ORIGINAL ARTICLE

Effects of early malnutrition and nutritional rehabilitation in rats

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Abstract

Objective: to verify the effects of malnutrition and nutritional rehabilitation regarding seizure threshold, body weight and brain weight in rats.

Methods: pregnant Wistar rats and their pups were used. Part of the rat pups were submitted to a malnutrition protocol and the rest served as nourished controls. At P15, malnourished and control rats were submitted to status epilepticus induced by flurothyl; and the rehabilitation period started after recovery from seizures. At P30, all rats were submitted to single flurothyl-induced seizures and the threshold was determined. After the seizures, the rats were sacrificed, the brain removed and weighed. Rat pups were weighed daily from age P2 to P30.

Results: significant differences as to body weight between malnourished and nourished rats were observed from P5 onwards. At P30, even after nutritional rehabilitation, there were still differences in terms of body weight. Nourished (mean 1.47 g ± 0.17) and male (mean 1.47 g ± 0.16) rats had brain weight slightly higher than that presented by malnourished (mean 1.42 g ± 0.17) and female (mean 1.38 g ± 0.12) rats; however, the difference was not significant. Differences observed in the threshold for the first clonic and tonic seizure at ages P15 and P30 between the groups were not statistically significant.

Conclusions: our results suggest that malnutrition does not influence seizure threshold in rat pups submitted to flurothyl-induced seizures. Early nutritional rehabilitation seems to have a protective effect on seizure threshold in previously malnourished animals.


Introduction

Malnutrition, both maternal and infant, is one of the major causes of the persistently high infant mortality rates in Brazil. Malnourished children are exposed to immune system deficiencies, greater risk for infection, and increased predisposition to delays in neuropsychomotor development.¹,²

Clinical and experimental data show that there is an increased susceptibility to seizures during the neonatal period. This increased susceptibility is probably due to a combination of enhanced excitation and diminished inhibition throughout the brain, and to developmental differences in subcortical circuits.³

Malnutrition and epilepsy are prevalent disorders in developing countries, and a cause-and-effect relationship has been suggested in studies with animal models.⁴⁻⁸ To date, no clinical studies have shown that malnutrition per se increases the risk for epilepsy; however, we have previously observed a trend towards increased incidence of epilepsy in malnourished children.⁹
Experimental studies show that perinatal malnutrition causes permanent physiological and morphological changes in the developing central nervous system (CNS), in addition to reducing seizure threshold.4-8 The effects of intrauterine malnutrition are especially severe during neuronal proliferation and growth, when specific CNS structures such as the cortex, hippocampus, and cerebellum are particularly vulnerable.10,11 Alterations on EEG and in the visual and sensorimotor cortices have also been described.4-8,12 Deficiency of specific nutrients, such as indispensable amino acids, activates the anterior piriform cortex (a highly seizure-prone limbic structure in rats), thus causing an increase in the severity of seizures and reduction in seizure threshold.7

Several paradigms of malnutrition have been proposed in the literature.4-5 The most widely employed include food restriction (separating pups from dams during the lactation period); protein restriction; and essential amino acid restriction. The experimental model of early, chronic malnutrition due to restriction of food intake13 is the model that best reflects the reality of our population of children, who often experience protein and calorie malnutrition during the first two years of life, starting after weaning.

There are significant age-related differences in the occurrence of seizures, and therefore developmental seizure models should be different from adult seizure models.14 The use of fluorothyl-induced seizures in developmental models is advantageous, since it allows seizures to be rapidly modulated and interrupted simply by controlling the intensity of the drug dripping in an airtight chamber.13

Although the concept of cerebral plasticity has been associated with recovery of nutritional status,15 experimental studies with rats submitted to a process of early malnutrition and later to nutritional rehabilitation have yielded controversial results concerning the recovery of previously damaged CNS structures.

The objective of the present study was to assess the effects of early malnutrition and nutritional rehabilitation on seizure threshold, body weight, and brain weight in developing rats.

Methods
We carried out a study with pregnant Wistar rats and their litter. After mating, the females received food and water ad libitum. They were kept in a 12-hour dark/12-hour light cycle in individual, clean cages at an appropriate ambient temperature. The litters were limited to a maximum of 10 pups per cage. The age of pups was expressed in postnatal days: the day of birth was defined as postnatal day P0, and subsequent days as P1 to P30. Part of the litter was submitted to the malnutrition paradigm and the remaining pups were used as controls. On P15, status epilepticus was induced in malnourished rats and controls; after recovery, nutritional rehabilitation began. On P30, the animals were again submitted to fluorothyl-induced seizures to determine the thresholds for the first clonic seizure and the first tonic seizure. After the seizures, the animals were sacrificed, and the brains were removed and weighed. The whole litter was weighed daily from P2 to P30. Both male and female pups were included in the experiment.

Malnutrition paradigm
The malnutrition paradigm employed consisted of limiting lactation by separating part of the litter from the lactating rat for increasingly longer periods. The period of privation started on P2 for two hours, and was increased daily in two hours for a maximum of 12 hours on P7; this was then maintained until P15. During separation, pups were maintained in heated cages to ensure that body temperature would remain constant (approximately 34°C). After the privation period, pups returned to their cages and had free access to the lactating female. Control animals remained in their cages with free access to the lactating female.13

Nutritional rehabilitation
After recovery from status epilepticus, the animals were returned to their cages and the period of nutritional rehabilitation began. Pups were allowed free access to suckling until P23, at which point they were weaned. After weaning, the rats had free access to water and food.

Fluorothyl-induced seizures
Fluorothyl [bis(2,2,2-trifluoroethyl) ether] is a volatile convulsant that rapidly stimulates the CNS, inducing generalized seizure attacks.16,17 On P15, rats were tested in a fluorothyl chamber in groups of three, with dripping at 6 drops per minute. Each group remained in the chamber for 30 minutes. Dripping was interrupted at 20 minutes. For each animal, the first clonic and the first tonic-clonic seizures were recorded to calculate seizure thresholds. Following the status epilepticus, the rats recovered in ambient air inside heated cages. They were returned to their original litters after complete cessation of the seizures. On P30, the animals were again submitted in pairs to fluorothyl exposure. This time, however, dripping was interrupted right after the first tonic-clonic seizure and the rats were removed from the chamber. Figure 1 shows the equipment used in the application of the fluorothyl technique.

After that, the animals (both malnourished and control rats) were sacrificed with high-dose barbiturates, submitted to transcardiac perfusion, and then guillotined. The brain was removed and weighed on a digital scale.

The results of seizure thresholds, animal and brain weight were compared using Student’s t test.
The study was approved by the Scientific and Research Ethics Committees at Hospital São Lucas (Pontifícia Universidade Católica do Rio Grande do Sul). International criteria for the care and use of animals in research were followed.18

Results

A total of 66 male and female rats from six different litters were included in the study. Forty animals were submitted to the malnutrition paradigm, and the remaining 26 were used as controls. Eighteen (28%) animals submitted to the malnutrition paradigm died, most (30%) on P8. During status epilepticus, six animals (three controls and three malnourished rats) died.

After P5, a statistically significant difference in body weight was observed between malnourished and control rats (Figures 2 and 3). The process of nutritional rehabilitation, which began on P16, was associated with weight gain, but the difference in weight between the groups persisted until P30. In both groups, body weight was greater in males (1g on average) than in females within the same group. During the period of nutritional rehabilitation, we observed that after P21 the body weight of malnourished females became greater than or equal to that of malnourished males; however, the difference in body weight between males and females within the same group was not statistically different throughout the entire period (from P2 to P30).

Brain weight after transcardiac perfusion, on P30, was not statistically different between the groups, though slightly higher in controls (average 1.47 + 0.17 g) and in male rats (average 1.47 + 0.16 g) in comparison to malnourished rats (average 1.42 + 0.17 g) and to female rats (1.38 + 0.12 g).

Seizure thresholds (in seconds) for onset of first clonic seizure and first tonic-clonic seizure were evaluated on P15 and P30. On P15, there were no statistically significant differences between the groups, although controls had a higher threshold for onset of clonic and tonic-clonic seizures in comparison to malnourished rats. An inverse trend (although not significant) was observed on P30: the rats that had been malnourished had a higher threshold for onset of both types of seizures. The difference in seizure threshold between males and females was not significant (Table 1).

Behavior and activity level during the seizures and post-ictal recovery period were similar, independently of nutritional status.

Rats presented similar behavior and activity levels during seizure attacks and the post-ictal recovery period, independently of nutritional status.

Discussion

The present results show that the paradigm of malnutrition employed is effective, since a significant difference in weight between nourished and malnourished animals was observed early on. The proposed paradigm - restriction of food intake - is similar to the process of malnutrition that affects underprivileged families; in these families, food intake is insufficient for several reasons, including unavailability of food. A possible effect of stress due to the separation of pups from their mothers was minimized by controlling for adequate ambient temperature; nonetheless, such an effect could also be extrapolated to a situation of malnutrition in human beings.

Figure 1 - Airtight chamber model for experiments with fluorothyl. The attached infusion pump allows constant dripping of the drug. The device for entrance of air can be seen on the front part of the chamber. Fluorothyl is eliminated by a vacuum-suction device.
Table 1 - Seizure thresholds (time in seconds + standard deviation) on P15 and P30 according to nutritional status and sex

<table>
<thead>
<tr>
<th>Type of seizure/age</th>
<th>Controls Males</th>
<th>Malnourished rats Males</th>
<th>Controls Females</th>
<th>Malnourished rats Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonic P15</td>
<td>567±152</td>
<td>495±187</td>
<td>538±174</td>
<td>538±163</td>
</tr>
<tr>
<td>Tonic P15</td>
<td>614±168</td>
<td>537±151</td>
<td>535±113</td>
<td>635±179</td>
</tr>
<tr>
<td>Clonic P30</td>
<td>521±70</td>
<td>536±152</td>
<td>554±152</td>
<td>501±72</td>
</tr>
<tr>
<td>Tonic P30</td>
<td>660±149</td>
<td>746±110</td>
<td>729±127</td>
<td>691±41</td>
</tr>
</tbody>
</table>

Note.: differences between groups were not statistically significant (P> 0.05).

We did not observe a significant difference between males and females in relation to either body or brain weight. Similarly, previous studies employing male and female rats have not reported differences in body and brain weight between the two sexes.4,5,19,20

In addition, we did not observe any differences between control and malnourished rats in terms of brain weight after nutritional rehabilitation, on P30. Wasterlain suggests that it is not malnutrition, but rather the occurrence of repeated seizures during critical developmental periods that causes a reduction in brain weight and in the number of brain cells.20 In a previous study13 with rats undergoing an early process of malnutrition and submitted to status epilepticus on P15, without nutritional rehabilitation, we observed a decrease in brain size in malnourished rats in comparison to controls (unpublished observation). Our opposite findings on P15 in that first study in relation to the findings observed on P30 in the present study may be related to both the occurrence of seizures during a period of significant growth and cell differentiation (age P15) and to the effect of nutritional rehabilitation.

We recorded constant weight gain during nutritional rehabilitation; however, on P30, the weight of malnourished rats was still significantly lower than the weight of controls. Sharma et al.21,22 have also reported persistent differences in body weight after a longer period of time (90 days) even after nutritional rehabilitation.

![Figure 2 - Graph showing differences in weight (+ standard error of mean) according to sex and age](image-url)
The effect of malnutrition on seizure thresholds is apparently age- and model-dependent. Animals suffering from malnutrition during intrauterine life or during the lactation period, submitted to seizures during adult life have been shown to be more susceptible to the development of seizures.\(^4\)\(^-\)\(^8\) Malnourished animals submitted to the convulsant kainic acid (KA) and kindling models have also been shown to be more susceptible to seizures. The hippocampal formation is one of the structures most affected by malnutrition, and these two seizure-induction models (KA and kindling) generally cause permanent alterations in hippocampal structures.\(^10\)\(^-\)\(^11\),\(^23\) With the fluorothyl model, (KA and kindling) generally cause permanent alterations in by malnutrition, and these two seizure-induction models hippocampal formation is one of the structures most affected have been shown to be more susceptible to seizures. The convulsant kainic acid (KA) and kindling models have also been shown to be more susceptible to seizures. The hippocampal formation is one of the structures most affected by malnutrition, and these two seizure-induction models (KA and kindling) generally cause permanent alterations in hippocampal structures.\(^10\)\(^-\)\(^11\),\(^23\) With the fluorothyl model, which induces acute seizures, we did not observe differences in seizure thresholds at any of the ages assessed (P15 and P30) even after nutritional rehabilitation. However, in comparison to other studies, we started nutritional rehabilitation at an early age (P16), which would be the equivalent to the age of 3 or 4 years in human beings.

Our results suggest that early malnutrition does not increase the susceptibility to seizures in developing rats submitted to a fluorothyl-induced seizure model. Early nutritional rehabilitation apparently has a protective effect against susceptibility to seizures in previously malnourished animals.

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References


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