1 **Danggui-Shaoyao-San, a Traditional Chinese Medicine, Relieved Depression and**
2 **Cognitive Disorder Induced by Chronic Restraint Stress Involved in**
3 **Regulating Dendritic Spines Remodeling**

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Abstract

Background: Clinical trails have revealed that patients with depression generally accompanied with learning and memory impairment, which critically impact on individual's health and development. Danggui-Shaoyao-San (DSS), a famous Chinese complex prescription, significantly overcame depression and relieved cognitive disorder based on previous research and publication. However, its effects and potential mechanism against chronic restraint stress (CRS) remained unknown.

Methods: CRS animal model was established and mice were divided to six groups while they were oral administrated with Danggui-Shaoyao-San at doses of 1.25, 2.5 and 5 g/kg for 14 days. Emotional and cognitive performances were detected by behavior tests, meanwhile neural plasticity and its molecular mechanism were examined by Dil staining, western blot and immunofluorescence.

Results: DSS treatment dose-dependently improved locomotion ability in open field test, overcame depression behavior in forced swimming test and elevated plus maze test, enhanced learning and memory ability in Morris water maze test. CRS decreased number of total spines and mushroom spines, while DSS treatment dose-dependently restored these by Dil staining. Expression of BDNF and GluR1 were significantly down-regulated in CRS group, which were significantly normalized by DSS.

Conclusions: DSS treatment dose-dependently reversed CRS-induced cognitive impairments by inducing structural remodeling of neurons.

Key word: Danggui-Shaoyao-San, chronic restraint stress, learning and memory, anxiety/depression, structural plasticity, three dimensional reconstructions

Introduction

Depression, one of the most severe and debilitating psychiatric illnesses, affects up to 350 million people worldwide according to statistical data from The World Health Organization [1]. Depression frequently presents with depressed mood, cognitive disorders, anhedonia, sleep disorders, and even suicide which usually occur in major depressive disorder patients [2, 3] and approximately two-thirds of them fail to respond fully to pharmacotherapy [3, 4]. It is surprising that cognitive impairment, as the same important as emotional abnormalities in depression, has not been given sufficient attention [5, 6]. Therefore, potential drugs are required for overcoming depression as well as relieving cognitive impairment.

Dendritic spines are small membranous protrusions from the dendrite shaft of neuron. They receive and storage signal from synapse and then help transmit information toward the neuron's cell body [7, 8]. Multiple studies have shown that the morphology and spine density of dendritic spine is correlated to learning and memory as well as depression [9-12]. Despite the well-known involvement of dendritic spine remodeling as a key factor in development of cognitive dysfunctions, few clinically therapeutic strategies worked. Low levels of brain-derived neurotrophic factor (BDNF) lead to specific functional and structural alterations, which cause depression based on neurotrophic hypothesis [13, 14]. Publications indicate that glutamate receptor 1 (GluR1) playing important roles in dendritic spines plasticity, long-term memory and possibly involving in depression [15, 16]. Stress down-regulates expression of BDNF and GluR1 while antidepressant drugs restored these in hippocampus from depressive mouse and depressed patients. Since BDNF and GluR1 participate in cognitive impairment and dendritic spine remodeling, we evaluate the content of BDNF and GluR1 in mouse hippocampus and Prefrontal Cortex (PFC) alterations in dendritic spine morphology and spine density.

Danggui-Shaoyao-San (DSS), it is also called Toki-shakuyaku-san in Japan, is a classic traditional Chinese complex prescription that firstly recorded in "JinKui Yao Lue" (early published in the Eastern Han Dynasty by Zhong-Jing Zhang). DSS is composed of six Chinese herbs: *Radix Angelica sinensis*, *Radix Paeoniae Alba*, *Rhizoma Chuanxiong*, *Rhizoma Alismatis*, *Rhizoma Atractylodis macrocephalae* and *Poriacocos* (3:16:8:8:4:4) [18]. For more than thousand years, DSS clinically applied in gynecological disorders such as infertility, amenorrhea, and dysmenorrhea for its blood-activating and stasis-eliminating activity without

obvious side effects [19]. Recent studies indicate that DSS play important roles in regulating hypothalamic–pituitary–adrenal system and immune-neuroendocrine functions, alleviating pain symptoms, ischemic stroke, post-menopausal and sleeping disorders [20-22]. Recent evidence shows that DSS reduced cognitive impairment induced by ischemia-reperfusion injury, ovariectomy, SAMP8 or chemical stimulus [23-26]. Our earlier study indicates DSS overcame depression on chronic unpredictable mild stress (CUMS) model, which was related to the central arginine vasopressin system [27]. Huang' group found that the anti-depression effects of DSS related to regulate central monoamine neurotransmitter systems and alleviate oxidative stress in CUMS model [28]. However, the effect of DSS on ameliorating depression related to cognitive impairment and dendritic spines remodeling remain uncertain. Therefore, present study aimed to investigate 1) the protective role of DSS in depression and cognition in the CRS model via an amelioration of dendritic spine morphology and spine density 2) mechanism underlying the protective role of DSS.

2. Methods

2.1 Animals

10-week old male C57/BL6 mice had free access to food and water in a temperature humidity controlled environment and maintained on a 12-h light/dark cycle. All experimental procedures were implemented in accordance with the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University (Xi'an, China).

2.2 DSS preparation and other reagents

DSS is composed of six medicinal materials described above in the dry weight ratio of 3:16:8:8:4:4. The 6 raw materials were purchased from Nanjing Medicinal Materials Company, Jiangsu Province, China, and authenticated by Dr. Boyang Yu (Jiangsu Key Laboratory of TCM Evaluation and Translational Research). The herbs were mixed and dipped in distilled water (1:8 w/v) for 1 h, and then boiled for 1 h. The filtrate was concentrated and the residues were refluxed twice with distilled water (1:6 w/v) for 1 h. After being concentrated and mixed, filtrates were condensed and dried with a vacuum dryer at 60 °C. DSS extract were stored at 4 °C suspended in distilled water before use. The concentration of active ingredient in DSS was determined by HPLC fingerprint [18].

2.3 Chronic restraint stress protocol

Mice were wrapped in clear plastic tubes with breathing hole for 8h (from 10A.M. to 6P.M.) daily for 14 days (Supplementary Fig 1). During chronic restraint stress (CRS), all mice were deprived of food and water and received no physical compression or pain experience. Ninety mice were randomly divided into six groups: (1) control group, (2) CRS group, (3) DSS 1.25 g/kg group, (4) DSS 2.5 g/kg group, (5) DSS 5 g/kg group, (6) Duloxetine 10 mg/kg group. All group were intragastric administrated with corresponding dose of DSS or Duloxetine or saline 1 h before restraint stress.

2.4 Open Field Test

The testing room remained quiet and dusk with indirect lighting during the experiment. Mice were softly placed at the center of the testing chamber [250 mm (W) × 250 mm (H) × 250 mm (D)] after 1 h acclimate to the testing room. The automated analyzing system (Shanghai Mobile datum Information Technology Co., Ltd) recorded the track of mice for 15 min [29]. The mean velocity, distance in zone percent and center time percent were evaluated the locomotion and anxiety/depression levels of mice.

2.5 Forced swim test

The forced swim test was performed without a pre-swim according to the methods reported previously [30]. The mice were placed at the center of a clear glass cylinder, which half-filled with clear water about 25 °C. Water will be replaced after each test. The water depth was 14 cm to avoiding the mice contact with the bottom of the cylinder [30]. The automated analyzing system recorded the video for 6 min, and the last 4 min was used for statistics. Two investigators blinded to the experiment analyzed the data according to the video. The mice remained immobility or slight movements to remain balance were recorded as immobility. The horizontal movements which involved two or more limbs were recorded as swimming. The vertical movements in which the front paws of mice touched the cylinder sides were recorded as climbing [31].

2.6 Elevated plus maze (EPM) test

The mice were placed at the central area of EPM, which constituted with two closed arms (50×10×40 cm), two open arms (50×10 cm) and a central area (10×10 cm). The bottom of the EPM was 50 cm above ground [32]. The automated analyzing system recorded the video for 5

min. The numbers of the mice entering each arms and the amount of time the mice spent on each arm was analyzed by two investigators blinded to the experiment. When four paws of the mice onto the open arm recorded an entry. OA entry time % and OA entries % were scored as described previously [32].

2.7 Morris water maze test

The Morris water maze test was performed according to the methods reported previously [33]. The mice were given learning ability test (four consecutive days) and memory ability test (the fifth day). The number of crossings the platform, the distance in the platform quadrant and swimming time in the platform quadrant were analyzed [33].

2.8 Spine Density Analysis

Dil dye staining was used to quantitatively analyze the dendritic spine density. Mice were anesthetized and perfused with 0.5% paraformaldehyde. The brain was sliced into 2 mm slices on a vibratory microtome. The slices were incubated with Dil (1 mg/mL) for 3 d at 37°C. After three more washes, the dendritic spines were observed with confocal microscope. To reconstruct 3D image for dendritic spine, serial pictures of dendrite were recorded and analyzed by Imaris 7.5 software. We divided the dendritic spines into two classes, Mushroom spines (head diameter > the length of the neck) and thin spines (head diameter $\leq$ the length of the spine neck).

2.9 Western blot

The lysates of PFC and hippocampus were prepared and protein concentrations were determined by BCA protein assay. Equivalent amounts of total proteins were separated by 10% SDS-PAGE, subjected to immunoblotting, probed with the respective antibodies(anti-BDNF, 1: 1000, Chemicon; anti-GluR1, 1: 500, Chemicon), and detected for signals using the corresponding fluorescence secondary antibody by the Bio-Rad ChemiDoc MP system (Bio-Rad, Hercules, CA).

2.10 Immunofluorescence

Mice were anesthetized and perfused with 4% paraformaldehyde. The brain was sliced into 30 μ m thick sections using a freezing microtome (Leica CM1800). Sections were permeabilized, blocked for 1 h and primary antibody was applied 48 h at 4 °C in a humid chamber. Then Sections were washed and incubated for 4 h at room temperature with Alexa Fluor antibody. Fluorescent images were observed with confocal microscope (FV1000, Olympus, Japan) and

processed using the FV1000 imaging software.

2.11 Data Analysis

All data were expressed as the mean $\pm$ SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Dunnett's test when the data involved three or more groups. $P < 0.05$ was defined as significant.

Dose-Effect Curve and ED₅₀ Calculation

The DSS dosages were transformed into alogarithm dose with Prism and the non-line fit was performed so as to build the dose-effect curve. The ED₅₀ of effects of DSS on locomotion, emotion, learning and memory ability were calculated based on the dose-effect curve. The reliability of ED₅₀ calculated from a specific dose-effect curve can be evaluated by the slope factor returned by the GraphPad Prism version 5.01 for Windows (San Diego California USA, www.graphpad.com) [34].

3. Results

3.1 Effect of DSS on the CRS-induced locomotion ability disorder in open field test

As shown in Fig. 1, CRS group showed remarkably decrease in mean velocity, central zone traveling percentage and central zone traveling time compared with normal group ($P < 0.01$). Comparing to CRS group, 2.5 and 5.0 g/kg DSS treatment group significantly increased mean velocity, central zone traveling percentage and central zone traveling time ($P < 0.01$), whereas DSS at dose of 1.25 g/kg showed improvements only in mean velocity and central zone traveling percentage. The anti-depression effects of DSS were calculated based on the log (dose) vs response curve (Fig. 2 D, 2 E and 2 F). The ED₅₀ of DSS on mean velocity was 1.92 g/kg, on time in central zone was 2.02 mg/kg, and on the percent of distance in zone/total distance was 2.04 mg/kg..

3.2 Effect of DSS on the CRS-induced emotion change in swimming test

The results shown that CRS increased the immobility time decreased the swimming and climbing time. Post-hoc analysis demonstrated that DSS (5 g/kg) reversed the CRS-induced increase of immobility time and decrease of swimming time and climbing time but DSS (2.5 g/kg) only has effect on increasing the climbing time and decreasing swimming time. The ED₅₀ of DSS on immobility time was 2.08 g/kg, swimming time was 2.27 mg/kg, and on climbing time was 2.12 mg/kg.

3.3 Effect of DSS on the CRS-induced emotion change in elevated plus maze test

In the elevated plus maze, mice in CRS day 14 group (stress control group) showed significantly decrease in OA entry time % and OA entries % ($P < 0.01$). DSS treatment group increased all of OA entry time % and OA entries % significantly ($P < 0.05$) (Fig. 3 A, 3 B). Based on the AUCs of EPM's change, dose-response in each observation was calculated. The ED_{50} of OA entry time% improvement was 1.88 g/kg and the ED_{50} of OA entries% improvement was 1.85 g/kg (Fig. 3 C and 3 D). These results suggested that DSS treatment observably increased the time spent in the open arms and the frequency of entry to the open after CRS stress.

3.4 Effect of DSS on the CRS-induced spatial learning and memory disorder in Morris water maze test

Compared with control group, CRS group needed long latent time to find the target platform and long length of path to reach the target platform. DSS at the dose of 2.5 and 5 g/kg treatment group ($P < 0.05$) or Duloxetine treatment group ($P < 0.01$) significantly decreased the escape latency and shortened the path length to reach the platform as compared to the CRS group. However, DSS treatment at doses of the 1.25 g/kg showed no significant effect on the escape latency or the path length to target platform. Based on the AUCs of spatial acquisition's change, dose-response in each observation was calculated. The ED_{50} for improvement of DSS on CRS induced increased escape latency was 1.79 g/kg and path length to reach the platform was 1.86 g/kg (Fig. 4 E and 4 F).

In the spatial memory test, we found that the number of crossings of the target platform, the percentage of the total swimming time spent in the target platform quadrant and the percentage of the total distance spent in the target platform quadrant were obviously decreased in CRS group as compared with the control group. Treatment with DSS (2.5 and 5 g/kg) can significantly increase the number of crossings of the platform, the percentage of the total swimming time spent in the target platform quadrant and the percentage of the total distance spent in the platform quadrant. And DSS 1.25 g/kg only show improvement on the last two indexes. Duloxetine treatment can achieve the same effect as the DSS 5 g/kg group. The ED_{50} for improvement of DSS on CRS induced decrease on number of crossings of the platform, percentage of the total time spent in the platform quadrant and percentage of the total distance spent in the platform quadrant were respectively 1.92g/kg, 2.03 g/kg and 1.89g/kg (Fig. 5 D, 5

E and 5 F).

3.5 Effect of DSS on the CRS-induced neuron structural plasticity change by Dil staining

To investigate the effect of DSS on neuron structural plasticity, the densities of dendritic spine in PFC, hippocampus CA1 and CA3 regions were analyzed. Dil staining revealed that CRS significantly lead to neural plasticity exchange based on the alternations of the shape and number of spine, which were improved with DSS treatment. We found that daily CRS significantly decrease total dendritic spine, mushroom spine and thin spine density of PFC and CA3 (Fig. 6B, 6D). DSS (5 g/kg) treatment significantly increased total dendritic spine and mushroom spine density of PFC and CA3 (Fig. 6B, 6D). In hippocampus CA1 regions, CRS remarkably decreased total dendritic spine and mushroom spine density of CA1, DSS (5 g/kg) treatment effectively increased mushroom spine density of CA1 (Fig. 6C).

3.6 Effect of DSS on the CRS-induced expression of BDNF

Western blot and immunofluorescence assays were performed to evaluate the protein level of BDNF in PFC and hippocampus. Expression level of PFC BDNF (Fig. 7 A, 7 B) ($P<0.05$), and hippocampus BDNF (Fig. 7 A, 7 C) ($P<0.05$) significantly decreased in CRS group when compared to control group. Treatment with DSS (5 g/kg) significantly restored the protein level of BDNF in PFC and hippocampus in comparison with CRS mice.

3.7 Effect of DSS on the CRS-induced expression of GluRI

As shown in Fig. 8, the results of western blot and immunofluorescence indicated that BDNF expression in PFC and hippocampus decreased in CRS group. Treatment with DSS (5 g/kg) significantly enhanced the protein level of BDNF in PFC and hippocampus in comparison with CRS mice (Fig. 8B, 8C).

Discussion

Our study systemically investigated the protective role of DSS indepression and cognitive on CRS model. In this paper, CRS induced a series of stress-related disorders including depression and impaired spatial learning and memory, which could be attenuated by DSS treatment. Apical dendritic of CA1, CA3 and PFC pyramidal neurons undergo dendritic remodeling after chronic stress while the anti-depression and cognitive protection properties of DSS may result from

increased dendritic spine density and enhanced proportion of mushroom dendritic spines. In addition, the underlying mechanism of DSS on CRS might be associated with BDNF and GluR1. Effects of DSS on motor ability and emotion were performed by OF, FST and EPM tests. Mean velocity, distance in the central area and the time spent in the central area within 5 min in the OF test were recorded while CRS mice displayed abnormal hypoactivity, compared with control group. This is consistent with a previous report that CRS mice spent less time in the central area and covered less distance in the central area in the OF test [35]. As expected, DSS treatment dose dependently increased mean velocity, distance in the central area and the time spent in the central area. These results further confirmed that CRS increased risk of depression while DSS could reduce it. In agreement with previous literatures, CRS group significantly increased immobility time, decreased swimming time and climbing time [36, 37], which were reversed by DSS treatment. In EPM test, the time spent on the open arms and the frequency of entry onto the open arms was significantly reduced in CRS group compared with control group. The time spent on the open arms and the frequency of entry onto the open arms in the CRS plus DSS group was significantly elevated compared with the CRS group, indicating that DSS alleviated the anxiety response.

In cued learning, the results shown that CRS significantly increased pathlength to the platform and escape latency. Meanwhile, treatment with DSS remarkably decreased pathlength to the platform and escape latency. We further found that the swimming speed the daily spatial learning had no effect on swimming speed (Supplementary Fig 2). Thus, CRS-induced pathlength to the platform and escape latency increase was owing to the impairment of the spatial acquisition ability rather than impairment in swimming ability [38]. In the spatial memory test, three-way ANOVA shown that CRS significantly decreased the number of crossings of the target platform, the percentage of the total swimming time and the total distance spent in the target platform quadrant, which is consistent with previous reports [39, 40]. Treatment with DSS (1.25-2.5 g/kg) effectively reversed CRS-induced spatial memory deficit. This is consistent with previous report that DSS could improve d-galactose-induced cognition deficits in senescent mice [25].

Another major novelty of this study is that a remarkably overall decreased in dendritic spine density in PFC and hippocampus neurons in CRS-stimulated mice. We performed dendritic

spine morphometric features by 3D analysis by digitally reconstructing and deconvolving dendritic segments in imaris software. Dendritic spines are tiny membranous protrusions from the dendrites shaft of various types of neurons. Dendritic spines can accept excitatory and inhibitory signals from axons [7, 41]. It is widely accepted that regulation of dendritic spine number, size, and shape is critical for synapses plasticity as well as learning and memory [41, 42]. Different subtypes of dendritic spines have different functions. For example, thin spines are regarded as immature and less involved in signal transmission, whereas mushroom spines represent a more mature and stable population of excitatory synapses [43, 44]. Hippocampus, which is the primary area of the brain, regulates cognitive function and plays an important role in the pathogenesis of depression [45, 46]. PFC is critical for cognitive integration, emotion-related information, modulation of subcortical system modulation [47, 48]. CRS-induced dendritic retraction and spine loss in the hippocampal and PFC neurons are accompanied by cognitive impairments, which are mediated by structural alteration respectively [49]. Dil staining revealed that CRS significantly lead to neural plasticity exchange based on the alternations of the shape and number of spine, which were improved with DSS treatment. We found that daily restraint stress for 14 days decreased total dendritic spine and mushroom spine density of PFC, CA1 and CA3 (Fig. 6A, 6 B and 6C). Treatment DSS increased mushroom-shaped dendritic spines and improved its function. Hippocampus dendritic spines reduction is not only an indicator of decreased axospinous synapses population, but also indicating that biochemical compartmentalization and plasticity of neuron was impaired. Improving dendritic atrophy and restraining dendritic spine loss in hippocampus may be important for treating stress-related psychiatric disorders.

Maintenance the structure of neuron and their dendritic arborizations, synaptic connectivity are required both in development and adulthood. Brain derived neurotrophic factor (BDNF) is a growth factor enriched in the rodent hippocampus and released from neurons in an activity-dependent manner by modulating both neuronal morphology and synaptic plasticity [50, 51]. Study has shown that paeoniflorin, an effective native compound of DSS, can increase the expression of BDNF to improve vascular dementia-induced learning and memory disorders [52]. In addition, there is a study shown that another active compound of DSS, Tetramethylpyrazine, can promote BDNF expression to improve depression symptom [53].

GluR1 also plays important role in regulating neuron plasticity. The results from behavioral, electrophysiological and biochemical studies have shown that GluR1 involved in neuron plasticity in brain regions including hippocampus, amygdala, striatum and cerebellum [54]. The expression of BDNF and GluR1 were significantly down-regulated in hippocampus of CRS group while those were significantly normalized by DSS (5 g/kg). Therefore, it is possible that DSS involved in regulating dendritic spine through BDNF/ GluR1 [55, 56].

In conclusion, we further demonstrated that CRS triggered anxiety/depression emotion accompanied by a loss in learning and memory, which could be overcome and restored by DSS *in vivo*. DSS is powerful in anti-depression through regulating neural plasticity enhancing BDNF/GluR1 expression in PFC and hippocampus, which provide new insights into the mechanisms of chronic stress-induced depression and appropriately targeted treatment in patients with depression and other neuron disorders.

Conflict of interest

All the authors report no conflicts of interest.

Acknowledgments

We thank Xiao-Nan Ma, Min-Hui Sun, and Ying-Jian Hou for technical support from Cellular and Molecular Biology Center of China Pharmaceutical University.

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Fig 1 Effect of DSS on the exploratory and locomotor activities in OF test. CRS was processed for 8h (from 10A.M. to 6P.M.) 14 days. DSS (1.25, 2.5 and 5 g/kg), duloxetine or saline, respectively, was intragastric administrated one hour before CRS. Mean velocity (A), distance in zone/total distance % (B) and time in central zone (C) were measured in open field test. Log (dose)-effect curves for therapeutic effects of DSS were shown in D (mean velocity), E (time in central zone) and F (distance in zone/total distance %). All data were presented as mean $\pm$ SEM. [#] $P < 0.05$, ^{##} $P < 0.01$ vs. Control group; ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. CRS group, $n = 6$.

Fig 2 Effect of DSS on the anxiety/depression behavior in forced swimming test. DSS dose-dependently reduce CRS-induced increase of time spent immobile (A) and enhance CRS-

induced decrease of time spent swimming (B), time spent climbing (C). Log (dose)-effect curves for therapeutic effects of DSS were shown in D (time spent immobile), E (time spent swimming) and F (time spent climbing). All data were presented as mean ± SEM. [#]*P*<0.05, ^{##}*P*<0.01 vs. Control group; **P*<0.05, ***P*<0.01 vs. CRS group, n=6.

Fig 3 Effect of DSS on the anxiety/depression behavior in Elevated Plus Maze. DSS dose-dependently enhance CRS-induced decrease of OA entry time % (A) and OA entries % (B). Log (dose)-effect curves for therapeutic effects of DSS were shown in C (OA entry time %), D (OA entries %). All data were presented as mean ± SEM. [#]*P*<0.05, ^{##}*P*<0.01 vs. Control group; **P*<0.05, ***P*<0.01 vs. CRS group, n=6.

Fig 4 DSS dose-dependently enhance CRS-induced learning disabilities during the Water Morris Maze test. The platform and escape latency from different groups were shown in A and B. The areas under curves for different groups were calculated to perform statistical analysis on the platform(C) and escape latency (D). Log (dose)-effect curves for therapeutic effects of DSS was shown in E (the platform) or F (escape latency). All data were presented as mean $\pm$ SEM. # $P < 0.05$, ## $P < 0.01$ vs. Control group; * $P < 0.05$, ** $P < 0.01$ vs. CRS group, $n = 6$.

Fig 5 Effect of DSS on memory impairment the Water Morris Maze test. DSS dose-dependently enhance CRS-induced decrease of number of crossing the platform (A), percentage of distance in the target quadrant (B) and percentage of time spent in the target quadrant (C). Log (dose)-effect curves for therapeutic effects of DSS were shown in D (number of crossing the platform), E (percentage of distance in the target quadrant) and F (percentage of time spent in the target quadrant). All data were presented as mean $\pm$ SEM. [#] $P < 0.05$, ^{##} $P < 0.01$ vs. Control group; ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. CRS group, $n = 6$.

Figure 6 Effects of DSS on CRS-induced decrease of spine density in the PFC, CA1 and CA3. After treatment with DSS, the spine density in the PFC, CA1 and CA3 are increased. $^{\#}P<0.05$, $^{##}P<0.01$ vs. Control group; $^{*}P<0.05$, $^{**}P<0.01$ vs. CRS group; n=10.

Figure 7 Effects of DSS on CRS-induced decrease of BDNF in PFC and hippocampus. After treatment with DSS, the expression levels of BDNF are increased. # $P<0.05$, ## $P<0.01$ vs. Control group; * $P<0.05$, ** $P<0.01$ vs. CRS group; $n=3$.

Figure 8 Effects of DSS on CRS-induced decrease of GluR1 in PFC and hippocampus. After treatment with DSS, the expression levels of Glur1 are increased. # $P<0.05$, ## $P<0.01$ vs. Control group; * $P<0.05$, ** $P<0.01$ vs. CRS group; $n=3$.

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