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ENVIRONMENTAL ENRICHMENT INDUCES NEUROPLASTIC CHANGES IN MIDDLE AGE FEMALE BALBC MICE AND INCREASES THE HIPPOCAMPAL LEVELS OF BDNF, P-AKT AND P-MAPK1/2

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Abstract—Hippocampus is one of the brain regions in which neuroplastic changes occur. Paradigms such as environmental enrichment (ENR) have been used to prevent or delay the neuroplastic changes of the hippocampus during aging. Here, we investigated the beneficial effects of ENR on dendritic spines and hippocampal neurogenesis in middle age BalbC mice. ENR increased the number of dendritic spines, cell survival, and intermediate stages of the hippocampal neurodevelopment process. Also, ENR alters the distribution of cells involved in the neurogenic process along the dorsal–ventral dentate gyrus. In addition, ENR increased the proportion of cells with more mature dendritic morphology and net hippocampal neurogenesis. Whole-hippocampus protein extracts revealed that ENR increases the levels of BDNF, phospho-Akt and phospho-MAPK1/2, suggesting that the positive effects of ENR on neuroplasticity in middle age BalbC mice involve the participation of these key-signaling proteins. Our results suggest that ENR is a relevant strategy to prevent neuroplastic decline by increasing the formation of both dendritic spines and new neurons in the hippocampus during middle age.

Key words: neuroplasticity, neurogenesis, hippocampus, aging, BDNF.

INTRODUCTION

Hippocampus, a brain region that belongs to the limbic system, is involved in learning and memory (Moser et al., 1995; Bannerman et al., 2004; Steffenach et al., 2005; Draganski et al., 2006; Garthe et al., 2009). During aging, it exhibits alterations of dendritic spines and neurogenesis in the dentate gyrus (Kuhn et al., 1996; Calhoun et al., 1998; Cameron and McKay, 1999; Foster, 1999; Kempermann et al., 2002; Bondolfi et al., 2004; Heine et al., 2004; Lawrenson et al., 2004; Kronenberg et al., 2006; Klempin and Kempermann, 2007; Couillard-Despres et al., 2009). Interestingly, during the age-related cognitive decline, dendritic spines change their structure and lose their synaptic function (Calhoun et al., 1998; Phinney et al., 1999; Hof and Morrison, 2004; Govoni et al., 2010). Moreover, in some pathological conditions such as schizophrenia, bipolar disorder, and depression, dendritic spines change their shape and show a decrease in size and density (Rudelli et al., 1985; Comery et al., 1997; Glantz and Lewis, 2000; Ramakers, 2002; Kolomeets et al., 2005; Carlson et al., 2011; Penzes et al., 2011).

Another form of brain plasticity affected by external factors or during aging is hippocampal neurogenesis (Kuhn et al., 1996; Kempermann et al., 2002; Bondolfi et al., 2004; Heine et al., 2004; Couillard-Despres et al., 2009). The changes observed in this type of plasticity have been related to the differences in the content of brain- and systemic-milieu found along aging (Villeda et al., 2011), but also to alterations in the neural stem cell population, at least in the hippocampus (Encinas and Sierra, 2012).

In order to delay or revert the effects of aging on neuroplasticity and hippocampal neurogenesis, several paradigms have been used such as the exposure to a variety of social and physical stimuli called environmental enrichment (ENR) (Ickes et al., 2000; van Praag et al., 2000; Kempermann et al., 2002; Brown et al., 2003; Schneider et al., 2006; Fabel et al., 2009; Goshen et al., 2009; Mirochnic et al., 2009; Ehninger et al., 2011). The benefits of this type of environment have been demonstrated in different mouse strains (Kempermann et al., 1997; Brown et al., 2003; Holmes et al., 2004; Mirochnic et al., 2009; Tanti et al., 2012). ENR promotes neurogenesis in the hippocampus of young and senescent C57Bl6 mice (Kempermann et al., 1998). Also, it promotes differential regulation of neurogenesis through the dorsal–ventral dentate gyrus

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Abbreviations: BDNF, brain-derived neurotrophic factor; CR, calretinin; DCX, doublecortin; EDTA, ethylenediaminetetraacetic acid; ENR, environmental enrichment; GCL, granular cell layer; NH, normal housing; PI3K, phosphatidylinositol-3-kinase; TTBS, Tween 20-TBS; ZT, Zeitgeber time.

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in young male BalbC mice (Tanti et al., 2012). Interestingly, young mice of this strain are widely used for studies related to chronic stress (Tanti et al., 2012). Recently, we observed a decline of the neurogenic process in BalbC mice at the age of 5–8 months (Ramirez-Rodriguez et al., 2012). However, the impact of ENR on the neuroplasticity of middle age female BalbC mice has not been explored.

Mediators of neuroplasticity have been described; among them is the brain-derived neurotrophic factor (BDNF) (Barde, 1994; Vaynman et al., 2004; Dijkhuizen and Ghosh, 2005; Hattiangady et al., 2005; Sairanen et al., 2005). This neurotrophin is involved in dendritic spine formation, survival of hippocampal newborn neurons, and shows a relevant participation in memory formation (Barde, 1994; Barnabe-Heider and Miller, 2003; Dijkhuizen and Ghosh, 2005; Babu et al., 2009; Minichiello, 2009).

Considering that dendritic spines and hippocampal neurogenesis are altered during aging and that a significant decline of the neurogenic process occurs around the age of 5–8 months in BalbC mice, we hypothesized that ENR may induce neuroplastic changes related to the increase of hippocampal BDNF levels concomitant to the modifications in dendritic spines density and in the different events of the neurogenic process along the dorsal–ventral regions in the dentate gyrus. Thus, we assessed middle age-dependent changes in dendritic spines density and the effects of ENR on middle age hippocampal neurogenesis in subpopulations of cells involved in this process in female BalbC mice.

EXPERIMENTAL PROCEDURE

Animals

Thirty-two female BalbC mice were obtained from Harlan (México, D.F. 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 (ZT 12; ZT12) at 1900 h. Mice had access to food and water *ad libitum* and were left to acclimatize in their environment until they were 6 months old. All institutional and legal regulations regarding animal ethics and handling were followed for the *in vivo* experiments (CEI/C/009/2013).

Normal housing (NH), ENR and BrdU labeling

At 6 months of age, mice were daily-administered 50 mg/kg of BrdU (Sigma, St. Louis, MO, USA) during the first 10 days of NH or ENR conditions (Fig. 1). Subsequently, mice were continuously exposed to NH or ENR for 35 additional days (45 days in total). For NH, 5 mice were housed in a normal cage, and for ENR, 10 mice were housed in a big box containing tunnels of different colors and shapes, a running wheel, pieces of wood, nesting materials and small plastic houses with

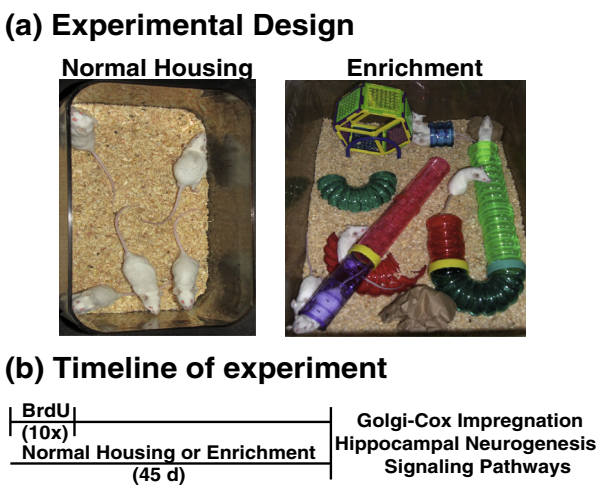

Fig. 1. Experimental design. (a) BalbC female mice were housed under normal housing or environmental enrichment as shown. (b) Timeline of experiment indicates that 6-month-old mice received 10 single injections of BrdU each of 50 mg/kg at the beginning of the experiment. Mice were exposed to the respective housing conditions for 45 days. On day 46, mice were sacrificed to perform the indicated analysis. The age of mice at the end of the experiment was 7 months and 16 days.

stairs. The complexity of the tunnels and the distribution of the other materials were changed every third day to avoid habituation. At day 36, the running wheel was taken out of the ENR and at day 46, mice were sacrificed by an overdose of ketamine and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and post-fixed for 24 h before they were stabilized in 30% sucrose in phosphate buffer (Kempermann et al., 2003; Ramirez-Rodriguez et al., 2009).

Golgi-Cox impregnation

For metal impregnation, brains were excised from the skull and immersed in a Golgi-Cox solution for 21 days. Every third day, the Golgi-Cox solution was replaced for fresh solution. Brains were transferred to a 30% sucrose solution and sliced on a microtome (Leica, Buffalo Grove, IL, USA). Coronal brain sections (100 μm) were collected in 3% sucrose solution and mounted in pre-gelatinized glass slides. Golgi-Cox impregnation was revealed with 18.7% ammonia and fixed in Kodak rapid fix (Kodak, Rochester, NY, USA). Brain slices were dehydrated and clarified in Neoclear (Merck) before they were coverslipped with Neomount medium (Merck, Whitehouse Station, NJ, USA) (Bessa et al., 2009). Twenty neurons in the granular cell layer (GCL) of the dentate gyrus were analyzed to determine dendritic spine density in mice exposed to either NH or ENR.

Tissue processing for immunohistochemistry

Brains were cut into 40-μm coronal sections on a sliding microtome (Leica). The sections were stored at 4 °C in cryoprotective solution containing 25% ethylene glycol and 25% glycerin in 0.05 M phosphate buffer. Sections were stained following the free-floating immunohisto chemistry method. For BrdU-immunodetection brain