Effect of Different Starvation Levels on Cognitive Ability in Mice

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Abstract. Objective: To study the effect of different starvation levels on cognitive ability in mice. Method: Mice were randomly divided into four groups: normal group, dieting group A, dieting group B, dieting group C. The mice of normal group were given normal feeding amount, the rest of groups were given 3/4 of normal feeding amount, 2/4 of normal feeding amount and 1/4 of normal feeding amount. After feeding mice four days, the weight was observed and T-maze experiment, Morris water maze test, open field test and Serum Catalase activity were detected. Result: Compared with the normal group, the correct rate of the intervention group in the T-maze experiment was decreased and dieting group A > dieting group B > dieting group C. In the Morris water maze test, Compared with the normal group, the correct rate of the intervention group was increased. Among these three intervention groups, dieting group A had the highest correct rate and the difference of dieting group B and dieting group C were similar. In the open field test, Compared with the normal group, the exploration rate of the surrounding environment in the intervention group was increased. In the Serum Catalase test, Compared with the normal group, the activities of serum peroxidase in the intervention groups were decreased and dieting group A > dieting group B > dieting group C. Conclusion: A certain level of starvation could affect the cognitive ability of mice. In a certain range, the level of starvation is inversely proportional to cognitive ability in mice.

1. Introduction
With the improvement of people's quality of life, people's diet structure has undergone tremendous changes that the dietary levels have continued to increase [1]. At the same time, the phenomenon of overweight appeared [2]. More and more people begin to lose weight. An important way to lose weight is to reduce appetite and increase physical activity. Excessive diet not only affects the body's ability to exercise, causing muscle atrophy, also has a certain impact on the brain. In this experiment, we explored the different starvation levels effects on learning and memory of mice and its preliminary mechanism.

2. Research Programs
2.1. Animals
Healthy Kunming mice, weighing 20~25g, male, were provided by the experimental animal center of Henan University of Chinese Medicine.
2.2. **Experimental Materials**

2.2.1. **Experimental Reagents.** Catalase (CAT) kit, that provided by Beijing Solei Bao Technology Co., Ltd.

2.2.2. **Experimental Equipment.** T box, Morris water maze rest, mouse open field test box (50cm×50cm×50cm), microplate reader.

2.3. **Animal Handling**

Mice were randomly divided into four groups: normal group (n = 6), dieting group A (n = 8), dieting group B (n = 8) and dieting group C (n = 8). Four groups of mice were given normal feeding amount, 3/4 normal feeding amount, 2/4 normal feeding amount, 1/4 normal feeding amount respectively for four days to prepare different degrees of starvation mice.

2.4. **Observations**

2.4.1. **Weighing the Quality of Mice.** The plastic plate was placed on the electronic scales, peeled, each mouse was placed in a plastic tray for weighing, and recorded.

2.4.2. **T-maze Experiment.** Put 30 mice into the T-box trunk arm in turn at the beginning, and opened their two arms. Let them be free for 10 minutes and familiar with the environment. Then put them back into the cage. The mice were placed alone in the beginning of the trunk of the T-box. Only made the left arm open. Placed a food pellet at the end of the left arm as a reward to direct the mice into the left arm, forcing the mice to select food and completing food intake. Then the mice were placed into the beginning of the trunk of the T-box once again. Only made the right arm open. Placed a food pellet at the end of the right arm as a reward to direct the mice into the right arm, forcing the mice to select food and completing food intake. Forced trials were conducted 4 times a day for 15 minutes each interval and the experiment lasted for four days. The final test was carried out on the fifth day. Firstly, put the mice into the T-maze to familiar the environment 10 min. Then the mice were placed in the main arm of the maze. It was correct for the mice to alternate into the left and right arms in turn, otherwise it was wrong. Record whether the mice were correct or not. In addition, the amount of food required was included in the daily intake of mice.

2.4.3. **Morris Water Maze Test.** Cleaned up the tool in the water maze and added water to submerge the platform 1cm. Keep the water temperature 18 to 22 degrees Celsius. Four equidistant markers were attached to the wall of the water maze. Each mouse was placed in water from four labeled direction in turn. Let the mouse find the platform in 60s. If the mouse find the platform, let the mouse stay on the platform for 10s to experience the feeling of standing on the platform. If the mouse did not find the platform within 60s, used the wood sticks guide to help the mouse find the platform. Then also let the mice stay on the platform for 10s. To ensure that each mouse had equal time to observe and obtain spatial information after each experiment. If mice stayed on the platform more than two seconds, we defaulted the mice had found the platform. If the mice fell off the platform or dived into the water and continued swimming, the mice were placed on the platform again and re-timing after thirty minutes. After training in all four directions, the mice were wiped dry and returned to the cage. The experimental data was recorded. The average of daily latency of four training sessions was used as the academic record on the day of the experiment. The experiment lasted 4 days, scheduled for a fixed period of time training 4 times a day.

2.4.4. **Open Field Test.** Placed the mice facing the wall into a fixed corner square and allow them to freely explore the environment for 5 min. Two experimental staffed, sitting in two corners of the wooden box respectively. One of them was in charge of the timing and the other was in charge of hidden time in the first grid and leg–raising times (legs off the ground). Finally, observed the fecal granules. After each mouse test, wiped the area with a damp cloth and wipe dry. Recorded hidden time...
in the first grid, leg-raising times (legs off the ground) and fecal granules.

2.4.5. Serum Peroxidase Activity Test. Blood samples were drawn from orbital vein. Mouse serum was separated. With the enzyme-labelling measuring instrument measured the initial absorbance A1 and measured absorbance A2 again after 1 minute. The activity of serum peroxidase was calculated according to the catalase kit instructions.

2.5. Statistical Methods
Data are presented as the mean ± SD. Differences were evaluated using Statistical Package for Social Science 19.0 (SPSS11.0, Chicago, IL, USA). Statistical analysis was performed using One-way ANOVA followed by least-significant difference (LSD). P< 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of Different Degree of Starvation on T-Maze Experiment of Mice
Compared with the normal group, T-maze experiment correct rate in the dieting group A, dieting group B, dieting group C was significantly reduced. Compared with the dieting group B, the T-maze experiment correct rate in dieting group A was increased, The T-maze experiment correct rate in dieting group C was decreased.

| group            | n  | Correct rate (%) |
|------------------|----|------------------|
| normal group     | 6  | 54.40            |
| dieting group A  | 8  | 50.00            |
| dieting group B  | 8  | 45.80            |
| dieting group C  | 8  | 16.70            |

Table 1. Effect of different extent of starvation on T-maze experiment of mice

3.2. Effect of Different Degree of Starvation on Morris Water Maze Test
Compared with the normal group, the percentage of found the platform within 1min in dieting group A
was increased, the percentage of found the platform within 1min in dieting group B and C was decreased.

Table 2. Effect of different degree of starvation on morris water maze test of mice

| group            | n  | Percentage of having found platform (%) |
|------------------|----|----------------------------------------|
| normal group     | 6  | 50.00                                  |
| dieting group A  | 8  | 75.00                                  |
| dieting group B  | 8  | 62.50                                  |
| dieting group C  | 8  | 62.50                                  |

Figure 2. Effect of different extent of starvation on Morris water maze test.

3.3. Effect of Different Degree of Starvation on Open Field Test of Mice

From table 3, Compared with the normal group, the vertical score of dieting group A was significantly increased (P<0.05). The vertical score of dieting group B and dieting group C was observably increased (P<0.01). Compared with the normal group, the hidden time in the first grid of dieting group A and dieting group C was increased (P<0.05). The hidden time in the first grid of dieting group B was increased (P<0.01)

Table 3. Effect of different extent of starvation on open field test (x±s)

| group            | n  | hidden time in the first grid (s) | vertical score  |
|------------------|----|----------------------------------|-----------------|
| normal group     | 6  | 2.98±0.17                        | 16.75±2.75      |
| dieting group A  | 8  | 4.67±0.13"                       | 31.25±5.12"     |
| dieting group B  | 8  | 7.99±2.12""                      | 37.20±5.40""    |
| dieting group C  | 8  | 4.24±1.31""                      | 55.20±8.87""    |

Note: Compared with the normal group #P<0.05, ##P<0.01
3.4. Effect of Different Extent of Starvation on Serum Catalase Activity

Compared with the normal group, serum catalase activity in the dieting group A, dieting group B and dieting group C was significantly reduced (P<0.05). The highest serum catalase activity was normal group. The serum catalase activity of dieting group A, dieting group B and dieting group C were descending in sequence.
Table 4. Effect of different extent of starvation on serum catalase activity (x±s)

| group                | n  | serum catalase activity(U/ml) |
|----------------------|----|------------------------------|
| normal group         | 6  | 19.26±3.81                   |
| dieting group A      | 8  | 13.64±3.53<sup>”</sup>        |
| dieting group B      | 8  | 9.88±2.65<sup>”</sup>         |
| dieting group C      | 8  | 7.78±1.56<sup>”</sup>         |

Note: Compared with the normal group, #P<0.05, # #P<0.01.

Figure 5. Effect of different extent of starvation on serum catalase activity

4. Discussion

4.1. T-maze Experiment Analysis and Discussion
T-maze experiment is one of the most commonly experiments to evaluate spatial memory ability. It is a classic behavioral method to detect spatial memory ability of rodents. We always use the model to research animal spatial memory ability. The correct rate of normal group, dieting group A, dieting group B and dieting group C in the experiment were significantly decreased one by one. In the T-maze experiment, the correct rate of the mice was decreased with the feed amount reducing. To a certain extent, it reflected that food intake influenced mice cognitive ability. The relationship between them was possibly positive correlation.

4.2. MorrisWater Maze Test Analysis and Discussion
Rodents are naturally good at swimming and hating underwater environment. Using the characteristic as driving force, the test forced mice to escape from the water and find the platform. After multiple training, mice learned to use the relationship between hidden platform and environmental markers to judge the location of the platform and form stable spatial awareness. In the test, the percent of finding the platform within one minute was gradually decreased. The order was dieting group A, dieting group B and dieting group C. The reason why the percent of normal group was lower than the others was that the number of normal group was too limited. Compared with the other three groups, the number of normal group is lower. Besides, the individual differences influenced the outcome to a large extent.
This experiment shows that over-starvation could lead to cognitive deficiency in mice.

4.3. Analysis and Discussion of Open Field Test
The open field test can reflect the autonomous activity, anxiety, memory and activity for exploring the environment of mice. Thus, the open field test reflected the excitability of central nervous system in mice. If the central nervous system of mice is excited, the exploring ability of mice will increased on environment. On the contrary, if the central nervous system of mice is inhibited, the exploring ability of mice is decreased on environment. The results shown that compared with the three dieting groups, both vertical cores and hidden time in the first grid in normal group was decreased. The results indicated that the more extent of starvation, the more anxiety of mice.

4.4. Analysis and Discussion of Serum Catalase Activity in Mice
Serum Catalase (CAT) has the function of clearing free radical and reducing damage. A large number of free radical damage can lead to metabolic disorders of nerve cells, degeneration and necrosis .Finally free radical can lead to cognitive dysfunction. CAT can scavenge free radicals. The results indicated that mice food-intake and measured average serum catalase activity was positive correlation. It shows that dieting reduced cognitive ability in mice. This experiment indicated that compared with the normal group, serum catalase activity in the three dieting groups was decreased. In dieting group A, dieting group B and dieting group C, the serum catalase activity was decreased in turn. Thus it can be inferred that the normal group was the highest in cognitive ability. With the decrease of food-intake, dieting group A, dieting group B and dieting group C, the serum catalase activity reduced in turn. This experiment shows that over-starvation could lead to cognitive deficiency in mice.

With the development of people’s living standard, people’s dietary level is gradually improving and fat is appearing. Then losing weight becomes fashionable. However, irrational dieting can cause damages of body. At present, study mostly focuses on the muscle movement ability rather than cognitive ability of brain[3]. This experiment was related in the social hot issues. The experimental results indicated that a certain degree of starvation affects the cognitive ability.

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6. References and notes
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