Article

Mechanism of Treadmill Exercise Combined with Rich Environmental Stimulation to Improve Depression in Post-stroke Depression Model Rats

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Abstract

Background: Post-stroke depression (PSD) is a common complication, occurring in approximately one-third of these patients. The neurological symptoms of PSD affect patients' daily life and subsequent recovery. Analyzing the pathogenesis of post-stroke depression from a psychological perspective, it was found that PSD patients often feel despair and anxiety, and it is crucial to explore non-pharmacological ways to improve post-stroke depressive symptoms. A combination of exercise and rich environmental stimulation (RES) has been found effective in improving post-stroke depressive symptoms. Therefore, this study aimed to explore the effects of exercise and rich environmental stimulation on PSD in rats and their potential underlying mechanisms and to provide a theoretical basis for managing PSD.

Methods: The PSD rat model was constructed, and the depression-like behaviors of rats in each group were evaluated using the open field test (OFT), sucrose preference test (SPT), and forced swimming test (FST). Moreover, changes in the morphological behavior of rat hippocampus were observed using hematoxylin-eosin (HE) staining and Nissl staining. The expression levels of 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in hippocampus tissues were assessed using enzyme linked immunosorbent assay (ELISA), and the levels of tryptophan-related proteins were determined employing western blot analysis. Additionally, a kynurenine-3-monooxygenase (KMO) inhibitor was administered to the combined stimulation group, and the levels of tryptophan (TRP), 5-HT, kynurenine (KYN), 3-hydroxy-kynurenine (3-HK), and quinolinic acid (QA) were evaluated using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS).

Results: Treadmill exercise combined with rich environmental stimulation significantly reduced the immobility time in the FST ($p < 0.01$), increased the exploratory behavior in the OFT ($p < 0.05$), and increased the sucrose water consumption in the SPT ($p < 0.01$), indicating that the depression-like behavior was improved. Treadmill exercise combined with rich environmental stimulation also improved the shape of the damaged hippocampus and increased the number of neurons in the hippocampus. Additionally, treadmill exercise combined with rich environmental stimulation significantly increased the levels of 5-HT and NE in hippocampus tissues ($p < 0.01$) and decreased KMO protein level ($p < 0.01$). In the KMO inhibitor group, the neural function was efficiently restored, the levels of 3-HK, QA, and KMO in the hippocampus were substantially reduced ($p < 0.01$), and the expression level of 5-HT was increased ($p < 0.01$).

Conclusions: Exercise stimulation combined with enriched environmental stimuli alleviates post-stroke depression in rats, and the underlying mechanisms may be related to TRP/KYN/3-HK/QA excitotoxicity pathways and increased 5-hydroxytryptamine levels.

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Keywords

post-stroke depression; kynurenine pathway; treadmill exercise; rich environmental stimulation

Introduction

With the global increase in aging, the elderly population is gradually growing. Stroke, a common condition among older people, often results in severe complications such as post-stroke depression (PSD) [1]. A study has shown that PSD can affect 20 to 50% of stroke survivors within the first year [2]. Unlike typical depression, PSD is manifested as emotional instability, elevated irritability, reduced interest, pessimism, and fatigue [3]. PSD not only affects the emotional well-being of patients but also significantly delays neurological recovery [4], reduces the quality of life, increases social function deficits [5], and even elevates mortality risk. Therefore, appropriate treatment for PSD is crucial in promoting neurological recovery and improving patient quality of life.

The hippocampus, an important brain region responsible for learning, memory, spatial coordination, and emotional changes, plays a crucial role in the mechanisms that trigger depression after a stroke [6]. During a stroke, neuronal damage occurs due to disrupted blood supply to brain tissue, and the hippocampus can be directly affected or indirectly damaged through inflammatory responses, neurotransmitter imbalances, and altered neuroplasticity [7]. The activation of the kynurenine pathway by inflammatory response may lead to the accumulation of neurotoxic metabolites, further damaging the hippocampus, thereby affecting neurological function and resulting in the development of PSD [8]. Effective management of PSD requires a combination of therapeutic approaches, and inadequate nonpharmacologic interventions have been associated with poor recovery, increased stroke recurrence, and higher mortality rates in these patients [9]. Treadmill exercise, a widely studied form of aerobic activity, has been proven to improve cognitive function and mood [10]. It positively affects the brain by promoting blood circulation, enhancing neuroplasticity, reducing inflammatory responses and other mechanisms [11].

Additionally, rich environmental stimulation, which provides various physical, social, and cognitive challenges, stimulates brain potential, supporting nerve regeneration and functional recovery [12]. In theory, combining these two approaches can more effectively promote physical and mental recovery after a stroke. This study aimed to investigate the effect of treadmill exercise combined with rich environmental stimulation on reducing depressive symptoms in a rat model of post-stroke depression. Additionally, this study sought to elucidate the underlying biological mechanism, especially by regulating the kynurenine pathway. The goal is to provide a new perspective for understanding the pathogenesis of PSD and offer a scientific basis for developing innovative and effective recovery strategies, ultimately advancing clinical practice.

Materials and Methods

Grouping of Experimental Rats

Healthy male sprague-dawley (SD) rats (n = 88), aged 8 weeks and weighing 230 ± 20 g, were obtained from the GLP Laboratory Center of Heilongjiang University of Traditional Chinese Medicine (SCXK(hei)2019-003). The rats were housed for one week with free access to food and water. Housing conditions included a clean-grade open animal room with suitable temperature (22–24 °C) and humidity (50–60%), natural lighting, and a 12-hour light and dark cycle (12L:12D).

The experiment was conducted in two phases. In the first experiment (n = 40), rats were divided into 5 groups: the control group (Control), which received sham operation; the model group (M), which was established as a model of post-stroke depression; the treadmill exercise group (Exe) and rich environmental stimulus group (RES), which received exercise and environmental stimulation, respectively, on the basis of M group; and the combination group (Com), which underwent both types of stimulation. In the second experiment (n = 48), the rats were divided into 6 groups, with the inhibitor group (Com+UPF-648) receiving the kynurenine-3-monooxygenase (KMO) inhibitor on the basis of the Com group, while the other groups were the same as the first experiment. The experimental design was approved by the Ethics Committee of Heilongjiang University of Chinese Medicine (approval number: 2023122934).

Establishment of a Stroke Rat Model

To establish the middle cerebral artery embolus occlusion (MCAO) model [13], the rats were fasted for 2 hours before the procedure and anesthetized with isoflurane (3% for induction and 1.5% for maintenance). A middle incision was created on the neck to expose and separate the right common carotid artery, external carotid artery, and internal carotid artery under an operating microscope. After this, micro-arterial clips were utilized to stop blood flow at the bifurcation of the inner and outer carotid arteries. Then, a V-

shaped incision was created in the common carotid artery, 3–5 mm from the bifurcation, using a 2 mL syringe needle. Meanwhile, a pre-prepared fishing line spigot (0.26 mm in diameter, 3 cm in length, with a 2 mm waxing coating) was inserted into the vessel and secured in a live knot. Then, the arterial clip was removed, and the spigot was advanced 18–22 mm from the bifurcation to reach the beginning of the middle cerebral artery, thereby blocking the blood flow. The flow blockage was confirmed using ultrasound Doppler. Finally, the fixation bolus was secured with a dead knot, the site was thoroughly sterilized, and the incision was sutured layer by layer.

Neurological Deficit Score

One day post-surgery, the neurological function of the rats was assessed using the Longa scoring method [14]: a score of 0 indicated no signs of neurological injury; 1 indicated incomplete extension of the contralateral forepaw when the tail was lifted off the ground; 2 indicated reduced resistance in the right forelimb, rightward walking, and circling to the right; 3 indicated leaning to the right and inability to walk; and 4 indicated loss of consciousness and the inability to walk spontaneously.

Establishment of PSD Rat Model

One day post-surgery, a composite model of PSD was established by combining the chronic unpredictable mild stress (CUMS) model [15] with the solitary rearing method. Each day, one of seven stimuli was randomly selected: 24-hour fasting, 24-hour water deprivation, 24-hour damp bedding, tilting the rat cage at a 45-degree angle, shaking the rat cage for 30 minutes per session, black-and-white inversion (black from 7:00 to 19:00 and white from 19:00 to 7:00 the next day), and 20 minutes of ultrasonic stimulation. The same stimulus was not repeated within one week. The CUMS method was applied for four consecutive weeks. Additionally, the rats were subjected to spatial behavioral restriction in a modified, well-ventilated individual cage from 9:00 to 11:00 daily. Cotton was used to block the back end of the cage, and a tail clip was placed on each rat to ensure immobility during this time.

Exclusion criteria included extreme physical weakness, resistance or inability to run on a treadmill, lack of statistically significant differences between pre- and post-CUMS outcomes in two or more behavioral tests, and death.

Depressive-like Behavioral Performance

The rats underwent the following behavioral performance assessment procedures:

Sucrose preference test: On the first day, rats were given two bottles of 1% sucrose solution. On the second day, one bottle of 1% sucrose solution was replaced with pure water. On the third day, water and food were withheld. On the fourth day, each cage was supplied with one bottle of 1% sucrose solution and one bottle of purified water, each containing 100 mL. The positions of the 2 bottles of water were changed in the middle of the day. After 24 hours, the sucrose solution consumed was calculated and recorded. The source preference was assessed by the proportion of sucrose solution consumed to the total liquid consumed.

Open field test [16]: Rats were placed in the center of an open box measuring 50 cm × 50 cm × 40 cm, with the floor evenly divided into 25 small compartments (10 cm × 10 cm). Their horizontal and vertical activities were recorded within 5 minutes. Horizontal activity primarily reflected the activity level of the rats, while vertical activity indicated their curiosity towards the new environment.

Forced swimming test: Rats were placed in a container with a diameter of 10 cm and a water depth of about 12 cm, with the water temperature between 20–25 °C. When each rat was tested, the height of the water surface was determined by the fact that the rat's hind paws did not touch the ground and its head was just exposed to the water. Video recordings were taken 6 minutes after the rats were immersed in the water, and the immobility time was analyzed for 4 minutes.

Treadmill Exercise Training Methods

Rats were given treadmill training [17] on the basis of the model group. The training intensity commenced at level 1 during the first week and was gradually increased each week. Treadmill running was performed 5 days a week for 6 weeks. Treadmill running intensity was determined based on the principle of overload (Table 1).

Environmental Stimulation Enrichment Methods

As shown in Fig. 1, the environmental enrichment [18] cages were constructed as Plexiglas boxes measuring 70 cm in length, 60 cm in width, and 50 cm in height, with a partition separating the box in the middle. Each side was equipped with tubes and movement-related items, such as

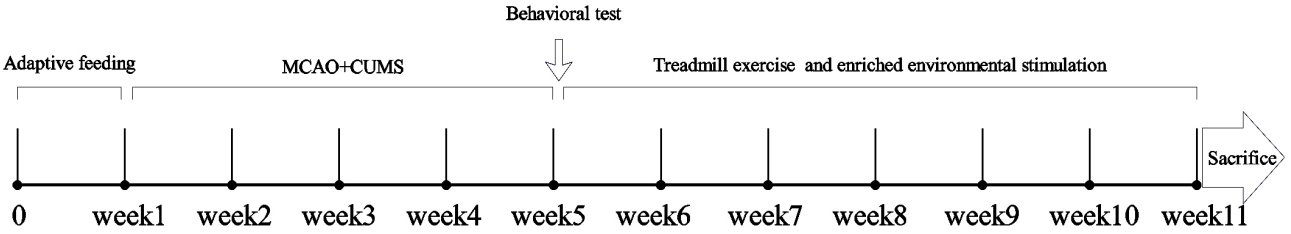

Fig. 1. A flowchart of the environmental enrichment approach. MCAO, middle cerebral artery embolus occlusion; CUMS, chronic unpredictable mild stress.

Table 1. Treadmill exercise intensity in rats.

Intensity level	Training intensity
1	16–20 min, 21 m/min
2	21–25 min, 22 m/min
3	26–30 min, 23 m/min
4	31–35 min, 24 m/min
5	36–40 min, 25 m/min
6	41–45 min, 26 m/min

an autonomous running wheel and a climbing ladder, allowing the rats to explore. The items in the box were rearranged daily, replacing some items to maintain novelty. This intervention was conducted once a day, 5 days a week, for 6 weeks.

Enzyme Linked Immunosorbent Assay (ELISA)

After anesthetizing with pentobarbital sodium (150 mg/kg), the rats were sacrificed, and the hippocampus was isolated. It was rinsed with normal saline at 4 ºC, then homogenized using a homogenizer, followed by centrifugation at 3000 rpm for 15 minutes to obtain the supernatant. The concentration of 5-hydroxytryptamine (5-HT) (cat. H104-1-1, Jiancheng Biotechnology, Nanjing, China) and Norepinephrine (NE) (cat. H096-1-1, Jiancheng Biotechnology, Nanjing, China) in the hippocampus of each rat group was determined using a specific kit. The optical density (OD) was then assessed at 450 nm employing an ELISA plate reader (Synergy LX, Bio Tek, Winooski, VT, USA).

Hematoxylin-Eosin (HE) Staining

The hippocampal tissue, after isolation, was fixed in 4% paraformaldehyde for 24 hours, dehydrated, and embedded in paraffin. The tissue was then sliced into 4 µm sections using a paraffin microtome (Leica RM2245, Witzler, Hessen, Germany) and subsequently dried at 37 °C for 48 hours. After this, these sections underwent dewaxing using xylene and ethanol at different concentrations. The slices were stained with hematoxylin (cat. C0105S, Beyotime, Nanjing, China) for 5 minutes, washed with water, differentiated with 1% hydrochloric acid ethanol, and then stained with eosin for 5 minutes. The slices were dehydrated in 85% and 95% graded alcohol for 5 minutes each and sealed with neutral balsam. Finally, histopathological changes were observed using a light microscope (Eclipse E100, Nikon, Tokyo, Japan).

Nissl Staining

The hippocampus, after isolation, was trimmed with a scalpel and placed in a dehydration box, where it was dehydrated with gradient alcohol. After this, the tissues were embedded using an embedding machine and sliced into 4 µm thick section with a paraffin microtome. The sections were dewaxed and stained with toluidine blue solution (cat. G1220, Solarbio, Beijing, China). In the next step, they were soaked in 70% alcohol for 1 minute and underwent differentiation with 95% alcohol. Nissl bodies were observed under a microscope (Eclipse E100, Nikon, Japan). Finally, the tissue sections were dehydrated with anhydrous alcohol and sealed using neutral gum.

Western Blot Analysis

The hippocampus tissue was lysed in RIPA lysate (cat. P0013B, Beyotime, Nanjing, China) containing 1% PMSF (cat. CW2200, CWBioTech, Beijing, China) for 10 minutes. Total proteins were quantified using a bicinchoninic acid (BCA) protein quantitative kit (cat. A55861, ThermoFisher, Waltham, MA, USA). The proteins were resolved by SDS-PAGE and transferred to a PVDF membrane (cat. IPVH00010, Millipore, Bedford, MA, USA). After this, the membranes were incubated overnight at 4 °C with corresponding primary antibodies, such as anti-5-HT (1:1000, cat.ab13898, Abcam, Shanghai, China), anti-KMO (1:1000, cat.ab167274, Abcam, Shanghai, China), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5000, cat.20536-1-AP, Proteintech,

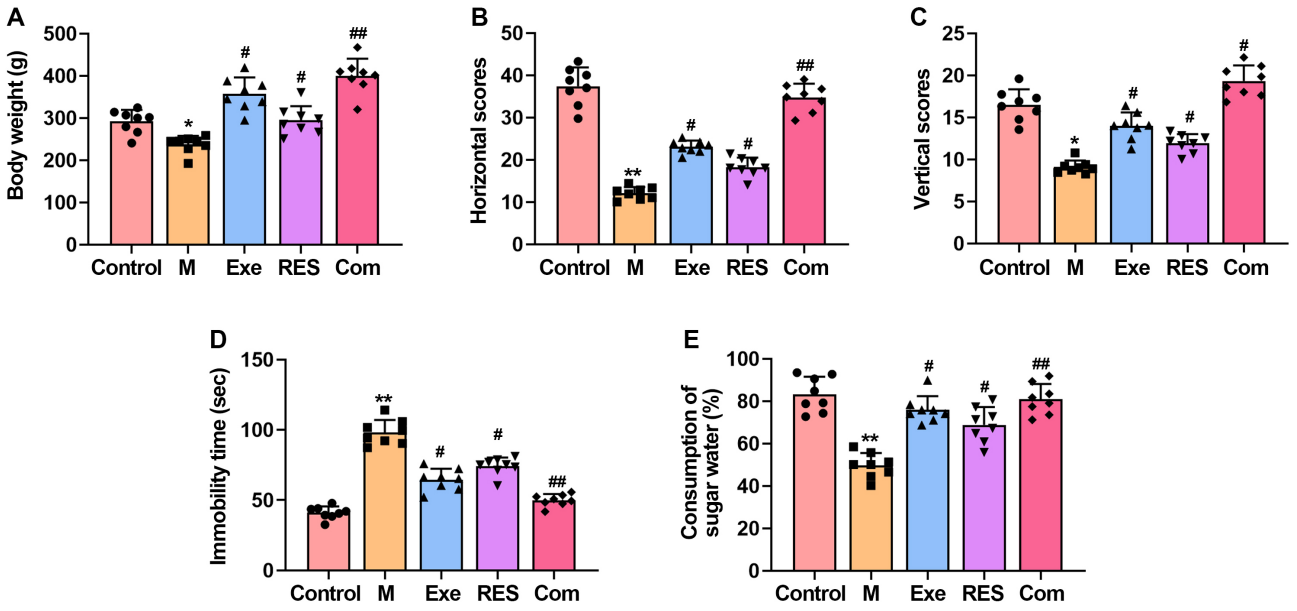

Fig. 2. Changes in body weight and neurological behaviors in rats. (A) Rat body weight (n = 8). (B) Horizontal scores in open field test (n = 8). (C) Vertical scores in open field test (n = 8). (D) Duration of immobility in forced swim test (n = 8). (E) Sucrose water preference test of each group (n = 8). * means $p < 0.05$ compared to the control group, ** means $p < 0.01$ compared to the control group; $^{\#}$ means $p < 0.05$ compared to the model group, and $^{\#\#}$ means $p < 0.01$ compared to the model group. M, model group; Exe, exercise group; RES, rich environmental stimulus group; Com, combination group.

Wuhan, China). The following day, the membrane underwent incubation with secondary antibodies, anti-rabbit (1:10,000, cat. CSA2115, Cohesion, Suzhou, China) or anti-mouse (1:10,000, cat. CSA2108, Cohesion, Suzhou, China), for immune reaction and 3,3′-diaminobenzidine (DAB) staining (cat. P0202, Beyotime, Nanjing, China). After drying, the membranes were photographed and analyzed using ImageJ (version 1.53k, NIH, Bethesda, MD, USA).

Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS)

The plasma was treated with precipitant 3 times to precipitate the protein, followed by centrifugation at 10,000 rpm for 10 minutes. A 100 µL of resultant supernatant was injected into 5 µL of liquid mass for LC-MS/MS analysis (Orbitrap-LTQ XL™ hybrid mass spectrometer, Thermo, Waltham, MA, USA). The analysis used a Thermo Syncronis C18 column (1.9 µm, 2.1 × 100 mm) with the following liquid phase conditions: (A) water containing 0.1% formic acid and 2 mmoL/L ammonium formate and (D) acetonitrile. Gradient elution was conducted with an analysis time of 0–8 minutes, an injection volume of 5 µL, and a flow rate of 0.25 mL/min. The electrospray ionization source was set to ESI (+) with a spray voltage of 2800 V, an evaporation temperature of 350 °C, sheath gas at 35 Arb, auxiliary gas at 10 Arb, and a capillary temperature of 320 °C. Data were obtained in secondary data-dependent scanning (Targeted-SIM) mode. The S-lensRF was set at 50, and the system was prepared for analysis.

Statistical Analysis

Statistical analysis was performed using SPSS18.0 (version 18.0; IBM, Armonk, NY, USA), while GraphPad Prism 7.0 was used for image processing (GraphPad Software Inc, San Diego, CA, USA). Data were expressed as mean ± standard deviation. For multiple group comparison, one-way-ANOVA was used, followed by least significant difference (LSD) test for post hoc. A comparison between the two groups was assessed employing a paired *t*-test. Statistical significance was achieved at a p-value < 0.05.

Results

Therapeutic Effect of Treadmill Exercise Combined with Rich Environmental Stimulation in PSD Rats

We developed a PSD rat model, and used the Longa score to assess neurological deficits in each group. As

Table 2. Neurological deficit scoring in different rat groups.

Group	n	Before treatment	After treatment	*t*-value	*p*-value
Control	8	0	0		
M	8	3.12 ± 0.18	3.09 ± 0.06	0.447	0.661
Exe	8	3.06 ± 0.16	2.82 ± 0.18*	2.81	0.013
RES	8	3.04 ± 0.17	2.87 ± 0.09*	2.5	0.025
Com	8	3.08 ± 0.08	1.93 ± 0.13**	21.3	<0.01

* means $p < 0.05$ compared to pre-treatment group, ** means $p < 0.01$ compared to pre-treatment group. M, model group; Exe, exercise group; RES, rich environmental stimulus group; Com, combination group.

shown in Table 2, all rats showed nerve damage before the intervention. However, after the intervention, the scale of the stimulation group was significantly reduced ($p < 0.01$), and the symptom of nerve damage was significantly alleviated. Subsequently, the body weight of rats in each group was compared. The body weight of rats in the model group decreased significantly ($p < 0.05$), compared to the model group, the body weight of rats in the Exe and RES groups increased significantly ($p < 0.05$), and the body weight of rats in the Com group increased significantly ($p < 0.01$, Fig. 2A).

Neurobehavioral experiments were conducted to evaluate the depressive state of the rats. The results of open field test (OFT) demonstrated a reduction in the vertical activity score ($p < 0.05$) and a substantial decrease in the horizontal activity score in the model group ($p < 0.01$). Compared to the model group, the rats in the Exe and RES groups exhibited an elevation in the horizontal activity score ($p < 0.05$), while the Com group showed a highly significant increase ($p < 0.01$). Similarly, the vertical activity score for the Exe, RES, and Com groups increased ($p < 0.05$) (Fig. 2B,C). The results of forced swimming test (FST) indicated that the immobilization time was significantly prolonged in the model group ($p < 0.01$). However, compared to the model group, the immobilization time was significantly reduced in the Exe and RES groups ($p < 0.05$), and the immobilization time of rats in the Com group was significantly reduced ($p < 0.01$, Fig. 2D). The sucrose preference test (SPT) demonstrated a substantial reduction in the proportion of sucrose water consumption in the model group ($p < 0.01$). Compared to the model group, the proportion of sucrose water consumption was elevated in the Exe and RES groups ($p < 0.05$) and highly elevated in the Com group ($p < 0.01$, Fig. 2E).

These results indicate the successful development of the post-stroke depression rat model and reveal that exercise and environmental stimulation can significantly reduce depression symptoms in rats, with the combined stimulation indicating the most effective outcomes.

Impact of Treadmill Exercise Combined with Rich Environmental Stimulation on the Morphology of Hippocampal Neurons in PSD Rats

The brain tissue was collected and weighed, indicating a substantial decrease in brain tissue weight in the model group ($p < 0.05$). Compared to the model group, the brain tissue weight was significantly elevated in the Com group ($p < 0.05$, Fig. 3A). Nissl staining results showed a substantially reduced number of Nissl bodies in the model group ($p < 0.01$), with lighter staining observed. However, the number of Nissl bodies was significantly higher in the Exe, RES, and Com groups ($p < 0.05$, Fig. 3B).

HE staining indicated the following observations: In the control group, the cell arrangement was dense, and the nucleolar membrane was clear. In the model group, the number of hippocampal neurons was decreased, the arrangement was loose, the nuclei of neurons were shrunk and vacuolated, and the hyperchromic cells were increased. In the Exe and RES groups, the number of neurons was increased, with a reduction in nuclear pyknosis and deep staining. The number of neurons in the Com group was comparable to that in the control group, and the cell gap was shortened (Fig. 3C).

Treadmill Exercise Combined with Rich Environmental Stimulation Improves PSD in Rats by Regulating Tryptophan (TRP) Metabolism

ELISA results indicated that the 5-HT content in the hippocampus of rats in the model group was significantly reduced ($p < 0.01$). Compared to the model group, the 5-HT content in the hippocampus of rats in the Exe and RES groups was increased ($p < 0.05$), with a substantial increase observed in the Com group ($p < 0.01$, Fig. 4A). NE content in the hippocampus was reduced in the model group ($p < 0.05$), while increased in the Com group ($p < 0.05$, Fig. 4B).

Western blot analysis indicated that the expression level of KMO protein was significantly increased in the

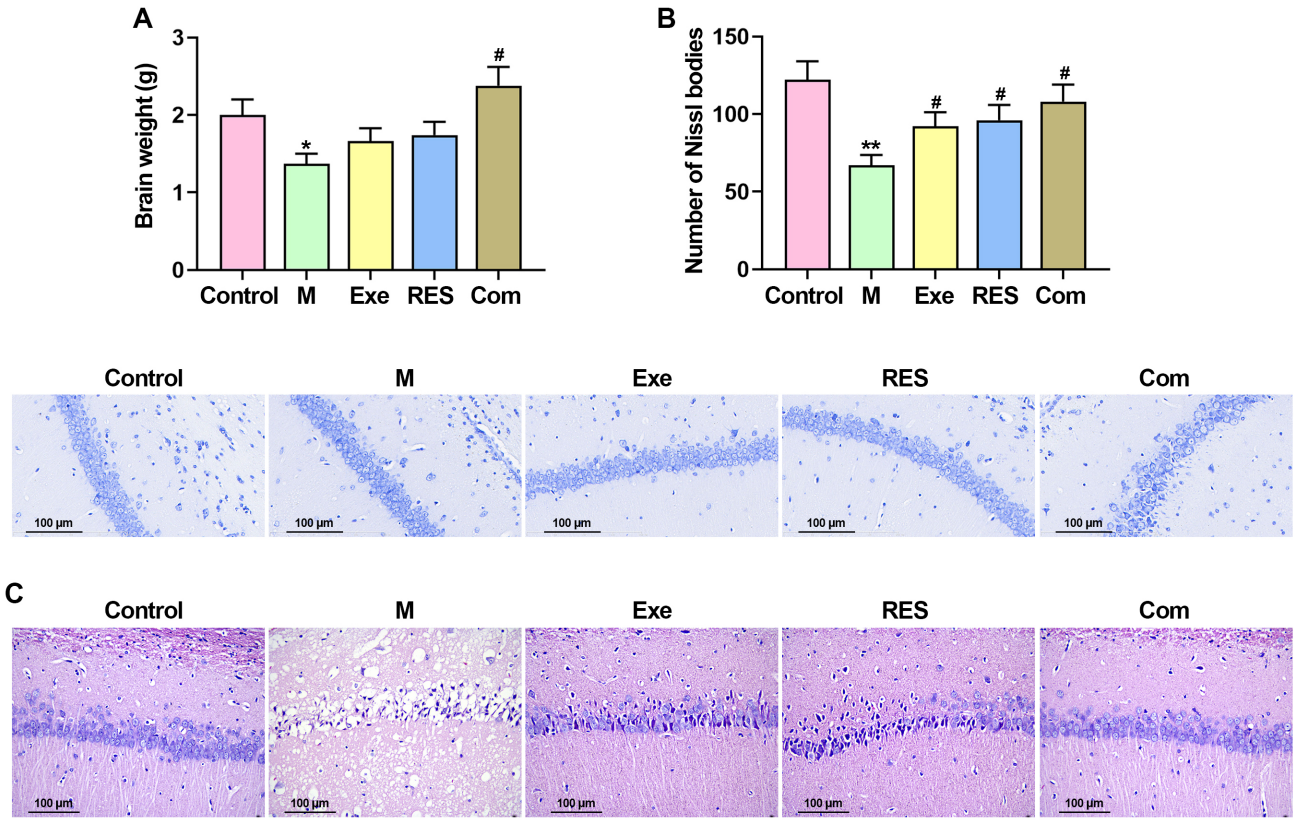

Fig. 3. Morphological changes in hippocampus tissues of rats. (A) Rat brain weight (n = 8). (B) Nissl staining to detect hippocampal neuron morphology and number. (C) Hematoxylin-eosin (HE) staining to detect pathological damage in hippocampus tissues. * means $p < 0.05$ compared to the control group, ** means $p < 0.01$ compared to the control group; # means $p < 0.05$ compared to the model group.

model group ($p < 0.01$), while the expression level of 5-HT protein was significantly decreased ($p < 0.01$). The expression of KMO in the Exe, RES, and Com groups was significantly alleviated ($p < 0.05$), and the level of 5-HT was significantly elevated in the Com group ($p < 0.01$, Fig. 4C).

These findings indicate that exercise stimulation combined with rich environmental stimulation affected the function of hippocampus neurons in PSD rats, and increased the expression level of 5-HT by stimulating KMO level.

Treadmill Exercise Combined with Rich Environmental Stimulation Improves PSD in Rats Possibly Related to the Kynurenine Pathway

Rats in the Com group were treated with 0.1 mM KMO inhibitor at a dose of 5 μL/d, injected every other day for 6 weeks. The OFT results revealed that the vertical and horizontal scores in the KMO inhibitor group were significantly higher than those in the model group ($p < 0.01$, Fig. 5A,B). Similarly, the SPT results showed that the proportion of sucrose water consumption significantly increased in the KMO inhibitor group compared to the model group ($p < 0.01$, Fig. 5C). Moreover, the FST results demonstrated that compared to the model group, the immobility time of rats in the KMO inhibitor group was significantly reduced ($p < 0.01$, Fig. 5D).

The TRP, 5-HT, kynurenine (KYN), 3-hydroxykynurenine (3-HK), and quinolinic acid (QA) levels in the hippocampus of rats were determined using LC-MS/MS. Compared to the model group, the levels of TRP and 5-HT were significantly increased in the hippocampus of the KMO inhibitor group ($p < 0.05$, Fig. 6A,B). Additionally, the levels of 3-HK, QA, and KYN were substantially reduced in the KMO inhibitor group compared to the model group ($p < 0.01$, Fig. 6C–E). Western blot analysis demonstrated significantly increased 5-HT expression level ($p < 0.01$) and decreased KMO expression level in the KMO inhibitor group ($p < 0.01$, Fig. 6F).

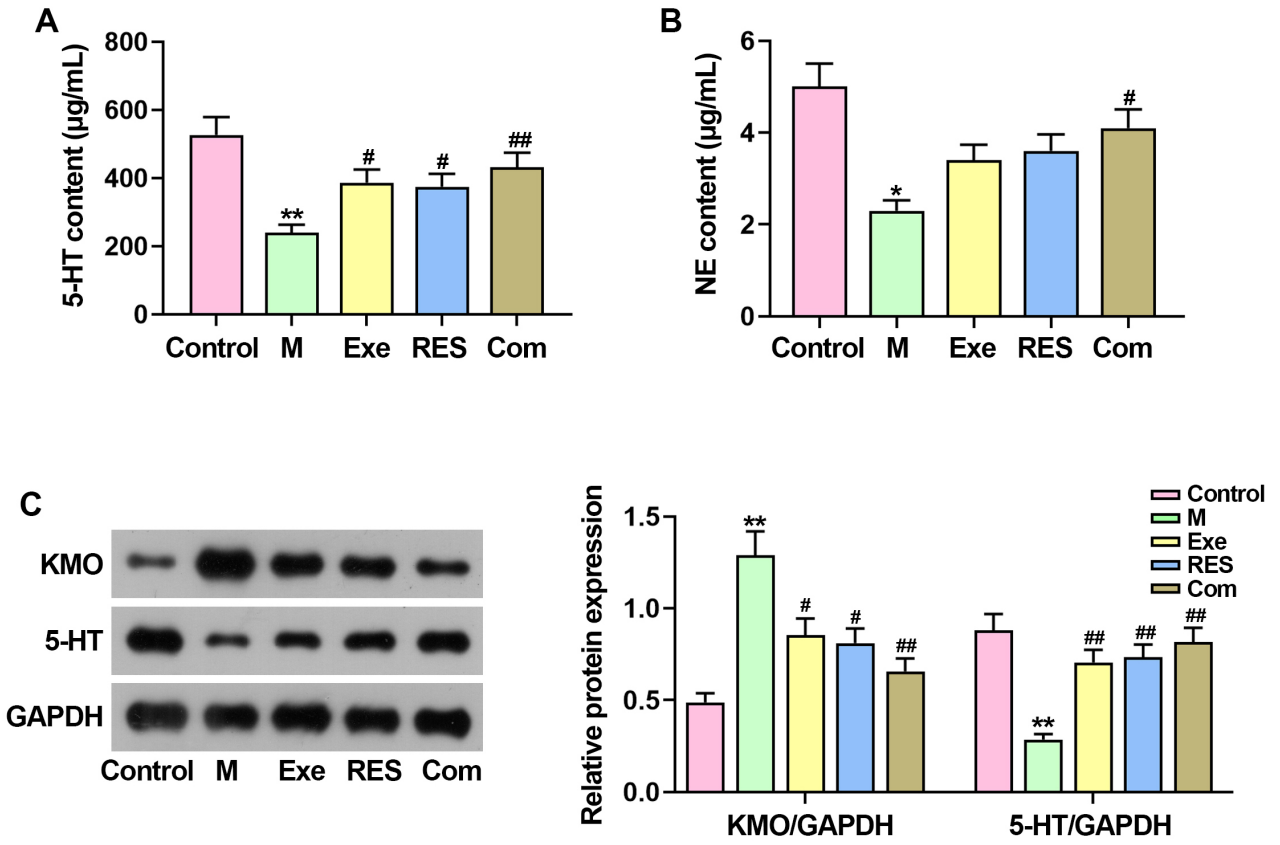

Fig. 4. Tryptophan (TRP) metabolite content in the hippocampus tissue of rats. (A) Enzyme linked immunosorbent assay (ELISA) results for 5-hydroxytryptamine (5-HT) (n = 8). (B) ELISA results for Norepinephrine (NE) (n = 8). (C) Western blot (WB) analysis results for 5-HT and kynurenine-3-monooxygenase (KMO) (n = 8). * means $p < 0.05$ compared to the control group, ** means $p < 0.01$ compared to the control group; # means $p < 0.05$ compared to the model group, and ## means $p < 0.01$ compared to the model group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

The above results represent that exercise stimulation combined with enriched environmental stimuli alleviates post-stroke depression in rats, and the underlying mechanisms may be related to TRP/KYN/3-HK/QA excitotoxicity pathways and increased 5-hydroxytryptamine levels.

Discussion

Post-stroke depression is a common complication after a stroke, resulting in substantial physiological and psychological challenges for patients [19]. According to Cai's study [20], 49.4% of stroke patients in China experience depressive symptoms, indicating that Chinese stroke survivors are more likely to develop PSD. While traditional treatments, such as antidepressants and psychological interventions, can be effective, there remain challenges like inconsistent treatment response rates and significant side effects [21,22]. Therefore, seeking for safer and more effective treatment has become a focus of current medical research.

In recent years, research has focused on the role of non-pharmacological treatments in improving PSD, especially lifestyle modifications such as exercise and environmental interventions [23]. A study has shown that non-pharmacological therapies can promote physiological and biochemical neural changes, reduce immune inflammatory responses, and decrease excitatory amino acid toxicity [15]. In this study, PSD was successfully modeled in rats using middle cerebral artery occlusion combined with chronic unpredictable middle stress [24]. Rats in the PSD group showed significant weight loss, likely due to a reduction in appetite-associated depressive symptoms [25]. Behavioral analyses demonstrated that PSD rats' reduced desire for vertical and horizontal exploration in the OFT, reduced sucrose consumption in the SPT, and behavioral immobility in the FST were interpreted as indicators of despair [26]. However, our study found that both exercise and rich environmental stimuli increased the anhedonic effect of PSD, increased the desire for exploration, and alleviated behavioral despair. These observations suggest that com-

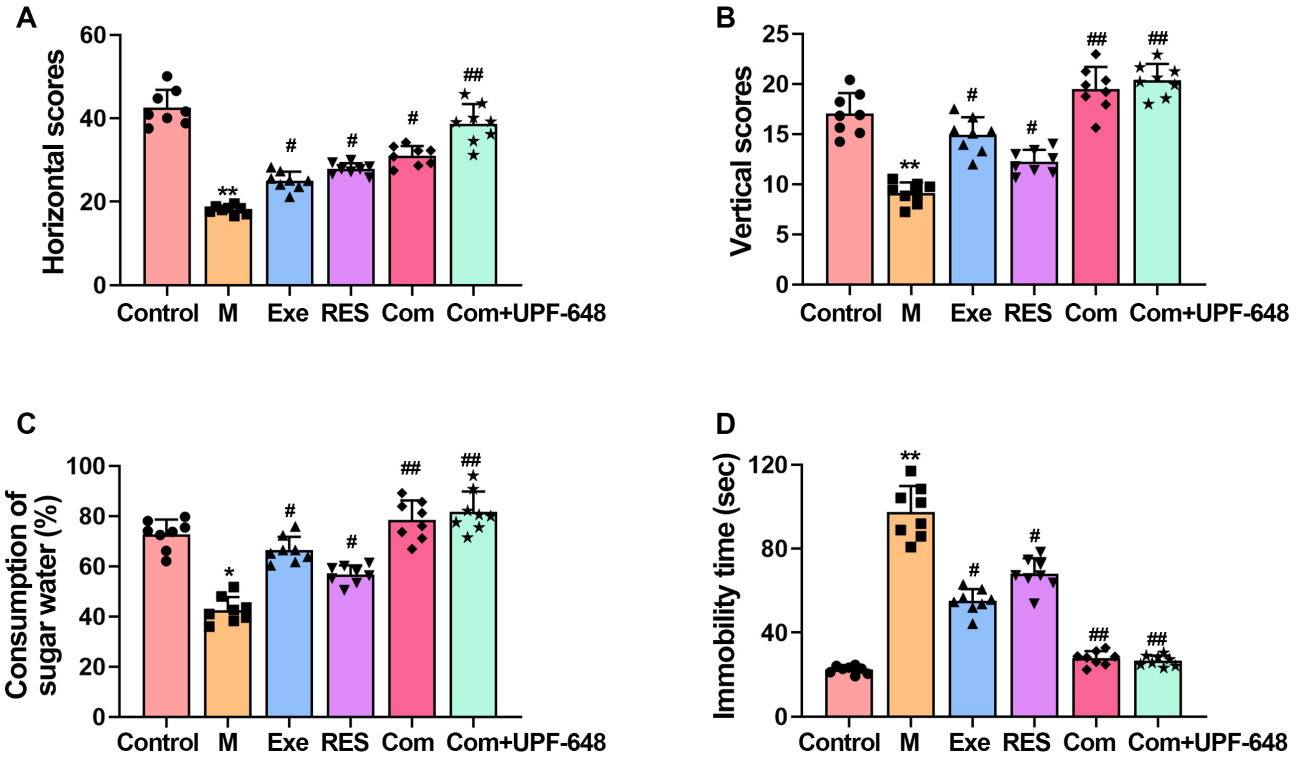

Fig. 5. Changes in the neurological behaviors of rats. (A) Horizontal scores in open field test (n = 8). (B) Vertical scores in open field test (n = 8). (C) Sucrose water preference test results (n = 8). (D) Forced swim test results (n = 8). * means $p < 0.05$ compared to the control group, ** means $p < 0.01$ compared to the control group; # means $p < 0.05$ compared to the model group, and ## means $p < 0.01$ compared to the model group.

bining exercise and rich environmental stimuli can help alleviate depressive-like symptoms. Subsequently, we performed Nissl staining and HE staining of the hippocampus and found that exercise and rich environmental stimulation increased the number of hippocampal neurons and reduced neuronal damage, indicating that the relief effect of exercise combined with rich environmental stimulation on depressive-like symptoms was closely related to the hippocampus.

Studies have shown that inflammation increases after stroke [27], pro-inflammatory cytokines interact with 5-HT, leading to 5-HT depletion [28]. On the other hand, it leads to the expansion of the inflammatory process and the activation of indoleamino-2, 3-dioxygenase (IDO) in the marginal region, which makes TRP to be metabolized through the kynurenine pathway and further converted into Kyn, 3-HK, QA and other substances, reducing the concentration of 5-HT [29]. These metabolites, especially QA, possess neurotoxic properties that cause neuronal damage and apoptosis, thereby exacerbating psychiatric symptoms such as depression and anxiety [30]. Our study showed that exercise combined with rich environmental stimulation significantly increased the levels of 5-HT and NE while decreasing the KMO in the hippocampus. This suggests the possibility that exercise, in combination with rich environmental stimuli, can exert antidepressant effects by inhibiting kynurenine pathways. Therefore, to further explore, KMO inhibitors were administered to the combined stimulation group. Behavioral tests showed that KMO inhibitors further reduced depressive symptoms of the rats. The study further analyzed the levels of TRP, 5-HT, KYN, 3-HK, and QA in the hippocampus using LC-MS/MS technology. The results showed that KMO inhibitor significantly reduced the levels of 3-HK, QA and KYN, confirming that the antidepressant effect of exercise combined with rich environmental stimulation may be related to affecting the kynurenine pathway.

As a comprehensive intervention, treadmill exercise combined with rich environmental stimuli has the potential to effectively ameliorate depressive symptoms in PSD rats by modulating the kynurenine pathway. This finding enhances our understanding of the pathophysiological mechanism underlying PSD and provides a scientific basis for developing novel and comprehensive rehabilitation strategies. However, the limitation of this study is due to the in-

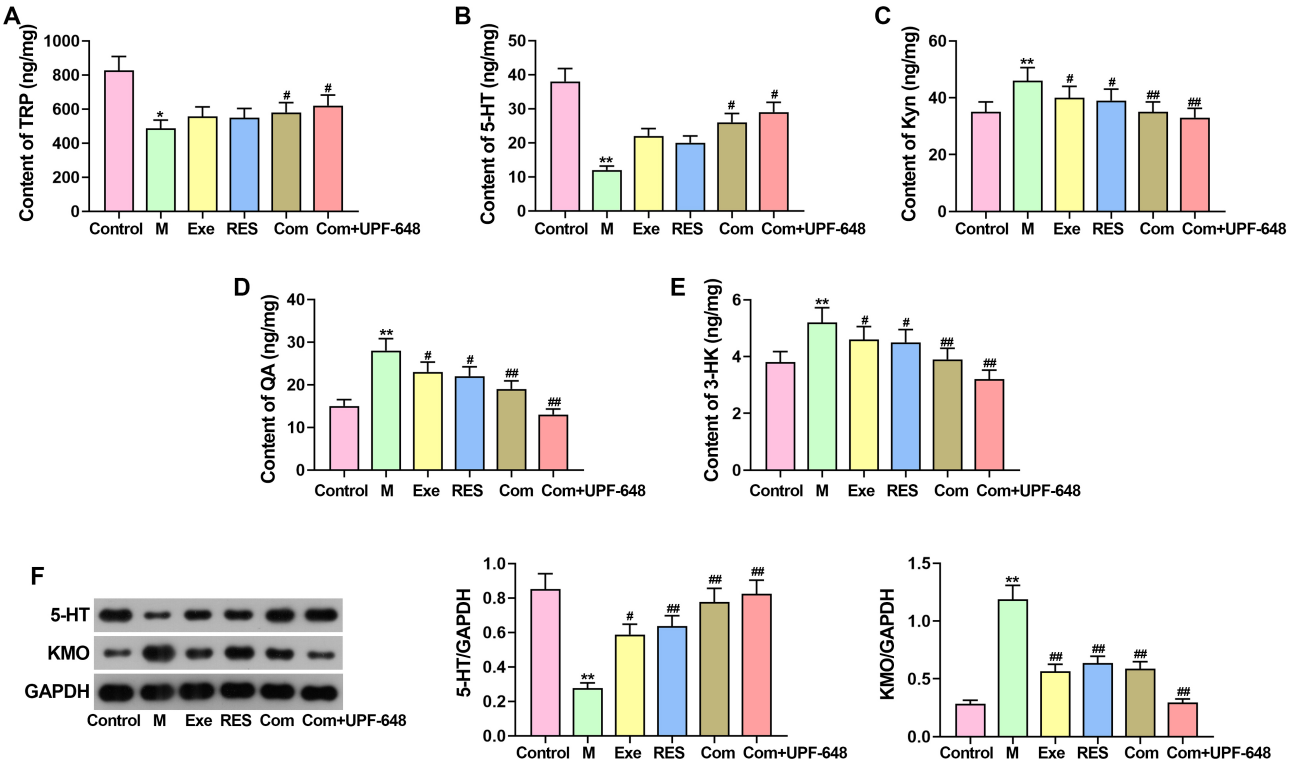

Fig. 6. Kynurenine pathway metabolite content in hippocampus tissues of rats. (A) TRP content in the hippocampus (n = 8). (B) 5-HT content in the hippocampus (n = 8). (C) Kyn content in the hippocampus (n = 8). (D) Quinolinic acid (QA) content in the hippocampus (n = 8). (E) 3-hydroxy-kynurenine (3-HK) content in the hippocampus (n = 8). (F) WB analysis of 5-HT and KMO (n = 8). * means $p < 0.05$ compared to the control group, ** means $p < 0.01$ compared to the control group; # means $p < 0.05$ compared to the model group, and ## means $p < 0.01$ compared to the model group.

herent differences between rats and humans, as well as the constraints in rat-based studies. Further research is needed to optimize these interventions and determine their applicability in human patients, ultimately aiding clinical practice.

Conclusions

Treadmill exercise combined with enriched environmental stimuli alleviates post-stroke depressive symptoms in rats, possibly by a mechanism that inhibits the kynurenine pathway and increases 5-hydroxytryptamine levels.

Availability of Data and Materials

The data analyzed in this study are available on request from the corresponding author.

Author Contributions

LNL designed the research study. WTX, LF and XYT performed the research. WGB, YMX and XLX collected and analyzed the data and made the manuscript preparation. WZ and BLL provided help and advice on the experiments. LNL analyzed the data and made the first draft. All authors contributed to important editorial changes in the manuscript. All authors have reviewed and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental design has been approved by the Ethics Committee of Heilongjiang University of Chinese Medicine with the approval number (2023122934).

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Conflict of Interest

The authors declare no conflict of interest.

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