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Swimming T-Maze and Radial Maze: two Cognitive Procedural Experiments to Determine the Influence of Acute Calorie Restriction over Learning Abilities in Wistar Rats

Radu MĂNĂLĂCHIOAE [1)], Orsolya SARPATAKI[1)], Iulia PRODAN[1)], Bogdan SEVASTRE[1)], Ioan MARCUS[1)]

[1)]University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Mănăştur Street, 400372, Cluj-Napoca, Romania; radumanalachioae@gmail.com

Abstract. Caloric restriction (CR) has long been known to increase median and maximal lifespan and to decreases mortality and morbidity in short-lived animal models, likely by altering fundamental biological processes that regulate aging and longevity. In rodents, CR was reported to delay the aging of the immune system (immune senescence), which is believed to be largely responsible for a dramatic increase in age-related susceptibility to infectious diseases. However, it is unclear whether CR can exert similar effects in long-lived organisms. In rodents subjected to CR, the onset of age-related pathology is delayed and its frequency is reduced. The mechanism by which simple under-nutrition exerts these beneficial effects is not understood. One of the major age-related pathological aspects refers to the regress of cognitive functions and it is currently believed that CR might delay or even cancel the onset of such disturbances. A modality to assess the cognitive is through using one or more mazes (swimming T-maze, radial maze). In our study, spanned over 6 week's period, we used 23 Wistar rats subjected to different levels of calorie intake (*ad libitum*, 100%, 75% and 25% of normal calorie intake). The rats subjected to different levels of CR were more successfully (statistically significant) at completing the tasks given by the types of mazes employed.

Keywords: acute calorie restriction, Wistar rats, swimming T maze, radial maze, cognitive effects.

INTRODUCTION

Calorie restriction (CR) is a scientific concept introduced in 1934 by McCay & Crowell from Cornell University, USA, (McCay and Crowell, 1934) and states that a lower than normal calorie intake will prolong the maximum lifespan of an animal organism in direct proportion to the level of the decrese in the calorie intake. Since then, this effect was established in numerous animal species, ranging from yeasts to primates, with the final goal, the human.

It has been observed that a calorie restriction of 30-60% of normal calorie intake, started immediately after weaning, will determine an increase of maximum life span up to 50% (Weindruch and Walford, 1988). Although this has been proven in many species, it has been yet to demonstrate similar effects in humans despite numerous positive signals from those who are experimenting on themselves.

Specifically in rats, concerning the length of CR period, it has been described 3 different stages of CR: 1) acute CR, if the diet spans over 30 days period (the individuals subjected to CR are lethargic, it is noticed a decrease in the digestive system activity and a decrease in the skeletal muscle); 2) sub acute CR, if the diet spans over 60 days period (the individuals subjected to CR are less lethargic and unresponsive as during the first CR month as their organisms are getting habituated to the reduced energy level); 3) chronic CR, if the CR diet spans at least 90 days period (the individuals subjected to CR are fully habituated with their current energy level, marked by a constant hypoglycemia and hipocholesterolemia) (Teske and Kotz, 2009).

Regarding the eventual cognitive impairments produced by the excess of calorie intake, the opinions are divided. Some studies imply these impairments to hypertriglyceridemia, hypercholesterolemia and hyperinsulinemia secondary to obesity but others do not (Morley, 2004, Reitz *et al.*, 2005, Rogers *et al.*, 1989, Atmon *et al.*, 2002, Farr *et al.*, 2008). However, a long-lasting hypertriglyceridemia may result in obesity and, as it has been demonstrated in recent studies on mice (Bank *et al.*, 2004, Farr *et al.*, 2000, 2001), triglycerides can impair the transport of leptin across the blood-brain barrier, which may account in part for the peripheral leptin resistance seen in obesity.

The purpose of the present study is to assess the influence of CR over memory capacity and cognitive functions in stress conditions and whether the swimming T-maze and radial maze with some constructive and procedural modifications are able to highlight cognitive and learning differences across groups of Wistar rats who received various caloric intake over during 30 days period (acute CR). Both experimental devices are intended to test the learning and memory capacity under stressful condition (Gregory, 2011).

MATERIALS AND METHODS

The animals. For this experiment we have used 23 male Wistar rats at 3 months age at the beginning of the experiment. The rats were obtained by breeding of our own Wistar line at the Faculty of Veterinary Medicine Cluj-Napoca Biobase, and were kept at a constant day-night cycle of 12/12 hours and at a constant temperature interval of 21 ± 2^0C. In the day 0 of the experiment, the rats were weighted and divided in 4 equal groups based on their initial weight. Each group was housed in 40/60/25 cm Plexiglas cage.

The chow was provided by Cantacuzino Institute, Bucharest, Romania, and consisted in combined granulated pellets, with 290 kcal/100 g, and recommended daily allowance of 20 g/rat. The animals had free access to drinking water. As bedding was used beech saw dust, which was changed once at every week during the experiment.

Group 1 (n=5) was considered as control and had unrestricted access to food. Group 2 (n=6) received 20 g/rat/day while groups 3 (n=6) and 4 (n=6) received 15 g/rat/day (mild CR) and 12 g/rat/day (moderate CR). The chow was provided daily between 8 and 10 o'clock in the morning. The experiment spanned over a 30 days.

Swimming T-maze. A swimming T-maze (Ardayfio *et al.*, 2008) was used to assess procedural learning. In this test, rats were placed in the base of an opaque, liquid-filled T-maze, with an escapable platform (height 10 cm, width 9 cm, length 10 cm) located in the left arm of the maze. T-maze dimensions: 20,32 cm in height, arms with 30,48 cm and 71,12 cm, 10,16 in width, water depth 16 cm. The maze was filled with tap water at 22 ± 2^0C to 18 cm height and was colored with methilen blue for the escapable platform could not be seen from the above water. Rats had to learn to turn left after reaching the top of the T to reach the platform directly. The platform was in the same location for each rat. Since the task was

performed in the dark and the platform is under water, rats must rely on egocentric navigation to remember de correct location of the platform. In the first day, each rat was subjected to a 10 minutes habituation trial, with the escape platform remove. If a rat has wanted to escape, then it would have been gently but firm hold into the maze until the time has past. In the next 3 days each rat was subjected to 4 trials per day, each one of them lasting up to 2 minutes and 3 minutes interval between two consecutive trials. A trial was ended sooner than 2 minutes is the rat had found the escape pod and after the rat has been forcibly kept on the platform for another 15 seconds. If the rat hadn`t found the platform in the allocated time, it would have been forcibly kept on the platform for another 15 seconds. After each trial the rat was dried up using a towel and put into a dry cage. In the 5th day of the experiment the escape platform was removed and each rat was subjected to one 2 minutes trial. In this time it was recorded the total time spent in each arm, the number of entries in each arm and what arm of the maze was chosen in first time. The rats were fed with their usual diet after finishing of trials.

Radial maze. The radial arm maze was designed by Olton and Samuelson in 1976 to measure spatial learning and memory in rats (Olton and Samuelson, 1976). Radial maze dimensions: 8 arms, lenght 46,99 cm, width 10,11 cm, height 20,32. The arms were counted from 1 to 8. The rat put in the arm no. 1 had to find the cocoa chocolate in the arm no. 4 in 2 minutes time. If the rat didn`t find the chocolate in the given time, then it was put in the arm no. 4 and kept there 15 seconds from when it started to eat the chocolate. In the first day at the end of each arm was placed a piece of cocoa chocolate and each rat was left to explore the entire maze for 15 minutes. For the next 3 days only in the arm no. 4 was left a piece of chocolate and each rat was subjected to 4 trials per day, each one of them lasting up to 2 minutes and 3 minutes interval between two consecutive trials. Each trial consisted in the search for chocolate placed in the arm no.4. A trial was ended sooner than 2 minutes if the rat had found the chocolate and after the rat was forcibly kept in the arm for another 15 seconds after it started to eat chocolate. In the 5th day chocolate from arm no. 4 was removed and each rat performed a 2 minutes trial. It has been recorded the total exploration time, the time from the start of the trial since the rat had entered arm no. 4 and the total time spent in the respective arm. The rats were fed with their usual diet after finishing of trials.

The radial maze experiment had been undertaken 2 weeks after swimming T-maze.

Statistics. The data were expressed as the mean and standard error of the mean (S.E.M.). ``t`` Student multiple range test from Excel Windows Software was used to assess the differences between groups. Differences at $0.01<p<0.05$ and $0.001<p<0.01$ were considered significant and respectively significantly distinct statistically, and $p<0.001$ was considered highly significantly statistically distinct.

RESULTS AND DISCUSSIONS

Weight evolution. The animals were weighted at the beginning and at the end of the experiment using an electronic scale with a 0,5 grams error. All animals gain in weight during the experiment (Fig. 1) but those comprised in the 3rd and 4th group had a smaller weight gain than the first two group (Tab. 1), with an accent on the 4th group who had the smallest weight gain ($p<0,001$ compared to control). This fact is explained by the fact that those animals were fed with just 60% of normal calorie intake recommended by the manufacturer of the food. None the less, the 4th group had the most uniform weight distribution at the end of the experiment (S.E.M= 2,11).

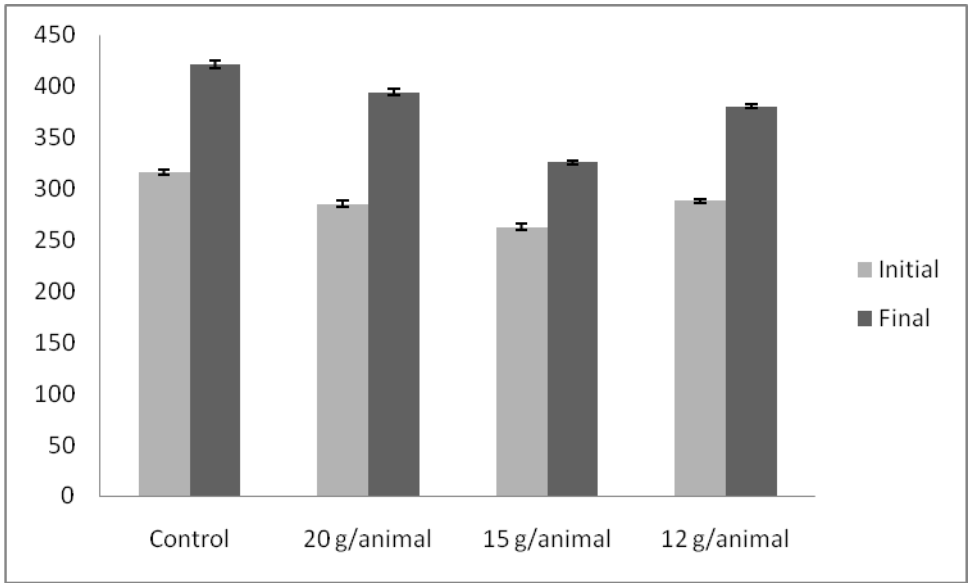

Fig. 1. Weight evolution during the experiment (mean ± S.E.M.) (grams).

An interesting fact is that the 2nd group had a bigger weight gain than the 1st group (control). This fact comes to confirm the fact that a calorie intake according to the individual needs rather than an *ad libitum* approach is more beneficial in realizing a weight gain in animals (Byung, 2006).

Tab. 1.

CR influence over weight gains.

	Procentual differences between initial and final weight (%)
Control	33,57 ± 2,46
20 g/animal	38,4565 ± 3,69
15 g/animal	24,07019 ±2,99 †‡
12 g/animal	9,542437 ± 2,11†††‡•

†= statistically significant at 0.01<p<0.05 as compared to Control group
‡= statistically significant at 0.01<p<0.05 as compared to 2nd group
†††= highly significantly statistically distinct at p<0.001 as compared to Control group
•= statistically significant at 0.01<p<0.05 as compared to 3rd group

Swimming T-maze. As shown in Fig. 2, animals comprised in group no. 3 and no. 4 spent far more time in the left arm (were the platform was in the previous 3 days) than the right arm during the 5th day of the experiment (0,001<p<0,01 compared to control in both groups). This fact can be explained that CR have a beneficial effect over the stress response behavior, resulting in a more accurate arm choosing by enhancing egocentric navigation to remember de correct location of the platform.

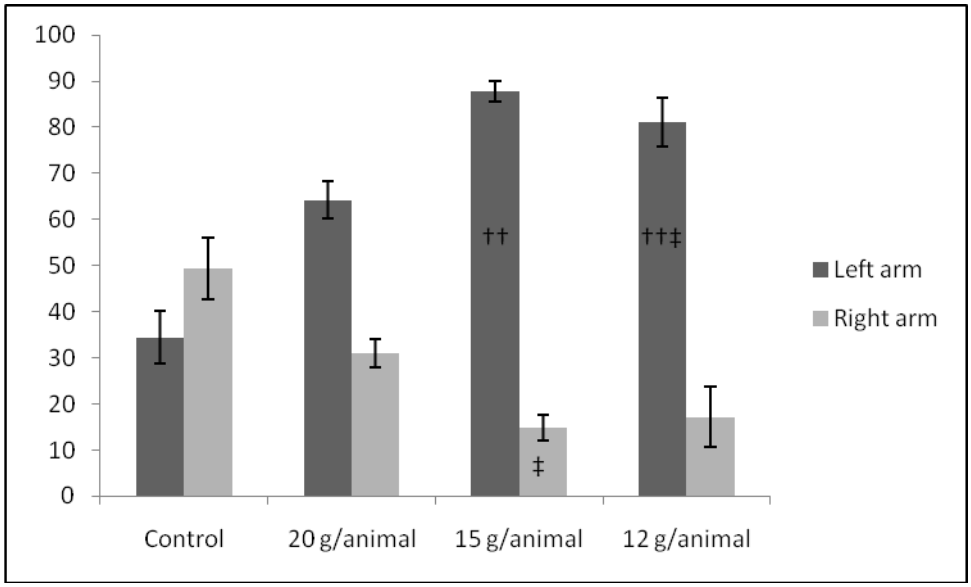

Fig. 2. Left arm/ right arm time spending in the 5th day of the experiment (seconds).
††= significantly statistically distinct at 0.001<p<0.01 as compared to Control group
‡= statistically significant at 0.01<p<0.05 as compared to 2nd group

Further more, the animals comprised in control group have made more mistakes in chosing the first arm entry, thus consolidating the above hipothesys.

Tab. 2.

Arm entries and preferences in the last day of the experiment (seconds).

	Left arm entries	Right arm entries	No. of animals who entered right arm first
Control	6	10	2
20 g/animal	21†	16	0
15 g/animal	15†	7‡	0
12 g/animal	16†	5‡	0

†= statistically significant at 0.01<p<0.05 as compared to Control group
‡= statistically significant at 0.01<p<0.05 as compared to 2nd group

Radial maze. During the last day of radial maze experiment, after removing the chocolate, the animals comprised in 1st and 2nd groups have registered a larger exploration time (Fig. 3) than the last two groups (0,001<p<0,01, 0,01<p<0,05). Perhaps an explanation may be that due to the fact that these animals, not being subjected to CR (control group) or being subjected to just a mild CR (20 g/animal group) they were not so interested in finding food as the animals subjected to a more severe CR (15 g/animal group and 12 g/animal group) and they kept exploring the maze during most of their trial.

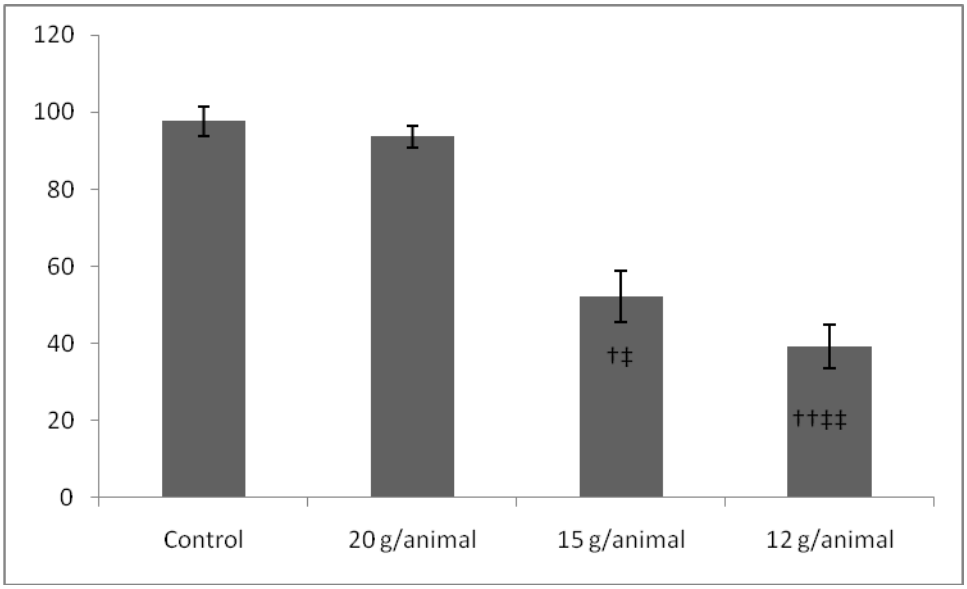

Fig. 3. Total exploration time in the last day of the experiment.
†= statistically significant at 0.01<p<0.05 as compared to Control group
††= significantly statistically distinct at 0.001<p<0.01 as compared to Control group
‡= statistically significant at 0.01<p<0.05 as compared to 2nd group
‡‡= significantly statistically distinct at 0.001<p<0.01 as compared to 2nd group

In contrast, the animals from the last two groups had a lesser tendency to explore (Fig. 4). Furthermore, they found the 4th arm more rapidly than the 1st and 2nd group (0.001<p<0.01) and remained longer in the hope to find the chocolate. This fact resembles the results from swimming T-test, were the same rats have spent more time in the left arm where the escaping platform has been before removing it.

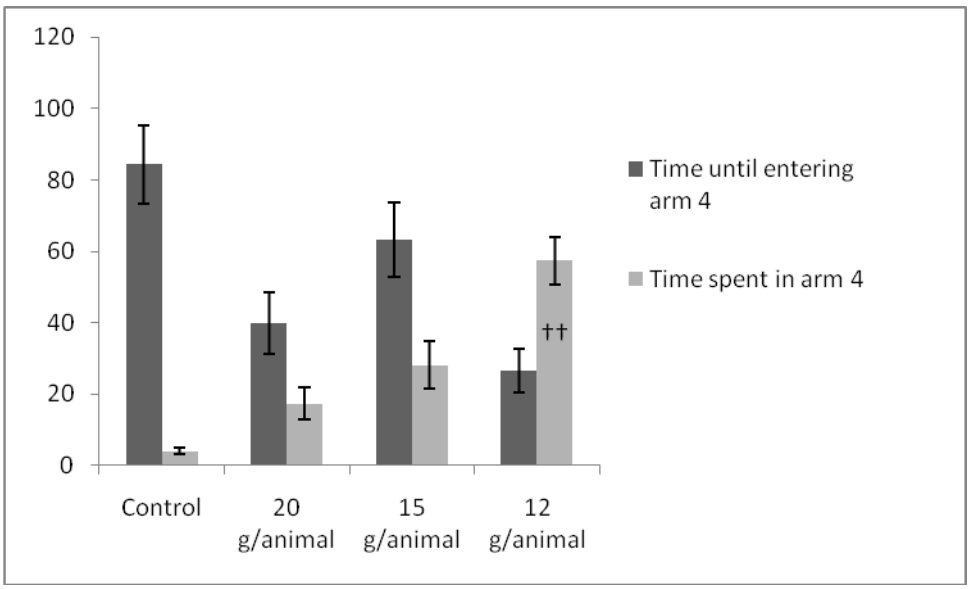

Fig. 4. Time from the trial started since the rat had entered arm no. 4 and the total time spent in the respective arm.
††= significantly statistically distinct at 0.001<p<0.01 as compared to Control group

Referring to the usability of swimming T-maze and radial maze, we appreciate that although the first one was slightly more difficult to operate due to the constant monitoring of water temperature, but in the end the results we had obtained were more accurate and more ``statistically significant`` than those obtained from radial maze. The radial maze was nonetheless less physically demanding on rats due to the nature of the task they had to complete (finding food in radial maze versus escaping by the means of swimming from the swimming T-maze).

CONCLUSIONS

This study proved that acute mild and moderate CR had a beneficial effect over stress response reactions and over memory capacity. The CR rats had remembered very accurately where the escaping platform previously had been in the swimming T-maze test, and which arm contains the piece of chocolate in the radial maze test. In both tests, CR rats remained in the target arm longer than the non-CR rats.

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REFERENCES

1. Ardayfio, P., M. jiSook, KaKa Amanda Leung, Y.H. Dong, K. Kwang-Soo (2008). Impaired learning and memory in Pitx3 deficient aphakia mice: A genetic model forstriatum-dependent cognitive symtoms in Parkinson`s disease. Neurobiology of Disease. 31: 406-412.
2. Atmon, G., I. Gabriely, W. Greiner, D. Davidson, C. Schechter and N. Barzilai (2002). Plasma HDL levels highly correlate with cognitive function in exceptional longevity. J. Gerontol. Med. Sci. 57:M712-M715.
3. Bank, W.A., A.B. Coon, S.M. Robinson, A. Moinuddin, J.M. Shultz, R. Nakaoke and J.E. Morley (2004). Triglyceride induces leptin resistance at the blood-brain barrier. Diabetes. 53: 1253-1260.
4. Byung, P.Y. (2006). Why calorie restriction would work for human longevity, Biogerontology. 7:179-182.
5. Farr, Susan A, W.A. Bank, M.E. La Scola, F.J. Flood and J.E. Morley (2001). Permanent and temporary inactivation of the hippocampus impairs T maze foot shock avoidance acquisition and retention. Brain. Res. 872:242-249.
6. Farr, Susan A., J.F. Flood and J.E. Morley (2000). The effect of cholinergic, GABAergic, serotonergic and glutamatergic receptor modulation on post-trial memory processing in hippocampus. Neurobiol. Learn. Mem. 73:150-167.
7. Farr, Susan A., K. Yamada, D.A. Butterfield, H.A. Mohammad, L. Xu, Nicole E. Miller, W.A. Banks and J.E. Morley (2008). Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology. 149(5):2628-2636.

8. Gregory, R.J. (2011). Psychological Testing: History, Principles, and Applications (Sixth ed.). Boston: Allyn & Bacon. ISBN 978-0-205-78214-7.
9. McCay, C.M., M.F. Crowell (1934). Prolonging the life span. Sci. Mon. 39:405–414.
10. Weindruch, R., R.L. Walford (1988). The retardation of aging and disease by dietary restriction. Springfield, IL: Charles C. Thomas.
11. Morley, J.E. (2004). The metabolic syndrome and ageing. J. Gerontol. Med. Sci. 59(A):139-142.
12. Olton, D.S. and R.J. Samuelson (1976). Remembrance of places passed: Spatial memory in rats. Journal of Experimental Psychology: Animal Behavior Processes. 2:97-116.
13. Reitz, C., J. Luchsinger, M.X. Tang, J. Manly and R. Mayeux (2005). Impact of plasma lipids and time on memory performance in healthy elderly without dementia. Neurology. 64: 1378-1383.
14. Rogers, R.L., J.S. Meyer, K. K. McClintic and K.F. Mortel (1989). Reducing hypertriglyceridemia in elderly patients with cerebrovascular perfusion. Angiology. 40: 260-269.
15. Teske, J.D. and C.M. Kotz (2009). Effect of acute and chronic caloric restriction and metabolic glucoprivation on spontaneous physical activity in obesity-prone and obesity-resistant rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 297:R176–R184.