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Effects of enriched environment in spatial learning and memory of immature rats submitted to early undernourish and seizures

Fabrício Simão, Juliana Antola Porto, Magda Lahorgue Nunes*

Neuroscience Laboratory, Institute of Biomedical Research, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil

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ABSTRACT

We recently reported that early undernourishment and seizures to the rat brain resulted in morphological changes and progressive learning and memory disability, which started at around 6 week later and is representative of human adolescence. The purpose of the present study was to examine whether enriched environmental can recovery this slowly progressing deficits in early undernourished and in two different models for seizures. Undernourished groups were maintained on a nutritional deprivation regimen from post-natal day 2 (P2) to P15. From P8 to P10, recurrent seizures (RS) groups were exposed to three seizures per day, while status epilepticus (SE) groups experienced status epilepticus at P16, both induced by flurothyl. Next, animals were exposed to enriched environment between P30 and P60. Beginning at P61, all groups were trained and tested in the Morris water maze (MWM). Enriched environment led to a significant benefit in learning and retention of visual-spatial memory, being able to reverse the cognitive impairment generated by undernourishment and SE.

1. Introduction

Undernourishment is responsible for serious physiological and morphological changes in the developing central nervous system (Huang et al., 2003). Structurally malnutrition results in tissue damage, growth retardation, disorderly differentiation, reduction in synapses and synaptic neurotransmitters, delayed myelination and reduced overall development of dendritic arborization of the developing brain. There are deviations in the temporal sequences of brain maturation, which in turn disturb the formation of neuronal circuits (Holmes and Ben-Ari, 2001). Long-term alterations in brain function have been reported which could be related to long lasting cognitive impairments associated with malnutrition (Levitsky and Strupp, 1995).

Seizures in the developing brain might affect memory and cognition (Liu et al., 2003; Lynch et al., 2000). As previously demonstrated in animal models, epilepsy and undernourishment are related, but a cause–effect relationship has not yet been established (Bronzino et al., 1997). Undernourishment does not seems to be a direct cause of epilepsy, however, several studies has reported a reduction of seizures threshold in malnourished pup rats or adult rats (Florian and Nunes, 2011; Hemb et al., 2010; Palencia et al., 1996). While seizure-induced cell loss in the hippocampus may account for some of the cognitive impairment in patients with temporal lobe epilepsy (Pauli et al., 2006), there are indications that seizures may lead to functional impairment without necessarily causing cell loss.

Environmental enrichment is defined as a combination of complex inanimate and social stimulation (Van Praag et al., 2000). It has been demonstrated to increase hippocampal synapse and spine density (Moser et al., 1994) and enhance long-term potentiation in the hippocampus, as well as improve spatial learning performance (Duffy et al., 2001; Van Praag et al., 2000). Beneficial effects of enriched environment following different types of seizures in the developing brain have been demonstrated by many authors such as improvement on cognition, enlargement of cerebral cortex, increased neurogenesis, increased dendritic sprouting, activation of transcriptor factors and enhanced visuo-spatial memory (Rosenzweig and Bennett, 1996; Van Praag et al., 2000).

While the immature brain appears to be less vulnerable to the adverse effects of prolonged seizures than the mature brain (Holmes et al., 2002), seizures early in life can be associated with later cognitive and behavioral disturbances, even in the absence of overt structural neuronal damage (Lynch et al., 2000; Stafstrom, 2002). The aim of this study was to verify the effects of environmental enrichment on spatial learning and memory in rats submitted to early undernourishment, and two models of seizures: repeated early-life seizures and *status epilepticus*.

* Corresponding author at: Division of Neurology, Hospital São Lucas PUCRS, Av. Ipiranga 6690/220, 90610-000 Porto Alegre, RS, Brazil. Tel.: +55 51 33203318; fax: +55 51 33203316.
E-mail address: nunes@pucrs.br (M.L. Nunes).

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2. Methods

2.1. Experimental procedures

The experiments were conducted under conditions approved by the Scientific and Research Ethics Committees of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) regarding animal welfare. Pregnant female Wistar rats from our breeding colony were maintained on a 12-h dark–light cycle with food and water freely available. After delivery, each dam with the litter was housed individually. Each litter was culled to 10 pups. The day of birth was counted as P0. All animals were weaned at P21. The entire litters were assigned to specific groups. The litters were randomly assigned under either nonenriched (six groups) or enriched (six groups) conditions using only male pups in the following groups: NC, nourished control; NRS, nourished recurrent seizures; NSE, nourished status epilepticus; UC, undernourished control; URS, undernourished recurrent seizures; USE, undernourished status epilepticus.

2.2. Flurothyl-induced seizures

To provoke early recurrent seizures and status epilepticus, we used flurothyl [bis(2,2,2-trifluoroethyl)ether] (99% min), a volatile convulsive agent that rapidly stimulates the central nervous system (CNS), inducing generalized seizures (Nunes et al., 2000). Animals were challenged with flurothyl (20 μL/min constant flow rate) in an airtight chamber (9.38 L) to provoke two different models of seizures: early recurrent seizures and status epilepticus. Latency to seizures was considered the time from first exposure to flurothyl until the onset of the first seizure. Animals from the NRS and URS groups were submitted to early recurrent seizures-3 exposures of flurothyl per day (1 h inter-exposure interval) from P8 to P10. Each time, the animals were exposed to the constant flow rate until the appearance of the first clonic seizure. For all animals, the exposure to flurothyl took place immediately after separation from their mother. Immediately after that, the animals were returned to their home cages. Rats from the NSE and USE groups were submitted to status epilepticus by exposure of flurothyl at P15. The animals were exposed to the constant flow rate for 20 min, after which they spent 10 more minutes inside the drug filled chamber. In all the SE groups, the time of the first clonic seizure was recorded in order to calculate the seizure threshold. However, while in the NRS and URS groups the animals were removed from the chamber right after the seizures, in the NSE and USE groups the animals continued to receive flurothyl for 20 min despite the seizures. Control animals were exposed to the same procedure except that water was used instead of. After the experiment, the animals were returned to their original litters.

2.3. Undernourishment paradigm

The undernourishment paradigm consisted of limiting the offspring's access to nutrition by removing the dams from the cage starting at P2. The deprivation period was increased by 2 h for 6 consecutive days, from 2 h on P2 to 12 h on P7. The deprivation period remained at 12 h/day for the next 8 days (P8–P15). During deprivation, pups remained in a light heated cage, with room temperature maintained at 34 °C (measured with a thermometer placed in the room). After the deprivation period, the pups were housed with their respective dams. Age-matched control rats remained with their dams. Body weights were measured daily, first thing in the morning, before any intervention. This method of food deprivation has been successfully used before, despite the neonatal isolation stress. Besides, it resembles very much the paradigm of the human preterm very low birth weight newborn (Florian and Nunes, 2011; Hemb et al., 2010; Nunes et al., 2000).

2.4. Environment enrichment

To test whether a period in an enriched environment has a beneficial as well as an enduring effect following SE and undernutrition, an enriched environment was introduced between P30 and P60. The enriched environment consisted of a large plastic cage measuring 100 cm (length) × 50 cm (width) × 40 cm (height). There were various toys, wooden blocks, climbing platforms, plastic tubes, small shelters, and a running wheel. These objects were rearranged every week to facilitate exploratory behavior. Enriched rats were housed in groups of 7–10, which permitted extensive social interactions between cagemates. The nonenriched group remained in plastic cages but were handled the same amount of time as the enriched group.

2.5. Training in the spatial version of the Morris water maze

To properly evaluate spatial learning and memory, we used the Morris water maze, which has often been used in the validation of rodent models for neurocognitive disorders. The observer was blind to the protocol. The maze consisted of a black circular pool (100-cm in diameter) conceptually divided into 4 equal imaginary quadrants for the purpose of data analysis. The water temperature was 24 °C. One and a half cm beneath the surface of the water and hidden from the rat's view was a black circular platform (8 cm in diameter). It had a rough surface, which allowed rats to climb onto it easily. The water maze was located in a well-lit white room with several posters and other distal visual stimuli hanging on the walls to provide spatial cues. A curtain separated the water maze room from the room where the computer was set up and where the animals were temporarily housed during the behavioral sessions. Training in the hidden platform (spatial) version of the Morris water maze was carried out during 5 consecutive days (P61–P65), as previously described by Hemb et al. (2010). On each day, rats received 5 consecutive training trials during which the hidden platform was kept in a constant location. A different starting location was used in each trial, which consisted of a swim followed by a 60-s platform sit. Any rat that did not find the platform within 60 s was guided to it by the experimenter. The inter-trial interval was 30 s. During the inter-trial interval, rats were carefully dried with a towel by the experimenter. Memory retention was evaluated in a 60-s probe trial carried out in the absence of the escape platform 24 h after the last training session (P66). Data (latency to reach the platform and time spent in each quadrant) were measured by a single person with a chronometer and analyzed using one-way or multi-way ANOVA followed by post hoc tests, as appropriate.

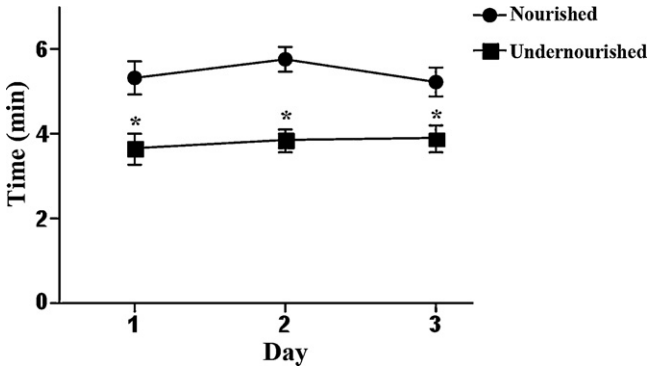

Fig. 1. Variation of seizure threshold between groups. Seizure threshold (mean ± SEM) was significantly decreased in undernourished group. Animals from the nourished and undernourished groups were submitted to early recurrent seizures-3 exposures of flurothyl per day (1 h inter-exposure interval) from P8 to P10. Each time, the animals were exposed to the constant flow rate until the appearance of the first clonic seizure. *$P < 0.001$ vs. nourished group.

2.6. Statistical analysis

The data from the seizures susceptibility experiments were analyzed with the aid of Student's *t*-test. Two-way ANOVA was used to analyze body weights. Two- and three-way ANOVA were employed to analyze spatial memory retention and acquisition, respectively. Values are expressed as mean ± SEM. Statistical significance was defined as $P < 0.05$ for all tests.

3. Results

3.1. Seizure threshold determination

To determine if there was an increased susceptibility to seizures in undernutrition, we assessed seizure threshold by flurothyl inhalation into postnatal rats at P8–P10. The time to onset of the seizures demonstrated that the undernutrition groups required fewer flurothyl inhalation to develop seizures (Fig. 1), compared with the nourished group ($P < 0.05$, $n = 5$). The behavioral changes induced by the flurothyl were similar in all groups. The rats recovered quickly and there were no deaths.

3.2. Body weight

The average body weight in RS group in the undernourished animals (270.6 ± 7.7 g, UC and URS; $P < 0.05$) weighed less than the nourished animals (295.5 ± 7.3 g, NC and NRS) without enriched environment and the nutritional rehabilitation procedure did not restore the body weight deficit of undernourished rats in P90. Likewise, in animals with enriched environment the undernourished animals (277.9 ± 6.2 g, UC and URS; $P < 0.001$) weighed less than the nourished animals (309.4 ± 6.0 g, NC and NRS) in P90. In the SE groups, the undernourished animals (271.3 ± 7.7 g, UC and USE; $P < 0.05$) weighed less than the nourished animals (295.8 ± 8.1 g, NC and NSE) without enriched environment and