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Research report

Environmental enrichment protects spatial learning and hippocampal neurons from the long-lasting effects of protein malnutrition early in life

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ABSTRACT

As early protein malnutrition has a critically long-lasting impact on the hippocampal formation and its role in learning and memory, and environmental enrichment has demonstrated great success in ameliorating functional deficits, here we ask whether exposure to an enriched environment could be employed to prevent spatial memory impairment and neuroanatomical changes in the hippocampus of adult rats maintained on a protein deficient diet during brain development (P0-P35). To elucidate the protective effects of environmental enrichment, we used the Morris water task and neuroanatomical analysis to determine whether changes in spatial memory and number and size of CA1 neurons differed significantly among groups. Protein malnutrition and environmental enrichment during brain development had significant effects on the spatial memory and hippocampal anatomy of adult rats. Malnourished but non-enriched rats (MN) required more time to find the hidden platform than well-nourished but non-enriched rats (WN). Malnourished but enriched rats (ME) performed better than the MN and similarly to the WN rats. There was no difference between well-nourished but non-enriched and enriched rats (WE). Anatomically, fewer CA1 neurons were found in the hippocampus of MN rats than in those of WN rats. However, it was also observed that ME and WN rats retained a similar number of neurons. These results suggest that environmental enrichment during brain development alters cognitive task performance and hippocampal neuroanatomy in a manner that is neuroprotective against malnutrition-induced brain injury. These results could have significant implications for malnourished infants expected to be at risk of disturbed brain development.

1. Introduction

Protein malnutrition is one of the most common health conditions, affecting human children during the most vulnerable stage of their development [1]. Adequate nutrition during this critical period is an essential factor that ensures the normal structural and functional development of the brain [2]; and while there is no doubt that all nutrients are essential for adequate neurodevelopment, proteins appear to play the most critical role in this period [3]. An insufficient protein diet during brain development may result in maladaptive structural changes and, consequently, behavioral deficits [4–8].

The effects of nutritional imbalance are aggravated when they occur during brain development and there is evidence that the earlier the malnutrition, the more severe, lasting, or permanent are its effects on brain maturation [9,10]. In fact, the effects of malnutrition on brain development can lead to severe long-term changes, which may last until adulthood [10,11]. Neuroanatomical changes due to malnutrition are not uniform throughout the brain; among the brain regions most affected in early postnatal life, the hippocampus is particularly vulnerable. The hippocampal formation has been widely studied for its importance in the acquisition and consolidation of memory, and neuroanatomical changes in the hippocampal formation are correlated with deficits in learning and memory, which include decreased neuronal density in the CA1 and CA3 regions caused by malnutrition [12–14]. It has also been demonstrated that malnourished rats were slower to learn an efficient search strategy on the Morris water maze (MWM), requiring more time to find the hidden platform, and showed retention deficits on the probe-trial indicated by impaired performance in recalling the former position of the platform [5,13]. Cordoba et al. [15] showed that malnutrition also induces deficits in efficient navigation even after a long period of nutritional recovery. In the same vein, Braga et al. reported that protein malnutrition in early life was responsible for impaired performance in recognition memory in adult rats and even after nutritional recovery they were unable to reach the

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recognition indices demonstrated by control animals [16]. Lukoyanov and Andrade [13] demonstrated that the total number of neurons in the granular layer of the dentate gyrus and pyramidal neurons in the CA1 region was significantly reduced even after a period of nutritional rehabilitation. All these results indicate that malnutrition may result in irreversible consequences to the normal development of the hippocampal formation and its function in learning and memory.

It is well known that the brain is remarkably responsive to its interaction with the environment and this can shape its development. Studies have shown consistently alteration in the morphological, biochemical, and molecular processes of brain development due to environmental stimulation, mainly when performed during the early days of life [17–21]. It has also been demonstrated that when animals are exposed to environmental enrichment, which is a combination of complex inanimate, social, visual, and sensorial stimulation, this may lead to neuroanatomical changes in the hippocampus that differ from those in animals living in standard conditions, which include increases in hippocampal thickness and volume [22–24], in the number of granule and glial cells [23,24], in dendritic spine density [25,26], hippocampal progenitor proliferation and neurogenesis [27,28], and alterations in receptor expression [29]. The beneficial effects of environmental enrichment are not limited to neuroanatomy, and it has been showed to induce improvements in spatial learning performance [24,30,31]. Studies have reported that the possible cause for these structural and behavioral changes is related to changes in brain neurochemistry. Indeed, environmental enrichment increases the expression of factors associated with enhanced spatial ability and neurogenesis, such as the brain-derived neurotrophic factor and vascular endothelial growth factor [32–36].

Although some effects of protein malnutrition have been shown to be permanent, a degree of protection can be obtained through an enriched early experience. Growing evidence reveals that malnourished rats can achieve some prophylaxis by exposure to an enriched environment [20,29,37,38]. However, there are no studies showing whether the protective effect of environmental enrichment on hippocampal neuroanatomy and its function in learning and memory can be extended to adult rats maintained on a protein malnutrition diet early in life. Thus, based on all the preceding studies examining the influence of environmental enrichment on brain and behavior, we hypothesized that it is plausible that such an experience could be protective against neuroanatomical changes in the hippocampus and memory impairment in protein-deficient rats exposed to an enriched environment, for 1 h per day, during the first 35 d of life. The purpose of this study was to investigate learning and memory performance and hippocampal morphology of adult rats maintained on a protein-deficient diet in the early postnatal period and the possible benefits produced by environmental enrichment under this condition. To elucidate the protective effects of environmental enrichment, we used the MWM and structural analysis to determine whether changes in spatial memory and the number and size of CA1 neurons, caused by early malnutrition, were significantly different between an enriched and non-enriched group.

2. Experimental procedures

2.1. Animals

Forty-eight male Wistar rats, from the animal colony at the Ribeirão Preto campus of the University of São Paulo, were used. According to the experimental protocol previously established in our laboratory, on the day of birth, each litter was culled to 6 male and 2 female pups housed with the dam in transparent plastic cages (40 × 25 × 20 cm) during the lactation period, from postnatal day (P) 0 to P21. During the lactation period, the dams and entire litters were weighed once a week. Weaning occurred on P21 and consisted of separation of the dams and female pups that were not used in this study to avoid possible result variation related to the estrous cycle. Male rats were reared in pairs in standard polypropylene cages (30 × 19 × 18 cm) and they were weighed individually once a week, until P70.

Rats were randomly divided into two dietary groups: well-nourished, with a diet containing 16% protein (W), and malnourished, with a diet containing 6% protein (M). Diets were prepared according to proportions recommended by the American Institute of Nutrition and the Association of Official Agriculture Chemists, with the addition of methionine and choline as described by Cambraia et al. [39]. Essentially, the low protein diet consisted of 6% protein (casein), 79.8% corn starch, 8% lipids (corn oil), 5% salt mixture, 1% vitamin mixture, 0.2% choline, and methionine (2 g/kg of casein). The W diet was composed of 16% protein (casein), 69.8% corn starch, 8% lipids (corn oil), 5% salt mixture, 1% vitamin mixture, 0.2% choline, and methionine (2 g/kg of casein). Rats were subjected to the experimental diet from birth to P35; during the lactation period, the diet was offered only to the mother and the pups were weaned onto the same diet as their respective mothers until P35. From P36 to the sacrifice day, they were maintained on a diet containing 16% protein.

The W and M groups were further subdivided into two groups according to the early environmental treatment: enriched (E) and non-enriched (N) environment.

Thus, we established four experimental groups: Well-nourished and Non-enriched (WN, n = 12 newborn rats/group); Well-nourished and Enriched (WE); Malnourished and Non-enriched (MN); Malnourished and Enriched (ME).

2.2. Environmental enrichment

In this condition, rats were exposed to environmental enrichment from P8 to P21 and from P22 to P35. The enrichment between P8 and P21 consisted of a transparent plastic cage (40 × 25 × 20 cm) which contained activity wheels, tunnels, plastic toys (with different shapes and textures), marbles, objects that emitted sounds (e.g., rattles), mirrors, and pieces of wood. Each litter that was assigned to the E group was placed in the enriched environment, without their mothers, for 1 h per day; litters were then returned to their mothers in their respective home cages. The groups not exposed to an enriched environment during the lactation period were kept in a home cage, but were separated from their mothers during the same period of time as the rats in the enriched condition. From P22 to P35, a cage (40 × 60 × 90 cm) with three floors, connected with ramps, containing an activity wheel, plastic toys, rubber balls, wooden objects with different shapes, textures and colors, objects that could emit sound when touched by the animals, and mirrors on the lateral walls were used for the E protocol [37,38,40]. Seven to ten rats from each enrichment group were placed in the enriched cage at the same time. These rats were exposed to the enriched environment for 1 h per day [37,38], after which each rat was returned to its home cage. The toys and objects in the enriched cage were changed weekly to guarantee a novelty effect [40–42].

2.3. Behavioral testing

Beginning at P70 rats (12 animals per group) were tested on the Morris Water Maze (MWM) using a procedure similar to that described by Fukuda at al. [43] based on the original task described by Morris [44]. Testing took place between 08:00 am and 10:00 am, in a room with controlled luminosity and temperature. The maze apparatus was a circular tank (1.5 m diameter × 39 cm height) with black walls, divided into four imaginary quadrants numbered in a clockwise fashion (quadrants 1, 2, 3, and 4). The tank was filled with water and a clear Plexiglas circular platform (12 cm diameter × 25 cm height) was placed at a fixed position inside the tank and completely submerged 1.5 cm below the water level. This apparatus was located in a room that contained distal visual cues such as pictures on the wall, door, and window. The swim path for each rat on every trial was recorded using a tracking system (Ethovision®, Noldus, Wageningen, the Netherlands),