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Response threshold to aversive stimuli in stimulated early protein-malnourished rats

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Abstract

Two animal models of pain were used to study the effects of short-term protein malnutrition and environmental stimulation on the response threshold to aversive stimuli. Eighty male Wistar rats were used. Half of the pups were submitted to malnutrition by feeding their mothers a 6% protein diet from 0 to 21 days of age while the mothers of the other half (controls) were well nourished, receiving 16% protein. From 22 to 70 days all rats were fed commercial lab chow. Half of the animals in the malnourished and control groups were maintained under stimulating conditions, including a 3-min daily handling from 0 to 70 days and an enriched living cage after weaning. The other half was reared in a standard living cage. At 70 days, independent groups of rats were exposed to the shock threshold or to the tail-flick test. The results showed lower body and brain weights in malnourished rats when compared with controls at weaning and testing. In the shock threshold test the malnourished animals were more sensitive to electric shock and environmental stimulation increased the shock threshold. No differences due to diet or environmental stimulation were found in the tail-flick procedure. These results demonstrate that protein malnutrition imposed only during the lactation period is efficient in inducing hyperreactivity to electric shock and that environmental stimulation attenuates the differences in shock threshold produced by protein malnutrition.

Key words
- Early protein malnutrition
- Enriched environment
- Shock threshold test
- Tail-flick test

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Introduction

Malnutrition early in life produces alterations of neurohistological (1-4), electrophysiological (5-9), biochemical (10), neurological (11) and behavioral (12-22) parameters. Regarding behavioral changes, a lower shock threshold has been consistently demonstrated in early malnourished animals compared to well-fed animals (21-24). We have previously demonstrated that protein malnutrition during lactation and post-lactation leads to a lower shock threshold in rats tested during the malnutrition period or later, after nutritional recovery (12,15). This lower shock threshold in malnourished animals has been used to interpret enhancement in inhibitory avoidance (12,15,21) and higher resistance to extinction after active avoidance learning in these animals (17,20,22,25). This hyperreactivity to aversive stimuli may lead to a stronger emotional response by malnourished rats, producing behavioral alterations in tests using electric shock as an aversive stimulus.

Since in our previous investigations (12,15) and other studies in the literature (21,25) protein malnutrition was used during both the lactation and post-lactation periods, it was interesting to investigate if a shorter period of malnutrition would result in the same hyperreactivity to electric shock. In addition, considering that early social or environmental isolation alters the emotional response in a fashion similar to that described after early protein malnutrition and that these changes in emotionality are enhanced by isolation and decreased by environmental stimulation in malnourished rats (25), the interactions between malnutrition and environmental stimulation represented another interesting point to investigate. In fact, it has been reported that environmental stimulation produces changes in structural, neurochemical and functional parameters in the central nervous system of the rat (26-29). It has been suggested that these effects of early protein malnutrition may be partially reversed by environmental stimulation offered during malnutrition, or later during the nutritional recovery period (26,30-33).

Thus, the main objective of the present investigation was to study the interactional effects of protein malnutrition (only during lactation**)** and environmental stimulation in two animal models of pain (shock threshold and tail-flick latency). In addition, since both malnutrition (7,8,15) and environmental stimulation (26) affect brain development, brain weights were also determined.

Material and Methods

Animals

Eighty male Wistar rats from the animal house of the Ribeirão Preto Campus, University of São Paulo, were used. Within 12 h of birth, the pups were weighed and randomly assigned to a litter of six per dam. From the same day on (day of birth), half the animals were suckled by mothers maintained on a 16% protein diet and the other half by mothers maintained on a 6% protein diet. During the lactation period the dams and pups were placed in transparent plastic cages (35 x 30 x 20 cm). The two diets were isocaloric and prepared according to Barnes et al. (34). The protein-deficient diet contained 6% protein (casein), 5% salt mixture, 1% vitamin mixture, 8% corn oil, 0.2% choline, and 77.8% cornstarch. The normoprotein diet contained 16% protein, 60.8% cornstarch, and the same percentages of the other constituents as the protein-deficient diet. The dams were fed *ad libitum* 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) with free access to a balanced lab chow diet until test day. The animals were weighed at birth (litter weight) and individually at 21 and 70 days of age.

Apparatus

For the shock threshold, a modified Mowrer cage (Funbec, São Paulo, Brazil) measuring 91 x 15 x 31 cm was used. The grid floor of the cage was made of stainless steel bars spaced 0.5 cm apart and connected to a shock generator (model 700, Grason-Stadler, West Concord, Massachusetts). The front of the box was of transparent plastic to allow the observation of animal reactions.

The Thermotimer tht 103 (Multieletrônica, Brazil) used for the tail-flick test consisted of a nickel chrome wire coil heated by the passage of electrical current. The current raised the temperature of the coil from room temperature (23 ± 1°C) at a rate of 9°C/s. An auxiliary tube (5 cm in diameter) was used to restrict animal movements.

Procedure

Environmental stimulation. Half the pups in both diet groups were exposed to daily 3-min individual handling from birth to 70

days of age. The other half was maintained without manipulation. The handling consisted of holding the animal in one hand and making cranio-caudal movements on the dorsal region with the thumb of the other hand. After eye opening, the handling was conducted outside the animal room in order to increase visual and auditory stimulation. After weaning (22 to 70 days of life) the animals were also exposed to olfactory stimulation during handling, consisting of the impregnation of the experimenter's hands with a deodorant (Miss France, Gessy Lever, Brazil). The stimulated animals were maintained in enriched metal cages with a variety of objects such as plastic platforms, stairs, mirrors and marbles. The non-stimulated animals were maintained in a similar metal cage without the objects. The environmental enrichment, as described by Lima (35), was an adaptation of the procedure of Rosenzweig and Bennett (36).

Four experimental groups were formed: well-nourished animals exposed to environmental stimulation by handling plus enrichment of their home cages (SW), non-stimulated well-nourished animals (NW), stimulated malnourished (SM) animals, and non-stimulated malnourished animals (NM). Twelve rats per group were used in the shock threshold and 8 rats per group were used in the tail-flick test. In both procedures independent groups of animals were tested at 70 days of age.

Shock threshold test. The testing procedure was similar to that described by Evans (37), and followed the same parameters previously used in our laboratory (2,5). Briefly, after a 2-min habituation to the test situation, the rats received 10 series of unavoidable shocks. Each series consisted of 10 different shock intensities (0.05, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 mA) for a total of 100 shocks. The series were presented in alternating ascending and descending order. The shock duration was 0.5 s and the interval between shocks was 30 s. The interval between series of shocks was 2 min.

During each shock three behavioral categories (flinching, vocalization and jumping) were scored. A flinching response was scored when the animal crouched raising 1 or 2 front paws from the grids. A jumping response was scored when the animal jumped or withdrew 3 or more paws from the grids, followed or not by running or locomotion. A vocalization response was scored when the rat made an audible squeak. The test was conducted by two experimenters blind to the nutritional and environmental conditions of the rat, and interobserver reliabilities were calculated by determining the correlation values (r_s) of >0.90 for each behavioral category (flinching, jumping and vocalization).

Tail-flick test. The tail-flick test procedure was adapted from Prado and Roberts (38). The temperature was adjusted before the test to ensure that the tail-flick baseline latency was between 2.5 and 3.5 s for the control animals. The rats were adapted to the restraining device in a habituation session of 3 trials and 3-min duration, with 5-min intertrial intervals. Twenty-four hours later the rats were returned to the restraining device, with their tail resting on the nickel chrome filament. Three tail-flick latencies were measured at 5-min intervals. If in any trial the animal failed to flick its tail after 6 s the equipment was automatically switched off to prevent damage to the skin.

Brain weight

At 21 and 80 days of age, animals from each nutritional group (N = 3) were perfused with 1% paraformaldehyde through the left heart ventricle. Immediately after perfusion the animals were decapitated and the brains were removed and weighed.

Statistical analysis

Three-factor (diet, environmental stimulation and age) analysis of variance

(ANOVA) with repeated measures on age was used to evaluate group differences in body weight. Brain weights (recorded in independent groups of rats at 21 and 80 days of age) were analyzed by a three-factor ANOVA (diet, environmental stimulation and age). The behavioral categories recorded in the shock threshold (flinching, vocalization and jumping) were analyzed by a three-way ANOVA (diet, environmental stimulation and behavioral category). The tail-flick response was analyzed by two-way ANOVA (diet and environmental stimulation). When appropriate, *post-hoc* analyses were conducted by the Newman-Keuls test (39).

Results

Body and brain weights

The analysis of body weight showed a significant effect of diet ($F(1,76) = 93.11$, $P<0.001$) and age ($F(1,76) = 3702.26$, $P<0.001$) and an interaction between diet and age ($F(1,76) = 7.49$, $P<0.01$). *Post-hoc* analysis showed that body weights of malnourished animals were significantly lower than those of control animals both at 21 (18.64 ± 1.21 g *vs* 47.27 ± 4.72 g) and 70 days of age (269.71 ± 31.32 g *vs* 321.98 ± 44.19 g) ($P<0.05$). The interaction between diet and age was due to a higher increase in body weight of control animals from 21 to 70 days of age as compared with the weight gain of malnourished animals. No effect of stimulation on body weight was found.

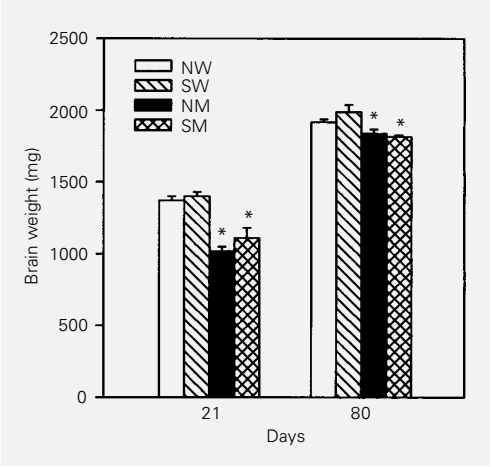

Figure 1 - Brain weight of pups at 21 and 80 days of age. The vertical bars indicate the SEM of 3 rats. NW, Non-stimulated well-nourished; SW, stimulated well-nourished; NM, non-stimulated malnourished; SM, stimulated malnourished. *P<0.05 compared to well-nourished groups of the same age (ANOVA followed by Newman-Keuls test).

Brain weights of rats were also significantly affected by diet ($F(1,23) = 51.56$, $P<0.001$), age ($F(1,23) = 466.83$, $P<0.001$) and diet x age interaction ($F(1,23) = 9.89$, $P<0.01$). *Post-hoc* analysis showed a higher brain weight of control animals as compared with malnourished animals both at 21 (1.38 ± 0.04 g *vs* 1.06 ± 0.06 g) and 80 days of age (1.96 ± 0.04 g *vs* 1.83 ± 0.02 g) ($P<0.05$). Additionally, both control and malnourished animals significantly increased the brain weight from 21 to 80 days of age ($P<0.05$). The significant interaction between diet and age factors was due to a faster increase in brain weight in malnourished animals than in controls after the beginning of nutritional recovery. However, at 80 days of age the malnourished animals still showed a lower brain weight than controls ($P<0.05$). No other main or interaction effects were found (Figure 1).

Shock threshold test

Statistical analysis showed a lower shock threshold of malnourished animals ($F(1,143) = 6.10$, $P<0.01$). Stimulated animals showed a higher shock threshold when compared to non-stimulated animals ($F(1,143) = 6.26$, $P<0.01$). Additionally, a significant effect of behavioral category was found ($F(2,143) = 50.05$, $P<0.001$), indicating differences in the shock threshold for the three categories analyzed (flinching, vocalization and jumping). *Post-hoc* analysis of shock threshold values showed that flinching was lower than vocalization and jumping ($P<0.05$), and vocalization was lower than jumping ($P<0.05$). No significant effects were observed for any of the interactions between factors (Figure 2).

Tail-flick test

Diet, environmental stimulation and diet

x environmental stimulation interactions had no statistically significant effects on this procedure.

Discussion

Protein malnutrition only during the lactation period impaired body and brain development in the present study. Nutritional recovery imposed after weaning produced an increase in the body and brain weights of previously malnourished animals, but not enough to equalize the weights of the two nutritional groups. The body and brain deficits produced by protein malnutrition during lactation are in agreement with previously reported data showing that malnutrition early in life causes impairment of development (40). In addition, the present data showed that even a short period of malnutrition was efficient to produce long-lasting effects on body and brain development. The prolonged period of nutritional rehabilitation was not sufficient to correct the impairment of normal brain development of malnourished animals. These results agree with a large number of previous reports (9,15,40). The significant effect of interaction between diet and age factors on brain weight demonstrated that the nutritional recovery period was efficient in producing a faster increase in the weight of the previously malnourished animals compared to the control group, but during adulthood the previously malnourished rats still had significantly lower brain weight.

The significant effect of diet on the shock threshold test showed that early protein malnutrition caused an increase in sensitivity to painful stimuli. This lower shock threshold after a period of malnutrition imposed only during lactation extends previously reported data from our laboratory (12,15) and others (21,22,24) which have demonstrated that postnatal plus postweaning malnutrition causes a decrease in the shock threshold. The lower shock threshold observed in the present study was consistent within the three behavioral categories recorded (flinching, vocalization and jumping) since no significant diet x behavioral category interaction was found. This consistently lower shock threshold in malnourished animals demonstrates that even short periods of malnutrition can induce hyperreactivity to electric shock delivered to the paws of the animals. The present data confirm previously reported data and point to the need for more caution in the analysis of behavioral changes in malnourished animals submitted to experimental models using painful aversive stimuli.

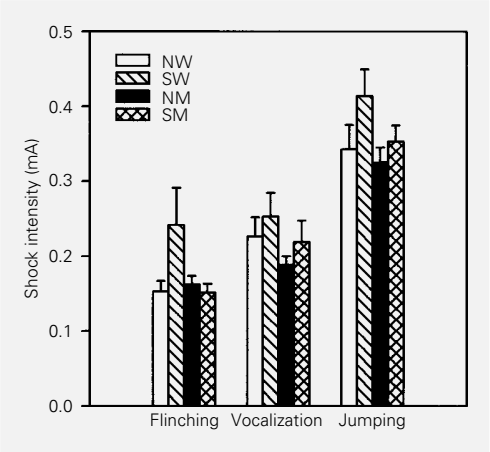

Figure 2 - Flinching, vocalization and jumping thresholds of the experimental groups submitted to electric shock at 70 days of age. The vertical bars indicate the SEM of 12 rats. NW, Non-stimulated well-nourished; SW, stimulated well-nourished; NM, non-stimulated malnourished; SM, stimulated malnourished. Flinching < vocalization < jumping thresholds ($P<0.05$, ANOVA followed by Newman-Keuls test).

Although a clearly lower shock threshold in malnourished rats was demonstrable by electric shock, it is difficult to explain why these animals did not differ from the well-nourished control group in the tail-flick test. It could be suggested that protein malnutrition may cause different changes in the mechanisms underlying the responses to these two different experimental models of pain. However, this possibility needs further experimental investigation.

Regarding the stimulation procedure, our results showing that environmental stimulation did not change brain weight agree with data obtained by Celedon et al. (41), Crnic (42) and Carughi et al. (32). The absence of a main effect of environmental stimulation or nutritional x environmental interaction on this parameter indicates that the impairment

of brain weight produced by malnutrition was not corrected by environmental stimulation. Certainly, brain weight is a gross measurement, and it would be desirable to perform a more detailed and specific morphological or neurochemical analysis of different brain regions in response to malnutrition and environmental factors since other studies have shown significant alterations in behavior-related regions of the brain (43-46).

Despite the absence of environmental stimulation effects on body and brain weights, this factor significantly changed the response of animals in the shock threshold test. Stimulated animals had a higher shock threshold indicating that this procedure reduced the shock sensitivity in these animals. However, the absence of interaction between diet and environmental stimulation factors demonstrates that animals submitted to both nutritional conditions reacted similarly to the environmental stimulation procedure. In addition, it is interesting to note that environmental stimulation of malnourished animals reduced the difference in shock threshold between the two nutritional groups. These data suggest that environmental stimulation attenuates the changes in sensitivity to shock produced by early protein malnutrition. The effect of environmental stimulation observed in the present study agrees with previously reported data showing that stimulation attenuates changes produced by protein malnutrition in a more natural situation (light-dark and elevated plus-maze tests) (47), as well as in experimental procedures designed to measure social and emotional behaviors (25).

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References

1. Andrade JP, Cadete-Leite A, Madeira MD & Paula Barbosa MM (1991). Long-term low-protein diet reduces the number of hippocampal mossy fiber synapses. *Experimental Neurology,* 112: 119-124.
2. Bedi KS, Massey RF & Smart JL (1989). Neuronal and synaptic measurements in the visual cortex of adult rats after undernutrition during normal or artificial rearing. *Journal of Comparative Neurology,* 289: 89-98.
3. Bedi KS & Warren KS (1990). Synapse-to-neuron ratios in rat cerebellar cortex following lengthy periods of undernutrition. *Journal of Anatomy,* 170: 173-182.
4. Faundez V, Cordero ME, Rosso P & Alvarez J (1990). Calibers and microtubules of nerve fibers: differential effect of undernutrition in developing and adult rats. *Brain Research,* 509: 198-204.
5. Austin KB, Beiswanger C, Bronzino JD, Austin-LaFrance RJ, Galler JR & Morgane PJ (1992). Prenatal protein malnutrition alters behavioral state modulation of inhibition and facilitation in the dentate gyrus. *Brain Research Bulletin,* 28: 245-255.
6. Austin-LaFrance RJ, Morgane PJ & Bronzino JD (1991). Prenatal protein malnutrition and hippocampal function: Rapid kindling. *Brain Research Bulletin,* 27: 815-818.
7. Bronzino JD, Austin-LaFrance RJ, Morgane PJ & Galler JR (1991). Effects of prenatal protein malnutrition on kindling-induced alterations in dentate granule cell excitability. I. Synaptic transmission measures. *Experimental Neurology,* 112: 206-215.
8. Bronzino JD, Austin-LaFrance RJ, Morgane PJ & Galler JR (1991). Effects of prenatal protein malnutrition on kindling-induced alterations in dentate granule cell excitability. II. Paired-pulse measures. *Experimental Neurology*, 112: 216-223.
9. Morgane P, Miller M, Kemper T, Stern W, Hall R, Bronzino J, Kissane J, Hawrylewicz E & Resnick O (1978). The effects of protein malnutrition and the developing central nervous system in the rat. *Neuroscience and Biobehavioral Reviews,* 2: 137-230.
10. Wiggins R, Fuller G & Enna S (1984). Undernutrition and the development of brain neurotransmitter systems. *Life Sciences,* 35: 2085-2094.
11. Eberhardt MJ & Hallas ES (1987). Developmental delays in offspring of rats undernourished or zinc deprived during lactation. *Physiology and Behavior,* 41: 309-314.
12. Almeida SS, De Oliveira LM, Bichuette MZ & Graeff FG (1988). Early malnutrition alters the effect of chlordiazepoxide on inhibitory avoidance. *Brazilian Journal of Medical and Biological Research*, 21: 1033-1036.
13. Almeida SS, De Oliveira LM & Graeff FG (1990). Decreased reactivity to anxiolytics caused by early protein malnutrition in rats. *Pharmacology, Biochemistry and Behavior,* 36: 997-1000.
14. Almeida SS, De Oliveira LM & Graeff FG (1991). Early life protein malnutrition changes exploration of the elevated plus-maze and reactivity to anxiolytics. *Psychopharmacology,* 103: 513-518.

15. Almeida SS, Soares EG, Bichuette MZ, Graeff FG & De Oliveira LM (1992). Effects of early postnatal malnutrition and chlordiazepoxide on experimental aversive situations. *Physiology and Behavior*, 51: 1195-1199.
16. Almeida SS, Garcia RA & De Oliveira LM (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.
17. Almeida SS & De Oliveira LM (1994). Acquisition and extinction of jumping two-way shuttle box and barpress avoidance responses in malnourished rats: effects of shock intensity. *Brazilian Journal of Medical and Biological Research*, 27: 2443-2452.
18. Almeida SS, Garcia RA, Cibien MMR, Araújo M, Moreira GMS & De Oliveira LM (1994). The ontogeny of exploratory behaviors in early-protein-malnourished rats exposed to the elevated plus-maze test. *Psychobiology*, 22: 283-288.
19. Brioni JD & Orsingher OA (1988). Operant behavior and reactivity to the anticonflict effect of diazepam in perinatally undernourished rats. *Physiology and Behavior,* 44: 193-198.
20. De Oliveira LM & Almeida SS (1985). Effects of malnutrition and environment on the acquisition and extinction of avoidance behavior in rats. *Physiology and Behavior,* 34: 141-145.
21. Lynch A (1976). Passive avoidance behavior and response thresholds in adult male rats after early postnatal undernutrition. *Physiology and Behavior,* 16: 27-32.
22. Smart JL, Whatson TS & Dobbing J (1975). Thresholds of response to electric shock in previously undernourished rats. *British Journal of Nutrition*, 34: 511-516.
23. Fernestron JD & Lytle LD (1979). Long-term consumption of low-protein corn-based diets: effect on serotonin synthesis in rat brain, and sensitivity to painful stimuli. In: Levitsky DA (Editor), *Malnutrition, Environment and Behavior. New Perspectives.* Cornell University Press, New York.
24. Levitsky DA & Barnes RH (1970). Effect of early malnutrition on the reaction of adult rats to aversive stimuli. *Nature*, 225: 468-469.
25. Levitsky DA & Barnes RH (1972). Nutritional and environmental interaction in the behavior development of the rat: Long-term effects. *Science,* 176: 68-71.
26. Bedi KS & Bhide PG (1988). Effects of environmental diversity on brain morphology. *Early Human Development,* 17: 107-143.
27. Szeligo F & Leblond CP (1977). Response of the three main types of glial cells of cortex and corpus callosum in rats handled during suckling and exposed to enriched, control and impoverished environments following weaning. *Journal of Comparative Neurology,* 172: 247-264.
28. Walsh RN, Budtz-Olsen QE, Penny JE & Cummins RA (1969). The effects of environmental complexity on the histology of the rat hippocampus. *Journal of Comparative Neurology*, 137: 361-366.
29. Wilson DA, Willner J, Kurz EM & Nadel L (1986). Early handling increases hippocampal long-term potentiation in young rats. *Behavioural Brain Research*, 21: 223-227.
30. Adaro L, Fernandes V & Kaufmann W (1988). Effects of nutrition environmental interactions upon body weight, body size and development of cortical pyramids. *Nutrition Reports International*, 33: 1013-1020.
31. Bhide PG & Bedi KS (1982). The effects of environmental diversity on well-fed and previously undernourished rats. I. Body and brain measurements. *Journal of Comparative Neurology,* 207: 403-409.
32. Carughi A, Carpenter KJ & Diamond MC (1989). Effect of environmental enrichment during nutritional rehabilitation on body growth, blood parameters and cerebral cortical development of rats. *Journal of Nutrition*, 119: 2005-2016.
33. Katz HB & Davies CA (1983). The separate and combined effects of early undernutrition and environmental complexity at different ages on cerebral measures in rats. *Developmental Psychobiology,* 16: 47-58.
34. Barnes RH, Neely CS, Kwong E, Labadan BA & Frankova S (1968). Postnatal nutritional deprivation as determinant of adult rat behavior toward food: Its consumption and utilization. *Journal of Nutrition,* 96: 467-476.
35. Lima JG (1988). Avaliação das alterações comportamentais, ponderais e bioquímicas do sistema nervoso central de ratos com restrição protéica, expostos à estimulação ambiental. Master's thesis, Universidade de São Paulo, Ribeirão Preto.
36. Rosenzweig MR & Bennett LE (1972). Cerebral changes in rats exposed individually to an enriched environment. *Journal of Comparative Physiology and Psychology,* 80: 304-313.
37. Evans OW (1961). A new technique for the investigation of some analgesic drugs on a reflexive behavior in rat. *Psychopharmacology*, 2: 318-325.
38. Prado WA & Roberts MHT (1985). An assessment of the antinociceptive and aversive effects of stimulating identified sites in the rat brain. *Brain Research*, 340: 219-228.
39. Bruning JL & Kintz BL (1977). *Computational Handbook of Statistics*. Scott, Foresman and Company, Glenview.
40. Dobbing J (1987). *Early Nutrition and Later Achievement.* Academic Press, London.
41. Celedon JM, Santander M & Colombo M (1979). Long-term effects of early undernutrition and environmental stimulation on learning performance of adult rats. *Journal of Nutrition*, 109: 1880-1886.
42. Crnic LS (1983). Effects of nutrition and environment on brain biochemistry and behavior. *Developmental Psychobiology,* 16: 129-145.
43. Blatt GJ, Chen JC, Rosene DL, Volicer L & Galler JR (1994). Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: an immunocytochemical, ligand binding, and neurochemical study. *Brain Research Bulletin,* 34: 507-518.
44. Cintra L, Díaz-Cintra S, Galvan A, Kemper T & Morgane PJ (1990). Effects of protein malnutrition on the dentate gyrus in rats of three age groups. *Brain Research,* 532: 271-277.
45. Díaz-Cintra S, Cintra L, Galvan A, Kemper T & Morgane PJ (1991). Effects of prenatal protein deprivation on the postnatal development of granule cells in the fascia dentata. *Journal of Comparative Neurology,* 310: 356-364.
46. Jordan TC & Clarck GA (1983). Early malnutrition impairs hippocampal long term potentiation in adult rats. *Behavioral Neuroscience,* 97: 319-322.
47. Santucci LB, Daud MM, Almeida SS & De Oliveira LM (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.