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Early life stress accelerates age-induced effects on neurogenesis, depression, and metabolic risk

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ABSTRACT

Early life stress (ELS) affects hippocampal neurogenesis, increases depressive-like behavior, and causes mild metabolic imbalance in early adulthood (2 months). However, whether these effects worsen in mid life remains unclear. To test whether age-dependent effects of ELS on hippocampal neurogenesis are related to deficient hypothalamic–pituitary–adrenal (HPA) axis feedback that causes increased comorbidity of depression and metabolic risk, we evaluated the effects of periodic maternal separation (MS180) in young (4-months-old) and middle-aged (10-months-old) adult rats. MS180 caused more severe depressive-like behavior in middle-aged adults than in young animals. There were no behavioral phenotypic differences between young MS180 and control middle-aged groups. MS180 similarly affected glucose tolerance, increased fasting corticosterone, insulin, and the quantitative insulin sensitivity check index (QUICKI) at both ages. However, middle-aged adult MS180 rats showed more severe age-induced obesity (> 40% BW) than controls (> 22% BW). MS180 differentially affected dorsal and ventral neurogenesis. In young adults, MS180 animals only showed a decrease in dorsal hippocampal neurogenesis as compared to their age-matched counterparts. In contrast, at 10 months of age, MS180 caused a similar decrease in both dorsal and ventral hippocampal neurogenesis as compared to age-matched controls, and a more severe decrease as compared to young animals. Taken together, our data indicate that MS180 animals show an early onset of age-induced alterations on depression and metabolic risk, and these effects relate to alterations in hippocampal neurogenesis.

1. Introduction

Metabolic syndrome (MetS) is a cluster of risk factors that include obesity, insulin resistance, hyperlipidemia, and hypertension. MetS increases the risk for cardiovascular disease and type 2 diabetes (T2DM), and shows a high comorbidity with depression (Zanoveli et al., 2016). However, this relationship is complex and poorly understood. Clinical evidence has shown that while T2DM did not predict symptoms of depression or anxiety, these symptoms emerged as significant risk factors for the onset of T2DM. This relationship was independent of established risk factors (Engum, 2007). Moreover, it has been shown that appropriate control of depressive symptoms in women can improve metabolic imbalances (Rotella and Mannucci, 2013). These findings suggest that there may be a common etiology underlying the comorbidity of these diseases, and that depression is not solely a complication of metabolic imbalances in the brain.

Both depression and MetS are stress-related pathologies that relate to high glucocorticoid (GC) levels. Hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis has been implicated as an important pathophysiological mechanism underpinning both diseases (Zanoveli et al., 2016). GCs increase insulin resistance, stimulate glucose production in the liver, and affect insulin secretion from pancreatic β-cells (Whirledge and DeFranco, 2017). Moreover, circulating GCs follow an age-related trajectory, which can be affected by both the external environment and internal physiological events (Zambrano et al., 2015). Stress-induced rises in GC levels accelerate aging processes and contribute to the development of age-related pathologies (McEwen, 2002). Both GC resistance and GC-enhanced aging have a strong influence on brain metabolic pathways (Chen et al., 2013). Furthermore, a MetS or T2DM phenotype can be rapidly induced in rats by combining

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exogenous administration of GCs and a high fat diet (Shpilberg et al., 2012).

Adverse early environmental experiences, such as child abuse and neglect, contribute to the development of both depression and metabolic disease (Morris et al., 2014). Early life stress (ELS) programs HPA activity by elevating basal GC levels (Doom et al., 2014) and increases individuals' vulnerability to anxiety and depression (Heim et al., 2008). However, there is a paucity of information on whether the age-enhanced trajectory of depression and metabolic risk is affected by early adverse experiences. Maternal separation during the first few weeks of life (MS180) is a widely used rodent model of ELS that mimics many of the long-term consequences observed in victims of child abuse (O'Mahony et al., 2011). Previous work from our group has shown that MS180 causes mild metabolic imbalances in combination with depressive-like behavior in early adulthood. Two-month-old MS180 animals showed a passive coping strategy in the forced swimming test; altered glucose homeostasis; and mildly increased fasting glucose, triglycerides, cholesterol, and body weight (< 10% increase). However, MS180 alone was not sufficient to induce MetS in juveniles (Vargas et al., 2016).

The hippocampus is one of the main structures related to HPA axis negative feedback. Additionally, the hippocampal formation is rich in GC receptors and is highly sensitive to developmental stress (McEwen et al., 1968). The subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) is one of the two neurogenic niches that remain active throughout life. In this region, neural precursors proliferate, differentiate, and migrate to be integrated as part of resident brain circuits (Altman and Das, 1965). Suppression of adult neurogenesis increases basal and stress-induced GC levels, suggesting that these newly generated neurons play a role in functional inhibition of the HPA axis by the hippocampus (Schloesser et al., 2009). In rats, ELS decreases hippocampal neurogenesis (Lajud et al., 2012; Mirescu et al., 2004; Suri et al., 2014). Therefore, it has been proposed that alterations in the activity rates of the neurogenic niche could also contribute to altered negative feedback of the HPA axis that is caused by ELS (Maccari et al., 2014). However, the relationship between MS180-induced effects on neurogenesis, behavioral consequences, and metabolic imbalances have not been studied.

Depression and its comorbidity with MetS or T2DM are considered a more significant health issue in middle-aged than in young individuals (Korten et al., 2012a; Park et al., 2003). Clinical evidence has shown that men tend to have onsets of obesity and hypertriglyceridemia in young adulthood, hypertension in middle age, and diabetes later in life (Tsay et al., 2016). In animal models, the effects of ELS have been mainly studied either before the onset of age-induced increases in metabolic risk and when hippocampal maturation is incomplete (2-month-old juveniles), or after the onset of age-induced metabolic imbalances and hippocampal deterioration (> 16-month-old senescent rats). This has resulted in poor bench-to-bedside translatability of results. Therefore, the aim of the present study was to test the hypothesis that ELS aggravates the effect of age on hippocampal neurogenesis, causing deficiencies in HPA axis feedback that lead to increased comorbidity of depression and metabolic risk. To assess this, we evaluated hippocampal neurogenesis in rats subjected to MS180 at two translationally relevant ages (4 and 10 months of age), and correlated the results with emotionality and metabolic risk.

2. Materials and methods

2.1. Animals

Time-pregnant female Sprague–Dawley E14 rats were obtained from the animal facility of the Neurobiology Institute—UNAM and habituated in our facility for 1week. Animals were maintained in standard temperature-controlled rooms with a 12-h light/dark cycle (lights on at 07:00 h), with free access to food and water (Purina, LabDiet, St. Louis, MO, USA). Day of birth was considered as postnatal day zero (P0). On P1, litters were cross-fostered, culled to eight pups (four to six males), and randomly assigned to the periodic maternal separation protocol (MS180 group) or left undisturbed with their mothers, except for routine cage cleaning (CONT group). This procedure was performed to ensure that each neonate, and not the whole litter, could be assigned to a different treatment group and be considered as an independent experimental unit (Festing, 2006). Pups were weaned at P21 and group housed (two to three siblings per cage) until adulthood. To avoid sex- and litter-dependent effects, only males were evaluated. Since cross-fostering may reduce, but not entirely eliminate, litter effects, each experimental group included animals from four to five different dams. We evaluated MS180 effects on behavior, stress response, metabolic risk, and neurogenesis in young (P120/4 M) or middle-aged (P300/10 M) adult rats.

All experiments were carried out in accordance with the official regulations for use and care of laboratory animals of Mexico (NOM-062-ZOO-1999), and the Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996). All efforts were made to minimize animal suffering and to reduce the number of animals used. The present protocol was approved by the local ethics committee (1602) of the Instituto Mexicano del Seguro Social.

2.2. Maternal separation

At 09:00 h, litters were removed and pups placed together in a separate small polymethyl methacrylate (Plexiglas®) cage filled with a mixture of clean sawdust and bedding from the nest, in a room separate from the main colony, at a controlled temperature (30–33 °C) for 180 min (MS180), as previously described (Lajud et al., 2012). The whole procedure was repeated daily from P1 to P14 using the same cages throughout the entire exposure period.

2.3. Forced swimming test

For the forced swimming test (FST), adult rats were placed individually into a Plexiglas® cylinder (35 × 50 cm) filled with water at 21 °C for 10 min. Behavior was recorded with a webcam. Struggling, swimming, and immobility behavior were defined as previously described (Lajud et al., 2012). The data were collected by an experimenter blinded to the conditions and were analyzed with EVENTLOG software.

2.4. Jugular vein catheterization and blood sampling

Animals were implanted with a 15-cm Silastic®/polyethylene (Dow Corning Corporation, MI, USA) catheter under ketamine (80 mg/kg)/xylazine (6 mg/kg) anesthesia, as previously described (Lajud et al., 2012; Neumann et al., 1998). After surgery, the rats were handled daily and left to recover for 1 week. Rats were fasted overnight and then subjected to the intravenous glucose tolerance test (IVGTT). The following day, we evaluated HPA axis reactivity. For sampling, rats were left to stabilize for 60 min. Blood samples were withdrawn (0.2 mL) and immediately substituted by sterile 0.9% saline. For the IVGTT, fasting samples were collected before intravenous infusion of 1 g/kg glucose (50%, DX-50; Pisa, Guadalajara, Mexico) and 30, 60, and 120 min after glucose administration. To evaluate HPA axis reactivity, baseline samples were collected after 120 min of stabilization. Animals were then subjected to a 2-min cold swim and samples were collected at 5, 15, and 60 min after this stress exposure. Samples were collected in tubes containing ethylenediaminetetraacetic acid (5% solution, 5 µL/200 µL blood) and aprotinin (0.039 units/tube; Sigma, St. Louis, MO, USA), and then centrifuged at 4000 rpm at 4 °C. Samples were stored at −30 °C until further analysis.