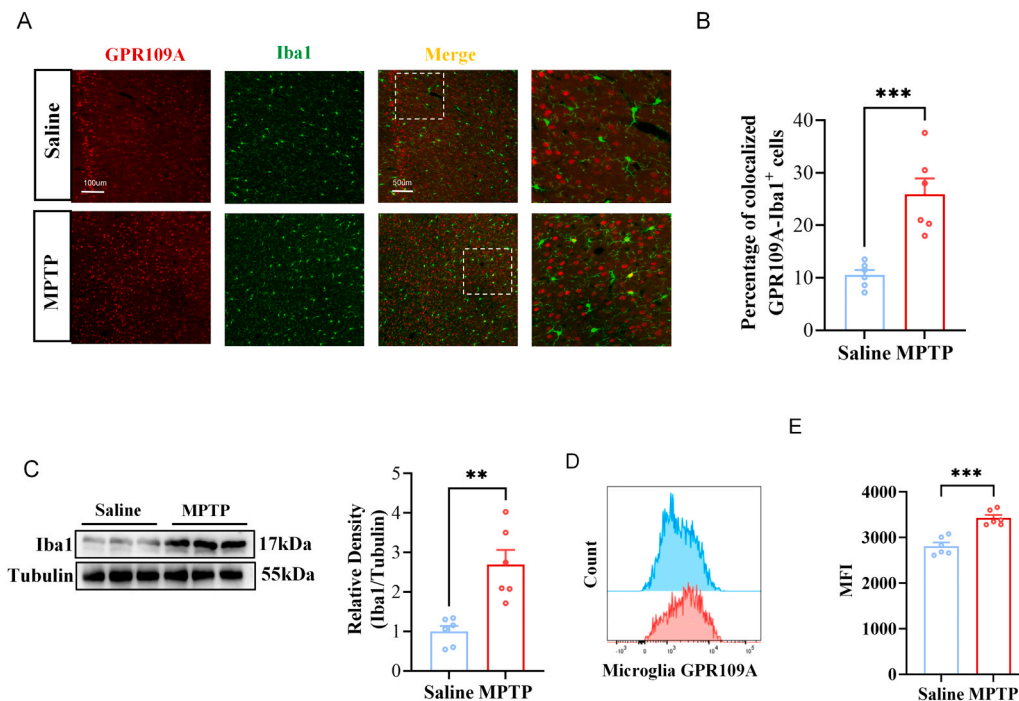


Fig. 3. GPR109A expression is significantly upregulated in microglia within the mPFC of PD model mice. (A) Immunofluorescence of the mPFC for GPR109A (red) and Iba1 (green) in saline and MPTP-treated mice. Scale bars = 100 μ m (rows 1–2); Scale bars = 50 μ m (rows 3–4). (B) Counts of GPR109A/Iba1 double-labeled neurons in the mPFC (n = 6). (C) Iba1 protein levels as determined by Western blot with quantification in mice with and without MPTP (n = 6). (D) Representative histograms of microglial GPR109A expression. (E) MFI of microglial GPR109A in the mPFC (n = 6). Data are shown as means \pm SEM. Significant differences between groups were assessed using independent t test (* P < 0.05, ** P < 0.01, *** P < 0.001).

esketamine in the SPT ($F(2, 33) = 17.33$, $P < 0.0001$, Fig. 4D). Collectively, these behavioral findings demonstrate that esketamine alleviates depressive in PD mice. To validate the behavioral results, WB quantification was performed to measure mPFC protein expression of IL-1 β , IL-6, TNF- α and GPR109A. Compared with that in control mice, GPR109A protein expression in MPTP-treated mice was increased. Notably, single administration of esketamine further increased GPR109A protein expression in the mPFC compared with that in the MPTP group. MPTP-treated mice presented elevated levels of inflammatory mediators (One-way ANOVA, $F(2, 15) = 19.46$, $P < 0.0001$ (Fig. 4E); $F(2, 15) = 8.333$, $P = 0.0037$ (Fig. 4F); $F(2, 15) = 32.64$, $P < 0.0001$ (Fig. 4G); $F(2, 15) = 23.81$, $P < 0.0001$ (Fig. 4H)). Post-hoc analysis using Tukey's test revealed that esketamine treatment in MPTP-induced mice (ESK+MPTP) resulted in a significant increase in GPR109A levels compared to the MPTP group ($P = 0.0116$). Acute esketamine administration significantly suppressed these proinflammatory markers, suggesting that an antineuroinflammatory mechanism underlies its antidepressant effects.

3.5. The GPR109A receptor mediates the antidepressant effects of esketamine

To investigate its efficacy as an antidepressant, we examined depressive-like behavior, as well as the presence of pro-inflammatory cytokines and GPR109A in the mPFC of MPTP-treated mice. We employed pharmacological agonism and antagonism of the receptor (Fig. 5A). Fig. 5B–D demonstrated that treatments significantly affected behavioral outcomes across tests (one-way ANOVA with Tukey's test). In TST ($F(3, 44) = 13.96$, $P < 0.0001$), both MMF and ESK groups reduced immobility versus MPTP group ($P < 0.0001$; $P = 0.0004$). In FST ($F(3, 44) = 8.430$, $P = 0.0002$), ESK decreased immobility versus MPTP ($P = 0.0076$), both esketamine and MMF showed lower immobility than MPN+ESK in PD mice ($P = 0.0005$; $P = 0.0057$). In SPT ($F(3, 40) = 41.59$, $P < 0.0001$), both esketamine and MMF showed higher

preference versus MPTP ($P < 0.0001$; $P < 0.0001$) and MPTP+MPN+ESK groups versus esketamine and MMF groups ($P < 0.0001$; $P < 0.0001$). Fig. 5E, F displayed the effect of MMF or esketamine treatments on IL-6 expression in MPTP-treated mice. One-way ANOVA revealed significant effects of different treatment (One-way ANOVA, $F(3, 20) = 25.69$, $P < 0.0001$). Post-hoc Tukey's test showed that both esketamine and MMF treatments showed pronounced reduction in IL-6 levels than MPTP group ($P < 0.0001$) and MPN+ESK group ($P = 0.0006$, $P < 0.0001$). Fig. 5E, G showed that MMF or esketamine treatments significantly altered IL-1 β expression (One-way ANOVA, $F(3, 20) = 9.117$, $P = 0.0005$). Post-hoc Tukey's test revealed: esketamine and MMF reduced IL-1 β vs MPTP ($P = 0.0025$; $P = 0.0022$), lower IL-1 β than MPTP+MPN+ESK ($P = 0.033$; $P = 0.0302$). Fig. 5E, H showed that TNF- α expression was significantly altered by treatments (One-way ANOVA, $F(3, 20) = 8.656$, $P = 0.0007$). Post-hoc Tukey's test showed: MPTP+ESK reduced TNF- α vs MPTP ($P = 0.0034$); MPTP+ESK and MPTP+MMF showed lower TNF- α than MPTP+MPN+ESK ($P = 0.0077$; $P = 0.0252$); MPTP+MPN+ESK vs MPTP: ns ($P = 0.9837$). Fig. 5E, I showed that GPR109A expression was significantly altered by treatments (One-way ANOVA, $F(3, 20) = 17.09$, $P < 0.0001$). Post-hoc Tukey's test showed: MPTP+ESK increased GPR109A vs MPTP ($P = 0.0012$); MPTP+ESK and MPTP+MMF showed higher GPR109A than MPTP+MPN+ESK ($P < 0.0001$; $P = 0.0002$); MPTP+MPN+ESK vs MPTP: ns ($P = 0.3732$). Fig. 5J–L showed that GPR109A expression was significantly altered by treatments (One-way ANOVA, $F(3, 20) = 17.54$, $P < 0.0001$). Post-hoc Tukey's test showed: MPTP+ESK increased GPR109A expression vs MPTP ($P = 0.0001$); MPTP+ESK and MPTP+MMF showed higher GPR109A expression than MPTP+MPN+ESK ($P < 0.0001$; $P = 0.0007$); MPTP+MPN+ESK vs MPTP: ns ($P = 0.9925$). These integrated behavioral, neuro-inflammatory, and molecular analyses collectively demonstrated that GPR109A activation is essential for mediating esketamine's antidepressant effects against MPTP-induced depressive-like behaviors, microglial inflammation.

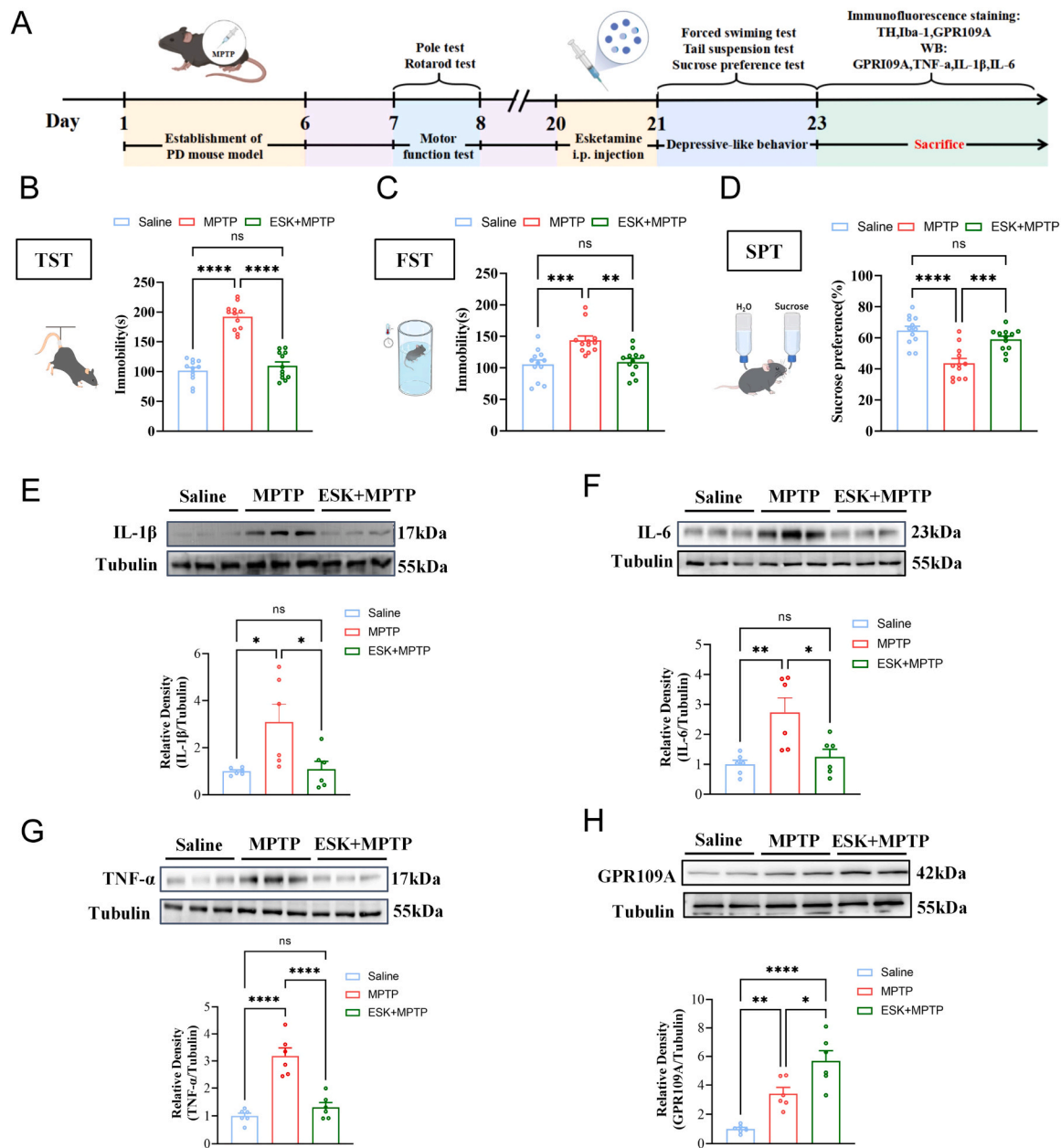


Fig. 4. Esketamine attenuates MPTP-induced depressive-like behaviors and modulates GPR109A and proinflammatory cytokines in the mPFC of PD mice. (A) Timeline of intervention: intraperitoneal administration of esketamine or saline 2 weeks after MPTP administration and 24 h before testing. (B, C) Immobility time of mice treated with saline, MPTP or esketamine+MPTP in the TST and FST ($n = 12$). (D) Sucrose preference (%) in the sucrose preference test (SPT, $n = 12$). (E-H) The protein levels of IL-1 β , IL-6, and TNF- α in the mPFC of mice injected with saline, MPTP or MPTP+esketamine were detected by Western blot ($n = 6$). (H) Western blot analysis of GPR109A in the mPFC of mice injected with saline, MPTP or MPTP+esketamine ($n = 6$). Data are shown as means \pm SEM. Significant differences between groups were assessed using one-way ANOVA and post-hoc Tukey's test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

4. Discussion

Depression is a highly prevalent non-motor symptom of PD, significantly affecting disease progression and quality of life. Epidemiological studies indicate that depressive symptoms occur in up to 50 % of PD patients, a prevalence higher than in many other chronic conditions (Ahmad et al., 2023). In this study, we demonstrated that a single sub-anesthetic dose of esketamine significantly alleviates MPTP-induced depressive like behaviors in mice. Furthermore, we identified the upregulation of GPR109A within the mPFC as a pivotal mechanism mediating the antidepressant and anti-inflammatory effects of esketamine. At a mechanistic level, our findings suggest that esketamine attenuates neuroinflammatory processes through enhanced GPR109A

expression, thereby contributing to its rapid antidepressant action.

Previous studies have consistently demonstrated that various pharmacologically-induced PD mouse models exhibit significant depressive-like behaviors (Mendonça et al., 2022). For instance, Ingrid Prata Mendonça et al. reported depressive phenotypes in rotenone-induced PD mice; D. Vecchia et al. observed similar behaviors in 6-hydroxydopamine (6-OHDA)-treated models; and Zhang, T., et al. reported similar findings in MPTP-induced PD mice. These studies, employing behavioral tests such as TST, SPT, and FST, have consistently revealed depressive-like behaviors, supporting the validity of these models for studying PD-related depression (Li et al., 2023). MPTP administration induced characteristic depressive-like behaviors in mice, which emerged 2–3 weeks after dopaminergic denervation (Savall et al.,

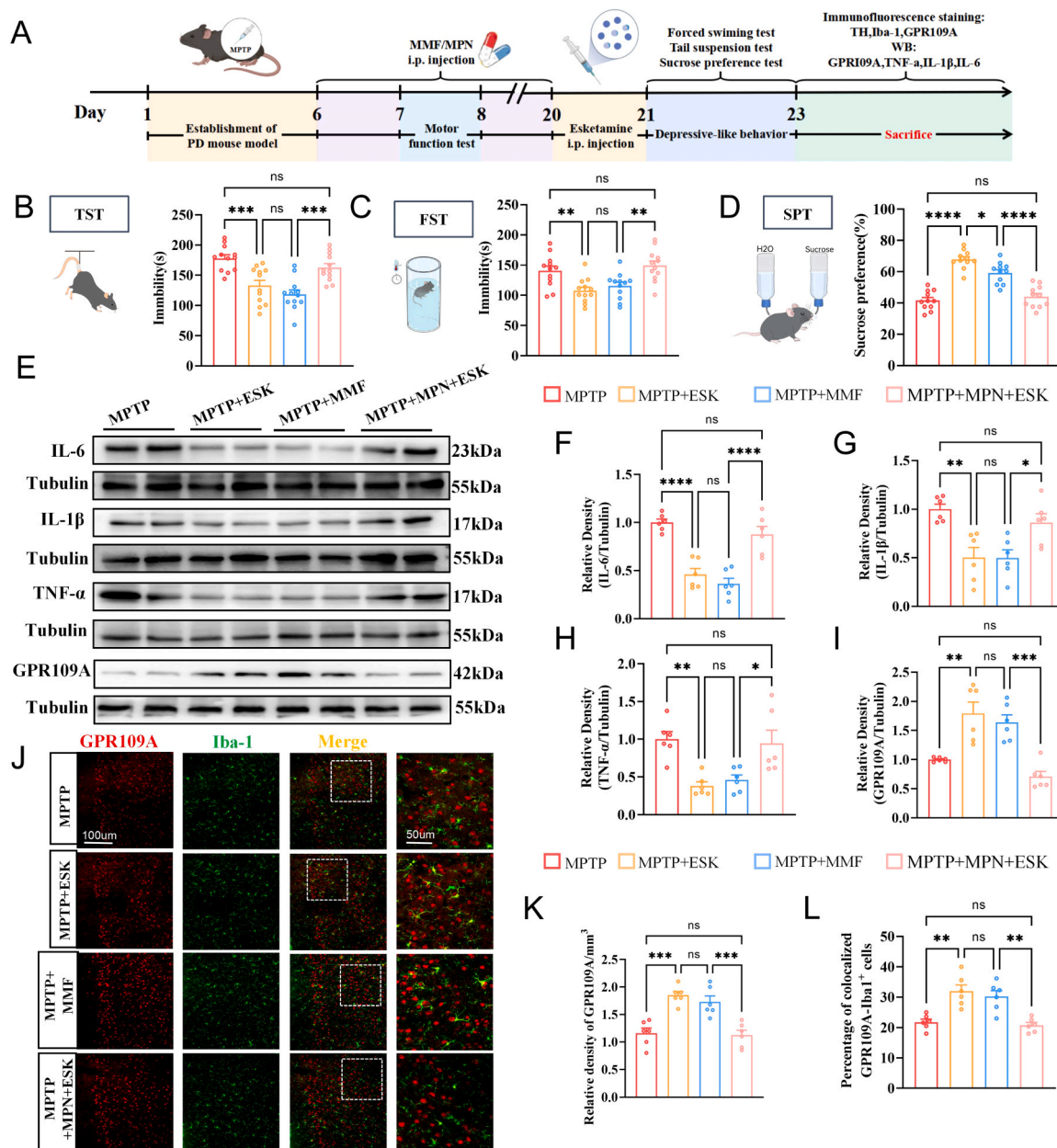


Fig. 5. The GPR109A receptor mediates the antidepressant effects of esketamine. (A) Design: Four groups were used: MPTP, MPTP+ESK, MPTP+MMF (a GPR109A agonist) and MPTP+ESK+MPN (a GPR109A antagonist). (B, C) Behavior test analysis of the immobility time of the mice in the four groups in the TST and FST ($n = 12$). (D) SPT results showing percentage sucrose preference across experimental groups ($n = 12$). (E–I) mPFC immunoblots with quantification ($n = 6$) for IL-1 β , IL-6, TNF- α and GPR109A following ESK, MMF or MPN+ ESK in the MPTP model. (J) mPFC immunofluorescence for GPR109A (red) and Iba1 (green) after ESK, MMF or MPN+ESK. Scale bars: 100 μ m (rows 1–3) and 50 μ m (row 4). (K, L) Relative density and numbers of GPR109A/Iba1 double-labeled neurons in the mPFC ($n = 6$). Data are shown as means \pm SEM. Significant differences between groups were assessed using one-way ANOVA and post-hoc Tukey's test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

2025). The behavioral profile of our study included: significantly increased immobility periods in both FST and TST, which is consistent with behavioral despair; and markedly decreased sucrose consumption, which is indicative of anhedonia. This comprehensive behavioral signature validates the successful establishment of an MPTP-induced model that recapitulates affective disturbances observed in PD. Although three classical assays (SPT, FST, and TST) were applied to evaluate depressive-like behaviors, we recognize the ongoing debate regarding their specificity in Parkinson's disease models, where motor deficits may confound interpretation. Notably, in the present study these behavioral assessments were performed at a stage when open field testing confirmed compensatory recovery of locomotor activity, thereby

minimizing interference of motor dysfunction with the evaluation of depressive-like behaviors (Sheta et al., 2024). Nevertheless, these assays remain indirect proxies for human depression, and future studies incorporating motor-independent paradigms (e.g., ultrasonic vocalization or social interaction tests) will provide valuable complementary evidence.

There is converging evidence that implicates microglia-driven neuroinflammation in the pathogenesis of PD (Chen et al., 2023). Immune mediators released from activated microglia can shift astrocytes towards neurotoxic states or reduce trophic/synaptotrophic support (Ghosh and Pearce, 2024). Furthermore, a meta-analysis of 48 studies identified an association between elevated levels of IL-6, TNF- α and IL-1 β , and

cognitive decline. TNF- α was linked to depression and anxiety, and IL-6 and IL-1 β were correlated with sleep disturbances severity (Qu et al., 2023). Our study demonstrates that elevated levels of IL-6, TNF- α , and IL-1 β in mPFC are closely associated with depressive-like behaviors in PD mice. These findings suggest that the amelioration of neuroinflammation may underlie the improvement in depressive-like phenotypes; however, the precise causal relationship requires further elucidation. It remains plausible that the reduction in pro-inflammatory cytokines reflects a downstream consequence of attenuated neurodegeneration rather than directly driving behavioral recovery. Future studies should extend these investigations to other key regions affected in PD, such as the SNpc, to provide a more comprehensive mapping of neuroinflammatory dynamics across neural circuits implicated in both motor and non-motor symptoms.

In 2019, the FDA approved esketamine for treating treatment-resistant depression in adults (Kim et al., 2019). Its antidepressant effects are attributed to multiple mechanisms, including suppression of microglial activation (Zhou et al., 2024), reduction of pro-inflammatory cytokine release (Halaris and Cook, 2023), and mitigating neuroinflammation (Wisłowska-Stanek et al., 2021). The rapid antidepressant action of esketamine is primarily linked to its antagonism of NMDA receptors (Moore et al., 2022), which induces a transient increase in synaptic glutamatergic neurotransmission (Wei et al., 2022). Emerging evidence also suggests that downstream activation of AMPARs also contributes to the antidepressant response (Aleksandrova et al., 2017). In addition, metabotropic glutamate receptors (mGluRs) (Henter et al., 2021) and the Nrf2 signaling pathway have been implicated in mediating the neuroprotective and anti-inflammatory effects of ketamine (Ma et al., 2025). The improvement of depressive-like behaviors by esketamine is unlikely to stem from nonspecific restoration of motor function. Its antidepressant effects typically emerge 24 h after administration (consistent with FDA clinical guidelines), which is clearly distinct from its short-lasting anesthetic and analgesic effects. Behavioral tests were conducted within this window of antidepressant efficacy. Previous studies have shown that esketamine does not enhance general locomotion (Ma et al., 2025) at this time point. Moreover, outside of neuropathic pain models, there have been no reports of sustained improvement in motor function induced by esketamine (Zhao and Li, 2024). Therefore, the observed behavioral improvements are more likely attributable to its specific antidepressant-like effects. A single administration of esketamine at 10 mg/kg, with behavioral assessments conducted 24 h post-injection—was carefully selected to align with both preclinical conventions and clinical relevance. This dose is well-established in rodent models of depression for eliciting rapid antidepressant-like effects and when adjusted for interspecies differences via body surface area normalization, corresponds approximately to a human equivalent dose of 0.8 mg/kg. This is comparable to the exposure achieved with the FDA-approved intranasal esketamine dose of 84 mg used in treatment-resistant depression, supporting its translational validity. Moreover, the use of a single injection allowed us to isolate the acute pharmacodynamic actions of esketamine from potential confounders associated with repeated dosing, such as tolerance or compensatory neuroadaptations. This approach mirrors clinical observations wherein a single dose of esketamine can induce rapid antidepressant effects within hours, sustaining efficacy for several days. Our studies demonstrate that esketamine upregulates GPR109A expression in the mPFC of PD mice, concomitant with a reduction in neuroinflammatory markers and the alleviation of depressive-like behaviors. GPR109A receptors are known to play pivotal roles in metabolic regulation, anti-inflammatory processes, and neuroprotection (Taing et al., 2023). Activation of GPR109A has been shown to mitigate inflammation-induced depressive-like behaviors (Wadie et al., 2023). A cross-sectional study revealed a U-shaped association between niacin intake (a GPR109A agonist) and the risk of depression (Zhao et al., 2023). Under physiological conditions in the central nervous system, endogenous GPR109A ligands such as nicotinic acid and

β -hydroxybutyrate are present at subthreshold concentrations for receptor activation (Boccella et al., 2019). In Parkinson's disease, neuroinflammatory processes further deplete nicotinic acid reserves (Giri et al., 2019), resulting in only partial activation of GPR109A that is insufficient to resolve neuroinflammation. This deficiency underscores the therapeutic potential of exogenous GPR109A agonists to restore anti-inflammatory signaling and produce clinically meaningful symptomatic improvement. Our findings establish GPR109A receptor activation as a pivotal mechanism underlying the antidepressant and anti-inflammatory effects of esketamine.

To define the contribution of GPR109A, we employed MPN to pharmacologically inhibit GPR109A signaling (Ibrahim et al., 2022). Notably, GPR109A inhibition significantly attenuated the protective effects of esketamine against both MPTP-induced depressive like behaviors and neuroinflammation in the mPFC. The results conclusively demonstrate that GPR109A activation is essential for the antidepressant and anti-inflammatory actions of esketamine in this PD model. The involvement of GPR109A in the therapeutic effects of esketamine suggests a promising pharmacological target for developing rapid-acting antidepressants. Moreover, GPR109A agonists may represent a promising intervention strategy not only for major depressive disorder but also for other neuropsychiatric conditions characterized by oxidative stress and neuroinflammation, warranting further preclinical and clinical investigation (Xu et al., 2022).

However, several important limitations of this study should be considered: Firstly: the acute MPTP mouse model used does not fully recapitulate the chronic and diverse pathogenesis of human Parkinson's disease. The exclusive use of male animal limits insight into sex-specific responses. Secondly: the pharmacokinetics and dynamics of esketamine and MPTP in mice differ from humans, these differences may limit the direct extrapolation of our findings to clinical settings. Thirdly: the downstream signaling pathways of GPR109A activation and their potential crosstalk with esketamine's NMDA receptor blockade remain to be fully elucidated, therefore, while our data suggest a role for GPR109A, the evidence remains indicative rather than conclusively causal. Fourthly: other established pathways—such as NMDA/AMPA modulation, BDNF signaling, and antioxidant mechanisms—may also contribute to esketamine's effects. Fifthly: this study used only male mice to control for hormonal variability, limiting generalizability. As sex influences PD and antidepressant response, future work should include both sexes to assess potential dimorphism in esketamine's effects. Finally: while our pharmacological and co-localization data strongly suggest a primary role for microglial GPR109A, the potential contribution of other cell types cannot be entirely ruled out. Future studies utilizing cell-type-specific knockout models will be essential to unequivocally resolve this question. Esketamine is not a first-line treatment for depression in PD. It is a powerful specialized tool reserved for the most severe, treatment-resistant cases where the potential benefit of rapid remission outweighs the significant risks and practical hurdles. Its use must be managed by specialists in a controlled setting, with rigorous monitoring for both its psychiatric and cardiovascular side effects. Further research specifically focused on PD patients is essential to better define its efficacy and safety profile in this vulnerable population and while motor confounds cannot be completely ruled out, the temporal dissociation of effects suggests the behavioral improvements primarily reflect true antidepressant activity, higher-level clinical evidence is needed to validate the involvement of GPR109A in esketamine's antidepressant effects in PD patients.

5. Conclusions

In summary, our study demonstrates that MPTP-induced PD mice exhibit elevated proinflammatory cytokine levels and increased GPR109A expression in the mPFC. Administration of a single sub-anesthetic dose of esketamine further upregulates GPR109A, suppresses neuroinflammatory responses, and ameliorates depressive-like

behaviors. Pharmacological blockade of GPR109A abolished both anti-inflammatory and antidepressant effects of esketamine, confirming that GPR109A activation is involved in esketamine's antidepressant action. These findings establish GPR109A as a key mechanism underlying esketamine's efficacy and provide a scientific rationale for developing novel GPR109A-targeting compounds to meet the urgent need for fast-acting treatments in Parkinson's disease associated depression.

CRediT authorship contribution statement

Lin Ji: Validation, Funding acquisition. **Wei Tan:** Methodology. **Wei Song:** Software, Methodology. **Yuanyuan Gao:** Supervision, Formal analysis. **Chen Wang:** Writing – review & editing, Conceptualization. **Shu Wang:** Writing – original draft, Project administration.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.brainresbull.2025.111595](https://doi.org/10.1016/j.brainresbull.2025.111595).

Data availability

Data will be made available on request.

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