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Anxiety-like behavior in weanling and young adult male and female malnourished rats

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ABSTRACT

The present study determined whether protein-calorie malnutrition alters anxiety-like behavior in weanling and young adult, male and female malnourished rats. On the day of birth, litters of Wistar rats were divided into Control (C) and Malnutrition (M) groups. In the C group, litters were fed by dams receiving *ad libitum* lab chow, whereas in the M group, litters were fed by dams receiving 40% of the total amount of the diet offered to dams in the C group. After weaning (PND21) until PND50, animals received the same food as theirs mothers (i.e., *ad libitum* access in the C group and 40% of the C group food in the M group). On PND21 and PND50, independent C (male [CM] and female [CF]) and M (male [MM] and female [MF]) groups were exposed to the elevated T-maze. The time taken to withdraw four paws from this arm was recorded (baseline latency [BL]). The same measurement was repeated twice at 30 s intervals (avoidance trial 1 [AT1] and avoidance 2 [AT2]). The cutoff time in each trial was 300 s. ANOVA indicated a four-way age × diet × sex × trials interaction. *Post hoc* comparisons revealed that PND50 rats had a lower BL and AT1 latency compared with PND21 rats. Training increased both AT1 and AT2 latencies compared with BL in both the CM and CF groups. Weanling malnourished rats exhibited reduced anxiety-like behavior and young adult male rats presented less anxiety-like behavior than young adult female rats in this experimental model.

1. Introduction

Protein and protein-calorie malnutrition imposed early in life is a well-known environmental factor that produces morphological, neurochemical, and neurophysiological alterations in the central nervous system and can affect functional aspects of the developing brain [1–4]. Studies have shown that postnatal protein malnutrition causes rats to become hyperactive to aversive painful stimuli [5,6], increase their motivation for food or water in response to deprivation [7], reduce avoidance latencies, and cause more resistance to extinguishing avoidance responses [8]. When animals are subjected to aversive tests, such as the elevated plus maze (EPM), malnutrition decreases anxiety-like behavior in adult rats [9].

Studies in developing malnourished animals have demonstrated delays in the ontogeny of play behaviors [10], reflex maturation, and locomotor activity [11], as well as a decrease in play and social behaviors [12]. Indeed, the effect of malnutrition on the weanling period – postnatal day 20–23 – might be particularly important for defensive behavior regulation. For example, it has been shown that malnourished weanling male rats showed decrease in anxiety-like behavior in the EPM [13].

There are also evidences in the literature indicating that gender is an important variable in the study of malnourishment in defensive behavior. For example, it has been shown that male and female rats postnatally protein-malnourished exhibited less anxiety-like behavior than control animals in the EPM [9,14–16]. However, other studies have reported that female rats displayed more [17] or did not differ from control rats [18] in the EPM test when malnutrition was imposed during the perinatal period.

Despite the literature showing differences between male and female malnourished rats, there are still some controversies regarding how these effects might interact across different development periods. Moreover, the use of ethological models of anxiety, such as the elevated T-maze (T-maze), to perform behavioral evaluations of malnourished rats has been shown to be beneficial because these tests do not involve food or water deprivation or painful aversive stimuli. Therefore, the present study investigated whether protein-calorie malnutrition alters T-maze defensive behavior in weanling (postnatal day 21 [PND21]) and young adult (PND50), male and female rats.

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2. Materials and methods

2.1. Animals

Litters of Wistar rats from the animal house of Estácio de Sá University at Resende were used. On the day of birth (PND0), pups were divided into Control (C) and Malnutrition (M) groups. All litters were composed of eight pups each (six males and two females). In the C group, the litters were fed by dams receiving *ad libitum* lab chow, whereas in the M group, the dams received 40% of the total amount of the diet offered to dams in the C group. Briefly, M group received 5 to 12 g/d in the first week, 12 to 15 g/d in the second week, and 15 to 20 g/d in the last week of the lactation period. The diet offered to C dams was weighed and replaced every morning so that we could evaluate daily ingestion and calculate the amount of food offered to the M dams. During the lactation period, the dams and pups were housed in polypropylene cages (41 cm × 33 cm × 16 cm). After weaning (PND21) until PND50, animals received the same food as theirs mothers (i.e., *ad libitum* access in the C group and 40% of the C group food in the M group). The animals were housed individually in polypropylene cages (30 cm × 18 cm × 13 cm) under a 12 h/12 h light/dark cycle (lights on at 6:00 AM) at 23 ± 1 °C and given free access to water throughout the experiment. The rats were weighed at birth and on the day of behavioral testing (PND21 or PND50) using an electronic balance. All experiments were performed according to the recommendations of the Brazilian Society of Behavioral Neuroscience, which are based on the United States National Institutes of Health Guide for Care and Use of Laboratory Animals [19].

2.2. Apparatus

The T-maze (modified from the EPM) was made of wood and had three arms of equal dimensions (50 × 12 cm). One arm, enclosed by 40 cm high walls, was perpendicular to two opposed open arms. To avoid falls, a 1 cm high Plexiglas edge surrounded the open arms. The entire apparatus was elevated 50 cm above the floor.

2.3. Procedure

Two days before the test, the rats were gently handled for 5 min. On PND21 or PND50, independent Control groups (male [CM] and female [CF]) and Malnourished groups (male [MM] and female [MF]) were subjected to the T-maze ($n = 8$ per group). Two rats of the same sex per litter were randomly selected for this study (two males and two females). For the T-maze testing, each rat was placed at the distal end of the enclosed arm facing the intersection of the arms. The time taken to withdraw the four paws from this arm was recorded (baseline latency [BL]). The same measurement was repeated twice at 30 s intervals (avoidance trial 1 [AT1] and avoidance trial 2 [AT2]). During the 30 s intervals between trials, the animals were placed in a polypropylene cage (30 cm × 19 cm × 11.5 cm). The cutoff time for each trial was 5 min. If the animal did not avoid or escape before this cutoff time, a latency of 300 s was recorded. Animals that fell from the maze or had 300 s BL were excluded from the data analysis.

3. Results

3.1. Body weight

Body weights on PND0 were analyzed using *t*-tests for independent samples. Body weights on PND21 and PND50 were analyzed using a three-way ANOVA (age × diet × sex) with age as the repeated measure. Despite the similar weights of the pups (C group: 6.48 ± 0.91 g; M group: 6.29 ± 0.18 g; $t_{38} = 0.89$; $p > 0.38$) the ANOVA showed a significant effect of diet ($F_{1,28} = 15.76$) on body weight on PND21 (C group: 30.92 ± 1.13; M group: 22.91 ± 1.59) and significant effects of diet ($F_{1,27} = 111.21$; C group: 178.77 ± 7.30; M group: 100.35 ± 5.42) and sex ($F_{1,27} = 16.06$; Male: 154.18 ± 12.00; Female: 126.57 ± 11.14) on body weight on PND50.

3.2. Elevated T-maze

Behavioral data in the T-maze were subjected to logarithm transformation to normalize the data and were analyzed using a 2 × 2 × 2 × 3 (age × diet × sex × trials) ANOVA, with trials (BL, AT1, and AT2) as the repeated measure. Fig. 1 shows the mean (± SEM) of BL, AT1, and AT2 latencies on the T-maze. The ANOVA indicated a four-way age × diet × sex × trials interaction ($F_{2,102} = 4.38$, $p < 0.05$). A two-way age × trials interaction ($F_{2,102} = 3.40$, $p < 0.04$) and main effects of trials ($F_{2,102} = 47.00$, $p < 0.001$) and age ($F_{1,51} = 26.25$, $p < 0.001$) were also found. No other differences were found in the ANOVA ($p > 0.05$). *Post hoc* comparisons were made using Fisher's least significant difference test. As shown in Fig. 1, PND50 animals had lower BL and AT1 latencies in the T-maze compared with PND21 animals ($p < 0.05$). This age difference remained during AT2 only among malnourished animals ($p < 0.05$). Training increased latencies between BL and AT1

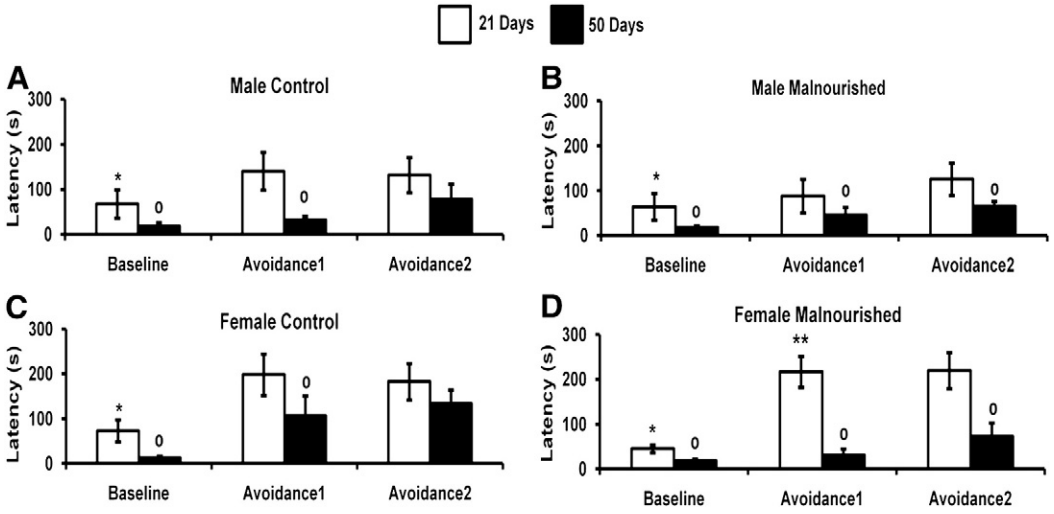

Fig. 1. Mean (± SEM) baseline latencies and latencies during inhibitory avoidance trials 1 and 2 in the elevated T-maze in male (A and B) and female (C and D) control (A and C) and malnourished (B and D) rats on postnatal day 21 (open bars) or postnatal day 50 (filled bars). o, PND21 compared with PND50; *, BL compared with A2 at PND21 and PND50; **, malnourished male avoidance trial 1 and malnourished female BL compared with malnourished female avoidance trial 1. $N = 8$ per group.

and between BL and AT2 in both male and female control animals ($p<0.05$). The effect of training among malnourished animals was observed only during AT2 ($p<0.05$). The only exception was found among malnourished PND21 female rats during AT1, which exhibited higher latencies compared with malnourished PND21 female rats during BL and compared with malnourished PND21 male rats during AT1 (both $p<0.05$).

4. Discussion

The lower body weight of malnourished animals is consistent with previous reports showing that caloric restriction or a protein-deficient diet during the lactation and post-lactation periods impairs physical development. Impaired growth of rats subjected to protein-calorie malnutrition has been well-established in the literature [20–22].

Numerous studies have shown that increasing age correlates with increased anxiety-like behavior in humans and rats [23–26]. In contrast, results from the present study indicated that weanling rats spent less time in the open arms of the T-maze compared with young adults. It is important to point out that our study compared weanling rats (PND21) and young adults (PND50), which contrasts with previous studies from the literature that demonstrated an increased anxiety-like behavior in adult animals that were at least 90 days old. However, our results are consistent with other studies showing that 60-day-old rats exhibited less anxiety-like behavior compared with (*i*) 30-, 90-, or 120-day-old rats exposed to the T-maze (25), (*ii*) 22- and 35-day-old rats exposed to the EPM (13), and (*iii*) 30-, 90-, and 120-day-old rats exposed to the EPM (26). As a whole, these results seem to indicate that PND50 rats were less afraid than younger or older rats, suggesting that young adult rats may adapt more readily to stressful situations. Future studies are needed to investigate more fully this developmental period.

Reexposure to the T-maze increased BL, AT1, and AT2 latencies compared with control animals. However, this effect was not observed between BL and AT1 when compared with malnourished animals, suggesting that lower anxiety-like behavior may result from early malnutrition. Previous studies also showed that prenatal or postnatal malnutrition led to a increase in open-arm exploration in the EPM [14,16] and T-maze [1]. The hypothesis that malnutrition decreases anxiety-like behavior in rats is supported by other studies showing that the anxiolytic drugs, such as diazepam, prevented the increase in avoidance latencies in saline-treated eutrophic animals [27].

Additionally, the data from AT1 and AT2 may be used as an index of learning. The control animals may have shown high latencies in these trials because previous experience with the T-maze might lead them to learn the safety of the enclosed arms. Thus, lower latencies in malnourished rats in these trials compared with control animals, might suggest impairment in the learning mechanism in these animals [1].

The hippocampus plays a fundamental role in learning and memory processes [28,29]. Moreover, it has been shown that malnutrition during the early developmental period damages the hippocampus since this structure undergoes a maturation phase during the lactation period [30]. Therefore, it is possible that hippocampal injury caused by early malnutrition may cause the learning deficit observed in the AT1 and AT2 latencies.

Another possibility to explain the lack of differences between AT1 and AT2 might be the fact that malnourished rats had deficits in their inhibitory response, as reflected in decreased latencies to visit the open arms of the T-maze. Indeed, it has been shown that malnourished rats have deficits in learning tasks that require response inhibition, such as differential low reinforcement task [8,17,20,31].

It is important to mention that malnourished rats at PND21 and PND50 presented a better performance in AT2 when compared to BL. These results are consistent with another study showing that malnourished animals had performance similar to controls when they were subjected to a greater number of sessions in an avoidance task [8]. Therefore, although malnutrition might impose some sort of learning deficit to avoidance of the aversive stimulus that requires inhibitory response, if the animals were submitted to a task to a longer period they might be able to reach the goal.

Weanling malnourished female rats learned to avoid the aversive situation during AT1 more efficiently than during BL, indicating that they learned to avoid the aversive environment and exhibited anxiety-like behavior when reexposed to the T-maze. Estrogen can either improve [32,33] or impair [34,35] learning and memory in a dose-dependent manner, depending on the type of task, and can alter hippocampal function by decreasing inhibition through γ-aminobutyric acid mechanisms [36]. Moreover, malnourished animals have been shown to have alterations in this system revealed by hyporeactivity to benzodiazepine drugs [14]. Further studies are necessary to more fully elucidate the effects of malnutrition in female rats because changes in estrogen levels during the estrous cycle may affect various behavioral measures.

Food restriction is a major stressor that can activate different neurohormonal systems. Pregnant or lactating mother exposed to food restriction have higher levels of adrenocorticotropin and corticosterone secretion which in turn might change the development of the neuroendocrine system of its offspring such as adrenal atrophy and hypothalamic-pituitary-adrenals (HPA) axis hypoactivity [37,38]. Therefore, it is possible that restricted diet imposed to the mothers in the malnourished groups of the present study might have caused a reduction in the hyperactivity of the HPA in malnourished animals and thus a reduction in the anxiety-like behavior of these animals when exposed to the T-maze.

Finally, it is important to recognized that there are various studies that showed that underfed mothers are less efficient in the maternal care given to the pups (poor retrieving, deficient licking and nursing pups, nest building, reduced physical contacts, etc.), that impair physical growth, brain neuronal organization and brain functioning of the litter at juvenile and adult stages [39,40]. However, this possibility might be taken with caution. For example, Riul et al. [41], employing an ethological analysis found that although a lower frequency of suckling was observed in malnourished animals, this parameter was not indicative of a lower amount of nursing of the malnourished dams, since they expend more time feeding their pups than the control mothers. Therefore, future studies are important to investigate whether expression of anxiety-like behaviors in the T-maze among malnourished animals is mediated through changes in maternal care.

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