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EFEITOS DE SUBNUTRIÇÃO PROTÊICA PRECOCE E TESTAGEM REPETIDA SOBRE COMPORTAMENTOS LOCOMOTORES E EXPLORATÓRIOS NO LABIRINTO EM CRUZ ELEVADO*

EFFECTS OF EARLY PROTEIN MALNUTRITION AND REPEATED TESTING UPON LOCOMOTOR AND EXPLORATORY BEHAVIORS IN THE ELEVATED PUS-MAZE

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ABSTRACT

An elevated plus-maze was used to investigate the effects of repeated testing on the locomotor and exploratory behaviors of malnourished rats. Pup malnutrition was induced during the lactation period (0 to 21 days of age) by feeding the dams a protein-deficient diet (6% protein) and the animals were allowed to recover from weaning to 70 days of age by eating a commercial lab chow diet. Control animals were suckled by dams receiving normal protein diet (16% protein) during the lactation period and were fed a commercial lab chow diet after weaning. At 70 days, malnourished and control animals were placed on the central platform of the elevated plus-maze facing an enclosed arm and allowed to explore for 5 min. This procedure was repeated at 24-h intervals of 6 days. The repeated testing in the elevated plus-maze did not change the total number of arm entries and attempts to enter open arms, but decreased the percentage of open arm entries, time spent in open arms, and total time spent on the central platform. These data suggest an increase in anxiety with repeated testing in the elevated plus-maze. In addition, the malnourished animals showed a large number of these animals as compared to control. The elevated plus-maze proved to be useful animal model to evaluate exploratory behaviors in early protein malnourished animals.

Keywords: Early protein malnutrition Elevated plus-maze Impulsiveness Exploration Anxiety Repeated testing

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Protein malnutrition early in life causes long-lasting structural and neurochemical changes in the central nervous system, as well as behavioral abnormalities (Dobbing, 1987; Morgane, et al., 1978; Wiggins, Fuller, & Enna, 1984). The more pronounced alteration of behavior is the hyperreactivity of malnourished animals submitted to aversive stimulation, indicated by lower shock threshold (Almeida, De Oliveira, Bichuette, & Graeff, 1988; Almeida, Soares, Bichuette, Graeff, & De Oliviera, 1992; Lynch, 1976; Smart, Whatson, & Dobbing, 1975) lower avoidance latency (De Oliveira & Almeida, 1985), more resistance to extinguishing avoidance responses (De Oliveira & Almeida, 1985), and higher postshock latency in an inhibitory avoidance task (Almeida et al., 1988; Almeida et al., 1992; Lynch, 1976). This hyperreactivity of malnourished animals has been observed mainly with experimental procedures using painful aversive stimuli such as electric shock. However, it is desirable to develop alternative procedures to electric shock because malnourished animals have consistently shown a lower shock threshold (Almeida et al., 1988; Almeida et al., 1992; Lynch, 1976; Smart et al., 1975). Recently reported results have demonstrated that in more naturalistic aversive situations, malnourished animals present lower anxiety contrasting with the hyperreactivity described above. This lower anxiety leads to a larger number of transitions in the light/dark transition test (Brioni & Orsingher, 1988) and to a larger number of open-arm entries in the elevated plus-maze test (Almeida, De Oliveira, & Graeff, 1991).

On the other hand, malnourished animals take longer to habituate to situation involving novelty, such as ambulation on the open-field test (Almeida et al., 1992) and exploratory of novel object (Barnes, Levitsky, Pond, & Moore, 1976; Wiener, Robinson, & Levine, 1983). However, the data in this area are controversial, and the usually employed procedures do not permit a separation of locomotor and exploratory behavior (Archer & Birke, 1983; Barnabé, Soares, & De Oliveira, submitted).

More recently, a novel anxiety model based on the exploratory behavior of rats has been developed. This model is based on the natural aversion of rodents to heights and open spaces (Montgomery, 1958), namely the elevated plus-maze (Handley & Mithani, 1984; Pellow, Chopin, File, & Briley, 1985), and has been validated for rats (Pellow et al., 1985) and mice (Lister, 1987). In the elevated plus-maze the percentage of entries and the time spent in the open arms are taken as a measure of anxiety, and the total arm entries (open + closed) provide a measure of overall activity. Placing the rat on the central platform of the elevated plus-maze can evoke both the exploratory drive and the fear drive, thus generating an approach-avoidance conflict behavior. Recently reported results have shown that, in addition to the basic preference for closed vs. open arms, animals spend a significant proportion of time on the central platform of the maze (Lee & Rodgers, 1990; Rodgers, Lee, & Sheperd, 1992; Trulla, Jackson, & Skolnick, 1989). It is important to note that, from this location, rats show high level of exploratory headdipping and anxious stretch attend/approach response toward the open arms (Rodgers et al., 1992), a behavioral description similar to the Blanchard concept of risk assessment (Blanchard, Blanchard, Tom, & Rodgers, 1990).

Thus, the objective of the present study was to investigate the effect of repeated testing on the locomotor and exploratory behaviors of well-nourished and malnourished rats in the elevated plus-maze.

METHOD

Animals

Twenty male Wistar rats from the animals house of the Ribeirão Preto Campus of the University of São Paulo were used. Within 12 h of birth, the male pups were weighed and randomly assigned to a litter of six per dam. The dams and pups were placed in wooden cages (30 x 30 x 15 cm) and randomly assigned to receive ad lib either an 8 or a 25% casein diet. The two diets were isocaloric and prepared according to Barnes et al. (Barnes, Neely, Kwong, Labadan, & Frankova, 1968). The protein-deficient diet contained 8% casein, 5% salt mixture, 1% vitamins mixture, 8% corn oil, 0.2% choline, and 77.8% cornstarch. The normoproteic diet contained 25% casein, 60.8% cornstarch, and the same percentage of the other constituents in the protein-deficient diet. The litters were maintained on these diets until the end of lactation (21 days). After weaning, the pups were maintained in individual metal cages (20 x 25 x 15 cm) and were fed a balance lab chow diet. During the experimental phase, starting at 70 days of age, the rats were maintained under a 12 h light-12 h dark cycle (lights on at 0700 h) and room temperature was kept at 23-25°C.

Apparatus

The elevated plus-maze consisted of two open arms (50 x 10 cm) opposite to each other, crossed by two enclosed arms (50 x 10 x 40 cm) with an open roof (Pellow et al., 1985). The maze was elevated 50 cm from ground floor.

Procedure

The animal was placed at the center of the maze facing an enclosed arm and allowed to explore for 5 min. During this period, the number of entries into open and enclosed arms, the time spent on the central platform of the maze, the attempts to enter open arms, and rearing in the central platform were recorded with a Sony videocamera, linked to a monitor and VCR in an adjacent room. The arm entries were defined as entry of all four paws into the arm and the attempts to enter open arm as entry of the two front two paws into the open arms. This procedure was repeated for 6 days at 24-h intervals.

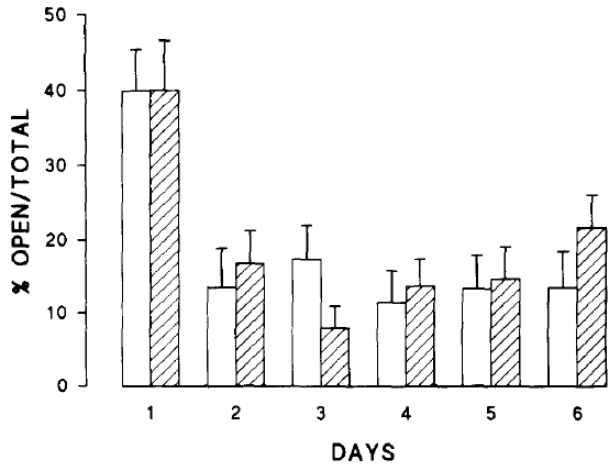

Figure 1. Effects of repeated testing on the percentage of open-arm entries by well-nourished (open bars) and malnourished (hatched bars) animals. Vertical bars represent the SEM of 10 rats.

Statistical Analysis

The body weights of the nutritional groups were compared by the Student's t-test. The behavioral data in the elevated plus-maze were analyzed by analysis of variance (ANOVA), with days as a repeated measure.

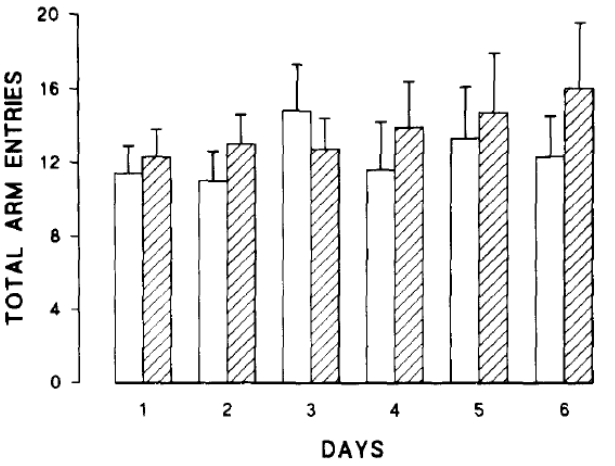

Figure 2. Effects of repeated testing on total arm entries. See legend of Figure 1.

RESULTS

Body weight at 70 days of age was 333.7 +- 7.9 and 296.9 +- 11.5 (mean +- SEM) for W and M rats, respectively. The difference was statistically significant, $t(18) = 2.64$, $p < 0.01$.

Both the percentage of open-arm entries and percentage of time in the open arms were decreased with repeated testing. The percentage of open/total arm entries is shown in Fig. 1. ANOVA demonstrated a significant effect of days, $F(5, 90) = 19.98$, $p < 0.001$, but no significant effect of diet or diet X day interaction. The same was observed for the percentage of open/total time (data not shown), with ANOVA showing a significant effect of days, $F(5, 90) = 13.56$, $p < 0.001$, and no significant effect of diet or diet X day interaction.

As illustrated in Fig. 2, no significant effect of diet, days, or diet X day interaction was observed on total arm entries.

Regarding the time spent on the central platform (Fig. 3), ANOVA indicated a significant effect of days, $F(5, 90) = 3.81$, $p < 0.003$, but no significant effect of diet or diet X day interaction. As observed for the percentage of open arm entries and time spent in the open arms, time spent on the central platform also decreased with repeated testing. Although no differences were observed between M and W rats in the time spent on the central platform, the number of rearings on this platform was significantly higher for M than for W animals, $F(1, 18) = 4.73$, $p < 0.04$. The number of rearings increased with repeated testing, $F(5, 90) = 2.89$, $p < 0.02$, but no diet X day interaction was observed (Fig. 4).

As illustrated in Fig. 5, M animals made more attempts to enter the open arms them W animals, $F(1, 18) =$ $p < 0.02$, but no significant effect of days or diet X day interaction was found.

DISCUSSION

Protein malnutrition during the lactation period did not affect the exploration of the plus-maze when we analyzed measures such as percentage of open/total arm entries, open/total time, or total arm entries. These data do not agree with previously reported results showing higher exploration of open arms in malnourished animals (Almeida et al., 1991). However, the shorter period of exposure to a low-protein diet.

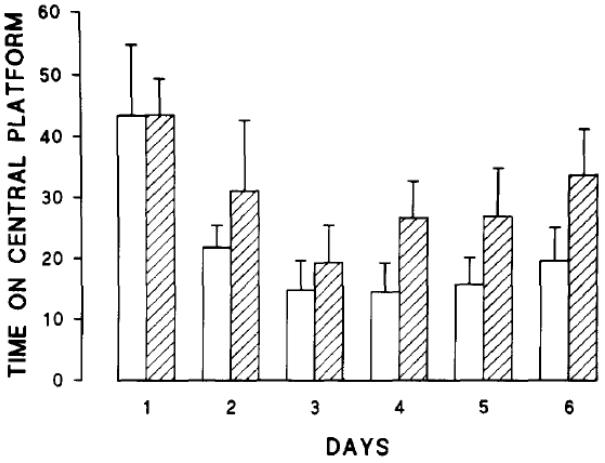

Figure 3. Effects of repeated testing on time spent on the central platform. See legend of Figure 1.

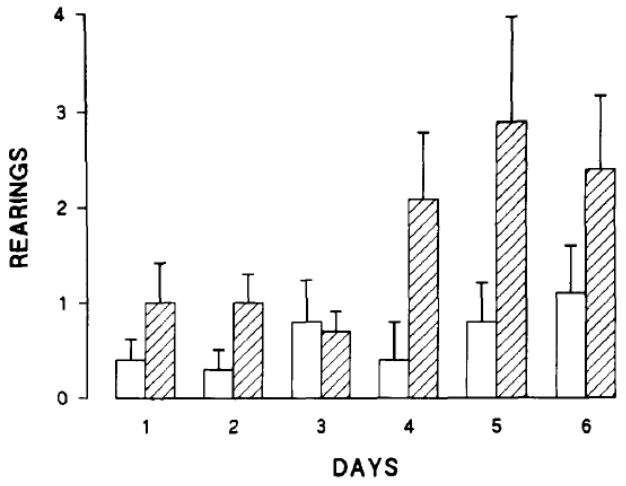

Figure 4. Effects of repeated testing on number of rearing on the central platform. See legend of Figure 1.

However, it is interesting to observe that repeated testing leads to a reduction in both open/total arm entries and open/total time ratios for both nutritional conditions. These data do not agree with previously reported results showing no variability of these parameters with repeated testing over 2 or 3 days (File, 1990; File, Mabbutt, & Hitchcott, 1990; Pellow et al., 1995), but agree with recently reported results showing that reexposure to the elevated plus-maze decreases the time spent in the open arms (Lee & Rodgers, 1990; Rodgers et al., 1992). In the present study, we found that on the second day of exposure to the elevated plus-maze rats submitted to both nutritional conditions reduced the exploration of open arms and maintained with lower levels of exploration on the subsequent days of testing. Total arm entries, on the other hand, did not change with repeated testing, indicating that the reduction of exploration on the subsequent days of testing. Total arm entries, on the other hand, did not change with repeated testing, indicating that the reduction of exploration of open arms was compensated by increasing closed-arm entries. These data show that repeated exposure to this test can produce per se a profile consistent with enhanced anxiety. As reported by others (File, 1990; File et al., 1990), previous exposure to the elevated plus-maze make the animals tolerant to the anticonflict effect of chlordiazepoxide in the second trial carried out 24 h after initial exposure (first trial). This tolerance suggests that previous experience with the experimental situation generates a new form of fear that is unresponsive to benzodiazepine treatment. File, Zangrossi, Viana, and Graeff (in press) have suggested that, because benzodiazepines are relatively ineffective against phobias, this raises the possibility that the behavior in the second trial may reflect a phobic anxiety (fear of heights).

Decreases in both open-arm entries and time spent on the central platform with repeated testing could also be an indication of a reduction of open arms novelty; however, this interpretation is impaired by the fact that neither rearings nor attempts to enter open arms were reduced, showing that there is no habituation to exploratory behaviors on the central platform with reexposure to the elevated plus-maze. The stability of the attempts to enter the open arms with repeated testing over 6 days may indicate that the conflictive situations continues to be present after reexposure. Thus, the interpretation of an increase in fear (enhanced anxiety) seems to be reinforced.

It has been also suggested (Lee & Rodgers, 1990) that this anxiogenic profile with repeated testing may reflect an anticipatory component. This proposal is based on the significant elevation of baseline tail-flick latency from day 1 (first trial) to day 9 (second trial) in the elevated plus-maze. As repeated tail-flick testing has either no effect upon or decreases latencies (Rodgers & Randal, 1985, 1986a, 1986b), it would seem reasonable to suggest that this anticipatory reaction is somehow related to the entire elevated plus-maze testing procedure (Lee & Rodgers, 1990).

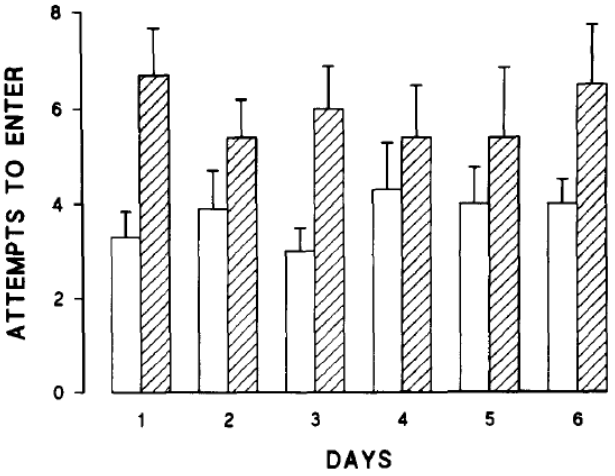

Figure 5. Effects of repeated testing on the attempts to enter the open arms. See legend of Figure 1.

The larger number of rearings and attempts to enter the open arms exhibited by M animals (Figs. 4 and 5) indicates a high level of exploration of these animals in a naturalistic conflictive situation, with no differences in locomotor activity, as demonstrated by an equal number of total arm entries. It is interesting to note that both rearing and attempts to enter the open arms are behaviors displayed on the central platform of the maze, a decision place, where the animals are exposed to a conflictive situation involving entry to open X closed arms. These data and others showing more transitions of M animals in the light/dark test (Brioni & Orsingher, 1988), and more open-arms entries in the elevated plus-maze (Almeida et al., 1991), are also indicative of high level impulsiveness of M animals in naturalistic conflictive situations. In addition, it has been shown (Brioni & Orsingher, 1988) that M rats make more errors in a DRL procedure, indicating a difficulty to inhibit operant response to wait for a food/water reward and reinforcing the proposition of high impulsiveness of these animals.

Because malnutrition early in life causes structural and neurochemical alterations in the CNS (Dobbing, 1987; Morgane et al., 1978; Wiggins et al., 1984), the high impulsiveness of M rats could be due to malnutrition-induced damage to the brain system regulating behavior inhibition in naturalistic aversive situations.

The higher impulsiveness of M rats in more naturalistic animal models of anxiety contrasts with a high timidity of these rats in some models of anxiety based upon painful aversive stimuli (Almeida et al., 1988; Almeida et al., 1992; De Oliveira & Almeida, 1985). This contrast may be due to a lower threshold of M animals to painful aversive stimuli, as demonstrated by other (Almeida et al., 1988; Almeida et al., 1992; Lynch, 1976; Smart et al., 1975). If this assumption is true, the higher timidity of M rats in animal models of anxiety with painful aversive stimuli is not due to higher levels of anxiety in stressful situations in general, but to a higher emotional response to painful stimuli. Thus, the development of more naturalistic animal models of anxiety, such as the elevated plus-maze, will contribute to the study of emotional responses of M animals, without the confounding factors due to a lower pain threshold of these animals or even due to differences in motivational state due to food/water deprivation.

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