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Basic nutritional investigation

Prolonged maternal separation induces undernutrition and systemic inflammation with disrupted hippocampal development in mice

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ABSTRACT

Objective: Prolonged maternal separation (PMS) in the first 2 wk of life has been associated with poor growth with lasting effects in brain structure and function. This study aimed to investigate whether PMS-induced undernutrition could cause systemic inflammation and changes in nutrition-related hormonal levels, affecting hippocampal structure and neurotransmission in C57BL/6J suckling mice.
Methods: This study assessed mouse growth parameters coupled with insulin-like growth factor-1 (IGF-1) serum levels. In addition, leptin, adiponectin, and corticosterone serum levels were measured following PMS. Hippocampal stereology and the amino acid levels were also assessed. Furthermore, we measured myelin basic protein and synapthophysin (SYN) expression in the overall brain tissue and hippocampal SYN immunolabeling. For behavioral tests, we analyzed the ontogeny of selected neonatal reflexes. PMS was induced by separating half the pups in each litter from their lactating dams for defined periods each day (4 h on day 1, 8 h on day 2, and 12 h thereafter). A total of 67 suckling pups were used in this study.
Results: PMS induced significant slowdown in weight gain and growth impairment. Significant reductions in serum leptin and IGF-1 levels were found following PMS. Total CA3 area and volume were reduced, specifically affecting the pyramidal layer in PMS mice. CA1 pyramidal layer area was also reduced. Overall hippocampal SYN immunolabeling was lower, especially in CA3 field and

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dentate gyrus. Furthermore, PMS reduced hippocampal aspartate, glutamate, and gamma-aminobutyric acid levels, as compared with unseparated controls.
Conclusion: These findings suggest that PMS causes significant growth deficits and alterations in hippocampal morphology and neurotransmission.

Introduction

Undernutrition during early postnatal life in children may be associated with a significant slowdown in the rate of central nervous system growth, with reduced brain weight, thinner cerebral cortex, diminished numbers of neurons, deficient myelination, poor neuritic arborization, and several changes in the microscopic features of dendritic spines such as reduction in their width and number, and also cognition impairment [1–4].

In rodents, undernutrition during early life may result in similar reduced brain weight, reduced total cell number, reduced and delayed myelination, loss of axon terminals in the cerebral cortical neurons, and substantial deficits in the synapse-to-neuron ratio in some brain regions, indicating a reduction in the total synapse number [5]. Furthermore, recent evidence indicates that long-lasting decrements in hippocampal plasticity occur in mice subjected to early environmental stress, such as maternal separation [6,7]. It has also been shown that undernutrition reduces peripheral circulating concentrations of insulin-like growth factor (IGF-1) [8].

Recently, a negative correlation has been found between IGF-1 serum levels and chronic inflammation markers in stunted Zimbabwean infants [9]. However, it remains elusive whether systemic inflammation coupled with low IGF-1 levels, induced by chronic undernutrition, could affect early hippocampal development.

We investigated whether prolonged maternal–offspring separation would cause systemic inflammation and poor growth and assessed its effects on the hippocampus structure and amino acid neurotransmitter levels in 2-wk-old mice, a time in which the hippocampus relies on a strong postnatal plasticity [10]. To relate systemic inflammation and nutritional status, we assessed circulating levels of C-reactive protein and nutrition-related hormones in the blood sera following undernutrition.

Materials and methods

Prolonged maternal separation model

C57BL/6J wild-type mice were purchased from Charles River laboratories. Either purchased pregnant mice or breeding pairs were used to obtain the study pups. Detectably pregnant mice (at ~12 d pregnant) were then caged individually, with free access to standard rodent chow and water, and were monitored daily for delivery, termed Day (D) 0. Newborn litters were adjusted to 6 to 8 pups. Undernutrition was induced by separating half the pups in each litter from their lactating dams for defined periods each day (4 h on D4; 8 h on D5 and 12 h thereafter). Pups were separated between 8:00-10:00 am daily: All pups were breastfed and rested in their cages. In rare cases of encountering breastfeeding, pups were left undisturbed until breastfeeding was finalized. Litters with about the same male:female ratio were selected for the study entry. The separation protocol was not conducted in newborn mice (<4 d old) to guarantee more colostrum intake and to avoid perinatal mortality. A total of 67 pups were used in this study.

The maternal separation protocol was adapted from Calikoglu et al [8]. This method has the advantage of providing littermate controls and well-nourished pups to compare with undernourished ones. Weight and tail length were recorded daily until the pups were humanely killed immediately before the separation handling. A thermal pad was used to warm the pups during daily measurements (27 ± 2°C). Care was taken to assure the same level of handling for all experimental mice. Protocols from this study were previously approved by the Institutional Animal Care and Use Committee at the University of Virginia and the University of Fortaleza (protocol #15005). All efforts were made to minimize the number of animals used and their suffering.

Nutritional status

To assess changes in growth and weight gain, daily tail length and body weight were analyzed, the former as a measure of linear skeletal growth. Experimental mice were monitored carefully by daily inspection of weight and tail length during the suckling time on days 4 to 14. Tail length was recorded by means of gently measuring the tail from the base to the tip, using a digital caliber and a card board (to the nearest 0.1 mm). All measurements were conducted before starting the procedures of daily mice separation. Care was taken to keep the same degree of handling during this process for all mice.

Neonatal reflex ontogeny

We assessed the ontogeny of selected neonatal reflexes along the first 2 wk of life, including cliff avoidance, surface righting, dorsal immobility, and swimming behavior [11,12]. The cliff avoidance reflex test is used to assess the integration of exteroceptive input (vibrissae) and locomotor output, providing information concerning motor skills as well as sensory function and/or processing [13,14]. The offspring is placed on a platform elevated 10 cm above a table top. The forelimbs and snout of the animals are positioned so that the edge of the platform passes just behind an imaginary line drawn between the eye orbits. Avoidance is scored by reflex latency between being placed on the edge and turning until it is parallel to the edge of the table (0 = no response, latency >60s; 1 = response <10s; 2 = <5 s). The surface righting reflex is a measure of the capacity to return to a prone position. Pups were placed on their backs on a smooth surface, and the time required to right themselves to a position where the four limbs touches the surface was measured. The time is scored as follows: 2 = righting ≤1 s, 1 = righting >1 s and ≤2 s, 0 = righting >2 s. The swimming behavior test is used to assess navigational and motor development. Pups are placed into a tank with water temperature maintained at 27 ± 1°C and swimming behavior was rated for direction (straight = 3, circling = 2, floating = 1) and head angle (ears out of water = 4, ears half out of water = 3, nose and top of head out of water = 2, and unable to hold head up = 1). Dorsal immobility (tonus immobility) was measured by suspending the mice by the back up to approximately 10 cm above a flat surface. The procedure is performed with the aid of a modified twissor, covered by rubber on its tip to avoid trauma. The dorsal immobility scores the latency time (in s) until the mouse moves to escape the grasping and is related to ventrolateral mesencephalic periaqueductal gray matter activity [15]. N = 8 for PMS and N = 6 unseparated mice were used for all behavioral tests done.

Systemic inflammation marker

To find whether prolonged maternal separation could cause systemic inflammation, we measured serum C-reactive protein (N = 10 for both PMS and unseparated mice), which is a liver acute phase protein released during unspecific inflammatory responses. High sensitivity C reactive protein was assessed using a commercial kit (Labstest and Bioclin, Belo Horizonte, Brazil).

Nutrition and stress-related hormone serum levels

To find whether prolonged maternal separation could cause changes in nutrition and stress-related hormones (leptin, adiponectin, and corticosterone), serum levels were assessed. On day 14, blood was drawn from some mice following decapitation (N = 10 per group). We also assessed IGF-1 serum levels as IGF-1 deficiency has been associated with stunting. These hormones were measured in serum samples using an enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Hippocampal structure and amino acid levels

Hippocampal stereology

A total of 14 male C57BL/6J mice were used in this experiment. Mice were divided into the following two groups: unseparated mice (n = 7) and prolonged