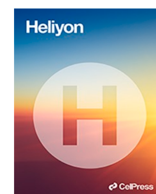
Heliyon 10 (2024) e28916

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Shenyu ningshen tablet reduced neuronal damage in the hippocampus of chronic restraint stress model rat by inhibiting A1-reactive astrocytes

Yaxin Wang [a,1], Shuran Li [a,1], Mengping Chen [a], Meihua Zeng [c], Lirun Zhou [a], Rongmei Yao [b], Bo Pang [a], Yingli Xu [a], Shan Cao [a], Shanshan Guo [a,**], Xiaolan Cui [a,*]

[a] *Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China*
[b] *Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, China*
[c] *Guangdong Si Ji Pharmaceutical Co., LTD, China*

ARTICLE INFO

Keywords:
SYNS tablet
Depression
CRS rat model
Neuron
A1 astrocyte

ABSTRACT

Context: Shenyu Ningshen (SYNS) tablet is the first pure Chinese medicinal small compound preparation approved for clinical trials for the treatment of depression in China. Clinical experiments confirmed that the formulation had a significant Improvement effect against depression due to the deficiency of both qi and yin. It has been shown to exhibit noticeable anti-inflammatory effect in an animal model of depression. Our previous study showed that SYNS could effectively inhibit the inflammatory response in a depression model.
Aim of the study: The purpose of this study was to investigate the protective effects of SYNS on neurons and explore whether the underlying mechanism was associated with A1s.
Materials and methods: The depression model of solitary raising-chronic restraint stress (CRS) rats was established; body weight examination, sugar water preference test, open field test, and histological analysis were performed to preliminarily verify the efficacy of the formulation. Subsequently, neuronal nucleus (NeuN) and synaptic-associated proteins (MAP2 and PSD95) were labeled, and the protective effect of SYNS on hippocampal neurons was observed based on the fluorescence intensity of the above indicators. Western blotting, histological examination, and immunofluorescence were used to evaluate the inhibitory effects of SYNS on neuroinflammation and activation of A1s in CRS depression model.
Results: SYNS improved behavioral indicators such as weight loss, pleasure loss, and reduced exercise volume in CRS rat model. SYNS restored the CRS-induced histopathological changes in the hippocampus. SYNS showed a certain degree of protective effect on synapses. Further, SYNS inhibited the activation of A1s by inhibiting neuroinflammatory factors in the hippocampus.
Conclusion: Our results showed that SYNS had a certain degree of neuroprotective effect, which might be related to its inhibition of the inflammatory response and A1s.

* Corresponding author.
** Corresponding author.
E-mail addresses: xiaolancui2812@126.com (S. Guo), ruochushan@163.com (X. Cui).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e28916
Received 30 October 2023; Received in revised form 26 March 2024; Accepted 27 March 2024
Available online 2 April 2024
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1. Introduction

Major depressive disorder (MDD) is a complex and common mental disorder, and its symptoms are mainly characterized by significant and persistent depressed mood, lack of pleasant sensation, and feeling of despair. It is often accompanied by cognitive and physical dysfunction [1]. Depression is currently the leading cause of disease and disability worldwide [2,3]. According to the World Health Organization [4], nearly 800,000 people die of suicide every day and more than 300 million people worldwide suffer from depression, causing substantial economic loss and social burden. It is estimated that depression will become the most significant global burden by 2030 [5]. According to epidemiological studies, more than 30% of patients with depression receive ineffective antidepressant treatment for a long time, with a lifetime prevalence of approximately 16.2% [6]. Therefore, there is an urgent need to develop safer and more effective antidepressants.

Scientists have proposed many hypotheses regarding the pathogenesis of depression. There are primarily monoamine neurotransmitter, neural plasticity, and inflammatory response mechanisms. The neural plasticity hypothesis states that chronic stress or increased glucocorticoid levels can induce atrophy or loss of mature neurons in the hippocampus [7]. The results of the research by Watanabe et al. have shown that after repeated restraint stress or administration of glucocorticoids, hippocampal neurons can be induced to shrink. For example, the length and density of dendritic spines can be reduced [8]. The inflammatory response mechanism suggests a close relationship between the immune and central nervous systems. A damaged immune system and increased peripheral inflammation increase the number of pro-inflammatory cells in the central nervous system and induce inflammatory reactions, which in turn can regulate a series of reactions in the central nervous system [9], such as neurotransmitter metabolism [10]. Corresponding research data support many of the above hypotheses, but they do not fully explain the disease pathophysiology, clinical presentation, and mechanisms of therapy [11].

The hippocampus plays important roles in cognitive activity, stress, and mood regulation in patients with depression. Depression in adult patients may be related to the destruction of synaptic connections between hippocampal neurons. Hippocampal structural atrophy and decreased neurogenesis, ultimately lead to depression [12]. An autopsy study found that patients with depression showed impaired plasticity of hippocampal neurons, manifested as a decrease in hippocampal gray matter density and reduction in the nerve fiber network and hippocampal neurogenesis [13]. The synapse is the main site of transmission of neuronal information, and its protein markers include postsynaptic density protein 95 (PSD95) and microtubule-associated protein 2 (MAP2) [14]. They play an important role in synaptic plasticity and participate in the regulation of the number of developing synapses, along with their formation and stability. The hippocampus in the brain can continuously produce neurons throughout life, and glial cells are essential for controlling nerve growth and conduction. With the deepening research on glial cells, we have realized that glial cells not only have the function of "adhesive," but also have vital effects on neuron survival, synapse generation, and nerve conduction in the process of brain development [15]. Microglia maintain nerve conduction by engulfing unnecessary synaptic structures, and astrocytes release growth factors to stimulate nerve growth.

Reactive astrocyte hyperplasia refers to changes such as dilation, hypertrophy, and increased expression of glial fibrillary acidic protein (GFAP) in early astrocytes after harmful stimulation or nerve injury, leading to the development of phenotypic characteristics of reactive astrocytes [16]. It has been proposed that neuroinflammation and ischemia induce two different types of reactive astrocytes, A1 and A2. Among them, A1, which is induced by neuroinflammation, secretes neurotoxins, which further accelerate the apoptosis of neurons and oligodendrocytes. Different types of recognition can be performed based on the genetic expression of individual reactive astrocytes [17]. For example, complement 3 (C3) is a characteristic and significantly upregulated gene in A1s that is not expressed in A2s, and can be used as a specific marker of A1s [18]. A1s are present in most human neurodegenerative diseases. Studies have found that more than half of the GFAP-positive astrocytes in the prefrontal cortex of patients with Alzheimer's disease are C3-positive. This suggests that A1s is likely one of the reasons for the progressive deterioration in neurodegenerative diseases [19].

At present, commonly used drugs for depression treatment in clinics include tricyclic antidepressants, selective 5-hydroxytryptamine reuptake inhibitors, norepinephrine, and dopamine reuptake inhibitors, which can improve depressive mood to a certain extent. However, most of them have the disadvantages of slow onset, low remission rate, and adverse reactions. Recently, progress has been made in the development of natural antidepressants and Chinese medicinal compounds [20]. Shenyu Ningshen (SYNS) tablets are composed of four Chinese medicinal products: *American Ginseng*, *Radix Curcumae*, *Semen Ziziphi Spinosae*, and *Schisandra Chinensis*. Each traditional Chinese medicine comprises the following components in parts by weight: *Radix Curcumae* 600–1200, Semen *Ziziphi Spinosae* 600–1200, *American Ginseng* 100–600, and *Schisandra Chinensis* 400–1000. It was granted an approval document for CFDA clinical research (approval document no. 2005L02629) in 2005 and a national invention patent (patent no. ZL031531040) in February 2007. This is the first pure Chinese medicinal small-compound preparation (Class 6.1) approved for clinical trials in China for the treatment of depression. It regulated the immune inflammatory response in a rat model of chronic restraint depression. Clinical experiments confirmed that it had a significant Improvement effect against depression due to the deficiency of both qi and yin (this refers to the pathological changes of qi and yin deficiency simultaneously; its symptoms include general fatigue, palpitations, shortness of breath, and hot flashes). Our previous experimental results confirmed good anti-inflammatory effect of SYNS in a CRS (Chronic Restraint Stress) rat model [21,22]. However, the mechanism of anti-inflammatory effect of the drug on related cells in the rat brain has not been clarified. Therefore, we studied the protective effects of SYNS on neurons and explored whether the underlying mechanism was associated with A1s.

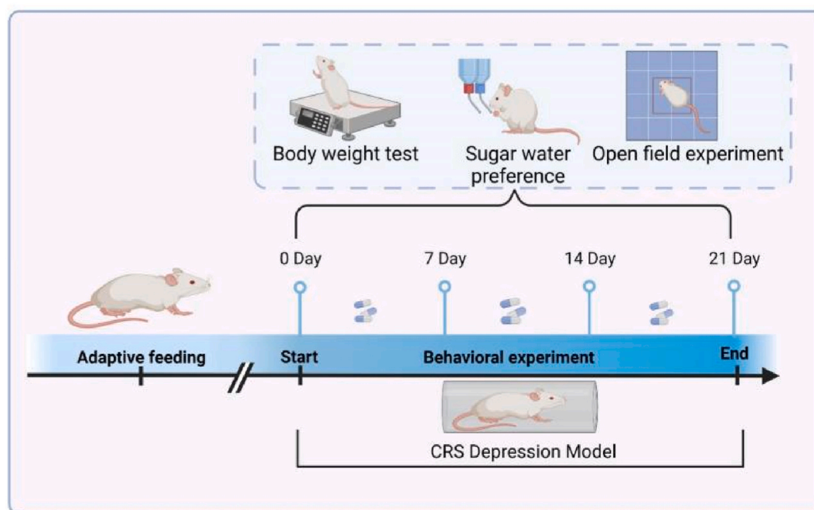

Fig. 1. The entire experimental plan. First, one week of adaptive feeding was conducted of the rats. Rats in each treatment group were dosed continuously throughout 21 days of chronic restraint stress (CRS). Body weight test, sucrose preference test (SPT) and open field test (OFT) were measured every 7 days to detect depressive-like behavior in rats after CRS modeling. After completing all behavioral tests, animals were sacrificed, and tissue was collected.

2. Materials and methods

2.1. Drugs

Shenyu Ningshen Tablets (SYNS) (for clinical trials only), provided by Guangdong Si Ji Pharmaceutical Co., LTD. The recommended clinical dose for adults is 7.2 g/60 kg/d. Clinical Research Approval No.: 2005L02629. Product Lot Number 190101. The fingerprint was established by HPLC to control the quality of SYNS (Supplementary material 1). Fluoxetine hydrochloride dispersion tablets (FLX), manufacturer: Lilly Suzhou Pharmaceutical Co., Ltd. Product Lot Number 9542A. Clinical recommended dose is 20mg/granule in adults. Indications: depression, obsessive-compulsive disorder, bulimia nervosa.

2.2. Animals and treatment

All operations during the experiment followed the regulations of the National Institutes of Health (NIH), Beijing Municipal Ethics Committee for Experimental Animals, and Beijing Municipal Experimental Animal Ethics Committee; all experimental animals received humane care based on the 3R principle. This study was approved by the Animal Ethics Committee of the Institute of Traditional Chinese Medicine of the Chinese Medical Sciences (Ethics number: 2021D001). A total of 66 male Sprague-Dawley (SD) rats (SPF level, 180–200 g) were provided by the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All rats were acclimated for a week and randomly selected as Normal Control group (NC), Depression model group (CRS), fluoxetine hydrochloride group (FLX 1.86 mg/kg/d), SYNS high-dose group (SY 1.34 g/kg/d), SYNS medium-dose group (SY 0.67 g/kg/d), SYNS low-dose group (SY 0.34 g/kg/d), 11 rats in each group. All dosing groups, as described above, were based on the body surface area equivalence of animals according to the recommended daily human dosage, whereas the other groups were administered equal amounts of normal saline. All groups were administered drugs through gavage for 21 days. The chronic restraint stress (CRS) procedure was performed between 9:00 and 15:00 [23], and each rat was restrained daily in a self-made soft wire mesh sleeve (25 cm long and 10 cm in diameter) for 21 consecutive days. Body weight, sucrose preference, and open field tests were performed every seven days (Fig. 1).

2.3. Body weight test

All rats were weighed on days 0, 7, 14, and 21 of the experiment, and the changes in the body weight of rat groups at different time points were compared.

2.4. Sucrose preference test (SPT)

The SPT was primarily used to evaluate the degree of loss of pleasure in rats, and performed using a two-bottle free-choice paradigm. Two days prior to the experiment, the rats were trained to adjust to drinking sugar water. First, two bottles of 1% sucrose solution were placed in each cage, and the rats were allowed to drink freely for 24 h. Then, a bottle of purified water and a bottle of 1%

sucrose solution were placed in each cage, and the rats were allowed to drink freely for 24 h. Notably, this was the training period (data not included). To assess sucrose intake, rats were deprived of water and food for 24 h before the experiment began. The following day, each mouse had free access to two bottles of sugar and water. The positions of the bottles containing water and sucrose were changed every half an hour. Finally, we recorded the weight of the sucrose solution and purified water before and after the experiment and calculated using the following formula: SPT = (sucrose consumption/water and sucrose consumption) × 100.

2.5. Open field test (OFT)

The rats were subjected to the OFT to evaluate the behavioral characteristics of spontaneous activity, exploration, and anxiety. To adapt to the environment, the rats were placed in the laboratory for 1 h before the start of the experiment and then placed in an open box of 100 × 100 × 40 cm (XR-XZ301). The bottom of each box was evenly divided into 25 squares. A camera was placed above the box to track and record the performance of rats. One rat was placed at the center of the floor, and its behavior was monitored for 3 min. Indicators such as total distance, central zone residence time, total number of grids spanned, and standing time covered by the rats were measured, and the device was thoroughly cleaned with 70% ethanol after each trial. This test was analyzed and recorded using Super Maze Software via the video analysis system software Visutrack (XR-VT; Shanghai Xinruan Information Technology Co., Ltd. Shanghai, China).

2.6. Sample collection and preparation

After the behavioral tests were completed, three rats were randomly selected from each group and their hearts were instilled *in vivo* with 4% paraformaldehyde after anesthesia. Whole brain paraffin sections were prepared for hematoxylin-eosin (HE) staining and immunofluorescence staining. The hippocampi of the remaining rats were removed and placed on ice for western blotting.

2.7. Histological examination (HE)

The prepared tissue sections were stained with HE, and then observed and photographed using a microscope at 20 × magnification to observe the arrangement of neuronal cells in the dentate gyrus of the rat hippocampus.

2.8. Western blotting analysis

Protein levels of NF-κB p65, TNF-α, and IL-1β in the hippocampus were identified by western blotting. After completion of behavioral tests, hippocampi of the rats were collected and treated with RIPA lysis buffer containing a protease inhibitor (R0010; Solarbio, CHN) at a certain proportion. Equal amounts of protein (50 μg/sample) were separated by SDS-PAGE (P0012A; Beyotime Biotechnology, Shanghai, CHN) on 12% gel electrophoresis and transferred onto 0.45 μm polyvinylidene difluoride membranes (Millipore, USA). After blocking with 10% milk solution at room temperature for 2 h, The following monoclonal primary antibodies were used for Western blot analysis overnight at 4 °C: anti-IL-1β (cat. no. ab254360; Abcam; 1:2000), anti–NF–κB p65 (cat. no. 66535-1-1g; Proteintech; 1:2000), anti-TNF-α (cat. no. ab66579; Abcam; 1:1500), and anti-Tubulin (cat. no. 10068-1-AP; Proteintech; 1:5000). The membranes were then washed three times (10 min/wash) in Tris-buffered saline with 0.1% Tween (1 × TBST, T1070-500; Solarbio, CHN) followed by incubation with secondary antibodies for 2 h at room temperature. The membranes were washed thrice with 1 × TBST for 10 min at room temperature. The membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin IgG (1:3000) or anti-mouse IgG (1:5000) secondary antibodies at room temperature for 1 h. The signals were visualized using a chemiluminescence (ECL) kit (Beyo ECL Plus; Beyotime Biotechnology). The protein bands were processed using a chemiluminescent gel imaging system (FluorChem FC3, ProteinSimple, USA). The relative intensities of the target bands were normalized to that of Tubulin. The density of the bands was determined using ImageJ software.

2.9. Immunofluorescence staining

Briefly, brain tissue sections (20-μm thick) were washed with phosphate-buffered saline (PBS) for 15 min (5 min × 3). After washing, the sections were treated with a blocking buffer (BSA; BIOFROXX, DAKO, Denmark) for 30 min at room temperature. After blocking, the tissue was treated with primary antibodies (C3, GFAP, NeuN (Neuron nucleus), MAP2, and PSD95) overnight at 4 °C. The next day, secondary antibodies (corresponding to the primary antibodies) were incubated at room temperature for 50 min. The sections were washed three times with PBS for 5 min each time. After washing, the tissues were dehydrated with absolute ethanol, and the sections were sealed with xylene-clear neutral gum. Images were captured using a panoramic tissue cell quantitative analysis system at 20 × magnification (Tissue FAXS Plus, AP194065, Austria).

2.10. Data analysis

All data were expressed as mean ± SD. Western blot bands were analyzed using ImageJ, and statistical analysis was performed using SPSS Statistics 25 (IBM, US) and GraphPad Prism 8 software. Statistical analyses were performed using one-way analysis of variance (ANOVA). The least significant difference (LSD) method was adopted for comparison between groups. *P*-value <0.05 was considered statistically significant difference.

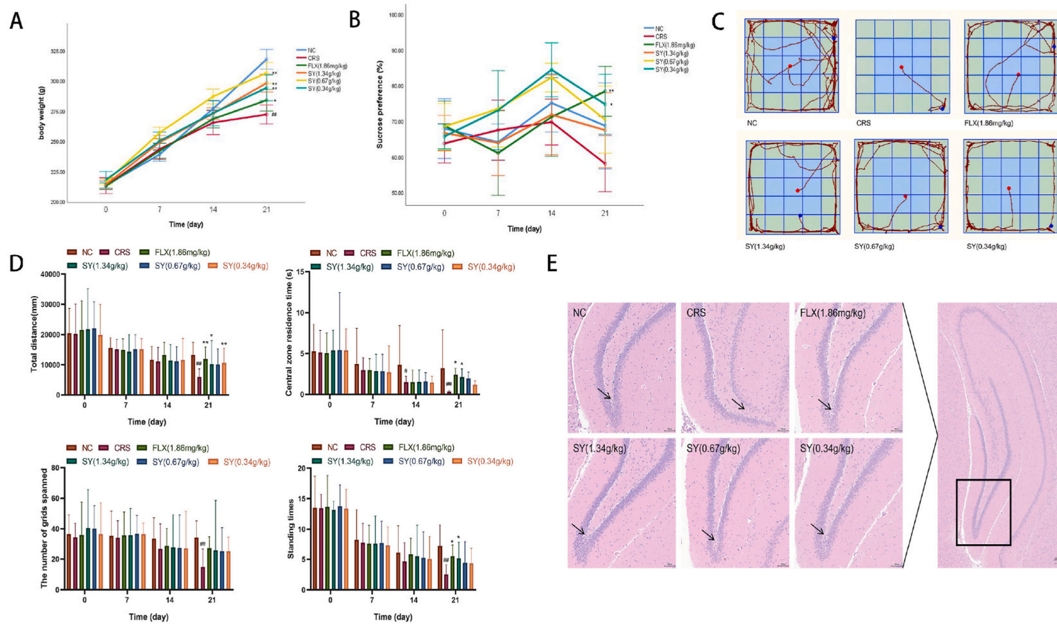

Fig. 2. **(A)** Changes in body weight during the CRS modeling period (n = 11); **(B)** Changes in sucrose preference rate during the CRS modeling period (n = 11); **(C)** the representative moving trails in OFT of rat in each group assessed by video tracking software; **(D)** Result of total move distance in OFT for 21 Days of Modeling (n = 11); Result of total grids spanned in OFT for 21 Days of Modeling (n = 11); Result of central zone residence time in OFT for 21 Days of Modeling (n = 11); Result of standing times in OFT for 21 Days of Modeling (n = 11); **(E)** Results of representative h and e staining of paraffin sections. Scale bar = 100 μm, (n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs the normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs the model group.

3. Results

3.1. SYNS improved body weight and depression-like behavior in CRS model rats

CRS causes decreased appetite and weight loss in rats. The results revealed that the CRS model group had significantly reduced animal body weights compared to the NC group. However, after 21 days of treatment, the SYNS groups showed improvement in CRS-induced weight loss ($P < 0.05$, $P < 0.01$; respectively), suggesting the antidepressant potential of SYNS (Fig. 2A).

To continue observing the behavior of CRS model rats, standard behavioral tests were carried out, including the SPT and OFT. CRS can cause a loss of pleasure in animals, which can be alleviated by antidepressants [24]. Our results showed that the sugar water preference rate of experimental animals in each group was the same before the start of the experiment, but on the 21st day, the sugar water preference rate of the CRS group was lower than that of the NC group. The sugar water preference rates of the other administration groups were increased compared with the CRS model group, and significant differences were observed between the CRS model group and the FLX and SYNS low-dose groups (SY 0.34 g/kg) ($P < 0.01$, $P < 0.05$; respectively). This finding further supported the positive effect of SYNS on depression (Fig. 2B).

The OFT test of rats was performed every seven days. At the beginning of the experiment (day 0), there were no significant differences in OFT results. However, after 21 days of drug treatment, we found that compared with the NC group, the total distance, central region residence time, number of grids spanned, and standing times were significantly reduced in the CRS group, and the FLX and SYNS high-dose groups (SY 1.34 g/kg) showed better Improvement effects based on the above indicators, with significant differences compared with the CRS model group ($P < 0.05$, $P < 0.01$; respectively) (Fig. 2C and D).

3.2. SYNS improved the hippocampal histopathological changes in CRS model rats

In the dentate gyrus regions of the hippocampus, neurons were arranged in an orderly manner in the NC group, with clear profiles and distinct nucleoli. However, hippocampal neurons in the CRS group displayed an irregular arrangement, characterized by enlarged pericellular spaces, unclear nuclear structure, and absence of clearly visible nucleoli (black arrows). After the treatment period, the above pathological phenomena improved in all groups compared with the CRS model group (Fig. 2E), suggesting that SYNS could improve the histopathological changes in the hippocampus caused by CRS to a certain extent.

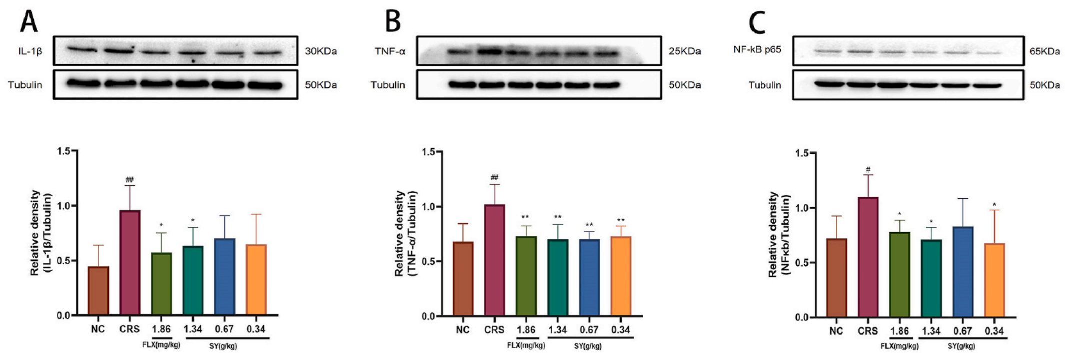

Fig. 3. Changes in the hippocampal of expressions of NF-κBp65, IL-1β and TNF-α in CRS model rats. **(A)** The protein results of IL-1β; Relative IL-1β/Tubulin protein (n = 4); **(B)**The protein results of TNF-α; Relative TNF-α/Tubulin protein (n = 4); **(C)**The protein results of NF-κBp65; Relative NF-κBp65/Tubulin protein (n = 4). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs the normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs the model group.

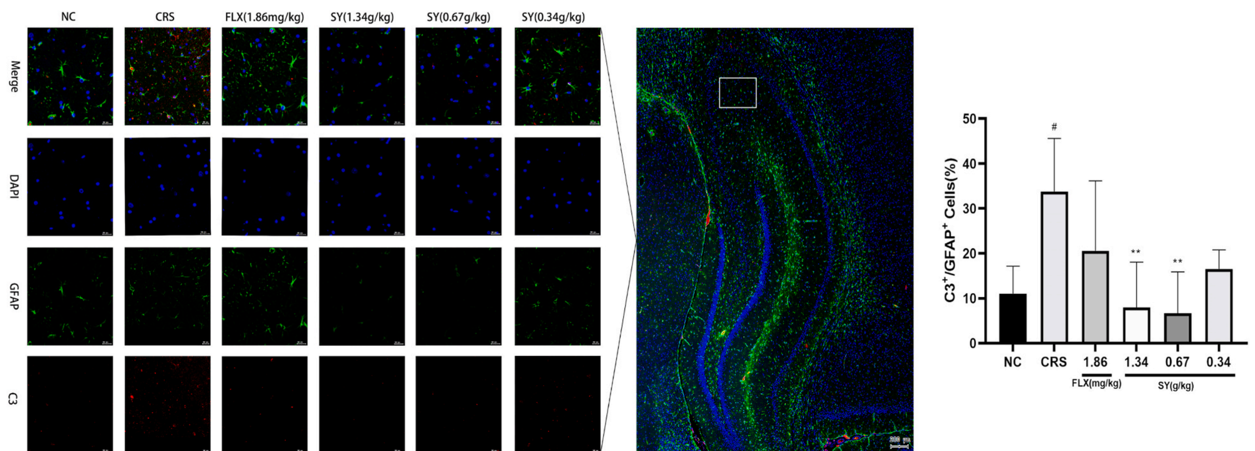

Fig. 4. SYNS Inhibited the Reactivity of A1s. IF staining of C3/GFAP in different groups. the percentage of C3+/GFAP + positive co-labeled cells (The percentage refer to the proportion of positive co-labeled cells to the total cells, Scale bar = 20 μm, n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs the normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs the model group.

3.3. SYNS reduced the expressions of NF-κB p65 and neuroinflammatory factors activated by CRS

After the completion of all behavioral experiments, hippocampi of the rats were removed and placed on ice. We measured the protein expression levels of NF-κB p65 and inflammatory factors (IL-1β and TNF-α). Compared with NC group, CRS model group demonstrated increased protein expression of IL-1β, TNF-α, and NF-κB p65 (Fig. 3A, B, C). Compared with the CRS model group, protein expression levels in the FLX and SYNS high-dose groups (SY 1.34 g/kg) were significantly decreased ($P < 0.05$, $P < 0.01$; respectively). These results suggested that SYNS inhibited the inflammatory response activated by CRS.

3.4. SYNS inhibits A1 astrocyte reactive activation in CRS model rats

Research has confirmed that C3 is one of the main characteristics of A1 astrocytes. We found that, compared with the CRS model group, the SYNS high- and medium-dose groups showed a reduced percentage of C3+ and GFAP + positive co-labeled cells (C3+/GFAP+) in the hippocampus of CRS rats (the percentage refers to the proportion of positive co-labeled cells to the total cells) ($P < 0.01$) (Fig. 4). This indicated that SYNS treatment decreased the reactive activation of A1s in the hippocampus of CRS model rats, which might have contributed to the antidepressant effect of this drug.

3.5. Effects of SYNS on the expression of neurons (NeuN) and synaptic-related proteins (MAP2 and PSD95) in the hippocampus of CRS model rats

For examining the role of SYNS in neuronal protection, we first counted the number of neurons in the hippocampus of rats in each group. The results showed that there was no significant difference in the number of neurons among the groups, indicating that the CRS model did not cause neuronal loss, and that each administration group did not provide a more effective treatment in this regard

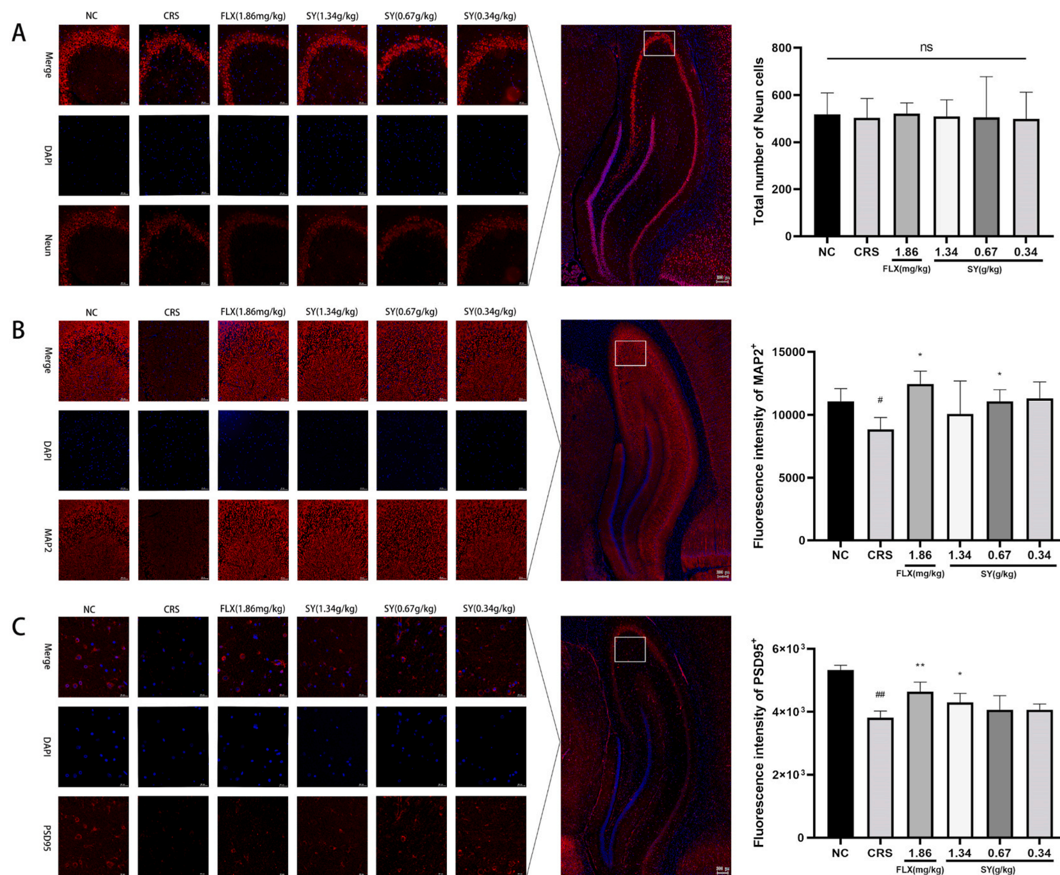

Fig. 5. CRS model and treatment groups did not affect the number of neurons, but SYNS Improved expression of synaptic related proteins (MAP2, PSD95) in Hippocampus of CRS Model Rats. **(A)** IF staining of NeuN in different groups, Scale bar = 50 μm. The number of NeuN positive cells in the hippocampus of CRS model rats (n = 3). **(B)** IF staining of MAP2 in different groups, Scale bar = 50 μm. Fluorescence intensity of MAP2 positive cells in the hippocampus of CRS model rats (n = 3). **(C)** IF staining of PSD95 in different groups. Scale bar = 20 μm. Fluorescence intensity of PSD95 positive cells in the hippocampus of CRS model rats (n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs the normal control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs the model group.

(Fig. 5A).

The morphological and structural stability of neuronal synapses is related to MAP2, and the stability of synapses can be reflected by observing the expression of MAP2 protein in neuronal synapses. The results showed that compared with the NC group, the CRS model group showed reduced fluorescence intensity of MAP2. The expression of MAP2 in each group was restored after treatment, and the FLX and SYNS medium-dose groups (SY 0.67 g/kg) showed significant increase in the fluorescence intensity of the above indicator ($P < 0.05$) (Fig. 5B). This indicates that SYNS has a positive effect on the structural stability of neuronal synapses.

PSD95 promotes synapse formation and is an important protein in the postsynaptic membrane. The density of neuronal synapses can be reflected by observing the fluorescence intensity of PSD95 protein. The results showed that after treatment, all groups recovered from CRS-induced decreased PSD95 fluorescence intensity, and the FLX and SYNS high-dose groups (SY 1.34 g/kg) showed a significant increase in the above indicator ($P < 0.01$, $P < 0.05$; respectively) (Fig. 5C). This indicates that SYNS has a positive effect on the density of neuronal synapses.

4. Discussion

Numerous studies have shown that rats exposed to CRS (consisting of a daily 6 h restraint period for 21 consecutive days) exhibit long-lasting depression-like behavior [25]. In this study, we constructed a rat model of solitary raising-CRS depression to explore the effects and mechanisms of action of SYNS against depression.

Animal models are of great significance in preclinical studies. It is crucial to construct an animal model that is suitable, effective, and capable of simulating clinical symptoms for research on drug mechanisms. Currently, there are many animal models of depression, including stress, drug, and surgical models. The depression model with solitary raising-CRS established in this study [26] has the advantage of not damaging the animal body during production of depressive behaviors due to psychological stress. In addition, the model better simulated the chronic, long-term, and low-intensity characteristics of clinical depression and has been used by many

researchers in the field of traditional Chinese medicine. Experiments on rodents under various types of chronic stress (such as chronic unpredictable stress, repeated constraint stress, or chronic social failure stress) have revealed that such stress models can lead to behavioral abnormalities, such as weight loss, inability to experience pleasant sensations, reduced exercise, and other typical clinical manifestations of depression [27,28]. Therefore, in this study, a CRS rat model was established to explore the effects of SYNS on depression. The results demonstrated that the above behavioral indicators of the CRS depression model were improved to different degrees after SYNS treatment; for example, SYNS had a positive effect on animal weight and sugar water intake, and it also showed higher activity than that of the model group in the open field experiment. However, due to the high concentration in the high-dose group, it may have some negative effects on animal diet, and therefore, the results of the high-dose group were not as good as those of the middle-dose group with regard to animal weight restoration and sugar water intake.

In addition, the CRS model can also affect the immune system of animals, and studies have shown that chronic stress enhances NF-κB signaling in the hippocampus, thereby increasing the expression of pro-inflammatory factors [29]. Here, we found that the SYNS treatment groups improved the inflammatory response caused by the CRS model, and the differences between the model group and the medication groups were observed in the subsequent hippocampal pathomorphological observation, suggesting anti-inflammatory effect of SYNS to a certain degree. Therefore, we continued to explore the pathological phenomena that may be caused by inflammation.

Glial cells perform extremely complex regulatory processes in both brain function and psychopathology [30]. Under pathological conditions, glial cells may be the primary cause of abnormal nerve conduction [31]. While studying neuronal changes, several studies have confirmed that non-neuronal (such as glial) cellular abnormalities also play an important role in stress-related diseases. Astrocytes are glial cells that are closely related to other types of cells in the brain. For a long time, astrocytes have been called “brain glue,” owing to their major role as neuronal supporting matrix [32], and also considered as “secretory cells” in the brain because they can release, increase, and store various factors necessary for maintaining the steady state of the brain environment [33]. In recent years, studies have found that astrocytes not only support cells but also support the nutritional and metabolic processes of neurons and provide a special microenvironment for the growth of adult neurons. Moreover, along with maintaining the stability of neurons, they promote neurons to play normal functions such as the regulation of extracellular fluid, ion homeostasis, ion transport, cerebral blood flow, synaptic remodeling, and energy supply [34,35]. However, various central nervous system diseases can affect astrocyte reactivity [33,36]. A1 reactive astrocytes induced by neuroinflammation can produce neurotoxicity, do not show many positive effects on neurons [17], and are involved in the process of brain aging and neurodegenerative diseases [37,38]. Our results show that SYNS reduced the expression of A1s to a certain extent, which may be due to the anti-inflammatory effect of the drug. Therefore, the decrease in A1s may be a key factor related to the disease alleviating effects of SYNS. To confirm this conclusion, we studied neuronal damage in the hippocampi of the experimental animals.

A1s do not perform many normal functions such as promoting the survival and growth of neurons, and the synaptic length and density of A1s are lower than those of normal early astrocytes. Synapses are important sites for the transmission of neuronal information. Protein markers include MAP2, PSD95, and synaptophysin (SYP), of which MAP2 is an important component of cytoskeletal microtubules that promotes the polymerization of tubulin into microtubules and provides stability to cells [39]. It plays an important role in the stability and plasticity of dendrites [40]. Changes in the neuronal synaptic structure can also be observed by labeling the MAP2 protein. PSD95 is the most important and abundant protein in the postsynaptic membrane. It not only guarantees the activity and stability of receptors on the postsynaptic membrane but also participates in the regulation of synaptic plasticity, thus promoting the formation of synapses [41], which is often used as an evaluation index of synaptic density. Pathological synaptic loss is a sign of several neurodegenerative diseases and various types of nerve damage [42]. Brain imaging studies have found that, in stressed animal models, phenomena such as neuronal atrophy, changes in synaptic density, and cell loss in the brain may occur. The chronic stress model induces damage to the length and branches of neuronal dendrites as well as a decrease in the number and function of spinous synapses of medial prefrontal cortex pyramidal neurons [43]. Autopsy has confirmed that pathological synaptic loss, an early pathological phenomenon of depression, continuously increases with the severity of clinical symptoms [44]. This phenomenon is extremely common in cases of nerve damage and often associated with cognitive dysfunction in Alzheimer’s disease and depression. Therefore, neuronal synaptic damage may be an important cause of depression and could be used as a therapeutic target for depression in drug research. Current treatment with typical antidepressants can improve synaptic plasticity, increase spinal column density, and reduce atrophy of the spine and dendrites [16]. For example, 5-hydroxytryptamine selective serotonin and norepinephrine reuptake inhibitors exhibit antidepressant effects by promoting the regulation of neural plasticity [45]. Our study confirmed that this model did not cause neuronal loss. In this case, SYNS could restore the fluorescence intensity of MAP2 and PSD95, and the evaluation indices of these synapses could prove the damage caused to the neurons. This suggests that SYNS plays an active role in protecting neurons, which may be related to the inhibition of A1s overactivation by SYNS, one of the important factors related to the antidepressant effect of the drug.

Through this research, we found that SYNS can protect neurons, which may further reduce the expression of A1s by inhibiting inflammatory reactions. This is of significance for the study of the mechanism of traditional Chinese medicine in the treatment of depression. However, this study had some limitations. For example, SYNS tablet used in this study was composed of four Chinese medicinal products rather than a single compound with the exact composition. It is not known whether these components interact with the body. This may be an important reason for the “non-dose dependent effect” of some indicators in this study. Further studies are needed to identify the targets related to SYNS for the inhibition of A1s overactivation and protection from neuronal synaptic damage. Our future studies will focus on solving these problems.

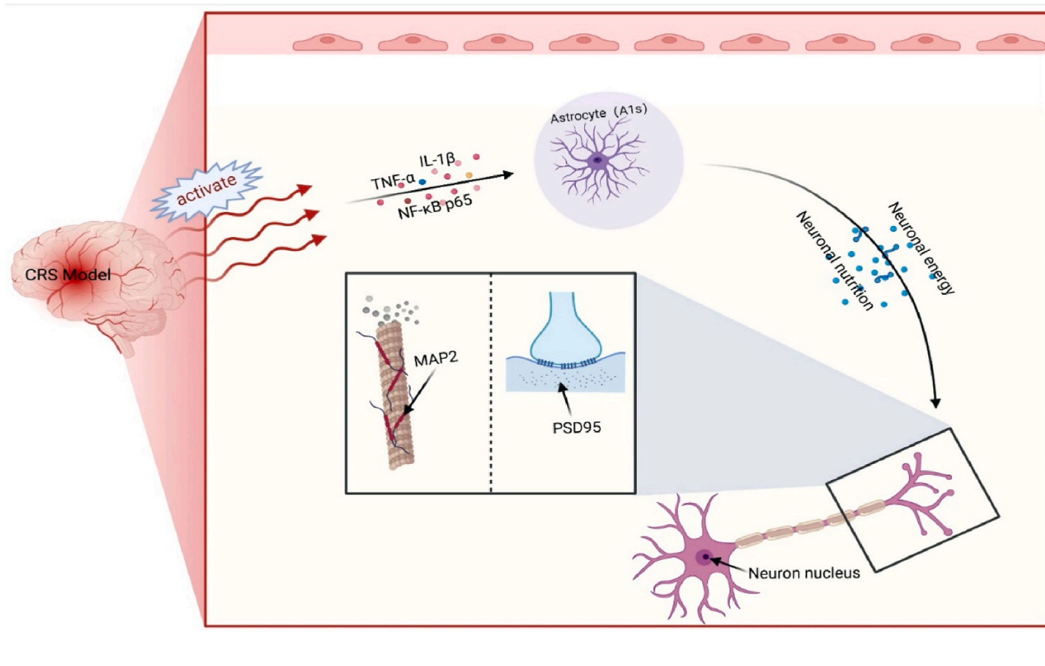

Fig. 6. Potential mechanism of SYNS on CRS Model.

5. Conclusion

In summary, this study provides sufficient evidence that SYNS can improve behavioral performance in solitary raising-chronic restraint stress depression. Our results demonstrate that SYNS may inhibit the reactive activation of A1s by inhibiting neuro-inflammatory factors to protect synapses from damage and relieve depression (Fig. 6).

Ethics statement

Animal Ethics Committee of the Institute of Chinese Materia Medica, No. 2021D001.

Funding statement

This study was supported by the grant from the National Major Scientific and Technological Special (Major New Drug Creation) Project: 2019ZX09301005.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yaxin Wang: Writing – original draft. **Shuran Li:** Data curation. **Mengping Chen:** Data curation. **Meihua Zeng:** Project administration. **Lirun Zhou:** Data curation. **Rongmei Yao:** Formal analysis. **Bo Pang:** Data curation. **Yingli Xu:** Data curation. **Shan Cao:** Data curation. **Shanshan Guo:** Writing – review & editing. **Xiaolan Cui:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like thank all authors have made a substantial, direct, and intellectual contribution to the work and approved it for

publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28916.

References

[1] Z. Jia, X. Huang, Q. Wu, T. Zhang, S. Lui, J. Zhang, N. Amatya, W. Kuang, R.C.K. Chan, G.J. Kemp, A. Mechelli, Q. Gong, High-field magnetic resonance imaging of Suicidality in patients with major depressive disorder, Am. J. Psychiatr. 167 (2010) 1381–1390, https://doi.org/10.1176/appi.ajp.2010.09101513.

[2] F. Ferrari, R.F. Villa, The Neurobiology of depression: an Integrated Overview from biological theories to clinical evidence, Mol. Neurobiol. 54 (2017) 4847–4865, https://doi.org/10.1007/s12035-016-0032-y.

[3] M.J. Friedrich, Depression is the leading cause of disability around the World, JAMA 317 (2017) 1517, https://doi.org/10.1001/jama.2017.3826.

[4] L. Kong, F. Wu, Y. Tang, L. Ren, D. Kong, Y. Liu, K. Xu, F. Wang, Frontal-subcortical volumetric deficits in single episode, medication-naïve depressed patients and the effects of 8 weeks fluoxetine treatment: a VBM-DARTEL study, PLoS One 9 (2014) e79055, https://doi.org/10.1371/journal.pone.0079055.

[5] C.D. Mathers, D. Loncar, Projections of global mortality and burden of disease from 2002 to 2030, PLoS Med. 3 (2006) e442, https://doi.org/10.1371/journal.pmed.0030442.

[6] R.C. Kessler, P. Berglund, O. Demler, R. Jin, D. Koretz, K.R. Merikangas, A.J. Rush, E.E. Walters, P.S. Wang, National comorbidity survey replication, the epidemiology of major depressive disorder: results from the national comorbidity survey replication (NCS-R), JAMA 289 (2003) 3095–3105, https://doi.org/10.1001/jama.289.23.3095.

[7] R.M. Sapolsky, Depression, antidepressants, and the shrinking hippocampus, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 12320–12322, https://doi.org/10.1073/pnas.231475998.

[8] Y. Watanabe, E. Gould, H.A. Cameron, D.C. Daniels, B.S. McEwen, Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons, Hippocampus 2 (1992) 431–435, https://doi.org/10.1002/hipo.450020410.

[9] J. Szelényi, Cytokines and the Central Nervous System - PubMed, 2001. https://pubmed.ncbi.nlm.nih.gov/11306183/. (Accessed 18 April 2022).

[10] A.M. Myint, Y.K. Kim, Cytokine-serotonin interaction through Ido: a neurodegeneration hypothesis of depression, Med. Hypotheses 61 (2003) 519–525, https://doi.org/10.1016/s0306-9877(03)00207-x.

[11] S. Boku, S. Nakagawa, H. Toda, A. Hishimoto, Neural basis of major depressive disorder: beyond monoamine hypothesis: neural basis of major depressive disorder, Psychiatr. Clin. Neurosci. 72 (2018) 3–12, https://doi.org/10.1111/pcn.12604.

[12] L. Schmaal, D.J. Veltman, T.G.M. van Erp, P.G. Sämann, T. Frodl, N. Jahanshad, E. Loehrer, H. Tiemeier, A. Hofman, W.J. Niessen, M.W. Vernooij, M.A. Ikram, K. Wittfeld, H.J. Grabe, A. Block, K. Hegenscheid, H. Völzke, D. Hoehn, M. Czisch, J. Lagopoulos, S.N. Hatton, I.B. Hickie, R. Goya-Maldonado, B. Krämer, O. Gruber, B. Couvy-Duchesne, M.E. Rentería, L.T. Strike, N.T. Mills, G.I. de Zubicaray, K.L. McMahon, S.E. Medland, N.G. Martin, N.A. Gillespie, M.J. Wright, G.B. Hall, G.M. MacQueen, E.M. Frey, A. Carballedo, L.S. van Velzen, M.J. van Tol, N.J. van der Wee, I.M. Veer, H. Walter, K. Schnell, E. Schramm, C. Normann, D. Schoepf, C. Konrad, B. Zurowski, T. Nickson, A.M. McIntosh, M. Papmeyer, H.C. Whalley, J.E. Sussmann, B.R. Godlewska, P.J. Cowen, F.H. Fischer, M. Rose, B.W.J.H. Penninx, P.M. Thompson, D.P. Hibar, Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group, Mol. Psychiatr. 21 (2016) 806–812, https://doi.org/10.1038/mp.2015.69.

[13] C.E. Sexton, C.E. Mackay, K.P. Ebmeier, A systematic review and meta-analysis of magnetic resonance imaging studies in late-life depression, Am. J. Geriatr. Psychiatry Off. J. Am. Assoc. Geriatr. Psychiatry. 21 (2013) 184–195, https://doi.org/10.1016/j.jagp.2012.10.019.

[14] J. Xu, F. de Winter, C. Farrokhi, E. Rockenstein, M. Mante, A. Adame, J. Cook, X. Jin, E. Masliah, K.-F. Lee, Neuregulin 1 improves cognitive deficits and neuropathology in an Alzheimer's disease model, Sci. Rep. 6 (2016) 31692, https://doi.org/10.1038/srep31692.

[15] C. Eroglu, B.A. Barres, Regulation of synaptic connectivity by glia, Nature 468 (2010) 223–231, https://doi.org/10.1038/nature09612.

[16] A.M. Magariños, B.S. McEwen, Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors, Neuroscience 69 (1995) 83–88, https://doi.org/10.1016/0306-4522(95)00256-i.

[17] S.A. Liddelow, K.A. Guttenplan, L.E. Clarke, F.C. Bennett, C.J. Bohlen, L. Schirmer, M.L. Bennett, A.E. Münch, W.-S. Chung, T.C. Peterson, D.K. Wilton, A. Frouin, B.A. Napier, N. Panicker, M. Kumar, M.S. Buckwalter, D.H. Rowitch, V.L. Dawson, T.M. Dawson, B. Stevens, B.A. Barres, Neurotoxic reactive astrocytes are induced by activated microglia, Nature 541 (2017) 481–487, https://doi.org/10.1038/nature21029.

[18] A. Fujita, H. Yamaguchi, R. Yamasaki, Y. Cui, Y. Matsuoka, K. Yamada, J. Kira, Connexin 30 deficiency attenuates A2 astrocyte responses and induces severe neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride Parkinson's disease animal model, J. Neuroinflammation 15 (2018) 227, https://doi.org/10.1186/s12974-018-1251-0.

[19] T. Li, X. Chen, C. Zhang, Y. Zhang, W. Yao, An update on reactive astrocytes in chronic pain, J. Neuroinflammation 16 (2019) 140, https://doi.org/10.1186/s12974-019-1524-2.

[20] Q. Mao, Z. Huang, Research progress on action mechanism of Chinese medicine in treatment of depression, Chin. J. Chin. Med. (2007) 877–880.

[21] Y. Tu, X. Cui, M. Zeng, Application of a traditional Chinese medicine composition for treating depression in regulating immune inflammatory response. https://kns.cnki.net/kcms/detail/detail.aspx?dbcode=SCPD&dbname=SCPD2019&filename=CN109602884A&uniplatform=NZKPT&v=tntoxAfnp6R8fFn5A9LV8PSJVxDySUmvbpYpPAeQNrKgOQyxohM5zycKVLQcDbIj, 2019. (Accessed 18 April 2022).

[22] H. Jiang, Y. Wang, Y. Tu, M. Zeng, J. Zeng, X. Cui, Effect of shenwei ningyu pills on immunoinflammatory response mediated by TLR4/MyD88 signaling pathway in Hippocampus of rats exposed to chronic restraint stress, Chin. J. Exp. Tradit. Med. Formulae 27 (2021) 8–15, https://doi.org/10.13422/j.cnki.syfjx.20211405.

[23] M.K. Seo, C.M. Choi, R.S. McIntyre, H.Y. Cho, C.H. Lee, R.B. Mansur, Y. Lee, J.-H. Lee, Y.H. Kim, S.W. Park, J.G. Lee, Effects of escitalopram and paroxetine on mTORC1 signaling in the rat hippocampus under chronic restraint stress, BMC Neurosci. 18 (2017) 39, https://doi.org/10.1186/s12868-017-0357-0.

[24] S. Chiba, T. Numakawa, M. Ninomiya, M.C. Richards, C. Wakabayashi, H. Kunugi, Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 39 (2012) 112–119, https://doi.org/10.1016/j.pnpbp.2012.05.018.

[25] H. Qiao, M.-X. Li, C. Xu, H.-B. Chen, S.-C. An, X.-M. Ma, Dendritic spines in depression: what we learned from animal models, Neural Plast. 2016 (2016) 8056370, https://doi.org/10.1155/2016/8056370.

[26] W. Wang, H. Guo, S.-X. Zhang, J. Li, K. Cheng, S.-J. Bai, D.-Y. Yang, H.-Y. Wang, Z.-H. Liang, L. Liao, L. Sun, P. Xie, Targeted metabolomic pathway analysis and validation revealed glutamatergic disorder in the prefrontal cortex among the chronic social defeat stress mice model of depression, J. Proteome Res. 15 (2016) 3784–3792, https://doi.org/10.1021/acs.jproteome.6b00577.

[27] N. Abe-Higuchi, S. Uchida, H. Yamagata, F. Higuchi, T. Hobara, K. Hara, A. Kobayashi, Y. Watanabe, Hippocampal sirtuin 1 signaling mediates depression-like behavior, Biol. Psychiatr. 80 (2016) 815–826, https://doi.org/10.1016/j.biopsych.2016.01.009.

[28] F. Higuchi, S. Uchida, H. Yamagata, N. Abe-Higuchi, T. Hobara, K. Hara, A. Kobayashi, T. Shintaku, Y. Itoh, T. Suzuki, Y. Watanabe, Hippocampal MicroRNA-124 enhances chronic stress r esilience in mice, J. Neurosci. Off. J. Soc. Neurosci. 36 (2016) 7253–7267, https://doi.org/10.1523/JNEUROSCI.0319-16.2016.

[29] S.-Y. Lee, W.-H. Cho, Y.-S. Lee, J.-S. Han, Impact of chronic stress on the spatial learning and GR-PKAc-NF-κb signaling in the Hippocampus and cortex in rats following cholinergic depletion, Mol. Neurobiol. 55 (2018) 3976–3989, https://doi.org/10.1007/s12035-017-0620-5.

[30] L. Rudzki, M. Maes, The microbiota-gut-immune-glia (MGIG) Axis in major depression, Mol. Neurobiol. 57 (2020) 4269–4295, https://doi.org/10.1007/s12035-020-01961-y.

[31] T. Iram, D. Trudler, D. Kain, S. Kanner, R. Galron, R. Vassar, A. Barzilai, P. Blinder, Z. Fishelson, D. Frenkel, Astrocytes from old Alzheimer's disease mice are impaired in Aβ uptake and in neuroprotection, Neurobiol. Dis. 96 (2016) 84–94, https://doi.org/10.1016/j.nbd.2016.08.001.

[32] A. Volterra, J. Meldolesi, Astrocytes, from brain glue to communication elements: the revolution continues, Nat. Rev. Neurosci. 6 (2005) 626–640, https://doi.org/10.1038/nrn1722.

[33] A. Verkhratsky, M. Matteoli, V. Parpura, J. Mothet, R. Zorec, Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion, EMBO J. 35 (2016) 239–257, https://doi.org/10.15252/embj.201592705.

[34] J.A. Stogsdill, J. Ramirez, D. Liu, Y.H. Kim, K.T. Baldwin, E. Enustun, T. Ejikeme, R.-R. Ji, C. Eroglu, Astrocytic neuroligins control astrocyte morphogenesis and synaptogenesis, Nature 551 (2017) 192–197, https://doi.org/10.1038/nature24638.

[35] B.S. Khakh, Astrocyte-neuron interactions in the striatum: insights on identity, form, and function, Trends Neurosci. 42 (2019) 617–630, https://doi.org/10.1016/j.tins.2019.06.003.

[36] M.V. Sofroniew, Astrocyte reactivity: subtypes, states, and functions in CNS innate Immunity, Trends Immunol. 41 (2020) 758–770, https://doi.org/10.1016/j.it.2020.07.004.

[37] B. Diaz-Castro, M. Gangwani, X. Yu, G. Coppola, B. Khakh, Astrocyte molecular signatures in Huntington's disease. https://www.science.org/doi/10.1126/scitranslmed.aaw8546, 2019. (Accessed 18 April 2022).

[38] S. Sun, Y. Sun, S.-C. Ling, L. Ferraiuolo, M. McAlonis-Downes, Y. Zou, K. Drenner, Y. Wang, D. Ditsworth, S. Tokunaga, A. Kopelevich, B.K. Kaspar, C. Lagier-Tourenne, D.W. Cleveland, Translational profiling identifies a cascade of damage initiated in motor neurons and spreading to glia in mutant SOD1-mediated ALS, Proc. Natl. Acad. Sci. USA 112 (2015), https://doi.org/10.1073/pnas.1520639112.

[39] G.A. Lammie, I.R. Piper, D. Thomson, F. Brannan, Neuropathologic characterization of a rodent model of closed head injury–addition of clinically relevant secondary insults does not significantly potentiate brain damage, J. Neurotrauma 16 (1999) 603–615, https://doi.org/10.1089/neu.1999.16.603.

[40] G.L. Pereno, C.A. Beltramino, Timed changes of synaptic zinc, synaptophysin and MAP2 in medial extended amygdala of epileptic animals are suggestive of reactive neuroplasticity, Brain Res. 1328 (2010) 130–138, https://doi.org/10.1016/j.brainres.2010.01.087.

[41] E. Kim, M. Sheng, PDZ domain proteins of synapses, Nat. Rev. Neurosci. 5 (2004) 771–781, https://doi.org/10.1038/nrn1517.

[42] M.M. Riccomagno, A.L. Kolodkin, Sculpting neural circuits by axon and dendrite pruning, Annu. Rev. Cell Dev. Biol. 31 (2015) 779–805, https://doi.org/10.1146/annurev-cellbio-100913-013038.

[43] Y. Watanabe, E. Gould, B.S. McEwen, Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons, Brain Res. 588 (1992) 341–345, https://doi.org/10.1016/0006-8993(92)91597-8.

[44] D.J. Selkoe, Alzheimer's disease is a synaptic failure, Science 298 (2002) 789–791, https://doi.org/10.1126/science.1074069.

[45] H. Xu, Z. Chen, J. He, S. Haimanot, X. Li, L. Dyck, X.-M. Li, Synergetic effects of quetiapine and venlafaxine in preventing the chronic restraint stress-induced decrease in cell proliferation and BDNF expression in rat hippocampus, Hippocampus 16 (2006) 551–559, https://doi.org/10.1002/hipo.20184.