

# Mechanisms by Which Physical Activity Modulates the Wnt/ $\beta$ -catenin Pathway to Alleviate Anxiety-like Depression

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**Abstract.** This study aimed to explore the effect of treadmill exercise on anxiety in rats. Thirty SPF male rats aged 2 months with a body mass of (225±25) g were randomly divided into control group (CG, n=10), chronic sleep deprivation group (CSD, n=10) and sleep deprivation exercise group (CSD+E, n=10) after adaptive feeding for 1 week. The CSD model of rats in CSD group and CSD+E group was made by multi platform water environment method. Sleep deprivation of 18 h per day (from 12:00 pm. to 6:00 am. the next day) for 8 weeks. The effect of running on the anxiety-like behaviour of CSD rats was examined in the open field test (OFT) and the elevated plus maze (EPM) experiment. Hematoxylin-eosin (HE) staining, Annexin V/PI flow cytometry, immunofluorescence staining, Western blot, RT-qPCR and other methods were used to detect the effects of treadmill exercise on the morphology of hippocampus, apoptosis related factors caspase-12, Bax, Bcl-1, inflammatory factors (IL-6, TNF-a), Wnt  $\beta$ -catenin, p- $\beta$ -catenin. The results of HE staining showed that the brain tissue of the control rats was structurally intact, with thick layers of cone cells, relatively dense, neatly arranged and compact, the cell edge structures were intact and clearly visible, with no obvious abnormal changes. The cone cell layer of brain tissue in the chronic sleep deprivation group was thin, with relatively low cell density, disorganized and sparse arrangement, and blurred cell edges. The symptoms of the above pathological changes in brain tissue of rats in sleep deprivation exercise group gradually alleviated. OFT results showed that compared with CG group, the number of activities in the central region of CSD group was significantly reduced ( $P<0.01$ ), and the total distance of exercise was significantly shortened ( $P<0.01$ ). EPM results showed that compared with CG group, OT and CE in CSD group decreased significantly ( $P<0.01$ ). CCK-8 results showed that compared with CG group, the activity of neurons in CA1 area of hippocampus in CSD group was significantly decreased ( $P<0.01$ ), while that in CSD+E group was significantly increased ( $P<0.01$ ); Annexin V/PI flow cytometry results showed that compared with CG group, the apoptosis of neurons in CA1 area of hippocampus in CSD group increased ( $P<0.01$ ), and that in CSD+E group decreased significantly ( $P<0.01$ ); Western blot results showed that caspase-12, Bax, IL-6, IL-1 $\beta$  and TNF-a were highly expressed and Bcl-1 was lowly expressed in hippocampal tissues of rats in the CSD group compared with the CG group ( $P<0.01$ ), Wnt,  $\beta$ -catenin and p- $\beta$ -catenin were lowly expressed in hippocampal tissues of rats in the CSD group, and Gsk-3 $\beta$  protein expression was significantly higher ( $p<0.01$ ). The results of RT-qPCR showed that caspase-12 mRNA, Bax mRNA, IL-6 mRNA, TNF-a mRNA and IL-1 $\beta$  mRNA were highly expressed and Bcl-1 mRNA was lowly expressed in hippocampal tissues of rats in the CSD group compared with the CG group ( $P<0.01$ ). Compared with the CG group, Wnt mRNA and  $\beta$ -catenin mRNA were significantly lowly expressed and Gsk-3 $\beta$  mRNA was significantly highly expressed in hippocampal tissue of CSD rats ( $P<0.01$ ). Our findings indicated that 8 weeks of aerobic exercise significantly improved anxiety-like depression in CSD rats by increasing neuronal activity, inhibiting apoptosis, reducing the inflammatory response and activating the Wnt/ $\beta$ -catenin pathway.

## 1. Introduction

Today's increasingly competitive society has caused individuals to work longer and sleep shorter. According to statistics [1], approximately 19% of adults work more than 48 h per week and 7% work more than 60 h per week. Professional and social demands have led to chronic sleep

deprivation and sleep disorders becoming the norm. The WHO estimates that more than 300 million adults worldwide suffer from sleep disorders (less than 6 h of sleep per day), with 30% to 50% of those suffering from sleep disorders being associated with social stress and neurological disorders. Mental disorders are directly related with it [2]. Adequate sleep plays an important role

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in cerebrospinal fluid circulation, brain development, synaptic plasticity and nerve recovery. Insufficient sleep or sleep disorder will cause negative effects on psychology, reduce the number of neurons, reduce cognitive function (including attention, decision-making and various types of memory) and immune dysfunction [3]. In addition, chronic sleep deprivation is considered to be a risk factor for various diseases (such as mental illness), and may even cause fatal consequences within a few months or years [4]. Alzoubi et al. (2017) confirmed through behavioral experiments that acute and chronic sleep deprivation could cause rodents to have memory deficits in some behavioral tasks. In addition, clinical and animal experiments showed that chronic sleep deprivation could lead to human morbid anxiety and animal anxiety behaviors [5-7]. Therefore, the relationship between chronic sleep deprivation and anxiety needs to be studied in depth. Recent studies have shown that psychiatric-related disorders such as depression and autism may be associated with abnormalities in the Wnt signalling pathway [8-10]. Cui JM, etc. [11] found that platform running significantly improved depressive behaviour in chronic sleep deprived rats by modulating the BDNF/TrkB signalling pathway. Chen LJ, etc. [12] found that low-intensity voluntary exercise improved post-stroke depression through the MFN2/BDNF signalling pathway. It is reported that [13] aerobic exercise can improve STAT3 and relieved postpartum anxiety in rats. On Motor Regulation Wnt/  $\beta$ - Catenin signaling pathway has not been reported to alleviate anxiety disorder in sleep deprived rats. In this study, we, therefore, used chronic sleep deprivation to develop an anxiety model of rats to explore the improvement effect of animal treadmill exercise on anxiety rats, and provided scientific and reasonable theoretical guidance for alleviating anxiety like depression caused by insufficient sleep.

## 2. Materials and Methods

### 2.1 Study Design

30 SPF grade SD male rats at the age of 2 months, with a body mass of ( $225 \pm 25$ ) g. After adaptive feeding for 1 week, this study was approved by the Experimental Animal Welfare and Ethics Committee of Yan'an University (License No.: 20210009), and carried out in accordance with the Guidelines for Raising and Using Experimental Animals (National Institutes of Health Publication No. 80-123, revised in 1996)[14]. The rats were randomly divided into control group (CG, n=10), chronic sleep deprivation group (CSD, n=10) and Chronic sleep deprivation exercise group (CSD+E, n=10). The CSD model of rats in CSD group and CSD+E group was established by multi platform water environment method. Sleep deprivation lasted for 18 hours every day (from 12:00 p.m. to 6:00 a.m. the next day) for 8 weeks. The control group did not receive special treatment. The specific scheme of CSD+E group was as follows: 5 days/week, 8 weeks in total. we would have a running platform training on every Monday to Friday, at 9:00~11:00 am and have a rest on Saturday and Sunday, 8

weeks in total. The running time of rats on the treadmill was 30 minutes on the first day, increasing by 10 minutes every day. After increasing to 50 minutes, the running time was maintained until the first weekend. The running time was 60 minutes every day in the second week, 70 minutes every day in the third week, 80 minutes every day in the fourth week, and 90 minutes every day after the fifth week until the end of the experiment.

### 2.2 Instruments and Equipment

Elevated cross maze: Shanghai Luowei Biotechnology Co., Ltd. (model: XR-XG201); PCR instrument, Thermo Thermo Micro 21R refrigerated micro centrifuge: Shanghai Baji Industrial Co., Ltd. (model: Micro 21R); Refrigerator and other common instruments; in vitro transcription kit: Fermentals; 7500 fast Real time PCR System kit: Applied Biosystems; 8 Orbital animal running platform (ZH-PT, Chuangbo Global (Beijing) Biotechnology Co., Ltd.).

### 2.3 Open Field Test

The experimenter randomly put the three groups of rats into the OFT test box (40 cm  $\times$  40 cm  $\times$  35 cm, Kunshan Danbo Instrument Co., Ltd.), adaptive activity of rats for 10 minutes. An experimental recording system was used to record the total distance of the rats and the number of entries into the central area by the number of times the rats interrupted the infrared rays. After the test of the first rat was completed, wiped the test box with 10% medical alcohol to remove the odor left. All tests were completed between 8:00 a.m. and 12:00 a.m.

### 2.4 Elevated Plus Maze Experiment

The Elevated Plus Maze consisted of two open arms (50cm  $\times$  10cm), two closed arms (50cm  $\times$  10cm), a central area (10cm  $\times$  10cm), the whole maze was 60cm above the ground. The closed arm baffle was 40cm high, and the open arm edge was 1cm high, to reduce the fall of experimental rats during the test. A video camera was installed at the top center of the maze to record the behavior of the experimental rats during all testing. The recorded video was analyzed with the animal behavior trajectory analysis software Nodus Etho Vision XT 8.5 (purchased from the Netherlands Nodus Company). The rats were acclimatized in the test laboratory for at least 30 minutes before the test. The temperature of the test laboratory was  $24 \pm 2^\circ\text{C}$  and the light intensity was approximately 10 Lux. The light intensity of the open arm of the elevated cross maze was approximately 6 Lux and that of the closed arm was approximately 5 Lux. The test was repeated 4 times per day for each rat. The time point of the first test was 30 minutes before the injection of sterile water (i.e. baseline), and the time points of the other three tests were 30 minutes, 100 minutes and 240 minutes after the injection. Every two test intervals, the experimental rats were put back into the original cage. The cage was placed in the test laboratory until the four behavioral tests were completed every day. All behavioural test videos recorded were analysed using animal behavioural analysis software to obtain the

following behavioural parameters: Open arm entry (OE), open arm time (OT), closed arm entry (CE) and closed arm time (CT), we also calculated the percentage of open arm entry ( $OE\% = OE / (OE + CE) \times 100\%$ ) and the percentage of open arm time [ $OT\% = OT / (OT + CE) \times 100\%$ ]. The decrease of CE and OT reflected the aggravation of anxiety in rats.

### 2.5 Hematoxylin-eosin Staining

The brain tissue paraffin section samples were prepared for the three groups of rats. The brain tissues were carefully stripped, fixed in 4% paraformaldehyde (PFA) for 48 hours, dehydrated in 50% alcohol for 30 minutes, and stored in 70% alcohol at room temperature for standby. Frozen section samples were prepared for the three groups of rats, and brain tissues were taken from the rats by heart perfusion. After being placed in 4% PFA solution for 48 hours, they were transferred to 30% sucrose for full dehydration, and 30µm thick sections of brain tissues were taken and stored at -20°C.

The above samples were dewaxed and hydrated first, and then the samples were soaked in hematoxylin for 5min. After the residual liquid was washed off with tap water, the hydrochloric acid alcohol differentiated for 30s. Then the samples were passed through eosin for 30s, and the residual liquid was washed off with tap water. Finally, after the samples were dried naturally, DPX was used for sealing. The prepared dyed samples were stored at room temperature for subsequent photos.

### 2.6 Cell viability assay

Cell Counting Kit-8 (CCK-8) was performed to detect cells activity. Laid brain tissue cells onto 96 well cell culture plate, when the cell density reached 80%, the cells were incubated with 100µmol/L for 48h. Depending on the time of cell treatment, the cell nutrient solution was changed 2h before the OD value was measured and 10µL of CCK-8 solution was added dropwise to the cell suspension of each treatment group, incubated for 2h in a suitable environment in vitro, and the OD value at 450nm was measured with an enzyme marker to determine the viability of the chondrocytes. 3 replicate wells of each group were averaged.

### 2.7 Detection of cell apoptosis

The cells were collected from rat brain tissue, removed from the culture flask, washed 3 times with PBS and digested with 0.125% trypsin for a few minutes. Gently blew down the nerve cells from the culture bottle with a pipette, and collected the suspended cells directly into a 15ml centrifuge tube, and centrifuge for 5 minutes with 1000r. The PBS was washed once and centrifuged for 5 min at 1000r. After repeating the previous step, the cells were resuspended with 200ul bonding buffer, added with 10ul Annexin V-FITC, and left for 20 min. Resuspended the cells again with 300ul bending-buffer, added 5ul PI, and stood in dark for 5min. Conducted flow cytometry analysis and determination to detect apoptotic cells in brain tissue and calculated the apoptosis rate: the excitation wavelength of flow cytometry was 488 nm, the

FITC fluorescence was detected with a passband filter with a wavelength of 515 nm, and the PI was detected with a filter with a wavelength greater than 560 nm.

### 2.8 Western blotting assay

Western blotting assays were performed to investigate protein expression. The hippocampal tissues of rats in the three groups were separated, the lysate was added, and then crushed by ultrasonic, centrifuged at 4 °C at 14000 r/min for 30 minutes, the supernatant was aspirated, and the protein concentration was determined by BCA protein kit. The equivalent amount of protein was transferred to polyvinylidene double oxide membrane (PVDF) by electrophoresis. The protein band was added with rat anti mouse caspase-12 antibody (1:200), rabbit anti mouse Bax (1:1000), rabbit anti mouse Bcl-1 (1:1000), rabbit anti mouse IL-6 (1:1000), rabbit anti mouse TNF-α (1:1000), rabbit anti mouse Wnt (1:2000), rabbit anti mouse β-catenin (1:1000). Incubated overnight at 4°C with slow shaking and the following day with HRP-labelled goat anti-rat IgG antibody (1:5000), 1:5000 for 1h at room temperature and ECL hypersensitive luminescent mix for gel imaging photography.

### 2.9 Real-time quantitative PCR

We used real-time quantitative PCR (RT-qPCR) to detect mRNA expression. We separated the hippocampus of three groups of rats, extracted RNA with Trizol reagent, and detected the RNA concentration with ultraviolet spectrophotometer. A reverse transcription reaction system was added to reverse transcribe the RNA to synthesize the first strand of cDNA, and then PCR pre-mix was added to the cDNA template for PCR amplification. Bio-Rad detected the relative expression level of the target gene, and obtained the relative expression level of the target gene by comparing CT values (Table 1).

**Table 1.** PCR primer sequences

Primer	Upstream Sequence( 5'–3')	Downstream Sequence( 5'–3')
IL-1β	CAGTGGCCTGGTCACTGGCGA	TGGCGTCTGGGTATTAAGATCGG
IL-6	GAGGCATCATGGGTATCCAGATCAAG	GAGCGGGGTGGAATGTAACCTTTAG
TNF-α	ATGTGGTCACTTCGTGAG	AGGTATCTAAGGCATTTGGTTCTG
caspase-12	CGG CAA TAG CTC TGT AT	CCT TGA AAC TTT GCC TCA
Bax	CAC AAG TCC GGA GAG GAG AC	CAG AAT TGC CAT TGC ACA AC
Bcl-1	AAAAGTGCTTACAGTGCAGGTAG	GGAAAAGTGCTTACAGTGCAGGT
Wnt	GCTTCGGCAGCACATATACTAAAAT	CGCTTACGAAATTTGCGTGTCAAT
β-catenin	CCCAGGGAGGAGCAATACAG	GGGAGGAGCCGATAACAACCT
GAPDH	ACGGCAAGTTCACGGCACAG	GAAGACGCCAGTAGACTCCACGAC

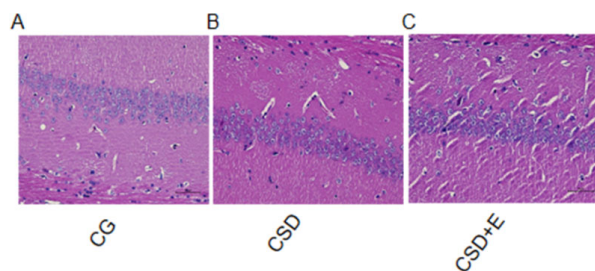
### 2.10 Statistical analysis

SPSS 23.0 statistical software was used for analysis, expressed as ( $\bar{x} \pm s$ ), and  $\chi^2$  test was used for comparison between groups. The difference was statistically significant with a value of  $p < 0.05$ .

### 3. Results

#### 3.1 Effect of aerobic exercise on brain histomorphology in the hippocampal CA1 region of CSD rats

The results of HE staining in the hippocampal CA1 region of rats in each group (Figure 1). The brain tissue structure of rats in CG group was complete, the pyramidal cell layer was thick, the cell density was relatively large, the arrangement was neat and compact, the structure of the cell edge was complete and clear, and there was no obvious abnormal change (Figure 1A). The pyramidal cell layer of brain tissue in the CSD group was thin, with relatively low cell density, disorganized and sparse arrangement and blurred cell edges (Figure 1B). The pathological symptoms of brain tissue of rats in CSD+E group gradually alleviated (Figure 1C). All of the above indicators were reversed in the CSD+E group. These results suggested that aerobic exercise significantly improved the pathomorphological changes in the brain tissue of rats with CSD anxiety-like depression.

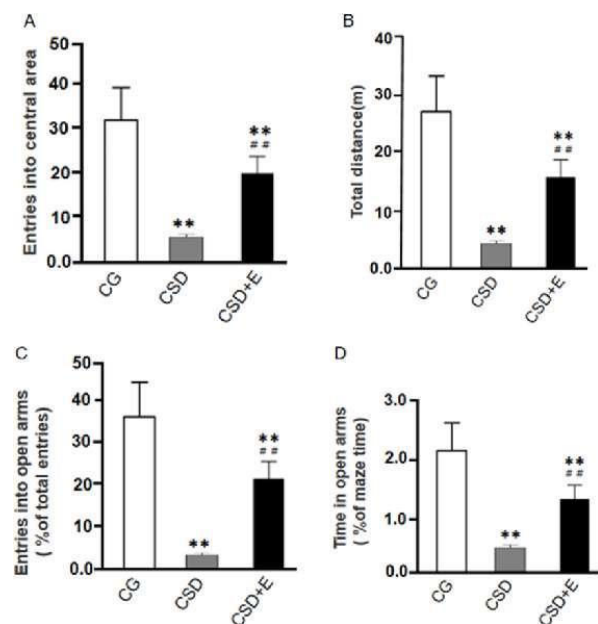


**Figure 1.** Effect of aerobic exercise on brain histomorphology in the hippocampal CA1 region of CSD rats A. HE staining results of brain tissue in hippocampal CA1 region of rats in CG group; B. HE staining results of brain tissue in hippocampal CA1 area of rats in CSD group C. HE staining results of brain tissue in hippocampal CA1 area of rats in CSD+E group

#### 3.2 Aerobic exercise could improve anxiety and depression like behavior of CSD rats

In OFT, the number of activities in the central area and the total distance of exercise were closely related to the level of anxiety in rats. Compared with the CG group, the CSD group showed a significant decrease in the number of activities in the central area ( $P < 0.01$ ) and a significant decrease in the total distance of movement ( $P < 0.01$ ) (Figure 2A, 2B). All of the above indicators were reversed in the CSD+E group. The above results suggested that 8-week aerobic exercise could significantly improve the anxiety like depression behavior of CSD rats.

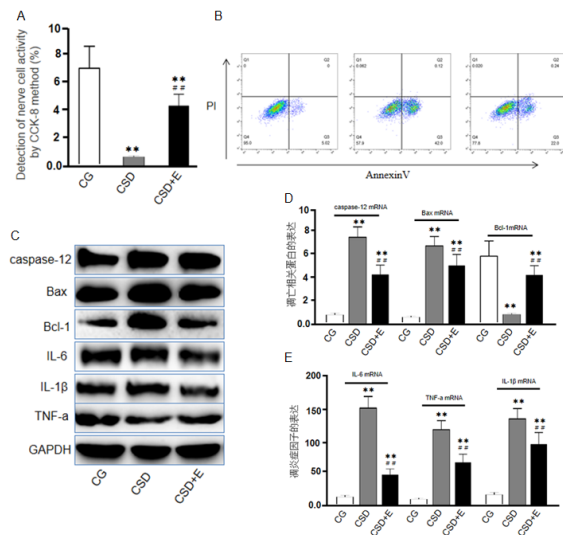
In EPM, the number and time of activities in the open arm of rats were closely related to the level of anxiety. Compared with CG group, OT and CE in CSD group decreased significantly ( $P < 0.01$ ) (Figure 2C, 2D). All of the above indicators were reversed in the CSD+E group. The above results suggested that 8-week aerobic exercise could significantly improve the anxiety like depression behavior of CSD rats.



**Figure 2.** Aerobic exercise could improve anxiety and depression like behavior of CSD rats. A.B. OFT experimental results. C. D. EPM experimental results. \*\* $p < 0.01$ , \* $p < 0.05$ , vs.CG. #  $p < 0.05$ , ##  $p < 0.01$ , vs.CSD.

#### 3.3 Effects of aerobic exercise on neuronal apoptosis and inflammatory response in CSD rats

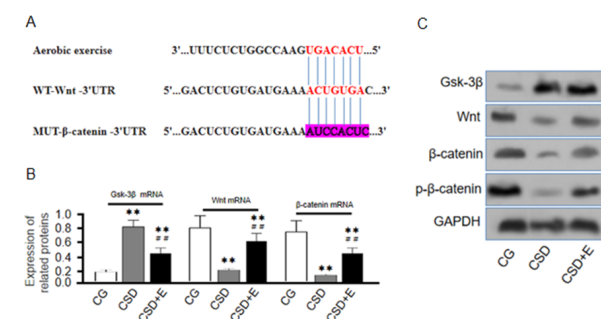
Neuroapoptosis was the root cause of neurological deficit after anxiety like depression in CSD. The results of CCK-8 in this study showed that compared with CG group, the activity of nerve cells in CA1 area of hippocampus in CSD group was significantly decreased ( $P < 0.01$ ), while that in CA1 area of hippocampus in CSD+E group was significantly increased ( $P < 0.01$ , Figure 3A). Annexin V/PI flow cytometry showed that compared with CG group, the apoptosis of neurons in CA1 area of hippocampus in CSD group was increased ( $P < 0.01$ ), while that in CA1 area of hippocampus in CSD+E group was significantly decreased ( $P < 0.01$ , Figure 3B). Western blot results showed that compared with CG group, caspase-12, Bax, IL-6, IL-1 $\beta$  and TNF- $\alpha$  were highly expressed in hippocampal tissues and Bcl-1 was low in the CSD group ( $P < 0.01$ , Figure 3C). RT-qPCR results showed that caspase-12 mRNA, Bax mRNA, IL-6 mRNA, TNF- $\alpha$  mRNA, IL-1 $\beta$  mRNA were highly expressed and Bcl-1 mRNA was lowly expressed in hippocampal tissues of rats in the CSD group compared with the CG group ( $P < 0.01$ , Figure 3D-E). All of the above indicators were reversed in the CSD+E group. The above results suggested that 8-week aerobic exercise could improve the activity of nerve cells, inhibited apoptosis, reduced inflammatory reaction, and improved anxiety like depression in CSD rats.



**Figure 3.** Effects of aerobic exercise on neuronal apoptosis and inflammatory response in CSD rats. A. CCK-8 assay for neuronal activity. B. Annexin V/PI flow cytometry for neuronal apoptosis. C. Western blot for expression of apoptosis-associated proteins. D. RT-qPCR to detect the expression of apoptosis-related genes. E. RT-qPCR for expression of inflammation-related factors. \*\* $p < 0.01$ , \* $p < 0.05$ , vs.CG. #  $p < 0.05$ , ##  $p < 0.01$ , vs. CSD.

### 3.4 Aerobic targeted activation Wnt/ $\beta$ - Catenin pathway improved anxiety-like depression in rats

Bioinformatics analysis showed that aerobic exercise could target and regulate Wnt and  $\beta$ - Catenin was the downstream target gene of Wnt (Figure 4A). Western blot results showed that compared with CG group, Wnt  $\beta$ -catenin, p-  $\beta$ - Catenin was low expression, Gsk-3  $\beta$  Significant increased in protein expression ( $P < 0.01$ , Figure 4B). RT-qPCR results showed that compared with CG group, Wnt mRNA,  $\beta$ - Catenin mRNA was significantly low expressed, Gsk-3 $\beta$  was Significantly high mRNA expression ( $P < 0.01$ , Figure 4C). All of the above indicators were reversed in the CSD+E group. These results suggested that 8 weeks of aerobic exercise could target the Wnt/ $\beta$ -catenin pathway and improved anxiety-like depression in rats.



**Figure 4.** Aerobic targeted activation Wnt/  $\beta$ - Catenin pathway improved anxiety like depression in rats. A. Aerobic targeted activation Wnt/  $\beta$ - Catenin pathway. B-C. Western blot, RT-qPCR detected the expression of Gsk-3 $\beta$ , Wnt,  $\beta$ -catenin and p- $\beta$ -catenin proteins and genes. \*\* $p < 0.01$ , \* $p < 0.05$ , vs.CG. #  $p < 0.05$ , ##  $p < 0.01$ , vs.CSD.

## 4. Conclusion

The sleep deprivation caused by the fast pace of life and increased stress has led to the increasing prevalence of "sleep deficit" and sleep insufficiency related diseases, which has brought more and more serious physiological and psychological burdens to human beings[15-16]. Sleep deprivation(SD) means that the sleep time is less than the average level of the population[17]. The acute sleep deprivation (ASD) of individuals deprived of more than 24-48 hours of sleep at one time and the chronic sleep deprivation (CSD) of individuals which deprives individuals of less than 5-6 hours of sleep every day for a long period of time, are two main patterns of sleep deprivation. SD is closely related to cancer [18], hypertension [19], coronary heart disease [20], diabetes [21], and stress reaction [22]. SD also increases the risk of depression or anxiety [23-24], and is recognized as an independent risk factor for suicide [25-26]. At present, nearly one quarter of women and one sixth of men in the world has experienced depression in their lives[27], and up to 65% of individuals has recurrent symptoms[28]. Depression has become the fourth largest disease in the world, ranking first in non fatal diseases, and also one of the main causes of functional disability of patients[29]. Clinical studies have shown that patients with depression are often accompanied by symptoms of anxiety [30]. The prevalence of sleep deprivation in patients with depression approaches 60% [31]. In addition, a large study of adolescents shows that sleep deprivation increases the risk of developing depression by 4-5 times [32], suggesting a strong relationship between sleep deprivation and depression. However, there is no animal experimental evidence to support the view that sleep deprivation can directly lead to depression and anxiety. Based on this, this experiment established a rat sleep deprivation model to explore whether sleep deprivation could directly induce depression and anxiety behavior from the perspective of animal experiments.

The development of depression is a very complex and multifactorial combination of factors, common hypotheses include 5-HT, NE, DA, etc [33]. In recent years, studies have found that changes in neuronal signal transduction pathways are also considered to be closely related to the occurrence of depression [34]. The mechanisms of Wnt/ $\beta$ -catenin signalling pathway involved in the development of depression are unclear, but some studies have shown that Wnt/catenin signaling pathway is involved in many complex biochemical reactions of cells, and then regulates the central nervous system, which is closely related to the occurrence of emotional related mental diseases [35].

Animal experiments showed that mood stabilizers and antidepressants improved neuronal plasticity by acting on Wnt signaling pathways [36]. Autopsy results of suicidal patients with depression showed that the prefrontal cortical  $\beta$ -catenin protein expression was decreased and Gsk-3 $\beta$  expression was increased, with a negative correlation between them [37]. Gsk-3 $\beta$  is highly expressed in brain tissue, especially in hippocampus, and participates in the regulation of neurogenesis, synaptic plasticity and homeostasis. Studies in depressed patients



found abnormal Gsk-3 $\beta$ -mediated Wnt/ $\beta$ -catenin expression [38-39], and in vitro studies also found that activation of the Gsk-3 $\beta$ / $\beta$ -catenin signalling pathway promoted proliferation of embryonic precursor neuronal cells [40].

In addition, an in vitro experiment found that Gsk-3 $\beta$ -mediated Wnt/ $\beta$ -catenin expression in brain tissue of anxious depressed rats was consistent with the results of aerobic exercise intervention [41]. Aerobic exercise intervention activated the Wnt/ $\beta$ -catenin pathway in SD rats consistent with Wnt/ $\beta$ -catenin activation in the presence of low Gsk-3 $\beta$  expression. We hypothesized that Gsk-3 $\beta$  expression was elevated in rats with depressive-like behavior, suggesting that Gsk-3 $\beta$  might be involved in the pathology of depression. Gsk-3 inhibitors significantly improved depressive behaviour in depressed rats and mice, consistent with the results of a forced swimming model in which Gsk-3 $\beta$  inhibitors produced rapid antidepressant effects and significantly reduced the duration of immobility in rats [41]. The above studies suggested that Gsk-3 $\beta$  played a crucial role in the pathogenesis of depression, possibly by regulating intracellular proteins and transcription factors, including  $\beta$ -catenin, through Gsk-3 $\beta$  phosphorylation, which in turn affected neuronal survival and plasticity or led to apoptosis, leading to depression [42].

From the perspective of physiology and biochemistry, physical exercise can promote blood circulation and nervous system, and have a positive impact on people's mood and physiology. Aerobic exercise means that during exercise, the body inhales as much oxygen as it needs to achieve a state of physiological equilibrium and is now recognized as the best form of exercise to improve depression [43]. In this study, a rat model of chronic sleep deprivation (CSD) was established by the multi-platform water environment method. The treadmill was used for exercise intervention for 8 weeks. The HE staining results showed that the brain tissue structure of CSD+E group rats was relatively complete, the pyramidal cell layer was thickened, the cell density was large, the arrangement was neat and compact, and the cell edge structure was relatively complete and clear. OFT results showed that rats in the CSD+E group had an increased number of activities and prolonged total distance of movement in the central region ( $P < 0.01$ ). EPM results showed that rats in the CSD+E group moved more often and for longer periods in the open arm ( $P < 0.01$ ). CCK-8 results showed a significant increase in neuronal activity in the CA1 region of the hippocampus in rats in the CSD+E group ( $P < 0.01$ ). The results of Annexin V/PI flow cytometry showed that neuronal apoptosis was reduced in the CA1 region of the hippocampus in the CSD+E group of rats. Western blot results showed that caspase-12 and Bax were lowly expressed and Bcl-1 was highly expressed in hippocampal tissues of rats in the CSD+E group ( $P < 0.01$ ). In addition, we found that IL-6, IL-1 $\beta$ , TNF- $\alpha$  and Gsk-3 $\beta$  were lowly expressed in hippocampal tissues of rats in the CSD+E group ( $P < 0.01$ ) and Wnt,  $\beta$  catenin, p- $\beta$ -catenin were highly expressed ( $P < 0.01$ ). RT-qPCR results showed that caspase-12 mRNA, Bax mRNA, Wnt mRNA and  $\beta$ -catenin mRNA were highly expressed in hippocampal tissues of rats in the CSD+E group ( $P < 0.01$ ), and IL-6

mRNA, TNF- $\alpha$  mRNA, IL-1 $\beta$  mRNA and Gsk-3 $\beta$  mRNA were significantly less expressed ( $P < 0.01$ ). Therefore, we can draw the conclusion that aerobic exercise can effectively alleviate the anxiety-like depression of rats with chronic sleep deprivation and has a good anti-anxiety effect. In the follow-up experiment, we will start with cell experiment and cell transfection to further study the anti-anxiety effect of aerobic exercise, effectively improve the quality of life of the majority of patients with sleep-deficient anxiety-like depression, and have greater guiding significance for clinical treatment of sleep-deficient anxiety-like depression.

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