Biomedical Research 2017; 28 (11): 5003-5007

ISSN 0970-938X
www.biomedres.info

Effects of nutrition style on neuro-behavior.

Hasan Basri Savas[1*], Fatih Gultekin[1], Duygu Kumbul Doguc[2]

[1]Department of Medical Biochemistry, Faculty of Medicine, Alanya Alaaddin Keykubat University, Alanya, Antalya, Turkey

[2]Department of Medical Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Abstract

Objective: The effects of meal frequency and caloric restriction on spatial memory based on hippocampus in rats were examined in our study.
Materials and methods: As a result of a four-weeks pilot study amount of food and meal times was determined with 9 rats. In the main study, Wistar albino, 12 weeks, male, 24 rats were divided into three groups as; Ad libitum control (AL) (n=8), two meals fed group (TM) (n=8), two meals fed and 20% calorie restriction of group (TM-CR) (n=8). All rats were kept individually in cages. According to the results of a pilot study in main study; 20 g/day; 10 g for the morning and evening meals were given to TM group, 16 g/day; for the morning and evening meals were given feed in the form of 8 grams to TM-CR group. At the end of the experiment, the hippocampus-based spatial memory training and testing was performed in the Morris Water Maze (MWM) and the experiment was terminated.
Results: There was no significant difference between groups about learning speed and memory assessment by WMT (p>0.05).
Conclusions: Thus, it was shown that TM and the TM-CR groups have no negative effect in terms of learning and memory. TM and the TM-CR can be presented as a healthy nutritional diet taking into metabolic benefits and no negative impact on learning.

Keywords: Nutrition, Meal frequency, Calorie restriction, Learning, Morris water mase, Neurobehaviour, Spatial memory.

Accepted on March 30, 2017

Introduction

Being in the first-place growth and development of creatures, receiving external nutrient and using effectively to realize viability function and maintaining a healthy life is called nutrition. According to Maslow's "Theory of Human Motivation" in 1943, the needs that people try to supply throughout their lives are defined as pyramids, from simple to complex. Among the most simple and basic needs which form the base of the nutrition pyramid is in the lead [1] Behavioral science is one of the sub-branches of neurology. Behavioral science is protecting animals' life, and investigating adaptation to environment to maintain their species and their behavioral patterns. It is looked for an answer to "what, how and why" questions with behavioral science. Behaviors can be observed in the spectrum which changes from very simple to too complex. When searching answer to "how" question in behavioral science, it is associated with all branches of basic medical sciences. Previous research has shown that information obtained from animal studies will also exist for humans, depending on the proximity between brain structures, functions, and behavior levels of different mammals [2,3]. One of the used tests to neurobehavior assessment is called as Water Maze Test (WMT) which designed by Morris at 1982 for experiments to search hippocampus-dependent spatial learning and memory research [4]. Although various researches which examine the relationship between nutrition and neurobehavior have been conducted, studies which investigate the association between meal frequency and calorie restriction have not been discovered [5-11]. Thus, how neurobehavior is influenced was investigated in this study by taking into consideration effects of meal frequency and calorie restriction on metabolism. Holistic view about the relationship of nutrition, meal frequency and calorie restriction and spatial memory was revealed.

Material and Methods

Animals

Thirty-three male Wistar albino rats weighing 200-250 g were used for all the experimental procedures. The ambient temperature and relative humidity of the animal room were 21 ± 1°C and 60 ± 7%, respectively. The room was illuminated with artificial light for 12/12 h in a dark/light cycle. The animals were allowed free access to tap water but standard pelleted food was consumed in a controlled environment. All studies were performed with the approval of the ethical committee of the Faculty of Medicine at Suleyman Demirel University (Date: 21.05.2015. Number: 21438139-172).

Animal experiment

Before the pilot and main studies, all rats were exposed to a light/dark living cycle for a week, where they were kept in the dark room for 12 h in order to reverse their active period in day time, and they were kept in illuminate room for 12 h to fall asleep at night. Each rat was accommodated in a single standard Eurotype 2 cage, which has a capacity to accommodate two rats at once. After 4 weeks of the pilot study, the 20-week main study was conducted by determining the amounts of food and lengths of meals. Rats were divided into three groups based on nutrition style as; ad libitum (AL), two meal (TM), and two meal and calorie restriction (20%) (TM-CR) groups. There were three rats for pilot study and eight rats in main study for all groups. As a result of a 1-month preliminary study, the average amount of food for a rat which would be consumed in a day and also the consumption time for each meal (1 h) was determined. In the pilot study, it was seen that the rats in the AL group consumed 0.062 g of food per a gram of body weight. Based on this result, when the necessary calculations were made regarding the weights of the animals in the main study, it was calculated that the TM and the TM-CR groups would consume 20 and 16 g/day of food, respectively. It was decided to provide the daily meals of the TM and TM-CR groups between 09:00-10:00 every morning, and between 16:00-17:00 every evening in the main study.

Morris water maze (MWM)

MWM is a task that evaluates spatial learning and memory, a measure of cognitive function. The tank was placed in a dimly lit, soundproof test room with various visual cues. This task uses a round pool of water in which a platform is submerged beneath the surface. When placed in the maze the animal's task is to find the hidden platform. The MWM consisted of a circular pool, 150 cm in diameter and 80 cm in height, with the interior painted white. The temperature of the water was checked daily by a thermometer and adjusted to 22 ± 2°C and was made opaque by the addition of nontoxic dark yellow paint. The pool was surrounded by four halogen lamps (300 W with adjustable power range), which were directed to the walls that surrounded the pool. Visible extra-maze cues including a picture on the wall, a chair, and a colored box were left at fixed positions around the water maze [4].

Experimental procedure

Each trial was tracked by using an overhead camera (Sony SSC-DC398P, Sony Corporation, Shinagawa, Japan) interfaced with a computer that recorded the training phase and probe trial test for all the runs in the maze. The tracking program we used was Smart Version 2.5 (Smart Video-Tracking, Panlab, Barcelona, Spain). The maze was divided into four equally sized virtual quadrants, designated as zones one to four. The platform remained in a fixed location for all runs and the target quadrant was the fourth quadrant. Throughout the experiment, animals were handled before the first trial of each day and then were released once from each of the four quadrants facing the center of the pool. Daily training consisted of five trials in which the rat was placed in the water from four random starting positions (1, 2, 3, and 4) and the latency of escaping onto the platform was recorded. Starting locations were equally spaced around the perimeter of the pool. This was conducted for 4 consecutive days. In a protocol modified from Morris, acquisition of place learning using spatial cues and navigational strategy was done on days 1-4 and then the test for memory called ‘probe trial’ was performed on day 5. On the first day of testing, animals were allowed to swim in the pool for 60 seconds. If a rat could not find the hidden platform, it was placed on the platform for 30 s to introduce the platform and show that the platform is the mean of escape from the water. On day 2, if a rat could not find the platform, it was placed on the platform by the experimenter and were left on the platform for 15 s and then removed to their home cages by the experimenter. There were five trials per day, with an intertrial interval of approximately 20 min for each rat. After trials, subjects were dried with a towel and warmed under a 40 W soft white bulb (Osram, OSRAM AG, Munich, Germany) before being returned to the home cages. At the end of the fourth day, each rat had been trained 19 times totally and the acquisition period had been completed. On day 5, the platform was removed and the rats were released from the three other quadrants which did not contain the hidden platform before. At this probe trial test, the time spent in the target quadrant where the platform had been during acquisition period was recorded. The percentage of swimming in the quadrant of the former platform was calculated as a measurement of spatial memory. At day 1 and following days if the time taken was greater than 60 s, it was recorded as 70 s. On day 5, after probe trial test, visible platform procedure was applied. This is the cued version of the Morris water escape task which is used to eliminate the direct or indirect effects of the food colorings such as decreased motivational factors, increased anxiety of animals in the water and therefore decreased the desire to escape. Each rat was released from the fourth quadrant all the time and visible platform was carried to the different quadrants for each trial except the fourth quadrant. Again escape latency to the visible platform was recorded.

Statistical analysis

Statistical evaluations were conducted with the use of package software “SPSS 21.0 for Windows”. Inter day comparison of first four days were assessed by with nonparametric Friedman Test and $p<0.05$ was accepted as significant. Wilcoxon Test was applied to find out which day was responsible for the significance and $p<0.001$ was accepted as significant. Comparisons between groups were made for each day of exercise with nonparametric Kruskall-Wallis Test. Results were given as mean ± standard deviation.

Results

Morris water maze test data

Results of learning experiment data were given as "mean ± SD".

Training period data

Intra group comparison of each group showed that each group learnt the task during the training period from day 1 to day 4, and all the groups started to learn the task by the second day of training period ($p<0.005$), (Graphic 1).

When separate comparisons for training day of each control and experimental groups, there were no significant difference in terms of finding platform period, swimming speed, travelled distance between control and experimental groups ($p<0.05$), but duration time at the outer zone of TM groups was significantly higher than control group on the 3rd and 4th Day ($p=0.006$, $p=0.009$ respectively). This data meant that during the learning period anxiety level of TM group was significantly higher than control group (Table 1).

*Table 1. The evaluated data of training period of all groups in the Morris Water maze task. Explanation: AL: Ad-Libitum group; TM: Two Meal group; TM-CR: Two Meal and 20% Calorie Restriction group. *Stated statistically significant difference as compared with AL group (p<0.05).*

Day	Group	finding target platform period (sec)	Average duration in external dial (sec)	Mean travelled distance (cm)	Average speed (cm/sec)
Day 1	AL	56.81 ± 10.53	41.03 ± 10.13	1000.79 ± 245.88	18.98 ± 3.13
	TM	61.41 ± 9.75	42.18 ± 8.12	1102.21 ± 326.02	19.37 ± 5.06
	TM-CR	51.28 ± 7.24	32.54 ± 8.49	888.41 ± 206.45	19.58 ± 2.68
Day 2	AL	21.38 ± 6.77	10.91 ± 5.11	418.40 ± 151.13	19.60 ± 1.46
	TM	26.70 ± 10.84	14.76 ± 7.51	552.01 ± 206.59	19.99 ± 3.38
	TM-CR	24.93 ± 5.45	10.06 ± 3.89	511.75 ± 135.25	19.24 ± 3.39
Day 3	AL	12.63 ± 7.39	2.53 ± 3.60	230.76 ± 142.67	17.23 ± 1.54
	TM	20.18 ± 7.14	7.89 ± 4.48*	358.11 ± 123.78	17.01 ± 2.17
	TM-CR	16.97 ± 6.61	3.47 ± 2.28	323.51 ± 169.16	18.24 ± 2.53
Day 4	AL	9.55 ± 5.19	1.49 ± 1.21	157.11 ± 74.28	17.79 ± 2.41
	TM	15.33 ± 7.24	6.46 ± 5.41*	245.54 ± 98.22	16.78 ± 2.31
	TM-CR	10.45 ± 3.37	2.52 ± 1.58	176.41 ± 64.47	17.45 ± 3.64

Probe test data

There were no statistically significant difference between groups in terms of average duration in target quadrant, average swim speed and travelled distance parameters $p>0.05$) (Table 2).

Table 2. Morris water maze probe test data. Explanation: AL: Ad-Libitum group; TM: Two Meal group; TM-CR: Two Meal and 20% Calorie Restriction group.

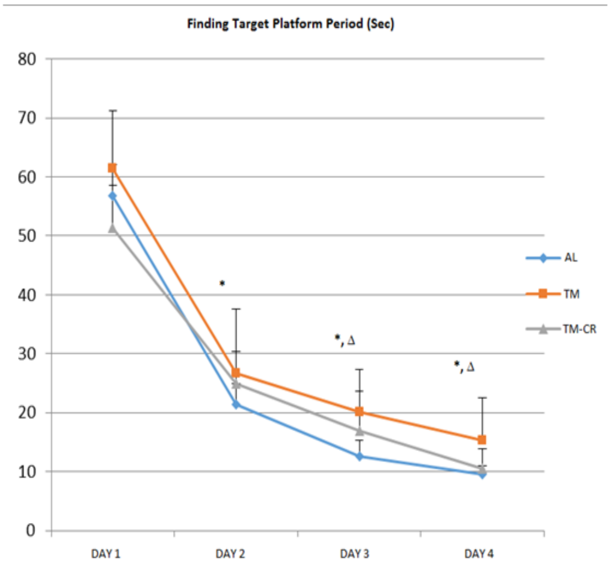
Groups	AL	TM	TM-CR
Average duration in target quadrant (sec)	15.19 ± 5.91	12.94 ± 8.79	16.33 ± 7.52
Average swim speed (sec)	19.38 ± 2.59	18.58 ± 4.26	19.79 ± 3.05
Travelled distance (cm)	1161.76 ± 155.19	1113.36 ± 255.16	1186.23 ± 182.74

Visible platform test data

In visible platform test, finding visible platform duration, average swim speed, and travelled distance data were evaluated and there was no statistically significant difference between groups ($p>0.05$) (Table 3).

Table 3. The comparisons on visible platform data of groups. Explanation: AL: Ad-Libitum group; TM: Two Meal group; TM-CR: Two Meal and 20% Calorie Restriction group.

Groups	AL	TM	TM-CR
Finding visible platform duration (sec)	4.92 ± 4.66	7.71 ± 9.36	5.42 ± 4.83
Average swim speed (sec)	20.54 ± 5.71	19.95 ± 7.07	20.46 ± 6.28
Travelled distance (cm)	94.25 ± 52.73	124.40 ± 113.90	98.55 ± 47.71



Graphic 1. *Evaluation of inter-day data of each group for the time spent to the find the target platform (sec) in the Morris water maze test during training period. Explanation: "*" expresses the statistically significant difference when compared by day 1 (p<0.05), 'Δ' expresses the statistically significant difference when compared day 2 (p<0.05). AL: Ad-Libitum group; TM: Two Meal group; TM-CR: Two Meal and 20% Calorie Restriction group.*

Discussion

Behavior is all the organisms' reactions in the face of stimulus and reflects temperament, personality and characteristic traits. Central nervous system is determinant in the behavior shaping and is influenced by nutrition as one of the environmental factors [5]. Nutrient content is a factor which affecting the behaviors in either prenatal and postnatal period or adulthood. In experimental and clinical studies, it was shown that deficiency of nutritional element such as vitamins, minerals, essential fatty acids and amino acids negatively affect neurobehaviors [6,7]. The studies which are conducted on children found that some kind of temperament determine or affect the diet [8,9]. There are also studies that investigate the effects of obesity on individual's psychology. On obese individuals, psychology is generally influenced with serious of problems such as susceptibility to depression, lack of self-esteem, being unpleasant [10]. Having a healthy body, normal body mass and accordingly normal appearance can directly influence on human psychology and behaviors. In conducted Water Maze learning experiment, when there was no significant difference between control and experimental groups in terms of finding target platform duration, floating speed, travelled distance when comparing groups with each other for each day of training period ($p<0.05$), but external dial duration of TM group was significantly higher than control group at 3rd and 4th days ($p=0.006$, $p=0.009$ respectively). When evaluating probe test and visible test results, there was no statistically significant difference between groups in terms of duration in target dial, finding target dial duration, speed and travelled distance parameters ($p>0.05$). Higher swimming duration in external dial of TM group at 3rd and 4th days meant that their anxiety level was significantly higher than control group when learning period continues. No significant difference on probe test and visible test results shows any difference between groups in terms of hippocampus-dependent spiritual memory and learning. In performed studies, it was shown that high-calorie diet interfered learning and memory in Mase test [11]. However, significant learning difference did not found. It is possible to reach significant results with increase on calorie restriction rate and nutrition regulation for long-term. There was no significant group difference in learning speed and memory evaluation tests. Thus, in TM and TM-CR groups, negative picture about learning and memory did not compose. TM and TM-CR nutrition can be presented as health nutrition having regard to no negative effect of them on metabolic benefit and learning.

Conflict of Interest

No conflict of interest was declared by the authors.

Funding

The study was supported by the Scientific Research Unit and Faculty Development Program (OYP Coordination Unit) at Suleyman Demirel University (Project Numbers: SDÜ BAP (4476-ÖYP-D2-15) and ÖYP (ÖYP 05333-DR-12) unit). (Thanks to SDÜ BAP and OYP).

References

1. Gurdol F. Bilimin Mum Isiginda Yemek; Beslenmenin Biyokimyasi. Istanbul Nobel Tip Kitabevleri 2014; 1-5.
2. Kesner RP, Hopkins RO. Mnemonic functions of the hippocampus: a comparison between animals and humans. Biol Psychol 2006; 73: 3-18.
3. Manns JR, Eichenbaum H. Evolution of declarative memory. Hippocampus 2006; 16: 795-808.
4. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Meth 1984; 11: 47-60.
5. Rosales FJ, Reznick JS, Zeisel SH. Understanding the role of nutrition in the brain and behavioral development of toddlers and preschool children: identifying and addressing methodological barriers. Nutr Neurosci 2009; 12: 190-202.
6. Fredriksson A, Schroder N, Eriksson P, Izquierdo I, Archer T. Neonatal iron exposure induces neurobehavioral dysfunctions in adult mice. Toxicol Appl Pharmacol 1999; 159: 25-30.
7. Gesch CB, Hamond SM, Hampson SE, Eves A, Crowder MJ. Influence of supplementary vitamins, mineral sand essential fatty acids on the antisocial behavior of young adult prisoners. Br J Psych Mental Sci 2002; 181: 22.
8. Wonderlich SA, Connolly KM, Stice E. Impulsivity as a risk factor for eating disorder behavior. Assessment implications with adolescents. Int J Eat Disord 2004; 36: 172-182.

9. Haycraft E, Farrow C, Meyer C, Powell F, Blissett J. Relationships between temperament and eating behaviours in young children. Appetite 2011; 56: 689-692.
10. Wardle J, Cooke L. The impact of obesity on psychological well-being. Best Pract Res Clin Endocrinol Metab 2005; 19: 421-440.
11. Xu BL, Wang R, Ma LN, Dong W, Zhao ZW, Zhang JS, Wang YL, Zhang X. Effects of Caloric Intake on Learning and Memory Function in Juvenile C57BL/6J Mice. Biomed Res Int 2015; 2015: 759803.

*Correspondence to

Hasan Basri Savas

Department of Medical Biochemistry

Faculty of Medicine

Alanya Alaaddin Keykubat University

Turkey