RNA-Seq analysis of Dendrobium nobile Lindl. alkaloids (DNLA) protection against CUMS-induced anxiety and depression in Fawn-Hooded (FH/Wjd) rats

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Research Article

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Abstract

Background: Dendrobium is the famous Chinese medicine. *Dendrobium nobile* Lindl. alkaloids (DNLA) is the active ingredient and has been shown to have anti-aging, anti-Alzheimer's disease, and neuroprotective properties. This study examined effect of DNLA on chronic unpredictable mild stress (CUMS)-induced anxiety and depression in Fawn-Hooded (FH/Wjd) rats, a depression model in comorbid of alcoholism.

Methods: FH/Wjd rats were subjected to CUMS for 49 days, followed by the treatment with DNLA (20 mg/kg/day, po) for 35 days. The anxiety/depression behaviors, Nissl bodies, neurotransmitters and hypothalamic-pituitary-adrenal axis were examined. RNA-Seq analysis was performed to explore the protection mechanisms.

Results: The CUMS-induced anxiety/depressive-like behaviors were ameliorated by DNLA in the elevated-plus-maze test, open-field test, and forced swimming test. DNLA alleviated. The CUMS reduced Nissl bodies in the hippocampal CA2 region and cortex was alleviated by DNLA. CUMS disrupted 5-hydroxytryptamine, dopamine and gamma-aminobutyric acid in the brain, which were attenuated by DNLA. Increases in serum adrenocorticotropic hormone and corticosterone levels were prevented by DNLA. RNA-Seq revealed CUMS-induced 1600 differentially expressed genes, which were ameliorated with DNLA treatment. KEGG showed the enrichment of Ribosome, Rap1 signaling, tight junction, and RNA transport pathways.

Conclusions: FH/Wjd rats subjected to CUMS were an anxiety and depression model. DNLA protected against CUMS-induced anxiety/depression behaviors and neuronal damage. Multiple mechanisms were involved in DNLA protection, including neurotransmitters, the hypothalamic-pituitary-adrenal axis, and hippocampal gene expressions.

Highlights

- FH/Wjd rats subjected to CUMS have been proposed as a model for depression/anxiety.
- DNLA improved CUMS-induced behavioral deficits in EPM, OPT, and FST tests.
- DNLA ameliorated CUMS-induced hippocampus and cortex neuron damage in FH/Wjd rats.
- DNLA reversed CUMS-induced disruption in GABA, DA, and 5-HT and the HPA axis
- RNA-Seq revealed CUMS-induced aberrant gene expression in brain and effect of DNLA.

1. Introduction

Comorbid anxiety and depression are the major cause of psychiatric disorders (Craske et al. 2017), which increased risk for many diseased and are significant public health burdens (Baxter et al. 2013; Machado et al. 2017). Therapies for anxiety and depression include non-pharmacological interventions and drug treatment, such as selective serotonin reuptake inhibitors, serotonin norepinephrine reuptake inhibitors,

azapirones (e.g., buspirone), mixed antidepressants (e.g., mirtazapine), antipsychotics, antihistamines, alpha- and beta-adrenergic medications, and natural remedies (Garakani et al. 2020). Herbal medicines have been shown to be a promising treatment choice (Sarris 2018).

Dendrobium species are traditional Chinese medicines in the treatment of various diseases including cognitive and psychiatric disorders (Lam et al. 2015). *Dendrobium nobile* Lindl. alkaloids (DNLA) is the main active ingredient and has been demonstrated to have anti-aging properties (Nie et al. 2020), neuroprotective effects against Alzheimer's disease animal models (Li et al. 2022) and Parkinson's disease models (Li et al. 2021). DNLA also exerts beneficial effects on hepatic lipid homeostasis (Huang et al. 2019) and RNA-Seq revealed the molecular mechanism of DNLA protection against CCl_4-induced liver injury (Zhang et al. 2021).

The Fawn-Hooded (FH/Wjd) rats are an inbred strain of rat that has been proposed to be a depression model in comorbid of alcoholism (Rezvani et al. 2002; Wei et al. 2020). FH/Wjd rats exhibit higher corticosterone levels and depressive-like behaviors in the forced swimming tests associated with a dysregulated HPA axis (Knapp et al. 2018). Consequently, the FH/Wjd rats have been used to test anti-anxiety and anti-depression drug candidates. For example, rolipram increased FH/Wjd rats to enter and stay in the open arms of the elevated plus maze test and reduced duration of immobility in both the forced-swim and tail-suspension tests associated with ethanol abstinence (Gong et al. 2017).

We have recently demonstrated that DNLA against chronic unpredictable stress-induced anxiety and depression in SD rats (Xiong et al. 2021). To further verify and extend the beneficial effects of DNLA against anxiety and depression, FH/Wjd rats undergoing chronic unpredictable mild stress (CUMS) were used to determine the effects of DNLA, from behavioral tests, Nissl staining of neurons, neurotransmitters, the levels of corticosterone, adrenocorticotropic hormone (ACTH) to gene expression via RNA-Seq. The beneficial modulating effects of DNLA against anxiety and depression in FH/Wjd rats and potential molecular targets were suggested.

2. Materials And Methods

2.1 DNLA

DNLA was extracted from *Dendrobium nobile*. The dried stems of the herb were extracted by 95% ethanol solution, followed by LC-MS characterization. Alkaloids accounted for 78.3%, including Dendrobine, Dendrobine-N-oxide, Nobilonine, Dendroxine, 6-Hydroxynobilonine and 13- Hydroxy-14-oxodendrobine (Nie et al. 2016; Li et al. 2017; Nie et al. 2018).

2.2 Animals

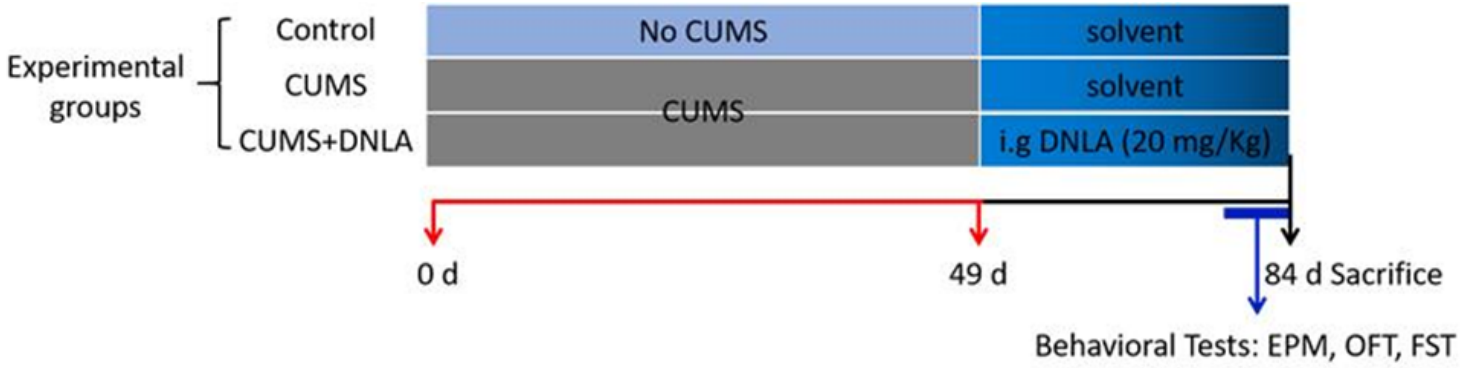
Male Fawn-Hooded (FH/Wjd) rats of 12-month old weighing 350–390 g were initially gifted by Prof. Jian-Hui Liang at Beijing University and maintained in the accredited animal facilities at Key Lab of Basic Pharmacology of Zunyi Medical University on a 12-h light/dark cycle under controlled temperature (22–

25°C). Rats had free access to feed and water. All animal procedures comply with Animal Use and Care Guidelines in China and were approved by the Institutional Animal Care and Use Committee of Zunyi Medical University (2017-08).

2.3 Experimental design

Fawn-Hooded (FH/Wjd) rats were randomly divided into two groups (8 rats in the Control group and 16 rats in the CUMS group). Chronic unpredictable mild stress procedures were performed as described previously (Bondi et al. 2008), with a slight modification. Briefly, rats were exposed to different stressors, namely, food deprivation, water deprivation, empty water bottles (after water deprivation), cage tilt, grouped housing, soiled cage, stroboscopic lighting, 5-min cold swimming (at 45 °C), 1-min tail pinch (1 cm from the end of the tail), physical restraint, illumination and white noise. One of these stressors (in random order) was given every day for 49 days. The Control rats were left undisturbed except for weekly clean cage changes.

CUMS rats were further divided into CUMS group treated with solvent (1% Tween 80, n = 8) and CUMS + DNLA group receiving DNLA 20 mg/kg, intragastrically (i.g.) for 35 days (n = 8). The dose and duration of DNLA administration were based on our recent publications (Li et al. 2020; Liu et al. 2020). Behavior tests were performed after 31 days of treatment during the daytime in the. At the end of 35-day treatment, rats were euthanized, the left brain was used for morphological examination and the right brain was used to perform biochemical and molecular tests. The diagram of the experimental design is shown below:



2.4. Behavioral tests

The elevated plus maze test is a common test for anxiety behaviors (Horii et al. 2018). The apparatus consisted of two open arms and two closed arms (31.25 cm L, 95 cm W, 91.45 cm H; Version 3.0 Topscan, Cleversys, USA). Rats were put in the central area and the head toward the open arm and allowed to explore freely for 10 min. The central area entries, travel distance, and the time stayed in open arms were recorded.

The open field test is a common method for locomotor activity and anxiety behaviors (Seibenhener et al. 2015). The apparatus consisted of square area (100 cm×100 cm×40 cm), and divided into the center and

periphery. The total distance, entries to the center, movement time in center and periphery area were recorded (Topscan, Cleversys, USA).

The forced swimming test (FST) is the most commonly used assays for the study of depressive-like behavior in rodents (Yankelevitch-Yahav et al. 2015). Rats were first habituated to the testing room 30 min prior to testing. A pre-swimming (10 min) was conducted initially 24 h prior to the experiment. On the test day, rats were subjected to the plexiglass cylinder (60 cm tall × 20 cm in diameter) filled with water (24°C) to a depth of 18 cm (ZH-QPT, Anhui Zhenghua, China). Test sessions (5 min) were recorded by a video camera to record the immobility time, which was defined as no additional movement required by the animal to maintain its head above water.

2.5 Nissl staining

The brain was fixed in 4% paraformaldehyde, embedded by paraffin, and cut into coronal sections of 5 μm thick for Nissl staining (Liu et al. 2020). The sections were deparaffinized in xylene and rehydrated using gradual alcohol, incubated with Nissl staining solution (Solarbio, Beijing, China) for 5 min, and mounted with neutral balsam. The cortical region and the hippocampal CA2 region were observed under a light microscope (Leica Microsystems Ltd., Wetzlar, Germany).

2.6. Measurement of neurotransmitters in cortex

2.6.1 Measurement of GABA by ELISA

Cortex tissues were homogenized with cooled PBS (1: 9), and centrifuged at 14000 × g for 10 min at 4 ◦C. The contents of GABA measured using rat enzyme-linked immune-sorbent assay (ELISA) kit (Shanghai Jianglai Biotechology, Shanghai, China) following the manufacture instructions (Zhang et al. 2019).

2.6.2. Measurement of $GABA_A$ receptor by Immunohistochemistry

Each brain was paraffin embedding and sectioned sagittally, with thickness 5 μm. The sections were dewaxed, rehydrated and incubated with 3% H_2O_2 solution for 30 min, then blocked with normal goat serum for 30 min, followed by rabbit polyclonal anti-$GABA_AR$ antibody (1:400 dilution, ab33299, Abcam, England) incubation for 14 h, then washed and subsequently reacted with the biotinylated secondary antibody (SP-0029, Bioss, Beijing, China), then chromogenic reaction, finally, light microscope (Oly-pus BX43, Japan) was used for capturing the image. The immunoreactive staining density was quantified by Image-Pro Plus 6.0 (Media Cybernetics, USA) and measured in integrated optical density (IOD) (Yang et al. 2016).

2.6.3 Measurement of 5-HT, DA and their metabolites

Approximate 80 mg of cortex tissues were homogenized with 1 ml of 5% $HClO_4$ on ice and centrifuged at 14000×g for 10 min at 4°C. The supernatants were mixed with the mobile phase (50 mmol/L $C_6H_8O_7$, 90 mmol/L NaH_2PO_4, 1.7 mmol/L OSA, 0.5 μmol/L Na_2EDTA, and ACN (92:8, v/v). The pH was adjusted to

3.2 with 50% NaOH) at 1:2 ratio and filtered through 0.22 um Nylon membrane, followed by ultrasound degassed for 15 min. The reverse-phase high-performance liquid chromatography with electrochemical detection (RP-HPLC-ECD). The injection volume was 20 µl, the flow rate (0.21 ml/min) and column temperature (30°C) were maintained. The detector parameters were channel I, 150 mV, channel II, 300 mV, Guard Cell 350 mV. The acquisition time and injection volume were 24 min, and 20 µl, respectively. Data acquisition was carried out by EZChrom SI Offline workstation software (Xiong et al. 2021). The content of 5-HT, 5-hydroxyindole acetic acid (5-HIAA), DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the cortical tissue was measured as previously described (Vermeiren et al. 2014).

2.7 Measurement of ACTH and CORT in serum

The contents of ACTH and CORT in serum (50 µl) were measured using rat enzyme-linked immune-sorbent assay (ELISA) kit (Shanghai Jianglai Biotechology, Shanghai, China) following the manufacture instructions as previously described (Gao et al. 2016).

2.8. RNA sequencing

Total RNA was isolated from the hippocampal tissues via Trizo and reverse transcribed with Oligo dT primer to produce cDNA, and dsDNA samples were generated on the ice under the action of DNA polymerase, RNase H enzyme and T4 DNA ligase. Then dsDNA was purified by magnetic separation and Tn5 marker was carried out. The samples are then coded according to the TruePrep DNA library preparation kit and P5/P7 adapter primers (Vazyme, cat. TD503), and purified by THE VAHTS™ DNA cleaning Bead kit (Vazyme, cat. N411-02) was used for enrichment PCR amplification. Finally, Trimmomatic data cleaning and filtering software was used to evaluate the quality of library. Illumina HiSeq platform was used for RNA-Seq data sequence analysis of 150-BP end-to-end model, and enrichment analysis was performed in GO database and KEGG database by Genesky Biotechnologies Inc., (Shanghai, China).

2.9. Bioinformatic Analysis

The raw RNA-Seq data (~43000/sample) was subjected to Principle Component Analysis (PCA) via Partek Flow (Partek Inc., St. Louis, MO), and grouped by Control, CUMS, CUMS + DNLA (3 sample/group), respectively to generate PCA images to visualize the distribution patterns.

The DESeq2 method ($p < 0.05$ and Log2fold > 2) was used to define differentially expressed genes (DEGs). The Gene Cluster version 3.0 was used to generate hierarchical complete linkage of DEGs, and imported into TreeView version 1.6 to generate the 2D-heatmap for visualization.

2.10. Statistical analysis

Statistical analysis was carried out using SPSS 20 software. All values are expressed as mean ± SEM. Comparisons among three groups were analyzed by one-way ANOVA, followed by Bonferroni test. The significance level was set at $P < 0.05$ in all cases.

3. Results

3.1. Animal body weight gain

Forty-nine days of CUMS slightly decreased animal body weight gain starting at Day 14, and the body weight remained less than Controls at the end of experiments. DNLA treatment for 5 weeks (from 49 to 84 days after CUMS) increased the body weight from Day 49 of 356 g to Day 84 of 383 g. At the end of the DNLA treatment, the body weights were 384 ± 9, 371 ± 13, and 380 ± 12 g for Control, CUMS + DNLA, and CUMS groups, respectively. DNLA treatment slightly increased the body weight by approximately 25 g. No mortality occurred during the experimental period.

3.2.Behavioral tests

Anxiety-related behavior was evaluated via the elevated plus maze (Fig. 2A and Fig. 2B). The percentage of the open arm entries was decreased from 31–18% in CUMS rats, and the time spent on the open arm was decreased from 6.1–2.9%, suggesting anxiety-like behavior. The percentage of open arm entries and the time spent on open arms were slightly increased by DNLA, although it was not statistically significant.

The spontaneous activity and learning/memory function were evaluated by the open field test (Fig. 2C and Fig. 2D). The percentage of travel distance in central zone was decreased from 3.8% in Control to 0.4% in CUMS rats; however, the total travel distance did not differ among groups (5.8, 5.6, and 5.5 m for Control, CUMS, and CUMS + DNLA, respectively)

The immobility time in forced swimming test is used to evaluate the depressive-like behavior. The immobility time tended to increase by 15% in CUMS rats as compared with the Control, DNLA treatment significantly reversed the CUMS-increased immobility time (Fig.2E).

3.3. Nissl staining

The hippocampus is responsible for cognitive functions, and hippocampal CA2 region is essential for social memory (Hitti et al. 2014). Consistent with our previous studies in the brain of SD rats with CUS (Xiong et al. 2021), FH/Wdj rats subjected to 49-day of CUMS also showed reduced Nissl bodies and foci of neuron condensation (arrows). DNLA treatment ameliorated these morphological changes (Fig. 3).

3.*4.Effects of DNLA on neurotransmitters in CUMS rats*

3.4.1Effects of DNLA on GABA and the GABA $_A$ receptor

The contents of brain GABA were measured using the ELISA kit for GABA. CUMS tended to decrease brain GABA by 10%, but not significant from Control. DNLA significantly increased cortex GABA content compared to CUMS only rats (Fig. 4A). To further examine the effect of DNLA on GABA system, the expression of $GABA_AR$ receptor in the brain was determined by immunohistochemistry with rabbit polyclonal antibody against anti-$GABA_AR$ (Fig. 4B). CUMS reduced $GABA_AR$ positive cells, which was recovered following DNLA treatment (arrows).

3.4.2Effects of DNLA on DA, 5-HT and their metabolites

Figure 5 shows the effects of DNLA on neurotransmitters in the cortex in CUMS induced FH/Wjd rats. CUMS for 49 days increased 5-HT and the metabolites 5-HIAA, DA and the metabolites DOPAC and HVA in the cortex compared to Control, DNLA treatment for 35 days was able to reverse the effects of CUMS on the 5-HT, DA and their metabolites.

3.5. DNLA attenuated hyperactivity of the HPA axis

CUMS for 49 days increased serum levels of ACTH by 20% (Fig. 5A) and CORT by 25% (Fig. 5B), suggesting the hyperactivity of the HPA axis. DNLA treatment attenuated such increases.

3.6. RNA-Seq analysis of transcriptome of hippocampus

3.6.1. Principle component analysis

All gene counts from RNA-Seq were imported into the Partek Flow Server to generate an overview by Principle component analysis (PCA), with PC1 of 21.69%, PC2 of 15.19%, PC3 of 12.79%, and an overall PCA score of 49.67% (Fig. 7). The gene expression profiles of CUMS (dark red square) were different from CUMS + DNLA (gold plus), and Control (blue circle) groups.

The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichments were performed to better understand CUMS-induced molecular events in FH/Wjd rats. The top 20 of GO enrichments include ribosome structure and function, GTPase mediated signal transduction and ncRNA metabolic process are top enrichments (Fig. 8A). Whereas the top 10 KEGG enriched pathways include Ribosome, Rap1 signaling, tight junction, and RNA transport (Fig. 8B).

3.6.2. Differentially expressed gene analysis

The DESeq2 method with Log2fold > 2 was used to identify differentially expressed genes (DEGs). CUMS vs Control had 1607 DEGs; CUMS + DNLA vs Control had 1586 DEGs; while CUMS + DNLA vs CUMS had 1933 DEGs. The DEGS were subjected to two-dimensional clustering analysis. An example of the enlarged 2D clustering was shown on right panel to visualize the differences among groups (Fig. 9). A 40-gene cluster is shown on the left, which were genes increased by CUMS but prevented by DNLA. Eight genes among them were discussed. The entire DEGs were provided in Supplementary Table 1.

4. Discussion

This study demonstrated anxiety and depression-like behavior produced by CUMS in FH/Wjd rats to verify this genetic rat model to study anti-anxiety/depression drug candidates. DNLA treatment improved behavioral deficits, alleviated neuron loss. The contents of GABA were increased, while the contents of DA, 5-HT and their metabolites in the cortex of CUMS FH/Wjd rats were decreased by DNLA. The hyperactivity of the HPA axis, evidenced by increased levels of CORT and ACTH in CUMS FH/Wjd rats, was also attenuated by DNLA. RNA-Seq showed aberrant gene expressions in FH/Wjd rats. The major

biological process and molecular events were reveals by GO analysis. KEGG showed the enrichment of Ribosome, Rap1 signaling, tight junction, and RNA transport. CUMS produced 1600 differentially expressed genes (DEGs) compared to controls. These DEGs were abolished or attenuated after DNLA treatment as seen in 2D-dimentional clustering comparison. Thus, this study on FH/Wjd rats confirmed the beneficial effects of DNLA, but the underlying molecular targets seem to be different from that observed in SD rats (Xiong et al. 2021).

The CUMS paradigm mimics stress events in life and is frequently used in studying anti-anxiety/depression drug candidates (Sequeira-Cordero et al. 2019). The FH/Wjd rats did not habituate to the repeated stressors over time in respond to CUMS stress. During the CUMS process, animals lost body weight gain as early as the 7th day of CUMS, and after 49 days of CUMS, the body weight gain was reduced by 15%. Anxiety behavioral measures such as the elevated plus maze (Horii et al. 2018), the open field test (Seibenhener et al. 2015), and the forced swimming test (Yankelevitch-Yahav et al. 2015) all points towards anxiety- and depressive-like behaviors in FH/Wjd rats with CUMS. DNLA treatment for 35 days ameliorated these behavioral changes, suggesting that DNLA not only improves anxiety- and depressive-like behavior in SD rats induced by CUS (Xiong et al. 2021), but also in FH/Wjd rats with CUMS. The water extract of *Dendrobium nobile* that contains DNLA as the major ingredient also showed beneficial effects agaibt anxiety behaviors in mice (Li E-L 2018).

The impaired GABA synthesis, uptake and release are associated with depression-like behaviors induced by CUMS (Ma et al. 2016). CUMS disturbs the glutamine-glutamate-GABA cycle in the striatum, hippocampus, and cerebellum (Xu et al. 2020). In the present study, the content of GABA tended to decrease in the cortex as evidenced by ELISA (Fig. 5A) and the expression of $GABA_AR$ receptor in the hippocampus was also decreased in CUMS FH/Wjd rats, as evidenced by immunohistochemistry (Fig. 5). Fortunately, DNLA treatment increased GABA and $GABA_A$ receptor in CUMS rats significantly.

Monoamine neurotransmitters (5-HT, NE and DA) play important roles in anxiety/depression disorders. Antidepressant drugs such as *Panax notoginseng* could increase the brain monoamine levels as a mechanism of action (Xie et al. 2018). However, not all 5-HT receptor agonists are effective against anxiety (Jastrzębska-Więsek et al. 2018; Wankhar et al. 2020). Under the current experimental conditions, CUMS not only produced depression but also anxiety. In rats receiving CUMS over 4 weeks, monoamine levels could be decreased, no change, or even higher (Cox et al. 2011; Xue et al. 2016; Peng et al. 2018; Yang et al. 2019). and anti-anxiety drug could bring the elevated monoamines towards the normal levels (Gao Z 2011), including DNLA in our prior observations (Xiong et al. 2021). Nonetheless, DNLA was able to reverse CUMS-induced abnormality in monoamine neurotransmitters in 12-month-old FH/Wjd rats receiving 49-day of CUMS, and 35-day of DNLA treatment.

CUMS-induced neuron condensation and neuron loss in the hippocampus and cortex (Fig. 3), which were ameliorated by DNLA. DNLA has been shown to improve age-associated reduction of Nissl bodies in the hippocampus and cortex in aged SAMP8 mice (Liu et al. 2020) and in Aβ25–35 induced Nissl body reduction (Nie et al. 2016). Hippocampal CA2 region is a critical hub of socio-cognitive memory

processing essential for social memory, and is an important brain region for anti-depression effects of the Chinese medicine XCHT (Zhang et al. 2016) and DNLA (Xiong et al. 2021).

The hyperactivity of HPA axis is associated with anxiety and depression (Tafet et al. 2020). Increased serum ACTH can stimulate the synthesis and release of corticosterone in the adrenal cortex (Boonen et al. 2015). The present study demonstrated that CUMS increased the levels of ACTH and corticosterone, consistent with hyper-reaction of the HPA axis. DNLA treatment ameliorated these adverse effects not only in CUS SD rats, but also in FH/Wjd rats subjected to CUMS.

RNA-Seq has provided opportunities to understand anxiety and depression associated gene expression changes as described recently (Xiong et al. 2021). In the present study, it is clear that gene expression patterns in CUMS group were dispatched away from Controls in PCA analysis, and DNLA treatment brought the aberrant gene expressions towards normal Controls (Fig. 7), indicating the beneficial effects of DNLA on aberrant gene expression associated with anxiety and depression.

GO enrichment analysis (Fig. 8A) pointed towards the top molecular events associated with biological processes, molecular functions, and cellular components as a clue for occurred molecular events (Ashburner et al. 2000). KEGG annotate genes to pathways (Kanehisa et al. 2010). In CUMS FH/Wjd rats, Ribosome, Rap1 signaling, tight junction, and RNA transport were the major molecular pathways affected.

To better view the DEGs among groups, the 2D-cluster was used to compare the gene expression pattern among groups (Yu et al. 2012; Xiong et al. 2021). In the present study, several clusters were evident for visualization. An example of the 40-gene cluster was shown on the left of Fig. 9. DNLA prevented CUMS-induced upregulation of these genes in this cluster. For example, Network pharmacology has identified nuclear receptor coactivator 2 (Ncoa2) as a key targets for Chinese medicines to treat depression (Shi et al. 2021); Accumulating evidence points towards the nerve growth factor (NGF) as a key modulator in the treatment of depression (Mondal et al. 2019); GNAS complex locus (Gnas) is associated major depression disorder (Yi et al. 2012); guanosine triphosphate cyclohydrolase 1 (Gch1) is associated with depressed mood and anxiety in labor pain (Pettersson et al. 2016); Transient receptor potential canonical channels 3 (Trpc3) mediates the induction of cerebellar long-term depression (Kim 2013); High expression of neurocalcin delta (Ncald) is evident in CUMS rats (Zhang et al. 2021); Neuronal colony stimulating factor 1 (Csf1) could modulate microglia function and behavioral consequences after CUMS (Wohleb et al. 2018); Mediator complex subunit 12 (Med12) is a candidate gene implicated in major depressive disorder and complex human behavioral disease. The entire clusters of DEGs were provided in Supplementary Table 1. Frankly speaking, roles of majority of genes in anxiety and depression remain unknown, and require further investigation.

In summary, the present study demonstrated that FH/Wjd rats subjected to CUMS were an anxiety and depression model. DNLA treatment for 35 days ameliorated CUMS-induced anxiety and depression behaviors, reduced pathological damage to the brain. The protection mechanisms may be related to

neurotransmitters, hyperactivity of the HPA axis, and modulation of gene expressions in the hippocampus.

Abbreviations

DNLA, *Dendrobium nobile* Lindl. alkaloids

CUMS, chronic unpredictable mild stress

FH/Wjd, Fawn-Hooded inbred strain of rats

ACTH, adrenocorticotropic hormone

EPM, the elevated plus maze test

OFT, the open field test

FST, the forced swimming test

GO, Gene Ontology

KEGG, Kyoto Encyclopedia of Genes and Genomes

PCA, Principle Component Analysis

DEGs, differentially expressed genes

Declarations

Declaration of Competing Interest

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Ting-Wang Xiong: Conceptualization, Methodology, Data curation, Visualization, Writing - original draft. **Bo Liu:** Writing - review & editing, visualization, **Qin Wu:** resources, review & editing, **Yunyan Xu:** Data curation, review & editing. **Jie Liu:** review & editing, **Jing-Shan Shi:** Supervision, project administration, funding acquisition, review & editing.

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Supplementary data

Supplementary materials (STable 1: 2-Dclustering of differentially expressed genes) related to this article can be found in the online version.

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Figures

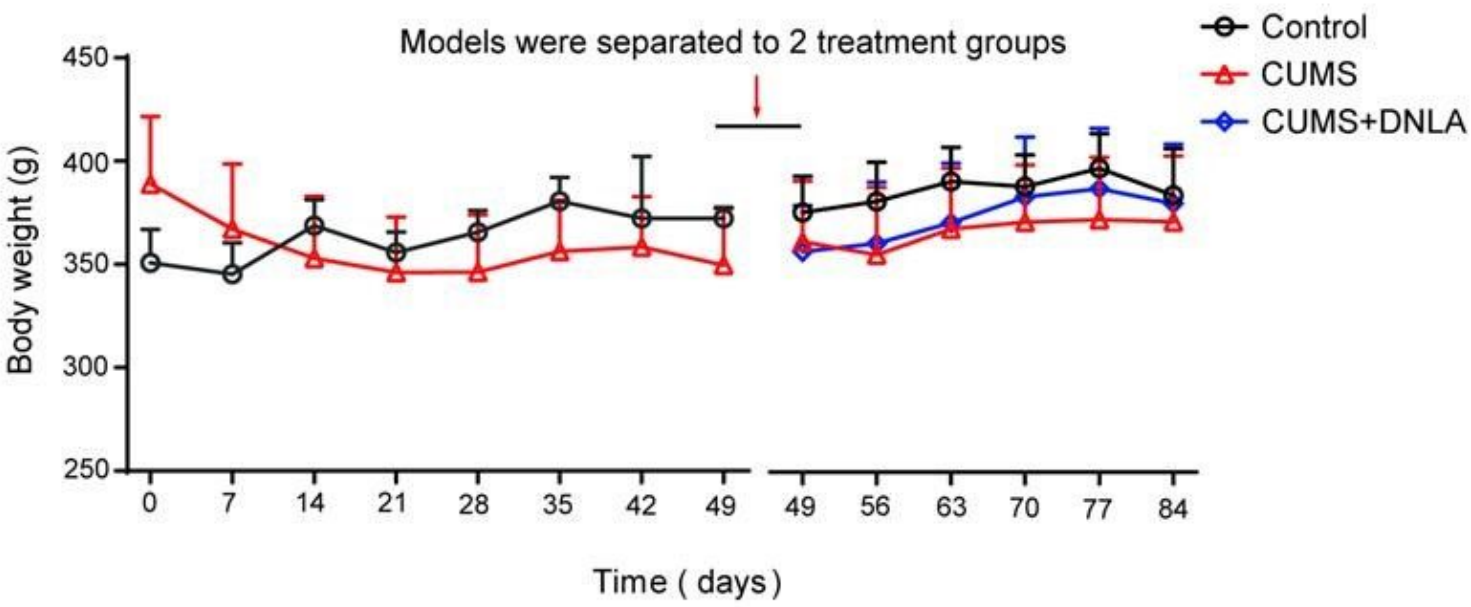

Figure 1

Animal body weights. Twenty-four FH/Wjd rats were randomly divided into two groups: Control group (8 rats), CUMS group (16 rats). Chronic unpredictable mild stress was applied to CUMS group for 49 days. Subsequently, CUMS group rats were further randomly divided into CUMS and CUMS+DNLA (20 mg/kg, po) groups (8 rats/ group). Data expressed as mean ± SEM, n=8-16 before treatment, n=8 after treatment.

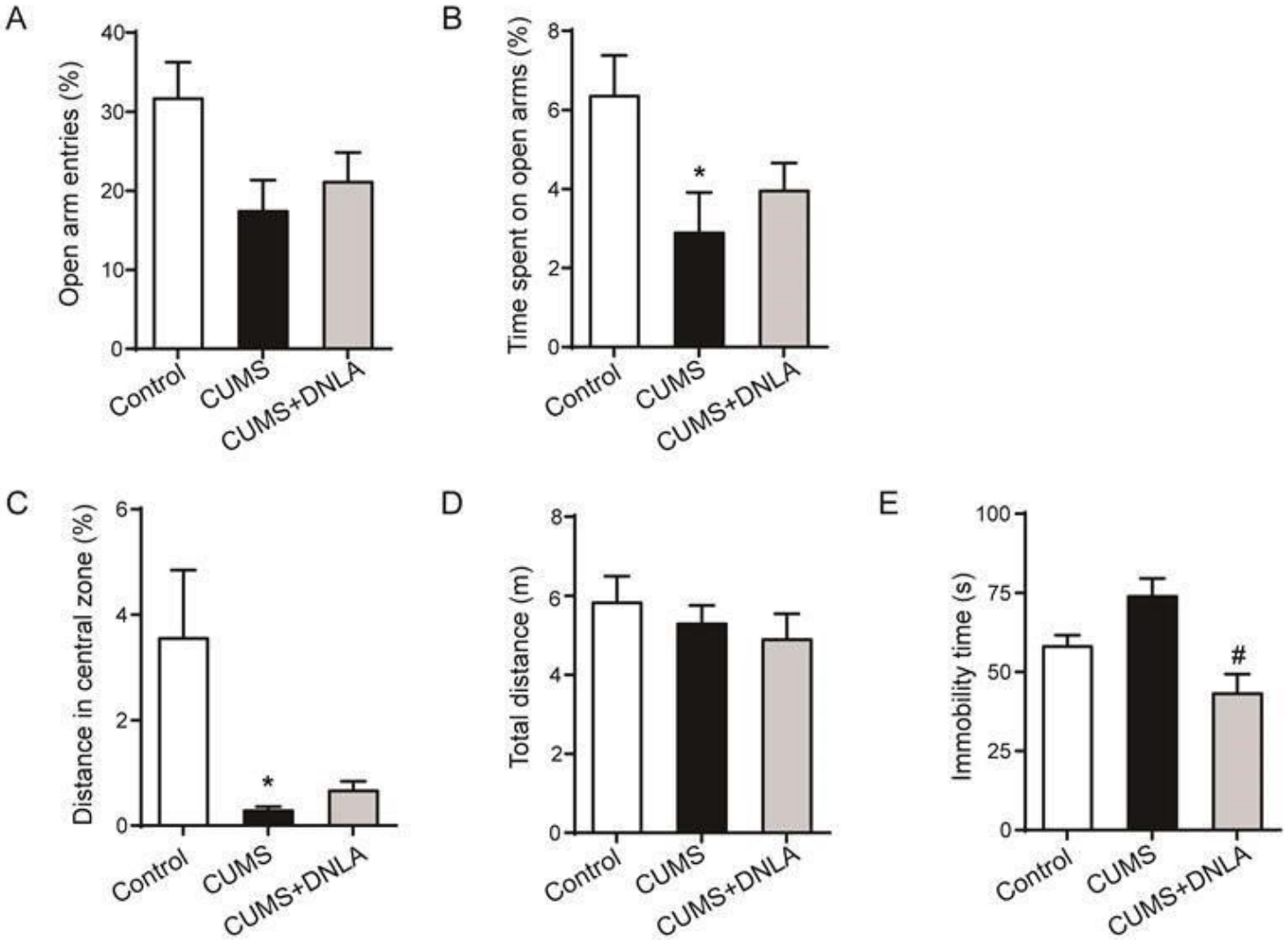

Figure 2

Effects of DNLA on elevated plus-maze test (A, B), open field test (C, D) and forced swim test (E) in CUMS rats. Data expressed as mean ± SEM, n=8, $^{*}P<0.05$ vs Control group; $^{\#}P<0.05$ vs CUMS group.

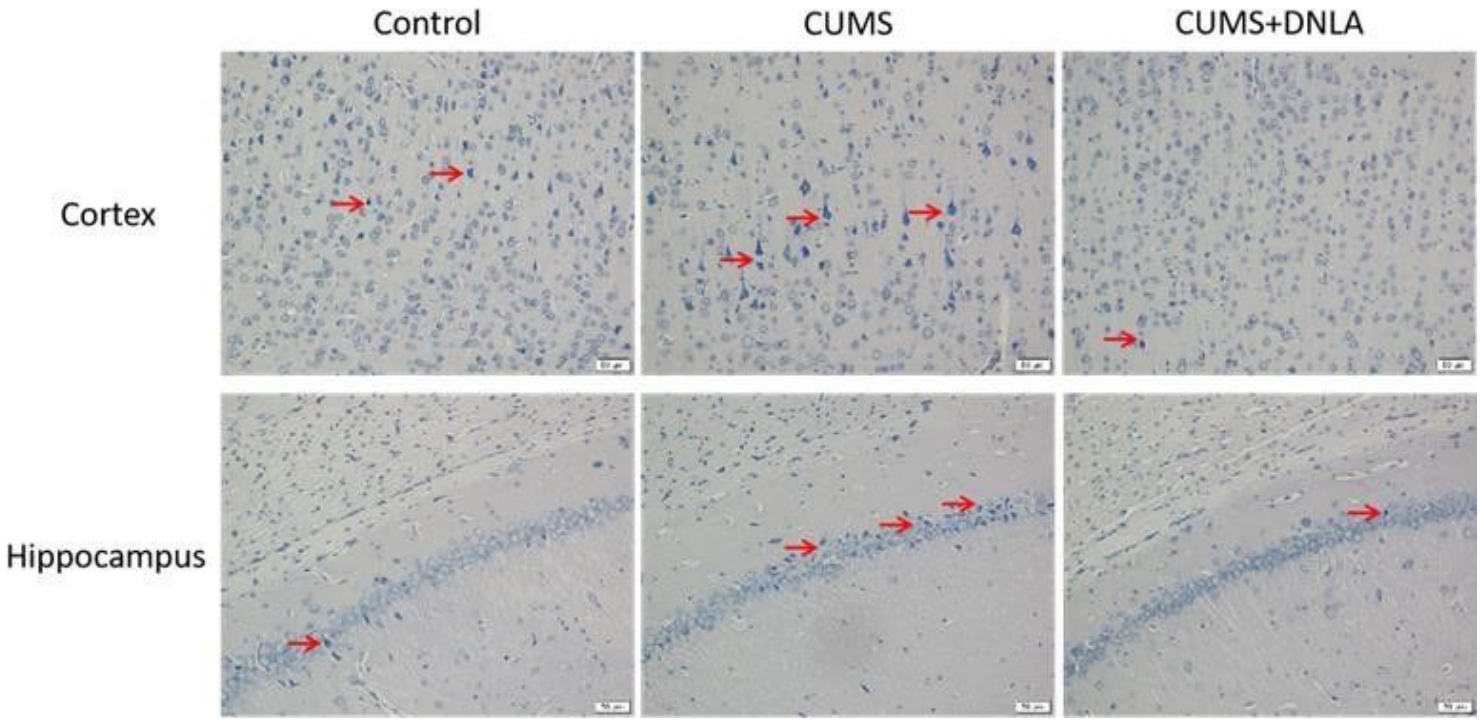

Figure 3

Effects of DNLA on cortical and hippocampal neurons in CUMS induced FH/Wjd rats. Sections of the cortex and the hippocampus CA2 region were stained with Nissl bodies. Arrows indicates neuron degeneration. Magnification 200×, scar bar = 50 μm.

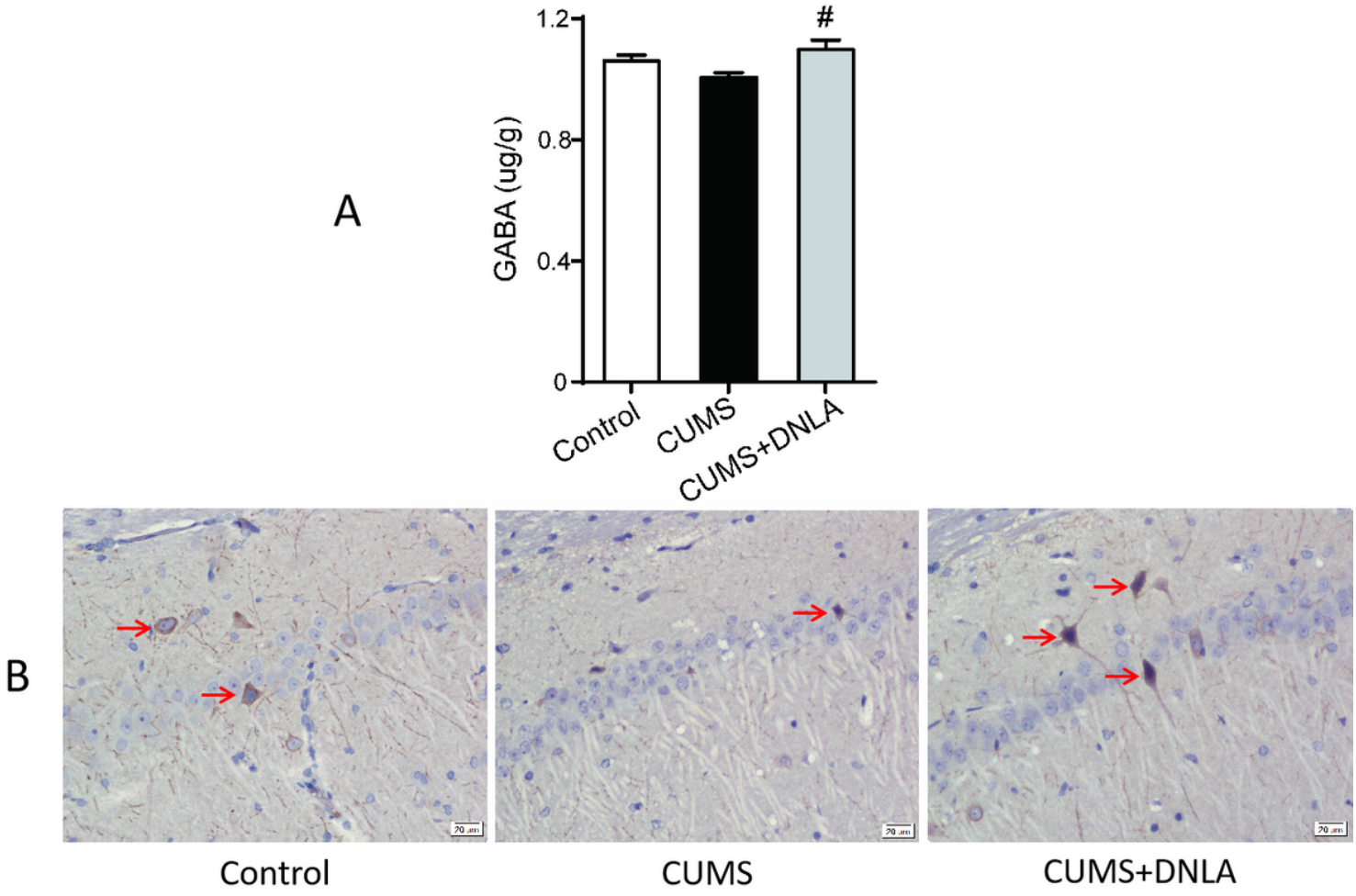

Figure 4

Effects of DNLA on GABA and $GABA_A$ receptor in CUMS rats. (A), GABA content in cortex tissues as determined by ELSIA. Data were mean ± SEM, n=8, $^{\#}P<0.05$ vs CUMS group; (B), immunohistochemistry of $GABA_AR$ staining in the hippocampus. Arrows indicate positive $GABA_AR$ cells. Magnification 400×, scar bar = 20 μm.

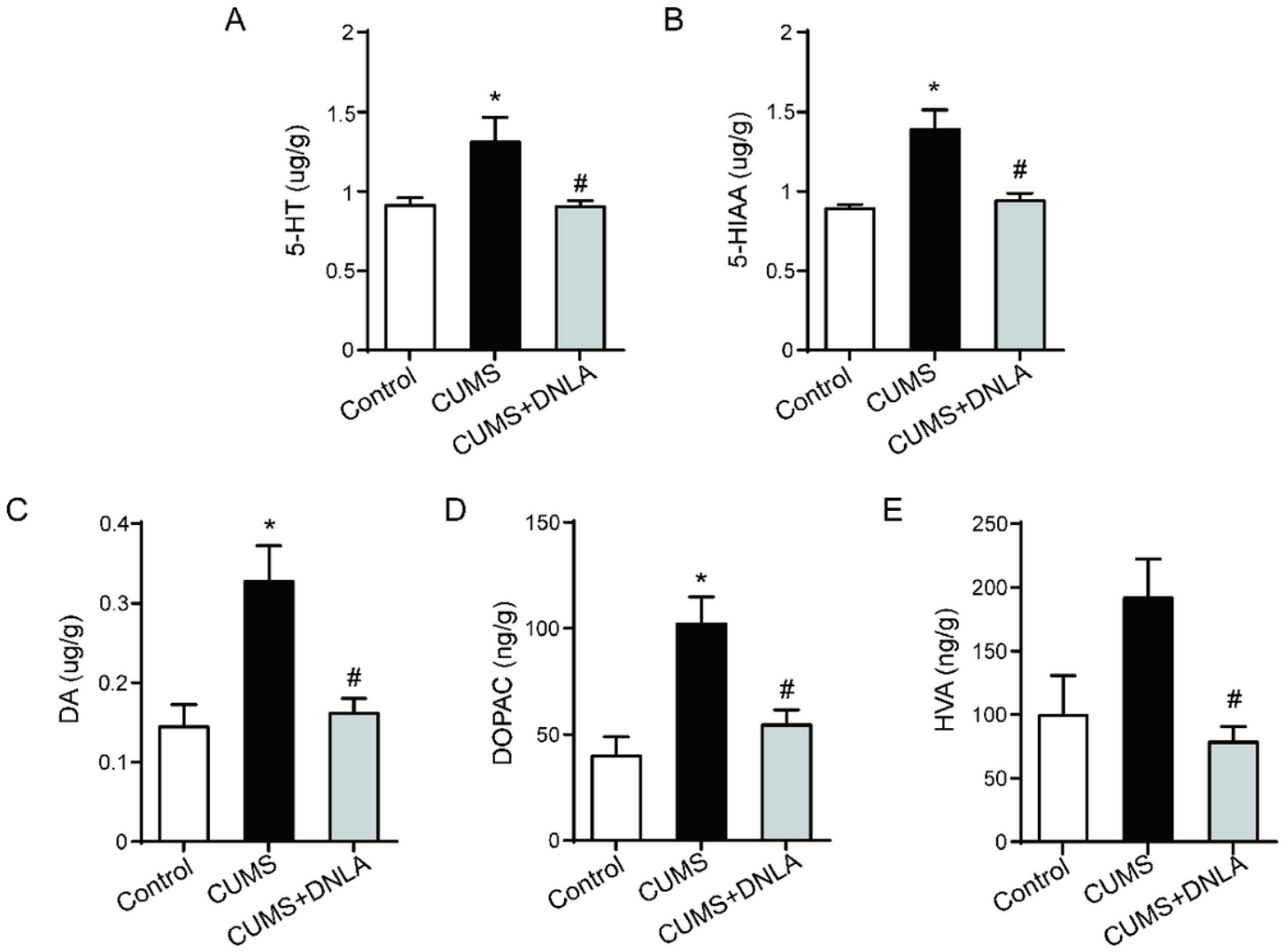

Figure 5

Effects of DNLA on 5-HT, DA and their metabolites in the cortex. (A) The contents of 5-HT(A), 5-HIAA(B), DA(C), DOPAC(D), HVA(E) determined by HPLC in the cortex are shown. Data expressed as mean ± SEM, n=8, *P < 0.05 vs Control group; #P <0.05 vs CUMS group.

Figure 6

Effects of DNLA on the HPA axis in CUMS induced FH/Wjd rats. The contents of CORT (A) and ACTH (B) in serum were measured by ELISA. Data expressed as mean ± SEM, n=8, $^{*}P$<0.05 vs Control group; $^{\#}P$<0.05 vs CUMS group.

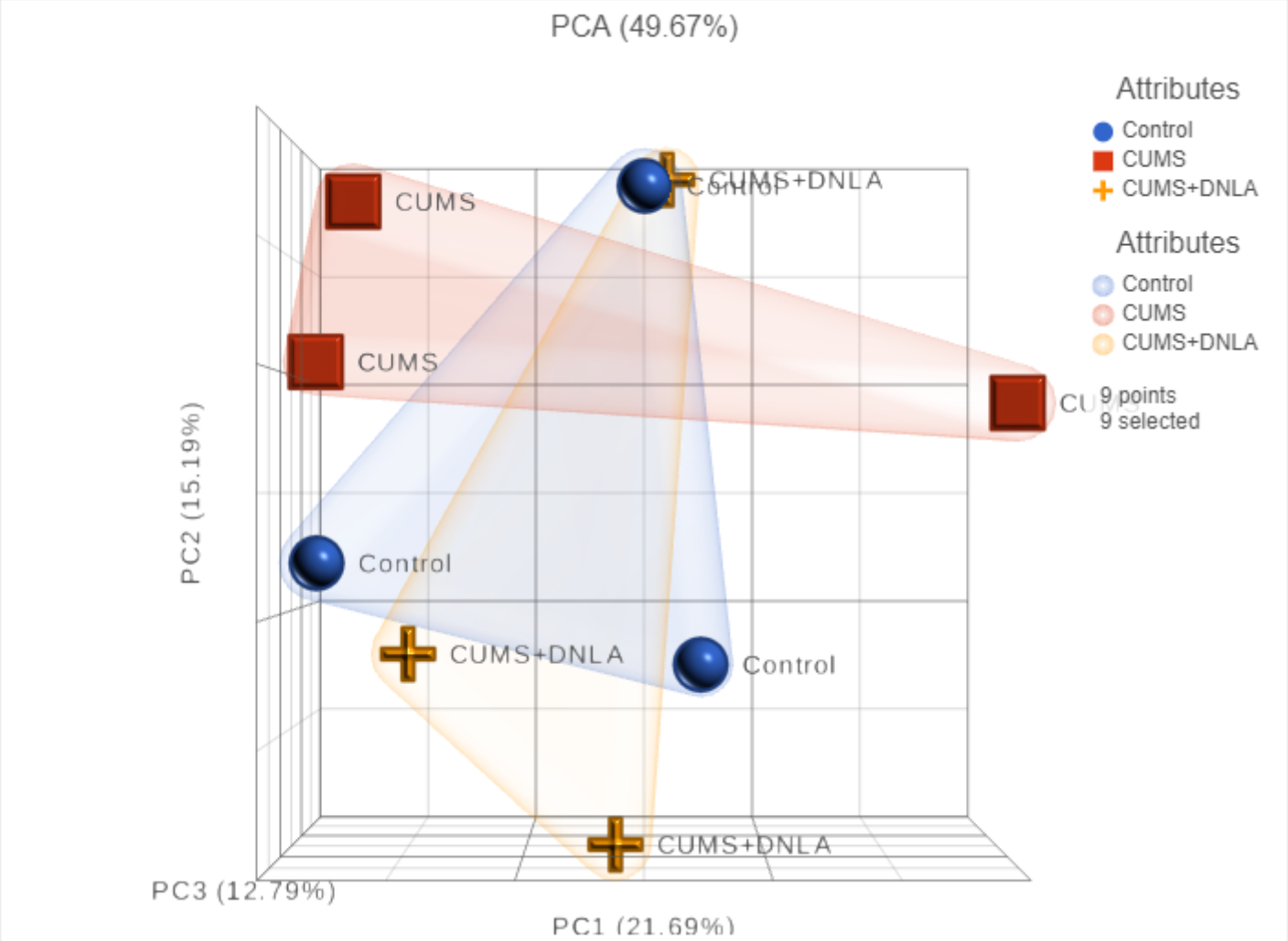

Figure 7

Principle Component Analysis (PCA) of gene expression. Total RNA from the hippocampus was extracted and subjected to RNA-Seq. There were Control, CUMS and CUMS+DNLA groups, 3 rats in each group. Approximately 43000 reads/sample were obtained and PCA shows distinct patterns among groups.

Figure 8

Gene functional enrichment of DEGs. (A) GO analysis shows the biological processes. (B) KEGG shows the signaling pathway.

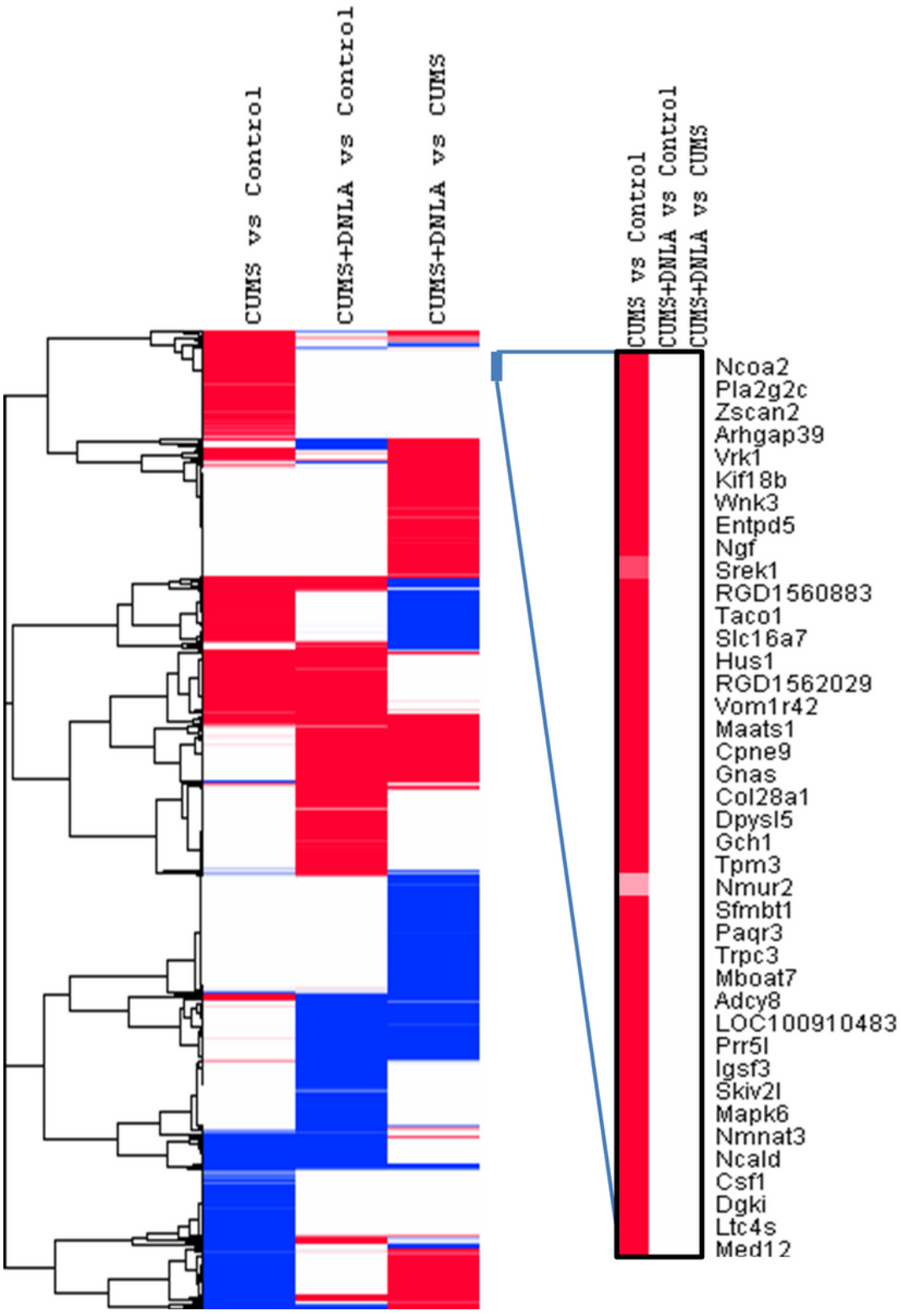

Figure 9

Two-dimensional clustering analysis of differentially expressed genes (Log2fold >2). Three example clusters were labeled for easy visual comparisons. (Red indicates up-regulation and blue indicates down-regulation).

Supplementary Files

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- GraphicalAbstract.png
- SupplementaryTable.pdf