Inhibition of indoleamine 2, 3-dioxygenase in prelimbic or infralimbic cortex of ICV-STZ rats can exert antidepressant effects through different pathways

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Abstract

Background and purpose Activation of indoleamine 2,3-dioxygenase (IDO) in prefrontal cortex (PFC) is closely related to depression. It has been proved that prelimbic (PrL) and infralimbic (IL) regions of medial PFC are involved in emotion regulation, and may play distinct roles in regulation of depression. However, the mechanism of how IDO in PrL or IL affects depressive behaviors remains unclear. Experimental approach IDO inhibitor 1-MT was directly injected into PrL and IL of depressive rats induced by ICV-STZ, respectively. Depressive- and anxiety-behaviors were evaluated in forced swim test, sucrose preference test, novelty-suppressed feeding test, novel object recognition test and open field test. HPLC-MS/MS detected kynurenine metabolites, ELISA detected cytokines, Western blot examined protein expression and Golgi staining assessed synaptic plasticity. Immunofluorescence staining was used to observe the expression and morphology of glial cells. Key results Dissimilar abnormalities were observed in PrL and IL of ICV-STZ depressed rats. In PrL, astrocyte defects were manifested, including reduced GFAP-positive cells, glial transporters and kynurenic acid, and morphological damage. In IL, microglial overactivation was manifested by increased cytokines, Iba1-positive cells and 3-hydroxy-kynurenine, accompanied by morphological alterations. Meanwhile, synaptic plasticity was decreased in both subregions. Microinjection of 1-MT at PrL or IL may improve depressive behaviors by reversing these different abnormalities in PrL and IL, respectively, without influencing anxiety behavior. Conclusions and implications Overall, the antidepressant effects of 1-MT by inhibiting IDO in PrL or IL are realized through different pathways, that is, by enhancing neuroprotective effects in PrL and attenuating neurotoxic response in IL.

Research article

Inhibition of indoleamine 2, 3-dioxygenase in prelimbic or infralimbic cortex of ICV-STZ rats can exert antidepressant effects through different pathways

Running title: 1-MT attenuates depressive behaviors in a distinct way in PrL and IL

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Author contributions

Yu Qin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing–original draft. Su-Ying Cui: Resources, Writing–review & editing, Funding acquisition, Project administration, Supervision. Xiao Hu: Formal analysis, Investigation, Methodology, Software. Hui-Ling Zhao: Data curation, Investigation, Validation. Nurhumar Kurban: Formal analysis, Investigation, Software. Xi Chen: Data curation, Formal analysis, Methodology. Jing-Kun Yi: Data curation, Formal analysis. Yuan Zhang: Formal analysis, Investigation. Yong-He Zhang: Conceptualization, Funding acquisition, Project administration, Supervision, Writing–review & editing

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

What is already known?

1. Both PrL and IL mediate depressive behaviors.

2. Activation of IDO in the PFC may induce depressive behaviors.

What does this study add?

1. Regionally specific pathological changes in the PrL and IL in ICV-STZ rats.

2. Inhibiting IDO in the PrL or IL may improve depressive behaviors through an independent mechanism.

What is the clinical significance?

1. Distinct mechanisms in PrL and IL provide potential new avenues for searching more effective antidepressants.

Abstract

Background and purpose

Activation of indoleamine 2,3-dioxygenase (IDO) in prefrontal cortex (PFC) is closely related to depression. It has been proved that prelimbic (PrL) and infralimbic (IL) regions of medial PFC are involved in emotion regulation, and may play distinct roles in regulation of depression. However, the mechanism of how IDO in PrL or IL affects depressive behaviors remains unclear.

Experimental approach

IDO inhibitor 1-MT was directly injected into PrL and IL of depressive rats induced by ICV-STZ, respectively. Depressive- and anxiety-behaviors were evaluated in forced swim test, sucrose preference test, novelty-suppressed feeding test, novel object recognition test and open field test. HPLC-MS/MS detected kynurenine metabolites, ELISA detected cytokines, Western blot examined protein expression and Golgi staining assessed synaptic plasticity. Immunofluorescence staining was used to observe the expression and morphology of glial cells.

Key results

Dissimilar abnormalities were observed in PrL and IL of ICV-STZ depressed rats. In PrL, astrocyte defects were manifested, including reduced GFAP-positive cells, glial transporters and kynurenic acid, and morphological damage. In IL, microglial overactivation was manifested by increased cytokines, Iba1-positive cells and 3-hydroxy-kynurenine, accompanied by morphological alterations. Meanwhile, synaptic plasticity was decreased in both subregions. Microinjection of 1-MT at PrL or IL may improve depressive behaviors by reversing these different abnormalities in PrL and IL, respectively, without influencing anxiety behavior.

Conclusions and implications

Overall, the antidepressant effects of 1-MT by inhibiting IDO in PrL or IL are realized through different pathways, that is, by enhancing neuroprotective effects in PrL and attenuating neurotoxic response in IL.

Keywords: IDO, prelimbic cortex, infralimbic cortex, microglia, astrocytes, ICV-STZ, depression

Abbreviations: IDO, indoleamine 2,3-dioxygenase; PFC, prefrontal cortex; mPFC, medial prefrontal cortex; PrL, prelimbic cortex; IL, infralimbic cortex; IL-6, interleukin-6; IL-1β, interleukin-1beta; Trp, tryptophan; Kyn, kynurenine; KA, kynurenic acid; 3-HK, 3-hydroxy-kynurenine; BDNF, brain-derived neurotrophic factor; STZ, streptozotocin; 1-MT, 1-Methyl-DL-tryptophan; Iba1, ionized calcium-binding adaptor molecule-1; GFAP, glial fibrillary acidic protein; GLAST, glutamate-aspartate transporter; GLT-1, glutamate transporter-1.

1 Introduction:

The prefrontal cortex (PFC), a critical brain region in the neural circuitry of stress and emotion regulation, undergoes severe pathological changes in psychiatric disorders(Carlen, 2017; Souza, Jesse et al., 2016). The prelimbic cortex (PrL) and infralimbic cortex (IL) are two major medial PFC (mPFC) areas in rodents thought to mediate the control of depressive behaviors(Wellman, Bollinger et al., 2020). Although the PrL and IL have similar projection patterns in certain aspects, a growing body of research has revealed that they are functionally distinguished in mediation of a variety of physiological and behavior processes in rodents, including fear expression and extinction(Sierra-Mercado, Padilla-Coreano et al., 2011), cocaine-seeking(Shin, Templeton et al., 2018) and anxiety(Suzuki, Saitoh et al., 2016). Fullana et al. demonstrated that the depressive-like phenotype may be associated with IL hyperactivity, likely leading to an excessive top-down inhibitory control of serotonergic activity through IL-midbrain descending pathways(Fullana, Covelo et al., 2019). However, the regional specificity of PrL and IL in the regulation of depressive behaviors remains poorly understood.

Proinflammatory cytokines, including interleukin-6 (IL-6) and interleukin-1beta (IL-1β), secreted by glial cells within the brain in response to injury and infection, impair the 5-HT system through activation of indoleamine-2,3-dioxygenase (IDO), the rate-limiting enzyme catalyzing the conversion of tryptophan (Trp) to kynurenine (Kyn) along the Kyn pathway(Garrison, Parrott et al., 2018). Consecutively, intermediates of Kyn are metabolized into kynurenic acid (KA) with neuroprotective effects mainly in astrocytes, while Kyn is metabolized into 3-hydroxy-kynurenine (3-HK) and quinolinic acid in microglia induces excitotoxic effects. Yet, it is unclear that the role of IDO-related glial alterations in the regulation of mood disorders.

Kyn and several downstream metabolites have been proposed to be highly correlated with depression(Deng, Zhou et al., 2021; Hestad, Alexander et al., 2022). Further evidence speculated that the homeostasis imbalance of downstream metabolites regulated by microglia and astrocytes might be the prominent mechanism of depressive behaviors after IDO activation induced by LPS(Tao, Yan et al., 2020). Intracerebroventricular injection of streptozotocin (ICV-STZ) triggered progressive mood and cognitive impairments by causing Aβ-Tau pathology, oxidative stress, insulin resistance and immune-inflammatory responses in the cerebral cortex and hippocampus of rats(Akhtar, Dhaliwal et al., 2021; Grieb, 2016). Souza et al. reported that ICV-STZ can induce up-regulation of pro-inflammatory cytokines and down-regulation of brain-derived neurotrophic factor (BDNF) in the hippocampus of mice at the early stage of the formation of a sporadic Alzheimer's disease (sAD) model(Souza, Filho et al., 2013; Souza, Jesse et al., 2017), and subcutaneous administration

of IDO inhibitor 1-MT improved depressive behaviors in ICV-STZ mice(Souza, Jesse et al., 2017). However, its mechanism in the central nervous system, especially in subdivided brain regions, has not been studied.

Therefore, this study aimed to investigate whether there are differences in the regulatory mechanisms caused by ICV-STZ activation of IDO in the PrL and IL of rats. For this purpose, we evaluated the effects of ICV-STZ on the Kyn pathway, microglia, astrocytes and synaptic plasticity in the PrL and IL. In addition, 1-MT was directly injected into the PrL and IL respectively, to study its antidepressant effect and its influence on the above pathological mechanisms.

2 Method :

2.1 Animals

Adult male Sprague-Dawley rats (RRID: MGI: 5651135) (250-280 g weight) were procured from the Animal Center of Peking University (Beijing, China). The rats were housed individually in plastic cages with ad libitum access to food and water at an optimum temperature of 25 ± 2°C and 55-65% relative humidity. The position of the bottle was in the middle of the cage cover to eliminate location bias. A 12 h/12 h light/dark cycle (lights on at 9:00 AM) was regulated in the animal house. All rats were allowed to acclimate for 7 d before receiving any experimental manipulation. All the experimental procedures were carried out under the guidelines of the "Animal Research: Reporting of In Vivo Experiments (ARRIVE)"(Percie du Sert, Hurst et al., 2020) and the recommendations made by the British Journal of Pharmacology (BJP)(Lilley, Stanford et al., 2020). The animal experiment protocol was approved by the Peking University Committee on Animal Care and Use (permission no. LA 2020279).

2.2 Surgery for cannula implantation

Rats were anesthetized with isoflurane anaesthesia (5% induction and 2% maintenance) and placed in a stereotaxic frame. A unilateral and a bilateral guide cannula (OD 0.64 mmxI.D. 0.25 mm, CC 1.2 mm, RWD Life science Co Ltd., Shenzhen, China) were chronically implanted in the lateral ventricle (-0.8 mm AP; -1.5 mm ML; -3.5 mm DV) and mPFC (PrL: +3.2 mm AP; +-0.60 mm ML; -2.6 mm DV, IL: +3.2 mm AP; +-0.6 mm ML; -3.8 mm DV) (Paxinos & Watson, 1986), respectively. The cannula and three anchor screws were bonded to the skull with dental acrylic and dental cement. After surgery, rats were placed in a heated pad and observed for at least 30 min, then returned to its original cage. Rats were handled and checked for signs of pain and distress at least once daily. Topical analgesic (lidocaine/prilocaine cream) was used to prevent signs of pain and/or discomfort for at least 2-3 days. The rats were intramuscularly injected with penicillin (400000 IU kg^{-1}) for at least 3 days and allowed to recover for 7 days before the experiments began.

2.3 Drugs and treatment

The ICV-STZ procedure was used as previously described(Cui, Song et al., 2018; Zhao, Cui et al., 2021). After 7 days of recovery from surgery, streptozotocin (Sigma-Aldrich, Cat# S0130, MO, USA) was administered on day 1 and day 3 at 3:00-5:00 PM at a dose of 3 mg kg^{-1}dissolved in 4 +- 1 μl artificial cerebrospinal fluid (aCSF; Tocris Bioscience, Cat# 3525/25 ML, Bristol, UK) according to body weight. Streptozotocin was slowly injected into the lateral ventricle using a Hamilton microsyringe fixed to the syringe pump (RWD Life Science Co Ltd., Shenzhen, China) and connected to the injection cannula at a rate of 0.8 μl min^{-1}. The needle was left in place for an additional 3 min to allow for diffusion. The vehicle treatment was with aCSF.

1-Methyl-DL-tryptophan (1-MT; Sigma-Aldrich, Cat# 860646, MO, USA) was dissolved in 1 M HCl and adjusted the pH to 6.5 using NaOH, then diluted using 0.9% sterile physiological saline to the final treatment concentration(Lawson, Parrott et al., 2013). 1-MT was injected once daily into the PrL or IL bilaterally (1 μl per side) at a rate of 0.2 μl min^{-1} at 9:00-11:00 AM from day 1 to day 8. The injection cannula was kept in place for another 2 min to allow the drug to diffuse from the tip entirely. According to O'Connor et al., the effective dose of 1-MT in the brain to produce antidepressant effects is 50 μg ml^{-1}(O'Connor, Lawson et al., 2009).

2.4 Nissl staining

Nissl staining was carried out using a Nissl stain Kit (Solarbio, # G1430, China). Brain sections were dried at 60°C and stained with Reagent A (Cresyl violet Stain) for 1 h at 56°C. Subsequently, the sections were washed with distilled water, immersed in Reagent B (Nissl Differentiation) for seconds to 2 min, and dehydrated in absolute ethanol. Finally, the sections were cleared in xylene before being cover-slipped with neutral balsam (Solarbio, # G8590, China).

2.5 Experiment design and behavioral assessment

Animals with surgery-related infections were excluded. Then, the rats were randomly grouped into six groups, with six animals (n=6) in each group presented in Figure 1A. The cannula placements of the lateral ventricle are shown in Figure 1B. Animal body weight and food consumption were measured daily. The water intake was measured daily from day 1 to day 5 before food and water deprivation. All behavioral tests except the novel object recognition test were conducted on day 7 and performed as previously described(Ding, Cui et al., 2021; Ye, Cui et al., 2018). On day 7, the open field test was performed at 9:00 AM, the sucrose preference test was performed at 12:00 PM, the novelty-suppressed feeding test was performed at 2:00 PM, and the forced swim test was performed at 4:00 PM. The novel object recognition test was performed at 9:00 AM on day 8. The rats were decapitated immediately after the behavioral tests on day 8, and the tissue was used for western blot and HPLC-MS/MS. The other three same experiments with six animals (n=6) in each group were used for ELISA, immunofluorescence staining and Golgi staining.

Open field test

The OFT was performed to evaluate locomotor activity and anxiety-like behavior. Rats were submitted individually to a Plexiglas chamber (40 cmx40 cmx65 cm), and behavior was recorded by an automated video tracking system (DigBehv-LM4, Shanghai Jiliang Software Technology, Shanghai, China). The video files were later analysed using DigBehv analysis software. In 10 minutes, locomotor activity is expressed as the total distance, and time in the center is considered an indicator of anxiety-behavior. The apparatus was wiped with 75% alcohol between tests to eliminate any smell.

Sucrose preference test

The SPT was used to determine anhedonia-like behavior, which is considered a core symptom of depression. Rats were habituated to drink from two bottles of 1% sucrose for 48 hours. After training, the rats were deprived of food and water for 24 hours before the test. On the test day, one bottle containing 1% sucrose solution in tap water and the other containing tap water alone were placed in the rat's home cages simultaneously, and rats were allowed to drink freely from both bottles for 1 hour. Water and sucrose consumption was measured by comparing the weight difference of the bottles before and after the test. Anhedonia was assessed as sucrose preference, which was calculated according to the following formula: sucrose preference=sucrose intake (g)/(sucrose intake [g] + water intake [g]) x 100%. To exclude the non-specific suppression of drinking, total fluid consumption was calculated as the sum of sucrose intake and water intake.

Novelty-suppressed feeding test

The NSFT is most widely used to check the efficacy and efficiency of chronic and sub-chronic antidepressant treatments in rodent models(Blasco-Serra, Gonzalez-Soler et al., 2017). The test was performed in an open field containing five to six food pellets placed in the middle of the arena. Rats were deprived of food at least 24 h before the test. After SPT, individual rats were placed in the corner of the arena and allowed to explore it freely for 10 minutes. The latency for the rat from leaving the corner of the arena to pick up food was recorded. The latency to feed was measured up to 10 minutes. The increase in latency to feed is considered a measure of anhedonia based on food. After the NSFT, the rats were returned to their home cages and allowed to eat food. Food consumption within 60 minutes across the groups was recorded to exclude the non-specific ingestive behavior.

Forced swim test

The FST procedure was conducted according to our previous study(Ding, Cui et al., 2021). On the pretest day, each rat was individually placed for 15 minutes into a 25cm diameterx60 cm high Plexiglas cylinder filled with 24+-1degC water to a depth of 40 cm. On the test day, the rat was placed into the same cylinder again and recorded for 5 minutes. The water was changed between testing sessions. Behavior was recorded by two video cameras (one on top and one on the side). After the experiment, rats were removed from the water, dried with a towel, and returned to their home cage. The videotapes were analysed by a researcher who was blinded to each rat's treatment condition. Immobility was defined as the minimum movement necessary to keep the rat's head above the water. Increased immobility time indicated a state of helplessness.

Novel object recognition test

The NORT is used to evaluate declarative memory and object recognition and has an essential application in the study of cognitive alterations. The test was performed in a Plexiglas box (40 cmx40 cmx65 cm) and the task procedures consist of three phases: habituation, training, and test. The rats were habituated to the test box for 20 min on the habituation phase (24 h before the training phase). During the training phase, two identical objects were placed in opposite walls of the testing box, and the animals were allowed to explore for 10 min. Then, we changed one object to a different shape and color in the test phase, and a test session of 10 min was performed after a retention interval of either 2 h. The behavior of the subjects in each trial was recorded on video, and the exploration time was scored by an observer blinded to the experimental conditions. Exploration was defined as sniffing, biting, licking or touching the object with the nose. Turning around or sitting on the object was not considered exploratory behavior. During the test session, a discrimination index (DI) was calculated using the formula (B-A)/(B+A), with B being the time spent exploring the novel object and A being the time spent exploring the familiar object.

2.6 Western blot

Western blot analysis was performed as required by BJP guidelines(Alexander, Roberts et al., 2018). After the rats were decapitated, the brains were immediately removed to a prechilled brain matrix with a 1.0 mm coronal slice thickness (RWD Life Technology, Shenzhen, China). PrL and IL tissues were harvested on ice guided by the Paxinos and Watson rat brain atlas(Paxinos & Watson, 1986) and stored separately in prechilled microcentrifuge tubes at -80degC until assayed. The tissue was homogenized in RIPA buffer (Solarbio, Cat# R0010, China) supplemented with protease inhibitors (Solarbio, Cat# P0100, China) and phosphatase inhibitors (Solarbio, Cat# P1260, China). Protein (45 μg) was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes (0.45 μm, Millipore, USA). The membranes were blocked with 5% skim milk for 1 h at room temperature and incubated with primary antibodies, including anti-β-actin (1:4000; Rabbit mAb, ABclonal, Cat# AC038, Wuhan, China), anti-β-Amyloid (Aβ) (1:50; Mouse mAb, Santa Cruz Biotechnology, Cat# sc-28365, RRID:AB_626669), anti-Tau (1:1000; Mouse mAb, Cell Signaling Technology, Cat# 4019, RRID:AB_10695394), anti-Phospho-Tau (Ser199) (1:1000; Mouse mAb, Cell Signaling Technology, Cat# 29957, RRID:AB_2798984), anti-glial fibrillary acidic protein (GFAP) (1:1000; Rabbit mAb, Cell Signaling Technology, Cat# 80788, RRID:AB_2799963), anti-glutamate-aspartate transporter (GLAST) (EAAT-1 in humans, 1:1000; Rabbit mAb, Cell Signaling Technology, Cat# 5684, RRID:AB_10695722), anti-glutamate transporter-1 (GLT-1) (EAAT-2 in human, 1:500; Rabbit mAb, Abcam, Cat# ab41621, RRID:AB_941782), anti-ionized calcium-binding adaptor molecule-1 (Iba1) (1:1000; Rabbit mAb, Cell Signaling Technology, Cat# 17198, RRID:AB_2820254) and anti-BDNF (1:500; Rabbit mAb, Abcam, Cat# ab108319, RRID:AB_10862052) in TBST buffer (Tris-buffered saline + 0.1% Tween-20) overnight at 4°C. The blots were then washed with TBST three times before incubation with HRP goat anti-mouse IgG (H+L) antibody (1:2000; ABclonal, Cat# AS003, Wuhan, China) or HRP goat anti-rabbit IgG (H+L) antibody (1:2000; ABclonal, Cat# AS014, Wuhan, China) for 2 h at room temperature. After 3 x 5 min TBST washes, an ECL Enhanced Kit (ABclonal, Cat# RM00021, Wuhan, China) was used for detection enhancement, and blots were visualized using ImageJ software. The results were normalized to the protein expression level of β-actin.

2.7 Immunofluorescence staining and Image analysis

Immunofluorescence staining was performed as described previously(Ye, Cui et al., 2018). Anesthetized rats were perfused slowly with 200 ml of 0.01 M phosphate-buffered saline (PBS) and 200 mL of 4% paraformaldehyde. Whole brains were immediately removed, soaked in 4% paraformaldehyde at 4°C for 24 hours, and then successively transferred to 20% sucrose and 30% sucrose at 4°C until tissues were sunk. The brains were rapidly frozen in liquid optimal cutting temperature compound (Sakura Finetek, Cat# 4583, CA, USA) cooled with a mixture of solid carbon dioxide and ethanol. Serial coronal brain sections were cut in 20 μm thickness on a cryostat microtome (Leica Microsystems UK, Leica CM1950, Milton Keynes, UK) and stored at -20 °C in cryoprotectant solution (48% PBS, 30% ethylene glycol, 20% glycerol, 2% DMSO). To label the astrocytes and microglia, the sections were first placed in PBS (3×5 minutes) to wash out the cryoprotectant solution. Then, the antigen retrieval procedure was conducted in citrate buffer (Solarbio, Cat# C1032, China) for 5 minutes at 96°C. After cooling to room temperature, the sections were washed in PBS (3 × 5 minutes) again. Then, the sections were permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, Cat# T9284, MO, USA) for 20 minutes and blocked with 5% donkey serum at room temperature for 40 minutes. The sections were then incubated with primary antibodies against GFAP (1:500; Rabbit mAb, Cell Signaling Technology, Cat# 80788, RRID: AB_2799963) and Iba-1 (1:500; Rabbit mAb, Cell Signaling Technology, Cat# 17198, RRID: AB_2820254) overnight at 4degC respectively. After washing in PBS (3x5 minutes), sections were incubated with fluorescent-conjugated secondary antibodies (donkey anti-rabbit IgG Alexa Fluor 488, Abcam, Cat# ab150073, RRID: AB_2636877, 1:2000; donkey anti-rabbit IgG Alexa Fluor 647, Abcam, Cat# ab150075, RRID: AB_2752244, 1:2000) for 2 hours at room temperature. Finally, nuclei were subsequently stained with DAPI.

The single images from the PrL and IL for each mouse were scanned by a Nikon DS-Ri2 microscope camera (Nikon, Tokyo, Japan) and processed by Fiji software. All images of each section were acquired at 200x magnification. The morphological changes in microglia were evaluated following a previously published protocol (Young & Morrison, 2018). The number of branches and end-points were examined to investigate microglial morphological changes. These morphological parameters of microglia can reflect microglia complexity and distinguish the state of microglia. Sholl analysis protocol was based on those described earlier(Codeluppi, Chatterjee et al., 2021). In brief, bidimensional images were converted to 8-bit images and adjusted for brightness-contrast before thresholding to minimize background noise. Following these steps, the images were processed with the despeckle function and skeletozine. Then, the ending radius was depicted with a straight line. Last, we run the Sholl analysis with a set radius step of 3 μm.

2.8 Golgi staining

The FD Rapid Golgi-Staining Kit (FD NeuroTechnologies, Columbia, MD, USA) was used to reveal the density of dendritic spines in pyramidal neurons in the PrL and IL. Brains were immersed in Golgi-staining impregnation solution for 2 weeks. Sections were cut at 150 μm on a cryostat at -20°C to -22°C. The spines on secondary or tertiary dendrites of pyramidal neurons were calculated at a dendritic segment length of approximately 50 μm. At least 3 dendrites per rat were traced, and a total of 6 rats per group were counted. The images were captured under Nikon Eclipse Ci-L microscope (Nikon, Tokyo, Japan) using DP controller software with 100×A/1.25 oil immersion lens. The density of dendritic spines and the dendritic spine morphologies in the PrL and IL were analysed using ImageJ software. Dendritic spine density was calculated as the total number of spines per 10 μm length of branch. Dendritic spine morphologies were classified into 4 main types: thin, filopodia, mushroom and stubby(Tackenberg, Ghori et al., 2009). The thin type has a narrow neck with elongated protrusion; the filopodia type was identified as long, thin structures; the mushroom type has a large irregular head with a neck diameter smaller than the head diameter; the stubby type has no obvious constriction between the protrusion and attachment to the neck. The proportion of each type was quantified as ([spine number with each type/total spine number] ×100).

2.9 Estimation of kynurenine metabolites

The concentrations of Trp, Kyn, 3-HK and KA were measured using an HPLC–MS/MS method as described by Han et al. (Han, Qin et al., 2019) with slight modifications. Briefly, tissue was transferred into a new EP tube, then mixed with 90 μl of prechilled (4 °C) methanol (0.1% formic acid)-aqueous (8:2, v/v) mixture and

10 µl of IS (2-Cl-Phe; 1 µg mL^{-1}). The mixture was homogenized using an ultrasonic homogenizer followed by centrifugation at 20,000 ×g for 20 min at 4degC. After centrifugation, the separated supernatant was transferred into a 2 ml autosampler, and 10 µl was injected into the system at a flow rate of 0.4 ml min^{-1}. The standard curve was prepared using the same procedure as the brain sample.

The HPLC–MS/MS system consisted of a Dionex UltiMate 3000 Ultra-HPLC system (Thermo, San Jose, CA, USA) and an API 4000Q Trap mass spectrometer (AB SCIEX, Foster City, USA) equipped with an electrospray ionization (ESI) source interface. The optimized mass spectrometric parameters were set as follows: curtain gas, 15 psi; collision gas, 2; ion spray voltage, 5500 V for positive mode or -4500 V for negative mode; ion source temperature, 600°C; ion source gas 1, 55 psi; ion source gas 2, 55 psi. Accurate quantification was operated in multiple reaction monitoring (MRM) mode; the transitions were m/z 205.1-146.1 for Trp (positive), m/z 209.1-94.1 for Kyn (positive), m/z 225.1-208.1 for 3-HK (positive), m/z 188.0-144.0 for KA (negative). The declustering potential (DP) was set at 40, 60, 45 and -40 V, and the collision energy (CE) was 24, 22, 14 and -22 V for Trp, Kyn, 3-HK and KA, respectively.

Chromatographic separation was performed on an Ultimate XB-C18 column (100 mm x 2.1 mm, 5 µm, Welch Materials, Inc.). Mobile phase A was water containing 0.1% formic acid, and mobile phase B was acetonitrile. The temperature of the autosampler was set at 4 °C. Gradient separation was set as follows: 0-1 min, 5% B; 1-3 min, 5-60% B; 3.1-5 min, 5% B for column equilibration. The analysis was performed in a total run time of 5 min. Under these conditions, the retention times were 3.53, 1.97, 0.89 and 3.68 min for Trp, Kyn, 3-HK and KA, respectively. The above data were recorded and analysed using AB SCIEX Analyst 1.6 software.

Tryptophan (Trp, purity[?]99.5%, Cat# 93659), kynurenine (Kyn, purity[?]98%, Cat# K8625), 3-hydroxy-DL-kynurenine (3-HK, purity[?]98%, Cat# 148776) and kynurenic acid (KA, purity[?]98%, Cat# K3375) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-Chloro-L-phenylalanine (2-Cl-Phe, purity=98%, Cat# C105993) used as an internal standard (IS) was purchased from Aladdin Inc. (Shanghai, China). HPLC-grade acetonitrile and methanol were obtained from Fisher Chemical (Fisher Scientific, Shanghai, China).

2.10 Enzyme-linked immunosorbent assay (ELISA)

Rat ELISA kits were used to measure the levels of interleukin-1β (IL-1β) (ABclonal, Cat# RK00009, Wuhan, China) and interleukin-6 (IL-6) (ABclonal, Cat# RK00020, Wuhan, China) in the PrL and IL. Briefly, the tissue was homogenized and centrifuged at 2000×g for 20 minutes at 4degC, then the supernatant was extracted and incubated in a 96-well plate. The cytokine levels were estimated by interpolation from a standard curve by colorimetric measurements at 450 nm (correction wavelength 630 nm) on an ELISA plate reader (Thermo Fisher Scientific Multiskan Mk3, MA, USA). The results are shown as pg mg^{-1} of protein.

2.11 Data and Statistical analysis

Data and statistical analysis complied with the recommendations of the BJP on experimental design and analysis in pharmacology(Curtis, Alexander et al., 2018). The sample size was the number of independent values, and statistical analysis was performed using these independent values. The sample sizes are indicated in the figure legends. The data from western blot were normalized to control group values and determined as 'Fold change' in figures. Statistical analysis of the results was done with the help of GraphPad Prism software (version 8; GraphPad Software, Inc., RRID: SCR_002798). Statistical analysis was undertaken only for studies where each group size was at least n=6. The data are presented as the means +- SEM. Unpaired t-test was used to test data for vehicle and ICV-STZ model of variance. One-way and two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied for comparison among several groups. For two-way ANOVA, the procedure (vehicle or ICV-STZ) and the treatment (saline or 1-MT) were taken as between-group factors. $P<0.05$ was considered statistically significant in all tests. The experimenter or observer was blind to the groups while analysing the results.

3 Result s

3.1 ICV-STZ could induce depressive- and anxiety-like behaviors.

Our previous studies demonstrated that ICV-STZ induced sleep disorder(Cui, Song et al., 2018) and memory impairments(Zhao, Cui et al., 2021) in rats on day 14. However, the non-cognitive behaviors in the early stage were still unknown. The results of FST exhibited a significant increase in immobility time, indicating helplessness in the ICV-STZ rats ($t_{(10)}$ =9.026, Figure 2A). Meanwhile, the ICV-STZ rats exhibited a significant decrease in sucrose preference in the SPT ($t_{(10)}$ =5.809, Figure 2B), representing anhedonia-like behavior. And a significant prolongation in latency time to feed in the NSFT ($t_{(10)}$ =4.730, Figure 2C) was found in ICV-STZ rats. Food consumption after NSFT was no difference across the groups excluded the ingestion bias (Figure 2D). Still, ICV-STZ rats showed a significant decrease in time in center in the OFT, representing anxiety-like behavior in certain aspects($t_{(10)}$ =5.580, Figure 2E). Locomotor activity did not reveal significant differences between the vehicle and ICV-STZ rats (Figure 2F). In the NORT, the discrimination ratio was found no significant difference in ICV-STZ rats compared with the vehicle rats, reflecting there were no impairments in recognition memory and discrimination ability under the treatment of ICV-STZ (Figure 2G). In addition, the Western blot image (Figure 2H) and quantitative analysis did not reveal a significant difference in the expression of Aβ (Figure 2I), Tau (Figure 2J), $pTau^{ser199}$ (Figure 2K) and the ratio of $pTau^{ser199}$/Tau (Figure 2L) in the mPFC of ICV-STZ rats.

3.2 Differential responses of PrL and IL to IDO activation and inflammation induced by ICV-STZ.

In order to investigate whether the ICV-STZ induced behavioral alterations are related to the activation of IDO in the PrL and IL, we examined the levels of kynurenine metabolites, and local injection of 1-MT was used to directly explore the role of IDO in the PrL and IL in ICV-STZ rats. The cannula placements of PrL and IL are shown in Figure 3A and S1A, B. The body weights of all groups maintained the same increasing trend to the end of the experiment (Figure 3B). Besides, ICV-STZ and 1-MT treatment generally had no effect on food and water consumption, as shown in Figure S1C, D. In the PrL, there was main effect of 1-MT treatment ($F_{(1,20)}$=89.37) for the levels of Trp. Regarding the levels of Kyn, significant effects of ICV-STZ ($F_{(1,20)}$=47.29), 1-MT treatment ($F_{(1,20)}$=15.16) and interactions between ICV-STZ and 1-MT treatment ($F_{(1,20)}$=16.88) were observed. For the ratios of Kyn/Trp, significant effects of ICV-STZ ($F_{(1,20)}$=29.37), 1-MT treatment ($F_{(1,20)}$=64.32) and interactions between ICV-STZ and 1-MT treatment ($F_{(1,20)}$=12.75) were also observed. Post hoc test showed that ICV-STZ did not cause significant alterations in the levels of Trp (Figure 3C) but induced an up-regulation of Kyn levels (Figure 3D) and Kyn/Trp ratios (Figure 3E), which are standardly used as a measure of IDO activity. Intra-PrL injection of 1-MT increased Trp levels and reversed the up-regulation of Kyn levels and Kyn/Trp ratios(Figure 3C-E) in the PrL induced by ICV-STZ. In the IL, there was main effects of ICV-STZ ($F_{(1,20)}$=23.85) for the levels of Trp. Regarding the levels of Kyn, significant effects of ICV-STZ ($F_{(1,20)}$=25.35), 1-MT treatment ($F_{(1,20)}$=18.80) and interactions between ICV-STZ and 1-MT treatment ($F_{(1,20)}$=19.38) were observed. For the ratios of Kyn/Trp, significant effects of 1-MT treatment ($F_{(1,20)}$=16.91) and interactions between ICV-STZ and 1-MT treatment ($F_{(1,20)}$=16.58) were also observed. Different with PrL, the post hoc test revealed that ICV-STZ increased the levels of Trp (Figure 3F), Kyn (Figure 3G) and Kyn/Trp ratios (Figure 3H) in the IL. Intra-IL injection of 1-MT has no effect on Trp levels for vehicle and ICV-STZ rats but normalized the up-regulation of Kyn levels and Kyn/Trp ratios (Figure 3F-H) in the IL induced by ICV-STZ. These results proposed that ICV-STZ induced behavior alterations are likely related to activating IDO in the PrL and IL.

Pro-inflammatory cytokines are the main factors that trigger IDO activation. Hence, we further tested the levels of cytokines to elucidate the involvement of neuroinflammatory response in the PrL and IL. Two-way ANOVA revealed significant effects of 1-MT treatment ($F_{(1,20)}$=9.711) and interactions between ICV-STZ and 1-MT treatment ($F_{(1,20)}$=14.92) on the levels of IL-1β in the IL. And there were also significant effects of 1-MT treatment ($F_{(1,20)}$=15.21) and interactions between ICV-STZ and 1-MT treatment ($F_{(1,20)}$=8.396) on the levels of IL-6 in the IL. The results showed that the levels of IL-1β (Figure 3I) and IL-6 (Figure 3J) in the PrL were not significantly altered by ICV-STZ and intra-PrL injection of 1-MT. In contrast, the neuroinflammatory response in the IL induced by ICV-STZ was characterized by a significant increase in

the levels of IL-1β (Figure 3K) and IL-6 (Figure 3L). Intra-IL injection of 1-MT prevented the increase of IL-1β and IL-6 levels in the IL induced by ICV-STZ.

3.3 Intra-PrL or IL injection of 1-MT blocks depressive behaviors induced by ICV-STZ.

To directly evaluate whether the activation of IDO in the PrL and IL was responsible for the depressive- and anxiety-like behaviors in ICV-STZ rats, we observed the effects of IDO inhibitor 1-MT on the behaviors of ICV-STZ rats. Depressive-like behaviors induced by ICV-STZ, including the increase in immobility time in the FST($F_{(5,30)}$=34.96, Figure 4A), the decrease in sucrose preference rate in the SPT($F_{(5,30)}$=11.37, Figure 4B) and latency time to feed in the NSFT ($F_{(5,30)}$=16.20, Figure 4C) were reversed by intra-PrL or IL injection of 1-MT. Food consumption after NSFT was no difference across the groups (Figure 4D). The result of time in center showed that intra-PrL or IL injection of 1-MT could not reverse the anxiety-like behavior in ICV-STZ rats (Figure 4E). Results of locomotor activity in total distance in the OFT did not reveal significant differences among the various groups (Figure 4F, G). Based on these results, intra-PrL or IL injection of 1-MT both prevented depressive-like behaviors in ICV-STZ rats but failed to produce an anxiolytic effect.

3.4 Differential responses of PrL and IL to microglial alterations induced by ICV-STZ.

Since neuroinflammation is regulated by glial cells in the central nervous system, we further observed the alteration of microglia in the PrL and IL to examine the potential target of 1-MT. Iba1 was considered as the microglia marker. As shown in Figure 5, the main effects of 1-MT treatment were observed in the Iba1-positive cell number ($F_{(1,20)}$=5.118), levels of Iba1 protein ($F_{(1,20)}$=11.20) and 3-HK ($F_{(1,20)}$=11.97) from PrL. The post hoc test revealed that there was no significant difference in the fluorescent images of immunostaining for Iba1 (Figure 5A) and the count of microglia (Figure 5B) in the PrL of ICV-STZ rats administered saline or 1-MT. Similarly, the expression of Iba1 (Figure 5C) and microglial morphology in the PrL (Figure 5D-F) were not influenced by either ICV-STZ or 1-MT. Levels of 3-HK (Figure 5G) in the PrL was insignificant difference across the groups, although intra-PrL injection of 1-MT induced an increasing trend. In the IL, the main effects of ICV-STZ were observed in the Iba1-positive cell number ($F_{(1,20)}$=8.092) and levels of Iba1 protein ($F_{(1,20)}$=7.915); the main effects of 1-MT treatment were observed in the Iba1-positive cell branches ($F_{(1,20)}$=8.960). Moreover, a significant interaction between ICV-STZ and 1-MT treatment was observed in the Iba1-positive cell number ($F_{(1,20)}$=8.092), levels of Iba1 protein ($F_{(1,20)}$=6.103), Iba1-positive cell branches ($F_{(1,20)}$=37.13), Iba1-positive cell end-points ($F_{(1,20)}$=7.035) and levels of 3-HK ($F_{(1,20)}$=6.667). Fluorescent images of immunostaining for Iba1 (Figure 6A) and the count of Iba1-positive cells number (Figure 6B) presented that the number of microglia in the IL was significantly increased by ICV-STZ, while intra-IL injection of 1-MT recovered the density of Iba1-positive cells to normal. Western blot analysis also revealed that the expression of Iba1 in the IL was significantly increased by ICV-STZ, and reversed by intra-IL injection of 1-MT (Figure 6C). Subsequently, the analysis of morphological features in microglia (Figure 6D-F) indicated that ICV-STZ remarkably decreased the branch number (Figure 6E) and end-point number (Figure 6F) of Iba1-positive cells in the IL, and intra-IL injection of 1-MT prevented these decreases. On the other hand, the levels of 3-HK increased with microglia activation in the IL, and this increase was reversed by intra-IL injection of 1-MT (Figure 6G).

3.5 Differential responses of PrL and IL to astrocyte alterations induced by ICV-STZ.

Then, we evaluated the expression and function of astrocytes in the PrL and IL separately. GFAP was considered as the astrocyte marker. In the PrL, the main effects of ICV-STZ were observed in the intersection number (12 μm: $F_{(1,20)}$=5.075; 15 μm: $F_{(1,20)}$=7.447), levels of GLAST ($F_{(1,20)}$=8.530), GFAP ($F_{(1,20)}$=8.883) and KA ($F_{(1,20)}$=20.43); the main effects of 1-MT treatment were observed in the intersection number (3 μm: $F_{(1,20)}$=5.057; 9 μm: $F_{(1,20)}$=4.648; 12 μm: $F_{(1,20)}$=7.488; 15 μm: $F_{(1,20)}$=18.27; 18 μm: $F_{(1,20)}$=24.75; 21 μm: $F_{(1,20)}$=17.39; 24 μm: $F_{(1,20)}$=7.569), levels of GLT-1 ($F_{(1,20)}$=9.437), GLAST ($F_{(1,20)}$=5.884), GFAP ($F_{(1,20)}$=10.77) and KA ($F_{(1,20)}$=38.04). A significant interaction between ICV-STZ and 1-MT treatment was observed in the GFAP-positive cell number ($F_{(1,20)}$=21.19), intersection number (12 μm: $F_{(1,20)}$=5.959; 15 μm: $F_{(1,20)}$=8.523; 18 μm: $F_{(1,20)}$=5.719; 21 μm: $F_{(1,20)}$=7.213), levels of GLAST

($F_{(1,20)}$=14.21) and KA ($F_{(1,20)}$=17.85). ICV-STZ significantly reduced the number of GFAP-positive cells in the PrL, which was reversed by intra-PrL injection of 1-MT (Figure 7A, B). Sholl analysis showed an attenuated number of intersections, suggesting the atrophy of astrocytes. Specifically, the ICV-STZ rats significantly decreased at steps 9 and 12 compared with the vehicle rats, and a significant increase in the number of intersections was found in the intra-PrL injection of 1-MT rats at steps 9 to 24 compared with ICV-STZ rats (Figure 7C, D). Consistent with above results, western blot analysis (Figure 7E) also revealed that ICV-STZ markedly decreased the expression of GFAP (Figure 7H) in the PrL, accompanied by dysfunctional changes including the reduction of GLT-1 (Figure 7F) and GLAST (Figure 7G). All these defects were restored by intra-PrL injection of 1-MT (Figure 7E-H). Levels of KA decreased with astrocyte deficits in the PrL, and this decrease was reversed by intra-PrL injection of 1-MT (Figure 7I). In the IL, the main effects of ICV-STZ were observed in the intersection number (12 μm: $F_{(1,20)}$=5.809; 21 μm: $F_{(1,20)}$=4.424) and levels of KA ($F_{(1,20)}$=10.61); the main effects of 1-MT treatment were observed in the levels of GFAP ($F_{(1,20)}$=17.08). ICV-STZ and intra-IL injection of 1-MT had no effect on the number of astrocytes per unit area (Figure 8A, B). In addition, there was no significant distinction in the number of intersections for all groups (Figure 8C, D). Western blot analysis (Figure 8E-H) did not reveal a significant difference in the expression of GLT-1 (Figure 8F), GLAST (Figure 8G) and GFAP (Figure 8H) in the IL across the groups. Intriguingly, the levels of KA also decreased in the IL, and did not reverse by intra-IL injection of 1-MT (Figure 8I).

3.6 Synaptic plasticity was impaired in both PrL and IL in ICV-STZ rats and reversed by 1-MT.

It has been acknowledged that neuroinflammation can influence both neurogenesis and neuroplasticity. Golgi staining can visually observe the dendritic spines, thereby reflecting changes in synaptic plasticity and function (Figure 9A). Additionally, BDNF is a dimeric protein used to delineate neuroplasticity. In the PrL, the main effects of ICV-STZ were observed in the total spine density ($F_{(1,20)}$=55.46), mushroom type ($F_{(1,20)}$=37.50) and levels of BDNF ($F_{(1,20)}$=11.73); the main effects of 1-MT treatment were observed in the total spine density ($F_{(1,20)}$=39.21), mushroom type ($F_{(1,20)}$=10.36) and levels of BDNF ($F_{(1,20)}$=19.42). A significant interaction between ICV-STZ and 1-MT treatment was observed in the total spine density ($F_{(1,20)}$=26.53), thin type ($F_{(1,20)}$=4.376), filopodia type ($F_{(1,20)}$=4.543), mushroom type ($F_{(1,20)}$=11.06) and levels of BDNF ($F_{(1,20)}$=23.22). For IL, the main effects of ICV-STZ were observed in the total spine density ($F_{(1,20)}$=16.10), mushroom type ($F_{(1,20)}$=5.068) and levels of BDNF ($F_{(1,20)}$=9.407); the main effects of 1-MT treatment were observed in the total spine density ($F_{(1,20)}$=27.29), mushroom type ($F_{(1,20)}$=21.27), stubby type ($F_{(1,20)}$=10.09) and levels of BDNF ($F_{(1,20)}$=7.88). A significant interaction between the ICV-STZ and 1-MT treatment was observed in the total spine density ($F_{(1,20)}$=32.82), mushroom type ($F_{(1,20)}$=21.57), stubby type ($F_{(1,20)}$=18.78) and levels of BDNF ($F_{(1,20)}$=5.012). The post hoc test showed that ICV-STZ rats displayed a drastic decrease in dendritic spine density and the proportion of mushroom in both PrL and IL, and these abnormalities were reversed by intra-PrL or IL injection of 1-MT (Figure 9B-G). In another aspect, the proportion of thin and filopodia was increased significantly in the PrL by ICV-STZ, intra-PrL injection of 1-MT reversed the increase of thin, but the increase of filopodia was slightly and not significantly blocked by 1-MT (Figure 9D). In contrast, the proportion of stubby was increased significantly in the IL by ICV-STZ, which was reversed by intra-IL injection of 1-MT (Figure 9G). The expression of BDNF in the PrL and IL were both remarkably decreased in ICV-STZ rats, and intra-PrL and IL administration of 1-MT can reverse the decreased BDNF levels induced by ICV-STZ (Figure 9H-K).

4 Discussion

Several studies on depression have compared PrL and IL based on differences in cytoarchitecture, function and connectivity(Fullana, Covelo et al., 2019; Garro-Martinez, Fullana et al., 2021; Liu, Ota et al., 2015). In this study, we found that ICV-STZ activates IDO in both PrL and IL, but its regulatory pathways of depressive behaviors in the PrL and IL are different, with a remarkable attenuation of astrocytes in the PrL and overactivation of microglia in the IL as shown in Figure 10. The alteration of Trp levels in the PrL and IL was different, which hinted at the regional specificity of Kyn pathways between two subregions.

Although the reason is unknown, a possible explanation is that the up-regulation of Trp levels in the IL was a compensatory increase induced by neuroinflammation. Furthermore, the increase of pro-inflammatory cytokines was observed only in the IL but not PrL, reflecting the neuroinflammatory region specificity in the ICV-STZ induced depression.

There is substantial evidence of depression emerging from abnormalities of astrocytes and microglia(Tao, Yan et al., 2020; Yang, Zhou et al., 2020), and in the central nervous system, glia can respond positively to neuroinflammation via regulating the kynurenine pathway balance(Dezsi, Tuka et al., 2015; Garrison, Parrott et al., 2018). Although no differences were observed in the structural distribution of glial cells in the PrL and IL(Banqueri, Mendez et al., 2019; Gosselin, Gibney et al., 2009), they appear to play different roles in the mediation of depressive-like behaviors(Fullana, Covelo et al., 2019). The present study illustrated that ICV-STZ induced depressive behavior was paralleled by astrocytic defects in the PrL and microglia activation in the IL. In the PrL, astrocytes were shown deficit and dysfunction characterized by the decrease in astrocyte number, complexity of branches and levels of GLT-1, GLAST and GFAP, with a concomitant reduction in the neuroprotective metabolite KA. In the IL, microglia activation was characterized by increased microglia numbers, Iba1 levels, pro-inflammatory cytokines and neurotoxicity metabolite 3-HK with shortening of cellular processes and enlargement of the soma. The level of KA in the IL was also reduced despite there were no astrocyte deficits, which might be due to Kyn being more metabolized into 3-HK induced by ICV-STZ. However, we did not observe activation of microglia in the PrL and defect of astrocytes in the IL in ICV-STZ rats. All these dysregulations were restored by regional selective inhibition of IDO, suggesting that the antidepressant effects of 1-MT in the PrL might be through normalizing astrocyte deficit and dysfunction, while the antidepressant effects of 1-MT in the IL might be mediated, at least for a part, by local anti-inflammatory processes.

Studies in human and animals indicated that the central inflammatory response in depression may be associated with the reduction of total volume, the shrinkage of dendrites and loss of spines within the mPFC(Fan, Song et al., 2018; Meier, Drevets et al., 2016). Some studies have shown that under chronic or acute stress, the dendritic branches of neurons in the PrL(Moench & Wellman, 2017) and IL(Moench, Maroun et al., 2016) can be seen retraction. In the present study, the dendritic spine density and proportion of mushroom type in the PrL and IL were significantly decreased, and this effect was reversed by local treatment with 1-MT. The mushroom type was identified as the main type involved in the regulation of synaptic plasticity because it is more stable and has larger postsynaptic densities and contact areas than other types(Helm, Dankovich et al., 2021). Both neuroplasticity and BDNF deficiency in the PrL and IL suggested an additional mechanism implicated with IDO activation in ICV-STZ rats and the effects of 1-MT on basal dendrites. Noteworthy, enhanced thin type and filopodia type proportions distributed on the dendrites of PrL were observed in ICV-STZ rats, while in the IL, a larger proportion of stubby type was mainly detected. Although ICV-STZ differentially altered structural plasticity in the PrL and IL, these structural alterations were reversed by local treatment with 1-MT, suggesting that 1-MT ameliorates depressive behaviors involving the structural remodeling of dendritic spines.

The model of ICV-STZ was highly prevalent and has been used to resemble sAD in decades(Kalafatakis & Zarros, 2014). Over time, ICV-STZ rats showed typical pathological changes similar to sAD, such as the deposition of Aβ and the hyperphosphorylation of Tau in the brain, and started to develop learning and cognitive impairment on day 14(Song, Cui et al., 2018). According to our study, although there were no such pathological changes and learning and memory impairments on day 7, depressive-like behaviors appeared. Evidence has reported that some patients with sAD have depression in the early stage, and depression might be a prodromal symptom of sAD(Burke, Cadet et al., 2018; Gatchel, 2021). Therefore, it is necessary to further study whether this animal model can simulate the clinical processes of sAD. Although a clear explanation of the mechanism of sAD formation is still lacking, the ICV-STZ model might provide a valuable contribution to the field of sAD model research. Additionally, our data show for the first time that the regionally selective blockade of IDO by 1-MT within the mPFC, either PrL or IL, protected rats from ICV-STZ induced depressive behaviors but failed to produce an anxiolytic effect, suggesting that the anxiety-like behaviors induced by ICV-STZ might not be due to the IDO activation in the PrL and IL, which

need further confirmation.

5 Conclusion:

Overall, the results presented are encouraging and provide clear evidence that exploring the subregional mechanisms of mPFC is of great significance for further understanding the pathogenesis and treatment of depression. In ICV-STZ rats, IDO is activated either in PrL or in IL, but the mechanisms involved in the regulation of depressive behaviors of the two subregions are obviously different. 1-MT, a selective inhibitor of IDO, can reverse ICV-STZ-induced depressive behavior by ameliorating astrocyte defects in the PrL or inhibiting IL microglial hyperactivation. These findings provide valuable information for further understanding the pathogenesis of depression and discovering a novel target for depression treatment.

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Figure legends

Figure 1 . Experimental design (A) and the location of intracerebroventricular injection sites (B).

Figure 2 . ICV-STZ induced depressive- and anxiety-like behaviors without cognitive impairment, with no significant difference in Aβ, Tau, $pTau^{Ser199}$ protein levels and $pTau^{Ser199}$/Tau ratio in the mPFC. Immobility time in the FST (A), sucrose preference in the SPT (B) and latency time in the NSFT (C, D) were used to evaluate depressive-like behaviors. Time in center in the OFT (E) was used to evaluate anxiety-like behavior. Total distance in the OFT (F) was used to detect locomotor activity. Discrimination ratio in the NORT (G) was used to detect recognition memory and discrimination ability (*n1, n2* = 6). Western blots (H) and quantification of Aβ (I), Tau (J), $pTau^{Ser199}$ (K) and $pTau^{Ser199}$/Tau ratio in the mPFC. β-actin is shown as a quantitative loading control (*n1, n2* = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; A-G, I-L, unpaired Student's t-test.

Figure 3 . ICV-STZ induced IDO activation in the PrL and IL, increased the level of pro-inflammatory cytokines in the IL. Location of the PrL and IL injection sites (A). Intra-PrL or IL administration of 1-MT had no effect on body weight (B) (n=6 per group). Levels of Trp (C) and Kyn (D) examined by HPLC–MS/MS and the Kyn/Trp ratio (E) were used as a measure of IDO activity in PrL (n1, n2, n3, n4 = 6). Levels of Trp (F) and Kyn (G) and the Kyn/Trp ratio (H) in the IL (n1, n2, n3, n4 = 6). Levels of IL-1β (I) and IL-6 (J) examined by ELISA were used to evaluate the neuroinflammatory response in the PrL (n1, n2, n3, n4 = 6). Levels of IL-1β (K) and IL-6 (L) in the IL (n1, n2, n3, n4 = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; C-L, two-way ANOVA followed by Tukey's multiple comparison test.

Figure 4 . Intra-PrL or IL administration of 1-MT prevented depressive-like behaviors induced by ICV-STZ. Immobility time in the FST (A), sucrose preference in the SPT (B) and latency time in the NSFT (C, D) were used to evaluate depressive-like behaviors. Time in center in the OFT (E) was used to evaluate anxiety-like behavior. Total distance in the OFT (F) was used to detect locomotor activity (G) (*n1, n2, n3, n4* = 6). The data shown are individual values with means +- SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; A-F, one-way ANOVA followed by Tukey's multiple comparison test.

Figure 5 . ICV-STZ and intra-PrL administration of 1-MT had no effects on microglia-related indicators in the PrL. Fluorescent images of immunostaining for Iba1 (A) and the count of microglia (B) were used to evaluate the expression of microglia in the PrL. Iba1 was labelled with Alexa Fluor 647 (red) and the cell nucleus was counterstained with DAPI (blue), scale bars=100 μm, *n1, n2, n3, n4* = 6. Western blots and quantification of Iba1 (C) in the PrL, β-actin is shown as a quantitative loading control (*n1, n2, n3, n4* = 6). Morphological images (D), the count of branches (E) and the number of end-points (F) were used to evaluate the state of microglia in the PrL (*n1, n2, n3, n4* = 6). Levels of 3-HK (G) examined by HPLC–MS/MS in PrL (*n1, n2, n3, n4* = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; B, C, E-G, two-way ANOVA followed by Tukey's multiple comparison test.

Figure 6 . Intra-IL administration of 1-MT blocked the activation of microglia in the IL induced by ICV-STZ. Fluorescent images of immunostaining for Iba1 (A) and the count of microglia (B) were used to evaluate the expression of microglia in the IL. Iba1 was labelled with Alexa Fluor 647 (red) and the cell nucleus was counterstained with DAPI (blue), scale bars=100 μm, *n1, n2, n3, n4* = 6. Western blots and quantification of Iba1 (C) in the IL, β-actin is shown as a quantitative loading control (*n1, n2, n3, n4* = 6). Morphological

images (D), the count of branches (E) and the number of end-points (F) were used to evaluate the state of microglia in the IL (*n1, n2, n3, n4* = 6). Levels of 3-HK (G) examined by HPLC–MS/MS in IL (*n1, n2, n3, n4* = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; B, C, E-G, two-way ANOVA followed by Tukey's multiple comparison test.

Figure 7 . Intra-PrL administration of 1-MT prevented astrocyte defects in the PrL induced by ICV-STZ. Fluorescent images of immunostaining for GFAP (A) and the count of astrocytes (B) were used to evaluate the expression of astrocytes in the PrL. GFAP was labelled with Alexa Fluor 488 (green) and the cell nucleus was counterstained with DAPI (blue), scale bars=100 μm, *n1, n2, n3, n4* = 6. Sholl analysis (C) and the line plot (D) revealed the number of intersections per 3 μm of astrocytes in the PrL, *n1, n2, n3, n4* = 6. Western blots (E) and quantification of GLT-1 (F), GLAST (G) and GFAP (H) in PrL, β-actin is shown as a quantitative loading control (*n1, n2, n3, n4* = 6). Levels of KA (I) examined by HPLC–MS/MS in PrL (*n1, n2, n3, n4* = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; B, F-I, two-way ANOVA followed by Tukey's multiple comparison test.

Figure 8 . ICV-STZ and intra-IL administration of 1-MT had no effects on astrocyte-related indicators in the IL. Fluorescent images of immunostaining for GFAP (A) and the count of astrocytes (B) were used to evaluate the expression of astrocytes in the IL. GFAP was labelled with Alexa Fluor 488 (green) and the cell nucleus was counterstained with DAPI (blue), scale bars=100 μm, *n1, n2, n3, n4* = 6. Sholl analysis (C) and the line plot (D) revealed the number of intersections per 3 μm of astrocytes in the IL, *n1, n2, n3, n4* = 6. Western blots (E) and quantification of GLT-1 (F), GLAST (G) and GFAP (H) in the IL, β-actin is shown as a quantitative loading control (*n1, n2, n3, n4* = 6). Levels of KA (I) examined by HPLC–MS/MS in IL (*n1, n2, n3, n4* = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; B, F-I, two-way ANOVA followed by Tukey's multiple comparison test.

Figure 9 . Intra-PrL or IL administration of 1-MT improved synaptic deficits in the PrL and IL induced by ICV-STZ. Golgi staining of PrL and IL pyramidal neurons (A). Representative images of dendritic spines in the PrL (B), scale bars=2 μm. Total dendritic spine number (C) and the proportion of each type (D) were used to evaluate the spine morphology alterations in the PrL, *n1, n2, n3, n4* =18 dendrites from 6 brains in each group. Representative images of dendritic spines in the IL (E), scale bars=2 μm. Total dendritic spine number (F) and the proportion of each type (G) were used to evaluate the spine morphology alterations in the IL, *n1, n2, n3, n4* =18 dendrites from 6 brains in each group. Western blots (H) and quantification of BDNF (I) in the PrL (*n1, n2, n3, n4* = 6). Western blots (J) and quantification of BDNF (K) in the IL (*n1, n2, n3, n4* = 6). The data shown are individual values with means ± SEM. *P < 0.05, significantly different from vehicle group; #P < 0.05, significantly different from ICV-STZ group; C, D, F, G, I, K, two-way ANOVA followed by Tukey's multiple comparison test.

Figure S1. The location of intra-PrL (A) and IL (B) injection sites. Food consumption (C) and water intake across the groups (D) (n=6 per group).

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