



OPEN Enriched environment alleviates NLRP3 inflammasome mediated neuroinflammation in diabetes complicated with depression rats

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Depression is a serious and common complication of diabetes, with a well-established link between the two conditions. Recent studies indicate that activation of the NLRP3 inflammasome in hippocampal microglia plays a key role in the pathogenesis of diabetes complicated with depression (DD). While environmental enrichment (EE) has demonstrated significant anti-neuroinflammatory effects, its potential to alleviate neuroinflammation specifically induced by DD remains unclear. The DD rat model was established using a combination of a high-fat diet, streptozotocin injection, and chronic unpredictable mild stress. The effects of EE on NLRP3 inflammasome activation in the hippocampal microglia of DD rats were examined through a series of behavioral tests (open field test, forced swim test, and elevated plus maze), along with enzyme-linked immunosorbent assays, hematoxylin and eosin staining, TdT-mediated dUTP nick-end labeling staining, Western blot analysis, and immunofluorescence. Compared to the control group, DD rats exhibited impaired glucose metabolism and increased depressive-like behaviors, alongside hippocampal neuronal damage and elevated apoptosis rates. Activation of the NLRP3 inflammasome in hippocampal microglia was observed, with upregulation of the NLRP3 signaling pathway and inhibition of the PI3K/AKT signaling pathway. EE significantly mitigated these effects, reducing hippocampal neuroinflammation and alleviating depressive behaviors in DD rats. Neuroinflammation in the hippocampus is a key mechanism underlying the pathogenesis of DD. This study demonstrated that EE reduces neuroinflammation, likely by inhibiting the activation of the NLRP3 inflammasome in hippocampal microglia.

Keywords Enriched environment, Diabetes mellitus with depression, Neuroinflammation, NLRP3, Hippocampus

Diabetes mellitus is a global public health concern. According to the 2021 Diabetes Atlas published by the International Diabetes Federation, the prevalence of type 2 diabetes among individuals aged 20 to 79 is estimated at 10.50% (536.6 million people), with this figure projected to rise to 12.20% (783.2 million people) by 2045¹. People with diabetes require lifelong medication and strict self-management to prevent complications. Additionally, they frequently experience psychological stress, such as diabetes-related distress, which increases their vulnerability to depression². Studies indicate that the risk of depression in people with diabetes is twice as high as in the general population³. A large systematic review and meta-analysis revealed that the prevalence of diabetes mellitus with depression (DD) is as high as 28% (95% CI 27–29%)⁴. Furthermore, patients with DD face poor prognostic outcomes, with increased risks of dementia⁵ cardiovascular diseases⁶ and mortality⁷. Therefore, the prevention and treatment of diabetes with comorbid depression deserve greater attention in the public health domain.

The hippocampus, a brain region closely associated with learning, memory, and emotional regulation⁸ plays a key role in DD. Microglia in the hippocampus constantly monitor their environment, rapidly responding to neuronal injury, infection, or other pathological stimuli by mediating inflammatory responses⁹. Studies have shown that in DD rats, the expression of microglial chemokine receptor I in the hippocampus is upregulated, along

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with an increase in pro-inflammatory cytokines, particularly tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1 β (IL-1 β)¹⁰. These inflammatory cytokines not only affect the immune system but also cross the blood–brain barrier, directly influencing neurons and neurotransmitter systems. The NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, a multiprotein complex, plays a central role in microglial inflammation and immune regulation. Research has shown that NLRP3 inflammasome activation in hippocampal microglia mediates depressive-like behaviors in streptozotocin (STZ)-induced diabetic mice¹¹. Furthermore, NLRP3 inflammasome activation is linked to several signaling pathways in the hippocampus. Under hyperglycemic conditions, the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is inhibited, weakening the anti-inflammatory response and facilitating NLRP3 inflammasome activation¹². This neuroinflammation in the hippocampus may exacerbate emotional and cognitive dysfunction. Based on this, the study hypothesizes that hippocampal neuroinflammation is a key driver in the pathogenesis of DD, with NLRP3 inflammasome activation playing a critical role, linked to the inhibition of the PI3K/AKT signaling pathway.

Environmental enrichment (EE) is a non-pharmacological intervention that combines inanimate objects and social stimuli to enhance health by improving environmental conditions¹³. EE consists of four basic components: cognitive, sensory, motor, and social stimulation. Cognitive stimulation enhances learning, memory, attention, and spatial perception, while sensory stimulation involves visual, olfactory, auditory, and tactile inputs. Motor stimulation requires space and equipment for physical activity, and social stimulation involves interactions between animals to improve communication¹⁴. Previous studies have shown that EE can reduce neuroinflammatory responses, improving cognitive and emotional regulation in the hippocampus. One study found that EE improved glucose and lipid metabolism in aged mice with metabolic disorders, increased brain-derived neurotrophic factor (BDNF) gene expression in the brain, reduced the production of inflammatory markers, and regulated microglial gene expression and morphology, thus protecting the central nervous system¹⁵. Similarly, another study conducted a 4-week EE intervention in a rat model of depression induced by chronic unpredictable mild stress (CUMS). Results showed that EE reduced peripheral serum levels of IL-1 β , IL-6, and TNF- α , decreased the expression of the hippocampal microglial activation marker Iba-1, inhibited microglial activation, and downregulated NF- κ B expression, ultimately reducing depressive behaviors¹⁶. However, the mechanisms by which EE modulates neuroinflammation in DD remain insufficiently understood. Therefore, this study aims to investigate the effects of EE on the NLRP3 inflammasome in the hippocampus to elucidate the neuroprotective mechanisms of EE in DD and provide new insights into its prevention and treatment.

Materials and methods

All the experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals. The ethics committee of Sichuan Nursing Vocational College approved the study. The study is reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

Reagents and materials

Streptozotocin (STZ, Cat# WXBD7906V) and sodium citrate buffer (0.1 mol/L, pH 4.5, sterile, Cat# PHR1416) were purchased from Sigma (USA). Fluoxetine (Flu, Cat# H20064844) capsules were obtained from China Sinochem Pharmaceutical Co., Ltd. (Suzhou, China), while metformin hydrochloride tablets were sourced from Sino-American Shanghai Squibb Pharmaceuticals Ltd. (Met, Cat# ACB2318). Enzyme-Linked Immunosorbent Assay (ELISA) kits for serotonin (5-HT, Cat# ZC-35959-J), dopamine (DA, Cat# ZC-36551), norepinephrine (NE, Cat# ZC-37121), fasting insulin (FINS, Cat# ZC-36048), Interleukin-1 β (IL-1 β , Cat# ZC-36391W), Interleukin-18 (IL-18, Cat# ZC-36389W), and Interleukin-6 (IL-6, Cat# ZC-36404W) were obtained from ZCIBio (Shanghai, China). The TdT-mediated dUTP Nick-End Labeling (TUNEL) kit was purchased from Roche (Switzerland; Cat# 1168479590). The tubulin β antibody (Cat# T0023) was sourced from Affinity (USA), while antibodies for PI3K (Cat# 20584-1-AP), AKT (Cat# 10176-2-AP), and caspase-1 (Cat# 22915-1-AP) were obtained from Proteintech (USA). The NLRP3 antibody (Cat# 381207) was purchased from Zen-Bio (Chengdu, China), and the Iba1 antibody (Cat# A19776) was sourced from ABclonal (USA).

Animals

This study used 4-week-old specific pathogen-free (SPF) male Sprague–Dawley (SD) rats, weighing 180–220 g, purchased from Dashuo Laboratory Animal Co., Ltd. (Chengdu, China; certificate number: SCXK [Chuan] 2020-030). The rats were housed under controlled conditions with a temperature of 20–25 °C, humidity of 40–60%, and a 12-h light/dark cycle. The experiment was approved by the Ethics Committee of Sichuan Nursing Vocational College (approval number: DW2022001).

Experimental grouping and model establishment

Thirty-two SD rats were acclimated for 3 days before the experiment. After acclimatization, the rats were weighed and ranked by body weight, then randomly divided using a random number table into two groups: 6 rats in the control group and 18 rats in the diabetes-depression (DD) group. The 18 rats in the DD group were fed a high-fat diet (25% lard, 2% cholesterol, 2% salt, 5% sugar, and 66% standard feed) for 4 weeks. After the final feeding, the rats were fasted for 12 h without food or water.

STZ was dissolved in citrate buffer at a concentration of 35 mg/kg in a light-protected environment and immediately injected intraperitoneally into the DD group rats. The control group received an equivalent volume of citrate buffer via intraperitoneal injection. Seventy-two hours post-injection, tail vein blood was collected for 3 consecutive days to measure random blood glucose levels. Diabetes was considered successfully induced if blood glucose levels were ≥ 16.7 mmol/L.

Following the successful induction of diabetes, chronic unpredictable mild stress (CUMS) was applied to induce depression in the rats. The stressors included: (1) Tail clamping for 1 min, (2) Cold-water swimming for 5 min, (3) Cage tilting for 24 h, (4) Reversal of the light/dark cycle, and (5) Restraint for 1 h. These stressors were applied randomly over 3 weeks, with one stressor administered daily, each repeated 4–5 times. Depression was confirmed using the open field test and the forced swimming test, with significant differences ($P < 0.05$) in horizontal and vertical scores in the open field test and immobility time in the forced swimming test compared to the control group.

The 24 successfully modeled DD rats were then randomly divided into three groups based on body weight using a random number table: the DD group, the EE group, and the positive drug treatment group (Met & Flu, Pos), with 8 rats in each group.

Experimental intervention

The intervention lasted 6 weeks. Both the control and DD groups received daily intragastric administration of 10 ml/kg saline. The Pos group received metformin (200 mg/kg) and fluoxetine (5 mg/kg) via intragastric administration, based on clinical dosing guidelines. The EE group continued on a high-fat diet, received saline intragastrically, and was housed in an enriched environment (EE). The EE setup, based on literature reviews, consisted of large transparent cages (90 cm × 48 cm × 55 cm) filled with thick bedding, chew stones, nests, and sensory stimulation toys such as platforms, tunnels, and running wheels, with four rats per cage. Toys were rotated daily, and new toys were introduced weekly to maintain novelty.

Behavioral testing

Behavioral tests were conducted the day after the final intervention. SD rats were acclimated to the testing environment for 30–60 min before testing. Equipment was cleaned with 75% ethanol between tests.

Open field test

The test was conducted in a black-bottomed box (100 cm × 100 cm × 40 cm). The floor was divided into 25 squares. Each rat was placed in the center, and horizontal (squares crossed) and vertical (rearing events) activities were recorded over 5 min using video tracking software.

Forced swimming test

Rats were placed in a cylindrical tank (18 cm diameter, 40 cm height) filled with 23 cm of water at 25 °C. During the 6-min test, immobility time was recorded in the last 4 min, with immobility defined as floating with minimal movements.

Elevated plus maze

The maze had two open arms and two closed arms, with each arm measuring 50 cm in length and 10 cm in width. OT% (time in open arms) and OE% (entries into open arms) were calculated using the formulas:

$$\text{OT}\% = (\text{Time in open arms}) / (\text{Total time in open} + \text{closed arms}) \times 100\%$$

$$\text{OE}\% = (\text{Entries into open arms}) / (\text{Total open} + \text{closed arm entries}) \times 100\%$$

Sample collection

After behavioral testing, SD rats were anesthetized with sodium pentobarbital (1%, 0.5 ml/100 g, intraperitoneal). Abdominal aortic blood (5 ml) was collected, centrifuged at 3500 rpm for 15 min, and serum was stored in cryovials. The hippocampus was dissected on ice post-decapitation, stored in 4% paraformaldehyde or dry cryovials, and frozen at – 80 °C.

ELISA

ELISA was performed following the manufacturer's instructions. Serum concentrations of 5-HT, DA, NE, and FINS, and hippocampal concentrations of IL-1 β , IL-6, and IL-18 were measured. Absorbance at 450 nm was used to calculate the concentrations.

Hematoxylin and Eosin (HE) staining

Hippocampal tissue was dehydrated, paraffin-embedded, and sectioned at 5 μ m. Sections were stained with hematoxylin, differentiated, blued, eosin-stained, and dehydrated. Pathological changes were observed using a digital slide scanner.

TUNEL staining

Sections underwent TUNEL staining to assess apoptosis. After three PBS washes, sections were incubated with the TUNEL reaction mixture for 1 h at 37 °C, washed, and stained with DAPI. Images were captured using a digital slide scanner, and apoptotic cells were analyzed in selected regions at 400 × magnification.

Immunofluorescence

Hippocampal paraffin sections were deparaffinized with xylene, dimethylbenzene, and ethanol, followed by ethanol gradient dehydration and distilled water rinsing. Antigen retrieval was done by microwave heating, then sections were washed with PBS. Blocking was done using 3% hydrogen peroxide and bovine serum. The primary antibody Iba1 (1:200) was applied, followed by overnight incubation at 4 °C. After PBS washes, HRP-labeled secondary antibodies were applied at room temperature, followed by TSA signal amplification (TSA, 1:1000).

A second antigen retrieval was performed, and the process was repeated for NLRP3 (1:200) for double staining. Finally, sections were counterstained with DAPI and mounted using anti-fluorescence quenching medium. Images were scanned, and positive cells were counted at 200 \times magnification using Image Pro Plus software (<https://xrayscan.com/software-image-pro-plus/>).

Western blotting

Hippocampal tissue was homogenized and lysed with RIPA buffer. Protein concentration was determined using a BCA assay, and denatured proteins were separated by SDS-PAGE. Proteins were transferred to a PVDF membrane, blocked in TBST with 5% skim milk, and incubated overnight at 4 °C with primary antibodies against Caspase-1 (1:2000), NLRP3 (1:1000), PI3K (1:1000), AKT (1:2000), and Tubulin β (1:50,000). After washing, the membrane was incubated with secondary antibodies (1:5000) for 2 h at room temperature. Bands were visualized using the ECL method and imaged using a Tanon Fluorescence Imaging System. The integrated optical density (IOD) of target proteins was calculated using Gel-Pro Analyzer 4 software (<https://gel-pro-analyzer.software.informer.com/>) to determine relative expression levels.

Statistical analysis

Statistical analysis was performed using SPSS 29.0 software (IBM Corp., Armonk, NY, USA, <https://www.ibm.com/products/spss-statistics>), with significance set at $P < 0.05$. Normally distributed data were expressed as means \pm standard deviation, while non-normally distributed data were expressed as medians and interquartile ranges. One-way ANOVA with LSD post hoc tests was used for comparisons of normally distributed data, and the Kruskal–Wallis H test was used for non-normally distributed data. Generalized estimating equations were applied for repeated measures data to assess group, time, and interaction effects.

Results

Effects of environmental enrichment on blood glucose and FINS in DD rats

This study investigated the effects of EE on random blood glucose levels from the tail vein and serum fasting insulin (FINS) in DD rats. The experimental timeline is shown in Fig. 1A, and the real-life setup of the enriched environment is shown in Fig. 1B. After six weeks of intervention, random blood glucose levels showed statistically significant differences between the groups ($F = 68.846$, $P < 0.001$, Fig. 1D). The DD group exhibited a significant increase in blood glucose levels compared to the control group ($P < 0.001$). In contrast, both the EE and Pos groups showed a significant reduction in blood glucose levels compared to the model group ($P < 0.001$). Additionally, serum FINS levels showed statistically significant differences between the four groups ($F = 14.225$, $P < 0.001$, Fig. 1C). The DD group had higher serum FINS levels compared to the control group ($P < 0.001$). In

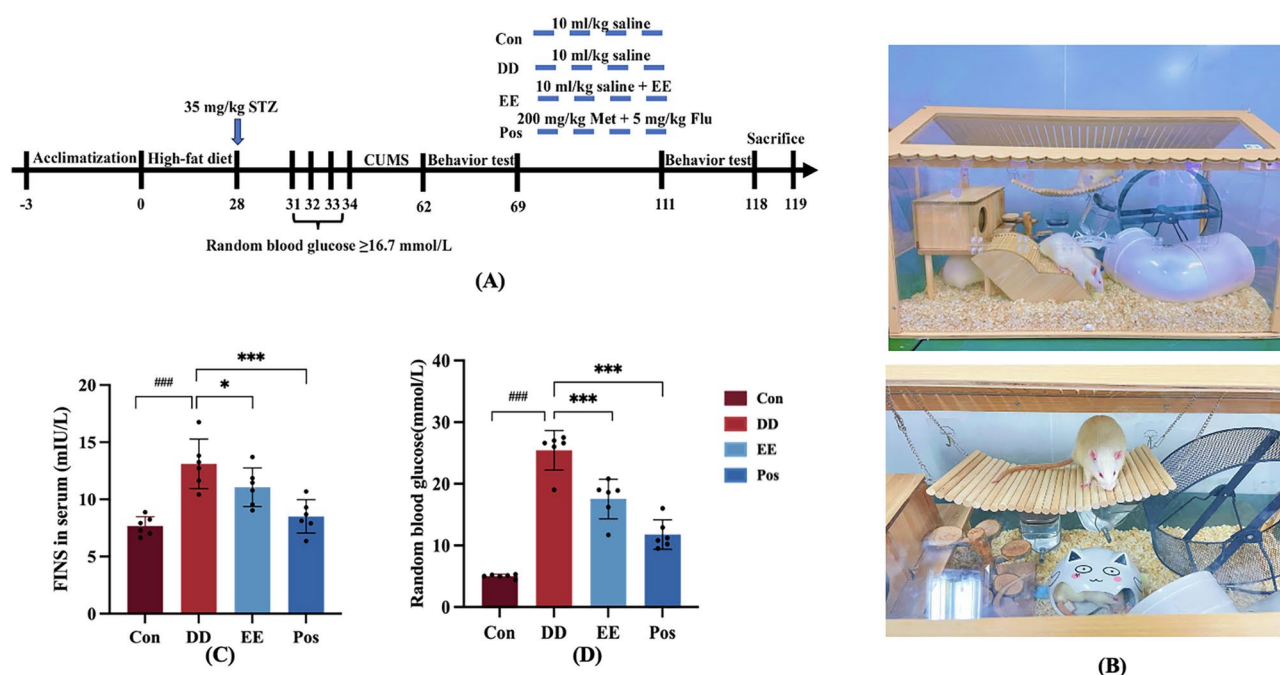


Fig. 1. Effects of environmental enrichment on blood glucose and FINS in DD rats. **(A)** Timeline of the experimental procedure. **(B)** Schematic representation of the environmental enrichment protocol. **(D)** Comparison of random blood glucose levels among the four groups of rats. **(C)** Comparison of serum FINS levels among the four groups of rats. Data are presented as mean \pm standard deviation, with 6 rats per group. Statistical analysis was performed using ANOVA. ### $P < 0.001$ compared to the control group. ** $P < 0.01$ compared to the DD group. *** $P < 0.001$ compared to the DD group.

contrast, FINS levels significantly decreased in both the EE ($P=0.04$) and Pos groups ($P<0.001$) compared to the DD group. These findings suggest that EE can partially improve glucose metabolism in DD rats.

Effects of environmental enrichment on depressive symptoms in DD rats

The open field test, forced swim test, and elevated plus maze were used to assess the antidepressant effects of EE. Statistical differences were observed between the four groups in the horizontal ($F=541.86$, $P<0.001$, Fig. 2A) and vertical scores ($F=151.03$, $P<0.001$, Fig. 2B), as well as the rest time ($F=383.597$, $P<0.001$, Fig. 2C) in the open field test, the immobility time of the forced swimming test ($F=53.21$, $P<0.001$, Fig. 2D), and the OT% ($F=264.237$, $P<0.001$, Fig. 2E) and OE% ($F=60.073$, $P<0.001$, Fig. 2F) in the elevated plus maze. The DD group showed significantly reduced vertical and horizontal exploration scores, as well as lower OT% and OE%, increased resting time in the open field test, and greater immobility time in the forced swim test compared to the control group ($P<0.001$). In contrast, the EE group exhibited increased exploration scores, higher OT% and OE%, and decreased rest and immobility times compared to the DD group ($P<0.001$). These findings suggest that the DD group displayed diminished exploratory behavior and reduced curiosity toward novel environments, while EE effectively alleviated depressive-like behaviors.

Effects of environmental enrichment on hippocampal neuroinflammation in DD rats

Neurotransmitter imbalance is a key mechanism in the pathogenesis of DD. This study measured serum levels of 5-HT, DA, and NE. The three indicators showed statistically significant differences between the groups ($F=23.916$, $P<0.001$, Fig. 2G; $F=20.1772$, $P<0.001$, Fig. 2H; $F=23.696$, $P<0.001$, Fig. 2I). Compared to the control group, the DD group had significantly lower levels of 5-HT, DA, and NE ($P<0.001$). However, both the EE and Pos groups exhibited elevated levels of 5-HT ($P<0.01$), DA ($P=0.04$ or $P<0.001$), and NE ($P<0.001$) compared to the DD group. This suggests that EE can enhance peripheral monoamine neurotransmitter levels in DD rats.

First, hippocampal neuronal damage was assessed using HE staining (Fig. 3A). Under light microscopy, the hippocampal pyramidal cell layer in the control group showed tightly arranged cells with clear structures, distinct nucleoli, and a moderate number of neurons. In the DD group, there was a reduction in neuronal count, along with degeneration, nuclear condensation, unclear boundaries, and disorganized cellular arrangements. Conversely, the EE and Pos groups exhibited increased neuronal numbers and more organized cell arrangements, with slightly reduced pathological damage. This suggests that EE can aid in the repair of hippocampal neuronal damage.

Next, Neuroinflammation can also induce cell apoptosis in the hippocampus, which was assessed using TUNEL staining (Fig. 3B). Statistical differences were observed in the hippocampal cell apoptosis rates among the groups ($F=256.46$, $P<0.001$). Compared with the control group, the hippocampal neuronal apoptosis rate

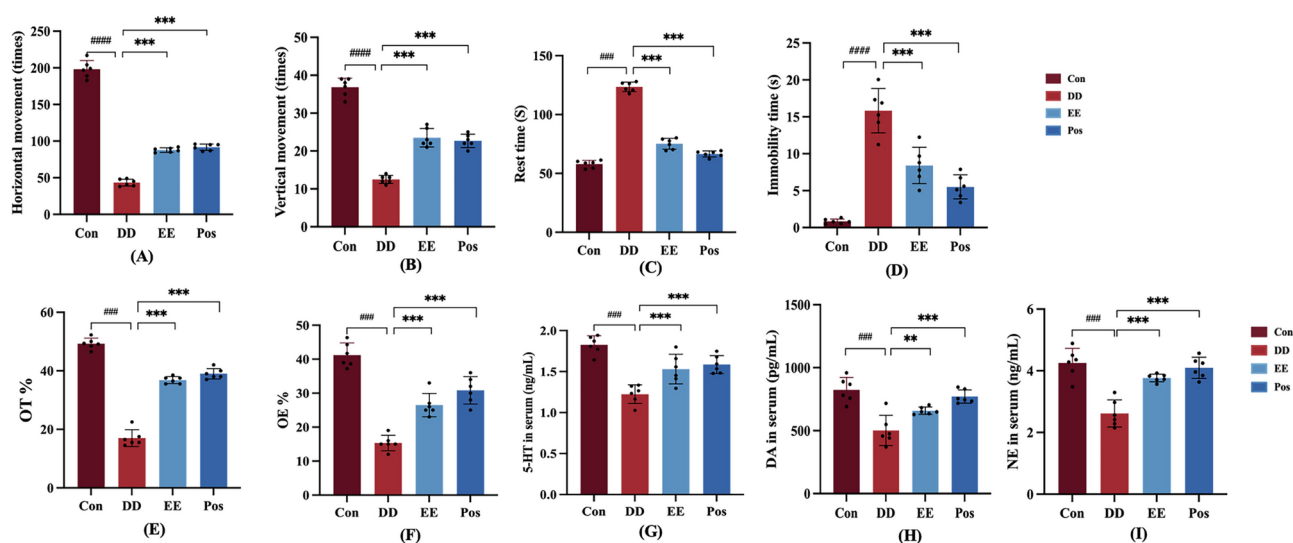


Fig. 2. Effects of environmental enrichment on depressive symptoms in DD rats. (A) Comparison of horizontal scores (number of grid crossings) in the open field test among the four groups of rats. (B) Comparison of vertical scores (number of rearings) in the open field test among the four groups of rats. (C) Comparison of rest time in the forced swim test among the four groups of rats. (D) Comparison of immobility time in the forced swim test among the four groups of rats. (E) Comparison of OT% in the elevated plus maze test among the four groups of rats. (F) Comparison of OE% in the elevated plus maze test among the four groups of rats. (G) Comparison of serum 5-HT levels among the four groups of rats. (H) Comparison of serum DA levels among the four groups of rats. (I) Comparison of serum NE levels among the four groups of rats. Data are presented as mean \pm standard deviation (SD), with 6 rats per group. Statistical analysis was performed using ANOVA. *** $P<0.001$ compared to the control group. ** $P<0.01$ compared to the DD group. * $P<0.05$ compared to the DD group.

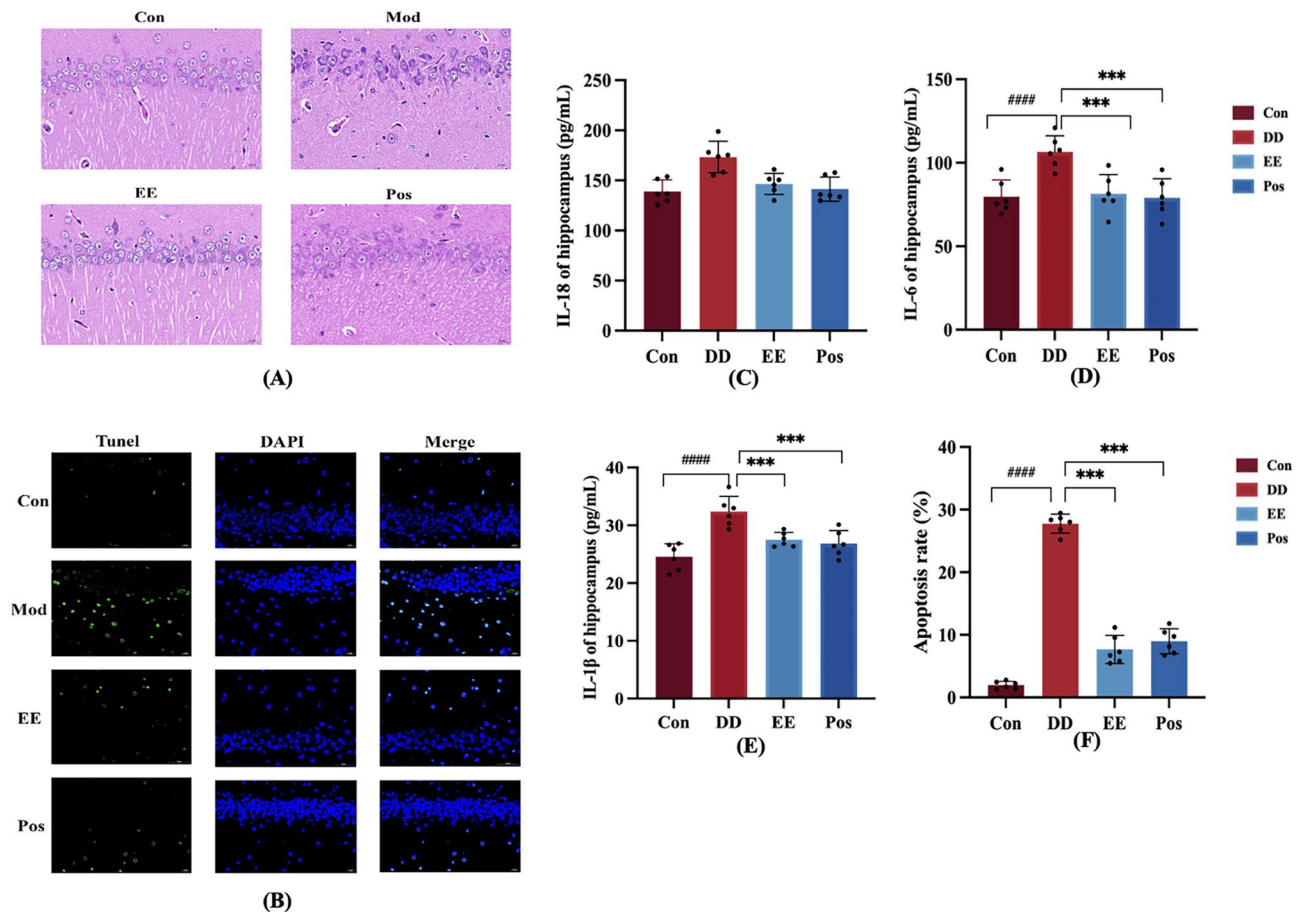


Fig. 3. Effects of enriched environment on hippocampal pro-inflammatory cytokines and structural morphology in DD rats. **(A)** HE staining images of the hippocampus from the four groups of rats (400×). **(B)** TUNEL staining images of the hippocampus from the four groups of rats (400×), with green indicating TUNEL staining and blue indicating DAPI nuclear counterstaining. **(C)** Comparison of hippocampal IL-18 levels among the four groups of rats. **(D)** Comparison of hippocampal IL-6 levels among the four groups of rats. **(E)** Comparison of hippocampal IL-1 β levels among the four groups of rats. **(F)** Comparison of hippocampal cell apoptosis rates among the four groups of rats. Data are presented as mean \pm standard deviation, with 8 rats per group. Statistical analysis was performed using ANOVA. #### $P < 0.001$ compared to the control group. ** $P < 0.01$ compared to the DD group. *** $P < 0.001$ compared to the DD group.

was elevated in the DD group ($P < 0.001$). However, the apoptosis rate significantly decreased in both the EE and Pos groups compared to the DD group ($P < 0.001$). These findings suggest that EE can reverse the hippocampal neuronal apoptosis observed in the DD group.

We measured the levels of inflammatory cytokines in the hippocampus. There were statistically significant differences in IL-18 ($F = 9.410$, $P < 0.01$, Fig. 3C), IL-6 ($F = 9.243$, $P < 0.001$, Fig. 3D), and IL-1 β ($F = 14.126$, $P < 0.001$, Fig. 3E) between the four groups. The DD group exhibited elevated levels of IL-1 β , IL-6, and IL-18 ($P < 0.001$) compared to the control group ($P < 0.001$). In contrast, both the EE and Pos groups showed decreased levels of IL-1 β ($P < 0.001$), IL-6 ($P < 0.001$), and IL-18 ($P < 0.01$ or $P < 0.001$) compared to the DD group. These results indicate that EE can suppress the increase in pro-inflammatory cytokines in the hippocampus.

Microglia are resident immune cells in the brain. When environmental changes occur, microglia become activated by various stimuli, upregulating the expression of the marker Iba1, releasing inflammatory cytokines, and inducing neuronal death, processes closely related to the NLRP3 inflammasome (Fig. 4A). Statistical differences were observed in the number of Iba1+ ($F = 49.372$, $P < 0.001$, Fig. 4B), NLRP3+ ($F = 35.379$, $P < 0.001$, Fig. 4C), and NLRP3-Iba1+ ($F = 59.43$, $P < 0.001$, Fig. 4D) cells. Immunofluorescence experiments revealed that, compared with the control group, the number of NLRP3+, Iba1+, and NLRP3-Iba1+ cells increased in the DD group ($P < 0.001$). However, these indicators were significantly reduced in both the EE and Pos groups compared to the DD group ($P < 0.001$).

Additionally, we measured the protein levels of NLRP3 ($F = 33.632$, $P < 0.001$, Fig. 4E), Caspase-1 ($F = 96.818$, $P < 0.001$, Fig. 4F), PI3K ($F = 8.321$, $P = 0.003$, Fig. 4H), and AKT ($F = 11.282$, $P = 0.003$, Fig. 4I), which are known to be dysregulated in metabolic diseases and may contribute to the overactivation of the NLRP3 inflammasome. The WB immunoblot images for the four proteins are shown in Fig. 4G, J.

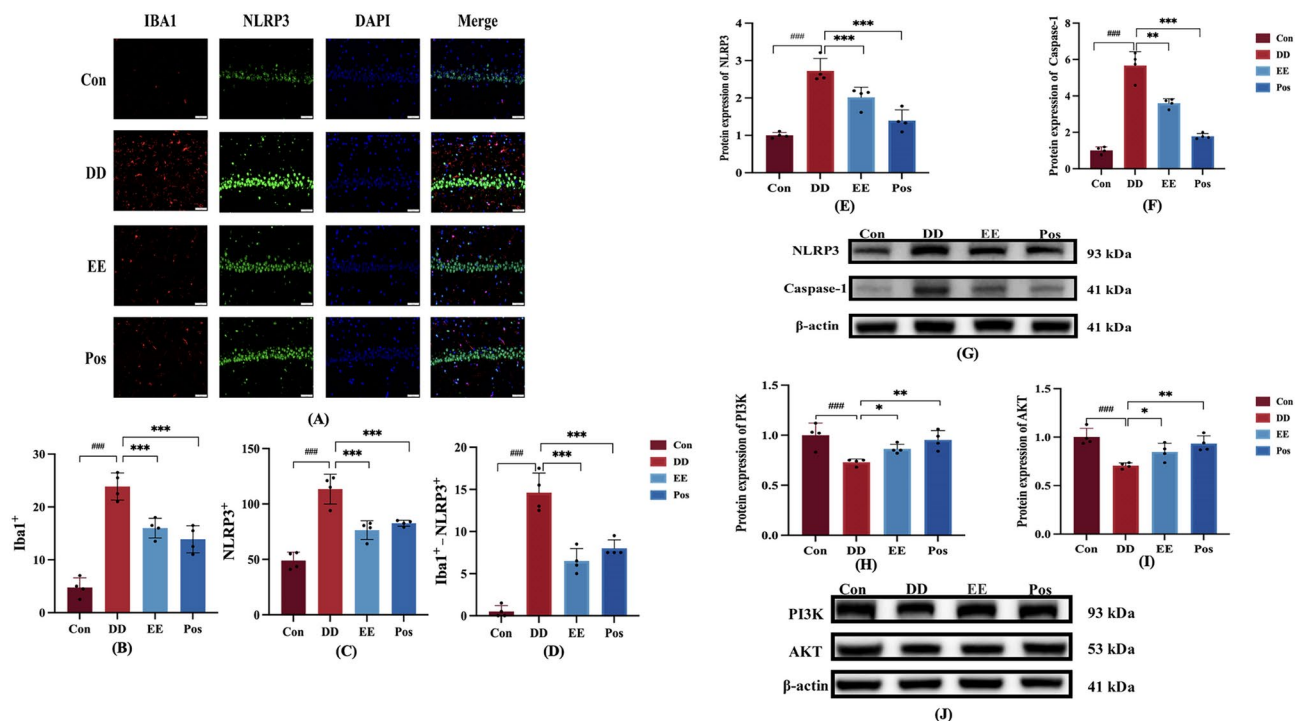


Fig. 4. Effects of environmental enrichment on hippocampal neuroinflammation in DD rats. **(A)** Immunofluorescence images of the hippocampus from the four groups of rats (400 \times), scale bar: 100 μ m, with red representing Iba1 and green representing NLRP3. **(B)** Comparison of Iba1⁺ cell counts in the hippocampus among the four groups. **(C)** Comparison of NLRP3⁺ cell counts in the hippocampus among the four groups. **(D)** Comparison of Iba1⁺—NLRP3⁺ double-positive cell counts in the hippocampus among the four groups. **(E)** Comparison of hippocampal NLRP3 protein expression levels among the four groups. **(F)** Comparison of hippocampal Caspase-1 protein expression levels among the four groups. **(G)** Representative Western blot images of NLRP3 and Caspase-1 protein expression in the hippocampus from the four groups. **(H)** Comparison of hippocampal PI3K protein expression levels among the four groups. **(I)** Comparison of hippocampal AKT protein expression levels among the four groups. **(J)** Representative Western blot images of PI3K and AKT protein expression in the hippocampus from the four groups. Data are presented as mean \pm standard deviation, with 4 rats per group. Statistical analysis was performed using ANOVA. ### P <0.001 compared to the control group. * P <0.05 compared to the DD group. ** P <0.01 compared to the DD group. *** P <0.001 compared to the DD group.

The results showed that, compared with the control group, the DD group exhibited elevated levels of NLRP3 ($P=0.002$) and Caspase-1 ($P<0.001$) and reduced levels of PI3K and AKT proteins ($P<0.001$). In both the EE and Pos groups, NLRP3 and Caspase-1 levels were reduced ($P<0.01$ or $P<0.001$), while PI3K ($P=0.042$ or $P=0.002$) and AKT ($P=0.022$ or $P=0.001$) protein levels increased ($P<0.05$ or $P<0.01$) compared to the DD group. These findings suggest that EE can inhibit neuroinflammation induced by the NLRP3 inflammasome by modulating the PI3K/AKT signaling pathway.

Discussion

Our study reveals that hippocampal neuroinflammation is a key mechanism underlying the pathogenesis of DD, with the NLRP3 inflammasome playing a significant role in this process. EE, as a non-pharmacological intervention that integrates cognitive, sensory, motor, and social factors, offers welfare benefits to DD rats. It not only improves glucose metabolism and reduces depressive behaviors but also alleviates hippocampal cellular damage and apoptosis. Additionally, EE inhibits the activation of the NLRP3 inflammasome in microglia and enhances PI3K/AKT signaling pathway expression, further suppressing NLRP3 activation and reducing hippocampal neuroinflammation.

Microglia, the brain's resident immune cells, are activated by hyperglycemia and inflammatory responses induced by diabetes, leading to NLRP3 inflammasome activation. Once activated, NLRP3 interacts with apoptosis-associated speck-like protein (ASC) and recruits Caspase-1, which cleaves pro-forms of IL-1 β and IL-18 into their active forms. These pro-inflammatory cytokines amplify the inflammatory response by activating nearby immune cells. Persistent activation of microglia is characterized by upregulation of markers such as Iba1 and morphological changes, including increased cell body size and shortened processes. Activated microglia also produce toxic molecules that further damage neurons, particularly in the hippocampus, potentially leading to neuronal apoptosis and synaptic damage. Research has shown that hyperglycemia-induced activation of the NLRP3 inflammasome occurs within hippocampal microglia in C57BL/6 mice and is associated with depressive

behaviors in diabetic mice. Additionally, studies have reported that NLRP3 inflammasome activation induces hippocampal neuroinflammation and impairs neuroplasticity in DD rats, contributing to the development of depressive behaviors.

The PI3K/AKT signaling pathway is crucial in regulating NLRP3 activation. Our study demonstrated that DD rats exhibited elevated random blood glucose and FINS levels, promoting NLRP3 inflammasome activation in hippocampal microglia and leading to depressive behaviors. Furthermore, we observed that the PI3K/AKT signaling pathway was suppressed.

EE comprises cognitive, sensory, motor, and social elements that enhance the structural and functional integrity of the hippocampus. Nest-building behavior, associated with the hippocampus, improves spatial memory and aids in repairing hippocampal structures. Moreover, studies have shown that providing experimental mice with shelters can reduce psychological stress, enhance immune function, and improve neurological performance. EE also promotes interaction and socialization, essential for animal welfare.

The primary exercise equipment in EE, such as running wheels, activates pathways that suppress inflammatory cytokine secretion and promote spatial learning and memory recovery. Sensory stimulation through various toys can reduce stress-related behaviors and enhance neuroendocrine function.

Furthermore, EE has been shown to reduce brain tissue damage from microglial activation and lower pro-inflammatory cytokine levels. Consistent with these findings, our study indicates that EE improves neuroinflammation in DD rats, subsequently reducing depressive-like behaviors. Although this study employed widely accepted behavioral paradigms to assess depressive-like symptoms, it did not include evaluations of non-motor depressive-like behaviors. The absence of tests such as the sucrose preference test or splash test limits the comprehensiveness of our behavioral assessment. Future studies are warranted to incorporate these paradigms to more fully validate the antidepressant-like effects of environmental enrichment.

Conclusion

This study identifies the NLRP3 inflammasome as highly active in hippocampal microglia of DD rats, with neuroinflammation as a potential mechanism of pathogenesis. EE intervention effectively regulates glucose metabolism, reduces depressive-like behaviors, and decreases neuroinflammation in DD rats, possibly through the inhibition of the NLRP3 signaling pathway. Additionally, EE enhances the PI3K/AKT signaling pathway, strengthening the suppression of the NLRP3 inflammasome. These findings highlight the role of EE in alleviating neuroinflammation in DD rats, offering a novel non-pharmacological approach for treating diabetes-related depression.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Sun, H. et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res. Clin. Pract.* **183**, 109119. <https://doi.org/10.1016/j.diabres.2021.109119> (2022).
2. Mangoulia, P., Milionis, C., Vlachou, E., & Ilias, I. The interrelationship between diabetes mellitus and emotional well-being: Current concepts and future prospects. In *Healthcare* (2024).
3. Farooqi, A. et al. A systematic review and meta-analysis to compare the prevalence of depression between people with and without Type 1 and Type 2 diabetes. *Prim. Care Diabetes* **16**(1), 1–10. <https://doi.org/10.1016/j.pcd.2021.11.001> (2022).
4. Khaledi, M., Haghighatdoost, F., Feizi, A. & Aminorroaya, A. The prevalence of comorbid depression in patients with type 2 diabetes: An updated systematic review and meta-analysis on huge number of observational studies. *Acta Diabetol.* **56**(6), 631–650. <https://doi.org/10.1007/s00592-019-01295-9> (2019).
5. Chow, Y. Y., Verdonchot, M., McEvoy, C. T. & Peeters, G. Associations between depression and cognition, mild cognitive impairment and dementia in persons with diabetes mellitus: A systematic review and meta-analysis. *Diabetes Res. Clin. Pract.* **185**, 109227. <https://doi.org/10.1016/j.diabres.2022.109227> (2022).
6. Farooqi, A. et al. Comorbid depression and risk of cardiac events and cardiac mortality in people with diabetes: A systematic review and meta-analysis. *Diabetes Res. Clin. Pract.* **156**, 107816. <https://doi.org/10.1016/j.diabres.2019.107816> (2019).
7. Wu, C. S., Hsu, L. Y. & Wang, S. H. Association of depression and diabetes complications and mortality: A population-based cohort study. *Epidemiol. Psychiatr. Sci.* **29**, e96. <https://doi.org/10.1017/s2045796020000049> (2020).
8. Surget, A. & Belzung, C. Adult hippocampal neurogenesis shapes adaptation and improves stress response: A mechanistic and integrative perspective. *Mol. Psychiatry* **27**(1), 403–421 (2022).
9. Gao, C., Jiang, J., Tan, Y. & Chen, S. Microglia in neurodegenerative diseases: Mechanism and potential therapeutic targets. *Signal Transduct. Target. Ther.* **8**(1), 359 (2023).
10. Li, Y. et al. The positive effects of running exercise on hippocampal astrocytes in a rat model of depression. *Transl. Psychiatry* **11**(1), 83 (2021).
11. Su, W. J. et al. Microglial NLRP3 inflammasome activation mediates diabetes-induced depression-like behavior via triggering neuroinflammation. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **126**, 110796. <https://doi.org/10.1016/j.pnpbp.2023.110796> (2023).
12. Guo, X. et al. Schisandrin A alleviates spatial learning and memory impairment in diabetic rats by inhibiting inflammatory response and through modulation of the PI3K/AKT pathway. *Mol. Neurobiol.* **61**(5), 2514–2529 (2024).
13. Sahini, S. N. M., Hazalin, N. A. M. N., Srikumar, B. N., Chellammal, H. S. J. & Singh, G. K. S. Environmental enrichment improves cognitive function, learning, memory and anxiety-related behaviours in rodent models of dementia: Implications for future study. *Neurobiol. Learn. Mem.* 107880 (2023).
14. Kimura, L. F., de Moura Mattaraia, V. G. & Picolo, G. Distinct environmental enrichment protocols reduce anxiety but differentially modulate pain sensitivity in rats. *Behav. Brain Res.* **364**, 442–446 (2019).

15. Ali, S. et al. CSF1R inhibitor PLX5622 and environmental enrichment additively improve metabolic outcomes in middle-aged female mice. *Aging (Albany N.Y.)* **12**(3), 2101–2122. <https://doi.org/10.18632/aging.102724> (2020).
16. Gu, J. Y. et al. Enriched environment mitigates depressive behavior by changing the inflammatory activation phenotype of microglia in the hippocampus of depression model rats. *Brain Res. Bull.* **177**, 252–262. <https://doi.org/10.1016/j.brainresbull.2021.10.005> (2021).
17. Bailoo, J. D. et al. Effects of cage enrichment on behavior, welfare and outcome variability in female mice. *Front. Behav. Neurosci.* **12**, 232 (2018).
18. Bayne, K. Environmental enrichment and mouse models: Current perspectives. *Anim. Models Exp. Med.* **1**(2), 82–90 (2018).
19. Chen, G. et al. Environmental enrichment attenuates depressive-like behavior in maternal rats by inhibiting neuroinflammation and apoptosis and promoting neuroplasticity. *Neurobiol. Stress* **30**, 100624. <https://doi.org/10.1016/j.ynstr.2024.100624> (2024).
20. Du Preez, A. et al. Chronic stress followed by social isolation promotes depressive-like behaviour, alters microglial and astrocyte biology and reduces hippocampal neurogenesis in male mice. *Brain Behav. Immun.* **91**, 24–47 (2021).
21. Guo, Y. S. et al. Effects of enriched environment on microglia and functional white matter recovery in rats with post stroke cognitive impairment. *Neurochem. Int.* **154**, 105295. <https://doi.org/10.1016/j.neuint.2022.105295> (2022).
22. Huang, Y., Xu, W. & Zhou, R. NLRP3 inflammasome activation and cell death. *Cell. Mol. Immunol.* **18**(9), 2114–2127. <https://doi.org/10.1038/s41423-021-00740-6> (2021).
23. Jirkof, P. Burrowing and nest building behavior as indicators of well-being in mice. *J. Neurosci. Methods* **234**, 139–146 (2014).
24. Kentner, A. C., Scalia, S., Shin, J., Migliore, M. M. & Rondón-Ortiz, A. N. Targeted sensory enrichment interventions protect against behavioral and neuroendocrine consequences of early life stress. *Psychoneuroendocrinology* **98**, 74–85 (2018).
25. Ko, Y. J. & Ko, I.-G. Voluntary wheel running improves spatial learning memory by suppressing inflammation and apoptosis via inactivation of nuclear factor kappa B in brain inflammation rats. *Int. Neurol.* **24**(Suppl 2), 96 (2020).
26. Li, P. et al. Pramipexole improves depression-like behavior in diabetes mellitus with depression rats by inhibiting NLRP3 inflammasome-mediated neuroinflammation and preventing impaired neuroplasticity. *J. Affect. Disord.* **356**, 586–596. <https://doi.org/10.1016/j.jad.2024.04.073> (2024).
27. Li, Z.-R. et al. GR/NF- κ B signaling pathway regulates hippocampal inflammatory responses in diabetic rats with chronic unpredictable mild stress. *Eur. J. Pharmacol.* **895**, 173861 (2021).
28. Mosher, C. E. et al. Family caregiving challenges in advanced colorectal cancer: Patient and caregiver perspectives. *Support. Care Cancer* **24**(5), 2017–2024. <https://doi.org/10.1007/s00520-015-2995-z> (2016).
29. Virijevic, K. et al. Chronic mild stress-induced dysregulation of MAPK and PI3K/AKT signaling in the hippocampus and medial prefrontal cortex of WKY female rats. *Neurosci. Lett.* **825**, 137709. <https://doi.org/10.1016/j.neulet.2024.137709> (2024).
30. Zhan, X., Li, Q., Xu, G., Xiao, X. & Bai, Z. The mechanism of NLRP3 inflammasome activation and its pharmacological inhibitors. *Front. Immunol.* **13**, 1109938. <https://doi.org/10.3389/fimmu.2022.1109938> (2022).

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Author contributions

L.Q.F. designed the study; M.W.L. and J.S.Y. performed the experiments and analyzed data; W.H.Y. and Z.H. wrote the manuscript. W.Z.F. further edited the manuscript; Z.X.G. supervised the study. All authors have read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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