REVISTA BRASILEIRA DE ANÁLISE DO COMPORTAMENTO / BRAZILIAN JOURNAL OF BEHAVIOR ANALYSIS, 2014, Vol. 10, Nº. 2, 106-111.

EFEITOS DE DESNUTRIÇÃO E AMBIENTE NA AQUISIÇÃO E EXTINÇÃO DE COMPORTAMENTO DE ESQUIVA EM RATOS*

EFFECTS OF MALNUTRITION AND ENVIRONMENT ON THE ACQUISITION AND EXTICTION OF AVOIDANCE BEHAVIOR IN RATS

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RESUMO

Twelve newborn rats were fed by mothers maintained on protein-deficient diet (12% casein, M) during lactation, and 12 rats fed by mothers maintained on a diet containing 25% casein were used as controls (C). After weaning, all animals were standard lab ration. Half of each group was housed individually (MI and CI), while the other half was allowed to live in pairs (MP and CP). When adult, all animals were trained to avoid footshocks by jumping onto a platform. Trainiing sessions consisted of 40 trials starting with a 20 sec light stimulus (CS) and followed by a 2 sec, 0.6 mA shock (US) with an average interval of 54 sec. When all animals displayed consistent avoidance behavior, the extinction phase was initiated. The produce was the same as for the training session except that shock generator was disconnected. Extinction continued until each animal showed a 50% reduction in avoidance performance. During acquisition, MI learned faster than CI and CI showed greater avoidance performance than CP, but no differences were observed between MP and CP. During extinction, group M responded more persistently than group C. The present acquisition results may explain the contradictory data reported in the literature with respect to the effects of malnutrition on avoidance performance, since environmental stimulation was shown to reduce the effects of early malnutrition. Individually housed animals showed greater avoidance performance during both phases.

Keywords: Protein malnutrition, avoidance response, acquisition, extinction, environmental stimulation.

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Early malnourished (M) rats show lower response thresholds to electric shock than control animals (C) [11, 19, 22). Contradictory data have been reported in the literature when C and M are compared in terms of the acquisition of avoidance behavior. Some investigators have shown that M learn avoidance behavior faster than C [18, 20], whereas others have detected no significant differences between C and M [1, 3, 13, 14]. Latency has also been found not to differ significantly, although malnourished animals showed more numerous intertrial responses [12]. In other studies, however, C animals showed better avoidance performance [6, 15].

These contradictory results may be due to variations in techniques and in time when undernourishment was started, or to changes in avoidance training procedures (shock intensity or response topography). Almeida and De Oliveira (unpublished results), using three different topographies and three different shock intensities, noted that the platform jumping response was learned faster than running or barpressing to avoid shock at all three intensities, although the differences between C and M in avoidance acquisition were statistically nonsignificant with any of the topographies used. On the other hand, even though percent avoidance did not differ between C and M animals at lower intensities, it was decreased in the M group at higher intensities, a fact showing disruption of M on going behavior.

Data obtained for the extinction of avoidance behavior, however, have been more consistent, with general agreement among investigators that responses learned during the conditioned stimulus (CS) by M animals persist longer during the extinction phase [1, 3, 12].

More recent studies have emphasized the difficulties in separating the effects of the diet per se from the effects of other environmental variables associated with malnutrition [9, 16]. The effects of malnutrition have been reported to be enhanced when associated with an unfavorable environmental, and to be reduced when some environmental stimulation is present [5]. Stimulation early in life reduces the effects of a restricted diet, whereas isolation enhances the effects of malnutrition [19].

The most frequently used experimental model is to submit the animal to malnutrition early in life and evaluate the effects of malnutrition on avoidance behavior during the animal's adult life (long-term-effects). This model may involve many interactions between malnutrition and changes in environmental stimulation occurring during nutritional recovery, which may eventually affect avoidance learning. Comments have been made in the literature on this subject [9, 16], but no experimental studies comparing the long-term effects of malnutrition with the effects of concurrent malnutrition on avoidance learning have been published.

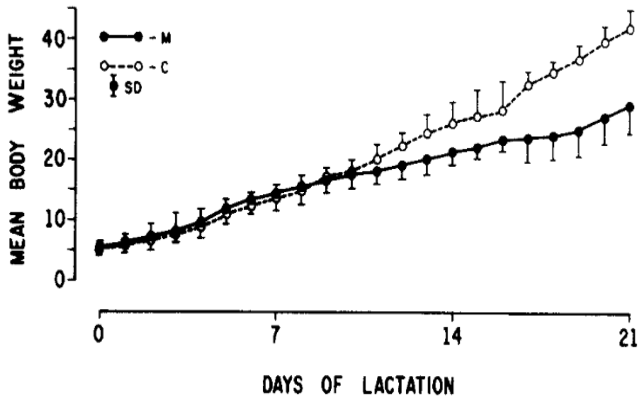

FIG. 1. Mean body weight of malnourished (●—●) and control (○——○) animals during the lactation period. The vertical bars represent the standard deviation.

The same difficulties in separating the effects of malnutrition from those of the environment are met in human studies, where socioeconomic and cultural variables are much more complex than in experimental studies. The importance of housing animals in groups or individually has been emphasized. In studies where animals were housed in groups after malnutrition, most investigators found no differences in behavior between M and C, whereas in studies where animals were housed individually, M animals were found to be more responsive [17].

The present study was designed to investigate the relationship between malnutrition and social environment in rats. The effects of a protein-deficient diet during lactation on the acquisition and extinction of avoidance behavior were studied on rats housed in pairs (MP and CP) or individually (MI and CI) during the rehabilitation period.

METHOD

Animals

Twenty-four male Wistar rats from the animal house of the Campus of Ribeirão Preto, University of São Paulo, were used. During the lactation period (21 days), each litter was culled to six pups, selected randomly at birth in order to avoid possible litter effects. Twelve animals were suckled by mothers maintained on a 25% casein diet (C) and the other twelve by mothers maintained on a 12% casein diet (M). The diet was prepared as described by Barnes, Neely, Kwong, Labadan, and Frankova [4]. After weaning, all animals were fed standard lab ration, but each group was subdivided into two subgroups of 6 animals each: one subgroup in each group was housed individually in standard 24 x 18.5 x 17.5 cm cages (malnourished individually, MI, and control individually, CI), whereas the other two subgroups were housed in pairs (malnourished pairs, MP, and control pairs, CP). The above housing conditions were maintained throughout the experiment. Avoidance training was started at 70 days of age.

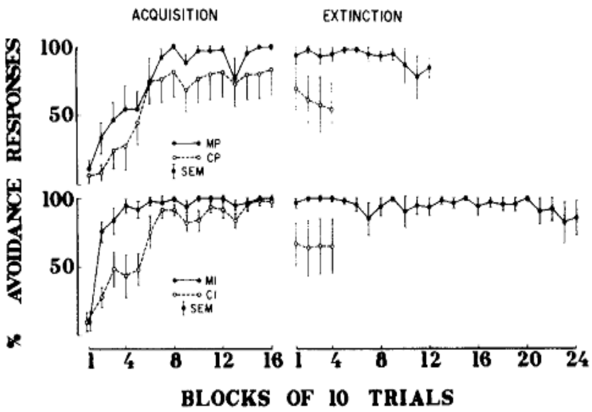

FIG. 2. Percentages of avoidance responses in blocks of 10 trials during acquisition and extinction. The upper part of the figure shows the performance of animals living in pairs (MP and CP), and the bottom part the results for individually housed animals (MI and CI). Vertical bars represent the standard error of the mean.

Apparatus

All animals were submitted to avoidance training in a 25x14x21 cm anodized aluminum box (Mawrer model, FUNBEC, São Paulo, Brazil) with a floor consisting of a grid of stainless bars (0.3 cm in diameter) spaced 1.2 cm apart and connected to a Grasson-Stadler shock generator (model 700) for delivery of a scrambled shock. The front side of the box was made of transparent plastic to permit animal observation. A 12 x 14 x 13 cm recessed chamber (platform) was placed inside the box on the left side, 8 cm above the floor. A 15 watt incandescent clear bulb installed on the ceiling of the cage was used as a conditioned stimulus (CS). The entire apparatus was connected to a panel for automatic programming of stimulus duration and data collection.

Procedure

Rats were submitted to daily sessions of 40 trials each during the white light period of the 14 hr red light/10hr white light cycle on which they were maintained. The trial was started by turning on the light (CS). After a CS of 20 sec duration, a footshock of 0.6 mA intensity and 2 sec duration was applied (US). Avoidance behavior (jumping onto the platform during CS) or escape response (jumping onto the platform during CS) or escape response (jumping onto the platform during US) and latency to avoidance were recorded for each trial. Intertrial intervals varied, as described by Catania and Reynolds [3], averaging 54 sec (range: 10 to 120 sec). Sessions were run until the criterion for stability (80% avoidance during three successive sessions) was satisfied. Extinction sessions were started when an animal satisfied the criterion for stability, regardless of the performance of other animals in the same group, and were continued until the animal showed 50% or less avoidance behavior in one session. The extinction procedure was the same as for the training sessions, except that no footshock was applied.

RESULTS

Body Weight

At the end of lactation (21 days of life), the average body weight of C animals was significantly higher than that of M animals ($p<0.005$, Student t-test), although no significant differences between groups were detected during nutritional recovery after weaning remained significantly lower up to 42 days of age ($p<0.005$) and continued to be significantly lower thereafter up to 63 days, although the difference was slightly reduced ($p<0.02$). No significant differences in body weight were detected between individually housed animals and animals housed in pairs.

Avoidance Training

Response percentages. M animals showed higher percentages of avoidance behavior both during the acquisition and extinction phase (Fig 2). When acquisition data for MP and CP were analyzed statistically by the Mann-Whitney U-test (Fig. 2, upper left) no significant differences were detected. Comparison between MI and CI, however (Fig. 2, bottom left), showed that the MI group acquired behavior more rapidly than CI ($p<0.01$).

When animals maintained on the same diet during lactation but under different living conditions after weaning were compared (MP vs. MI and CP vs. CI), no differences were detected between MP and MI, except that the MI group showed higher percentages of avoidance responses starting during the second block of 10 trials (first sessions), whereas more than seven blocks were needed for MP to reach the same level of performance. Individual housing conditions facilitated avoidance acquisition even among the controls, with statistically significant differences between CP and CI ($p<0.02$).

During the extinction phase (Fig. 2) the M group maintained avoidance behavior during CS longer than C animals, ($p<0.01$) and also between MI and CI ($p<0.001$). No significant differences were detected between CP and CI, whereas MI took a significantly longer time to extinguish the response than MP ($p<0.05$).

The data presented in Fig. 2 are only for sessions (each including 4 blocks of 10 trials each) during which the group consisted of 6 animals (16 blocks of 10 trials each during acquisition and 24 blocks during extinction), so that comparisons could be made even though the animals learned at different rates. Mean values for each session are given in Table 1. It can be seen that, during acquisition, most M animals reached 80% avoidance by the first or second session. During the extinction phase, the number of responses by CP and CI was rapidly reduced, whereas most M animals maintained very high percentages up to the 6th session. It is interesting to observe that all MI animals maintained more than 80% avoidance behavior up to the 6 th session during the extinction phase.

TABLE 1

AVERAGE PERCENTAGES OF AVOIDANCE RESPONSE IN EACH SESSION

	Acquisition				Extinction			
Sessions	MP	CP	MI	CI	MP	CP	MI	CI
1	36.7 (6)	17.0 (6)	66.0 (6)	54.9 (6)	95.0 (6)	61.2 (6)	99.7 (6)	65.4 (6)
2	80.0 (6)	69.5 (6)	96.2 (6)	76.7 (6)	96.2 (6)	51.9 (4)	92.9 (6)	70.0 (4)
3	95.0 (6)	76.6 (6)	98.3 (6)	87.5 (6)	86.3 (6)	97.5 (1)	94.6 (6)	62.5 (4)
4	93.0 (6)	79.1 (6)	98.4 (6)	92.9 (6)	78.5 (5)	87.5 (1)	97.1 (6)	85.8 (3)
5	97.5 (1)	48.7 (2)		92.5 (2)	69.4 (4)	75.0 (1)	96.6 (6)	85.8 (3)
6	97.5 (1)	67.5 (1)			69.2 (3)	22.5 (1)	87.5 (6)	43.3 (3)
7		87.5 (1)			85.0 (2)		85.5 (5)	22.1 (1)
8		90.0 (1)			27.5 (1)		55.0 (4)	
9		92.5 (1)					50.0 (1)	

Number of animals are given in parentheses. These numbers are decreasing because the sessions were interrupted when each animal reached the stability criterion during the acquisition and extinction phases.

TABLE 2

AVERAGE NUMBERS OF SHOCKS RECEIVED BY MALNOURISHED AND CONTROL ANIMALS DURING AVOIDANCE TRAINING

Control Mean ± SEM		Malnourished Mean ± SEM	
CP	CI	MP	MI
73.50 ± 12.33	44.67 ± 2.69	38.83 ± 3.56	16.50 ± 1.09

Statistical significances: CP vs. MP—$p<0.01$; CI vs. MI—$p<0.001$; CP vs. CI—n.s.; MP vs. MI—$p<0.01$.

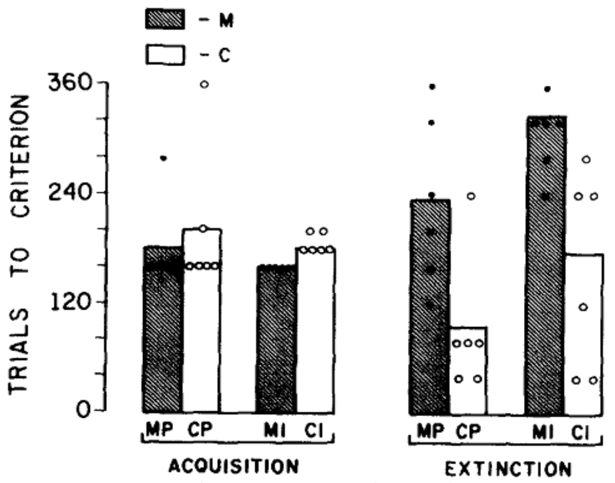

FIG. 3. Number of trials to criterion during acquisition and extinction of avoidance response for malnourished (MP and MI—hatched bars) and control animals (CP and CI—open bars). The open and filled dots represent individual data.

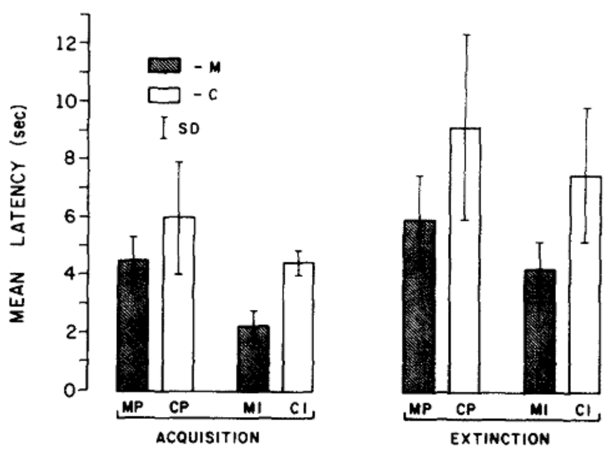

FIG. 4. Latency of avoidance response during acquisition and extinction in malnourished (MP and MI—hatched bars) and control animals (CP and CI—open bars). Vertical lines represent the standard deviation.

Trials to criterion

No differences were detected between M and C animals in the number of trials required to reach the criterion of stability during the acquisition phase (Fig. 3, left). During extinction, however (Fig 3, right), analysis by the Mann-Whitney U-test showed a significant difference between MP and CP ($p<0.001$) and between MI and CI ($p<0.001$). No significant differences were detected within groups (MP vs. MI and CP vs. CI) but M animals showed higher values than C animals and individually housed rats took longer to reach stability during extinction than rats housed in pairs.

Latency of avoidance behavior

M animals responded with shorter latency booth during acquisition and extinction (Fig. 4). Statistical analysis of latency to avoidance by the Mann-Whitney U-test showed significant differences between MI and CI ($p<0.001$), between MP and M ($p<0.001$), and between CP and CI ($p<0.05$), but no significant differences between MP and CP. During the extinction phase, statistically significant differences were also observed between MP and CP ($p<0.02$) and between MI and CI ($p<0.001$). Living conditions within groups had no significant effect on latency during extinction, although individually housed animals showed shorter latency than paired animals.

Number of shocks

M animals received fewer shocks than C animals during the acquisition phase. Statistical analysis by the Mann-Whitney U-test showed that CP received significantly more shocks than MP ($p<0.01$) and CI significantly more than MI ($p<0.01$). Comparison within groups showed that living conditions had no significant effect on the number of shocks in the C groups, whereas MP received significantly more shocks than MI ($p<0.01$) during the acquisition phase.

DISCUSSION

In the present study, no significant differences in avoidance acquisition were detected between M and C animals housed in pairs, but differences were significant between individually housed M and C animals. These data confirm observations by several researchers [5, 10, 16] who suggested that the effects of malnutrition may be enhanced when animals are maintained under conditions of poor stimulation, but decreased when animals exposed to proteindeficient diet early in life are later allowed to live under environmentally stimulating conditions.

The contradictory results obtained by several investigators with respect to avoidance learning by M animals may be better understood on the basis of the present results. In studies where no differences were detected between M and C during the acquisition of avoidance behavior [1, 14], animals were housed in pairs. Barnes et al. [3], who also found no differences in avoidance learning between malnourished and control pigs, maintained their animals in groups of three. Other investigators [6, 15] observed more rapid acquisition in the C groups but their C and M mice were maintained in groups of eight both during lactation and after weaning. In contrast, those authors who maintained their animals individually during the rehabilitation period [12, 18, 20] reported faster avoidance learning or a higher rate of avoidance response in M animals. Guthrie, however [13], found no differences between M and C even when she housed animals individually.

These discrepant results may be partially explained by differences in time of nutritional insult or in the parameters of the avoidance training procedure. Substantial differences in the rate of avoidance acquisition have been shown when the US is manipulated, with malnourished animals being affected much more than control animals by shock intensities of more than 0.6 mA, or when different topographies are used: M and C learned to jump onto a platform faster than to run in a shuttle-box or to press a bar (Almeida and Oliveira, unpublished results). For better analysis of the differences in the acquisition of avoidance learning, standardization of the malnutrition model or of the parameters of the avoidance procedure in needed, since factors such as resistance to electric shock or part of the animal to which the chock is delivered may introduce variations in sensitivity to electric shock [7].

Although all of these variables should be better evaluated, the data obtained in the present study on the basis of standardized malnutrition and avoidance acquisition procedures clearly showed that individually housed M animals acquired avoidance behavior more rapidly. Even the CI group learned avoidance behavior significantly faster than the CP group.

Malnutrition is always accompanied by many other concurrent environmental changes that make it almost impossible to interpret the effects of malnutrition alone on later learning [2, 9]. At the human level, the effects of malnutrition are enhanced by concomitant psychosocially unfavorable variables such as isolation or poor environmental stimulation. According to Barnes [2], however, these interactions also occur at the animal level because “we have consistently developed long-lasting behavioral abnormalities in early malnourished rats and pigs but have also noted that these changes in behavior are modified or abolished by social or environmental stimulation” (p. 913). Eckert, Levitsky and Barnes [10] demonstrated that even choline acetyltransferase activity was decreased in malnourished animals but was prevented in animals which were handled after being exposed to malnutrition: “This biochemical change in the brain, which has been associated with malnutrition and correlated with changes in behavior, has been reversed by an environmental change” ([2], p. 916).

In the present study, M animals took longer to satisfy the extinction criterion and showed shorter latency than C in both the acquisition and extinction phases, in agreement with reports by several investigators [1, 3, 12]. Both results may be interpreted as higher sensitivity to the aversive stimuli, as also shown by several other investigators [11, 17, 19, 22].

The results of the present study help explaining the contradiction in the literature concerning acquisition and the consistently reported longer extinction in M animals. Also, the significantly faster avoidance learning shown by our CI animals in relation to CP animals shows the importance of the interaction between environmental and nutritional variables in behavioral studies. The environmental stimulation used in the present experiment was housing animals in pairs. In future studies we intend to use the same kind of experimental design to compare the avoidance behavior of M and C animals submitted to manipulation of other nonsocial environmental variables.

AKNOWLEDGEMENTS

This research was supported by funds from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 78/1507 and FAPESP - 79/1231 – 3R)

REFERENCES

Almeida, S. S., & De Oliveira, L. M. (1981). Sous-alimentation proteique et effet de trois intensites de choc sur l'acquisition et l'extinction de responses d'evitement. *Med Nutr, 17,* 459-466.

Barnes, R. H. (1976). Dual role of environmental deprivation and malnutrition in retarding intellectual development. *Am J Clin Nutr, 29,* 912-917.

Barnes, R. H., Moore, A. U., Reid, I. M., & Pond, W. G. (1967). Learning behavior following nutritional deprivations in early life. *J Am Diet Assoc, 51,* 34-39.

Barnes, H. H., Neely, C. S., Kwong, E., Labadan, B. A., & Frankova, S. (1968). Postnatal nutritional deprivations as determinants of adult rat behavior toward food, its consumption and utilization. *J Nutr, 69,* 467-476.

Brozek, J. (1979). Nutrição, desnutrição e comportamento. *Cadernos de Pesquisa*. São Paulo, Brasil: Fundação Carlos Chagas. 29: 11-30.

Bush, M. S, & Leathwood, P. D. (1975). Effect of different regimens of early malnutrition on behavioral development and adult avoidance learning in Swiss white mice. *Br J Nutr, 33,* 373-385.

Campbell, B. A. & Masterson, F. A. (1969). Psychophysics of punishment. In B. A. Campbell and R. M. Church Eds. *Punishment and Aversive Behavior*. New York: Appleton – Century-Crofts, pp. 3-42.

Catania, A. C., & Reynolds, G. S. (1968). A quantitative analysis of the responding maintained by interval schedules of reinforcement. *J Exp Anal Behav, 11,* 327-383.

Crnic, L. S. (1976). Effects of infantile undernutrition on adult learning in rats: methodological and design problems. *Psychol Bull, 83,* 715-728.

Eckhert, C. D., Levistsky, D. A., & Barnes, R. H. (1975). Postnatal stimulation: the effects on cholinergic enzyme activity in undernourished rats. *Proc Soc Exp Biol Med*, *149,* 860-863.

Fernstron, J. D., & Lytle, L. D (1979). Long-term consumption of low-protein corn-based diets: effect on serotonin synthesis in rat brain, and sensitivity to painful stimuli. In D. A. Levitsky Ed. *Malnutrition, Enviroment, and Behavior: New perspectives*. New York: Cornell University Press, pp. 78-93.

Frankova, S., & Barnes, R. H. (1968). Effects of malnutrition in early life on avoidance conditioning behavior of adult rats. *J Nutr, 96,* 485-493.

Guthrie, H. A. (1968). Severe undernutrition in early infancy and behavior in rehabilitated albino rats. *Physiol Behav, 3,* 619-623.

Howard, D. E., Olton, S. D., & Johnson, C. T. (1976). Active avoidance and brain after postnatal food deprivation in rats. *Dev Psychobiol, 9,* 217-221.

Leathwood, P. D., Bush, M. S., Berent, C. D., & Mauron, J. (1974). Effects of early malnutrition on Swiss with mice avoidance learning after rearing in large litters. *Life Sci 14*, 157-162.

Levitsky, D. A. (1975). Malnutrition and animals models of cognitive development. In: *Malnutrition and Mental Function*, edited by G. Serban, New York: Plenum Press, pp. 75-89.

Levitsky, D. A. (1979). *Malnutrition, Environmental and Behavior*: New Perspectives New Yourk: Cornell University Press, pp. 271-272.

Levitsky, D. A., & Barnes, R. H. (1970). Effects of early malnutrition on the reaction of adult rats to aversive stimuli. *Nature, 225*, 468-469.

Levitsky, D. A., & Barnes, R. H. (1972). Nutritional and environmental interaction in the behavioral development of the rat: long-term effects. *Science, 176,* 68-71.

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

Morris, C. J. (1974). The effect of early malnutrition on one-way and two-way avoidance behavior. *Physiol Psychol, 2*, 148-150.

Smart, J. L., Whatson, T. S., & Dobbin, J. (1975). Thresholds of response to electric shock in previously undernourished rats. *Br J Nutr, 34,* 511-516.