Evaluation-based Stereological Study of Oligodendrocytes in Hippocampus of Depressive Rats

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Abstract: Purpose:To investigate the changes of CNPase + oligodendrocyte in hippocampal subareas of chronic unpredicTable stress depression model rats. Methods: Four to six weeks male rats were randomly divided into 15 control groups and 20 rats used to establish depression model. The depression rat model was established by CUS combined with orphan model for four weeks. Ten adult rats were selected by sugarwater preference test. At the same time, the behavioral level of control group and CUS model group was evaluated by forced swimming and open field experiment. Accurate three-dimensional quantitative study of the number of CNPase+ oligodendrocytes in rat hippocampus was performed by immunohistochemistry combined with modern stereology. Results :The results showed that the body weight, percentage of syrup and water, and open field test scores of the CUS model group were significantly lower than those of the control group, and the forced swimming time was significantly increased ($p<0.05$, $p<0.05$, $p<0.05$, $p< 0.05$). Conclusion: The total number of synapses and synaptic density in hippocampus DG of depression model rats are decreased, which indicates that oligodendrocyte changes may be one of the important neural structural bases for depression onset.

1. Introduction

Depression is a mental disease that seriously endangers human health. Its core symptoms are depression and loss of pleasure, and it has become one of the ten recognized causes of human death or disability in the world [1]. With the acceleration of urbanization and modernization in our country, our population will face fast pace, high competition, high congestion and widening gap between rich and poor, so the stress in life will increase, the incidence of depression will be higher and higher, and the social burden of depression will be greater and greater [2]. However, the current clinical first-line antidepressants can improve the symptoms of depression patients, but they are only effective for some patients, with slow onset, high recurrence rate and long-term treatment [3]. Therefore, it is of great significance to explore the pathogenesis of depression and explore new targets for the treatment of depression in order to improve the disease state and quality of life of patients with depression. Neuroplasticity changes including structural changes and functional damage occur in some brain regions of the limbic system of patients with depression. The antidepressant effect of existing antidepressants such as fluoxetine can be realized by increasing the survival and number of hippocampal newborn neurons and improving nerve regeneration [4]. This study will explore the changes of oligodendrocyte number in the hippocampus of depressive rats, in order to further understand the pathological changes of depression, and provide important scientific basis for finding structural targets and entry points for the treatment of depression in the future.

2. Materials and Methods

2.1 Laboratory animals and groups

Male SD rats were provided by the Animal Experimental Center. Adjust the sucrose baseline of the experimental animals after 4 weeks in a cage and provide enough food and water for 2 weeks at room temperature (22 ± 1) ℃ in a day and night alternate environment. Then, 8 experimental

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animals with unqualified (such as taste loss, dyskinesia, etc.) were removed by the sugar water preference test and the open field test. The remaining rats were randomly divided into the control group of 15 and the model group of 20 rats. Then the unqualified experimental animals were eliminated by open field test and sugar preference test, and the remaining rats were randomly divided into control group (15 rats) and model group (20 rats). Animal feeding and treatment shall follow the instructions of laboratory animal protection and use.

2.2 Behavioral experiment

Sugar water preference test. This test is the gold standard to evaluate the degree of animal pleasure deficiency. During the experiment, each rat was raised in a single cage and given sufficient food. The test was conducted once every 6 days and the sugar preference percentage of each group was recorded. In order to prevent animals from adapting, we use random arrangement of stress intensity and types to achieve the goal of modeling. This experiment adopts a combination of solitary rearing model and chronic unpredicTable model. Open field experiment. This experiment is to evaluate the anxiety level and activity ability of rats. The test was conducted twice in total, before the start of stress and after the end of stress at week 4. The rats were placed in the center of the square field test box, and the number of vertical and horizontal activities of the rats in the open field test chamber within 5 minutes was recorded by a computer software monitoring system. Forced swimming. This experiment was used to assess animal anxiety and despair behavior. The animals were placed in a cylindrical transparent organic container having a diameter of 20 cm and a height of 50 cm at a water temperature of (25 ± 2) ℃ and a water depth of 40 cm. After 24 hours, the formal forced swimming test was started. Each rat was tested for 5 minutes, and the immobility time was recorded. After each test, the next rat was tested with clean water. The experiment was conducted at the end of the fourth week of stress.

2.3 Specimen fixation and sampling

Five animals in each group were randomly selected and anesthetized by intraperitoneal injection of 1.0% sodium pentobarbital (0.3ml/100g). The thoracic cavity was opened quickly to expose the heart. The needle was inserted from the apex of the left ventricle. The needle was fixed with hemostatic forceps. The right ear was cut open and the perfusion pump was opened for perfusion. After the liver was lightened, the perfusion of about 85ml saline was continued with 4% polyformaldehyde until the neck was stiff. Synaptic photographs were analyzed to measure the length of the active region, the thickness of dense substance in the presynaptic and postsynaptic membranes, and the width of synaptic gap. Open the cranial cavity, remove the skull and remove the complete brain tissue. The brain was divided into left and right hemispheres along the cerebral fissure. One hemisphere was randomly selected and fixed with 4% paraformaldehyde for 24 h.

2.4 Section preparation and immunohistochemical staining

The fixed brain tissue was taken out and placed in a sucrose solution configured with PBS for dehydration treatment for 3 days, and the concentrations of the solutions were 10%, 20%, and 30%, respectively. Brain tissue was embedded in OCT, and 60 μm serial sections were made on a cryostat, and all sections were floated in 0.01 M PBS solution. The sections were washed with 0.01 M PBS for 5 min x 3 times at room temperature (21-23 ℃) and gently shaken. The slice was placed in a citrate solution for 30 min in a hot water bath, and the temperature was controlled at 95 ℃ to 98 ℃. Finally, the slides were placed in a blocking solution (10% goat serum SP-9002, 1% fetal bovine serum, 0.3 ml Triton X100) in a 37 ℃ water bath for 2 hours. Rinse with 0.3% Tween-20 for 5 min ×12 times and incubate overnight at 4℃ with 1: 200 immune colloidal gold reagents. Incubate three antibodies (SP-9002 C horseradish enzyme labeled streptavidin) in a 37℃ water bath for 2 hours, and wash the slices with 0.01MPBS for 15min×4 times. DAB color rendering (light shielding), color rendering time according to the degree of each film, about 30 seconds to 1 minute. Finally, it was re-dyed with hematoxylin, dehydrated and sealed.

2.5 Stereological analysis

After the above staining steps, the final staining thickness of the sections was about 20-35 um. Under the stereological equipment, CNPase + cells were counted by professional stereological software. The boundaries of CA1, CA3 and DG in each subarea of hippocampus of rats were delineated under 4-fold objective lens (Fig. 1). The counting stereo frame is set at 80%, the sampling area is set at 8%, the height of the stereo frame is 17 m, and there is a protective area of 3 m. The system randomly selected a point as the starting point of sampling. About 12 to 30 visual fields were selected in each area of each slice, and about 400 visual fields were selected in each subarea of hippocampus of each animal. When the field of vision under the mirror is clearly focused for the first time, it is the upper surface, set to 0; When the slice is clearly focused on the lower surface for the last time, the scale displayed on the Z axis is the thickness of the slice. Count under a 100-fold oil mirror (N.A.1.40) (Fig. 2). Stereological software will automatically record and count the number of CNPase+ cells in each subarea of hippocampus marked by each slice. The counting process of CNPase+ cells follows the forbidden line rule of optical stereoscope.

The number of CNPase+ cells is calculated by optical fractionation, and the formula is as follows:

$$N = \sum Q^{-} \times 1/ssf \times 1/asf \times 1/stsf$$

Where ∑Q- represents the total number of CNPase+ cells counted per rat, ssf represents the slice sample score, asf represents the area sample score, and stsf represents the height sample score.

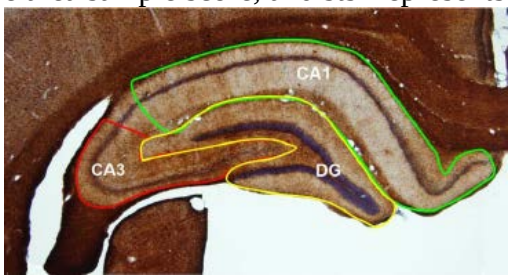

Fig.1. Division of CA1, CA3 and DG areas in the hippocampus

According to the counting principle of the optical prompt frame, the number of CNPase+ cells falling in the stereoscopic frame was counted under a 100-fold oil mirror. The height of the stereo frame is 17 μm and the height of the protected area is 3 μm m. The arrow refers to CNPase+ cells.

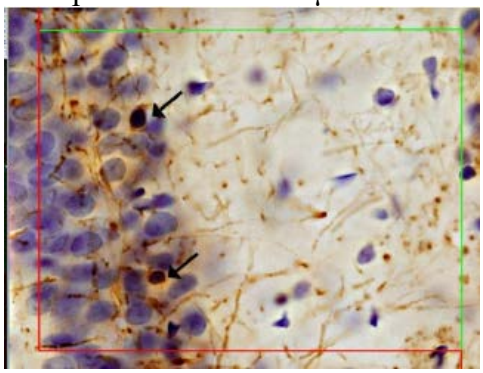

Fig.2. Determination of the total number of CNPase+ cells in hippocampus

2.6 statistical treatment

The experimental data were expressed as mean ± standard deviation (± s), and statistical analysis was performed using SPSS 19.0 statistical software. After statistical software analysis, the data all conform to normal distribution and meet the homogeneity of variance. Therefore, independent sample T test is used to compare the data between the two groups. Variance analysis of repeated measurement data was conducted on body mass and sugar water data. Test level α=0.05.

3. Result

3.1 Body mass

There was no difference in body mass between the control group and the model group before the experiment began. By the end of the 4th week stress, the body mass of CUS model group was significantly lower than that of the control group, and the difference was statistically significant (grouping principal effect F=25.159, P = 0.000; Time main effect F=141.397, p=0.000; grouping * time interaction F=51.805, p=0.000) (Fig. 3).

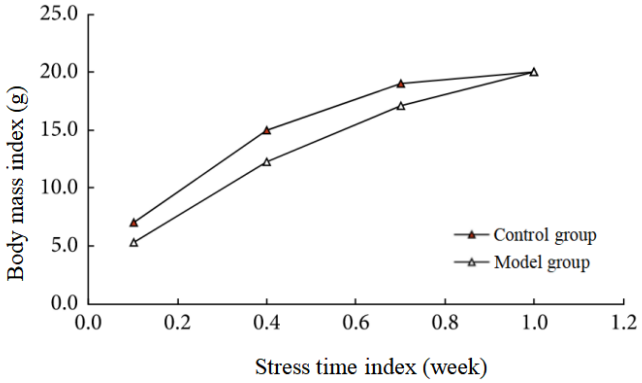

Fig.3. Comparison of body mass changes between control group and CUS group

3.2 Sugar water preference experiment

Before starting stress, the baseline of sugar water was adjusted for one week. After random grouping, there was no difference in sugar water preference percentage between the control group and CUS model group. In the third week of stress, the percentage of sugar preference in CUS model group was significantly lower than that in control group. By week 4, the percentage of sucrose preference in the CUS model group was significantly lower than that in the control group (group main effect F = 15.20, P = 0.001; time main effect F = 2.682, P = 0.38; group × time main effect F = 3.171) , P = 0.19) (see Figure 4).

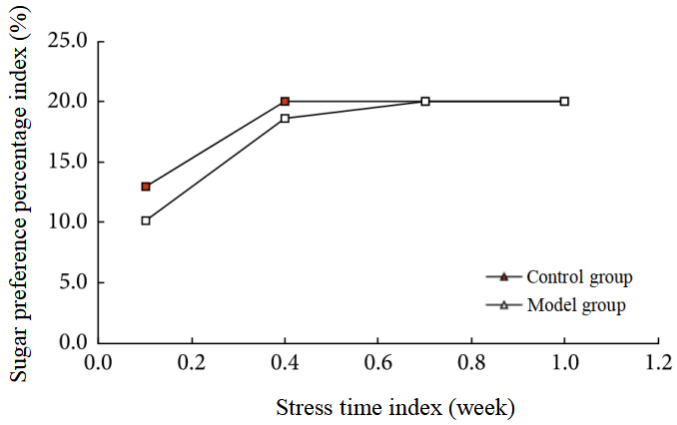

Fig.4. Comparison of percentage changes of sugar preference between control group and model group

3.3 Open field experiment

Before stress, there was no difference in the total score of open field test between control group and model group. The total score was divided into the sum of horizontal score and vertical score of each animal. However, the central area distance, central area time ratio and central area distance ratio in CUS group were significantly lower than those in control group. Four weeks after stress, the total score of open-field test in CUS model group was significantly lower than that in control group (t = 4.305, P = 0.000).

3.4 Forced swimming

After four weeks of stress, the forced swimming time of the CUS model group was significantly longer than that of the control group (t=-2.924, p=0.009).

3.5 Stereological results

The following figure shows the immunohistochemical staining in each area of hippocampus. As can be seen from the figure, the cells in each area of the control group are closely arranged, with clear structure and smooth and complete cell edges, while the cells in the model group are loosely arranged, with different shapes and unclear cell edge contours. Moreover, the number and density of CNPase + oligodendrocyte decreased compared with the control group (Fig. 5).

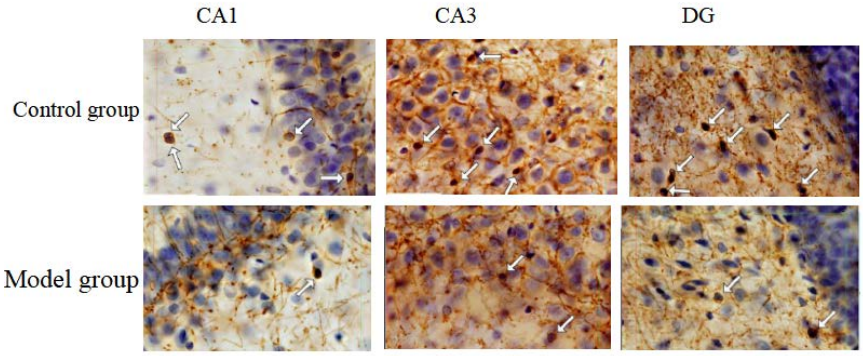

Fig.5. The staining of CA1, CA3 and DG CNPase + cells in hippocampus of the two groups was observed under 100-fold oil microscopy. The arrow zh indicates CNPase + cells

4. Discussion

When people are facing unexpected accidents or natural disasters, the most classical stress response of the brain is activation of the autonomic nervous system and HPA axis. However, the above conditions, the secretion of too low or too high adrenal hormones, and the over-activation of the autonomic nervous system will all affect health [5]. The animal's loss of pleasure is manifested by a reduction in the ability to respond to reward behavior. Behavioral evidence is that rats' intake of palaTable sugar water is reduced, and they are even unwilling to drink water. However, when a person is in such a “chronic stress” environment for a long time, he will have depression, depression, despair and even death, and gradually develop into depression [6]. Therefore, among many depression models, we chose the chronic unpredicTable stress model [7]. In this experiment, the percentage of preference for sugar and water in some SD rats after chronic stress intervention was significantly lower than that in the control group, suggesting that these SD rats had typical depressive symptoms of pleasure loss and were successful CUS models. In this experiment, the growth rate of body mass of CUS model rats was significantly lower than that of control group. At the same time, this study found that the total score of open-field test of CUS model rats was significantly lower than that of control group. The body mass of rats in CUS group decreased significantly, which was consistent with the clinical manifestations of depression patients. The above behavioral experiments show that the CUS model is successful and can well simulate the core symptoms of human depression.

We used unbiased stereological method to study quantitatively the changes of CNPase+ cells in each subarea of hippocampus in CUS model group. The results showed that CNPase+ cells in CA1 region of hippocampus in CUS model group had no significant changes compared with control group, while CNPase+ cells in CA3 and DG regions were significantly decreased compared with control group. However, the brain is a three-dimensional structure. Different sections, different parts and even different sampling fields may lead to inaccurate results. We cannot confirm changes in quantity simply from changes in density. In this experiment, a new unbiased stereological method was used to measure the total volume atrophy of DG in CUS model rats with depression after 4 weeks of stress [8]. The results showed that CNPase+ cells in CA1 area had no significant changes

compared with the control group, while CNPase+ cells in CA3 and DG areas were significantly lower than the control group [9]. Therefore, this result suggests that chronic unpredicTable stress failed to reduce the total amount of mature oligodendrocytes in the CA1 region of rats, but the oligodendrocytes in the CA3 and DG regions were significantly lower than the control group. The subject of this study was a depression CUS model rat, which mimicked early changes in depression, at which time the DG volume may not have changed. Therefore, this result suggests that chronic unpredicTable stress failed to reduce the total amount of mature oligodendrocytes in the CA1 region of rats, but the total amount of oligodendrocytes in CA3 and DG was significantly lower than that in the control group. Therefore, we speculate that ultrastructural changes such as synapses in the hippocampus of depression may be more important than the shrinkage of hippocampal volume [10].

5. Conclusion

In summary, the chronic unpredicTable stress depression model provides a good basis for animal research. The use of unbiased stereology combined with immunohistochemical techniques can more accurately understand oligodendrocytes in CUS depression model rats. The change in the hippocampus. CUS model rats have a feeling of pleasure, depression and other depression-like symptoms, and running exercise can effectively improve the above symptoms of CUS depression model rats. The results showed that DG synapses decreased and synaptic density decreased in the hippocampus of rats, but the volume of DG did not atrophy significantly, suggesting that DG synapses decreased in the hippocampus of rats may be an important neurostructural basis for depression. The results showed that oligodendrocyte was involved in the pathogenesis of depression, and the oligodendrocyte in hippocampal CA3 and DG was more susceptible to chronic stress. This suggests that oligodendrocyte may be one of the important structural bases for the treatment of depression. The results of this experiment provide morphological basis for further exploring the mechanism of anti-depression.

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