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RESEARCH ARTICLE

LPM570065 ameliorates anxiety-like and depressive-like behaviors in CUMS rats through regulating DNA methylation in hippocampus

Shengmin Ji[1] **Chunmei Li**[1] **Wei Zhang**[1] **Linyao Yu**[1] **Yue Yang**[1] **Yaoqin Shi**[1] **Hongbo Wang**[1] **Jingwei Tian**[1*]

[1] School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, Shandong 264005, China

Correspondence to: Jingwei Tian, School of Pharmacy, Yantai University, Yantai, Shandong 264005, China; Email: tianjingwei618@163.com

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Abstract: Objective: This study aims to analyze the effects and underlying mechanisms of LPM570065 on behavioral phenotypes in rats with generalized anxiety disorder (GAD). Methods: The chronic unpredictable mild stress (CUMS) rats were used to observe the results of LPM570065. Total 72 male Sprague Dawley rats were divided into control, vehicle (0.5% CMC-Na), LPM570065 (32 mg/kg) and diazepam (3 mg/kg) groups, 12 rats in each group. Anxiety-like behaviors of rats were observed by elevated zero maze test and novelty-suppressed feeding test. Depressive-like behaviors of rats were detected by forced swimming test. DNA methylation in hippocampi of rats were measured by reduced representation bisulfite sequencing (RRBS). In hippocampi of rats, expressions of DNA methyltransferase (DNMT) 1 and DNMT3a proteins were measured by western blot, and density of dendritic spines was observed by Golgi staining. Results: Compared with the control group, the weights of rats were obviously decreased ($p < 0.001$) and the rats showed anxiety-like and depressive-like behaviors ($p < 0.001$) in the vehicle group. Compared with the vehicle group, the weights of rats were significantly increased ($p < 0.001$) and the anxiety-like and depressive-like behaviors were improved ($p < 0.001$) in the LPM570065 group. The results of RRBS showed that there were 49964 promoters showed hypermethylation in the LPM570065 treatment rats contrasted to the vehicle treatment rats. In addition, these promoters were enriched in signal transduction and immune function. Furthermore, the expressions of DNMT1 and DNMT3a were significantly decreased, the density of dendritic spines was significantly increased in hippocampi of LPM570065 treatment rats compared with the vehicle treatment rats. Conclusions: LPM570065 ameliorates anxiety-like and depressive-like behaviors in CUMS rats, and its mechanism is possible associated with downregulating DNA methylation in hippocampus.

Keywords: LPM570065, generalized anxiety disorder, western blot

1 Introduction

Generalized anxiety disorder (GAD), clinically called chronic anxiety disorder, is the most common manifestation of anxiety disorder, which endangers both the physical and psychological health of people [1]. The GAD of onset process is long, charactering with excessive worry, tension, fear, dry mouth, sweating, palpitation and other symptoms [2]. However, the mechanisms of GAD have been fully elucidated.

Accumulating evidences have showed that GAD is related to biochemical and psychological factors, and the decreases of presynaptic release of monoamine neurotransmitters norepinephrine (NE), 5-hydroxytryptamine (5-HT) and dopamine (DA) are considered to be the main cause. The three monoaminergic neurotransmitter systems interact with each other and regulate different human emotions. In patients with depression order and anxiety disorder, due to the dysfunction of brain regions, including hippocampus, amygdala and prefrontal cortex, the dysfunction of neurotransmitter system leads to a variety of anxiety emotions, including negative emotions, decreased attention, cognitive impairment, *etc.* [3] At present, there are three kinds of first-line drugs for the treatment of GAD, including benzodiazepines (BZD), representing diazepam and alprazolam; 5-hydroxytryptamine reuptake inhibitors (SSRI), representing paroxetine and escitalopram; and 5-hydroxytryptamine and norepinephrine reuptake inhibitors (SNRI), representing duloxetine and venlafaxine [4]. However, clinical data show that these drugs can cause various neurological diseases, such as headache, mania, sedentary disorder, restlessness and insomnia, and may increase the suicidal tendency of some individuals. Sexual dysfunction

and intestinal adverse reactions are also the defects of these drugs [5]. The search for safer antidepressant therapies is becoming an urgent need.

LPM570065 is a new chemical entity and a 5-HT/NE/DA triple reuptake inhibitor, and approved for listing in June 2021 [6]. LPM570065 showed a high binding affinity with serotonin transporter (SERT), norepinephrine transporter (NET) and dopamine transporter (DAT), and increased the release of 5-HT, NE and DA in striatum after oral administration [7]. The results of its phase III clinical trial showed that LPM570065 could improve depressive symptoms, especially rapidly improve anxiety, pleasure loss and cognitive function, and do not cause drowsiness, do not affect sexual function, weight and lipid metabolism.

In this study, we aimed to analyze the effects of LPM570065 on anxiety-like and depressive-like behaviors in CUMS rats, and to further study its potential epigenetic mechanism.

2 Materials and methods

2.1 Animals

Seventy-two male Sprague Dawley rats, specific pathogen free grade, weighting 140-180 g, were purchased from Jinan Pengyue Experimental Animal Breeding Co,. Ltd. (Approval: SCXK(Lu)20190003). All animals were adapted to the laboratory environment for 3 days. All experiments were conducted in accordance with the Guidelines for the Care and Use of Experimental Animals issued by the National Institutes of Health, and were reviewed and approved by the Ethics Committee of Experimental Animals of Yantai University (Approval: YTU20200226).

2.2 Drugs

LPM570065 (Luye Pharma Group, China) wassuspended with 0.5% sodium carboxymethylcellulose (SCMC). The 0.5%SCMC was obtained through 5 g CMC-Na solid power (Shanghai Shengguang Edilble Chemicals Co., Ltd, China) dissolved in 1 L of sterile deionized water by heating and stirring. Diazepam (Northeast Pharmaceutical Group Shenyang No.1 Pharmaceutical Co., Ltd, China) was also suspended with 0.5% SCMC.

2.3 CUMS model

The animals were received chronic unpredictable mild stimulation for 21 days, 5-6 animals per cage. Select one kind of stimulation to deal with the rats every day, and arrange it in order without repetition in order to rats could not predict the occurrence of the stimulation (Table 1). The body weight was measured once a week.

Table 1 Chronic stress stimulation process

Time	9:00	Time	9:00
Day 1	Swimming in cold water	Day 12	Shake cage 10 min
Day 2	Binding for 2 h	Day 13	Fasting for 12 h
Day 3	Wet cage	Day 14	Without water for 12 h
Day 4	Clip tail	Day 15	Clip tail
Day 5	Shaking for 10 min	Day 16	Binding for 2 h
Day 6	Fasting for 12 h	Day 17	Wet cage
Day 7	Without water for 12 h	Day 18	Clip tail
Day 8	Swimming in cold water	Day 19	Shaking for 10 min
Day 9	Fasting	Day 20	Fasting for 12 h
Day 10	Wet cage	Day 21	Without water for 12 h
Day 11	Clip tail		

2.4 Animal treatment

The rats were randomly divided into control, vehicle (0.5% CMC-Na), LPM570065 (32 mg/kg) and diazepam (3 mg/kg) groups, 12 rats in each group. The rats were treated with drugs by oral gavage once daily for 4 weeks beginning from 15 days after stimulation.

2.5 Elevated zero maze (EZM)

The EZM test was performed in a room with dim light and quiet environment at 25°C. The maze (Gene&I, Beijing, China) is a black circular runway (100 cm × 10 cm), elevated 70 cm from the floor, consisting of two equal closed arms and two equal open arms. During the test, the rats were placed with the closed arm facing the open arm. The residence time of the rats in the open arm and the closed arm within 5 mins was recorded and analyzed by TopScan monitoring system. After the test of each animal, the table was wiped and cleaned with alcohol. During the

test, the groups were kept parallel, and the experimental process was always quiet under low light conditions. The test is controlled in the morning.

2.6 Novelty-suppressed feeding (NSF)

The NSF test was performed in a cuboid test box with an open top, covering with padding about 2 cm thick and placing 6 feed pellets in the center. After fasting for 24 h, the rats were put into the box from the same corner and began to count. The time taken to start chewing food was recorded as the standard, which was defined as the feeding incubation period. The test lasted for 5 min, and the incubation period of food intake for those who did not fast within 5 min was recorded as 5 min.

2.7 Forced swimming test (FST)

The test was performed in a glass cylindrical tank (100 cm $\times$ 40 cm), filling with fresh water (23$\pm$2°C) to the depth of 40 cm. The duration of immobility was considered as the time when the rat made only the small movements necessary to keep the head above water. Total duration of immobility was record during the last 4 min of a 6-min time frame.

2.8 Reduced representation bisulfite sequencing (RRBS)

DNA was extracted from the hippocampi of rats in the vehicle group and the LPM570065 group using the QIAamp Fast DNA Tissue Kit (Qiagen, Dusseldorf, Germany). The MspI restriction endonuclease cleaves the DNA sequence at the location of the methylated cytosine, with a clear methylation site, and enriches the CpG site through enzymatic digestion. The DNA was modified and connected by methylation connectors at both ends, and CpG enriched DNA fragments were selected to prepare a sequencing library. PCR technology was used to amplify the library fragments. The data was sequenced and analyzed to determine differential methylation regions (DMRs).

2.9 Western blot

The rats were sacrificed through decapitation to obtain brain tissues. The hippocampi were put into a RIPA buffer (#R0010, Solarbio, China) for 5 min, then centrifuged (140000 r/min, 15 min) to collect the supernatants. The protein concentration was determined by BCA method. Proteins (30 μg) were separated using 12% SDS-PAGE, then transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore). The membranes were blocked with 5% milk then incubated with primary antibodies diluted in 5% BSA overnight at 4 °C. The primary antibodies were included rabbit anti-DNMT1 (1:1,000, #AG1775, Beyotime, China), rabbit anti-DNMT3a (1:1,000, #AF1732, Beyotime), and rabbit anti-GAPDH (1:1,000, #AG8015, Beyotime). The membranes were then incubated with HRP-linked anti-rabbit IgG (H+L) (1:1,000, #A0208, Beyotime) for 120 min at 37°C. The signals were visualized using an enhanced chemiluminescence substrate detection kit (#P0018M, Beyotime). The quantitative analysis of results was analyzed by ImageJ software [8].

2.10 Golgi staining

The Rapid Golgi Staining kit (FD, NeuroTechnologies, MD, US) was used to observe the density of dendritic spines. Briefly, fresh brain tissues were placed in impregnating solutions (A and B) for 24 h without light, then changed the impregnating solutions (A and B) and stored for 14 days. After that, the brains were transferred to solution C for 3 days. Sections (150 μm) of brain were mounted on gelatin-coated microscope slides and immersed in a mixture of solutions D and E. After elution and dehydration, the sections were coated with resin mounting medium. The results were observed under a German ZEISS microscope, and the numbers of dendritic spines in hippocampal CA1 were analyzed by ImageJ software [9].

2.11 Statistical analysis

Data were analyzed by GraphPad Prism 8 software, and showed as mean$\pm$SEM. One-way ANOVA with Dunnett test were used for data analysis. P-value less than 0.05 was considered statistically significant.

3 Results

3.1 LPM570065 improved CUMS-induced weigh loss

As shown in Table 2, the weights of rats were obviously decreased after the CUMS stimulation compared with the control group ($p < 0.001$). After treatment with LPM570065 (32 mg/kg) or Dizaepam (3 mg/kg), the weights of rats were significantly improved when contrasted to the

vehicle treatment ($p < 0.001$). Additionally, no significant difference was found between the LPM570065 group and the Dizaepam group.

Table 2 Weight of rats in each group

Groups	n	Weights
Control	12	352.25±14.73
Vehicle	12	243.42±23.71###
LPM570065	12	292.25±10.91***
Dizaepam	12	297.67±17.80***

Note: N = 12, mean±SEM. Compared with the control group, ### $p < 0.001$; compared with the vehicle group, *** $p < 0.001$.

3.2 LPM570065 improved anxiety-like behaviors in CUMS-induced rats

The anxiety-like behaviors were evaluated by EZM test (Figure 1) and NSF test (Figure 2). As shown in Figure 1, the open time of rats in the vehicle group was significantly decreased contrasted to the control group ($p < 0.001$). Compared with the vehicle group, the open time was clearly increased in the LPM570065 group or Dizaepam group ($p < 0.001$). Similarly, the CUMS treatment resulted a significant decrease in feeding incubation period ($p < 0.001$), and the effect was prevented by LPM570065 administration in CUMS-treated rats ($p < 0.001$), suggesting that LPM570065 improved anxiety-like behaviors induced by CUMS.

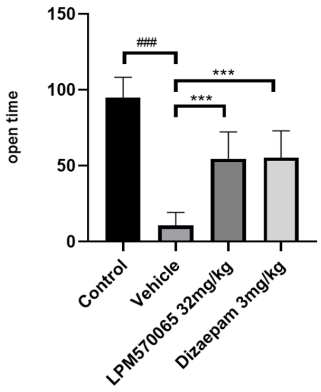

Figure 1 The results of elevated zero maze (EZM) test in each group. N = 12, mean±SEM. Compared with the control group, ### $p < 0.001$; compared with the vehicle group, *** $p < 0.001$.

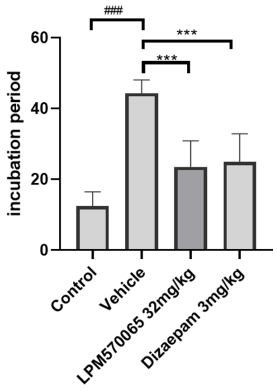

Figure 2 The results of novelty-suppressed feeding (NSF) test in each group. N = 12, mean±SEM. Compared with the control group, ### $p < 0.001$; compared with the vehicle group, *** $p < 0.001$.

3.3 LPM570065 improved depressive-like behaviors in CUMS-induced rats

The depressive-like behaviors were evaluated by FST test. As shown in Figure 3, the immobility time of rats in the vehicle group was significantly increased contrasted to the control

group ($p < 0.001$). Compared with the vehicle group, the immobility time was clearly decreased in the LPM570065 group or Dizaepam group ($p < 0.001$), suggesting that LPM570065 improved depressive-like behaviors induced by CUMS.

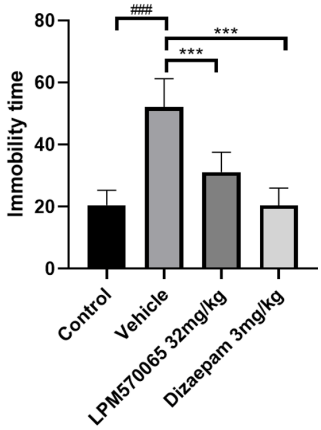

Figure 3 The results of forced swimming test (FST) in each group. N = 12, mean±SEM. Compared with the control group, $^{###}$ $p < 0.001$; compared with the vehicle group, *** $p < 0.001$.

3.4 LPM570065 regulated the level of DNA methylation in hippocampi of CUMS-induced rats

As shown in Figure 4, there were 49964 promoters DMRs showed hypermethylation and 14619 promoters DMRs showed hypomethylation in hippocampi of the LPM570065 treatment rats (C group) contrasted to the vehicle treatment rats (V group). By performing KEGG enrichment and GO enrichment on both groups (Figure 5A and Figure 5B), more differential DNA methylation are enriched in signal transduction (17%) and immune function (15%). In terms of molecular biological function, more differential DNA methylation are enriched in DNA replication and transcription.

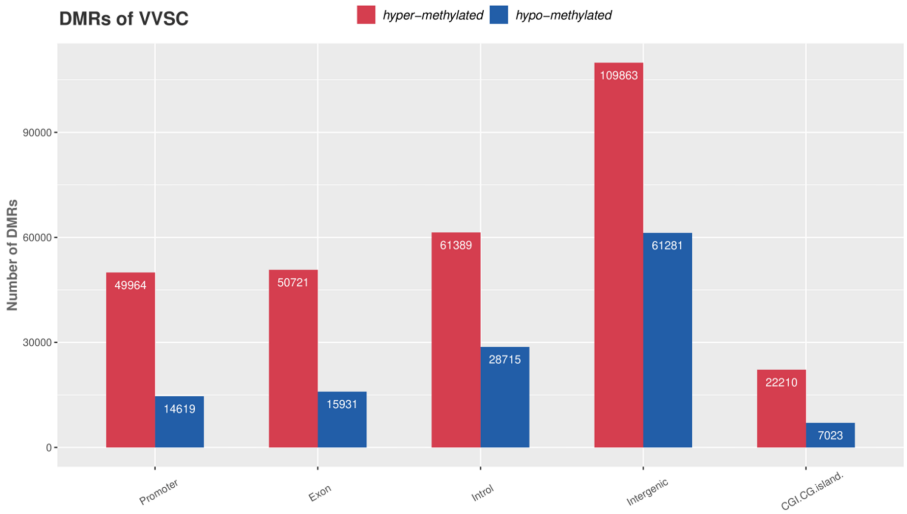

Figure 4 Changes of DMRs in hippocampi between the LPM570065 treatment rats (C group) and the vehicle treatment rats (V group).

3.5 LPM570065 decreased the expressions of DNMT1 and DNMT3a proteins in hippocampi of CUMS-induced rats

The DNA methylation in hippocampi was observed in the control, vehicle and LPM57005 groups by western blot (Figure 6). The expressions of DNMT1 and DNMT3a proteins in hippocampi were obviously increased in the vehicle group compared with the control group ($p < 0.001$). Importantly, the LPM570065 treatment was clearly downregulated the levels of DNMT1 and DNMT3a proteins ($p < 0.001$).

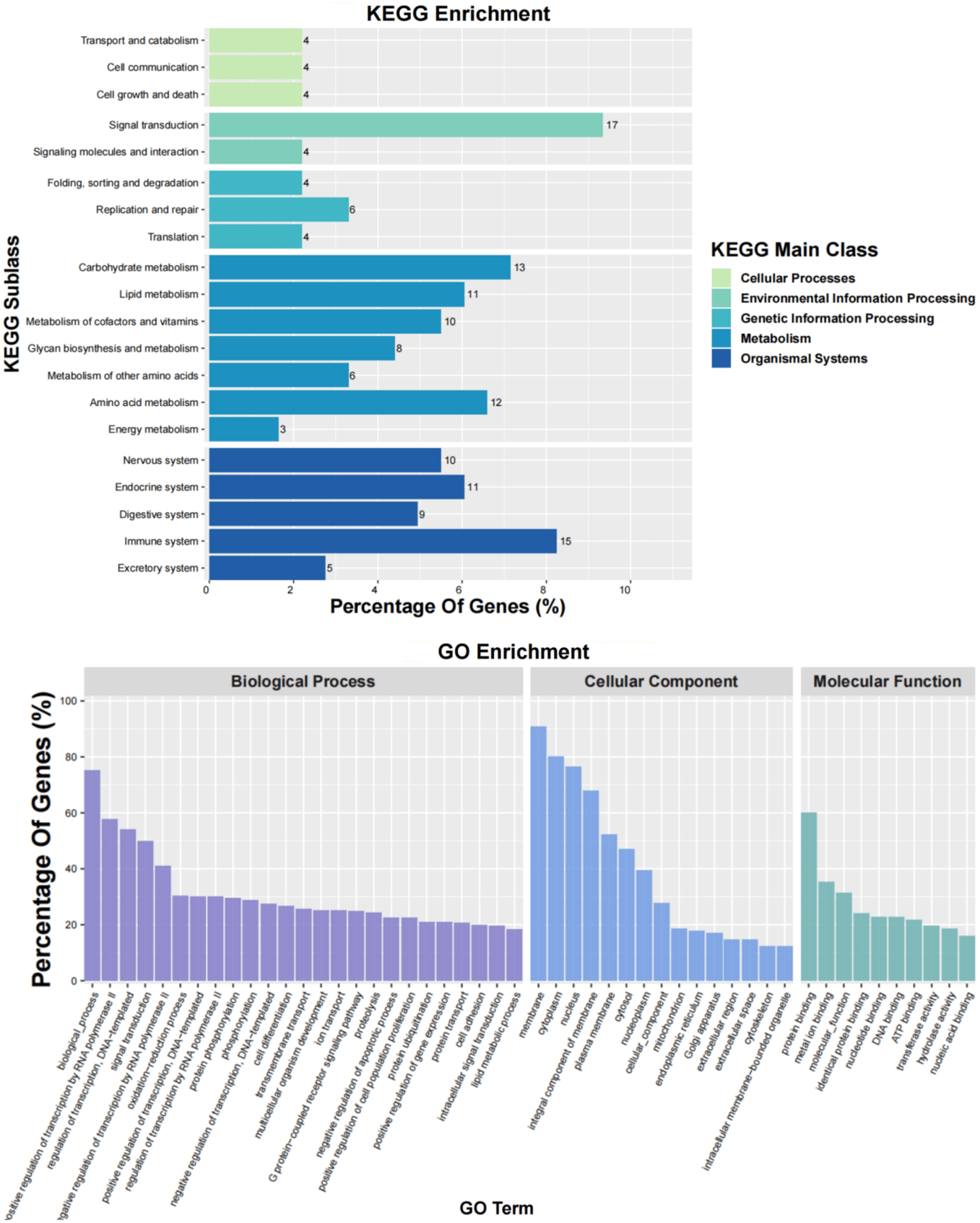

Figure 5 KEGG enrichment and GO enrichment. (A) Differential DNA methylation are enriched in signal transduction (17%) through KEEG enrichment; (B) Differential DNA methylation are enriched in DNA replication and transcription through GO enrichment.

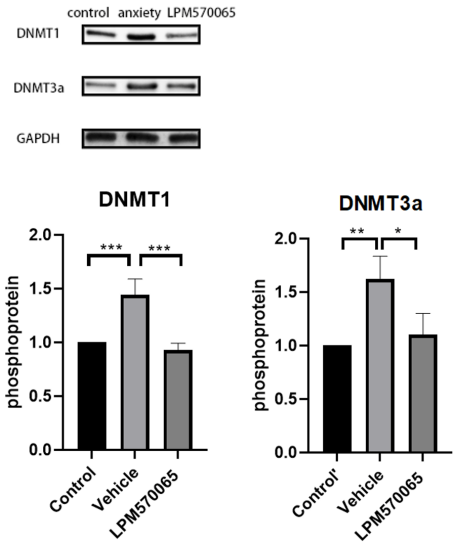

Figure 6 Expressions of DNMT1 and DNMT3a protein in hippocampi of each group were analyzed by western blot. N = 3, mean±SEM. Compared with the control group, $^{\#\#\#}$ $p < 0.001$; compared with the vehicle group, *** $p < 0.001$.

3.6 LPM570065 increased numbers of dendritic spines in hippocampi of CUMS-induced rats

The density of dendritic spines in hippocampi was observed in the control, vehicle and LPM57005 groups by Golgi staining test (Figure 7). The density of dendritic spines in hippocampi were obviously decreased in the vehicle group compared with the control group ($p < 0.001$). Importantly, the LPM570065 treatment was clearly upregulated the density of dendritic spines ($p < 0.001$).

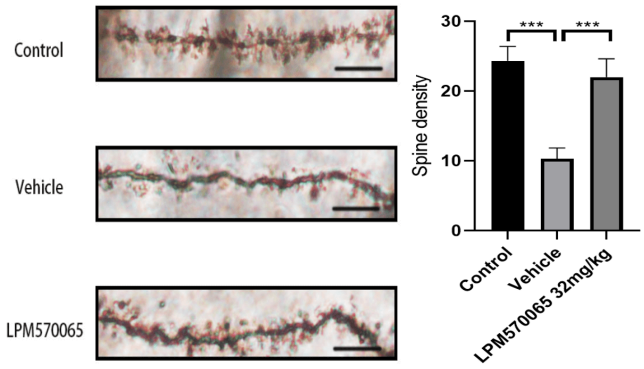

Figure 7 Density of dendritic spines in hippocampi of each group was analyzed by Golgi staining. N = 3, mean±SEM. Compared with the control group, $^{\#\#\#}$ $p < 0.001$; compared with the vehicle group, *** $p < 0.001$.

4 Discussion

Environmental factors of long-term chronic unpredictable sexual stimulation are considered as important inducements of behavioral abnormalities and mental disorders. The clinical manifestations of GAD are anxiety and annoyance, and patients are often worried about some dangerous or unfortunate events that may occur in the future. However, the degree of events is quite different from reality, which is the main symptom of GAD. Patients with GAD are difficult to concentrate so that seriously impact the daily learning and life. Although anxiety-related diseases have been confirmed to be related to the epigenetic mechanism of individuals [10], little is known about the epigenetic mechanism of 5-HT/NE/DA triple reuptake inhibitor mediated its anti-anxiety effect. At present, the first-line treatment drugs are 5-HT reuptake inhibitors (SSRIs), such as fluoxetine and paroxetine [4].

In order to explore the anti-anxiety effects of LPM570065, we chose the CUMS-induced rats to simulate the disease state of patients with GAD. The CUMS belongs to the conditional reflex

model [11], which is easy to operate with high stability. The model uses the psychological experience of fear and contradiction to induce anxiety-like and depressive-like behaviors. As the internationally recognized behavioral evaluation method of anxiety model, EZM is widely used in the study of the pathogenesis of GAD and the screening of anti-anxiety drugs. The EZM is an improved version of the elevated cross maze, avoiding the phenomenon that the central area of the elevated cross maze [12]. In recently years, the NSF test has also been widely used in behavioral evaluation of anxiety models [13]. To make the experimental results more accurate, we chose the EZM test and NSF test to evaluate the anxiety-like behaviors of CUMS-induced rats [14]. For the evaluation of depression, the FST test is used to study the pathogenesis of depression and screen antidepressants [15]. In this study, our data showed that LPM570065 ameliorated anxiety-like and depressive-like behaviors in CUMS rats.

RRBS is an efficient sequencing technology for analyzing methylation levels at the single nucleotide level of the genome [16, 17]. GO enrichment is a database established by the Gene Ontology Consortium, and its annotation classification is usually divided into molecular biological functions, biological processes, and cytological components. KEGG enrichment is a comprehensive database that integrates genomic, chemical, and system functional information, which is commonly used to enrich signal pathways.

As known, DNA methyltransferase (DNMT) plays an important role in maintaining methylation in DNA replication and repair [18, 19]. The changes of DNMT might be related to the change of DNA methylation in the promoter region of stress and generalized anxiety related genes. DNA methylation is mainly caused by DNMT, including DNMT1, DNMT3a and DNMT3b [20]. DNMT1 mainly maintains methyltransferase to establish DNA methylation at the genome, while DNMT3a and DNMT3b can methylate CpG, making it semi-methylated and then fully methylated. In this study, we found that the expressions of DNMT1 and DNMT3a were significantly increased in the hippocampi of CUMS-induced rats, and LPM570065 treatment was clearly suppressed the two proteins expressions, suggesting that LPM570065 might to improve CUMS-induced anxiety-like and depressive-like behaviors by inhibiting DNA methylation in hippocampi. In addition, we found that LPM570065 administration increased the density of dendritic spines in hippocampi of CUMS-induced rats [21, 22], indicating that LPM570065 might to improve GAD by regulating synaptic plasticity.

5 Conclusion

In this study, our results showed that the LPM570065 played a significant anti-anxiety and anti-depression role in rats with CUMS, which mechanisms might be related with decreasing DNA methylation and increasing synaptic plasticity in hippocampi. Our data extend previous preclinical studies of LPM570065 and supporting that LPM570065 could be a useful therapy for GAD.

References

[1] Andreescu C and Lee S. Anxiety disorders in the elderly. Anxiety Disorders: Rethinking and Understanding Recent Discoveries, 2020, **1191**: 561-576. http://doi.org/10.1007/978-981-32-9705-0_28

[2] Arango-Dávila CA and Rincón-Hoyos HG. Depressive disorder, anxiety disorder and chronic pain: multiple manifestations of a common clinical and pathophysiological core. Revista Colombiana de Psiquiatría (English ed.), 2018, **47**(1): 46-55. https://doi.org/10.1016/j.rcp.2016.10.007

[3] Liu Y, Zhao J and Guo W. Emotional roles of mono-aminergic neurotransmitters in major depressive disorder and anxiety disorders. Frontiers in psychology, 2018, **9**: 2201. https://doi.org/10.3389/fpsyg.2018.02201

[4] Murrough JW, Yaqubi S, Sayed S, *et al*. Emerging drugs for the treatment of anxiety. Expert opinion on emerging drugs, 2015, **20**(3): 393-406. https://doi.org/10.1517/14728214.2015.1049996

[5] Zhu J, Klein-Fedyshin M and Stevenson JM. Serotonin transporter gene polymorphisms and selective serotonin reuptake inhibitor tolerability: review of pharmacogenetic evidence. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2017, **37**(9): 1089-1104. https://doi.org/10.1002/phar.1978

[6] Li C, Jiang W, Gao Y, *et al*. Acute, subchronic oral toxicity, and genotoxicity evaluations of LPM570065, a new potent triple reuptake inhibitor. Regulatory Toxicology and Pharmacology, 2018, **98**: 129-139. https://doi.org/10.1016/j.yrtph.2018.07.011

[7] Meng P, Li C, Duan S, *et al*. Epigenetic mechanism of 5-HT/NE/DA triple reuptake inhibitor on adult depression susceptibility in early stress mice. Frontiers in Pharmacology, 2022, **13**: 848251. https://doi.org/10.3389/fphar.2022.848251

[8] Pillai-Kastoori L, Schutz-Geschwender AR and Harford JA. A systematic approach to quantitative Western blot analysis. Analytical biochemistry, 2020, **593**: 113608. https://doi.org/10.1016/j.ab.2020.113608

[9] Du F. Golgi-Cox staining of neuronal dendrites and dendritic spines with FD rapid GolgiStain™ kit. Current protocols in neuroscience, 2019, **88**(1): e69. https://doi.org/10.1002/cpns.69

[10] Bartlett AA, Singh R and Hunter RG. Anxiety and epigenetics. Neuroepigenomics in Aging and Disease, 2017, **978**: 145-166. https://doi.org/10.1007/978-3-319-53889-1_8

[11] Liu W, Xue X, Xia J, *et al*. Swimming exercise reverses CUMS-induced changes in depression-like behaviors and hippocampal plasticity-related proteins. Journal of Affective Disorders, 2018, **227**: 126-135. https://doi.org/10.1016/j.jad.2017.10.019

[12] Kulkarni SK, Singh K and Bishnoi M. Elevated zero maze: a paradigm to evaluate antianxiety effects of drugs. Methods and findings in experimental and clinical pharmacology, 2007, **29**(5): 343-348. https://doi.org/10.1358/mf.2007.29.5.1117557

[13] Pellow S, Chopin P, File SE, *et al*. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of neuroscience methods, 1985, **14**(3): 149-167. https://doi.org/10.1016/0165-0270(85)90031-7

[14] Blasco-Serra A, González-Soler EM, Cervera-Ferri A, *et al*. A standardization of the novelty-suppressed feeding test protocol in rats. Neuroscience Letters, 2017, **658**: 73-78. https://doi.org/10.1016/j.neulet.2017.08.019

[15] Yankelevitch-Yahav R, Franko M, Huly A, *et al*. The forced swim test as a model of depressive-like behavior. Journal of Visualized Experiments, 2015, **97**: e52587. https://doi.org/10.3791/52587

[16] von Känel T and Huber AR. DNA methylation analysis. Swiss Med Wkly, 2013, **143**: w13799. https://doi.org/10.4414/smw.2013.13799

[17] Nakabayashi K, Yamamura M, Haseagawa K, *et al*. Reduced representation bisulfite sequencing (RRBS). Epigenomics: Methods and Protocols. New York, NY: Springer US, 2022, **2577**: 39-51. https://doi.org/10.1007/978-1-0716-2724-2_3

[18] Poh WJ, Wee CP and Gao Z. DNA Methyltransferase Activity Assays: Advances and Challenges. Theranostics, 2016, **6**(3): 369-391. https://doi.org/10.7150/thno.13438

[19] Jin B and Robertson KD. DNA methyltransferases, DNA damage repair, and cancer. Epigenetic alterations in oncogenesis, 2012, **754**: 3-29. https://doi.org/10.1007/978-1-4419-9967-2_1

[20] Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nature Reviews Genetics, 2018, **19**(2): 81-92. https://doi.org/10.1038/nrg.2017.80

[21] Magee JC and Grienberger C. Synaptic plasticity forms and functions. Annual review of neuroscience, 2020, **43**: 95-117. https://doi.org/10.1146/annurev-neuro-090919-022842

[22] Citri A and Malenka RC. Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology, 2008, **33**(1): 18-41. https://doi.org/10.1038/sj.npp.1301559