EFFECT OF FEED RESTRICTION ON LEARNING, MEMORY AND STRESS OF RODENTS

EFEITOS DA RESTRIÇÃO ALIMENTAR SOBRE O APRENDIZADO, MEMÓRIA E ESTRESSE EM ROEDORES

Frederico Rogério FERREIRA[1], Vanessa Beatriz Monteiro Galassi SPINI[1], Ederaldo José LOPES[2], Renata Ferraz Fernandes LOPES[2], Eduardo Antônio MOREIRA[2], Marco Antônio Franco AMARAL[2], Ana Lúcia Souto CUNHA[1], Ana Paula de Souza BORGES[1], Luciane Borges MARSON[1], Gabriel de Camargos Cunha RIBEIRO[1]

ABSTRACT: Feed restriction (FR) has been recognized as the major experimental assay able to retard aging and degeneration diseases, although the effects of dietary manipulation at Central Nervous System (CNS) superior functions as learning and memory remain controversial. The aim of the present study was to evaluate the FR effects in CNS superior functions focusing in learning and memory in rodent models. Male Wistar rats were divided into three groups (n = 9/group) according to dietary conditions: *ad libitum* (AL) with unlimited access to standard rat diet (22% of protein, 8% fiber, 4% fat, 10% minerals, 1.4% Calcium, 0.8% Phosphorus); FR30 and FR60 groups were fed with 70% and 40%, respectively, of total rat diet offered to the AL group for 15 weeks. The animals FR30 and FR60 showed significant increase of learning performance in Y maze compared to animals fed *ad libitum*. The FR30 group, in memory task, also showed strong preference to the correct arm in Y maze at the first trials and greater rightness probability, in relation to the AL and FR60 groups. The AL group had significantly higher exploratory activity at the open field test than both FR groups. These findings implicate the FR on modulation of CNS functions as learning, suggesting, also, a possible anxiogenic-like effect of stress caused by FR.

UNITERMS: Feed Restriction; Learning; Memory; Stress; Rat.

INTRODUCTION

Feed restriction (FR) has been recognized as the major experimental assay able to retard aging and degeneration diseases. The increase in longevity may be explained, in summary, by decreasing in oxidative damages, enhance of genomic stability and the protein turnover (GUO et al., 2002).

Reporting to the central nervous system (CNS), recent observations suggest that long term reduced caloric intake stimulates neurogenesis and has neuroprotective effects (MATTSON et al., 2002), decreasing the risk of developing chronic neuropathologies, such as Alzheimer's and Parkinson's diseases (MAYEUX et al., 1999; CONTESTABILE; CIANI; CONTESTABILE, 2004). These beneficial effects may result in the production of neurotrophic factors and cytoprotective protein chaperones (MATTSON; DUAN; GUO, 2003).

However, the majority of studies have focused on the beneficial effects of FR on biochemistry and physiological roles, neglecting the influence of dietary manipulation at aspects of CNS superior functions as perception, cognition, learning and memory (WU; SUN; LIU, 2003). Studies in rats evaluating the spatial memory by radial arm maze could not confirm the preventive effect of long-term dietary restriction in aging impairments (BOND; EVERITT; WALTON, 1989). Also, it has been reported that long-term feed restriction may cause irreversible negative effects on cognitive functions (YANAI; OKAICHI; OKAICHI, 2004), and food deprivation during the neonatal period is associated with a decrease in neurogenesis and mild impairment of visual spatial memory (AKMAN et al., 2004). Moreover, it was

[1] Laboratory of Neuroscience, Institute of Biomedical Sciences, Federal University of Uberlândia
[2] Laboratory of Experimental Psychology, Institute of Psychology, Federal University of Uberlândia
Received: 04/06/05 Accepted: 06/11/05

found learning increase in male but not in female rats, that suggest a sex-dependent effect of FR on cognition (WU; SUN; LIU, 2003).

Nevertheless, controversies found between different studies raised doubts about the effects of dietary restriction on human approaching (THORNTON, 2002). The aim of the present study is evaluate the FR effects in learning and memory of rodent models, and its possible implications as anxiogenic-like effects associated to dietary stress.

MATERIALS AND METHODS

Animals

Wistar rats (age, 7 weeks; weight, 120–150 g) were housed in groups of three rats in acrylic cages (35 x 56 x 19 cm) in environmentally controlled room (temperature 23-25ºC; relative humidity 50-60%; light-dark cycle, 12:12 h; lights on at 3:00 pm). All experimental procedures were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and approved by the local Animal Care Committee.

Procedures

Rats were divided into three groups (n = 9/group) according to dietary conditions: *ad libitum* (AL) with unlimited access to standard rat diet (containing: 22% of protein, 8% fiber, 4% fat, 10% minerals, 1,4% calcium, 0,8%, phosphorus); FR30 and FR60 that were fed with 70% and 40%, respectively, of total rat diet consumed by control. Dietary manipulation was performed as previous described (HUBERT et al., 2000), and water was available freely for all groups. The body weight of animals was measured weekly, and after 15 weeks of feed restriction the tests were performed.

The open field test, a test with high predictive value of anxiety-like behavior (KALUEFF; TUOHIMAA, 2004), was performed in an open arena, which 1 x 1 m quadratic box with 16 equally sized squares and four 20 cm walls. A video camera set above the center of the open field recorded images of each rat placed in the center of the open-field arena. To statistical analyses were measured the length of path and number of rearings of the 5 min at the beginning of the test, as previously described (WU; SUN; LIU, 2003).

The learning and memory tests were performed in a Y maze consisting of three equal arms (40 x 15 x 15 cm) with a stainless-steel grid floor. Before the learning test, was made a habituation in the Y maze, where the animals had access to water on both arms after 24 h of water privation. There were given 30 trials; whereas rats entered into the left arm (error), they received water followed by foot-shock punishment (1.0 – 1.3 mA); and whereas rats entered into the right arm (correct), they received water without punishment. The same procedures were used to evaluate the memory of the learning training after 48 h.

Statistical analyses

All data were expressed in tables and analyzed according to dietary condition variance. For each parameter measured, a one-way analysis of variance (ANOVA) was performed. Post hoc comparisons between individual groups were performed by means of the Tukey HSD test (HO; EICHENDORFF; SCHWARTING, 2002; WU; SUN; LIU, 2003). Data are expressed as the mean values ± SD. Probabilities of less than 5 % ($p < 0.05$) were considered significant.

Results

After 15 weeks of feed restriction, the body weight was lower in FR animals when compared to the control, showing a restriction level-dependent response, according to previous studies (Figure 1) (HUBERT et al., 2000).

The open field test revealed that the AL group had higher exploratory activity when compared to the other groups in 5 minutes. The length of path average of control was two times greater ($p < 0.05$) in AL group (1,400±452cm) than in FR30 (683±29cm) and FR60 (558±152cm) (Figure 2). The number of rearings in AL (17.66±8.62) did not differ among the others groups (FR30, 9.33±2.08 and FR60, 11.33±4.51). However, the animals on FR expended more time (FR30, 225.33±57.06s and FR60, 266.33±14.15s) on the corners at the open field compared to AL (186.33±50.12s). These results suggest that FR induces anxiety-like behavior in restriction groups.

In the learning test, the FR groups were significantly better than the AL group (Table 1). The FR30 also showed higher rightness progression than the FR60 and AL at the first eight attempts (Figure 3).

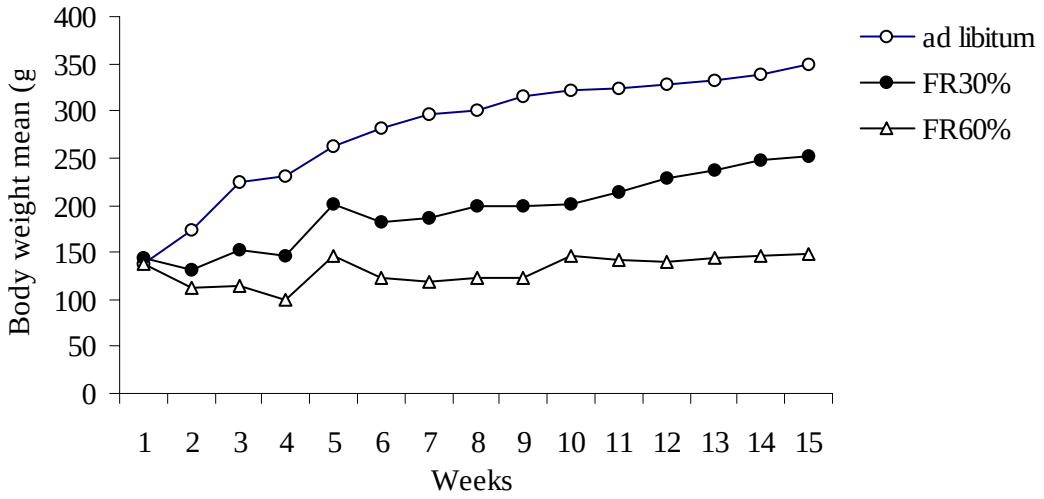

Figure 1. Body weight mean measured weekly according to feed conditions.

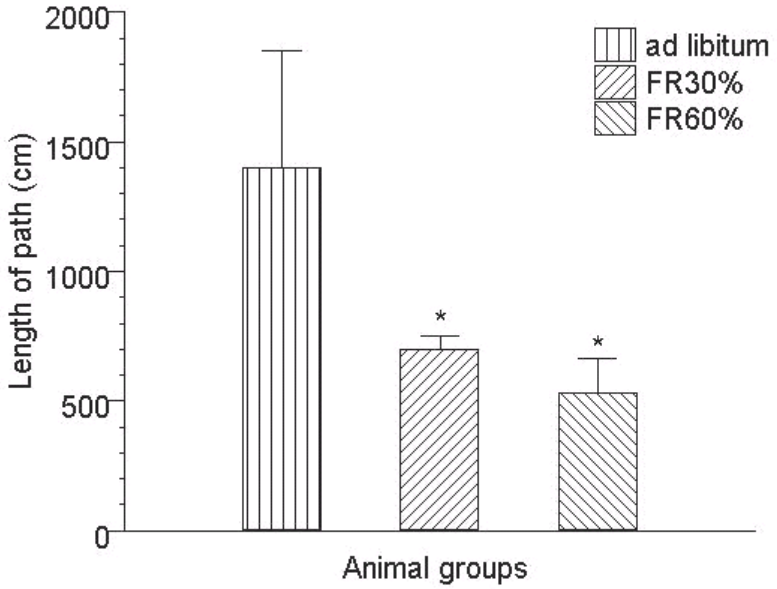

Figure 2. Performance of animals in different feed conditions evaluated according to the length of path at the open field arena. * $p < 0.05$.

Table 1. Evaluation of training and retention according to the number of trials and feed conditions

	Control		FR 30%		FR 60%	
	X	±SD	X	±SD	X	±SD
Learning	6.33	1.15	3.33*	1.15	3.66†	0.57
Memory	6.00	3.00	2.33	1.52	3.66	1.52

Learning and memory were tested in a Y maze. X represents the average of error numbers and ±SD represents standard deviation. *$p = 0.0335$ and †$p = 0.0232$ in comparison to AL group.

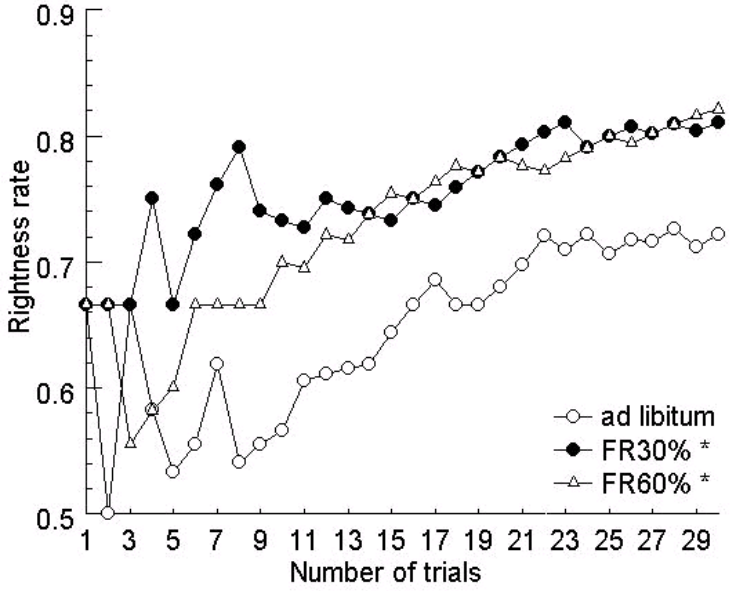

Figure 3. Learning performance of animals in different dietary conditions evaluated according to the rightness rate and the number of trials in Y maze. * $p < 0.05$.

When we compared the animals performance at the memory test, i. e., 48 h after learning test, they did not present significant increase at the rate of rightness in all groups (Table 1); however, the FR30 group presented strong preference by the correct arm in the first trials, suggesting a training retention at the learning test (Figure 4). Otherwise, the DR60 animals did not show training retention after 48 hours, which may be verified by the great number of faults at the first 5 attempts. Interestingly, in this group, the rightness rate tends to equalize to the curve of the FR30 animals (Figure 4).

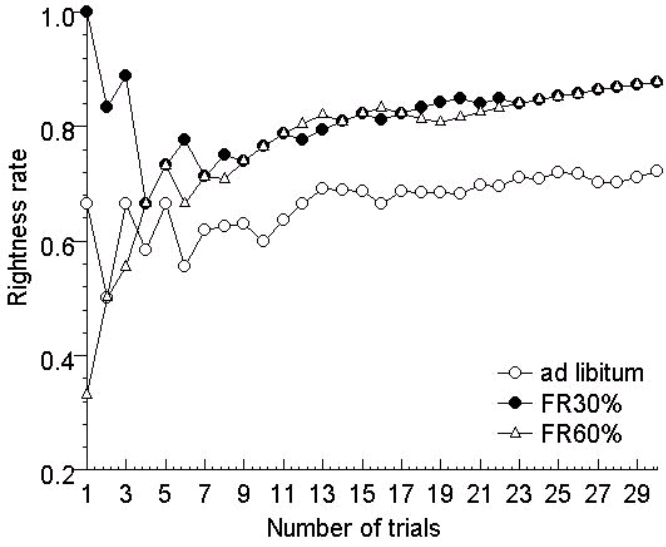

Figure 4. Memory performance of animals in different feed conditions evaluated according to the rightness rate and the number of trials in a retention Y maze.

DISCUSSION

At the present study we verified a learning increase on both animal groups submitted to the mild (30% of restriction) and intense (60% of restriction) FR, while memory improvement was found only in animals under mild FR. This memory and learning increment of these animals can be explained by 2 factors. Firstly, it has been verified that FR can decrease the expression of glucocorticoids receptors preventing neuron damages associated to stress as dendritic remodeling of the pyramidal neurons and hippocampal atrophy (GOULD et al. 1999; JOCA; PADOVAN; GUIMARÃES 2003).

On the other hand, 30% of dietary restriction is recognized as mild stress agent with beneficial hipocampal effects, as previously discussed by SHARMA (2004). During the restriction period, the hypothalamus-pituitary-adrenal axis (HPA) was stimulated possibly due to a feeling of stress by underfeeding, leading to an increased level of circulating glucocorticoids (PATEL; FINCH, 2002). This transient increase in glucocorticoids facilitates hippocampal long-term potentiation (LTP) causing improvements in memory and learning tasks (KORZ; FREY, 2003).

The fact of the animals with 60% of FR present the same rightness level in the training sessions compared to the animals under moderate restriction, i. e., 30% underfeeding, subsidy the hypothesis that the beneficial effects of FR upon the hippocampus surpassed the deleterious effects of glucocorticoids, although theses animals failed at the long term memory task. Probably the nutritional quality had greater influence on long-term memory than the effects of glucocorticoids (SHARMA, 2004).

The hippocampus has important role in spatial memory and learning in rodents as also in humans. In experiments with primates models, lesions in fornix (a major input/output pathway of the hippocampus) did not impair short delay memory (10 sec) but it was harmful to long delays (70 and 130 sec), suggesting the role of hippocampus on recognition (GOOD, 2002). Even, amnesic syndrome and impairments on the ability of place discrimination has been observed in monkeys with lesions in hippocampus and amygdale. Nevertheless, in humans, studies have appointed that the hippocampus, the prefrontal cortex, and the ventral striatum form interconnected neural circuits that may underlie several aspects of spatial cognition and memory (SEAMANS; FLORESCO; PHILLIPS, 1995; SEAMANS; PHILLIPS, 1994).

Despite the benefits of moderate FR on learning showed in this experiment, the results also suggest anxiogenic-like effects of FR and possible implications on emotional disorders, such as fear and depression, which need future investigations.

ACKNOWLEDGEMENTS

We thank PhD Antônio Vicente Mundim and Sebastião Sirmiano de Araújo, Clinical Pathology of Veterinary Hospital, Uberlândia Federal University by clinical analyses of animals, and M.Sc. Felipe Antônio Chegury Viana by software technical assistance.

RESUMO: A restrição alimentar (RA) tem sido reconhecida como a mais potente manipulação experimental capaz de retardar o envelhecimento e doenças degenerativas relacionadas à idade, contudo os efeitos da manipulação da dieta sobre as funções superiores do Sistema Nervoso Central (SNC), como o aprendizado e a memória, permanecem pouco explorados. O objetivo do presente estudo foi avaliar os efeitos da RA sobre o aprendizado e memória de roedores. Ratos machos (n = 9/grupo) Wistas EPM foram distribuídos aleatoriamente em três grupos, segundo as condições dietárias: *ad libitum* (AL), com livre acesso à dieta padronizada de roedores (22% de proteína; 8% fibras; 4% ácido graxo; 10% sais minerais; 1,4% cálcio; 0,8% fósforo); grupos RA30 e RA60 alimentados com 70% e 40%, respectivamente, do total da ração consumida pelo grupo AL. O grupo AL apresentou maior atividade exploratória pelo teste do campo aberto do que ambos os grupos RA. Os animais RA30 e RA60 apresentaram aumento significativo do aprendizado no labirinto em Y comparado aos alimentados AL. O grupo RA30, no teste de memória, também mostrou grande preferência pelo braço correto no labirinto em Y nas cincos primeiras tentativas e maior probabilidade de acerto, em relação aos grupos AL e RA60. Estes resultados mostram que a RA modula as funções do SNC como o aprendizado, e sugerem um possível efeito ansiogênico do estresse associado à RA.

UNITERMOS: Restrição alimentar; Aprendizado; Memória; Estresse; Rato.

REFERENCES

AKMAN, C.; ZHAO, Q.; LIU, X.; HOLMES, G. L. Effect of food deprivation during early development on cognition and neurogenesis in the rat. **Epilepsy Behav.,** New York, v. 5, n. 4, p. 446-54, Aug. 2004.

BOND, N. W.; EVERITT, A .V.; WALTON, J. Effects of dietary restriction on radial-arm maze performance and flavor memory in aged rats. **Neurobiol Aging**., Sydney, v. 10, n. 1, p. 27-30, Jan. 1989.

CONTESTABILE, A ; CIANI, E.; CONTESTABILE, A. Dietary restriction differentially protects from neurodegeneration in animal models of excitotoxicity. **Brain Research**, Bologna, v. 1002 , n. 1-2, p. 162-166, Mar. 2004.

GAFFMAN, D.; PARKER, A. Interaction of perirhinal cortex with the fornix-fimbria: memory for objects and "object-in-place" memory. **J. Neurosci**, Oxford, v. 16, n. 18, p. 5854-5869, Sept. 1996.

GOOD, M. Spatial memory and hippocampal function: where are we now? **Psicológica**, Cardiff, v. 1, n. 23, p. 109-138, Apr. 2002.

GOULD. E.; TANAPAT, P.; HASTINGS, N. B.; SHORTS, T. J . Neurogenesis in adulthood: a possible role in learning. **Trends Cogn Sci**, Princeton, v. 3, n. 5, p. 186-192, May 1999.

GUO, Z. M.; MITCHELL-RAYMUNDO, F.; YANG, H.; IKENO, Y.; NELSON, J.; DIAZ, V.; RICHARDSON, A.; REDDICK, R. Dietary restriction reduces atherosclerosis and oxidative stress in the aorta of apolipoprotein E – deficient mice. **Mech. Ageing Dev.,** San Antonio, v. 123, n. 8, p. 1121–1131, Apr. 2002.

HO, Y.; EICHENDORFF, J.; SCHWARTING, R. Individual response profiles of male Wistar rats in animal models for anxiety and depression. **Behav Brain Res**., Taiwan, v. 136, n. 1, p. 1-12, Oct. 2002.

HUBERT M. F.; LAROQUE, P.; GILLET, J. P.; KEENAN K, P. The effects of diet, *ad libitum* feeding and moderate and severe dietary restriction on body weight, survival, clinical pathology parameters, and cause of death in control Sprague-dawley rats. **Toxicol. Sci**., Oxford, v. 58, n. 1, p. 195-207, Nov. 2000.

JOCA, S. R. L.; PADOVAN, C. M.; GUIMARÃES, F. S. Estresse, depressão e hipocampo. **Rev. Bras. Psiquiatr**., São Paulo, v. 25, n. 1, p. 46-51, Jan. 2003.

KALUEFF, A. V.; TUOHIMAA, P. Experimental modeling of anxiety and depression. **Acta Neurobiol Exp**., Tampere, v. 64, n. 4, p. 439-48, Apr. 2004.

KORZ, V.; FREY, J. U. Stress-related modulation of hippocampal long-term potentiation in rats: Involvement of adrenal steroid receptors. **J. Neurosci.,** Magdeburg, v. 23, n .19, p. 7281-7287, Aug. 2003.

MATTSON, M. P.; DUAN, W.; GUO, Z. Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. **J. Neurochem**., Baltimore, v. 84, n. 3, p. 417-431, Feb. 2003.

MATTSON, M. P.; DUAN, W.; CHAN, S. L.; CHENG, A.; HAUGHEY, N.; GARY, D. S.; GUO, Z.; LEE, J.; FURUKAWA, K. Neuroprotective and neurorestorative signal transduction mechanisms in brain aging: modification by genes, diet and behavior. **Neurobiol. Aging.,** Baltimore, v. 23, n. 5, p. 695-705, Sept. 2002.

MAYEUX, R.; COSTA, R.; BELL, K.; MERCHANT, C.; TANG, M. X.; JACOBS, D. Reduced risk of Alzheimer's disease among individuals with low calorie intake. **Neurology**, Baltimore, v. 52, n. 6, s. 2, p. A296-A297, Apr. 1999.

PATEL, N. V.; FINCH, C. E. The glucocorticoid paradox of caloric restriction in slowing brain aging. **Neurobiol. Aging**, New York, v. 23, n. 5, p. 707-717, Sept. 2002.

SHARMA, R. Dietary restriction and its multifaceted effects. **Curr. Sci.,** Bangalore, v. 87, n. 9, p. 1203-12010, Nov. 2004.

SEAMANS, J. K.; PHILLIPS, A. G.; Selective memory impairments produced by transient lidocaine-induced lesions of the nucleus accubens in rats. **Behav. Neurosci**., Washignton, v. 87, n. 3, p. 456-468, June, 1994.

SEAMANS, J. K.; FLORESCO, S. B.; PHILLIPS, A. G. Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. **Behav. Neurosci**., Washington, v. 109, n. 6, p. 1063-1073, Dec. 1995.

THORNTON, R. Calorific restriction: can we extrapolate from animals to humans?, **Food Chem. Toxicol.**, Exeter, v. 40, n. 6, p. 743-746, June 2002.

WU, A.; SUN, X.; LIU, Y. Effects of caloric restriction on cognition and behavior in developing mice. **Neurosci. Lett.**, Limerick, v. 339, n. 2, p. 166-168, Mar. 2003.

YANAI, S.; OKAICHI, Y.; OKAICHI, H. Long-term dietary restriction causes negative effects on cognitive functions in rats. **Neurobiol. Aging**, New York, v. 25, n. 3, p. 325-332, Mar. 2004.

ZOLA-MORGAN, S.; SQUIRE, L. R.; MISHKIN, M. The neuroanatomy of amnesia: amygdale-hippocampus versus temporal stem. **Science**, Washington, v. 218, n. 4579, p. 1337-1339, Dec. 1982.