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Schisantherin B Improves the Pathological Manifestations of Mice Caused by Behavior Desperation in Different Ages-Depression with Cognitive Impairment

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Abstract

Depression is a major mood disorder. Abnormal expression of glial glutamate transporter-1 (GLT-1) is associated with depression. Schisantherin B (STB) is one bioactive of lignans isolated from *Schisandra chinensis* (Turcz.) Baill which has been commonly used as a traditional herbal medicine for thousands of years. This paper was designed to investigate the effects of STB on depressive mice induced by forced swimming test (FST). Additionally, we also assessed the impairment of FST on cognitive function in mice with different ages. FST and open field test (OFT) were used for assessing depressive symptoms, and Y-maze was used for evaluating cognition processes. Our study showed that STB acting as an antidepressant, which increased GLT-1 levels by promoting PI3K/AKT/mTOR pathway. Although the damage is reversible, short-term learning and memory impairment caused by FST test is more serious in the aged mice, and STB also exerts cognition improvement ability in the meanwhile. Our findings suggested that STB might be a promising therapeutic agent of depression by regulating the GLT-1 restoration as well as activating PI3K/AKT/mTOR pathway.

Key Words: Schisantherin B, FST, Depression, GLT-1, Cognitive impairments

INTRODUCTION

Depression is regarded as a major mood disorder, characterized by either the altered mood or cognitive functions (Dillon and Pizzagalli, 2018). Late life depression, characterized by a pervasive and persistent low mood in elderly people, is invariably accompanied by numbers of cognitive impairments, including executive function, memory, processing speed, attention and visuospatial skill domains (Jeon and Kim, 2017; Liao *et al*., 2017). It could be assured that the depression can cause cognitive impairment (Aydin *et al*., 2018; Morgan *et al*., 2018). Besides that, there exist various limitations of current antidepressant treatment. Monoaminergic antidepressants serve crucial roles in depression therapy. However their efficacy is only 60 and 70 percent, whereas, and the side effects include sexual problems, drowsiness, fatigue, sleep difficulties, nausea, weight gain, nervousness, dry mouth and blurred vision. In the light of reports regarding the limitations associated with the current antidepressant agents, it is necessary to develop novel antidepressant agents and explore the pathophysiology of depression clearly as well (Chen *et al*., 2017; Zhang *et al*., 2018b).

GLT-1 is termed as the major Na^+-driven glutamate transporter. Maladaptation of GLT-1 has been recommended as a contributor to several neuropathological conditions related to neuro (Rodriguez-Kern *et al*., 2003; Rimmele and Rosenberg, 2016). Significant functional loss of GLT-1 has been reported to correlate with synaptic degeneration and severity of cognitive impairment. Previous studies also proposed that pharmacological inhibition of GLT-1 in the central amygdala induced depressive-like symptoms (Soni *et al*., 2014; Weng *et al*., 2014).

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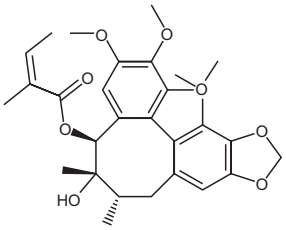

Fig. 1. Chemical structure of Schisantherin B.

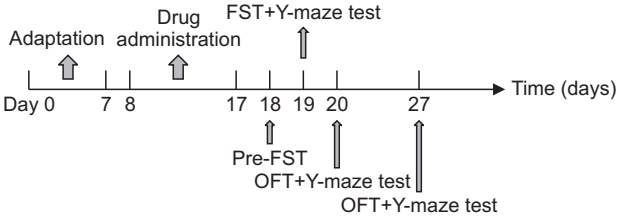

Fig. 2. The experimental schedule.

The PI3K pathway is known for regulating metabolism, together with cell growth and cell survival. Akt is one of the key molecules activated downstream of the PI3K. This kinase has been shown to play multiple roles in the cellular regulation and has emerged as a critical mediator of mammalian target of rapamycin (mTOR) activity (Sheppard *et al*., 2012; Lima, 2017). Activation of PI3K/AKT signaling pathway exerts its pro-survival effects by activating its downstream target mTOR to regulate GLT-1 expression. It is also involved in chronic unpredictable mild stress (CUMS) which could induce depressive behavior (Lima *et al*., 2017). These findings provide crucial insight into potential clinical strategies that target GLT-1, which is likely to present a novel effect for the treatment of depression, in addition to its comorbid psychiatric disorders.

Schisandra chinensis (Turcz.) Baill has been commonly used as a traditional herbal medicine in Korea, Japan and China to treat deficiency-syndrome. Many studies have confirmed that Schisandra has a variety of pharmacological activities which include antioxidant effect, anti-cough, relief of menopausal symptoms, anti-osteoporosis (Dilshara *et al*., 2013; Zhong *et al*., 2015; Cho *et al*., 2018). Recently, there were many reports revealed that total lignans and other active components from *Schisandra chinensis* (Turcz.) Baill could ameliorate cognition in rodents with depression (Li *et al*., 2014; Yan *et al*., 2016). Our previous studies have showed that Schisandra chinensis was involved in protecting against depressive-like behavior and cognitive deficits. Schisandrin, which is one of the major lignan components of Schisandra chinensis, produced an antidepressant effect in CUMS-induced mice (Wan *et al*., 2017). Schisantherin B (STB, Fig. 1), which is one of lignans isolated from Schisandra chinensis (Turcz.) Baill, combined with the earlier studies in our laboratory, which has reported about its potential effects on GLT-1. Taking into account its role in the pathogenesis of depression (Xu *et al*., 2016), we first investigated the effects of STB on depressive mice induced by FST with different ages. FST and CUMS are classic methods for modeling depressed mice. Previous studies reported that CUMS could simultaneously impair cognitive function (Hashimoto *et al*., 2011). However, there were few reports on whether FST were lead to a certain degree of cognitive impairment. Moreover, we considered that animal's own age was also an important factor affecting cognitive function in addition to outside stress. Thus, FST may have different effects on animals of different ages. In this study, we also evaluated the effect of STB on cognitive impairment induced by FST. Our goal of the present study was to evaluate the potential of STB as an antidepressant, and provide a possible mechanism of its action.

MATERIALS AND METHODS

Animals

Experiment was carried out in compliance with the National Institutes of Health and institutional guidelines for the humane care of animals. Experimental procedure was approved by the Animal Care Committee of Shenyang Pharmaceutical University.

Male KM mice (The joint venture of Charles River Laboratories in China) were used in experiments. The mice at 10-week old (20-25 g) and 11-month old (50-60 g) were maintained on a 12-h light/dark cycle (lights on 07:00 to 19:00 h) with *ad libitum* access to food and water. The mice were allowed to be kept at a regulated room temperature (22 ± 2°C) and humidity (55 ± 5%). Mice were group housed and habituated to environmental conditions for 7 days.

Drugs

STB was purchased from The National Institute for the Control of Pharmaceutical and Biological Products in China (Shenyang, China), with purity above 98%. Before the experiment, we used three mice per group to screen the dose of STB (10, 15 and 20 mg/kg) through FST. It was found that the STB of 15 mg/kg had better anti-depression effect in mice. Thus, the suspension solution was diluted with physiological saline to 1.5 mg/mL. Citalopram as the positive control drug was obtained from Melone Pharmaceutical Co (Dalian, China). It was dissolved in physiological saline at a concentration of 1 mg/mL. All other chemicals and reagents were of analytical grade.

Experimental design

Following 7 days of acclimation to the vivarium and housing conditions, mice were divided into 8 experimental groups shown as below, and 15 in each group, the experimental operation were parallel:

(1) Normal group-I (non-FST treated, 10-week old, n=15), (2) Control group-I (physiological saline, FST treated, 10-week old, n=15), (3) Citalopram group-I (FST treated; 10-week old, n=15), (4) STB group-I (FST treated; 10-week old, n=15), (5) Normal group-II (FST treated, 11-month old, n=15), (6) Control group-II (physiological saline, FST treated, 11-month old, n=15), (7) Citalopram group-II (FST treated; 11-month old, n=15) (8) STB group-II (FST treated, 11-month old, n=15). Citalopram (10 mg/kg, i.p.) and STB (15 mg/kg, i.p.) were administered daily between 09:00 and 10:00 h for 10 days. Except for the Normal group, FST was carried out on day 19. 30 min after FST, Y-maze test was implemented to assess immediate spatial working memory of animals. On the following day and the 27th day, Y-maze test was carried out again. 30 min before Y-maze test, OFT was carried out. After each Y-maze

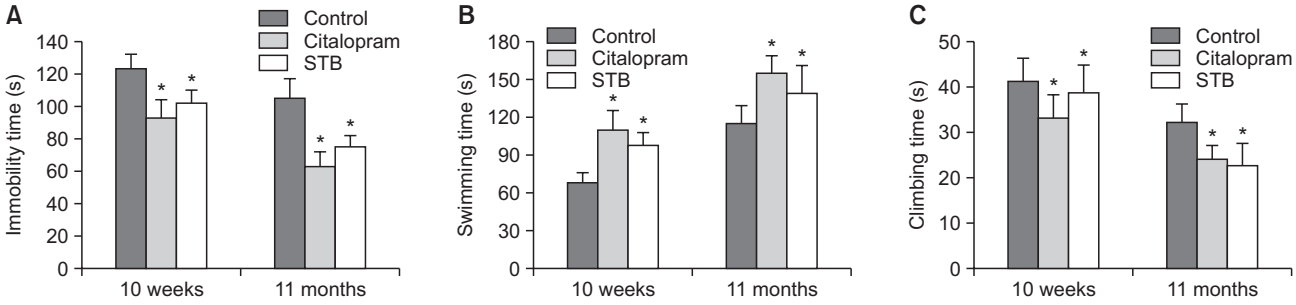

Fig. 3. Effects of STB administration on mice in FST. Control group: mice were given physiological saline. Citalopram group: mice were given Citalopram 10 mg/kg. STB group: mice were given STB 15 mg/kg. The duration of immobility time (A), swimming time (B) and climbing time (C) were measured during the 4 min test session. Values indicated mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey`s multiple comparison test (n=15). **p*<0.05, compared with control group.

test, 5 mice in each group were sacrificed by cervical dislocation and the brain was immediately removed. The tissues were stored at –80°C. Brief experimental design is explained in Fig. 2.

Forced swim test (FST)

Mice were subjected to the FST as described previously (Rojas *et al*., 2011). Previous studies have shown that a depressed state could be induced in mice by forcing them to swim in a narrow cylinder from which they cannot escape (Porsolt *et al*., 1977). FST is not only a classic method of modeling rodents depression, but also the most frequently used acute stress behavioral tests for measuring depressive-like response in rodents (Llorens-Martin *et al*., 2016). Briefly, mice were placed individually in a clear glass cylinder which the height is 25 cm and the diameter is 10 cm that containing 10 cm of fresh water at 23 ± 2°C. 24 h before testing, the mice were placed in the test room to adapt the environment. The first swimming session had 15 min duration. The mice were exposed to another swimming session for 6 min 24 h later. In both sessions, the behavior of mice was not allowed to interfere after they were placed in water. Three predominant behaviors were measured in the second session by an expert observer and expressed in seconds: immobility time (The mice were considered immobile when floating in the water without struggling and making only those movements necessary), swimming time (The mice moves horizontally in the swim cylinder), and climbing time (Struggling and upward-directed movement of the forepaws, usually against the side of the swim cylinder). All the behaviors were recorded within the last 4 min of the session. The mice were dried immediately after the experiment to reduce the impact of FST-induced discomfort on subsequent experiments.

Y-maze test

Y-maze test was carried out to assess immediate spatial working memory which is a form of short-term memory (Cognato Gde *et al*., 2012). The Y-maze is consisted of three arms at equal angles (30 cm length×5 cm width×12 cm high). Mice were placed at the end of one arm and allowed to move freely through the maze for 6 min. An arm entry was counted when the hind paws of the mouse were completely within the arm. The series of arm entries were recorded visually and the percentage alternation was calculated. A spontaneous alternation was defined as successive entries into the three arms, i,e., ABC, CAB, or BCA but not CBC. The percentage alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two) multiplied by 100 as showed by the following equation: Alternation%=[Number of alternations/(Total arm entries–2)]×100. In order to test the effect of FST on the learning and memory of mice and its relationship with time, we performed three times of Y-maze test at different time periods.

Open-field behavior test (OFT)

OFT was conducted in a camera obscura (30 cm long×30 cm wide×30 cm high) which was made of black plexiglas and with digital auto photometer to detect the depressive-like response of mice (Xie *et al*., 2017). The apparatus was placed in a darkened, and sound attenuated testing room. 24 h before testing, the mice were placed in the test room for habituation. 2 min before test, the mice were placed in the center of the apparatus to adapt the activity chambers and its activity including the total distance, time in the central area and marginal area during the subsequent 5 min only was assessed.

Western blot

Hippocampal tissues were homogenized in RIPA (150 mM sodium chloride, 50 mM Tris (pH 8.0), 0.5% sodium deoxycholate, 0.1% SDS, 1% Triton X-100) and PMSF (Dalian Melonepharma, Dalian, China) and kept on ice for 30 min. The tissue homogenate was centrifuged at 10 000 g for 20 min at 4°C. The supernatant was obtained and used as the total hippocampal protein extract measured by BCA assay kit (Dalian Melonepharma) to determine protein concentration and stored at –80°C until use. Samples were diluted with an equal volume of loading buffer (Beyotime Biotech Co., Shanghai, China), and boiled at 95°C for 5 min. Approximately 50 μg of protein was loaded in each well and separated in 10% or 12% SDS-PAGE gels. The proteins were transferred onto nitrocellulose membranes. The membranes were saturated and blocked with 5% fat-free powdered milk at 37°C for 1.5 h and incubated overnight at 4°C in one of the following primary antibodies, which were diluted in 5% fat-free powdered milk in TBS: GLT-1 (1:1000, Abcam, San Francisco, CA, USA), PI3 Kinase p85 (19H8) (1:1000, CST, USA), Akt (pan) (C67E7) (1:1000, CST, USA), mTOR (7C10) (1:1000, CST, USA), β-actin Rabbit mAb (1:1000, CST, USA). Anti-PI3K p85 (phosphor Y607) (1:1000, Abcam), Phospho-Akt (Ser473) (1:2000, CST, USA), Phospho-mTOR (Ser2448) (1:1000, CST, USA). Blots were

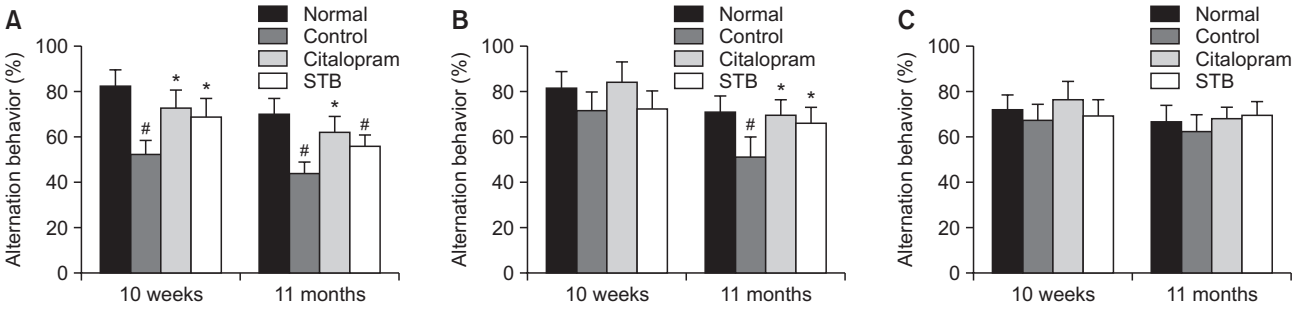

Fig. 4. Effect of Citalopram and STB on mice in Y-maze test after FST. Normal group: mice without FST and drug administration. Control group: mice were given physiological saline. Citalopram group: mice were given Citalopram 10 mg/kg. STB group: mice were given STB 15 mg/kg. The alternation behavior during 6 min was measured in 19th day (A), 20th day (B) and 27th day (C). Values indicated mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (n=15 in 19th day, n=10 in 20th day, n=5 in 27th day). $^{\#}p<0.05$, compared with the normal group, $^{*}p<0.05$, compared with the control group.

washed three times for 30 min in TBST at room temperature and then incubated for 1.5 h in one of the following HRP-conjugated antibodies which were diluted in 5% fat-free powdered milk in TBS: Anti-rabbit IgG (1:2000, CST, USA) for detection of target proteins, β-actin. After three times washes for 30 min in TBST, immunolabeled protein bands were detected using the ECL western blot detection kit (Dalian Melonepharma). Graphs of blots were obtained in the linear range of detection and were quantified for the level of specific induction by scanning laser densitometry.

Statistical analysis

Results are expressed as mean ± SEM. The significances between different groups were assessed using one-way ANOVA, followed by Tukey HSD post-hoc test when significant main effects were indicated. In all calculations, $^{*}p<0.05$ was considered to be statistically significant. Statistical analysis was performed with SPSS software 19.0 (IBM Corp., New York, NY, USA).

RESULTS

FST

Fig. 3 showed the duration of immobility times (A), swimming times (B) and climbing times (C) measured during the 4 min test session. Indeed, whether for 10-week or 11-month old mice, compared with the control group, 10 days treatments of Citalopram (10 mg/kg; i.p.) or STB (15 mg/kg; i.p.) could significantly increase swimming time, reduce immobility time and climbing time ($p<0.05$; Fig. 3).

Y-maze test

To detect whether FST lead to a certain degree of cognitive impairment, we performed a Y-maze test after 30 min of FST and on day 1 (20th day of the total experiment) and day 7 (27th day of the total experiment) after FST, respectively. On the first test, we found that stress induced by FST significantly decreased the spontaneous alternation behavior of mice compared with normal group whether for 10-week or 11-month old mice, which indicated FST could impair short-term memory of mice, and Citalopram or STB treatment could protect mice from FST for 10-week old mice ($p<0.05$; Fig. 4A). However, different from Citalopram, STB treatment showed no effect on 11-month old mice on the 19th day ($p>0.05$; Fig. 4A).

On the second time of Y-maze, the short-term learning and memory of control group had been restored to that of the normal group for 10-week old mice ($p>0.05$; Fig. 4B). However, control group of 11-month old mice still showed a significant difference compared with normal group ($p<0.05$; Fig. 4B). Interestingly, there was no difference between the STB group and the normal group, which meant treatment of STB could accelerate the memory recovery and this was consistent with our previous research (Xu *et al*., 2016).

To further study how many days the impact of FST on learning and memory could last, we carried out the Y-maze test again on the 27th day. As shown in Fig. 4C, there was no difference between all groups, which meant impairment from FST could restore to normal within 7 days.

OFT

As shown in Fig. 5A, on the 20th day, FST procedure resulted in significantly reduced total distance travelled and time in the central area in the OFT as compared to normal group for 11-month old mice ($p<0.05$; Fig. 5A-1, Fig. 5A-2), which meant the depressive-like behavior in mice induced by FST could last more than one day for older mice. What' more, Citalopram and STB treatment were effective in increasing the total distance travelled as compared to the control group. There was no difference in total track between control group and normal group in 10 weeks mice. This was consistent with our results of FST.

In the 27th day, as shown in Fig. 5B, we found no difference in total track and time in the marginal area among all groups of mice (Fig. 5B-1, Fig. 5B-3), which meant the depressive-like behavior in mice induced by FST could last more than one day for the older mice. However, we still found a significant decrease of control group in the time in the central area compared with normal group, and there was no significant recall after STB or Citalopram administration ($p<0.05$; Fig. 5B-2).

Western blot

In order to research the molecular changes in the brain, western blot was used to detect the effects of FST and STB treatment on GLT-1/PI3K/AKT/mTOR signaling pathways in the 19th, 20th, 27th day. From the results of the 19th day, compared with the control group, the FST procedure could significantly decrease the levels of GLT-1, P-PI3K/PI3K, P-AKT/AKT and P-mTOR/mTOR in hippocampus ($p<0.05$; Fig. 6),

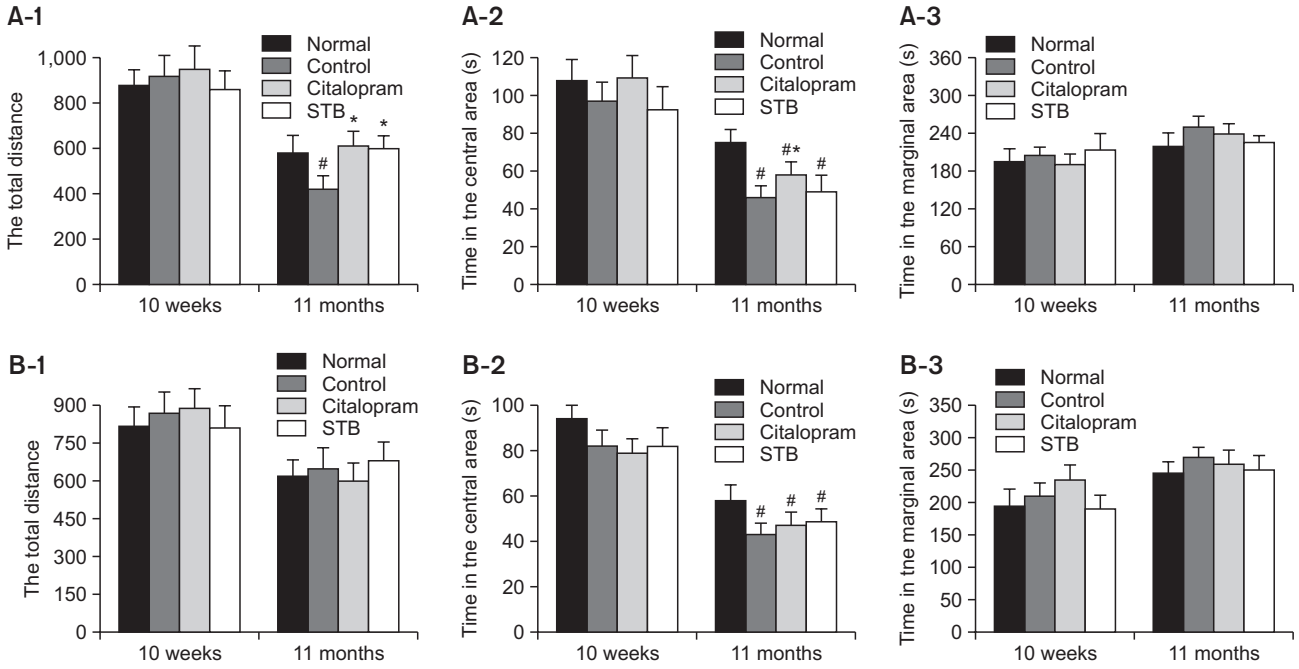

Fig. 5. Effects of Citalopram and STB on mice in OFT after FST. Normal group: mice without FST and drug administration. Control group: mice were given physiological saline. Citalopram group: mice were given Citalopram 10 mg/kg. STB group: mice were given STB 15 mg/kg. The total distance during 5 min session was measured in 20th day (A-1) and 27th day (B-1); Time in the central area was measured during 5 min session in 20th day (A-2) and 27th day (B-2); Time in the marginal area was measured during 5 min session in 20th day (A-3) and 27th day (B-3); Values indicated mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey`s multiple comparison test (n=15 in 19th day, n=10 in 20th day, n=5 in 27th day). $^{\#}p<0.05$, compared with the normal group, $^{*}p<0.05$, compared with the control group.

and STB treatment could protect 10-week old mice from damage of FST, which showed different performance in 11-month old mice. This result indicated that the short-term learning and memory impairment caused by FST test was more serious in the aged mice.

STB treatment could restore learning and memory more rapidly for 11-month old mice in 20th day ($p<0.05$; Fig. 7). Expectedly, the pathway indicators were restored automatically in the 27th day. There was no difference between control group and normal group in both 10-week and 11-month old mice on the 27th day (Data not shown).

DISCUSSION

Glutamate-related therapies are emerging as the new path for the treatment of major depressive disorder (MDD) (Miyagishi *et al*., 2017). Reductions in hippocampal volume that putatively reflect hippocampal atrophy associated with impaired neurogenesis and disrupted glutamatergic transmission are widely reported in MDD. The pathological changes in hippocampus partially signify the pathological development of depression (Doolin *et al*., 2018). Recent reports reviewing the use of glutamate transporters modulators in the treatment of resistant depression advocate the importance of understanding the alterations of the diverse components of this complex system in mood disorders (Liu *et al*., 2017). GLT-1 is the predominant central nervous system (CNS) glutamate transporter subtype and pharmacological activation of GLT-1 would be a powerful tool for reducing glutamate in pathological conditions. Hence, it may serve as a novel therapeutic target for the treatment of various neurological disorders and other diseases, such as depression (Liu *et al*., 2016; Kang *et al*., 2018). Our previous research found that STB might be a potential regulator of GLT-1 expression (Xu *et al*., 2016). Therefore, the purpose of our current study was to investigate the effect of STB on depressive-like behavior and pathological alteration of GLT-1 level of mice. Previous studies suggested that the mice showed increased immobility time and decreased climbing time in the FST were in behavioral despair. What' more, swimming behavior relies on the serotonergic system and climbing behavior on the noradrenergic system in mice. However, STB did not affect climbing time of 10-week old mice. This could be caused by the greater influence of age on the norepinephrine system, which is more sensitive to the norepinephrine system in 10-week old mice. Taking into account the time-effectiveness of FST, we used the method of pre-administration which could also detect the protective effect of STB on mice. Compared to CUMS, FST is a more convenient method of modeling depression, and it can also detect the degree of depression in mice (Yan *et al*., 2017). In addition, we hope to examine the impact of FST on cognitive function of mice in this article. Therefore, we finally decided to use FST instead of CUMS (Borsoi *et al*., 2015). The results were consistent with our expectations. We initially confirmed the antidepressant effect of STB in the current study. CUMS will be used to further verify the antidepressant effect of STB in future experiments. In addition to FST, OFT is mainly used to detect depressive-like

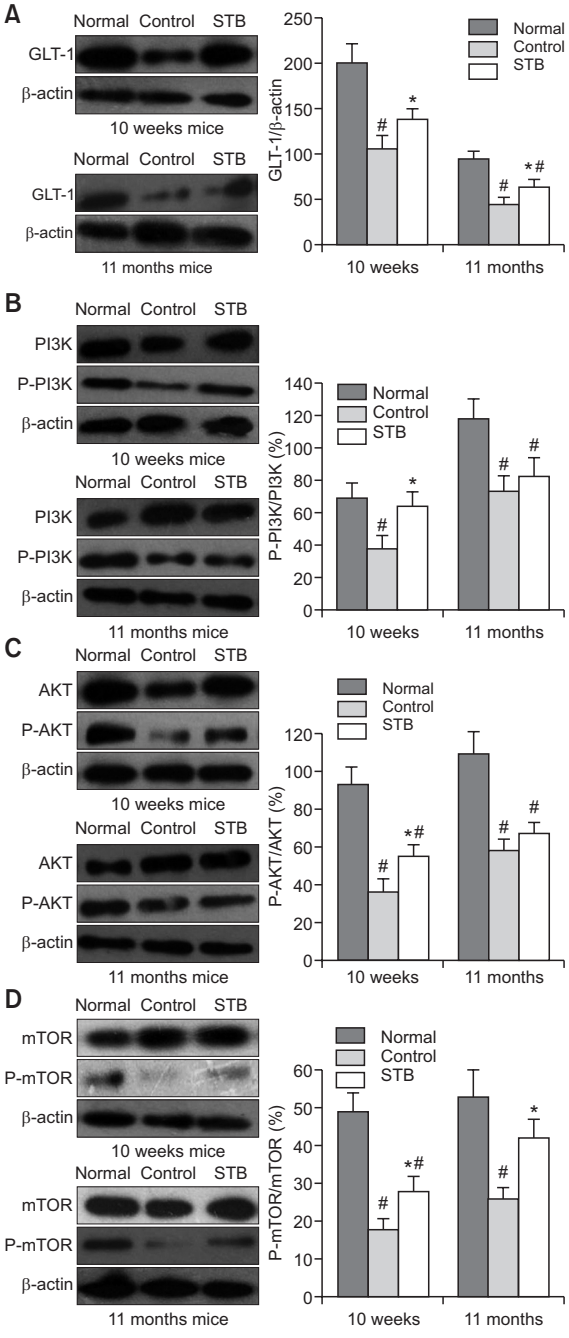

Fig. 6. Effects of STB administration on GLT-1 (A), P-PI3K/PI3K (B), P-AKT/AKT (C), and P-mTOR/mTOR (D) levels of hippocampus in 19th day. Values indicated mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey`s multiple comparison test (n=5). $^{\#}p<0.05$, compared with the normal group, $^{*}p<0.05$, compared with the control group.

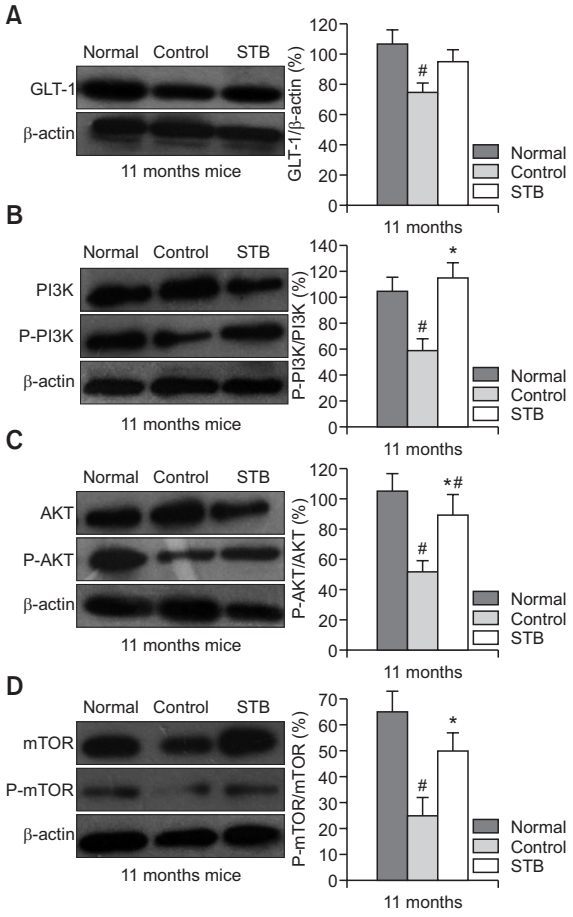

Fig. 7. Effects of STB administration on GLT-1 (A), P-PI3K/PI3K (B), P-AKT/AKT (C) and P-mTOR/mTOR (D) levels of hippocampus in 20th day. Values indicated mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey`s multiple comparison test (n=5). $^{\#}p<0.05$, compared with the normal group, $^{*}p<0.05$, compared with the control group.

behavior in mice after FST, time in central and marginal areas was revealed depression in mice in OFT. Determine the antidepressant effect of STB, time in the central can also evaluate the anxiety of mice to a certain extent. Anxiety is a complication of depression and the direct relationship between these two diseases has not been fully understood. From the results of OFT, we concluded that neither Citalopram nor STB has an excellent therapeutic effect on anxiety-like behavior in mice. We observed that FST-induced depressive symptoms and consequent PI3K/AKT/mTOR signaling down-regulation reversed by STB treatment, which indicated that STB could increase GLT-1 expression by affecting PI3K/AKT/mTOR pathway to exert antidepressant-like effect (Wu *et al*., 2010; Wang *et al*., 2014). Previous study showed that GLT-1 needed the activation of PI3K/AKT pathway to exert its action. The PI3K pathway is known for regulating metabolism, cell growth and cell survival (Lima, 2017; Wan *et al*., 2017). Some studies suggested that psychotropic agents such as antidepressants exerted their pharmacological effects by activating some signaling pathways, including the PI3K pathway (Fruman *et al*., 2017). AKT is one of the key molecules activated downstream of the PI3K signaling pathway, and its dysregulation plays key roles in the pathogenesis of many diseases (Goodwani *et al*., 2015). This serine/threonine kinase is ubiquitously expressed and its activity is central to the regulation of translation initiation and, consequently, protein synthesis required for long-term potentiation and new synaptic connections (Tao *et al*.,

2016). AKT has been shown to play multiple roles in cellular regulation and has emerged as a critical mediator of mammalian target of mTOR activity, a protein kinase which plays an important physiological and pathological role in the central nervous system, and has been proposed to have a role in GLT-1 regulation (Gubern *et al*., 2014; Rehnitz *et al*., 2017). In this paper, we focus on investigating the changes of brain indexes in mice treated with FST and STB. Interestingly, we found that treatment of STB not only affected the phosphorylation of PI3K/AKT/mTOR pathway proteins, but also had an effect on the expression of pathway proteins, its specific role remains to be studied. Moreover, the changes of GLT-1 and PI3K/AKT/mTOR pathways induced by age in mice need to be studied in the future.

Previous studies proposed that pharmacological up-regulation of GLT-1 could also ameliorate the age-dependent pathological tau accumulation (de Calignon *et al*., 2012). Tau could spread synaptically via connected glutamatergic neurons, which meant glutamate dyshomeostasis could be one of the driving forces of tau pathology (Liao *et al*., 2009). GLT-1 plays an important role in keeping synaptic glutamate concentrations in control, down-regulation of GLT-1 is suspected to be related to neurodegenerative diseases such as Alzheimer's disease (AD) (Ji *et al*., 2011). Depression and Alzheimer's disease are both common diseases in the elderly. Clinicians and researchers have observed close links between these two diseases, although the link between depression and AD is not easy to characterize. Depression is an important predisposing factor of AD, and cognitive dysfunction is also an important pathological manifestation of depression (Ishijima *et al*., 2018; Xue *et al*., 2018). Consistent with our current research, Y-maze was used to evaluate the cognitive impairment induced by FST, especially mice of different ages. We dried the mice immediately after the FST to further reduce the effect of the discomfort caused by FST stress on mice on the results of the Y-maze. After half an hour, we performed Y-maze test. Results showed stress of FST had a certain effect on cognitive function in mice, but this effect was not irreparable. Furthermore, in both behavior and biochemical indicators, young mice recovered more rapid than older ones. Meanwhile, the depressive behavior caused by FST also improved along with the recovery of cognitive dysfunction. Our study found that depressive-like behavior and cognitive dysfunction caused by FST in 10-week old mice could last less than one day, but the duration of this effect of FST may be last longer in older mice. In this paper, Citalopram also exerted positive effect on protect cognitive dysfunction in Y maze test. Studies have reported that Citalopram could restore short-term memory deficit and non-cognitive behaviors in APP/PS1 mice while halting the advance of Alzheimer's disease pathology. This is in line with our findings (Zhang *et al*., 2018a).

In summary, our research shows that the STB acting as an antidepressant increases GLT-1 levels by promoting PI3K/AKT/mTOR pathway, and also exert cognition improvement ability in the meanwhile. Our findings suggest that STB might be a promising therapeutic agent of depression, and further research is worth to be invested.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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