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Environmental enrichment preconditioning prevents chronic social defeat stress-induced memory impairment and hippocampal neurodegeneration

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Abstract. The World Health Organization anticipated major depressive disorder to become the primary contributor to the global burden of disease by 2023. Chronic psychological stress directly impacts synaptic plasticity and the formation of stress-associated memories, contributing to a predisposition to depression. Conversely, research suggests that environmental enrichment, promoting physical activity, social interaction, and sensory stimulation, positively influences neurocognitive functions. However, there is a paucity of studies examining the effects of environmental enrichment on learning and memory in chronic social defeat stress (CSDS) models. Male C57BL/6N mice were (n=5) housed in enriched environments for 30 days before undergoing daily social defeat stress by ICR strain aggressor mice over 10 days. Depressive-like behavior was assessed using the forced swim test (FST) and tail suspension test (TST). Memory and learning functions were evaluated through the Y-maze and novel object recognition (NOR) tests. Brain tissues were subjected to Hematoxylin and Eosin staining to examine histological alterations in the hippocampal region. Environmental enrichment significantly alleviated depressive-like behavior in mice subjected to CSDS. Dysfunctions in spatial working memory and recognition memory as well as hippocampal degeneration induced by CSDS were notably improved in mice exposed to environmental enrichment as compared to those without exposure. This study highlights the significance of environmental enrichment preconditioning in ameliorating memory and learning impairments induced by CSDS.

Keywords: chronic stress, environmental enrichment, hippocampal, memory, neurodegeneration, social defeat

INTRODUCTION

Chronic social defeat stress (CSDS) in humans can be understood through experiences of sustained social stressors such as bullying, workplace harassment, and social exclusion, which are prevalent worldwide, including in Asian countries. In the workplace, bullying is a significant issue, with studies indicating that approximately 15% of employees in Japan experience workplace bullying, leading to chronic stress, anxiety, and depression (Tsuno *et al.*, 2015). In South Korea, about 3 in 10 employees report workplace bullying, which significantly affects their mental health ("3 In 10 Workers Experience Workplace Harassment: Poll," 2024). School bullying is also a critical concern in Asia, with 32% of students in India reporting being bullied,

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contributing to mental health issues such as depression and suicidal ideation (UNESCO, 2019). In China, nearly 17% of students experience bullying, which similarly impacts their mental health (Zhang *et al*., 2019). Additionally, social isolation, exacerbated by the COVID-19 pandemic, poses serious mental health risks globally, including in Asian countries, leading to loneliness and increased stress levels (Holt-Lunstad, 2021).

The significance of chronic social defeat stress lies in its profound impact on mental health, neurobiology, and physical health. CSDS is linked to mental health disorders including major depressive disorder, anxiety disorders, and post-traumatic stress disorder (PTSD) due to the persistent state of social stress and perceived defeat (Murthy, 2024). Neurobiological changes associated with CSDS in humans include alterations in brain regions critical for emotional regulation, such as the prefrontal cortex and hippocampus (McEwen, 2017). Chronic stress also affects physical health, increasing the risk of cardiovascular diseases, weakening the immune system, and contributing to metabolic disorders (Cohen *et al*., 2007). In the workplace, the effects of chronic social stress led to decreased productivity, higher absenteeism, and increased turnover rates, impacting both individual well-being and economic outcomes (Hoel *et al*., 2004). Social and interpersonal relationships suffer as well, with individuals experiencing social defeat stress often struggling with trust issues and social withdrawal (Heinrich & Gullone, 2006). Furthermore, the intergenerational effects of chronic social stress, potentially transmitted through epigenetic mechanisms, suggest that the children of affected individuals may also be at higher risk for mental health problems (Yehuda *et al*., 2014).

One such intervention, environmental enrichment, has emerged as a promising strategy to promote cognitive resilience and protect against neurodegeneration. Environmental enrichment (EE) entails providing animals with a complex and stimulating environment, rich in social, cognitive, and physical stimuli. Prior studies have indicated that environmental enrichment positively impacts synaptic plasticity, neurogenesis, and cognitive function, among other aspects of brain function. In clinical populations, environmental factor modification appears to have comparable beneficial benefits. Human exposure to various EE components has been shown to have beneficial impacts on mental illnesses, such as physical exercise in depressive patients, cognitive activities to lower the risk of dementia, and social contact for bipolar disorder. (Craft *et al*., 2012; Morres *et al*., 2019; Viola *et al*., 2011; Najar *et al*., 2019; Stanton *et al*., 2013; Frank *et al*., 2007). However, its potential to mitigate the cognitive deficits and neurodegenerative histological changes induced by chronic social defeat stress remains relatively unexplored. Therefore, this study aims to investigate the impact of environmental enrichment preconditioning on memory impairment and neurodegeneration induced by chronic social defeat stress. Through a comprehensive battery of behavioural tests and histological analyses, we seek to elucidate the neuroprotective effects of environmental enrichment and unravel the underlying mechanisms mediating its beneficial effects.

MATERIALS AND METHODS

Animals

Fifteen male C57BL/6N mice (8–16 weeks old) were divided into three groups; 1) environmental enriched cage and later subjected to social defeat stress (EE-CSDS, n=5), 2) normal standard cage and later subjected to social defeat stress (CSDS, n=5), and 3) normal standard cage without being subjected to social defeat stress (normal, n=5). To promote territorial aggressive behaviour for the social defeat stress paradigm later, male ICR mice (older than 5 months) were kept separately in different cages. The animals were kept under controlled environmental conditions, with a temperature of 21±1°C and humidity maintained at 65±5%. The mice were kept in a 12-hour cycle of light and dark conditions, with food and water provided at all times.

Environmental enrichment

As previously described by de Souza *et al*. (2019), the environmental enrichment cage (44 cm × 32 cm × 18 cm) comprises various stimuli including a running wheel, plastic tubing, ladders, rubber

balls, and a wooden shelter. The C57BL/6N (n = 5) were kept in the enriched environment settings for consecutive 30 days.

Chronic social defeat stress

Chronic social defeat stress was conducted as previously described by Toyoda *et al.* (2017). The social defeat paradigm is initiated when the experimental mice (C57BL/N mice) are introduced into the home cage compartment of an older, aggressive, dominant aggressor (ICR mice). According to Miczek *et al.* (1982), the intruder (C57BL/N mice) is considered to be "socially defeated" when they exhibit behavioral submission towards the dominant aggressor (ICR mice). On day-31, each C57BL/6N mouse was placed into the home cage of ICR mouse (resident) where it was physically defeated for duration of five minutes. After the physical interaction, both mice were maintained for 24 hours in separate compartments of the same cage, divided by a perforated plexiglass partition. The same paradigm was repeated for ten consecutive days using different resident ICR mice. On the other hand, the control group C57BL/6N mice were housed in pairs in similar cages for 10 days.

Assessment of stress and anxiety

Force swim test (FST)

The forced swim test was utilized to evaluate the mice's active coping mechanisms in the face of unavoidable stress. On day-41, mice were individually placed in a glass cylinder (30 cm height, 20 cm width) which was filled with water (23–25°C) for duration of six minutes. Given that mice naturally float on water, immobility is defined by the absence of movement, except for that required to maintain the animal's nose above the water level. The total duration of immobility was measured during the last 4-minutes of the test (Lee *et al.*, 2017).

Tail suspension test (TST)

The tail suspension test was used to evaluate depressive-like behaviour on day-42. Each mouse's tail was individually strung by adhesive tape from a horizontal ring-stand bar at 25 cm from the floor using the tails as a point of attachment. Throughout a 5-minute test session, the time spent by the mice being immobile was recorded. The total duration of time when the mice hang passively and motionlessly was measured (Lee *et al.*, 2017).

Cognitive function assessment

Y-maze Test

The Y-maze task was performed on day-43 to assess spatial memory capacity. With one arm closed by using a divider, the mouse was given 15 minutes to freely explore the other two arms of the labyrinth. After a period of 24 hours of inter-trial interval, the mouse was let to freely explore all three arms of Y-maze for 5 minutes. The sequence (such as ACBCAB, etc.) and the number of arm entries were then recorded for each mouse. The percentage alternation score for each mouse was calculated by the number of alternations divided by the total number of arm entries and multiplied by 100 (Sanderson *et al.*, 2009).

Novel object recognition test

A novel object recognition test was used in this experiment to assess recognition memory in mice using a square open area made of white polyvinyl plastic experimental setup. On day-45, the mice were placed in the experimental apparatus with two identical items and given five minutes to explore the items. The mice were placed back into their housing cage for a 20-minute retention period. Afterwards, the mice were returned to the apparatus for duration of 5 minutes, with one of the two objects replaced by a novel item. The duration of the time spent exploring each object was recorded. The discrimination ratio was calculated was calculated as described by Lee *et al.* (2017).

Haematoxylin and eosin staining

Following the behavioural tests, the mice were anaesthetized with a mixture of ketamine and xylazine and were euthanized via decapitation. The brains that were extracted underwent fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS) with a pH of 7.4, followed by embedding in paraffin. The brain tissues were then sectioned with a Leica Biosystems microtome (Wetzlar, Germany) at a thickness of 5 μM in the coronal plane. Haematoxylin and eosin (H&E) staining of the resultant sections allowed for the easier visualization of pathological alterations at 4x and 20x magnification, which were subsequently inspected under an Olympus

light microscope located in Tokyo, Japan. The extent of the hippocampus damage was assessed by a neuropathologist, blindly, using a light microscope (Olympus 4009).

Data analysis

Data were averaged across animals and were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to measure the significant difference in terms of the outcomes between the three groups. Data were analyzed with the software SPSS where the level of significance was set at *p < 0.05, **p<0.01, and ***p<0.001.

RESULTS

The effect of environmental enrichment preconditioning on depressive-like behaviour in chronic social defeat stress mice

Force swim test

Based on Figure 1, the depression resulting from 10 days of social defeat stress was evident as shown by the significant difference (p<0.001) in the duration of immobility during force swim test between the control CSDS mice (156 ± 35.08 s) and normal mice (45 ± 8.6 s). Meanwhile preconditioning with environmental enrichment significantly improves (p<0.05) this condition as observed in lower duration of immobility in EE-CSDS mice (102 ± 43.44 s).

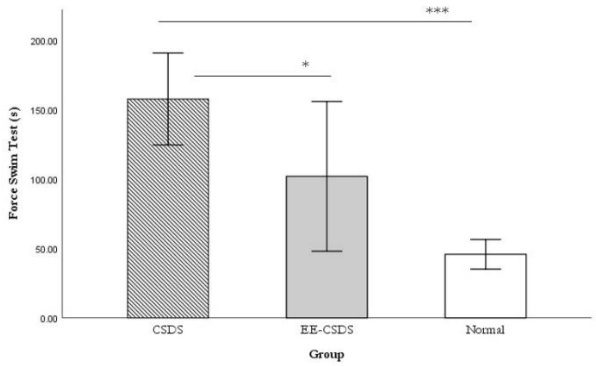

Figure 1. Immobility duration during force swim test, *p<0.05, ***p<0.001.

Tail suspension test

Similarly, during the tail suspension test shown in Figure 2, the control CSDS mice displayed a significant increase in the immobility time signifying helpless behaviour as compared to the normal mice (245 ± 31.36 s vs 154 ± 30.15 s, p<0.01). On the contrary, immobility was seen to be significantly reduced when the mice were subjected to environmental enrichment prior to CSDS as compared to the CSDS mice group (172 ± 29.18 vs 245 ± 31.36, p<0.01).

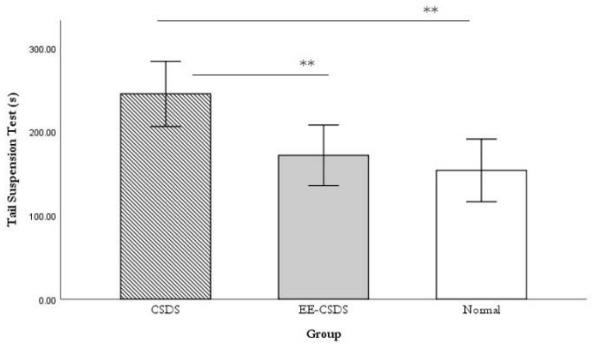

Figure 2. Immobility duration during tail suspension test, **p<0.01.

The effect of environmental enrichment preconditioning on spatial memory in chronic social defeat stress mice

Figure 3 shows that the control CSDS mice exhibited a significantly lower alternation rate compared to the normal mice group (p<0.05). However, one month of environmental enrichment preconditioning markedly improved this reduction in mice subjected to CSDS (p<0.01). A higher alternation percentage is generally considered indicative of good spatial memory in rodents, as they naturally explore the previously unvisited arm, sequentially visiting all three arms. This is further supported by the comparable rate of total arm entries (p>0.0.05) observed between the groups (Figure 4).

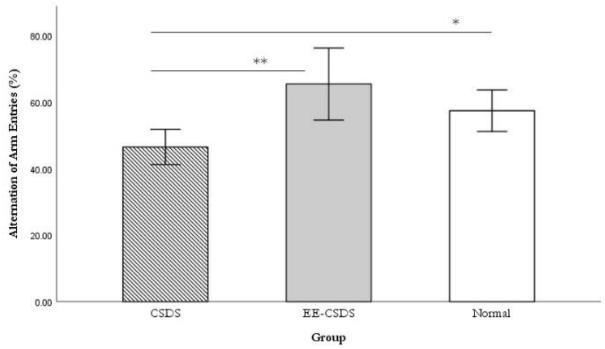

Figure 3. Percentage of Y-Maze Arm Alternations, *p<0.05, **p<0.01.

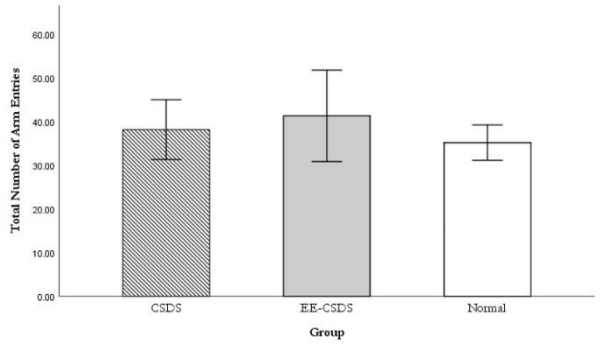

Figure 4. Number of Y-Maze Total Arm Entries.

The effect of environmental enrichment preconditioning on recognition memory in chronic social defeat stress mice

Based on Figure 5, the control CSDS mice showed significant impairment in recognition memory ability as compared to the normal mice (p<0.001). Meanwhile, preconditioning with environmental enrichment significantly reverses this impairment as seen in the EE-CSDS group as compared to the control CSDS group (p<0.001).

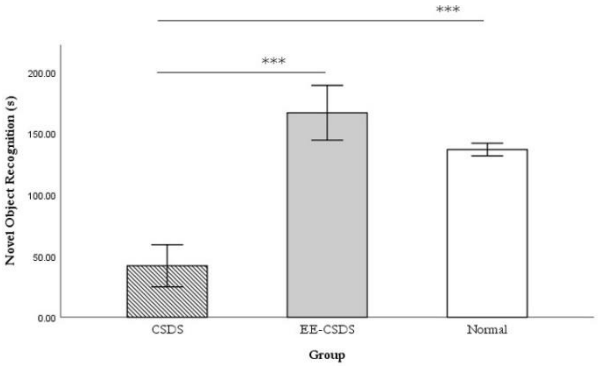

Figure 5. Novel object exploratory time, ***p<0.001.

The effect of environmental enrichment preconditioning on hippocampal neuro-degeneration in chronic social defeat stress mice

As shown in Figure 6, the histopathological examination of the hippocampal cornu ammonis subfields (CA1, CA3) and dentate gyrus (DG) revealed displacement of many pyramidal cells, reduction in the pyramidal cell layer thickness, and prominent necrosis of pyramidal neurons evident by shrunken cells and nucleus as well as acidophilically stained cytoplasm in the control CSDS mice as compared with the normal mice. Meanwhile, EE preconditioning was shown to salvage the neuronal loss in the EE-CSDS mice.

DISCUSSION

Chronic stress is a major risk factor for depression, and memory deficits are a common symptom of depressive disorders (Krishnan & Nestler, 2011). Additionally, it has been suggested that persistent stress hastens the course of neurodegenerative illnesses including Parkinson's and Alzheimer's diseases (Bisht *et al.*, 2018, Juszczyk *et al.*, 2021). Therefore, elucidating the impact of CSDS on memory function and neuronal integrity may provide valuable insights into the early pathophysiological changes that contribute to depression and neurodegeneration. Given the growing interest in non-pharmacological interventions for stress-related disorders, such as environmental enrichment, there is a compelling rationale to explore its potential as a preventive measure against the deleterious effects of chronic stress on brain health (Han *et al.*, 2022). The present study aims to investigate the potential protective effects of environmental enrichment preconditioning against memory impairment and neurodegeneration induced by chronic social defeat stress.

The trait of helplessness, often observed in response to chronic social stress, serves as a key indicator of depressive-like behaviour in preclinical models. Chronic social stressors, such as social defeat, can induce a state of perceived uncontrollability and hopelessness, leading to maladaptive coping strategies reminiscent of

human depression (Anisman & Merali, 2001). Rodents subjected to chronic social stress often exhibit reduced interest in previously rewarding activities, heightened sensitivity to negative stimuli, and increased immobility in behavioural despair tests, reflecting a learned helplessness phenotype (Anisman & Merali, 2001). This behavioural manifestation aligns with the core features of depressive-like behaviour, suggesting that the trait of helplessness serves as a reliable indicator of the depressive-like state induced by chronic social stress.

In this study investigating the effect of environmental enrichment preconditioning on depressive-like behaviour and spatial memory in mice subjected to chronic social defeat stress, significant findings emerged from two behavioural assays. Firstly, following 10 days of CSDS, EE-CSDS mice displayed significantly reduced immobility during the force swim test compared to control CSDS mice, indicating a potential amelioration of depressive symptoms in the EE-preconditioned group. Similarly, in the tail suspension test, EE-CSDS mice exhibited significantly less immobility compared to CSDS mice, further suggesting a beneficial effect of EE preconditioning on depressive-like behaviour. These results underscore the potential of environmental enrichment as a preventive strategy against the development of depressive symptoms in the context of chronic social stress.

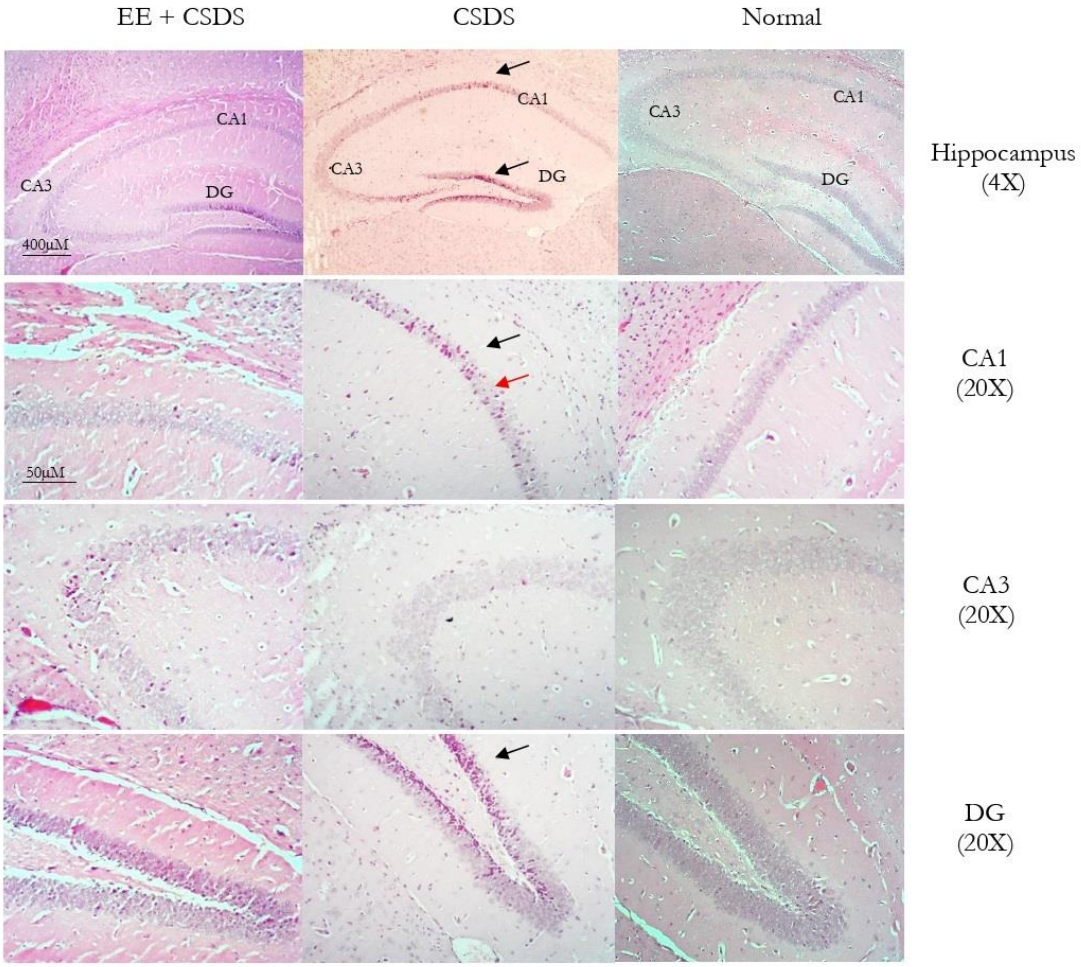

Figure 6. Histology of three hippocampal subfields (CA1, CA3, DG). Black arrow indicates prominent necrosis of pyramidal neurons evident by shrunken cells and acidophilic stained cytoplasm. Red arrow indicate reduction in the thickness of pyramidal cell layer. CA1: cornu ammonis regions 1, CA3: cornu ammonis regions 3, DG: Dentate gyrus

In a research article written by *Hendershott et al.* (2016), they declared that environmental enrichment improves rodent activity and reduces anxiety-like behaviour in female C57BL/6J mice while our findings show a similar pattern of enhanced activity and reduction of anxiety-like behaviour in male C57BL/6N mice. This demonstrates that environmental enrichment does have a positive impact on the exploratory mechanism and emotional behaviour of rodents, despite the gender and strain. A previous study also stated that an enriched environment increased the number of transitions between two compartments in a Light-Dark Transition Test which indicates that environmental enrichment does increase the exploratory activity despite being induced with chronic social defeat stress (Benaroya-Milshtein *et al.*, 2004; Roy *et al.*, 2001).

Chronic social defeat stress has been extensively studied for its detrimental effects on memory function and neuronal integrity. Rodent models subjected to CSDS consistently exhibit impairments in various memory tasks, including spatial memory, object recognition, and contextual fear conditioning (McEwen *et al.*, 2015; Bagot *et al.*, 2016). Structural changes in brain areas important for memory development and consolidation, like the prefrontal cortex and the hippocampus, frequently accompany these impairments in memory (Herman *et al.*, 2012). Mechanistically, chronic stress-induced dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis leads to prolonged elevation of glucocorticoid levels, impairing hippocampal function and promoting neuronal damage (McEwen, 2017). Additionally, chronic stress-induced inflammatory responses and oxidative stress contribute to synaptic dysfunction and neuronal loss (Ramirez *et al.*, 2017; McKim *et al.*, 2016). Elevated free radicals are associated with oxidative damage to the cells, affecting cognitive and psychological function. Therefore, inhibiting oxidative stress by introducing antioxidant agents could provide an alternative mechanism to prevent neurodegenerative effects (Aidiel *et al.*, 2024). Epigenetic modifications, including changes in DNA methylation and histone acetylation, may further mediate long-lasting alterations in gene expression underlying memory deficits and neuronal vulnerability to stress (Golden *et al.*, 2013).

Environmental enrichment (EE) preconditioning has emerged as a promising intervention strategy for mitigating the deleterious effects of chronic stress on cognitive function and neuronal integrity. EE involves providing animals with enhanced sensory, cognitive, and social stimulation in their living environment, typically through the provision of toys, running wheels, social interaction, and varied housing structures (Sale *et al.*, 2007). Preclinical studies have demonstrated that exposure to EE prior to stress exposure can confer resilience against stress-induced cognitive decline and neuronal damage, highlighting its potential as a preventive measure against the negative consequences of chronic stress on brain health (Gelfo & Petrosini, 2022; Schoentgen *et al.*, 2020).

Several lines of evidence support the neuroprotective effects of EE against stress-induced cognitive decline and neuronal damage. Studies have shown that animals housed in EE exhibit preserved cognitive function and reduced neuronal loss in response to chronic stress compared to those housed in standard laboratory conditions (Han *et al.*, 2022; Xu *et al.*, 2016). Moreover, EE has been shown to enhance neurogenesis, synaptic plasticity, and dendritic branching, which are important mechanisms underlying cognitive function and neuronal resilience (Kimura *et al.*, 2021). Additionally, EE promotes the secretion of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1), which play key roles in promoting neuronal survival, growth, and synaptic plasticity (Yolanda *et al.*, 2019). Collectively, these findings provide compelling evidence for the neuroprotective effects of EE against stress-induced cognitive decline and neuronal damage.

The protective effects of EE against stress-induced cognitive decline and neuronal damage may be mediated by several mechanisms, including neuroplasticity, neurotrophic factors, and inflammatory pathways. EE has been shown to promote structural and functional neuroplasticity in the brain, leading to enhanced synaptic connectivity, dendritic arborization, and neurogenesis (Sale *et al.*, 2007). These changes contribute to improved cognitive function and resilience against stress-induced neuronal damage. Furthermore, EE upregulates the expression of

neurotrophic factors such as BDNF and IGF-1, which promote neuronal survival, growth, and synaptic plasticity (Yolanda *et al.*, 2019). Additionally, EE exerts anti-inflammatory effects, reducing the production of pro-inflammatory cytokines and microglial activation, which contribute to neuroinflammation and neuronal damage (Kimura *et al.*, 2021). Overall, the multifaceted effects of EE on neuroplasticity, neurotrophic factors, and inflammatory pathways collectively contribute to its neuroprotective effects against stress-induced cognitive decline and neuronal damage

CONCLUSION

This study highlights the significance of environmental enrichment preconditioning in ameliorating memory and learning impairments as well as histopathological changes induced by chronic social defeat stress.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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