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ISOLAMENTO SOCIAL DE CURTO PRAZO NÃO REDUZ EXPLORAÇÃO NO LABIRINTO EM CRUZ ELEVADO EM RATOS COM SUBNUTRIÇÃO PROTEICA PRECOCE*

SHORT-TERM SOCIAL ISOLATION DOES NOT REDUCE ELEVATED PLUS-MAZE EXPLORATION IN EARLY PROTEIN MALNOURISHED RATS

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ABSTRACT

An increased number of visits and time spent in the open arms of the elevated plus-maze by malnourished rats has been used as indicative of lower anxiety or impulsiveness. In order to study how this behavior profile responds to an anxiogenic procedure (short-term social isolation), control (16% protein) and malnourished (6% protein) rats were socially isolated prior to the test in the maze. Litters (dam plus 6 male 2 female pups) were fed the diets from birth to 49 days of age. From 50 days on, all rats were fed a lab chow diet. Social isolation consists in removing the rats from the group and placing in individual cages for 2h before the test. During the test each rat was individually placed on the center of the maze and allowed to explore for 5 min. The results showed higher open arms exploration and lower attempts to enter open arms by the malnourished rats than by the controls. Social isolation decreased open arm exploration and increased time spent on the central platform in control animals, but had no effect on the malnourished rats. The results reinforce the lower anxiety or higher impulsiveness of malnourished rats, as well as the anxiogenic effect of social isolation in control rats. However, the malnourished rats were unresponsive to the anxiogenic effects of social isolation, indicating that protein deficiency early in life not only induces lower anxiety or higher impulsiveness in the maze, but also changes the behavior of these animals in response to another environmentally-induced procedure of anxiety (social isolation).

Keywords: Early protein malnutrition, Social isolation, Anxiety, Impulsiveness, Stress, Rats.

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It has been consistently demonstrated in our laboratory that postnatal protein or proteincalorie malnutrition causes an increased exploration of the open arms of the elevated plus-maze. This increased exploration has been interpreted as lower anxiety and/or increased impulsiveness in previously malnourished animals (Almeida, De Oliveira, & Graeff, 1991; Almeida, Garcia, & De Oliveira, 1993; Almeida, Garcia, Cibien, et al., 1994; Santucci, Daud, Almeida, & De Oliviera, 1994; Moreira, De Araújo, De Oliveira, & Almeida, 1997). In addition, prenatal protein malnourished rats showed both an increased exploration of the open arms of the elevated plus maze and a lower inhibitory avoidance latency in the elevated T-maze test (Almeida, Tonkiss, & Galler, 1996a,b), supporting the suggestion that early protein malnutrition induces a lower anxiety and/or higher impulsiveness in rats. This interpretation also finds support in the study of Brioni and Orsingher (1988) who described an increased exploration by previously protein malnourished rats in the classical light – dark transition test.

Other studies in our laboratory have also shown differences in the ontogeny of the exploration in the elevated plus-maze between malnourished and control rats, with the anxiety generated by the open arms of the maze appearing at later ages in malnourished rats than in controls (Almeida et al., 1993). Futhermore, an ethological analysis showed that early protein malnutrition changes several behaviors indicative of anxiety in the plus-maze addition to the traditional open arms exploration measures (Almeida et al., 1993; 1994; 1996a; Santucci et al., 1994; Moreira et al., 1997).

If the reason for the increased exploration of the elevated plus-maze by the malnourished rats is the lower anxiety of these animals, then the introduction of an anxiogenic procedure before the test in the maze may reduce the open arm exploration by malnourished rats and help to reduce differences due to diet. However, this anxiogenic procedure should be based on naturalist models to avoid the use of painful aversive stimuli, since early protein malnutrition has consistently proved to decrease shock threshold in rats (Smart, Whatson, & Dobbing, 1975; Lynch, 1976; Almeida, De Oliveira, Bichuette, & Graeff, 1988; Almeida, Soares, Bichuette, Graeff, & De Oliveira, 1992; Rocinholi, Almeida, & De Oliveira, 1997).

It has been demonstrated that social isolation for both long (Jankowska, Pucilowski, & Kotowsk, 1991; Wright, Upton, & Marsden, 1991; Motta, Maisonnette, Castrechini, & Brandão, 1992) and short periods of time (Maisonnette, Morato, & Brandão, 1993) is an efficient anxiogenic procedure, inducing significant reduction in the exploration of the open arms of the elevated plus-maze. It was demonstrated that even 2h of social isolation significantly increased the anxiety in rats exposed to the elevated plus-maze (Maisonnette et al., 1993).

Thus, the main objective of the present study was to determine whether an anxiogenic procedure (short-term social isolation) introduced before the test in the elevated plus-maze, can be efficient in reducing the exploration of the open arms of the maze by malnourished rats and, consequently, in decreasing the well documented differences due to diet.

MATERIALS AND METHODS

Subjects

Thirty-five male Wistar rats from the animal colony of the Ribeirão Preto Campus of the University of São Paulo were used. During the lactation period (21 days), each litter was culled to six male and two females on the day of birth. From the same day on, the dams and pups were placed in transparent plastic cages (35 x 30 x 20 cm) and randomly assigned to receive either a 6% or a 16% protein diet ad libitum. The two diets were isocaloric and prepared according to Almeida et al. (1994). The proteindeficient diet contained 6% protein (casein), 5% salt mixture, 1% vitamin mixture, 8% corn oil, 0.2% choline, and 77.8% cornstarch. The normal protein diet contained 16% protein, 60.8% cornstarck, and the percentage of the other constituents as in the protein-deficient diet. The two diets were supplemented with L-methionine (2.0 g/kg of protein) since casein is deficient in this amino acid. The litters were maintained on these diets until the end of lactation (21 days). After weaning (21 days of age), one male pup from each litter was randomly chosen to compose groups of four animals per cage; and all groups were fed the same diets that were administrated to their dams during the lactation phase until 49 days of age. From 50 to 70 days of age (nutritional recovery period) all animals were fed a balanced lab chow diet (Purina, Brazil). The rats were maintained on a 12L: 12D cycle and room temperature was kept at 23-25°C.

Apparatus

The elevated plus-maze was made of wood and 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 50cm from the ground floor. Fluorescent ceiling lights (2 x 60 W) provided the only illumination in the experimental room.

Procedure

At 70 days of age, half of the animals were maintained in groups of four per cage and the other half was socially isolated in individual transparent plastic cages (31 x 20 x 13 cm) for 2h before the test in the maze (Maisonnette et al., 1993). The groups consisted of 7 – 10 animals per experimental condition. Only one rat per litter was used. Each animal was placed individually in the center of the maze facing an enclosed arm and allowed to explore during a 5-min session. The test session was recorded by a vertically mounted videocamera linked to a monitor and VCR in an adjacent room. The videotapes were analyzed by an experimenter blind to the treatments, and the following data were recorded: (a) % open arm entries (number of open arm entries/total number of open + closed arm entries x 100); (b) % time spent in the open arms (time in open arms/total time in open + closed arms x 100); (c) closed arm entries; (d) number of attempts to enter open arms (entering an open arm with the forepaws only and returning to the central platform or closed arm); and (e) time spent in the central platform.

Statiscal Analysis

The body weights of the nutritional groups were compared by the Student t-test and the behavioral data in the elevated plus-maze by two-way (nutrition by social condition) analysis of variance (ANOVA). *Post-hoc* analysis was conducted by Newman – Keuls Tests.

RESULTS

Body Weight

Body weight at 70 days of age was 325.7 $\pm$ 6.7 and 293.3 $\pm$ 10.3 (mean $\pm$ SEM) for well-nourished and previously malnourished rats, respectively. The difference was statistically significant, $t(33) – 2.89$, *p*. <0.01.

Elevated Plus-Maze

Previously malnourished animals showed a higher percentage of open arm entries, as indicated by a significant main effect of diet $F(1,31)=115.24$, $p<0.001$. Animals socially isolated for 2h before the test showed a lower percentage of open arm entries, as indicated by a significant main effect of social condition $F(1,31)=37.45$, $p<0.001$. However, the great reduction in open arm entries in socially isolated control animals contrasted with almost no effect on malnourished animals, resulting in a significant nutritional by social condition interaction, $F(1,31)=26.73$, $p<0.001$. As illustrated in Figure 1, post-hoc analysis showed that social isolation produced a significant decrease in open arm entries only in control animals ($p<0.05$).

ANOVA showed the same results about the percentage of time spent in open arms, as indicated by a significant effect of diet $F(1,31)=278.44$, $p<0.001$; social condition $F(1,31)=42.05$, $p<0.001$ and diet by social condition interaction $F(1,31)=32.02$, $p<0.001$. As evidenced by the percentage of open arm entries, the percentage of time spent in the open arms was reduce by social isolation only in control animals ($p<0.05$) (Figure 2).

As illustrated in Figure 1, only social isolation affected the number of closed arm entries. Socially isolated animals decreased the number of closed arm entries, as indicated by a significant main effect of social condition $F(1,31)=4.74$, $p<0.05$. No effects of diet or diet by social condition interactions were observed.

Malnourished animals spent less time in the central platform, as indicated by a significant main effect of diet $F(1,31)=57.37$, $p <0.001$. Animals socially isolated for 2h before the test spent more time in the central platform, as indicated by a significant main effect of social condition $F(1,31)=15.65=p<0.001$. However, social isolation increased the time spent in the central platform for control animals and did not affect the behavior of malnourished animals, resulting in a significant nutrition by social condition interaction, $F(1,31)=9.75$, $p<0.01$. As illustrated in figure 2, post-hoc analysis showed that social isolation produced a significant increase in time spent in the central platform only control animals ($p>0.05$).

Malnourished animals showed a lower number of attempts to enter the open arms, as indicated by a significant main effect of diet $F(1,31)=25.58$, $p<0.001$. Animals socially isolated for 2h before the test showed an increase in the number of attempts to enter open arms, as indicated by a significant main effect of social condition $F(1,31)=32.05$, $p<0.001$. However, social isolation produced a higher increase in the attempts to enter open arms for the control animals as compared to malnourished animals, resulting in a significant nutritional by social interaction, $F(1,31)=4.64$, $p<0.05$. As illustrated in figure 2, post-hoc analysis showed that social isolation produced a significant increase in the attempts to enter open arms in both nutritional groups ($p<0.05$).

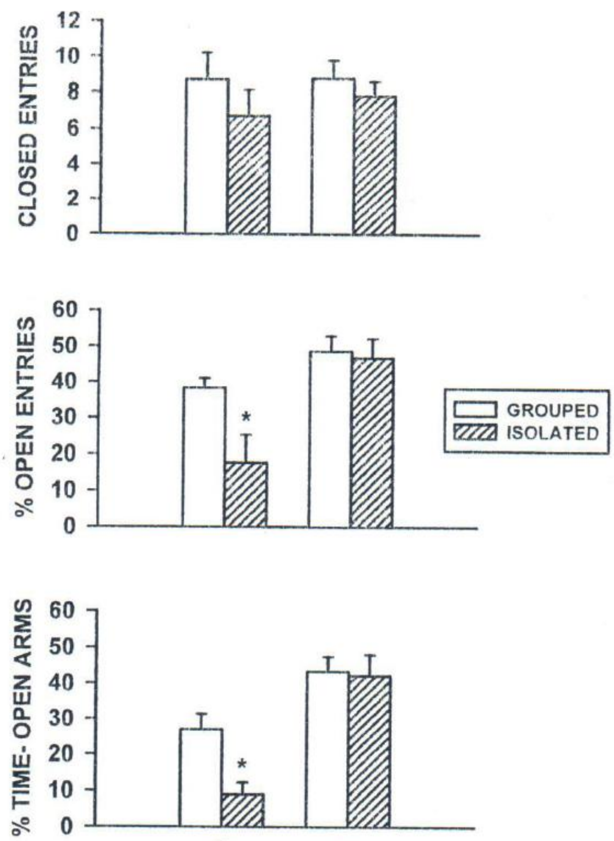

Figure 1. Number of closed arm entries (top), percentage of open arm entries (middle) and percentage of time spent in the open arms (bottom) of the elevated plus-maze. Animals were well-nourished © or previously malnourished (M) from birth to 49 days of age and maintained in groups (open bars) or socially isolated for 2h (hatched bars) before the test in the elevated plus-maze. Data represent the mean + SEM of 7-10 animals per condition. *p <0.05 compared to grouped rats in the same nutritional condition.

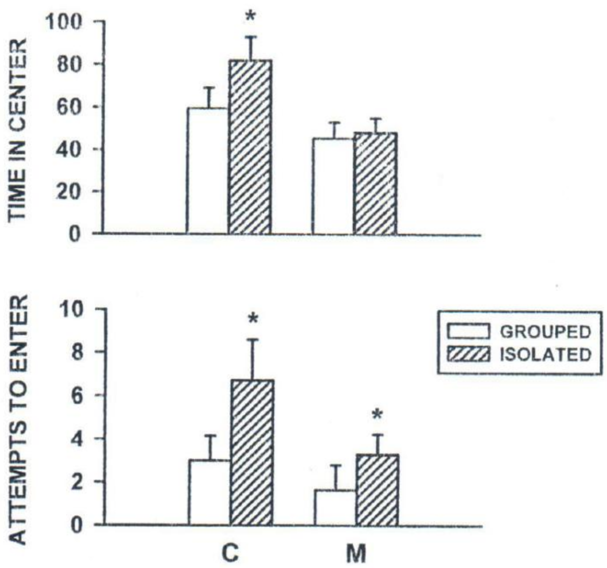

Figure 2. Time on the center platform (top) and attempts to enter open arms (bottom) in the elevated plus-maze. Animals were well-nourished (C) or previously malnourished (M) from birth to 49 days of age and maintained in groups (open bars) or socially isolated for 2h (hatched bars) before the test in the elevated plus-maze. Data represent the mean = SEM of 7-10 animals per condition. *p <0.05 compared to grouped rats in the same nutritional condition.

DISCUSSION

Early postnatal protein malnutrition led to significant decreases in rat body weight as previously reported by our group (Santucci et al., 1994; Rocinholi et al., 1997) and by others (Rocha & Mello, 1994).

Concerning the behavioral in the elevated plus-maze, the first observation was that short-term social isolation is an effective anxiogenic procedure as previously described in the literature (Maisonnette et al., 1993). Social isolation decreased the percentage of entries and time spent in the open arm entries, increased the time spent in the central platform and increased the number of attempts to enter the open arms.

Protein malnutrition increased the percentage of open arm entries and the time spent in the open arms. These results are in accordance with previous studies conducted in our laboratory; indicating a lower anxiety or higher impulsiveness in previously malnourished animals (Almeida et al., 1991; 1993;1994; Santucci et al., 1994; Moreira et al., 1997). In addition, the shorter time spent in the central platform and the lower number of attempts to enter the open arms displayed by malnourished rats support the suggestion of lower anxiety and/or higher impulsiveness. Since the central platform is considered as a “decision area” where the animal chooses to enter open or closed arms (Trullas, Jackson, & Skolnick, 1989), then a shorter time spent in this area could indicate lower conflict in the situation. This lower conflict is in accordance with the higher percentage of open arm entries and with the lower attempts to enter the open arms. While well-nourished rats assess the risk of entering the open arms by placing the two front paws is the open arms and returning to the two front paws in the open arms and returning to the central platform or enclosed arms, malnourished animals spend less time in this assessment and decide, more frequently, to enter the open arms. Taken together, these results suggest that early protein malnutrition changes the response of the animals in the direction of a lower anxiety or higher impulsiveness in the elevated plus-maze test. The suggestion of a higher impulsiveness is an interesting one because early malnourished animals exhibited a lower performance in a classical experimental model of behavioral inhibition named DRL (differential reinforcement of low rates), as described by Brioni and Orsingher (1988) and Tonkiss, Galler, Formica, Shukitt-Hale, and Timm (1990).

Short-term social isolation significantly decreased the exploration of open arms by the well-nourished animals as previously described in the literature (Maisonnette et al., 1993), but did not affect the behavior of malnourished animals tested under the behavior of malnourished animals tested under the same conditions. The differential effect of social isolation on malnourished animals indicates that even the introduction of a proven anxiogenic procedure prior to the test in the maze was not sufficient to reduce the exploration on the open arms. The exact mechanism by which social isolation increases the anxiety and decreases the exploration of the elevated plus-maze is not fully known, but previous studies have demonstrated that isolation also produces hypertension (Naranjo & Fuentes, 1985), neurochemical changes (Blanc et al., 1930), maze learning deficits (Wood & Greenough, 1974), and greater tail-flick latencies (Gentsch, Lichsteiner, Frischknecht, Feer, & Siegfried, 1988). Concerning the neurochemical changes, it has been demonstrated that social isolation alters the serotonergic neurotransmitter system (Popova & Petkiv, 1990; Valzelli, 1984).

Protein malnutrition per se has been consistently shown to produce alterations in the activity of the serotonergic neurotransmitter system (Almeida, Tonkiss, & Galler, 1996c). Thus, the malnourished animals could be reacting differentially to the social isolation as a result of such changes in the activity of the serotonergic system.

Another possible explanation for the unresponsiveness of the malnourished animals to the anxiogenic effect of social isolation could be that the malnutrition procedure per se is considered as an environmental factor causing stress (Adlard & Smart, 1972). Thus, once the malnourished animals experienced the stress of malnutrition early in life, it could help them, in the future, to cope more efficiently with another stressful situation, such as social isolation.

Finally, the fact that a proven anxiogenic procedure did not change the behavior of malnourished animals in the elevated plus-maze, in contrast with a true anxiogenic effect on wellnourished rats, could also suggest that higher exploration of open arms by malnourished rats should be mainly due to a higher impulsiveness rather than to a lower anxiety. Thus, the high impulsiveness of these animals, as suggested by previous works from our laboratory (Almeida et al., 1996a,b; Moreira et al., 1997) and by DRL produces (Brioni & Orsingher, 1988; Tonkiss et al., 1990), could be the main reason for the higher open arm exploration. This hypothesis is particularly interesting since early protein malnutrition changes the morphology (Cintra, Diaz-Cintra, Galvan, Kemper, & Morgane, 1990; Jordan, Howells, Mcnaugthon, & Heatlie,1982; Lewis, Patel, & Balaz, 1979), neurophysiology (Bronzino, Austin-Lafrance, & Morgane, 1990; 1991a,b; Jordan & Clark, 1983) and neurochemistry (Almeida et al., 1996c; Chen, Tonkiss, Galler, & Volicer, 1992; Blatt, Chen, Rosene, Volicer, & Galler, 1994) of the hippocampal formation, a brainn region described as especially important in the modulation of behavioral inhibition in mammals (Graeff, 1994; Gray, 1982a,b). The lower attempts to enter the open arms of the maze by the malnourished rats in the present study reinforce the suggestion of a deficit in behavioral inhibition. Malnourished animals do not assess properly the risk of entering the open arms of the elevated plus-maze when compared with well-nourished control rats.

Despite the uncertainty about the cause of higher open arm exploration in the maze observed in malnourished animals in the present study (lower anxiety vs higher impulsiveness), it was clearly shown that early protein malnutrition causes unresponsiveness to an environmentally-induced anxiety procedure (short-term social isolation). Whether this unresponsiveness is caused, at least in part, by structural and neurochemical changes in serotonergic brain systems in malnourished animals is a point that needs further investigation.

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REFERENCES

Adlard, B. P. F, & Smart, J. L. (1972). Adrenocortical function in rats subjected to nutritional deprivation in early life. *Journal of Endocrinology*, *54*, 99-116.

Almeida, S. S., De Oliveira, L. M., Bichuette, M. Z., & Graeff, F. G. (1988). Early malnutrition alters of chlordiazepoxide on inhibitory avoidance. *Brazilian Journal of Medical and Biological Research, 21*, 1033-1036.

Almeida, S. S., De Oliveira, L. M., & Graeff, F. G (1991). Early life protein malnutrition changes exploration of the elevated plus-maze and reactivity to anxiolytics. *Psychopharmacology, 103*, 513-518.

Almeida, S. S., Garcia, R. A., & De Oliveira, L. M. (1993). Effects of early protein malnutrition and repeated testing upon locomotor and exploratory behaviors in the elevated plus-maze. *Physiology and Behavior, 54*, 749-752.

Almeida, S. S., Garcia, R. A., Cibien, M. M. R., De Araújo, M., Moreira, G. M. S, & De Oliveira, L. M. (1994). The ontogeny of exploratory behaviors in early-protein-malnourished rats exposed to the elevated plus-maze test. *Psychobiology, 22*, 283-288.

Almeida, S. S., Soares, E. G., Bichuette, M. Z., Graeff, F.G., & De Oliveira, L. M. (1992). Effects of early postnatal malnutrition and chlordiazepoxide on experimental aversive situation. *Physiology and Behavior, 51*, 1195-1199.

Almeida, S. S., Tonkiss, J., & Galler, J. R. (1996a). Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus-maze test. *Physiology and Behavior, 60*, 675-680.

Almeida, S. S., Tonkiss, J., & Galler, J. R (1996b). Prenatal protein malnutrition affects avoidance but not escape behavior in the elevated T-maze test. *Physiology and Behavior, 60*, 191-195.

Almeida, S. S., Tonkiss, J., & Galler, J. R. (1996c). Malnutrition and reactivity to drugs acting in the central nervous system. *Neuroscience and Biobehavioral Reviews, 20*, 389-402.

Blanc, G., Herve, D., Simon, H., Lisoprawski, A., Glowinski, J, & Tassin, J. (1980). Response to stress of mesocorticofrontal dopaminergic neurons in rats after long-term isolation. *Nature 284*, 265-267.

Blatt, G. J., Chen, J., Rosene, D. L., Volicer, L., & Galler, J. R. (1994). Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: an immunocytochemical, ligand binding, and neurochemical study. *Brain Research Bulletin, 34*, 507-515.

Brioni, J. D. & Orsingher, O. A. (1988). Operant behavior and reactivity to the anticonflict effect of diazepam in perinatally undernourished rats. *Physiology and Behavior*, *44*, 193-198.

Bronzino, J. D., Austin-Lafrance, R. J., & Morgane, P. J. (1990). Effects of prenatal protein malnutrition on performant path kindling in the rat. *Brain Research, 515*, 45-50.

Bronzino, J. D., Austin-Lafrance, R. J., & Morgane, P. J. (1991a). Effects of prenatal protein malnutrition on kindlinginduced alteration in dentade granule cell excitability: I. Synsptic transmission measures. *Experimental Neurology*, *112*, 206-215.

Bronzino, J. D., Austin-Lafrance, R. J., & Morgane, P. J. (1991b). Effects of prenatal protein malnutrition on kindling-induced alteration in dentate cell excitability: II. Paired-pulse measures. *Experimental Neurology, 112*, 216-223.

Chen, J., Tonkiss, J., Galler, J. R., & Volicer, L. (1992). Prenatal protein malnutrition in rats enhances serotonin release from hippocampus. *Journal of Nutrition, 122*, 2138-2143.

Cintra, L., Diaz-Cintra, S., Galvan, A., Kemper, T., & Morgane, P. J. (1990). Effects of protein undernutrition on the dentate gyrus in rats of three age groups. *Brain Research, 532*, 271-277.

Gentsch, C., Lichsteiner, M., Frischknecht, H. R., Feer, H., Siegfried, B. (1988). Isolation induced locomotor hyperactivity and hypoalgesia in rats are prevented by handling and reversed by resocialization. *Physiology and Behavior, 43*, 13-16.

Graeff, F. G. (1994). Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. *Brazilian Journal of Medical and Biological Research, 27,* 811-829.

Gray, J. A. (1982a). The neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system. Oxford, Oxford: University Press.

Gray, J. A. (1982b). Multiple book review of the neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system. *Behavioral Brain Sciences, 5,* 469-534.

Jankowska, E., Pucilowski, O., & Kotowski, W. (1991). Chronic oral treatment with diltiazem or verapamil decreases isolation-induced activity impairment in elevated plus-maze. *Behavioral Brain Research, 43,* 155-158.

Jordan, R. C., & Clark, G. A. (1983). Early undernutrition impairs hippocampal long-term potentiation in adult rats. *Behavioral Neuroscience, 97,* 319-322.

Jordan, R. C., Howells, K. F., Mcnaugthon, N., & Heatlie, P. L. (1982). Effects of undernutrition on hippocampal development and function. *Research in Experimental Medicine, 180,* 201-207.

Lewis, P. D., Patel, A. J., & Balaz, R. (1979). Effect of undernutrition on cell generation in the rat hippocampus. *Brain Research, 168,* 186-189.

Lynch, A. (1976). Passive avoidance behavior and response thresholds in adult male rats after early postnatal undernutrition. *Physiology and Behavior, 16,* 27-32.

Maiosonnette, S., Morato, S., & Brandão, M. L. (1993). Role of resocialization and of 5-HT1a receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze test. *Physiology and Behavior, 54,* 753-158.

Moreira, G. M. S., De Araújo, M., De Oliveira, L. M., & Almeida, S. S (1997). The behavior of protein-caloriemalnourished rats on the elevated plus-maze test: an ethophartmcological analysis. *Psychobiology, 25,* 180-185.

Motta, V., Maisonnette, S., Castrechini, P., & Brandão, M. L. (1992). Effects of blockade of 5-HT2 receptors and activation of 5-HT1a receptors on the exploratory activity of rats in the elevated plus-maze. *Psychopharmacology (Berlin), 107,* 135-139.

Naranjo, J. R., & Fuentes, J. A. (1985). Association between hypoalgesia and hypertension in rats after short-term isolation. *Neuropharmacology, 24,* 167-172.

Pellow, S., Chopin, P., File, S., & Briley, M. (1985). Validation of open: closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods 14,* 149-167.

Popova, J. S., & Petkov, V. V. (1990). Changes in 5-HT, receptors in different brain structures of rats with isolation syndrome. *General Pharmacology, 21,* 223-225.

Rocha, J. B. T., & Mello, C. F. (1994). Handling during sucking alters the behavior but does not reserve the deleterious effects of undernutrition on naltrexone-induced inhibition of exploratory activity. *International Journal of Vitamin and Nutrition Research, 64,* 152-156.

Rocinholi, L. F., Almeida, S. S., & De Oliveira, L. M. (1997). Response threshold to aversive stimuli in stimulated early protein-malnourished rats. *Brazilian Journal of Medical and Biological Research, 30,* 407-413.

Santucci, L. B., Daud, M. M., Almeida, S. S., & De Oliveira, L. M. (1994). Effects of early protein malnutrition and environmental stimulation upon the reactivity to diazepam in two animal models of anxiety. *Pharmacology Biochemistry and Behavior, 49,* 393-398.

Smart, J. L., Whatson, T. S., & Dobbing, J. (1975). Thresholds of response to electric shock in previously undernourished rats. *British Journal of Nutrition, 34,* 511-516.

Tonkiss, J., Galler, J. R., Formica, R. N., Shukitt-Hale, B., & Timm, R. R. (1990). Fetal protein malnutrition impairs acquisition of a DRL task in adult rats. *Physiology and Behavior, 48,* 73-77.

Trullas, R., Jackson, B., & Skolnick, P. (1989). Anxiolytic properties of 1-aminocyclopropanecarboxylic acid, a ligand at strychnine-insensitive glycine receptors. *Pharmacology Biochemistry and Behavior, 34,* 313-316.

Valzelli, L. (1984). Reflections on experimental and human pathology aggression. *Progress Neuro Psychopharmacology and Biology Psychiatry, 8,* 311-325.

Wood, W. E., & Greenough, W. T. (1974). Effect of grouping and crowding on learning in isolation-reared adult rats. *Bulletin of Psychonomic Science, 3,* 65-67.

Wright, I. K, Upton, N., & Marsden, C. A. (1991). Resocialization of isolation-reared rats not alter their anxiogenic profile on the elevated X-maze model of anxiety. *Physiology and Behavior, 50,* 1129-1132.