Study on cognitive impairment in diabetic rats by different behavioral experiments

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Abstract: Object recognition test and Y maze test are widely used in learning and memory behavior evaluation techniques and methods. It was found that in the new object recognition experiment, the diabetic rats did more slowly than the normal rats in the discrimination of the old and new objects, and the learning and memory of the rats in the diabetic rats were injured. And the ratio of retention time and the number of errors in the Y maze test was much higher than that in the blank control group. These two methods can reflect the cognitive impairment in diabetic rats.

1. Introduction
Learning is a process of obtaining information about the outside world, and memory is the process of processing, storing and reproducing this information. In this paper, we established diabetes mellitus model and normal rats for different behavioral experiments to obtain cognitive impairment in diabetic rats. In 1950, Berlyne reported that rats had a preference for the natural properties of novel objects [1-2], whereas in 1988 Ennaceur and Delacour used this nature of rattan like novelty, tested and then reported a new method, the Object Recognition Task (OTR), is used to detect behavioral detection methods for rat memory [3-4]. The new object recognition experiment is a new type of one-time learning experiment mode, which can be used to study the two aspects of the acquisition and recovery of memory.

The new object recognition experiment is a widely used learning and memory behavior evaluation technique and method. And Y maze is widely used in the international classic learning and memory behavior evaluation techniques and methods [5-6]. The new object recognition experiment is a kind of fine and sensitive behavior detection method. It mainly uses the principle that rats are born close to and explore the characteristics of novel objects. [7].

Y maze experiment is used to study the ability of rodents in the recognition and memory ability, Y maze is the use of rodents on the natural environment of natural exploration of natural new nature, do not need to learn any rules to avoid disadvantages, can be effective Reflects the animal's ability to recognize and remember the new environment [8].

1.1 Progress and type of diabetes
As lifestyle changes, diabetes and its complications have become a major cause of morbidity and mortality. We need to reasonably predict the incidence of diabetes and its complications, with the
aging of the population, adult obesity rates, and other risk factors\textsuperscript{[9]}. Diabetes is a systemic disease that can destroy any organ of the body has been widely concerned in recent years. \textsuperscript{[10]}. When the insulin secretion is insufficient, can lead to elevated blood sugar. Long-term chronic hyperglycemia can damage the human body, such as capillary basement membrane thickening, so that the lumen narrowing, diabetes, lipid metabolism disorders, will increase blood viscosity, blood flow can cause cerebral blood Flow reduction. Studies have also shown that the brain's blood flow reduction can make the brain a series of information on the process of obstacles, cognitive and processing capacity of memory and learning ability are reduced, and ultimately lead to learning and memory impairment. In addition, high blood sugar can also make senile dementia rapid onset, studies have shown that in elderly patients with diabetes, the possibility of dementia than the normal control group increased by about 2 times, of which type 2 diabetes in the relationship between senile dementia more closely. These studies have found that cognitive impairment in diabetes reflects the process of accelerated decay in many ways\textsuperscript{[11-12]}.

1.2 Animal behavior methods
Y maze is considered to be one of the closest to the primate spatial delay response task model, often using delayed sample matching - non-matching task paradigm to test the animal's learning and memory performance, Y maze is a resolution of the initiative to avoid learning, So the Y maze experimental model commonly used in drug screening.

Y maze experiment This food species reward mechanism maze task can detect diabetic rats in the hippocampus or prefrontal brain area associated with spatial working memory and spatial reference memory. When the rats find food in the Y maze, the animals need to follow the maze around the graphics and color, remember it has been looking for the labyrinth arm, so as not to repeat into the same labyrinth arm, so fast access to food, this memory can guide The ongoing act, known as working memory. It is common to use the Y maze experiment to study the animal's working memory, which is useful information for determining the animal's useful information only during the current operation, that is, any day of the experiment.

Y maze experiment is mainly used in animal identification line learning, working memory and reference memory test, it is composed of three identical arms, each arm at the end of the food supply device, according to the analysis of rats feeding strategy The number of times, time, correct number of times, number of errors and so on can reflect the spatial memory ability and learning ability of experimental animals. Compared to other terms, Y maze experiment has a certain degree of practicality, is now used to study memory function evaluation.

1.3 The purpose and significance of this study
Animal behavior on animal learning ability and cognitive ability and other aspects of research, and the relevance of neuroscience. In this study, the cognitive impairment of diabetic rats was evaluated by the new object experiment and Y maze test. The difference between learning ability and memory ability of diabetic rats was evaluated. So the degree of cognitive impairment in diabetic rats can be determined by different behavioral studies. Therefore, it is of great significance to study the different behavioral methods to determine the cognitive impairment in diabetic rats. But also through these two different behavioral methods to evaluate the cognitive ability of diabetic rats.

2. experimental part
2.1 Experimental content and methods
2.1.1 Grouping of animals. The rats were fed with conventional feed and free to drink. After the rats were fed for a period of time. (Control, n = 10) and model group (model, n = 50) were randomly divided into blank group.
2.1.2 Replication of the diabetes model.
Male Wistar rats 50, fasting without water 16h, with ice bath of sodium citrate - sodium citrate buffer STZ powder dissolved, the above experiment in the dark operation, and then freshly set 1% of the STZ solution for abdominal cavity injection. The model group was injected intraperitoneally according to the dose of 55mg.kg-1, and the injection was carried out within 10min to avoid the failure of STZ solution. The control group was injected intraperitoneally with the same dose of citric acid-sodium citrate buffer. After 72 hours of injection, blood glucose was measured and rats with fasting blood glucose $\geq 16.7$ mmol / L were selected for the next experiment.

2.1.3 General status and metabolic statistics of animals.
In the course of the experiment every day to observe the general state of the rats and recorded every 30 days using metabolic cages to determine the rat's drinking water, food intake, fecal volume and urine output, each rat 19:00 given a given amount Food and water, the next day at 7:00 am to measure the remaining amount, the difference between the two is every 12h of each diet, water, and then through the metabolic cage weighing rats fecal weight and urine volume.

2.1.4 Dynamic determination of fasting blood glucose in rats.
Before performing the experiment, replace the litter, the rats fast for 16h, let the free drinking water. Rats were anesthetized with ether and 0.5 ml of blood from the fundus venous plexus, with heparin anticoagulation, centrifugation (3000rpm / min, 10min), separation of plasma to be measured. The supernatant was taken and the glucose was measured by glucose oxidation. The specific operation is carried out according to the kit instructions. Glucose Oxidase Assay Kit Detection Principle: Glucose oxidase in the role of glucose in the production of hydrogen peroxide, hydrogen peroxide decomposition of oxygen by the free radicals of oxygen, it will be phenol and 4-amino Bioline is red-based quinoid compounds, and absorbance values are measured at a wavelength of 510 nm.

2.1.5 Detection of Fasting Blood Glucose (FPG) in Diabetic Rats.
STG intraperitoneal injection 72h after the determination of FPG, the model group a total of 20 rats, of which blood glucose higher than 16.7 mmol.l-1 of 16, can be the next experiment, the remaining rats, of which 2 rats died And 2 rats blood glucose is not up to standard. During the course of the experiment, FPG was monitored every 30 days, see Table 3-2. Compared with the blank group, the blood glucose of the model group was significantly increased at 30d, 60d and 75d (P <0.01).

2.2 Experimental results

2.2.1 Experimental results of fasting blood glucose (FPG) in diabetic rats

| Table 1 Diabetic rats FPG dynamic changes (mmol / l, n = 10,) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group | 0d | 30d | 60d | 75d |
| Blank group | 4.78±0.77 | 4.94±0.91 | 4.71±0.91 | 4.95±0.60 |
| Model group | 21.13±3.71** | 22.78±3.57** | 22.34±2.73** | 22.56±5.89** |

Compared with the blank group, * P <0.05 was significantly different, ** P <0.01, there was a very significant difference. From the table can be seen, the model group of blood glucose is high and blank group and 0 ~ 75d blood glucose stability. Indicating that the model is stable.

2.2.2 Y maze experiment results
Experiments from 0 to 75 days to test Y maze test results are as follows.
Table 2 Y maze test results data

| Group          | Retention time ratio(%) | The number of errors | The number of passes |
|----------------|-------------------------|----------------------|---------------------|
| Model group (n=25) | 68.46±9.76*            | 12.69±1.95*         | 9.37±3.17          |
| Blank group (n=10)   | 35.30±8.17             | 7.00±3.00           | 10.60±2.88         |

Compared with the blank group, * P <0.05 was significantly different, ** P <0.01, there was a very significant difference. In the Y maze experiment, it can be seen from the data, diabetic rats in the Y maze of the number of errors increased significantly.

2.2.3 Experimental results of new object recognition experiments. The new object contact time used in this article refers to the time the rat used to reach new foreign bodies. And the number of physical contact refers to the head of the rat, especially the nose and mouth of the familiar objects and new objects of behavioral activities, these behavioral activities, including rats on the object sniffing, licking or tentacles, etc., when the rats sit Familiar or new objects on the nose and mouth does not directly point to the familiar or new objects do not think it is the number of exploration of the experimental object.

Table 3 Preliminary data on new object recognition experiments

| Group       | Animal number | New object contact time (s) | New object contact times | Old object contact time (s) | Old object contact times |
|-------------|---------------|-----------------------------|--------------------------|----------------------------|--------------------------|
| Diabetes    | 1             | 39.51                       | 5                        | 149.6                      | 5                        |
|             | 2             | 50.31                       | 1                        | 111.8                      | 1                        |
|             | 3             | 60.32                       | 1                        | 152.999                    | 3                        |
|             | 4             | 50.199                      | 3                        | 139.302                    | 3                        |
|             | 5             | 56.001                      | 3                        | 122.003                    | 9                        |
|             | 6             | 134.599                     | 8                        | 69.501                     | 4                        |
| Blank       | 1             | 41.198                      | 3                        | 102.701                    | 4                        |
|             | 2             | 146.199                     | 2                        | 66.196                     | 11                       |
|             | 3             | 81.8                        | 1                        | 160.603                    | 1                        |
|             | 4             | 158.704                     | 2                        | 77.904                     | 1                        |
|             | 5             | 120.32                      | 3                        | 87.32                      | 3                        |
|             | 6             | 50.32                       | 2                        | 70.21                      | 5                        |

Table 3 above experimental data through the application of SPSS 21.0 For Windows statistical software analysis of variance data after data processing Table 4

Table 4 New object recognition experiment experimental data results

| Group    | New object contact time | New object contact times | Old object contact time | Old object contact times |
|----------|-------------------------|--------------------------|-------------------------|--------------------------|
| Diabetes | 79.82±33.21*            | 3.67±2.53                | 111.24±2.19*            | 3.42±2.19                |
| Blank    | 94.81±34.98             | 2.67±1.30                | 91.38±32.05             | 3.42±2.81                |

Compared with the blank group, * P <0.05 was significantly different, ** P <0.01. There is a very significant difference. It can be seen from the experimental data of the new object. Compared with the blank group, the contact time of the diabetic group changed with the old and the new body, but the contact number of the old and the old body did not change much.

2.2.4 Discussion.
In this paper, the object recognition (Object recognition) task is a lot of memory types, rodents can distinguish between different familiar objects of new objects used in this article the ability of non-spatial memory of rats, rat Able to distinguish between familiar with the previous position of the object and the current location, this device is also used to detect the spatial memory of rats, rats can
identify the background of the object information. In recent years, many studies in the use of these methods at the same time continue to improve, and some researchers on its evaluation indicators, statistical methods were studied. Some scholars have studied the factors that may affect the experiment, such as animal species, sex, age and so on. Provide a valuable reference for this method. The establishment of automated object recognition can guarantee the objectivity and reliability of the method.

3. Conclusion
In this study, the number of errors in diabetic math rats was significantly increased by 75 days after establishment of the diabetic rat model by the STZ method, using the Y maze experiment and the new object recognition experiment. In the new object recognition experiment, the diabetic rats in the exploration of the old objects, the exploration time is also significantly increased, there are significant differences. Therefore, Y maze experiment and new object recognition experiment can reflect the difference of cognitive impairment in diabetic rats.

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