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Neurotoxic effects of nanoplastics exposure on depression-like behavior and cognitive function in mice under chronic unpredictable mild stress.

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Keywords: Microplastics, Nanoparticles; Nanoplastic; BDNF/TrkB; Chronic unpredictable mild Stress; Depression-like behavior; Disease Models, Animal; Hippocampus.

Abstract. The aim was to investigate the effects of gavage exposure to nanoplastics (NPs) on cognitive decline and depression-like behavior was investigated in mice subjected to chronic unpredictable mild stress (CUMS). BALB/c mice were randomly assigned to four experimental groups: Control (Ctrl), nanoplastics (NPs), Mod (subjected to CUMS), and NPs+Mod (nanoplastics + CUMS). We evaluated the role of the brain-derived neurotrophic factor (BDNF) and its receptor, the tyrosine kinase receptor B (TrkB), signaling pathway in the hippocampus of mice. Behavioral assessments included the sucrose preference test, the open field test, the forced swim test, and the Morris water maze. Nissl staining was used to assess hippocampal neuronal morphology. BDNF and TrkB mRNA levels and protein expression in the hippocampus were measured by qPCR and Western blotting, respectively. Mice in the NPs, Mod, and NPs+Mod groups showed reduced body weight, lower sucrose preference, poorer performance in the open field test, and prolonged immobility in the forced swim test. Additionally, there was a reduction in hippocampal

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neurons and deficits in spatial learning and memory compared with the control group. BDNF mRNA and TrkB protein levels were decreased. Compared with the Mod group, the NPs+Mod group exhibited increased depression-like behaviors and cognitive impairments, greater hippocampal neuronal damage, and further reductions in BDNF and TrkB mRNA and protein levels. In conclusion, NP exposure has neurotoxic properties that can exacerbate CUMS-induced depression-like behavior and cognitive deficits, likely by further suppressing the hippocampal BDNF/TrkB signaling pathway.

Efectos neurotóxicos de la exposición a nanoplásticos sobre el comportamiento similar a la depresión y la función cognitiva en ratones sometidos a estrés crónico leve impredecible.

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Palabras clave: Nanoplásticos; Microplásticos; Nanopartículas; BDNF/TrkB; Estrés Crónico; Comportamiento similar a la depresión; Hipocampo.

Resumen. En el presente estudio se evaluó el impacto de la exposición a nanoplásticos sobre el deterioro cognitivo y el comportamiento depresivo en ratones sometidos a estrés moderado crónico e impredecible (CUMS). Ratones BALB/c fueron asignados aleatoriamente a cuatro grupos experimentales: Control (Ctrl), nanoplásticos (NPs), Mod (sometidos a CUMS) y NPs+Mod (nanoplásticos + CUMS). Se determinó el papel del factor de crecimiento derivado del cerebro (BDNF) y su vía de señalización a través del receptor de la tirosina quinasa B (TrkB) en el hipocampo. Las pruebas de comportamiento incluyeron la prueba de preferencia por la sacarosa, la actividad motora en campo abierto, el nado forzado y el laberinto de Morris. Se evaluaron la morfología neuronal y la expresión de BDNF y TrkB mediante técnicas de PCR cuantitativa y Western blot. La exposición a los nanoplásticos, así como el estrés crónico, indujeron una disminución de la actividad en las pruebas de conducta. Además, se observó una disminución de las neuronas del hipocampo y de la memoria espacial en ambos tratamientos. La combinación de estrés crónico y la exposición a los nanoplásticos tuvo un efecto sumativo sobre las pruebas conductuales y cognitivas, así como sobre la expresión de BDNF y del receptor Trak B. En conclusión, la exposición a los nanoplásticos tiene efectos neurotóxicos que pueden potenciar los efectos del estrés crónico a nivel cognitivo y conductual. Dichos efectos se mediaron mediante la supresión de la vía de señalización de BDNF y del receptor TrkB en el hipocampo.

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INTRODUCTION

Nanoplastics (NPs), an emerging class of environmental contaminants, are attracting increasing attention due to their potential health hazards. Owing to their minute size, NPs can cross biological barriers, distribute widely throughout the organism, and adversely affect various physiological functions, including entry into the nervous system [1]. Accumulating evidence from experimental studies shows that exposure to

NPs can lead to heightened anxiety and depression-like phenotypes in animals [2]. While direct human epidemiological data linking NPs to specific mental health disorders remain limited, these controlled experimental models are critical because they provide a practical tool to elucidate the underlying neurotoxic mechanisms. For example, they allow precise dose control, detailed pathological examination, and the isolation of causal pathways that are not feasible in human studies. On the other hand, depression is a debilitating mental disorder characterized by low mood and significant impairments in cognition and motivation, representing a major public health concern [3,4]. Given unavoidable human exposure to plastic pollution, it is imperative to employ such experimental approaches to comprehensively investigate the impact of NPs on mental health, specifically their mechanisms for disrupting emotional regulation and cognitive function under conditions such as chronic unpredictable mild stress (CUMS) [5].

The hippocampus is a crucial brain region that regulates emotional responses and cognitive functions, and its dysfunction has been implicated in the pathogenesis of related disorders [6]. The "neuronal damage hypothesis" of depression posits that an insufficiency of neurotrophic factors plays a pivotal role in pathological changes within the hippocampus and in the pathogenesis of the disorder [7]. These neurotrophic factors are vital for neuronal survival and synaptic plasticity. Chief among them is brain-derived neurotrophic factor (BDNF), which, by binding to its receptor, the tyrosine kinase receptor B (TrkB), activates a critical signaling pathway for neuronal growth, development, and maintenance [8]. Disruption of the BDNF/TrkB signaling pathway can impair neuronal function, thereby adversely affecting emotional regulation and cognitive capabilities.

This study aims to investigate the combined neurotoxic effects of NPs and CUMS in mice, with a specific focus on depression-like behaviors and cognitive deficits. The effects were evaluated using a battery of behavioral tests, histopathological examinations, and molecular biological techniques. Furthermore, the study analyzes the involvement of the BDNF/TrkB signaling pathway in these processes, thereby providing a scientific basis for understanding the mechanisms underlying NPs-induced neurotoxicity and informing the development of potential intervention strategies.

MATERIALS AND METHODS

Experimental animals

We purchased a total of thirty-two female BALB/c mice. They were SPF-grade and 6 to 8 weeks old. The overall body weight of the mice was approximately 20-25 grams. The mice were obtained from the Hubei Provincial Research Center for Laboratory Animals (animal qualification certificate no. 42000600056335). The animal room's environmental parameters were set to maintain a temperature of 24-26°C and a humidity of 45-50%. Additionally, a 12-hour light/dark cycle was implemented. Mice had unrestricted access to food and water. This study was approved by the Ethics Committee of Hubei University of Science and Technology (Approval Certificate ID: HBUST-IACUC-2024-11-010), which is available upon request.

Reagents and kits

Polystyrene nanoparticles (PS-NPs) with a diameter of 25 nm were obtained from Zhongke Leiming Technology Co., Ltd. (Product No. PS000025). The Nissl staining solution was obtained from Shanghai Biyuntian Biotechnology Co., Ltd. (Product No. C0117). For RNA extraction, we used a kit from Shenzhen Dakewei Biotechnology Co., Ltd. (Product No. 8034111). The quantitative PCR (qPCR) kit was obtained from Beijing Quanshijin Biotechnology Co., Ltd. (Product No. Q601-02). Furthermore, antibodies against BDNF and TrKB were obtained from Abcam, USA (Product Nos. ab108319 and ab187041).

The equipment used in this research included the following: EthoVision XT Version 12.0 by Noldus (Netherlands) for recording neurobehavioral data in experimental animals; an Olympus dp73 biological microscope (Japan); a Leica RM2245 paraffin microtome (Germany); a Sartorius BAS124S electronic balance (Germany); a Sanyo MDF-U53V ultra-low-temperature freezer operating at -80 °C (Japan); an Eppendorf 5415R low-temperature centrifuge (Germany); a BioTek Epoch microplate reader (USA); a Bio-Rad CFX Connect real-time PCR system (USA); and a Bio-Rad PowerPac Basic system for Western blot electrophoresis (USA).

Animal grouping and modeling

After a week of adaptive feeding, 32 BALB/c mice were randomly assigned to four groups (n=8 per group): the control group (Ctrl), the nanoplastics group (NPs), the model group (Mod), and the combined nanoplastics and model group (NPs+Mod). The NPs and NPs+Mod groups received oral gavage with NPs (5 mg/kg/d, 10 μL/g) [9], whereas the Mod and NPs+Mod groups underwent CUMS to establish a depression model. Mice in the Ctrl group received an equivalent volume of 0.9% sodium chloride solution via gavage.

The CUMS protocol for the Mod and NPs+Mod groups consisted of a variety of unpredictable mild stressors applied over 4 weeks [10]. The stressors included tail clamping for 10 minutes, a 24-hour fast, a 24-hour water deprivation, inversion of the circadian rhythm, overnight light exposure, placement in an empty cage for 12 hours, contact with damp bedding for 12 hours, cage shaking for 15 minutes, foot shocks for 15 minutes, stroboscopic lighting for 10 minutes, refrigeration for 10 minutes, and swimming in cold water at 10°C for 10 minutes. Mice experienced 1-2 stressors each day, with the type of stressor varying daily to avoid repetition and ensure variability over a three-day period. One or two different stressors were applied daily in a random order to prevent habituation. The experimental design is shown in Fig. 1.

General condition inspection

The eating patterns, fur quality, and body weight of mice were monitored before the first exposure and after the final exposure to the experimental protocol.

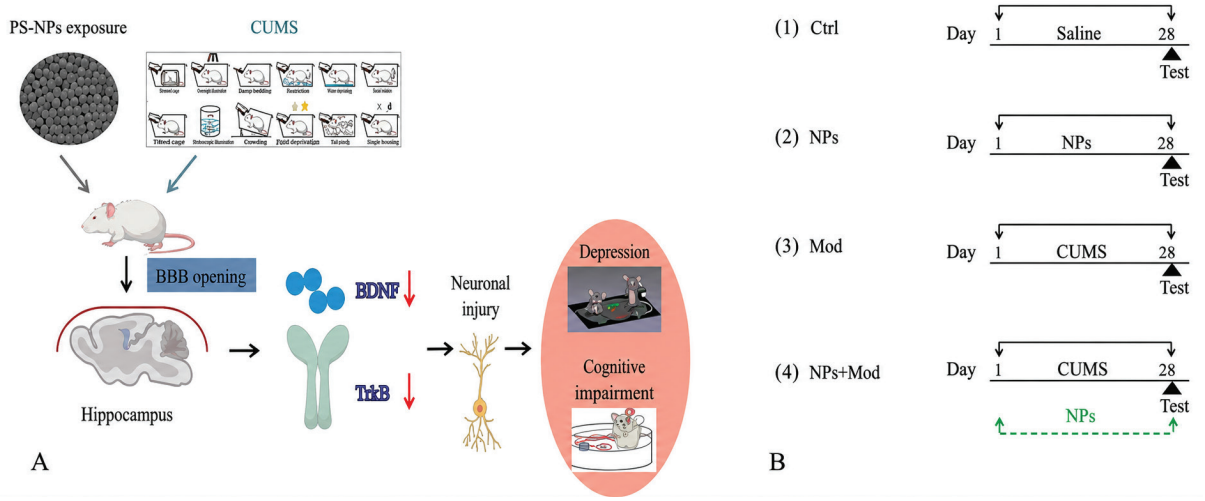

Fig. 1. Neurobiological effects of NPs on stressed mice. **A:** Scientific hypothesis of the study. **B:** Experimental groups and treatment protocol.

Behavioral testing

Sucrose preference test experiment

Before this experiment commenced, mice underwent a 3-day training phase that included sucrose water. Each cage contained two identical bottles in appearance and volume, which were rotated every 12 hours to prevent the formation of a routine memory. On the first day, each bottle was filled with a 1% sucrose solution. The next day, one bottle was replaced with an equivalent amount of distilled water. By the third day, the mice were denied access to any food or water. The evaluation took place on the fourth day, when each cage received one bottle of distilled water and another containing the 1% sucrose solution. After 24 hours, the weights of the bottles (in grams) were recorded. Sucrose solution and water consumption were determined by measuring the weight loss of each bottle. The sucrose preference rate (%) was calculated as: sucrose solution consumption / (sucrose solution consumption + water consumption) × 100%, which indicated the proportion of sucrose solution consumed by each group of mice prior to and following the final exposure.

Open field test

After the final NP administration, mice were placed at the center of an open-field reaction chamber measuring 100 cm in length, 100 cm in width, and 40 cm in height. At the same time, video recording and timing were started, and behavior was recorded for 5 minutes. The experimental data were collected and analyzed using EthoVision XT Version 13.0 software.

Forced swim test

Mice were placed in a clear acrylic cylinder, 50 cm high and 30 cm in diameter, filled with water to a depth of 30 cm at a temperature of (24±2) °C. Each mouse was carefully positioned upright in the water, ensuring that its limbs did not touch the bottom of the cylinder as it struggled and swam. The first 2 minutes were not timed, and the duration of immobility during the subsequent 4 minutes was measured. Immobility was defined as the state in which mice ceased struggling and made only minimal movements necessary to keep their heads above water. Following the swimming session, the animal was swiftly removed from the water, dried, and placed in a warm setting.

Morphological detection

After the final NPs administration, mice were anesthetized intraperitoneally with 1% sodium pentobarbital (40 mg/kg) and placed in a supine position on the surgical table. An incision was made in the chest cavity to expose the heart, and an infusion needle was carefully inserted 5-6 mm into the cardiac apex, aligned with the heart's axis. Once perfusion commenced, the right atrium was cut. The blood vessels were quickly flushed with 50 mL of warm physiological saline, followed by fixation perfusion with 450 mL of pre-cooled 4% paraformaldehyde. The whole brain was then removed and immersed in the same fixative for an additional 6 to 12 hours. Following standard paraffin embedding procedures, sections approximately 5 μm thick were prepared. These sections were stained with Nissl for 10 minutes to 1 hour, rinsed with double-distilled water, differentiated with 70% alcohol, dehydrated through a graded alcohol series, cleared with xylene, and finally mounted. Morphological alterations in hippocampal neurons were examined under an optical microscope and documented for archival records.

Cognitive function assessment

This study used the Morris water maze, a widely recognized method for assessing spatial learning and memory in rodents. The apparatus consisted of a cylindrical water tank with a diameter of 100 cm and a depth of 20 cm, maintained at a water temperature of 24±2 °C. The tank was artificially divided into four quadrants: northeast (NE), southeast (SE), northwest (NW), and southwest (SW). The escape platform was positioned at

the center of the SE quadrant, submerged 1 cm below the water's surface.

During the spatial acquisition phase, mice were placed in the water, facing the wall, at one of four designated starting points (N, E, S, W) and allowed to locate the hidden platform in the southeast quadrant. After the final trial, the swimming routes of mice from the different experimental groups, as well as the time spent in the target quadrant, were recorded. The experimental data were then collected and analyzed using EthoVision XT Version 13.0 software.

Detection of mRNA transcription levels

Total RNA was isolated from hippocampal cells of mice according to the Trizol kit instructions, and RNA concentration was measured. The extracted RNA was reverse-transcribed into complementary DNA (cDNA) using Oligo(dT) primers. Primers targeting mouse BDNF, TrkB, and β-actin were designed based on their complete GenBank sequences. The primer sequences are as follows: BDNF: forward, 5′-GTGTGACAGTATTAGCGAGTGGG-3′; reverse, 5′-ACGATTGGGTAGTTCGGCATT-3′. TrkB: forward, 5′-AGACAAACCCAAATTACCCTGA-3′; reverse, 5′-ACTTTTGTTCGTAGTATCCCCA-3′. β-actin: forward, 5′-CTCATGCCATCCTGCGTCT-3′; reverse, 5′-ACGCACGATTTCCCTCTCA-3′. These primers were synthesized by Beijing Tsingke Biotech Co., Ltd. Using the reverse-transcribed cDNA as a template, PCR amplification was performed according to the qPCR kit protocol. The reaction mixture (15 μL) consisted of 1.5 μL of cDNA, 7.5 μL of 2×SYBR Green Realtime PCR Master Mix, 0.5 μL of each primer (10 μmol·L^{-1}), and 5 μL of nuclease-free water. The PCR amplification protocol consisted of an initial denaturation at 95°C for 30 s, followed by 39 cycles of denaturation at 95°C for 5 s and annealing/extension at 60°C for 30 s.

Detection of protein expression levels

To obtain total protein from hippocampal tissue, we measured protein concentration using the BCA method. Next, protein samples were mixed with 5× SDS-PAGE loading buffer and heated for 8 minutes to denature them. Following this step, proteins were separated by electrophoresis on a 12% SDS-polyacrylamide separating gel with a 5% stacking gel. Proteins were transferred to a PVDF membrane by wet transfer for 90 minutes. The membrane was blocked with 5% skim milk for 90 minutes at room temperature with shaking. Subsequently, the membrane was incubated overnight at 4°C with primary antibodies against BDNF (1:1000) or TrkB (1:5000). After washing five times for 8 minutes each with TBST, the membrane was incubated with an HRP-conjugated secondary antibody (1:10000) for 60 minutes at room temperature. After a subsequent wash, the signal was detected using the improved chemiluminescence technique, with β-actin serving as a reference control.

Statistical analysis

Data were collected from six biological replicates, and results were reported as mean ± standard deviation (Mean ± SD). Statistical analyses were conducted in SPSS version 28.0, and GraphPad Prism 10.0 was used for data visualization. For comparisons between two groups, an independent-samples Student's t-test was used. For comparisons involving three or more groups, one-way analysis of variance (One-way ANOVA) was applied, followed by Tukey's post hoc test for multiple comparisons. A probability value (p) below 0.05 was considered statistically significant.

RESULTS

General condition of mice in each group

Before the experiment, all groups of mice had similar overall conditions. After the final exposure, mice in the Ctrl group exhibited normal food intake, shiny fur, and a significant increase in body weight (Fig. 2). In sharp contrast, mice in the NPs, Mod, and NPs+Mod groups showed reduced food intake and rough, lackluster fur. Although the

NPs and Mod groups showed a slight increase in body weight, the gain was significantly less than that in the Ctrl group. The NPs+Mod group showed little to no increase in body weight (Fig. 2).

Behavioral experiments

The effect on the sucrose preference rate in mice

A significant reduction in sucrose preference was observed in mice from the NPs, Mod, and NPs+Mod groups after the final exposure, compared with the Ctrl group ($p<0.05$). Furthermore, mice in the NPs+Mod group showed a marked decline in sucrose preference compared with the Mod group ($p<0.05$) (Fig. 3A).

The effect on the spontaneous activity of mice in the open field test

After the final exposure, mice in the NPs, Mod, and NPs+Mod groups showed reduced spontaneous activity in the open field test compared with the Ctrl group (Fig. 3B). This reduction was evidenced by a significant decrease in time spent in the central area, a concomitant increase in time spent near the walls, and reductions in total distance traveled and average speed ($p<0.05$). Moreover, compared with the Mod group, the NPs+Mod group exhibited a more pronounced decrease in spontaneous activity, characterized by an even greater preference for the periphery and further reductions in total distance traveled and average speed ($p<0.05$) (Fig. 3C).

The effect on immobility time during the forced swim test in mice

After the final exposure, the NPs-treated group, the Mod group, and the combined NPs+Mod treatment group exhibited behaviors indicative of despair. This was evidenced by a significantly longer immobility duration in these groups than in the control group. The statistical analysis confirmed that these differences were significant ($p<0.05$). Furthermore, when comparing the NPs+Mod group to the Mod group, the mice in the NPs+Mod group exhibited a longer duration of immobility, and this difference was also statistically significant ($p<0.05$) (Fig. 3D).

Morphological changes in hippocampal neurons

After the last exposure, hippocampal neurons in the control group of mice were organized in an orderly manner, with normal intercellular spaces, consistent morphology, intact structures, numerous Nissl bodies, and

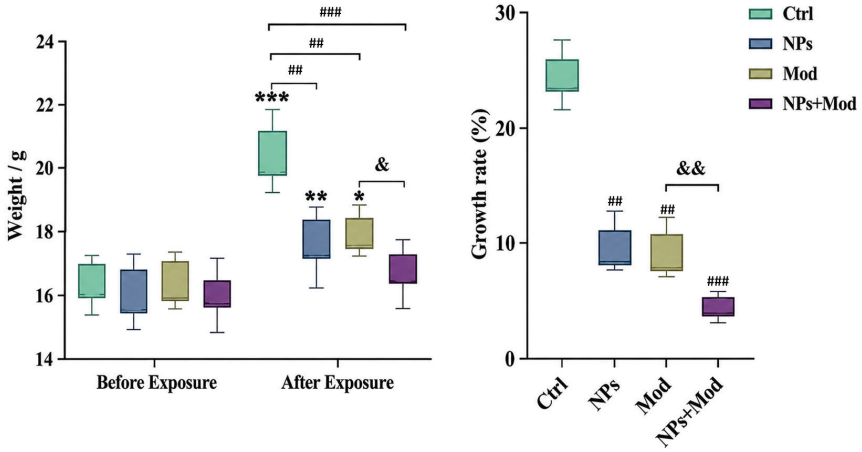

Fig. 2. Changes in body mass in each group. Note: *vs.* before poisoning, ** $p<0.01$, *** $p<0.001$; *vs.* Ctrl group, ## $p<0.01$, ### $p<0.001$; *vs.* Mod group, & $p<0.05$, && $p<0.01$.

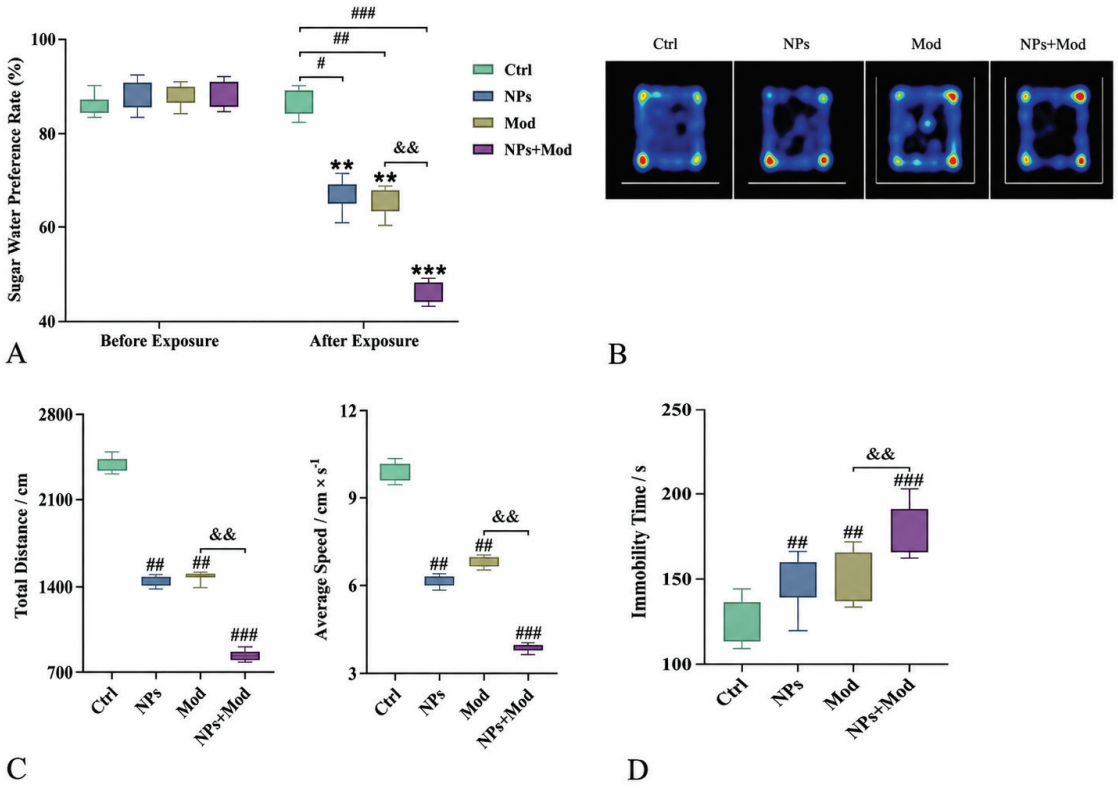

Fig. 3. Behavioral changes in each group. **A:** Sugar water preference test. **B:** Open field test (blue line: mouse path; absence of line: mouse did not enter that area). **C:** Autonomous activity. **D:** Immobility time. Note: *vs.* Ctrl group, ## $p<0.01$, ### $p<0.001$; *vs.* Mod group, && $p<0.01$.

uniform, pronounced staining. In contrast, the NPs group, Mod group, and NPs+Mod group showed disordered cell arrangements, enlarged intercellular spaces, substantial neuronal loss, and lighter staining of Nissl bodies. Importantly, the NPs+Mod group exhibited more pronounced alterations than the Mod group (Fig. 4).

The Morris water maze experiment

After the final exposure to the toxin, mice in the NPs, Mod, and NPs+Mod groups showed a significant decline in spatial memory capacity compared with the Ctrl group. This decline was evidenced by a significantly shorter time spent in the target quadrant and fewer platform location crossings during the probe trial ($p<0.05$). Furthermore, compared with the Mod group, the NPs+Mod group exhibited even more severe spatial memory impairment, with a further significant reduction in both time spent in the target quadrant and the number of platform crossings ($p<0.05$) (Figs. 5 and 6).

Changes in the expression levels of BDNF and TrkB in the hippocampal tissue

After the final exposure, reductions in BDNF and TrkB mRNA transcription and protein expression were observed in the hippocampal tissue of mice in the NPs, Mod, and NPs+Mod groups compared with the Ctrl group, demonstrating statistically significant differences ($p<0.05$). Furthermore, the NPs+Mod group showed significantly greater reductions in BDNF and TrkB mRNA and protein levels than the Mod group ($p<0.05$) (Fig. 7).

DISCUSSION

Global ecological and health implications of NPs exposure

In recent years, the widespread presence and ongoing release of NPs have become a major global environmental issue. Their high mobility enables migration across aquatic environments, terrestrial ecosystems, and atmospheric layers, ultimately disrupting ecological balance and posing significant threats to human health through bioaccumulation and trophic transfer. A notable concern is the confirmed neuroinvasive capability of NPs, as evidenced by their ability to cross the blood-brain barrier and accumulate in the central nervous system [11]. The distinct physicochemical characteristics of NPs, including a remarkably high specific surface area and surface energy, promote strong interactions with receptors on neural membranes (Fig. 2). Such interactions may impair neuronal function, leading to mood changes and cognitive challenges [12].

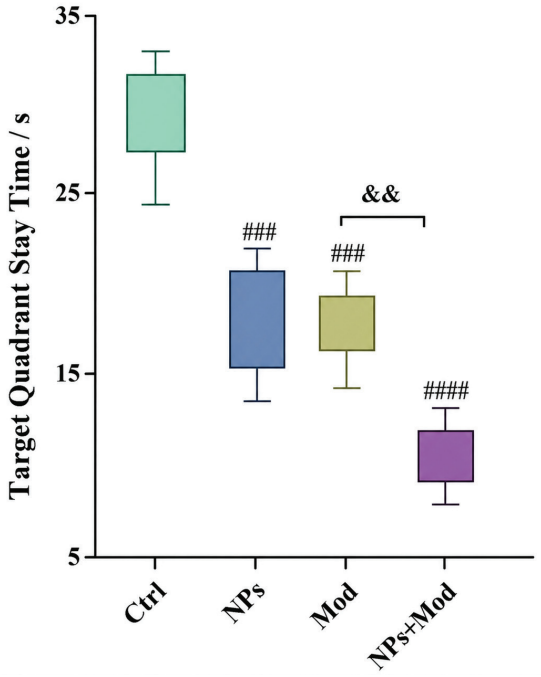

Fig. 6. Target quadrant dwell time in each group. Note: *vs.* Ctrl group, ## p<0.01, ### p< 0.001; *vs.* Mod group, && p<0.01.

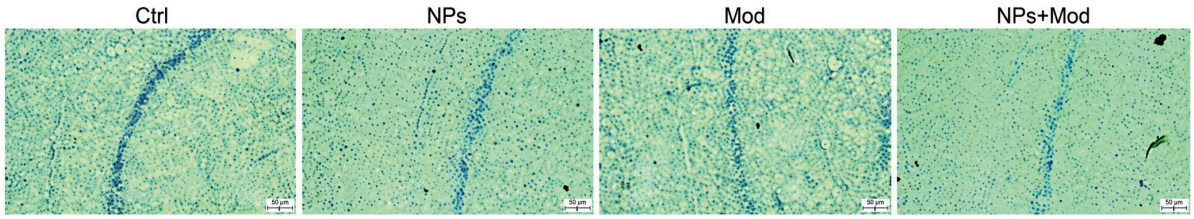

Fig. 4. Pathological changes of hippocampal neurons in each group. Nissl staining, ×100.

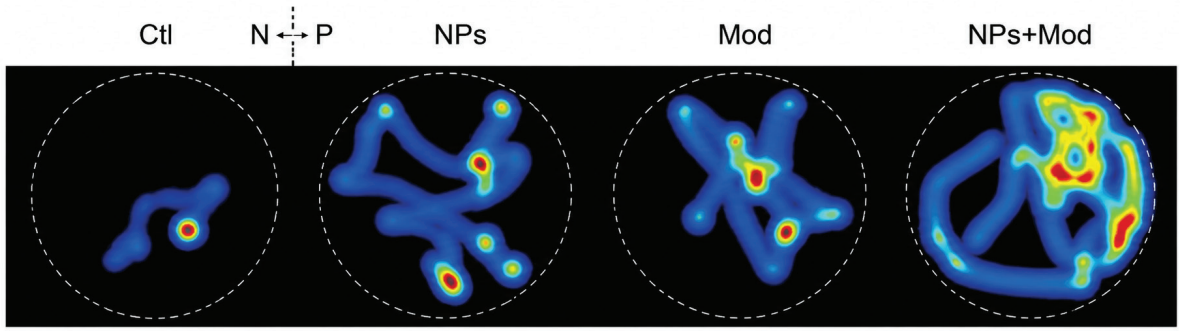

Fig. 5. Morris water maze test in each group. Blue line: mouse path; absence of line: mouse did not enter that area.

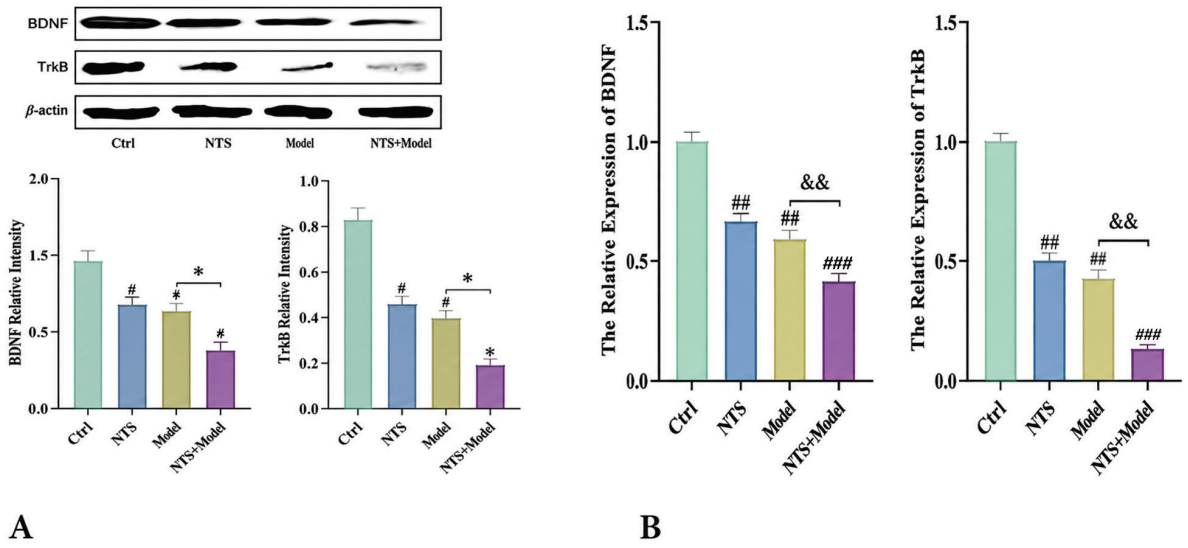

Fig. 7. Changes in the expression levels of BDNF and TrkB. **A:** Protein expression levels. **B:** mRNA transcription levels. Note: *vs.* Ctrl group, # p<0.05, ## p<0.01, ### p<0.001; *vs.* Mod group, & p<0.05, && p<0.01.

Given the neuroinvasive potential of NPs, there is growing concern about their role in mental health disorders, particularly depression [13]. The implications of depression for public health are significant; the global prevalence of depression increased by 34%, and the prevalence of major depressive disorder rose to 8%. The proportion of adolescents suffering from depression increased from 24% during 2001-2010 to 37% between 2011-2020 [14]. According to the report, in 2016, depression was among the leading global causes of disability. We therefore use the CUMS model, a widely used preclinical research framework with good construct validity and lasting behavioral outcomes. This model is highly appropriate for studying the pathogenesis of depression[8]. The objective of this study was to investigate the neurotoxicological effects of NPs and to assess their impact on depression-like behaviors and cognitive performance in CUMS mice. We also evaluated the role of the BDNF/TrkB signaling pathway in these processes.

Behavioral alterations induced by NPs exposure in mice

The current study employed an extensive behavioral battery to systematically investigate the effect of NPs exposure on depression-like behavior in mice [15]. Our results reveal that exposure to NPs causes substantial behavioral changes that correlate with states analogous to depression, as determined by three established behavioral assays. Reduced sucrose preference indicates that exposure to NPs may disrupt the reward-processing machinery, a core feature of anhedonia (Fig. 3). The changes in movement and reduced exploration of the center zone in the open field test may also reflect increased generalized psychomotor slowing or anxiety-like behaviors. Clinical reports of psychomotor agitation or retardation are consistent with these results among depressed persons he changes in movement and reduced exploration of the center zone in the open field test may reflect either generalized psychomotor slowing or anxiety-like behaviors, which is consistent with clinical

reports of psychomotor disturbances in depressed individuals (Fig. 3). The increased immobility time in the forced swim test, often interpreted as behavioral despair, further supports the emergence of a depression-like phenotype following NPs exposure (Fig. 3). Collectively, the consistent findings across these diverse behavioral paradigms robustly demonstrate that NPs exposure induces a depression-like state in mice. Our finding that NPs exposure intensifies the behavioral outcomes of CUMS is particularly significant, indicating that NPs exposure not only triggers depression-like behaviors but also amplifies the effects of CUMS. These results indicate that exposure to NPs may interfere with emotional regulation in mice and exacerbate characteristics associated with stress-induced depression.

Cognitive impairment induced by NPs exposure in mice

The hippocampus is a pivotal structure within the limbic system, widely connected to brain regions such as the prefrontal cortex and amygdala, and is critically involved in emotional processing and cognitive functions, particularly learning and memory[16]. Our histological observations show that exposure to NPs causes severe neuropathological alterations in the hippocampus, including disorganized neuronal arrangement, widened intercellular spaces, extensive neuronal loss, and markedly reduced Nissl staining. These morphological alterations were more severe in the NPs+Mod group than in the Mod group alone, strongly implying that NPs exposure exacerbates CUMS-induced hippocampal neuropathology, thereby contributing to the observed emotional dysregulation (Fig. 4).

Consistent with the hippocampus's critical role in cognition, hippocampal impairment also substantially affects spatial memory [17]. The Morris water maze test assesses spatial learning and memory in mice. Findings showed that, compared with the control group, the NPs group, Mod group, and NPs+Mod group spent less time in the target quadrant, took fewer paths within that quadrant, and exhibited reduced goal-directed movement. Additionally, compared with the Mod group, the NPs+Mod group spent even less time in the target quadrant and took fewer paths. This strongly indicates that exposure to NPs alone impairs hippocampal-related learning and memory functions and compounds cognitive deficits caused by CUMS (Figs. 5 and 6).

NPs exposure causes neuronal damage in mice

BDNF is a signaling protein essential for neuroplasticity and is primarily found in the central nervous system. It regulates neuroregeneration and neuroprotection by activating the TrkB receptor. Evidence shows that activating the BDNF/TrkB signaling pathway can reduce ischemic stroke damage while promoting the production of vascular endothelial cells [18,19]. BDNF exerts proliferative, trophic, and maturational effects on diverse neurons, promoting neuronal growth and the repair of neural structures; its expression levels can serve as a direct indicator of neural functional recovery [20,21]. Research by Liu et al. [20] indicated that enhancing the BDNF/TrkB signaling pathway may reduce neurotoxicity caused by tetrahydropalmatine, which can manifest as depression, anxiety, and cognitive deficits. Decreased expression of BDNF is associated with neuronal injury that could disrupt brain function and cause depression [22]. In this research, qPCR and Western blotting were employed to investigate mRNA transcription and protein expression levels of BDNF and TrkB in hippocampal tissues from mice across groups following the final exposure. The findings indicate that exposure to NPs significantly decreased the expression of both BDNF and TrkB. Furthermore, compared to the Mod group, the NPs+Mod group exhibited a more pronounced reduction in BDNF and TrkB levels. This downregulation of the BDNF/TrkB signaling pathway likely underlies the

observed neuronal damage in the hippocampus and, consequently, the exacerbated impairment of neuronal function, as well as the associated depression-like behavior and cognitive deficits, in CUMS mice exposed to NPs (Fig. 7).

In conclusion, this study demonstrates that NPs exposure exacerbates neurotoxicity in CUMS mice, worsening depression-like behavior and cognitive impairment. Mechanistic analysis indicates that these effects are mediated primarily through disruption of the BDNF/TrkB signaling pathway, resulting in significant hippocampal neuronal damage. These findings provide crucial insight into how NPs affect the central nervous system, offer a scientific basis for understanding NPs-induced neuropathology, and suggest potential therapeutic targets for stress-related mood and cognitive disorders.

The present study has several limitations that warrant consideration. We used a specific polystyrene NP model with a defined size, which does not capture the vast heterogeneity of environmental NPs; thus, our findings may not be fully generalizable to other particle types. Furthermore, although the study focused on the BDNF/TrkB pathway, the observed neurotoxicity is likely multifactorial, potentially involving interrelated processes such as oxidative stress, neuroinflammation, and cell death that were not fully elucidated here. Future studies should use more diverse NP models and employ multiomics approaches to systematically delineate the complex interplay of downstream mechanisms.

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Author's contributions

DC, MX, YS and JL conceiving and designing the study; DC, MX, WS, YH, ZW and ZN collecting the data; DC, MX, WS, YH, ZW and ZN analyzing and interpreting the data; DC, MX, WS, ZW, ZN and YS writing the manuscript; DC, MX, WS, YH, YS and JL providing critical revisions that are important for the intellectual content. All authors approving the final version of the manuscript.

Conflict of interests

All authors declare that they have no conflicts of interest.

Ethics approval

This study was approved by the Ethics Committee of Hubei University of Science and Technology (Approval Certificate ID:

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Availability of data and materials

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

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