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Research report

The neurogenic effects of an enriched environment and its protection against the behavioral consequences of chronic mild stress persistent after enrichment cessation in six-month-old female Balb/C mice

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HIGHLIGHTS

- Pro-neurogenic effects of environmental enrichment (ENR) persist after cessation.
- ENR cessation does not decrease doublecortin-positive cells in six-month-old mice.
- ENR induces behavioral protection against chronic mild stress.

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ABSTRACT

Because stress may underlie the presence of depressive episodes, strategies to produce protection against or to reverse the effects of stress on neuroplasticity and behavior are relevant. Preclinical studies showed that exposure to stimuli, such as physical activity and environmental enrichment (ENR), produce beneficial effects against stress causing antidepressant-like effects in rodents. Additionally, ENR induces positive effects on neuroplasticity, neurochemistry and behavior at any age of rodents tested. Here, we analyzed whether ENR exposure prevents the development of depressive-like behavior produced by unpredictable, chronic mild stress (CMS) exposure as well as changes in hippocampal neurogenesis in a six-month-old female Balb/C mice, strain that shows low baseline levels of hippocampal neurogenesis. Mice were assigned to one of four groups: (1) normal housing-normal housing (NH-NH), (2) NH-CMS, (3) ENR-NH, or (4) ENR-CMS. The animals were exposed over 46 days to ENR or NH and subsequently to NH or CMS for 4 weeks. ENR induces long-term effects protecting against CMS induction of anhedonia and hopelessness behaviors. Independent of housing conditions, ENR increased the number of proliferative cells (Ki67), and CMS decreased the number of proliferative cells. ENR increased the newborn cells (BrdU) and mature phenotypes of neurons; these effects were not changed by CMS exposure. Similarly, the number of doublecortin-positive cells was not affected by CMS in ENR mice, which showed more cells with complex dendrite arborizations. Our study suggests that ENR induces protection against the effects of CMS on behavior and neuroplasticity in six-month-old Balb/C mice.

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1. Introduction

Hippocampal neurogenesis is considered a highly dynamic process involving a series of events that together result in the production of new neurons [1]. The function of the new neurons is related to learning and memory processes. However, altered neurogenesis is present in preclinical models of psychiatric disorders [2–4]. In this sense, several studies have theorized that a decrease in hippocampal neurogenesis induced by negative factors, such as stress, could be implicated in the pathogenesis of depression and anxiety [4–9]. With regard to these theories, psychological stress induces signs of depression, such as anhedonia, despair and corticosterone hypersecretion [6,10–13] that underlie a variety of neuroplastic changes in the dentate gyrus of the hippocampus in rodents [10,14–16]. Additionally, alterations in the expression of certain factors associated with different steps of the neurogenic process, such as growth factors (FGF, EGF) and neurotrophins (BDNF, NT3), decrease after stress [17–19].

Moreover, other factors, such as aging, can negatively modulate neuroplastic changes in the brain (i.e., [20]). Aging is a complex physiological process associated with an increase in the incidence of cognitive dysfunction or neurodegenerative disease [20,23,24]. During age-related cognitive decline, the hippocampus exhibits a marked decrease in neurogenesis in the dentate gyrus [21,24–32]. Recently, we observed that a significant decline in the neurogenic process occurs an approximate age of 5–8 months in Balb/C mice [22].

In addition, several investigations have suggested that the exposure to a variety of social, cognitive and physical stimuli could act as a positive factor in the treatment of a variety of behavioral alterations related to chronic stress exposure [14,33–36]. Among these paradigms, environmental enrichment (ENR) is considered a strong inducer of hippocampal neurogenesis [20,31,37–40] and neuronal plasticity [41]. Preclinical studies based in animal models of depression have shown that ENR improves cognitive functions [20,42,43], reversing depressive-like behavior and conferring a restoration of decreased neurogenesis and hippocampal plasticity induced by exposure to stress [14,34,44,45]. In fact, it has been shown that exposure to ENR during a period of chronic stress is able to block the detrimental effects induced by chronic restraint stress on neuronal plasticity and hippocampal function [33,35].

Despite great interest in the neurobiological, behavioral and protective effects of ENR, few studies have focused on the persistence of its effects, particularly during the critical period of life where a significant decline of the neurogenic process, such as aging, is presented [21,25,46]. Thus, in this work we assessed the persistence of the neurogenic and protective effects of ENR against depressive behavior induced by CMS in six-month-old female Balb/C mice.

2. Materials and methods

2.1. Animals

Female BalbC mice were obtained from Harlan (Ciudad de México, México). They were housed in standard laboratory cages under 12-h light/12-h dark cycles at a temperature of 23 ± 1 °C in the animal facilities of the National Institute of Psychiatry "Ramón de la Fuente Muñiz". The light/dark cycle corresponded to the timing of lights on (Zeitgeber time 0; ZT0) at 0700 h and to the timing of lights off (Zeitgeber time 12; ZT12) at 1900 h. Mice had access to food and water ad libitum and were left to acclimate to their new environment until the animals reached the age of 6 months. All institutional and legal regulations regarding animal ethics and handling were followed for in vivo experiments; animal experiments were approved by the Ethical Committee of the National Institute of Psychiatry (IACUC: CEI/C/02/06/20/09). The age of mice at the end of the experiment was approximately 8.5 months old (Fig. 1).

2.2. BrdU labeling, normal housing and environmental enrichment

To label proliferating cells, mice were administered 50 mg/kg of BrdU (Sigma, Toluca, Estado de México, México) daily during the first 10 of a total of 45 days in normal housing (NH) or ENR (Fig. 1). NH mice were housed in a normal cage. However, ENR mice were housed in a large box (34 × 44 × 20 cm) containing tunnels of different colors and shapes, two-running wheels, pieces of wood, nesting materials and small plastic houses with stairs. The ENR complexity was changed every third day to avoid habituation. After housing conditions were ended (day 45), mice were habituated to 1% sucrose solution to determine basal preference (46, 48 and 50 days). Mice were divided into 4 groups: (1) NH plus 4 additional weeks of NH (NH-NH); (2) ENR plus 4 additional weeks of NH (ENR-NH); (3) NH plus 4 additional weeks of CMS (NH-CMS) or (4) ENR plus 4 additional weeks of CMS (ENR-CMS; Fig. 1). In all case, ENR did not persist during the CMS period. Thus, mice were housed in standard laboratory cages during the periods that they were not exposed to stress. Eight mice per group were used.

2.3. Chronic mild stress

The CMS was adapted from the original protocol in accordance to a recent report [47–49]. Briefly, mice in groups 3 and 4 were exposed to a different semi-random type of stressor (Table 1) for 4 weeks in accordance with a previous report (Fig. 1) [48–50].

In order to evaluate anhedonia during the course of the 4 weeks (Fig. 1), mice were deprived of food and water one day of every week for 18 h before the sucrose preference test, and then animals were exposed for 1 h to two-flasks, one containing 1% sucrose and the other containing drinking water. To avoid place preference, flask positions were exchanged during every weekly test. Sucrose preference was calculated according to the following formula: Percentage of preference = ((sucrose solution intake/total intake) × 100%) as previously reported [36]. Anhedonia was reflected as a reduction in sucrose preference reaching a value equal or lower than 50% [36].

2.4. Porsolt test

One day after the end of the CMS protocol, mice were exposed to the Porsolt's forced swim test (FST) [50]. Mice were habituated to the testing room for 30 min before behavioral recording. Behavioral testing was performed at the end of the dark phase of the light/dark cycle (ZT23). The testing area was dimly lit to reduce additional stress or anxiety. During the analysis of the FST,

Table 1
Stressors used in the CMS.

Stressor	Exposure time
Grouped housing (8 mice per cage)	8 h
Water deprivation	18 h
Food deprivation	18 h
Continuous light	24 h
Cold room (4 °C)	15 min
Stroboscopic light	3 h
Constant motion (100 rpm)	30 min
White noise	12 h
Movement restriction	1 h
Dirty or wet cage	12 h

During the CMS protocol two or three stressors were applied daily. The sequence and combination were changed every week. The time duration of stressors is shown in the right column of the table.