Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 2, pp. 393–398, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0176-I

Effects of Early Protein Malnutrition and Environmental Stimulation Upon the Reactivity to Diazepam in Two Animal Models of Anxiety

L. B. SANTUCCI, M. M. DAUD, S. S. ALMEIDA[1] AND L. M. DE OLIVEIRA

Laboratory of Nutrition and Behavior, FFCLRP, Campus of the University of São Paulo, 14040-901 Ribeirão Preto, SP, Brazil

Received 15 June 1993

SANTUCCI, L. B., M. M. DAUD, S. S. ALMEIDA AND L. M. DE OLIVEIRA. *Effects of early protein malnutrition and environmental stimulation upon the reactivity to diazepam in two animal models of anxiety.* PHARMACOL BIOCHEM BEHAV 49(2) 393–398, 1994. – In order to investigate the effects of early protein malnutrition and environmental stimulation upon the response to the anxiolytic properties of diazepam, two animal models of anxiety (elevated plus-maze and light–dark transition tests) were used. Rats were malnourished by feeding their dams a 6% protein diet during the lactation period (0–21 days of age) while well-nourished controls received a 16% protein diet. From 21 to 70 days of age all rats received a balanced lab chow diet. Environmental stimulation consisted of 3-min daily handling from birth to 70 days of age. Additional stimulation was provided from 21 to 70 days of age by rearing the rats in an enriched living cage. Eight groups of rats were studied in a 2 (malnourished or well-nourished) × 2 (stimulated or nonstimulated) × 2 (diazepam or vehicle) design. At 70 days of age, independent groups of rats treated with diazepam (2.5 mg/kg, IP) or vehicle were submitted to testing in the elevated plus-maze or light–dark transition procedures. The results showed that both diazepam and environmental stimulation reduced anxiety in the elevated plus-maze; stimulation changed the anxiolytic response to diazepam and the two diet conditions altered differentially the response to both pharmacological and stimulation procedures. These results suggest that environmental stimulation can affect differentially the behavioral response of malnourished and well-nourished rats treated with diazepam.

Early protein malnutrition Enriched environment Handling Anxiety Diazepam Rats

THE interaction between early malnutrition and environmental manipulations upon behavior has received increased attention in recent years. Previously reported results have shown body and brain growth deficits after malnutrition. These effects are more intense to developing brain, especially if malnutrition occurs during the neonatal period, leading to deficits that are not fully recovered by subsequent nutritional rehabilitation (29,45–47). These long-term effects are evidenced by alterations of neurohistological (7,12,13,31), electrophysiological (8,9,22,23,47), and biochemical parameters (53), as well as neurological (30) and behavioral manifestations (2–6,21,28,41,50).

The behavior of adult rats in tests of learning and anxiety is dependent, to a great extent, on their experiences during early life. Animals reared in a complex environment show both superior performance in problem solving (26,28,51) and alterations of morphological parameters (15,16,35,52) compared to groups reared in restricted environment or isolated conditions. Furthermore, the stimulation produced by daily handling of the neonatal rats has been reported to decrease emotionality in the open-field test when tested as adults (33). This group had a lower defecation and urination score and a greater degree of exploratory activity in a novel situation. On the other hand, early social or environmental isolation in-

[1] Requests for reprints should be addressed to S. S. Almeida at his current address: Center for Behavioral Development and Mental Retardation, M921B, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

creases the emotional response of rats (42). In addition, malnutrition early in life combined with environmental isolation, reduces exploratory behavior. The effect of malnutrition on emotionality is exaggerated by environmental isolation and decreased by environmental stimulation (38). Thus, it has been observed that the effects of early malnutrition can be reversed by environmental stimulation concurrent with the malnutrition, or later, during the nutritional recovery period (1,11,14, 25,35).

The great majority of studies concerning the long-term effects of protein malnutrition on animal models of anxiety have utilized painful aversive stimulation and/or food and water deprivation procedures. However, because malnourished animals have consistently demonstrated a lower shock threshold (5,41,50) and a higher motivation for food and water rewards (10,49), the use of these procedures with malnourished animals lead to problems of interpretation.

An anxiety model that uses the natural exploratory behavior of rats has been developed based on the natural aversion of rodents to heights and open spaces (44). This procedure has been termed the elevated plus-maze test (34,48), and has been validated for rats (48) and mice (39). The percentage of entries and the time spent in the open arms are taken as measures 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. Jointly with the classical light–dark transition test (21), the elevated plus-maze test was chosen for the present study, because it does not involve training, the use of painful stimuli, or food/water deprivation.

Early protein malnourished rats have previously been reported to show increased exploration of the elevated plus-maze (4,6), increased number of transitions in the light–dark transition test (21), and lower reactivity to the anxiolytic effects of benzodiazepines (2,3,4,5,20,21). Thus, the objective of the present study was to investigate the effects of environmental stimulation upon these same measures of anxiety following protein malnutrition.

METHOD

Animals

Two hundred male Wistar rats from the animal house of the Campus of Ribeirão Preto 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 transparent plastic cages (35 × 30 × 20 cm) and randomly assigned to receive ad lib either a 6% or a 16% protein diet. The two diets were isocaloric and prepared according to Barnes et al. (10). 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 normal protein diet contained 16% protein, 60.8% cornstarch, and the same 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, the pups were maintained in individual metal cages (20 × 25 × 15 cm) and were fed a balanced lab chow diet (Purina, Brasil). The rats were maintained under 12 L : 12 D cycle (lights on at 0700 h) 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 × 10 cm) opposite to each other, crossed by two enclosed arms (50 × 10 × 40 cm), with an open roof (48). The maze was elevated 50 cm from the ground floor. Fluorescent ceiling lights (2 × 60 W) from the ceiling provided the only illumination in the experimental room.

A two-chambered wooden cage (80 × 40 × 20 cm) was used (21). The cage was divided in the middle to provide a lighted and a dark chamber. Its floor was painted white and divided into 10 × 10 cm squares by black lines. Fluorescent ceiling lights (2 × 60 W) provided the only illumination for the lighted side.

Procedure

Environmental stimulation. Half of the pups of each diet condition were submitted to daily handling from the day of birth until 70 days of age. The other half was maintained without manipulation. The handling consisted in placing the animal in one hand and, with the thumb finger of the other hand, making cranio-caudal movements upon its dorsal region for 3 min. After eye opening, the handling was conducted outside of the animal room to provide additional visual and auditory stimulation. After weaning (22–70 days of age) the animals were also exposed to olfactory stimulation by coating the hands with a deodorant (Miss France, Gessy Lever Ltd.) prior to handling. Also after weaning, the handled animals were maintained in individual metal cages enriched with a variety of objects such as wooden blocks, plastic platforms, stairs, mirrors and marbles. The nonstimulated animals were maintained in similar metal cages without these objects. Thus, eight experimental groups were constituted: well-nourished animals submitted to environmental stimulation by handling plus enrichment of their home cages and treated with vehicle (WSV) or diazepam (WSD); well-nourished nonstimulated and treated with vehicle (WNV) or diazepam (WND); malnourished stimulated and treated with vehicle (MSV) or diazepam (MSD) and malnourished nonstimulated treated with vehicle (MNV) or diazepam (MND).

Elevated plus-maze test. At 70 days of age the animals of each group were placed individually at the center of the maze facing an enclosed arm and allowed to explore for 5 min. During this period, the number of entries and time spent in open and enclosed arms 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.

Light–dark transition test. At 70 days of age the animals of each group were placed individually in the light compartment of the cage and allowed to explore for 10 min. During this period, the number of transitions from one side to the other, the number of squares crossed in the light compartment, and the number of attempts to enter the light compartment were recorded as in the elevated plus-maze test. A transition was recorded when the animal crossed with all four paws from one side to the other, independently of the direction of the transition, and attempts to enter were recorded when the animal placed its two front paws in the light compartment before returning to the dark compartment.

Drugs

Diazepam (Roche) was suspended in distilled water containing 2% Tween 80 and injected IP (2.5 mg/kg) in a volume of 1 ml/kg. Vehicle was prepared adding 2% of Tween 80 in distilled water. Vehicle and diazepam were injected 15 min before the elevated plus-maze and light–dark transition tests.

Statistical Analysis

The weight of animals was analyzed by the Student's *t*-test. The behavioral measures were analyzed by an Analysis of Variance (ANOVA) followed by the Tukey test for multiple comparisons (24).

RESULTS

Body Weight

Body weights of early malnourished animals were significantly lower than those of control animals. At 21 days of age, the body weight (mean ± SEM) was 45.14 ± 0.53 g and 18.08 ± 0.25 g for control and malnourished rats, respectively. This difference was statistically significant, $t(198) = 46.48$, $p < 0.001$. Following 7 weeks of nutritional recovery, differences in body weight remained. Body weights at 70 days of age were 316.54 ± 4.86 g and 263.55 ± 3.66 g for control and malnourished rats, respectively. The difference remained statistically significant, $t(198) = 8.71$, $p < 0.001$. Environmental stimulation did not affect the body weights of the rats.

Elevated Plus-Maze

Analysis of the percentage of open arms entries showed significant effects of environmental stimulation, $F(1, 88) = 16.77$, $p < 0.001$ and pharmacological treatment, $F(1, 88) = 29.90$, $p < 0.001$ but no significant effects of diet, $F(1, 88) = 1.70$, $p > 0.05$. Environmental stimulation and diazepam both increased exploration of the open arms. Multiple comparisons showed that the diazepam increased open arms entries and the time spent in the open arms in the well-nourished nonstimulated animals ($p < 0.05$), but did not change the behavior of malnourished nonstimulated animals. On the other hand, diazepam did not increase the exploration of open arms in well-nourished stimulated animals, but increased the time spent in the open arms in malnourished stimulated animals ($p < 0.05$). The increase in the time spent in the open arms caused by the environmental stimulation in the malnourished animals treated with diazepam also reached statistical significance ($p < 0.05$) (Fig. 1). The analysis of total arm entries showed only a main significant effect of pharmacological treatment, $F(1, 88) = 4.34$, $p < 0.05$, indicating that diazepam increased locomotor activity in the maze (Fig. 2).

Light-Dark Transitions

The analysis of light-dark transitions showed no main effects of diet, environmental stimulation, or pharmacological treatment. However, significant diet × environmental stimulation, $F(1, 98) = 10.36$, $p < 0.002$, and environmental stimulation × pharmacological treatment, $F(1, 98) = 6.49$, $p < 0.01$, interactions were found. These data indicate that malnourished and well-nourished animals reacted differently to the environmental stimulation, and that environmental stimulation modified the rat's response to the diazepam treatment. Multiple comparisons showed that environmental stimulation decreased light-dark transition in well-nourished animals treated with diazepam ($p < 0.05$) and that malnourished rats showed more transitions as compared with well-nourished animals (Fig. 3) in the stimulated groups treated with diazepam.

When the locomotor activity (measured by the number of square entries in the light compartment of test cage) was nalyzed, no significant main effects of diet, environmental timulation, or pharmacological treatment were found. However, significant diet × environmental stimulation, $F(1, 98) = 4.63$, $p < 0.03$, and environmental stimulation × pharmacological treatment, $F(1, 98) = 4.94$, $p < 0.02$, interactions were demonstrated. These data indicate that malnourished and well-nourished animals reacted differently to the environmental stimulation procedure, and that environmental stimulation modified the response to diazepam treatment. Multiple comparisons showed that environmental stimulation reduced the locomotor activity of well-nourished rats treated with diazepam ($p < 0.05$) but did not modify this response in the mal-

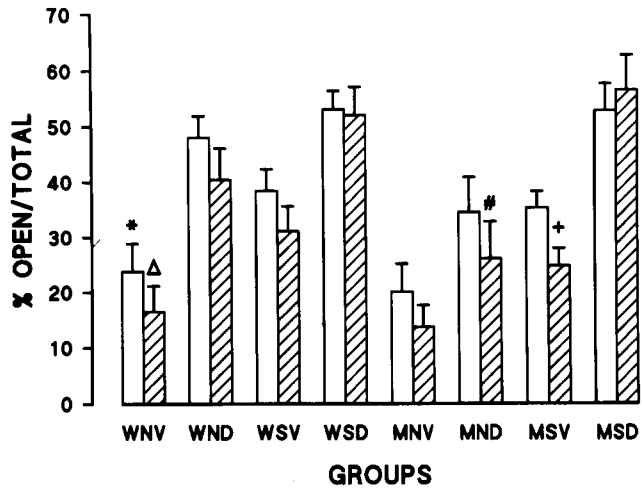

FIG. 1. The effects of malnutrition, environmental stimulation, and diazepam (2.5 mg/kg) on the percentage of open arms entries (open bars) and the percentage of time spent in the open arms (hatched bars) of the elevated plus-maze. Vertical bars represent the SEM of 12 rats. WNV = well-nourished nonstimulated treated with vehicle; WND = well-nourished nonstimulated treated with diazepam; WSV = well-nourished stimulated treated with vehicle; WSD = well-nourished stimulated treated with diazepam; MNV = malnourished nonstimulated treated with vehicle; MND = malnourished nonstimulated treated with diazepam; MSV = malnourished stimulated treated with vehicle and MSD = malnourished stimulated treated with diazepam. *$p < 0.05$ WNV × WND; Δ$p < 0.05$ WNV × WND; #$p < 0.05$ MND × MSD; +$p < 0.05$ MSV × MSD (Tukey tests).

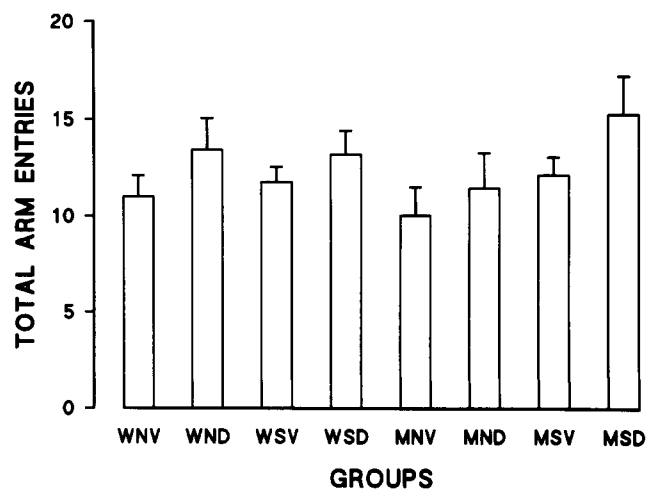

FIG. 2. Effects of malnutrition, environmental stimulation, and diazepam on total arm entries. See legend of Fig. 1.

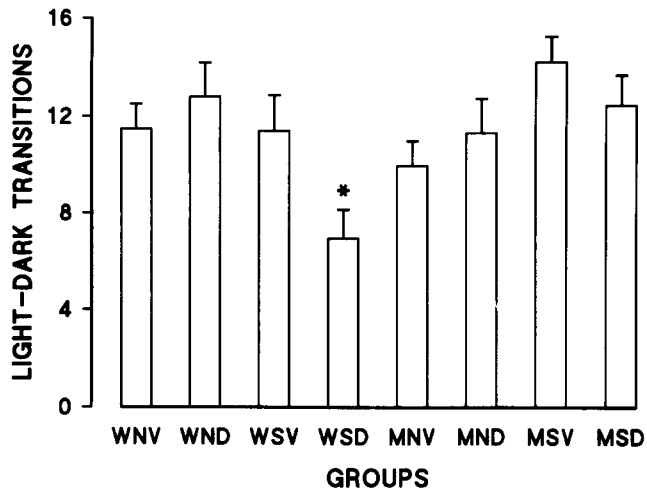

FIG. 3. Effects of malnutrition, environmental stimulation, and diazepam on number of light-dark transitions. See legend of Fig. 1. *$p < 0.05$ WSD × MSD (Tukey tests).

nourished animals treated with diazepam (Fig. 4). The analysis of attempts to enter the light compartment of the test cage showed only a significant effect of environmental stimulation, $F(1, 98) = 29.92$, $p < 0.01$, indicating that stimulated animals made more attempts to enter the light box as compared with nonstimulated animals.

DISCUSSION

The lower body weight of malnourished animals is consistent with previous reports showing that a protein deficient diet during the lactation period impairs normal development, and that even a prolonged nutritional recovery period is not sufficient to equalize the body weights of malnourished and well-nourished animals (10,28,29,38,47,49).

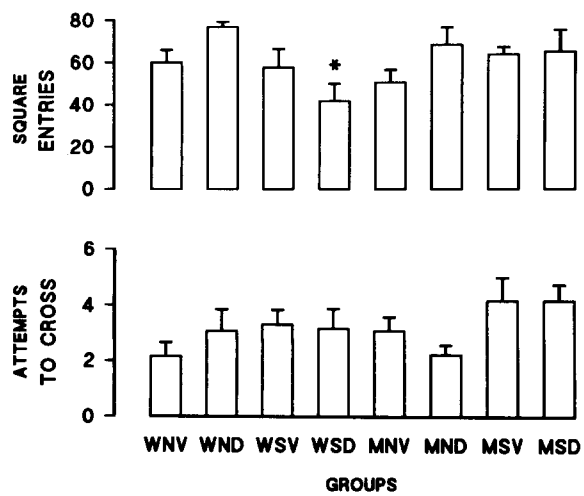

FIG. 4. Effects of malnutrition, environmental stimulation, and diazepam on number of squares entries and number of attempts to enter the light compartment of the light-dark cage. See legend of Fig. 1. *$p < 0.05$ WSD × WND (Tukey tests).

The overall effect of environmental stimulation was to increase the exploration of open arms in the elevated plus-maze. This finding confirms previous results showing that both handling and prolonged exposure to an enriched environment reduce the fear response of rats in several animal models of anxiety (18,32,40). Reduction of fearfulness in adulthood following environmental stimulation has also been shown by fewer defecation, increased ambulation in an open field test, lower novelty-induced corticosterone response (36,43), and better two-way active avoidance acquisition (37). Chronic handling also results in less hyponeophagia and higher [^{3}H]flunitrazepam binding in the whole rat brain, fewer learning and memory-related deficits, and less hippocampal neuronal death with aging (18,43). This fear-reducing effect of environmental stimulation is further confirmed by the higher number of attempts to enter the light compartment of the light-dark cage in the animals subjected to handling plus enriched environment in this study.

Multiple comparisons showed that diazepam increased both the numbers of entries and time spent in the open arms in well-nourished nonstimulated rats, but did not affect open arm exploration in the malnourished nonstimulated animals. These results are consistent with previously reported results showing that early protein malnutrition leads to a lower reactivity to the anxiolytic effects of benzodiazepine (2,3,4,5, 20,21) and nonbenzodiazepine anxiolytics (3).

The statistically significant interaction between diet and environmental conditions in the light-dark transition test showed that environmental stimulation differentially affected the behavior of malnourished and well-nourished animals. Environmental stimulation increased total transitions and locomotor activity in malnourished rats while it decreased this measures in well-nourished animals. On the other hand, the increased number of attempts to cross to the light compartment of the light-dark cage in stimulated animals (Fig. 4), indicate more attempts to interact with the aversive stimulus (white light). Because animals present a stretched attend/approach posture similar to that described by Blanchard et al. (17), this may represent an increase in the risk assessment behavior.

The significant interaction between environmental stimulation and pharmacological treatment observed in the current study also confirms previously reported results showing that stimulation reduces the anxiolytic effects of benzodiazepines in animal models of anxiety (19). This reduction of anxiolytic effects of benzodiazepines in environmentally stimulated animals has been interpreted as an alteration in the GABA-benzodiazepine neurotransmitter system produced by environmental stimulation (19). Thus, postnatal handling has been observed to reduce the novelty-induced fear response and increase [^{3}H]flunitrazepam binding in the brain (18). It has been also showed (27) that handling can modify the GABA-BZ-Cl^-ionophore receptor complex increasing the number of low affinity [^{3}H]GABA binding sites. As pointed out by Brett and Pratt (19), these data suggest that rats habituated to handling represent a normal unstressed state, and that the ability of diazepam to modify GABA function may depend on the emotional state of the animal. This suggestion was affirmed by their finding that handled rats did not react to the anxiolytic effects of diazepam in the elevated plus-maze. These and our data indicate that chronically handled well-nourished rats were less stressed in the plus-maze situation than unhandled animals.

The opposite was observed in malnourished animals exposed to environmental stimulation. Diazepam increased the time spent on the open arms in these animals. These data suggest that environmental stimulation may be acting differ-

ently in malnourished rats than in well-nourished rats, leading to an alteration in the response of GABA-BZ-Cl^-ionophore receptor complex. Such an alteration could play a significant role in determining the differences in reactivity to the anxiolytic effects of diazepam between malnourished and well-nourished rats subjected to environmental stimulation. Thus, in further studies, it will be interesting to investigate how the GABA-BZ-Cl^-ionophore receptor complex of malnourished rats reacts in response to environmental stimulation procedures.

ACKNOWLEDGEMENTS

We thank Dr. John Tonkiss for comments and english revision on a draft of this manuscript, and Dalmo C.P. Nicola for technical assistance. S. S. Almeida and L. M. de Oliveira were recipients of Research Fellowships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico. L. B. Santucci and M. M. Daud were the recipients of a Scholarship from Fundação de Amparo à Pesquisa do Estado de São Paulo. This study was supported by a research grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, proc. no. 90/3474-0).

REFERENCES

1. Adaro, L.; Fernandes, V.; Kaufmann, W. Effects of nutritional environmental interactions upon body weight, body size and development of cortical pyramids. Nutr. Rep. Int. 33:1013–1020; 1986.
2. Almeida, S. S.; De Oliveira, L. M.; Bichuette, M. Z.; Graeff, F. G. Early malnutrition alters the effect of chlordiazepoxide on inhibitory avoidance. Braz. J. Med. Biol. Res. 21:1033–1036; 1988.
3. Almeida, S. S.; De Oliveira, L. M.; Graeff, F. G. Decreased reactivity to anxiolytics caused by early protein malnutrition in rats. Pharmacol. Biochem. Behav. 36:997–1000; 1990.
4. Almeida, S. S.; De Oliveira, L. M.; Graeff, F. G. Early life protein malnutrition changes exploration of the elevated plus-maze and reactivity to anxiolytics. Psychopharmacology (Berlin) 103:513–518; 1991.
5. Almeida, S. S.; Soares, E. G.; Bichuette, M. Z.; Graeff, F. G.; De Oliveira, L. M. Effects of early postnatal malnutrition and chlordiazepoxide on experimental aversive situations. Physiol. Behav. 51:1195–1199; 1992.
6. Almeida, S. S.; Garcia, R. A.; De Oliveira, L. M. Effects of early protein malnutrition and repeated testing upon locomotor and exploratory behaviors in the elevated plus-maze. Physiol. Behav. 54:749–752; 1993.
7. Andrade, J. P.; Cadete-Leite, A.; Madeira, M. D.; Paula Barbosa, M. M. Long-term low-protein diet reduces the number of hippocampal mossy fiber synapses. Exp. Neurol. 112:119–124; 1991.
8. Austin, K. B.; Beiswanger, C.; Bronzino, J. D.; Austin-LaFrance, R. J.; Galler, J. R.; Morgane, P. J. Prenatal protein malnutrition alters behavioral state modulation of inhibition and facilitation in the dentate gyrus. Brain Res. Bull. 28:245–255; 1992.
9. Austin-LaFrance, R. J.; Morgane, P. J.; Brozino, J. D. Prenatal protein malnutrition and hippocampal function: Rapid kindling. Brain Res. Bull. 27:815–818; 1991.
10. Barnes, R. H.; Neely, C. S.; Kwong, E.; Labadan, B. A.; Frankova, S. Postnatal nutritional deprivation as determinants of adult rat behavior toward food: Its consumption and utilization. J. Nutr. 96:467–476; 1968.
11. Bedi, K. S.; Bhide, P. G. Effects of environmental diversity on brain morphology. Early Hum. Dev. 1:107–143; 1988.
12. Bedi, K. S.; Massey, R. F.; Smart, J. L. Neuronal and synaptic measurements in the visual cortex of adult rats after undernutrition during normal or artificial rearing. J. Comp. Neurol. 289: 89–98; 1989.
13. Bedi, K. S.; Warren, K. S. Synapse-to-neuron ratios in rat cerebellar cortex following lengthy periods of undernutrition. J. Anat. 170:173–182; 1990.
14. Bhide, P. G.; Bedi, K. S. The effects of environmental diversity on well-fed and previously undernourished rats. I. Body and brain measurements. J. Comp. Neurol. 207:403–409; 1982.
15. Bhide, P. G.; Bedi, K. S. The effects of a lengthy period of environmental diversity on well-fed and previously undernourished rats. I. Neurons and glial cells. J. Comp. Neurol. 227:296–304; 1984.
16. Bhide, P. G.; Bedi, K. S. The effects of a lengthy period of environmental diversity on well-fed and previously undernourished rats. II. Synapse-to-neuron ratios. J. Comp. Neurol. 227: 305–310; 1984.
17. Blanchard, D. C.; Blanchard, R. J.; Tom, P.; Rodgers, R. J. Diazepam changes risk assessment in an anxiety/defense test battery. Psychopharmacology (Berlin) 101:511–518; 1990.
18. Bodnoff, S. R.; Suranyi-Cadotte, B.; Quirion, R.; Meaney, M. J. Postnatal handling reduces novelty-induced fear and increases [^{3}H]flunitrazepam binding in rat brain. Eur. J. Pharmacol. 144: 105–107; 1987.
19. Brett, R. R.; Pratt, J. A. Chronic handling modifies the anxiolytic effect of diazepam in the elevated plus-maze. Eur. J. Pharmacol. 178:135–138; 1990.
20. Brioni, J. D.; Cordoba, N.; Orsingher, O. A. Decreased reactivity to the anticonflict effect of diazepam in perinatally undernourished rats. Behav. Brain Res. 34:159–162; 1989.
21. Brioni, J. D.; Orsingher, O. A. Operant behavior and reactivity to the anticonflict effect of diazepam in perinatally undernourished rats. Physiol. Behav. 44:193–198; 1988.
22. Bronzino, J. D.; Austin-LaFrance, R. J.; Morgane, P. J.; Galler, J. R. Effects of prenatal malnutrition on kindling-induced alterations in dentate granule cell excitability. I. Synaptic transmission measures. Exp. Neurol. 112:206–215; 1991.
23. Bronzino, J. D.; Austin-LaFrance, R. J.; Morgane, P. J.; Galler, J. R. Effects of prenatal malnutrition on kindling-induced alteration in dentate granule cell excitability. II. Paired-pulse measures. Exp. Neurol. 112:216–223; 1991.
24. Bruning, J. L.; Kintz, B. L. Computational handbook of statistics. Glenview: Scott, Foresman and Company; 1977.
25. Carughi, A.; Carpenter, K. J.; Diamond, M. C. Effect of environmental enrichment during nutritional rehabilitation on body growth, blood parameters and cerebral cortical development of rats. J. Nutr. 119:2005–2016; 1989.
26. Celedon, J. M.; Santander, M.; Colombo, M. Long-term effects of early undernutrition and environmental stimulation on learning performance of adult rats. J. Nutr. 109:1880–1886; 1979.
27. Corda, M. G.; Biggio, G. Stress and GABAergic transmission: Biochemical and behavioral studies. In: Biggio, G.; Costa, E., eds. GABAergic transmissionand anxiety. New York: Raven Press; 1986:121–136.
28. De Oliveira, L. M.; Almeida, S. S. Effects of malnutrition and environment on the acquisition and extinction of avoidance behavior in rats. Physiol. Behav. 34:141–145; 1985.
29. Dobbing, J. Early nutrition and later achievement. London: Academic Press; 1987.
30. Eberhardt, M. J.; Hallas, E. S. Developmental delays in offspring of rats undernourished or zinc deprived during lactation. Physiol. Behav. 41:309–314; 1987.
31. Faúndez, V.; Cordero, M. E.; Rosso, P.; Alvarez, J. Calibres and microtubules of nerve fibers: differential effect of undernutrition in developing and adult rats. Brain Res. 509:198–204; 1990.
32. Fernández-Teruel, A.; Escorihuela, R. M.; Núñez, J. F.; Gomà, M.; Driscoll, P.; Tobeña, A. Early stimulation effects on novelty-induced behavior in two psychogenetically selected rat lines with divergent emotionality profiles. Neurosci. Lett. 137:185–188; 1992.

33. Hall, C. S.; Whiterman, P. H. The effects of infantile stimulation upon later emotional stability in the mouse. J. Comp. Physiol. Psychol. 44:61–66; 1951.
34. Handley, S. L.; Mithani, S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of "fear"-motivated behavior. Naunyn Schmiedebergs Arch. Pharmacol. 327:1–5; 1984.
35. Katz, H. B.; Davis, C. A. The separate and combined effects of early undernutrition and environmental complexity at different ages on cerebral measures in rats. Dev. Psychobiol. 327:47–58; 1983.
36. Levine, S.; Haltmeyer, G. C.; Karas, G. G.; Denenberg, V. H. Physiological and behavioral effects of infantile stimulation. Physiol. Behav. 2:55–59; 1967.
37. Levine, S.; Wetzel, A. Infantile experiences, strain differences and avoidance learning. J. Comp. Physiol. Psychol. 56:879–881; 1963.
38. Levitsky, D. A.; Barnes, R. H. Nutritional and environmental interaction in the behavior development of the rat: Long-term effects. Science 176:68–71; 1972.
40. Loscher, W.; Stephens, D. N. Chronic treatment with diazepam or the inverse benzodiazepine receptor agonist FG7142 causes differential changes in the effects of GABA receptor stimulation. Epilepsy Res. 2:253–259; 1988.
41. Lynch, A. Passive avoidance behavior and response thresholds in adult male rats after early postnatal undernutrition. Physiol. Behav. 16:27–32; 1976.
42. Mason, W. A.; Davenport, R. K.; Menzel, E. M. Early experience and the social development of rhesus monkey and chimpanzees. In: Newton, G.; Levine, S., eds. Early experience and behavior. Springfield: Charles C. Thomas; 1968:440–480.
43. Meaney, M. J.; Aitken, D. H.; van Berkel, C.; Bhatnagar, S.; Sapolsky, R. M. Effect of neonatal handling on age-related impairments associated with the hippocampus. Science 239:766–768; 1988.
44. Montgomery, K. C. The relation between fear induced by novelty stimulation and exploratory behavior. J. Comp. Physiol. Psychol. 48:254–260; 1958.
45. Morgane, P. J.; Austin-LaFrance, R.; Bronzino, J.; Tonkiss, J.; Diaz-Cintra, S.; Cintra, L.; Kemper, T.; Galler, J. R. Prenatal malnutrition and development of the brain. Neurosci. Biobehav. Rev. 17:91–128; 1993.
46. Morgane, P. J.; Austin-LaFrance, R. J.; Bronzino, J. D.; Tonkiss, J.; Galler, J. R. Malnutrition and the developing central nervous system. In: Isaacson, R. L.; Jensen, K. F., eds. The vulnerable brain and environmental risks. vol. 1. Malnutrition and hazard assessment. New York: Plenum Press; 1992:3–44.
47. Morgane, P.; Miller, M.; Kemper, T.; Stern, W.; Forbes, W.; Hall, R.; Bronzino, J.; Kissane, J.; Hawrylewicz, E.; Resnick, O. The effects of protein malnutrition and the developing central nervous system in the rat. Neurosci. Biobehav. Rev. 2:137–230; 1978.
48. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
49. Smart, J. L.; Dobbing, J. Increased thirst and hunger in adult rats undernourished as infants: An alternative explanation. Br. J. Nutr. 37:421–430; 1977.
50. Smart, J. L.; Whatson, T. S.; Dobbing, J. Thresholds of response to electric shock in previously undernourished rats. Br. J. Nutr. 34:511–516; 1975.
51. Wells, A. M.; Geist, C. R.; Zimmermann, R. Influence of environmental and nutritional factors on problem solving in the rat. Percept. Mot. Skill. 35:235–244; 1972.
52. Wesa, J. M.; Chang, F. F.; Greenough, W. T.; West, R. W. Synaptic contact curvature: Effects of differential rearing on rat occipital cortex. Dev. Brain Res. 4:253–257; 1982.
53. Wiggins, R.; Fuller, G.; Enna, S. Undernutrition and the development of brain neurotransmitter systems. Life Sci. 35:2085–2094; 1984.