

# Long-term or short-term corticosterone treatment suppresses adult hippocampal neurogenesis leading to anxiety-like behaviors and cognitive deficits by activating autophagy and depleting Brain-derived neurotrophic factor (BDNF)

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**Abstract.** Autophagy is regarded as a self-protective procedure of cells, however, it can also cause the death of cells under certain conditions and thereby cause the death of neural stem cells and lead to cognitive deficits. Previous studies hold different opinions of the way autophagy leads to cognitive deficits. One opinion is that corticosterone directly (CORT) directly suppresses AHN and lead to anxiety-like behaviors and cognitive deficits, another opinion is that CORT treatment causes the depletion of BDNF, and thereby lead to anxiety-like behavior and cognitive deficits. In our study, we conducted several behavioral tests on mice with short-term and long-term corticosterone treatment to determine the reason of the death of neural stem cells, in both short-term and long-term corticosterone treatments, the mice are injected with corticosterone, AAV, and BrdU regularly, and our conclusion is that in short-term treatment, it is the decline of BDNF that cause cell death, while in long-term treatment, both the decline of BDNF and autophagy itself contribute to cell death..

**Keywords:** autophagic cell death, adult hippocampal neurogenesis, corticosterone, brain-derived neurotrophic factor, stress.

## 1. Introduction

### 1.1. Autophagy

Autophagy refers to a lysosome-dependent catabolic process, it has the feature of increased formation of double-membraned autophagosomes for sequestration of cytoplasmic components. It is a crucial part of development and physiology and it also a significant cell survival mechanism, which provides nutrients and eliminate obsolete cells. However, autophagy may also cause cell death under certain conditions. Previous studies showed that the deprivation of insulin can cause autophagic cell death. However, these studies were conducted in vitro, the physiological importance of ACD in vivo still remains unclear.

### 1.2. Adult Hippocampal Neurogenesis (AHN)

Adult hippocampal neurogenesis is related to learning, memory, and mood regulation. However, it can easily be affected by stress and major stress hormone, glucocorticoid. As a result, psychological

disorders such as anxiety and depression can cause the autophagic cell death and therefore suppress AHN and cognitive ability. The effects of PCD on the balance between the survival and death of adult NSCs and therefore mediates stress to suppress adult neurogenesis. Previous studies had different conclusions about what causes cognitive deficits. One opinion is that corticosterone directly (CORT) directly surpasses AHN and lead to anxiety-like behaviors and cognitive deficits, another opinion is that CORT treatment causes the depleting of BDNF, and thereby lead to anxiety-like behavior and cognitive deficits. In this study, we aim to investigate the effects of both long-term and short-term corticosterone treatment on adult hippocampal neurogenesis, anxiety-like behaviors, and cognitive performance, and we also aim to find whether the increasing autophagy level itself or the decline of BDNF caused by autophagy increase leads to death of NSCs in both of the long-term and short-term CORT treatment. Our hypothesis is in short-term treatment, it is the decline of BDNF that cause cell death, while in long-term treatment, both the decline of BDNF and autophagy itself contribute to cell death.

### *1.3. AAV1/2 guide RNAs*

AAV is a widely used vector for gene therapy, and gRNA is a significant component in CRISPR-based genome editing. The gRNA directs the CRISPR-associated (Cas) protein, like Cas9, to a specific location in the genome where it can introduce cuts or modifications. AAV1/2 guide RNA is a guide RNA that is designed to be delivered using an adeno-associated virus (AAV) vector, it is a hybrid of AAV1 and AAV2, offers advantages in tissue targeting, infection efficiency, immune response reduction and so on. In the corticosterone (CORT) treatment of mice, it is used in CRISPR-based experiments to investigate the role of specific genes in response to CORT. We use the AAV1/2 to deliver CRISPR components such as guide RNAs into specific tissues or brain areas of mice to knock out or modify genes that are involved in stress response, behavior, or other biological pathways influenced by CORT.

## **2. Experimental design**

### *2.1. CORT treatment*

Corticosterone (CORT) is one of the major forms of glucocorticoid released during stress. By studying its effects on anxiety-like behaviors and neural stem cells in mice and comparing it with the effects of BDNF, we can determine whether the cell death is caused by autophagy itself or the declining of BDNF that caused by autophagy. The experiment will be divided into two groups: short-term CORT treatment (8 days) and long-term CORT treatment (8 weeks). For short-term CORT treatment, AAV1/2-guide RNAs are injected into the brain of S-Cas9 KI mice on the first day, CORT injections are conducted everyday, and BrdU injection are from day 5 to day 7. After 4 days of CORT administration, the mice were injected with AAV1/2-guide RNAs again in the brain. For long-term CORT treatment, AAV1/2-guide RNAs are injected in week 1, CORT injections are conducted every week, and BrdU injections are from week 5 to week 7. After 4 weeks of CORT administration, the mice were injected with AAV1/2-guide RNAs again in the brain (Figure 1). The S-Cas9 KI will be divided into six groups, short-term CORT treatment and long-term CORT treatment will each have a control group, CORT (EV) group, and CORT (BDNF+) group respectively. (Figure 2)

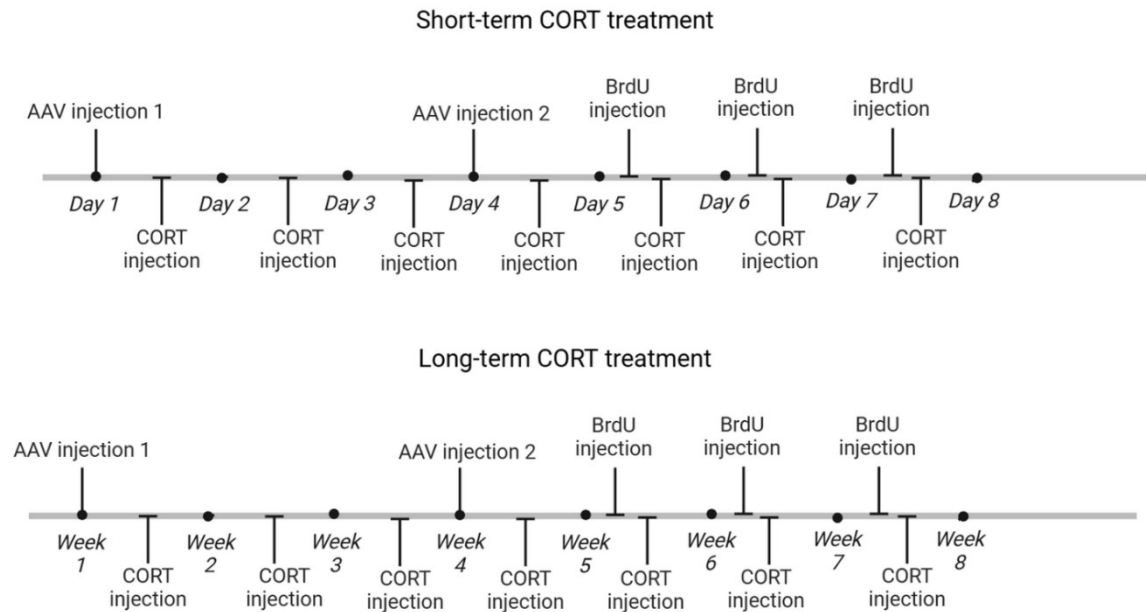


**Figure 1.** Short-term CORT treatment and long-term CORT treatment of S-Cas9 KI mice.

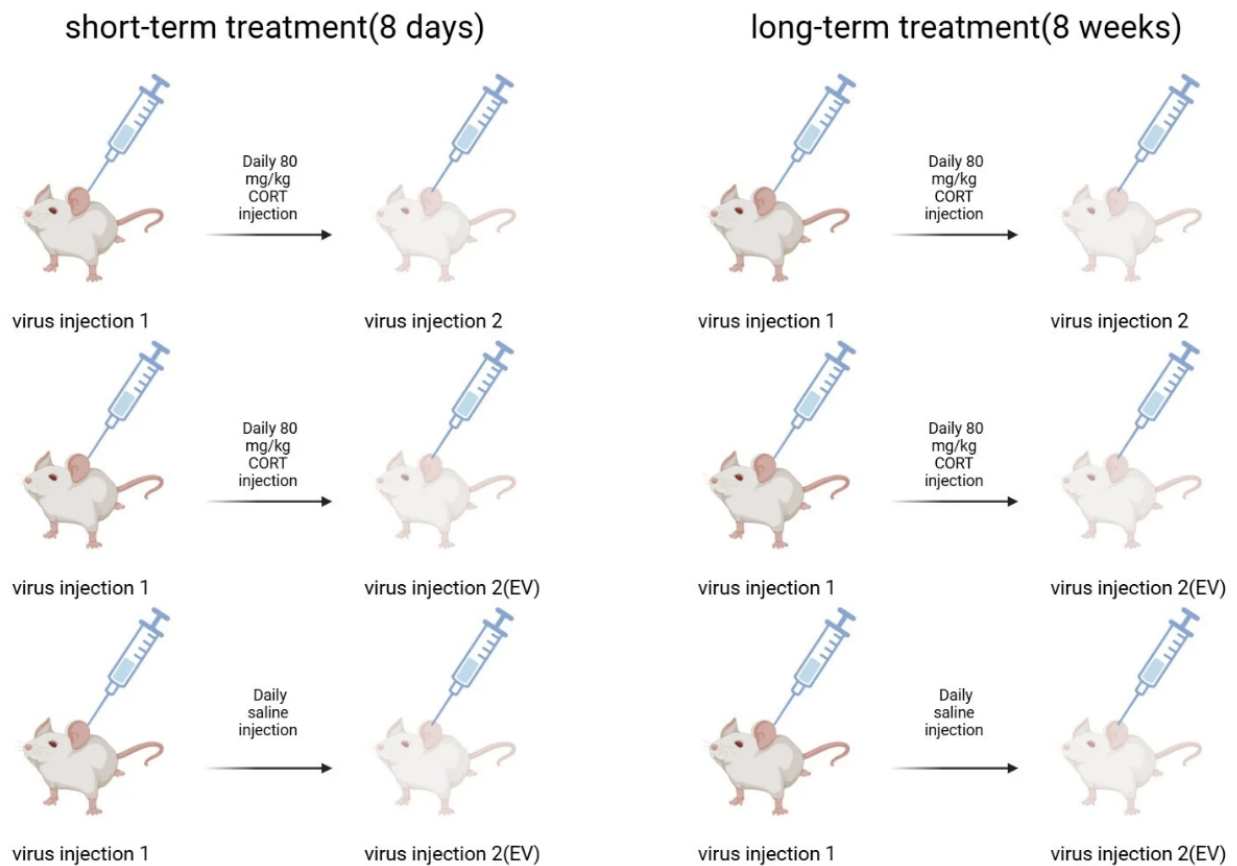


**Figure 2.** Different treatments each group of mice receive.

### *2.2. Open field test and elevated plus maze test (EPM)*

The open field test aims to examine mice's anxiety-like behaviors. Each mice were individually putted in a corner of a box ( $50 \times 50 \times 30 \text{ cm}^3$ ) in a room with dim light. The mice were permitted to move freely in the box for 6 min. A camera was placed right above the box to monitor the movement and location of each mouse. The total distance the mice travelled, the velocity of the mice, and time mice spent at the side, non-side, and center of the box were recorded. The apparatus was sanitized with 75% ethanol after each test in order to remove residual odors. The time each mouse spend in the center of the box determines the anxiety level of it.

The elevated plus maze (EPM) test was conducted to test the anxiety-like behavior of mice. The EPM was a plus-cross-shaped apparatus consisted by four arms: two arms with walls and two arms without walls. In the center of the cross was an open platform ( $5 \times 5 \text{ cm}^2$ ). The maze was placed 50 cm above the floor, and a camera was placed at the top of the apparatus to film the behaviors of each mouse. Each mouse was placed in the zone around the center and facing the closed arm where they were allowed to move freely for 5 min. We measure the total distance and time each mouse moved and record them. The apparatus was cleaned with 75% ethanol after each test in order to remove residual odors. The time mice spend in open arms determines the anxiety level of the mice.

### *2.3. Sucrose preference test and Y-maze test*

Sucrose preference test aims to detect the depression behavior. Two days before the test, sucrose solution with 1% concentration was provided to let the mice adapted the taste of sucrose. After the experiment began, 1% concentration sucrose solution and pure water were filled in two bottles respectively and labeled. Mice were packed in cages and bottles filled with two different kinds of liquids were placed at the top of the cages. The amount of both liquid consumed was measured every 12 hours and the position of bottles was switched every 12 hours. Higher preference of sucrose means lower depression level.

Y-maze test can examine the learning ability and spatial working memory of mice. Before the experiment, grains were placed at the end of the maze as rewards, mice were placed in a room without disturbance in order to let the mice get used to new environment and lower the impact of pressure during the experiment. After the experiment began, mice were placed at the starting point of the maze's arm, the time each mouse spent in each arms, each mouse's turning decisions, and time each mouse took to find the food were recorded. The experiment was repeated for ten times to examine mice's alternation actions.

### *2.4. Tail suspension test and forced swimming test*

Tail suspension test was conducted to test the depression-like behavior of mice. Before the experiment, mice were brought to the experimental environment to adapt for at least an hour. After the experiment began, mice were hang on the experimental hook, at the height of about 1 centimeter above the ground. The experiment lasted for six minutes, first two minutes were adapting period, and the last four minutes were actual experiment. During the experiment, the mobility time and immobility time were recorded. The experiment was repeated for ten times on different mice. Longer immobility time refers to higher depression level.

The forced swim test aims to test the depression-like behaviors of mice. Before the experiment, the mice were brought to the testing environment for adaption. During the experiment, each mouse was placed in a jar, filled with water by which the mouse cannot touch the bottom of the jar. The experiment lasted for six minutes, the immobility time, struggling time, and swimming time were recorded by an infrared HD camera, higher immobility time means higher depression level.

## **3. Expected results**

CORT increases antophagy level, while the extra expression of BDNF would not affect autophagy level (Figure 3); CORT decrease the amount of old neural stem cells in DG. While extra expression of BDNF prevents the decline in short-term treatment, it could only alleviate this decline in long-term treatment (Figure 4); CORT would not affect cell division rate of neural stem cell in short-term treatment, but

would decrease it in long-term treatment. Meanwhile, this decline could be alleviated by extra expression of BDNF (Figure 5); the differentiation of neural stem cells is not affected by CORT (Figure 6); CORT have a significant effect on anxiety of mice. By over-expression of BDNF in mice, the anxiety level of mice treated with CORT can be reversed in the short-term treatment condition. But in the condition of long-term treatment, anxiety can only be partially reversed (Figure 7). The spatial working memory affected by CORT treatment is similar to the anxiety-like behavior (Figure 8). Extra expression of BDNF can prevent depression in short-term CORT treatment, but it can only relieve depression in long-term CORT treatment (Figure 9). Overall, in short-term treatment, it is the decline of BDNF that cause cell death, while in long-term treatment, both the decline of BDNF and autophagy itself contribute to cell death.

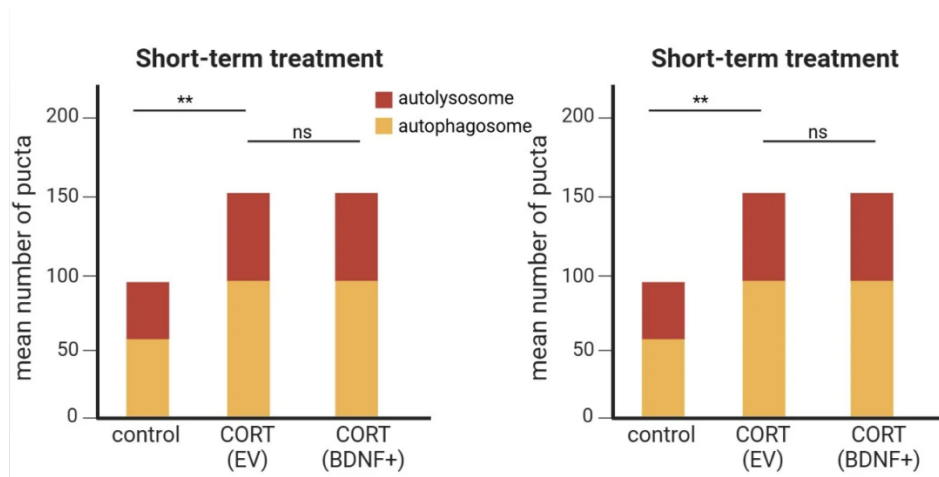


**Figure 3.** The autophagy level of NSCs.

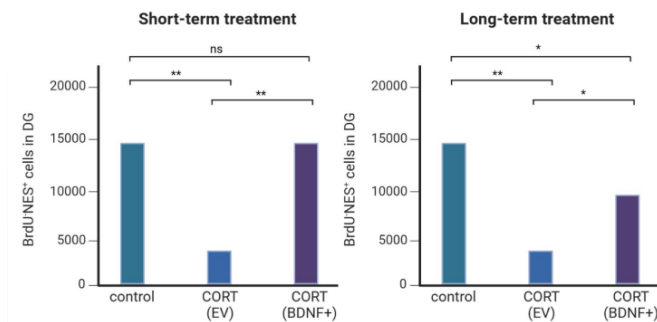


**Figure 4.** The amount of old NSCs in dentate gyrus.

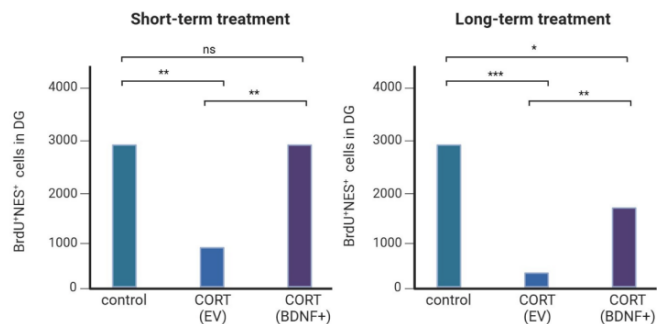


**Figure 5.** The cell division rate of NSCs.

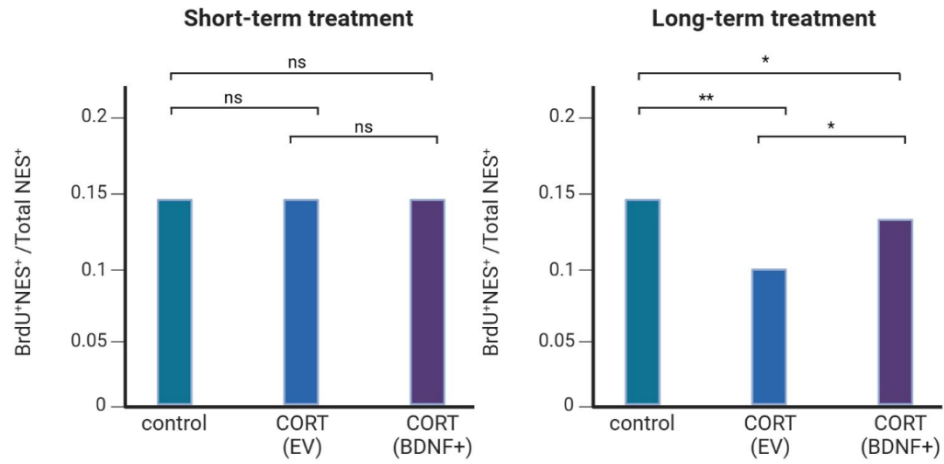


**Figure 6.** The differentiation rate of NSCs.

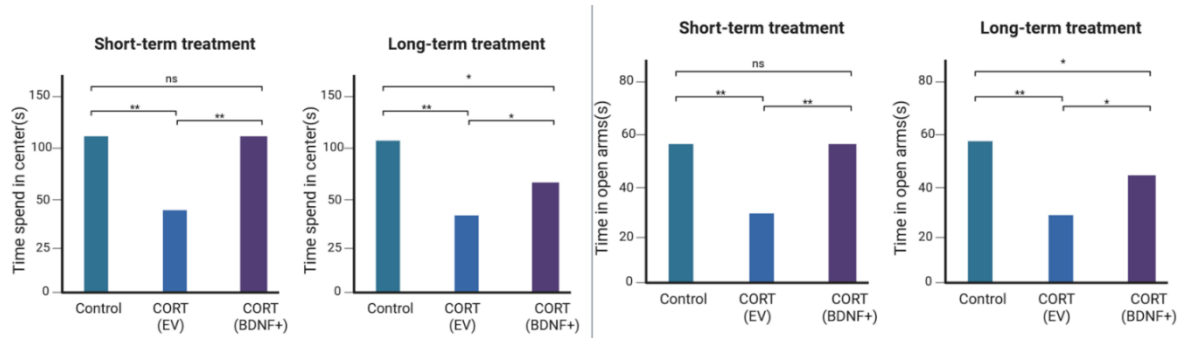


**Figure 7.** The anxiety level of mice.

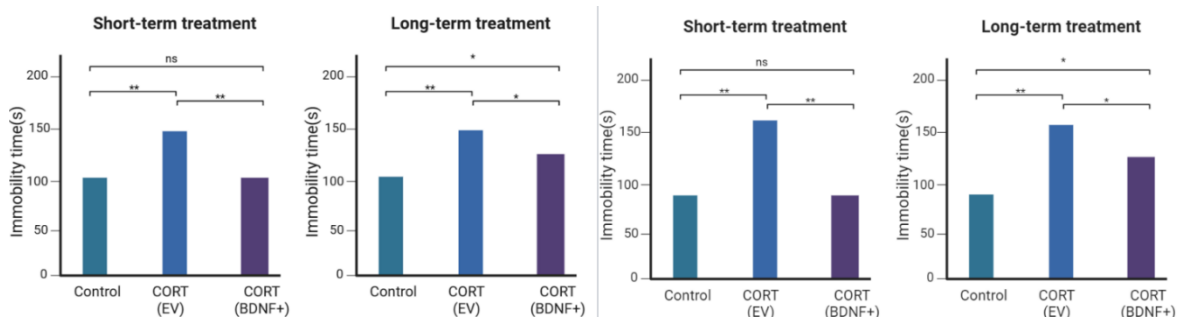


**Figure 8.** The results of anxiety level and spatial working memory of mice.

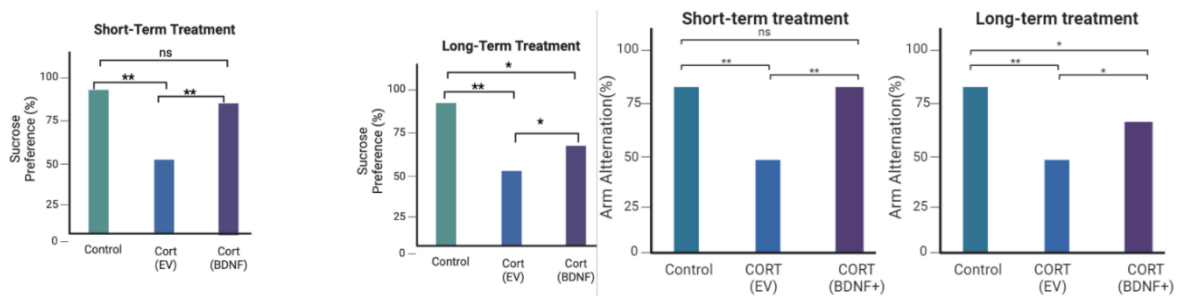


**Figure 9.** The result of depression level of mice

#### 4. Discussion

Admittedly, there are some controversies towards autophagy. For instance, some people say that ACD opposes a generally cytoprotective role of autophagy, and was regarded as a misinterpretation from the use of pharmacological and genetic tools to manipulate autophagy. Therefore, it was claimed that ACD may be absent in mammalian cells under conditions related to physiology. Moreover, there are critiques such as there is no evidence that autophagy-deficient cells is resistant to cell death and survive for a long time in vivo. In our study, we conducted an in vivo study by doing experiments of the autophagy level, the anxiety level, and spatial working memory. The results suggest that in short-term treatment, the decline of BDNF causes cell death, while in long-term treatment, both the decline of BDNF and autophagy itself contribute to cell death.

In addition, although we conducted experiments testing the anxiety-like behaviors and depression-like behaviors of mice and concluded that CORT and BDNF are associated with these, the mechanisms that cause these results still remain unclear. Further studies are required to reveal the mechanisms of how CORT and the declining of BDNF causes mental issues.

Overall, we investigated the effects of both long-term and short-term corticosterone treatment on adult hippocampal neurogenesis, anxiety-like behaviors, and cognitive performance, and we also aim found whether the increasing autophagy level itself or the decline of BDNF caused by autophagy increase leads to death of NSCs in both of the long-term and short-term CORT treatment, and concluded that in short-term treatment, it is the decline of BDNF that cause cell death, while in long-term treatment, both the decline of BDNF and autophagy itself contribute to cell death.

#### 5. Conclusion

Overall, this paper determines whether mammal's cognitive deficits and anxiety-like behaviors are induced by the surpassing of AHN directly caused by autophagy or these symptoms are caused by the depleting of BDNF due to CORT. Several behavioral experiments were conducted on mice to test the anxiety and depression-like behaviors. Based on the data of the experiments, we can conclude that: in short-term treatment, it is the decline of BDNF that cause cell death, while in long-term treatment, both the decline of BDNF and autophagy itself contribute to cell death. Looking forward, further studies need to be done to reveal the mechanisms of how CORT and the decline of BDNF cause these anxiety and depression-associated behaviors.

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