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Original Research

Protective Effects of Shi-Zhen-An-Shen Decoction on the Cognitive Impairment in MK801-Induced Schizophrenia Model

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Abstract

Background: Cognitive dysfunction is a core feature of schizophrenia that strongly correlates to the patients' difficulties in independent living and occupational functioning. Synaptic dysfunction may result in cognitive and behavioral changes similar to what have been identified in schizophrenia. Shi-Zhen-An-Shen Decoction (SZASD) is the empirical formula of traditional Chinese medicine adopted in treating psychiatric symptoms, especially the cognitive impairment in schizophrenia patients, with proven efficacy in the long term of clinical practice in Beijing Anding Hospital, Capital Medical University. However, the mechanisms of SZASD on the cognitive improvement in schizophrenia is still unclear. Here, we aim to investigate the underlying mechanisms of the impact of SZASD on the cognitive impairment in MK801-induced schizophrenia-like rats. **Methods**: Six rat groups (n = 12 per group) were subjected to different treatments for 14 days. All the six groups were injected intraperitoneally with a given volume of 0.9% saline and MK801 (0.2 mg/kg) for consecutive 14 days for modelling. And the rats in the SZASD-treated groups and the clozapine-treated group were given SZASD (low, middle, and high doses) or clozapine, respectively, by intragastric administration. Then, we performed behavioral tests after the treatments, and the rats were sacrificed on the 19th day for biological analysis. **Results**: Behavioral tests indicated that SZASD mitigated the aberrant motor activity and improved schizophrenia-like rats' spatial reference memory and sensory gating ability. Furthermore, SZASD significantly increased the expressions of PSD95, BDNF, and synapsin I in the hippocampus of MK801-induced schizophrenia-like rats. **Conclusions**: Our findings suggest that SZASD may ameliorate cognitive impairment by restoring the levels of synaptic proteins in the hippocampus.

Keywords: schizophrenia; Shi-Zhen-An-Shen decoction; MK801; cognitive dysfunction; mechanisms; the hippocampus

1. Introduction

Schizophrenia typically appears in early adulthood with multiple causes, eventually reducing life expectancy [1]. Cognitive dysfunction is a hallmark of schizophrenia, leading to difficulties in independent living and occupational functioning [2]. It has been acknowledged that improving cognitive functions is now a pivotal treatment goal in schizophrenia [3]. Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Initiative also supports the development of pharmacological agents for ameliorating the cognitive deficits in schizophrenic patients [4]. There are seven primary cognitive regions involved in schizophrenia: attention/vigilance, speed of processing, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition [5]. The pathogenesis of cognitive impairments in schizophrenia involves several signaling pathways and neurotransmitter systems [6]. It is recognised that the disturbance of brain synaptic connections and functions may contribute to cognitive impairments in schizophrenia patients, especially in the prefrontal cortex and hippocampus [7]. Notably, recent studies have reported that the alterations of parvalbumin interneuron are initially restricted to the hippocampal CA1/subiculum, corresponding to cognitive dysfunction in late adolescence [8]. The hippocampus CA1/subiculum may be an important targeted brain region in preventing schizophrenia.

Synapses are components of neuronal cells, providing the sites of releasing neurotransmitter to modulate neurological activities [9]. The normal synaptic function is mediated by several synaptic related proteins, such as postsynaptic density-95 (PSD-95), brain-derived neurotrophic factor (BDNF), and synapsin I [10]. PSD-95 is important for shaping the PSD structure and stabilising the postsynaptic spine structure [11]. Molecular studies have reported that the level of PSD-95 is downregulated in schizophrenia subjects [12]. BDNF can interact with PSD-95 to regulate synaptic plasticity, which is critical for cognitive function in schizophrenia patients [13]. With a high affinity of tropomyosin receptor kinase B (TrkB), BDNF can acti-

vate the downstream TrkB-MAPK-Erk1/2 pathway to modulate synaptic plasticity [14]. Furthermore, previous studies have illustrated that exogenous BDNF can upregulate the expression of synapsin I, which extremely correlates with the morphology and function of synapses [15]. Synapsin I acts as a presynaptic marker localised on the surface of synaptic vesicles, located in the inhibitory synapses [16]. Increasing evidence have displayed that a reduction of the level of synapsin I is found in the hippocampus of the schizophrenia-related rodent model [17]. Synapsin I may be the potentially important target for enhancing the functions of synapses in schizophrenia.

With no available agents targeting the improvement of cognitive functions, there is still a demand for cognition-protective agents treating patients in clinical settings [18]. Notably, cognitive remediation programs are continuously proceeded for improving functional outcomes of patients in schizophrenia. Shi-Zhen-An-Shen Decoction (SZASD) is an empirical Chinese medical formula to effectively treat psychiatric symptoms and ameliorate cognitive dysfunction in patients with schizophrenia at Beijing Anding Hospital, Capital Medical University [19]. SZASD is composed of components, such as *Danshen* (*Radix et Rhizoma Salviae Miltiorrhizae*), *Juhua* (*Flos Chrysanthemi*), *Heshouwu* (*Radix Polygoni Multiflori*), *Shanzhuyu* (*Fructus Corni*), *Dihuang* (*Radix Rehmanniae*) and other ingredients. The active components of SZASD include cornel iridoid glycoside and tetrahydroxystilbene glucoside; these both produce a protective effect on neurons and cognitive functions [20, 21]. Our previous study has revealed that SZASD has beneficial effects on reversing the schizophrenia-like behaviors and demyelination of cuprizone-induced mice [22]. Mechanically, SZASD can improve the expression of myelin basic protein in the hippocampus to ameliorate demyelination. However, the effect of SZASD on cognitive dysfunction of the schizophrenia-related animal model remains unclear. It has been reported that dizocilpine (MK801) could induce cognitive dysfunction and other schizophrenia-like behaviors in the rodent model by non-competitive blocking of N-methyl-D-aspartic acid (NMDA) receptors [23]. Thus, the purpose of this study was to investigate the mechanism of effects of SZASD on cognitive impairment in MK801-induced schizophrenia-like rats and synaptic proteins including PSD-95, BDNF, and synapsin I.

2. Methods

2.1 Animals and Treatment

Adult male Sprague-Dawley rats (8 weeks old, 180–200 g, Vital River, Beijing, China) were reared in the Specific Pathogen Free (SPF) with a 12 h light/dark cycle (22 ± 1 °C and 55 ± 5% humidity). All rats were divided in groups of three per cage free to food and water. A total of seventy-two rats were randomly divided into six groups (n = 12 per group): the control group (saline-treated group), the model group (MK801-treated group), SZASD low-dose group (SZASD 6 g/kg), SZASD medium-dose group (SZASD 12 g/kg), SZASD high-dose group (SZASD 18 g/kg), and clozapine-treated group (clozapine 5 mg/kg). The control group received regular chow and water. The model, SZASD-treated, and clozapine-treated groups were injected intraperitoneally with MK801 (0.2 mg/kg) once per day for two consecutive weeks. At the same treated duration, the rats in SZASD-treated groups were administered intragastrically with SZASD (6 g/kg, 12 g/kg and 18 g/kg). And the rats in the clozapine-treated group were administered intragastrically with clozapine (5 mg/kg). We performed behavioral tests from 15 to 18 days with no treatment on rats, and the rats were sacrificed on the 19th day for biological evaluation (the experimental procedures were shown in Fig. 1). Experimental processes were carried out based on the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The ethical statement was approved by the Institutional Animal Care and Use Committee of Capital Medical University (No. AEEI-2018-047). We made every effort to lower animals' suffering.

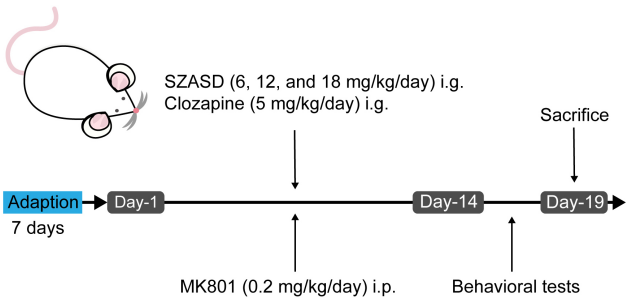

Fig. 1. The experimental procedure in the present study.

2.2 Chemicals and Antibodies

MK-801, isoflurane, paraformaldehyde, protease inhibitor cocktail, and TritonX-100 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Clozapine was purchased from Jiangsu Nhwa Pharmaceutical Co., Ltd (Xuzhou, Jiangsu, China). Laemmli sample buffer and clarity western ECL substrate were purchased from Bio-Rad (Hercules, CA, USA). Phosphate-buffered saline was purchased from Zhongshan Golden Bridge Biology Company (Beijing, China). And bovine serum albumin was purchased from Becton, Dickinson and Company (Franklin Lake, New Jersey, USA). Commercial antibodies used in this research were listed as follows: anti-PSD95 (Abcam, Cambridge, MA, USA, ab2723), anti-synapsin I (Abcam, Cambridge, MA, USA, ab64581), anti-BDNF (Abcam, Cambridge, MA, USA, ab108319), and horseradish peroxidase- (HRP-) conjugated secondary antibodies (Zhongshan Golden Bridge Biology Company, Beijing, China). SZASD is a traditional Chinese empirical formula, which is composed of *Danshen (Radix et Rhi-*

zoma Salviae Miltiorrhizae), Juhua (Flos Chrysanthemi), Heshouwu (Radix Polygoni Multiflori), Shanzhuyu (Fructus Corni), Dihuang (Radix Rehmanniae) and other components. All of the herbs in this formula were purchased from Beijing Tong Ren Tang Group (Co., Ltd., Beijing, China). The extraction procedure of the formula was performed as previously described [22].

2.3 Open Field Test

We used an open field test to estimate the rat's motor activity and exploratory behaviors. We performed this test in a square grid (100 × 100 × 45 cm^3) divided into 16 squares in a quiet room. Each rat was placed in the open field centre to test individually for 10 min [24]. And we recorded the behaviors of rats by a camera connected to a computer. The parameters were detected by the SuperMaze animal behaviour analysis system, including total distance, average speed (mm/s), centre time, centre/total time, centre/total time, center/total distance, and corner distance. After testing, the equipment was cleaned thoroughly with 70% ethyl alcohol.

2.4 Y Maze Test

The Y maze test is adopted to investigate spatial reference memory of rats by specifically using the spatial cognition test, which is hippocampal-dependent [25]. We used the Y maze with three arms (containing novel arm, start arm, and other arm) of equal dimensions (25 cm height, 35 cm length, 10 cm width) and a central area in an equilateral triangular shape. In the training phase, a guillotine door blocked one arm (novel arm), and each rat was placed in one of the two possible start arms (labelled start arm and other arm) in a randomized sequence for 10 min. After 1 h, each rat was placed back in the start arm with access to three arms. We recorded data in the process of rats entering the arms in 5 min, including total distance and the ratio of novel/total arm distance. After testing, the equipment was cleaned with 70% ethyl alcohol.

2.5 Prepulse Inhibition (PPI)

We performed a PPI test to evaluate rats' auditory startle reflex and sensory gating in this study. All test sessions were conducted in a single-chamber startle apparatus (MED-ASR-PRO1, MED Associates Inc., St. Albans, VT, USA). After the rats were allowed to adapt for 5 min, the rats were exposed to a series of startle pulses with white noise (68 dB). In this experiment, eight different levels of stimuli were supplied: no stimulus, a single 40 ms startle pulse stimulus (120 dB), prepulse stimulus (74 dB), prepulse stimulus (80 dB), prepulse stimulus (86 dB), prepulse stimulus (74 dB) + startle pulse stimulus (120 dB), prepulse stimulus (80 dB) + startle pulse stimulus (120 dB), and prepulse stimulus (86 dB) + startle pulse stimulus (120 dB). The test of each rat was completed within 30 min. The results of the PPI test were calculated automatically by the software, and the percentage of PPI was calculated as [1 – (startle amplitude on prepulse trial/startle amplitude on pulse alone)] × 100% [26].

2.6 Tissue Preparation

After the behavioral tests were completed, we conducted further analysis to observe the effects of SZASD on the specific brain region. All rats were anaesthetized with isoflurane for the biological analysis and euthanized using cervical dislocation. The brain tissue of the hippocampus was dissected and stored at –80 °C for Western blotting's use.

2.7 Western Blot Analysis

The brain samples were lysed by Tris-EDTA lysis buffer (1 mM EDTA, 20 mM Tris, pH 7.5, 1% Triton X-100, and 10% glycerol), which contained the protease inhibitor. The bicinchoninic acid protein assay determined the concentrations of protein. Afterwards, protein samples (20 μg/μL per group) were separated by 10% SDS-PAGE gels. And the samples were transferred to a polyvinyl difluoride membrane (Millipore, Boston, MA, USA). The membranes were blocked by Tris-buffered saline containing 0.1% Tween-20 and 5% nonfat milk and then probed with specific antibodies, including anti-PSD95 (1:1000) and anti-synapsin I (1:3000), and anti-BDNF (1:500), overnight at 4 °C. After three washes with PBST, the membranes were incubated with PBST/5% milk containing HRP-conjugated secondary antibodies (1:5000) for two hours. Finally, the protein bands (n = 5 or 6 for each group) were visualised by enhanced chemiluminescence (Bio-Rad, Hercules, CA, USA). And the blots were quantified by the software Quantity One (Version 4.6.2, Bio-Rad, Hercules, CA, USA) [27].

2.8 Statistical Analysis

Values were expressed as the mean ± standard error of the mean (SEM), and the differences among means were evaluated using one-way variance analysis followed by Tukey's test. The statistical analysis was conducted by the software GraphPad Prism (Version 7.0, San Diego, CA, USA). The $p < 0.05$ was considered as the threshold of statistically significant [28].

3. Results

3.1 SZASD Mitigated Spontaneous Activities and Anxious Behaviors of Rats in Open Field Test

Open field test aimed to explore the effect of SZASD on locomotor activity in MK801-induced schizophrenia-like rats. The representative movement traces of rats in six groups are shown in Fig. 2. The results showed that MK801 has significantly increased the total distance ($F = 8.738$, $p < 0.001$) and average speed ($F = 9.533$, $p < 0.001$) of rats compared with the control group (Fig. 2B,C). SZASD-treated groups (12 and 18 mg/kg) remarkedly reversed the total distance of rats compared with the model group

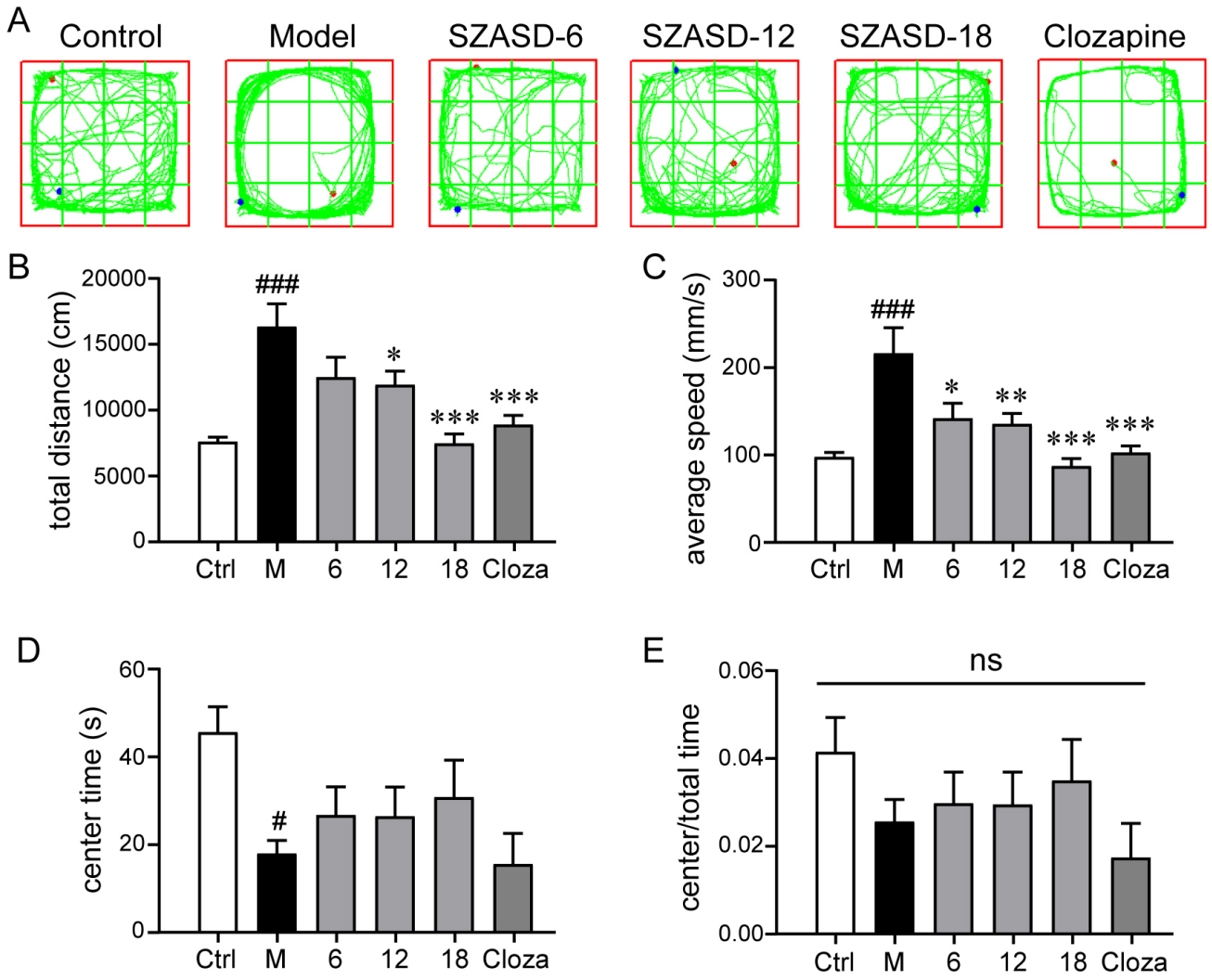

Fig. 2. The effect of SZASD on motor activity and anxious behavior of rats in open field test. (A) Representative movement traces of rats in each group. (B) Total distance, (C) average speed, (D) center time, and (E) center/total time were recorded and analyzed for the differently treated rats (n = 12 for each group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. model group; ns, not significant statistically; Ctrl, the control group; M, the model group; 6, the SZASD 6 mg/kg treated group; 12, the SZASD 12 mg/kg treated group; 18, the SZASD 18 mg/kg treated group; Cloza, the clozapine-treated group).

(Fig. 2C). SZASD-treated groups (6, 12 and 18 mg/kg) significantly decreased the average speed of rats compared with the model group (Fig. 2C). In the clozapine-treated group, rats' total distance and average speed were significantly lower than in the model group ($p < 0.001$). Furthermore, we investigated the anxious behavior of rats by recording the time spent in the central area ($F = 2.878$). MK801 remarkably reduced the time spent in the central area of rats compared with the control ($p < 0.05$). Nevertheless, neither SZASD nor clozapine alleviated the anxious behavior of MK801-induced schizophrenia-like rats (Fig. 2D,E).

3.2 SZASD Improved Spatial Reference Memory of Rats

We conducted a Y-maze test to assess the spatial reference memory of rats. Our results displayed that MK801 significantly upregulated the total distance ($F = 4.721$) and downregulated the ratio of novel arm distance and total distance ($F = 8.061$) of rats (Fig. 3B,C, $p < 0.001$). However, SZASD treatment (6, 12 and 18 mg/kg) has remarkably reduced the total distance of rats ($p < 0.001$), and also improved the ratio of novel arm distance and the total distance of rats compared with the model group ($p < 0.05$).

3.3 SZASD Improved the Auditory Startle Reflex and Sensory Gating of Rats

PPI test was performed to estimate the sensory gating function of rats, which is the typically behavioral test in schizophrenia-related models [29]. In the model group, the percentage of PPIs (74 dB + 120 dB, 80 dB + 120 dB, 86 dB + 120 dB) was significantly lower than the control group (Fig. 4, 74 dB + 120 dB: $F = 4.984$; 80 dB + 120 dB: $F =$

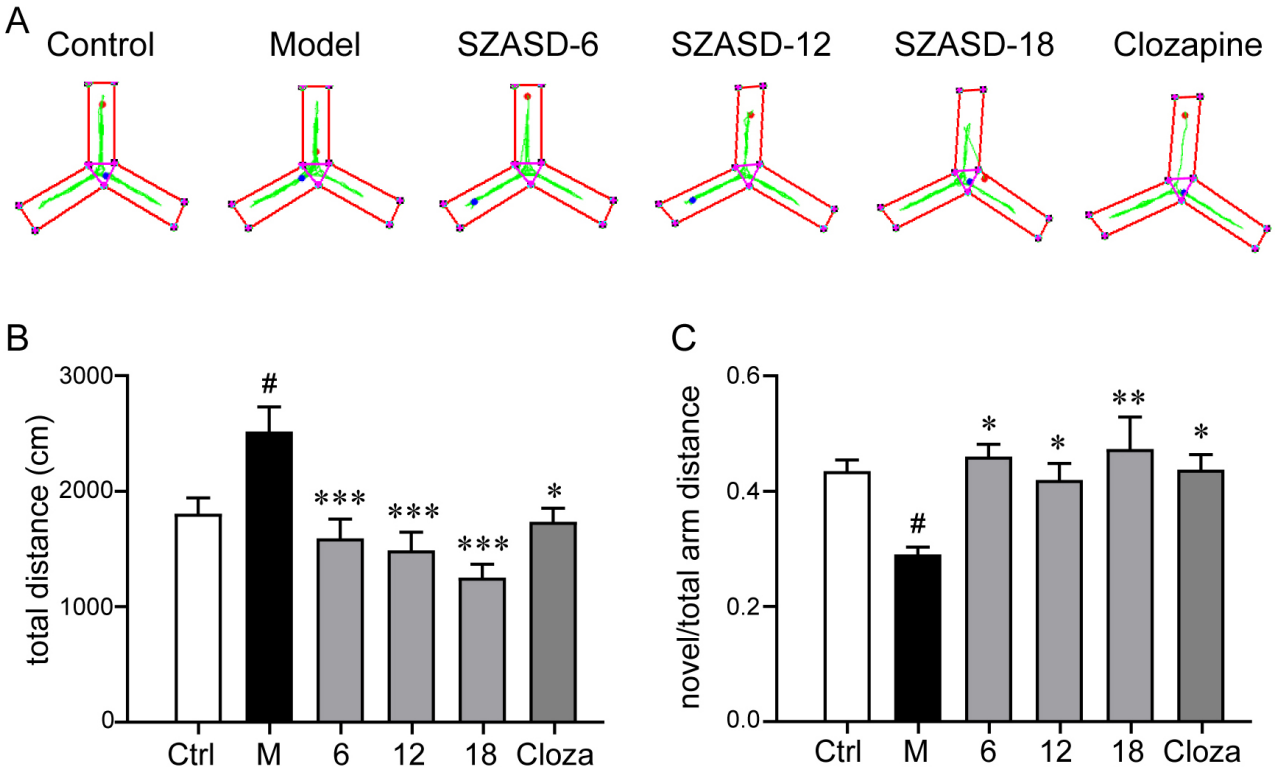

Fig. 3. The effect of SZASD on spontaneous motor activity and spatial memory of rats in Y-maze test. (A) Representative movement traces of rats in each group. (B) Total distance and (C) novel/total arm distance were recorded and analyzed in rats of each group (n = 12 for each group; $^{\#}p < 0.05$ vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. model group; Ctrl, the control group; M, the model group; 6, the SZASD 6 mg/kg treated group; 12, the SZASD 12 mg/kg treated group; 18, the SZASD 18 mg/kg treated group; Cloza, the clozapine-treated group).

5.184; 86 dB + 120 dB: $F = 6.524$; $p < 0.01$). Nonetheless, SZASD administration (12 and 18 mg/kg) significantly improved the percentage of PPIs (74 dB + 120 dB, 80 dB + 120 dB, 86 dB + 120 dB) in MK801-induced schizophrenia-like rats. Compared with the model group, clozapine treatment also significantly reversed the percentage of PPIs ($p < 0.01$).

3.4 SZASD Enhanced the Expressions of BDNF in the Hippocampus of Rats

BDNF exerts an important effect on the cognitive function of rodent models and human models [30]. We further estimated the effect of SZASD on the levels of BDNF in the hippocampus of rats by western blot. The results displayed that MK801 has significantly lowered the level of expression of BDNF in the hippocampus compared with the control group (Fig. 5A, $F = 4.869$, $p < 0.01$). And the treatments of SZASD (18 mg/kg) and clozapine have significantly enhanced the level of BDNF compared with the model group ($p < 0.05$).

3.5 SZASD Enhanced the Expressions of PSD95 and Synapsin I in the Hippocampus of Rats

The interaction of PSD95 and BDNF could effectively improve the cognitive functions in schizophrenia [31]. Specifically, PSD95 is critical for synaptic plasticity and morphology, participating in the glutamate transmission [32]. Our results showed that the expression of PSD95 in the model group was significantly lower than that in the control group (Fig. 5B, $F = 4.407$, $p < 0.05$). We observed that SZASD (12 and 18 mg/kg) and clozapine treatments remarkably increased the level of PSD95 in the hippocampus of rats ($p < 0.05$). Moreover, we observed that MK801 has significantly downregulated the expression of synapsin I in the hippocampus compared with the control (Fig. 5C, $F = 3.314$, $p < 0.05$). SZASD treatment (12 mg/kg) remarkably upregulated the level of synapsin I compared with the model group ($p < 0.05$); there was no statistical significance in the synapsin I level in the clozapine-treated group compared to the model group ($p > 0.05$).

4. Discussion

Our previous clinical study proved that SZASD could improve the symptoms and cognitive impairments of clinical high-risk individuals with schizophrenia [33]. Furthermore, we explored the mechanisms of SZASD on the treatment of schizophrenia with cuprizone-treated mice, we found that SZASD exert its therapeutic effect on schizophrenia independent with the neuregulin-1 signaling

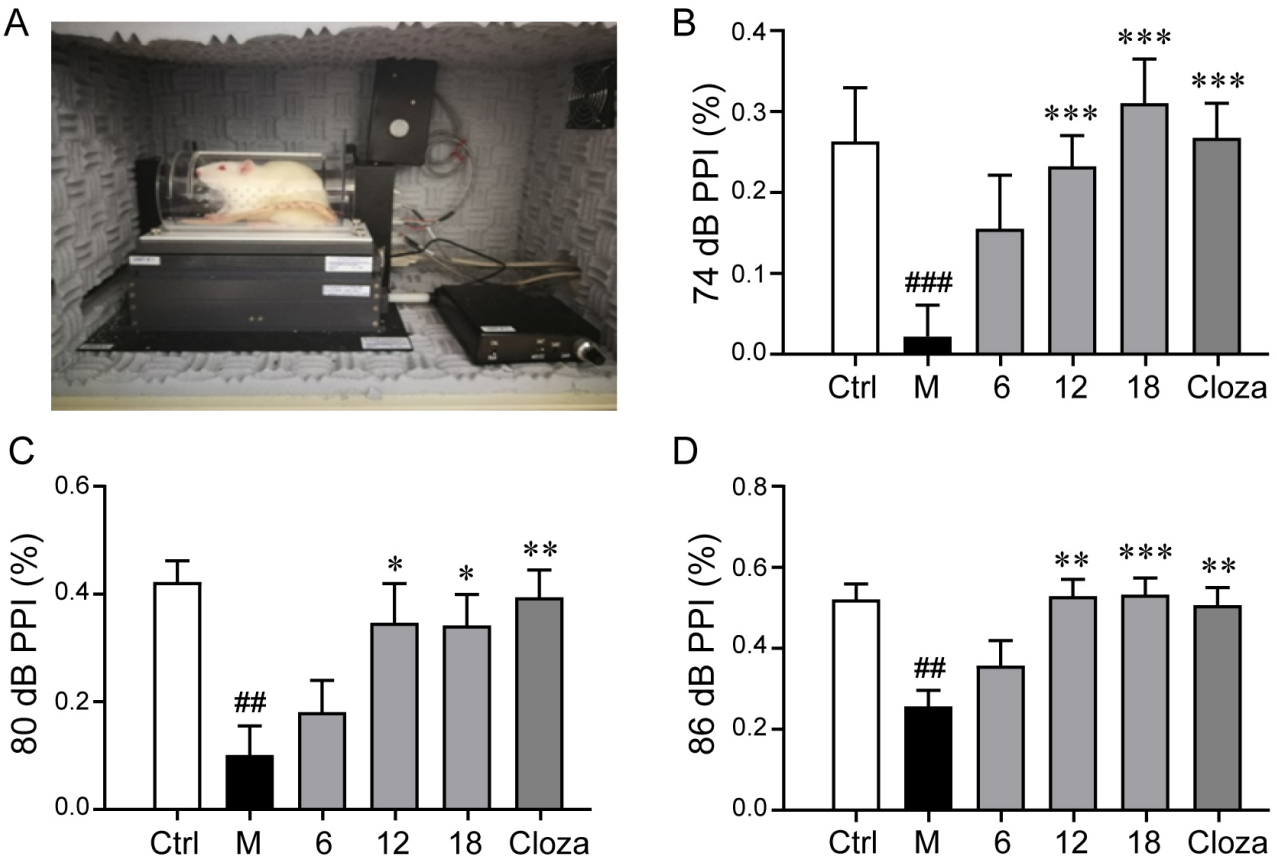

Fig. 4. The effect of SZASD on the auditory startle reflex and sensory gating of rats in PPI. (A) We performed PPI to observe the effect of SZASD on the auditory startle reflex and sensory gating of rats, and recorded (B) 74 dB PPI%, (C) 80 dB PPI%, and (D) 86 dB PPI% in this test (n = 12 for each group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. model group; Ctrl, the control group; M, the model group; 6, the SZASD 6 mg/kg treated group; 12, the SZASD 12 mg/kg treated group; 18, the SZASD 18 mg/kg treated group; Cloza, the clozapine-treated group).

pathway [22]. In the present research, we investigated the effects of SZASD on the cognitive impairment of MK801-induced schizophrenia-like rat model. We observed that SZASD effectively improved motor activities, spatial reference memory, and the sensory gating function of MK801-induced schizophrenia-like rats. Importantly, we found that SZASD had a beneficial effect on improving BDNF levels in the hippocampus of rats. The interaction of BDNF and synaptic proteins could regulate synaptic functions, contributing to restoring cognitive impairments in the rodent model [14]. In addition, SZASD has improved the levels of PSD95 and synapsin I in the hippocampus of rats to preserve synaptic functions in the MK801-induced model. The overall results suggested that SZASD potentially restored the cognitive impairment of schizophrenia-related animal models by modulating synaptic proteins' expressions at least.

MK801 is a commonly used intervention to induce schizophrenia-related animal models [34]. Although the pathogenesis of schizophrenia remains elusive, N-methyl-D-aspartic acid receptor (NMDAR) hypofunction has been hypothesised as a contributing factor in the pathology of schizophrenia for decades [35]. It has been reported that inhibition of NMDAR may result in schizophrenia-like behaviors in animals, such as enhanced spontaneous motor activities and impaired cognition [36]. With a high affinity of the NMDAR channel pore, MK801 can traverse the blood-brain barrier, resulting in histopathological changes related to schizophrenia [37]. According to the current hypotheses of pathogenesis in schizophrenia, we performed an open field test, Y-maze test, and PPI test to evaluate behavioral abnormalities of Sprague-Dawley rats in this study. Consistent with previous studies [38], our data showed that repeated injection of MK801 for consecutive 14 days led to motor dysfunction, the impairment of spatial reference memory, and the sensory gating deficiency.

The hippocampus abnormalities have been reported in schizophrenic patients and the rodent model. The hippocampal volume decrease in schizophrenic patients is associated with cognitive impairments, critical for spatial working memory in the animals' model [4,39]. Our findings also indicated that SZASD might significantly reverse the hyperactivity of MK801-induced schizophrenia-like rats in the horizontal direction and exploratory motiva-

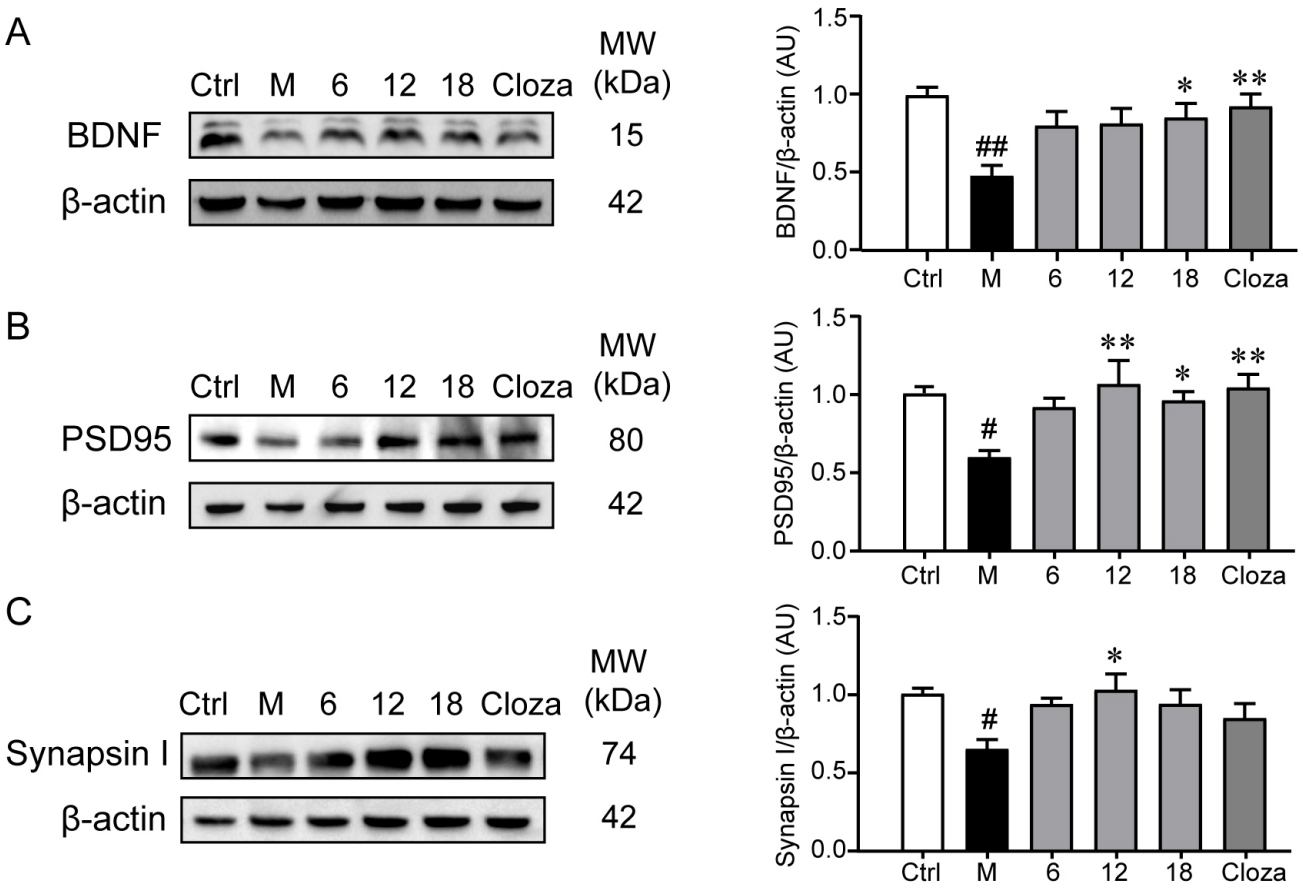

Fig. 5. The expressions of PSD95, synapsin I, and BDNF were upregulated by SZASD in hippocampus of rats. (A) Representative bands and quantitative analysis of BDNF level (n = 5 for each group). β-actin was used as the loading controls (representative blot). (B) Representative bands and quantitative analysis of PSD95 level (n = 5 for each group). β-actin was used as the loading controls (representative blot). (C) Representative bands and quantitative analysis of synapsin I level (n = 6 for each group). β-actin was used as the loading controls (representative blot) ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. model group; AU, arbitrary units; MW, molecular weight; Ctrl, the control group; M, the model group; 6, the SZASD 6 mg/kg treated group; 12, the SZASD 12 mg/kg treated group; 18, the SZASD 18 mg/kg treated group; Cloza, the clozapine-treated group).

tion, which is associated with the damage of monoaminergic activity. And we further explored the effects of SZASD on cognitive impairment of MK801-induced schizophrenia-like rats. The results illustrated that SZASD could restore the spatial reference memory and the sensory gating deficits, suggesting that SZASD might be beneficial to the improvement of cognitive function in schizophrenia. Clozapine is the atypical antipsychotic agent that antagonises the NMDAR [40]. Clozapine has the extraordinary efficacy on both negative and cognitive symptoms of schizophrenia [41]. Recent studies have reported that clozapine could improve MK801-induced schizophrenia behaviors, including locomotor hyperactivity and cognitive impairment [42]. Our data also suggested that clozapine had remarkable efficacy in improving motor dysfunction and cognitive impairment in MK801-induced schizophrenia-like rats.

MK801 administration can cause structural changes in rats' hippocampus, associated with behavioral abnormalities and cognitive dysfunction [43]. Besides, MK801 may induce a loss of hippocampal synaptic plasticity, which is associated with the NMDAR-hypofunction hypothesis [37]. Previous studies have reported that MK801 can downregulate the expression of BDNF in the hippocampus of rats [44]. Consistent with previous studies [44], our findings also verified the impact of MK801 on BDNF in the hippocampus. Mechanically, BDNF can relieve the synaptic injury via regulating the TrkB-MAPK-Erk1/2 signaling pathway [15]. BDNF receptor is presented at the postsynaptic density, indicating that BDNF may have an interaction with PSD95 to mitigate glutamate signaling dysfunction in schizophrenia [45,46]. Besides, previous studies have indicated that synapsin I has a pivotal role in BDNF-induced neurotransmitter release [47]. Growing evidences suggested that a reduction of BDNF, PSD95, and synapsin I was found in the schizophrenia-related animal model, relating to changes in the animal's cognitive function [48]. Our data suggested that MK801 significantly induced de-

pletion of BDNF, PSD95, and synapsin I levels in the hippocampus of rats. And we further explored the effect of SZASD on these synaptic proteins. The results displayed that SZASD improved the expressions of BDNF, PSD95, and synapsin I in the hippocampus of rats. We propose that SZASD may potentially ameliorate cognitive impairment by restoring the reduced levels of synaptic proteins and protecting synaptic plasticity. Furthermore, clozapine can downregulate the NMDAR level in the hippocampus, contributing to improved cognitive function [49]. In this research, we used clozapine as the positive control to probe the effects of clozapine on the expressions of synaptic proteins in the hippocampus. Our findings illustrated that compared with synapsin I, clozapine had remarkably enhanced the levels of BDNF and PSD95. The specific mechanisms of clozapine's impact on synaptic plasticity are still needed to explore in future research.

The present study also has limitations to some extent. Firstly, the optimal dosage is still needed to explore the best efficacy in improving cognitive function in schizophrenia. Secondly, we used the subacute model in which the rats were injected to MK801, which was insufficient to replicate the full spectrum of symptoms of schizophrenia completely. Moreover, other behavioral tests were also needed to conduct future research. Thirdly, we did not perform immunofluorescence or transmission electron microscopy to observe synaptic structure changes. And we would conduct more experimental methods to verify the effects of SZASD on synaptic plasticity in the hippocampus of the animal model. Finally, the signaling pathways related to BDNF and synaptic proteins were needed to investigate in further research.

5. Conclusions

Our study demonstrates that SZASD positively affects schizophrenia-like behaviors and hippocampal-dependent cognitive impairment in the MK801-induced schizophrenia-like rat model. Besides, SZASD can improve the expressions of synaptic proteins in the hippocampus of MK801-induced schizophrenia-like rats, including BDNF, PSD95, and synapsin I. Therefore, we propose that SZASD can have a preventive effect on ameliorating the cognitive dysfunction of schizophrenia by modulating the synaptic proteins in the hippocampus. Future research will investigate the effects of SZASD on signalling pathways related to BDNF and synaptic proteins to verify the underlying mechanisms.

Author Contributions

All authors had complete access to all research data and assume complete responsibility for the data integrity and accuracy of the data analysis. HJ and ZS conceived and designed the study, revised the draft. HJ acquired fundings. XLiu and ZF conducted the experiments. SF wrote the first draft and revised the draft. CM, YH, YN led statistical analysis and revised the draft. XLi led the revision of the draft. All authors reviewed and approved the submitted version of the manuscript.

Ethics Approval and Consent to Participate

The experimental animal protocol was approved by the Institutional Animal Care and Use Committee of Capital Medical University (No. AEEI-2018-047).

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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