NEUROSCIENCE
RESEARCH ARTICLE
J. Lu et al. / Neuroscience 430 (2020) 1–11

Tanshinone IIA Improves Depression-like Behavior in Mice by Activating the ERK-CREB-BDNF Signaling Pathway

Jiaqi Lu, [a†] **Hang Zhou,** [a†] **Danyang Meng,** [b†] **Junjun Zhang,** [c†] **Kailing Pan,** [a] **Bo Wan** [a,d*] **and Zhigang Miao** [a,d*]

[a] *Institute of Neuroscience, Soochow University, Suzhou City, China*

[b] *Department of Neurology, the Second Affiliated Hospital of Soochow University, Suzhou City, China*

[c] *Department of Radiotherapy and Oncology, the Second Affiliated Hospital of Soochow University, Suzhou City, China*

[d] *Jiangsu Key Laboratory of Neuropsychiatric Diseases, Soochow University, Suzhou, Jiangsu 215123, China*

Abstract—Depression is a serious global affective disorder and one of the most common neurological diseases. Tanshinone IIA (TSA) is the mainly active constituent of *Salvia miltiorrhiza* and has diverse biological effects, including anti-inflammatory and antioxidant effects and significant neuroprotective effects against cerebral ischemia and Alzheimer's disease. However, whether TSA has an antidepressant effect remains unknown. The present study attempted to explore the antidepressant effects and the mechanism of TSA by examining the brain-derived neurotrophic factor (BDNF) expression in the hippocampus of depressive mice. The tail suspension test (TST) and forced swim test (FST) showed that TSA can significantly reduce the immobility time of depressed mice. Chronic administration of TSA increased p-ERK and p-CREB, BDNF proteins in mice hippocampus. We further explored the potential mechanism of TSA' antidepressant effect. TSA significantly increased the expression of p-ERK, p-CREB and BDNF proteins in dexamethasone-treated PC12 cells, and this enhancement was suppressed by pretreatment with the extracellular signal-regulated kinase (ERK) inhibitor SL327. Moreover, we observed that SL327 treatment markedly suppressed the increased levels of p-ERK, p-CREB and BDNF in mice hippocampus induced by TSA, preventing the antidepressant effects of TSA. Taken together, our results suggest that the antidepressant-like effects of TSA were mediated by ERK-CREB-BDNF pathway in mice hippocampus.

Key words: TSA, antidepressant, depression, BDNF.

INTRODUCTION

Depression is a serious global affective disorder and one of the most common neurological diseases whose clinical manifestations are low mood, disproportionate to the situation, loss of interest, anhedonia, loss of energy, and fatigue (Bousser, 2000). People with severe depression also have suicidal thoughts and behaviors, which place a heavy psychological and economic burden on families and society (O'Leary et al., 2001; Kessler et al., 2003). Depression is caused by genetic abnormalities or an emotional dysfunction caused by a major change in the environment (Lesch, 2004). The worldwide prevalence of depression is estimated to be approximately 3–5%, according to the World Health Organization. Affecting approximately 1–200 million people by 2020, depressive disorders will become the most serious disease burden in modern society and depression will become the second leading cause of death (Sonnenberg et al., 2000).

Recently, a high safe Chinese herbal medicine with antidepressant effects has become a novel drug therapy for depression (Butler and Pilkington, 2013; Li et al., 2016b; Wang et al., 2017). Tanshinone IIA (TSA) is the mainly active constituent of *Salvia miltiorrhiza*, and has a variety of pharmacological effects, such as anti-inflammatory, antioxidant and neuroprotective effects (Imanshahidi and Hosseinzadeh, 2006; Hamidpour et al., 2014). Many signaling pathways have been implicated in the mechanism of TSA functionality, including MAPK signaling, NF-κB, and TGF-β (Sui et al., 2017; Cheng et al., 2018; Guan et al., 2018; Lu et al., 2018). The extracellular signal-regulated kinase (ERK)-cAMP response element binding protein (CREB) signaling pathway is implicated in learning, memory, and neuroplasticity and plays an important role in regulating many brain

*Corresponding authors. Address: Institute of Neuroscience, Soochow University, Suzhou City, China.
E-mail addresses: bowan@fudan.edu.cn (B. Wan), sxmiaomiao@163.com (Z. Miao).
† Co-first authors.
Abbreviations: BDNF, brain-derived neurotrophic factor; CREB, (ERK)-cAMP response element binding protein; Dex, dexamethasone; EDTA, ethylenediaminetetraacetic acid; FST, Forced swim test; Q-PCR, quantitative real-time polymerase chain reaction; TSA, Tanshinone IIA; TST, tail suspension test.

https://doi.org/10.1016/j.neuroscience.2020.01.026
0306-4522/

functions, including cell growth, apoptosis, differentiation, and cellular responses to stress (Ying et al., 2002; Liu et al., 2015). Presently, the vital role of the ERK signaling system in response regulation has become the focus of many studies, ERK1 and ERK2 are present in significant amounts in the hippocampus (Ortiz et al., 1995; Flood et al., 1998), most likely in relation to the stress response and depression (Feng et al., 2003; Guan et al., 2013; Yang et al., 2013). Previous studies have shown that brain-derived neurotrophic factor (BDNF) can improve depressive symptoms by activating the ERK pathway (Shirayama et al., 2002; Chen et al., 2015; Wang et al., 2016). These data suggest that the ERK signaling pathway may be a potential target for antidepressants and may participate in the molecular mechanism of depression.

There are growing evidences indicating that neurotrophic factors, such as BDNF, play vital roles in promoting neural growth and survival during development (Huang and Reichardt, 2001). Numerous studies have shown that neurotrophic factors are powerful candidates in the development of several neuropsychiatric disorders including depression (Castren et al., 2007; Bjorkholm and Monteggia, 2016). For example, clinical results showed that depressive patients or those who experienced prolonged exposure to stressful conditions have decreased levels of BDNF in the hippocampus (Karege et al., 2002; Karege et al., 2005; Wysokinski, 2016).

The exact mechanisms of Tanshinone IIA's antidepressant effects has not been fully defined. Many reports have show that 60 min after intraperitoneal injection of Tanshinone IIA (16 mg/kg), the blood brain barrier reached a peak concentration of 0.41 nmol/g brain wet weight (Lam et al., 2003), and exhibited beneficial effects in experimental models of some diseases (Wang et al., 2010; Zhang et al., 2010). In the current study, we aimed to investigate the antidepressant effects of Tanshinone IIA and explore the role of the ERK-CREB-BDNF pathway in the antidepressant mechanism of Tanshinone IIA.

EXPERIMENTAL PROCEDURES

Animals

Six-week-old male adult ICR mice were obtained from the SLAC Company (Shanghai, China). Mice were maintained at a temperature of 25 ± 2 under a normal 12-h light/dark schedule. All procedures were approved by the Soochow University Animal Care and Use Committee and were in accordance with the animal use and care guide of the National Institutes of Health. The weights of the mice were measured every three days with an electronic balance.

Spatial restraint stress

Chronic spatial restraint stress is extensively used to cause depression phenotypes in mice. Mice were placed in well-ventilated 50-ml tubes for 2-h from 9 to 11 am daily for 3 weeks. Mice were unable to move in the tube.

Animal drug administration

The dose of Tanshinone IIA (TSA, SelleckChem, Houston, TX, USA, 40 mg/kg body weight) was chosen based on a previous report (Liu et al., 2016). Tanshinone IIA was dissolved in DMSO/PEG300/Tween 80/H_2O, 5/30/2/63, v/v/v/v. The dose of SL327 (a selective MEK1/2 inhibitor, SelleckChem, Houston, TX, USA, 30 mg/kg) was chosen based on a previous report (Li et al., 2016a). SL327 was dissolved in DMSO/PEG300/Tween 80/H_2O, 2/30/5/63, v/v/v/v. As shown in Fig. 1A, Mice adaptation for 3 days, after stressed for 3 weeks, these mice were then subjected to behavioral test. After behavioral test, the mice in the control group and stress group were randomly assigned to four groups: control group, TSA treated control group, stress group, TSA treated stress group. Tanshinone IIA was injected intraperitoneally (i.p.) for 14 days, SL327 was injected intraperitoneally (i.p.) for 7 days and dissolved in corn oil before use. Control mice were injected with same volume of vehicle (5% DMSO + 30% PEG300 + 2% Tween80 + ddH_2O dissolved in corn oil).

Tail suspension test (TST)

TST protocol was based on the methods described in previous reports (Steru et al., 1985). The medical tape fixed the tail of the mouse at a height of 45 cm from the ground, and recorded the immobility time of the mouse within six minutes (Mice were considered immobile when hung passively and motionlessly without escape-oriented behavior). The experiment was double-blind.

Forced swim test (FST)

FST protocol was based on the methods described in previous reports (Porsolt et al., 1977). The mice were placed in a glass cylinder (20 cm high, 15 cm in diameter) containing water (25 , water depth 15 cm), and the immobility time of the mice was recorded for six minutes (defined as floating or the absence of active behaviors such as swimming or struggling to escape, was measured. Slight movements of the feet and tail necessary to keep the head above water were excluded as mobility). The experiment was double-blind.

Cell culture & drug administration

PC12 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and grown in Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, UT, USA), 10% FBS (Gibco BRL Co. Ltd, USA), and 100 μg/ml penicillin/streptomycin. PC12 cells were kept at 37 °C under 5% CO_2 in a humidified atmosphere. Drug administration was performed when cells reached 50–60% proliferation, the concentration of dexamethasone (Dex) is 20 μM for 48 h, TSA was administered at 3 μM for 24 h, and SL327 was administered at 5 μM for 24 h.

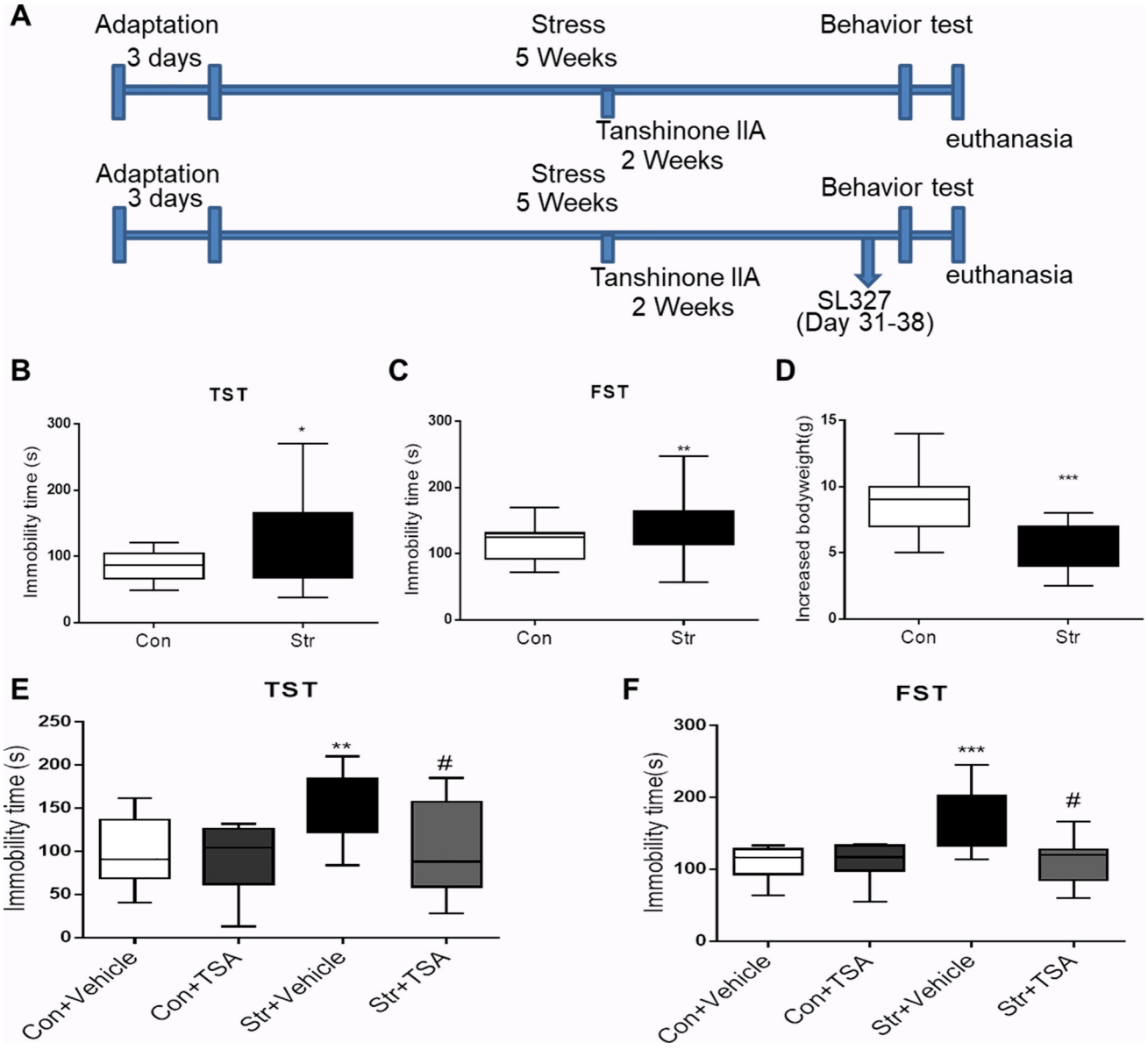

Fig. 1. TSA provided antidepressant effects in spatial restraint-induced depressive mice. **(A)** Schematic diagram of treatment plan. **(B, C)** After 3 weeks of spatial restraint stress, the immobility time in the tail suspension test (TST, $N = 23$) and the forced swim test (FST, $N = 23$) were tested in mice (Student's *t*-test). **(D)** Body weight was reduced in the stress group compared with control group (Student's *t*-test, $N = 23$). **(E, F)** Mice were treated with TSA (40 mg/kg) or vehicle for 2 weeks, The TST and the FST were performed 2 weeks after treatment. Data were presented as mean ± SEM ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Con + Vehicle, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus Str + Vehicle, $N = 10$–13). (Con: Control, Str: Stress, Tanshinone IIA: TSA).

Western blot analysis

At the end of the experiment, mice were euthanized by rapid decapitation. The whole brain tissue was removed immediately and snap-frozen in liquid nitrogen and stored at −80 °C. Total protein from hippocampal tissue and cells was extracted using RIPA lysis buffer (1% Triton X-100, 1% SDS, 500 mM NaCl, 1 mM EDTA and 50 mM Tris-HCl, pH 7.4) with a cocktail of protease inhibitor and phosphatase inhibitor. Protein concentration was determined using a BCA Protein Assay Kit (Pierce, Bonn, Germany). The samples were heated at 96 for 10 min, then electrophoresed on 12% Bis-Tris polyacrylamide gels and subsequently electro-transferred to nitrocellulose membranes. Blocked in blocking buffer (Tris-buffered saline containing 5% skim milk) for 1 h at 25 , the membranes were incubated with primary antibodies: anti-phospho-ERK1/2 (Thr 202/Tyr 204; 1:1000, Cat: A5036), anti-ERK1/2 (1:1000, Cat: A5029), GAPDH (1:1000, Cat: A5036), anti-CREB (1:1000, Cat: A5014, Selleckchem, Houston, TX, USA), anti-phospho-CREB (Ser133, Cat: YP0075 Immunoway), anti-BDNF (1:1000, Santa Cruz Biotechnology, CA, USA), anti-beta-tubulin (RRID: AB_477556, 1:20,000, Sigma-Aldrich, Saint Louis, MO, USA) in the same buffer overnight at 4 °C. On the second day, the samples were washed three times with

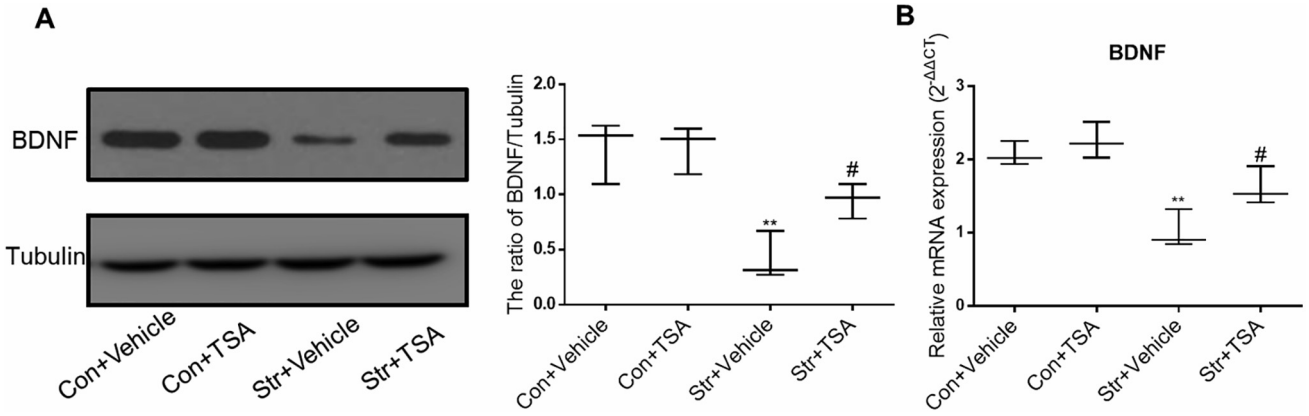

Fig. 2. Effect of Tanshinone IIA on BDNF protein, mRNA expression level in hippocampus of mice. The expression of BDNF protein, mRNA among groups was detected by Western blot and real-time RT-PCR. **(A)** Immunoblot images of BDNF protein. **(B)** BDNF mRNA levels determined by real-time RT-PCR. Data were analyzed with a one-way ANOVA, All values were presented as mean ± SEM (*$P < 0.05$, **$P < 0.01$ versus Con + Vehicle, $^{\#}P < 0.05$ versus Str + Vehicle). (Con: Control, Str: Stress, Tanshinone IIA: TSA).

TBST, then incubated with the secondary antibodies at 25 for 1 h, and detected using an Odyssey infrared imaging system (LI-COR Biosciences).

Quantitative real-time polymerase chain reaction (Q-PCR)

Isolate of total RNA with an RNAiso Plus kit (TaKaRa, Japan) according to the manufacturer's protocol. One microgram of total RNA was reverse transcribed with M-MLV (H−) Reverse Transcriptase (Vazyme, Nanjing, China). Q-PCR was performed using FastStart universal SYBR Green Master (Roche, Indianapolis, IN, USA) and Applied Biosystems StepOnePlus instrument (Thermo Fisher, Singapore), according to the manufacturer's instructions. The sequences of *Bdnf* forward primer: 5′-GCGGCAGATAAAAAGACTGC-3′, reverse primer 5′-G CAGCCTTCCTTGGTGTAAC-3′ (Chen et al., 2017), β-actin forward: 5′-ACCTCCTACAATGAGCTGC-3′, Reverse: 5′-TGCCAATAGTGATGACCT-3′.

Data analysis

Statistical analyses were performed using GraphPad Prism software (Version 6; La Jolla, CA, USA). The difference between the two groups was determined by Student's *t*-test. Differences between multiple groups were carried out by one-way analysis of variance (ANOVA). Significance was set at $P < 0.05$. All data are expressed as the mean ± SEM.

RESULTS

TSA administration alleviated depression-like behaviors induced by spatial restraint stress mice

To elucidate whether the neuroprotective effects could serve as a basis for their antidepressant effects, we first examine the ability of TSA to improve depression-like behavior in spatial restraint stress mouse model of depression. After 3 weeks of spatial restraint stress, the immobility time in the stress group was higher than control group in the TST ($n = 23$, $P = 0.0127$, Fig. 1B) and FST ($n = 23$, $P = 0.0046$, Fig. 1C). Additionally, the weight of mice in the stress group was significantly reduced ($n = 23$, $P < 0.001$, Fig. 1D). Mice were chronic pretreatment with Tanshinone IIA (TSA, 40 mg/kg body weight, i.p.) or vehicle for two weeks. The results revealed that animals treated with TSA significantly decreased the immobility time compared with vehicle treated animals in TST ($n = 10–13$, F (3, 41) = 4.862), $P = 0.0055$, Fig. 1E) and FST ($n = 10–13$, F (3, 41) = 10.73, $P < 0.001$, Fig. 1F). There were no significant differences between the vehicle-treated control group and TSA-treated control group with regard to struggling behavior. Overall, our results showed that TSA has a potential antidepressant effects in spatial restraint stress-induced mice.

TSA treatment increased the expression of BDNF in stressed mice

Many studies have shown that BDNF level are highly correlated with depression (Hashimoto, 2006; Chen et al., 2017). Therefore, we detected BDNF levels within the hippocampus of mice. Our results revealed that the expression of BDNF protein ($P = 0.0086$) and mRNA ($P = 0.0042$) was reduced in the stress group. After treatment with TSA, the levels of BDNF protein ($P = 0.027$) and mRNA ($P = 0.0488$) were significantly increased in the TSA treated stress group compared with the vehicle treated stress group (Fig. 2A, B). This result suggests that TSA can up-regulate BDNF levels in the hippocampus of stressed mice.

Effects of TSA on the ERK-CREB-BDNF signaling pathway in PC12 cells

Previous reports have shown that patients with major depressive disorder have high levels of glucocorticoids and that chronic treatment with Dex (a synthetic glucocorticoid steroid) can lead to depressive-like behavior in mice(Ridder et al., 2005; Chen et al., 2017). To explore the effects of TSA, PC12 cells were treated with Dex for 48 h and then treated with TSA for 24 h.

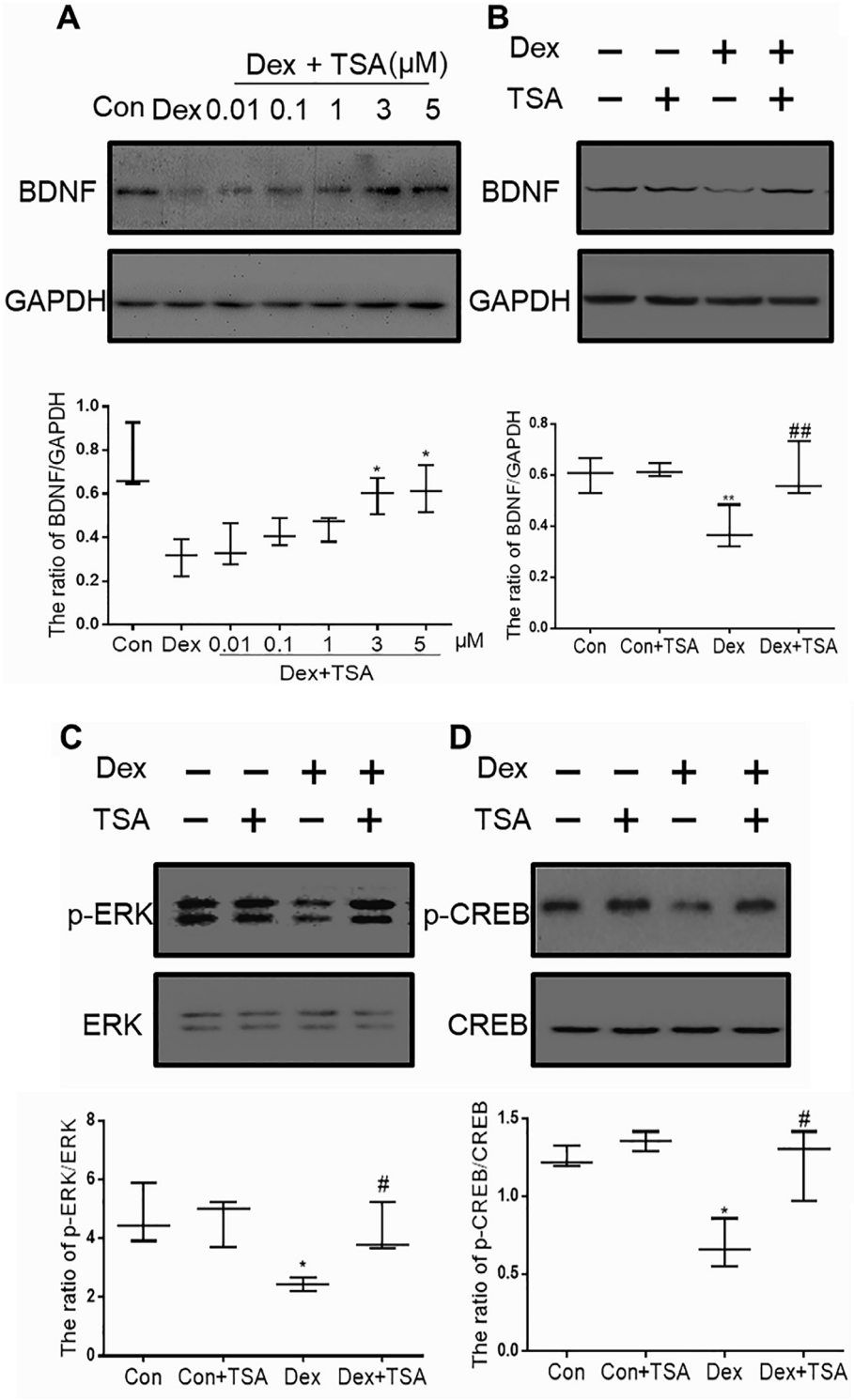

Fig. 3. TSA up-regulates the expressions of BDNF, p-ERK and p-CREB in dex-induced PC12 cells. **(A, B)** PC12 cells were exposure with dex (20 μM) for 48 h and then treated with different concentrations of TSA (0.01 μM, 0.1 μM, 1 μM, 3 μM, or 5 μM) for 24 h. Representative immunoblot images of BDNF protein and quantitative date. **(C, D)** Western blot was used to analyze the levels of p-ERK and p-CREB. Data were analyzed with a one-way ANOVA, All values were presented as mean ± SEM (*P < 0.05, **P < 0.01 versus Con + Vehicle, $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 versus Str + Vehicle). (Con: Control, Str: Stress, Tanshinone IIA: TSA, Dexamethasone: dex).

The results revealed that Dex reduced the expression of BDNF (P = 0.0149) and TSA (3 μM) enhanced the content of BDNF protein (P = 0.0148, Fig. 3A). As shown in Fig. 3B, a significant difference in BDNF protein content among the four groups [F (3, 12) = 11.72, P = 0.0007]. The expression of BDNF were up-regulated in Dex and TSA treated group, compared to the vehicle and TSA treated group (P = 0.0099, Fig. 3B). To further determine the possible involved with ERK-CREB signaling pathway in the anti-depressant effects induced by TSA. Significant differences in ERK phosphorylation levels were obtained among the four groups [F (3, 8) = 5.449, P = 0.0246, Fig. 3C], our results showed that p-ERK levels were reduced after treatment with Dex (P = 0.0193), and TSA reversed this reduction (P = 0.0262, Fig. 3C). Similarly, the results showed significant differences in CREB phosphorylation levels between the four groups in Fig. 3D [F (3, 8) = 12.32, P = 0.0023]. The level of p-CREB protein was also reduced in the Dex-treated group (P = 0.0049), and TSA treatment enhanced the content of p-CREB protein (P = 0.0281, Fig. 3D). No significant between the control group and con group with TSA treatment group. These results indicated that the ERK-CREB-BDNF signaling pathway in the possible mechanisms of antidepressant-like effects of TSA.

Treatment with TSA increased the expression of p-ERK and p-CREB in the hippocampus

Next, we investigated whether MAPK signaling pathways is activated in the hippocampus and are involved in the antidepressant effect induced by TSA. As shown in Fig. 4, the results revealed that there was an overall difference among the four groups with regard to the phosphorylation

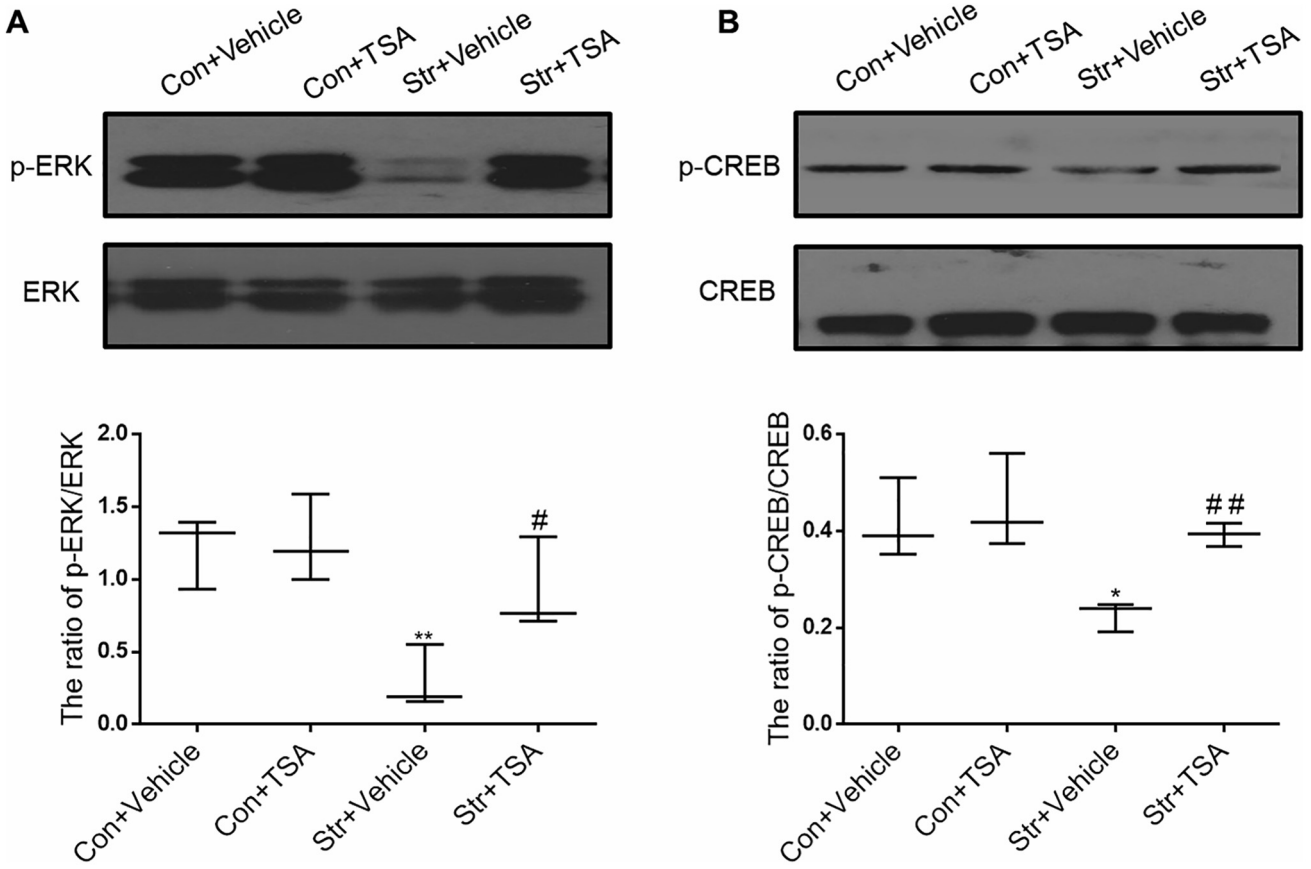

Fig. 4. TSA ameliorated p-ERK and p-CREB levels in hippocampus of mice. **(A, B)** Mice were treated with TSA (40 mg/kg) or vehicle for 2 weeks, and p-ERK and p-CREB protein levels in the hippocampus were detected by western blot analysis. Data were analyzed with a one-way ANOVA, all values were presented as mean ± SEM. (*$P < 0.05$, **$P < 0.01$ as compared with the con + Vehicle group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ as compared to the Str + Vehicle group). (Con: Control, Str: Stress, Tanshinone IIA: TSA).

levels of ERK [F (3, 8) = 7.780, P = 0.0093], and the phosphorylation levels of CREB within hippocampus regions [F (3, 8) = 6.783, P = 0.0137]. Western blot showed that the expression of p-ERK protein was reduced in the Vehicle treated stress group compared with vehicle treated control group (P = 0.0087). The expression of p-ERK protein was enhanced significantly after treatment with TSA compared to levels in the stress group (P = 0.0493, Fig. 4A). Similarly, the expression of p-CREB in the stress group was significantly lower than vehicle treated control group (P = 0.0197), and the levels of p-CREB was increased in the hippocampus after treatment with TSA (P = 0.0016, Fig. 4B). These results indicated that the ERK signaling pathway in the hippocampus is involved in the antidepressant effects induced by TSA, as assessed by the TST and FST in mice.

SL327 blocked the ERK-CREB-BDNF signaling pathway in PC12 cells

To further prove our hypothesis, PC12 cells were treated with SL327. As shown in Fig. 3C, the expression of p-ERK in Dex-treated PC12 cells was enhanced significantly after treatment with TSA. Therefore, we treated Dex-treated PC12 cells with TSA and SL327. We examined the content of p-ERK and p-CREB, the expression of p-ERK [F (3, 8) = 10.96, P = 0.0033] and p-CREB [F (3, 8) = 12.09, P = 0.0024] and BDNF [F (3, 8) = 24.99, P = 0.0002] were significantly different among the four groups. The results showed that the expression level of p-ERK protein was significantly decreased in the SL327 treatment group compared with that in the TSA group (P = 0.0306, Fig. 5A). Similarly, the expression levels of p-CREB (P = 0.0307, Fig. 5B) and BDNF (P = 0.0075, Fig. 5C) were significantly reduced after treatment with SL327 (Fig. 5B, C). These results provide strong evidence indicating that SL327 blocked the effects of TSA on the ERK-CREB-BDNF signaling pathway in PC12 cells.

SL327 blocked the activation of the MAPK signaling pathway and abolished the antidepressant effect of TSA in mice

We next investigated whether the activation of the MAPK signaling pathway within the hippocampus is involved in the antidepressant-like effects induced by TSA. SL327 was injected daily along with TSA administration for 7 days. In Fig. 6, the statistical analysis of the levels of expression of p-ERK [F (4, 10) = 17.46, P = 0.0002] and p-CREB [F (4, 10) = 25.21, P < 0.0001] and BDNF

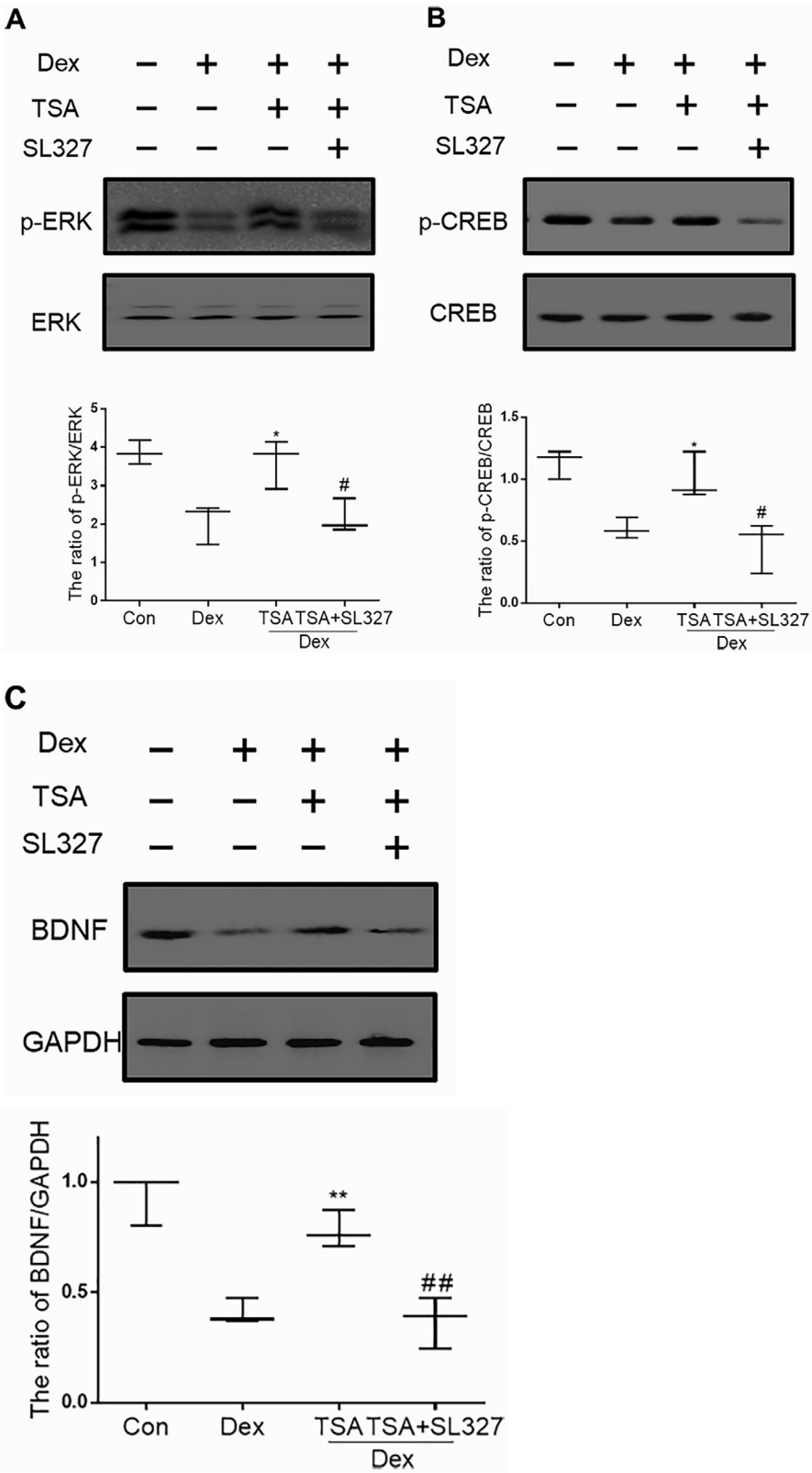

Fig. 5. SL327 blocked the effects of TSA in dex-induced PC12 cells. **(A–C)** Dex-induced PC12 cells were treated with TSA (3 μM) or SL327 (5 μM) for 24 h. Western blot was used to analyze the levels of p-ERK, p-CREB, BDNF. Data were analyzed with a one-way ANOVA, all values were presented as mean ± SEM. (*$P < 0.05$, **$P < 0.01$ compared with the control group $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the Dex + TSA group). (Con: Control, Str: Stress, Tanshinone IIA: TSA, Dexamethasone: dex).

[F (4, 10) = 7.358, P = 0.005] showed significant differences among groups. The results showed that the activation of p-ERK by TSA was significantly abolished by SL327 (P = 0.0023, Fig. 6A). Similarly, as expected the levels p-CREB (P = 0.004, Fig. 6B) and BDNF (P = 0.041, Fig. 6C) protein were significantly reduced after SL327 treatment. Consistent with these finding, SL327 treatment increased the immobility time of mice in both the TST [n = 9–11, F (4, 43) = 6.391, P = 0.0004, Fig. 6D] and FST [n = 9–11, F (4, 43) = 7.657, P = 0.0001, Fig. 6E] compared with the times of mice treated with TSA. SL327 attenuated the antidepressant effects of TSA by blocking the MAPK signaling pathway. These results provide strong evidence indicating that the hippocampus MAPK signaling pathway is involved in TSA-induced antidepressant effects as assessed in the TST and FST in mice (see Fig. 7).

DISCUSSION

In the present study, the effects of Tanshinone IIA in the treatment of a spatial restraint stress mouse model of depression were examined. Our results revealed that 3 weeks of spatial restraint stress induced depressive-like behavior in mice, which was characterized by increased the immobility time and decreased weight gain. These indices of depression were accompanied by decreased BDNF, p-ERK and p-CREB levels in hippocampal. Mice were chronic pretreatment with Tanshinone IIA significantly ameliorated all these behavioral alterations associated with stress-induced depression. To further explore the therapeutic effects of Tanshinone IIA on the ERK-CREB-BDNF signaling pathway, we treated mice and PC12 cells with SL327 (a specific inhibitor of p-ERK). SL327 not only reduced p-ERK, p-CREB and BDNF levels *in vitro* and *in vivo* but also abolished the therapeutic effects of TSA in stressed mice. Therefore, these findings suggest that the antidepressant-like effects

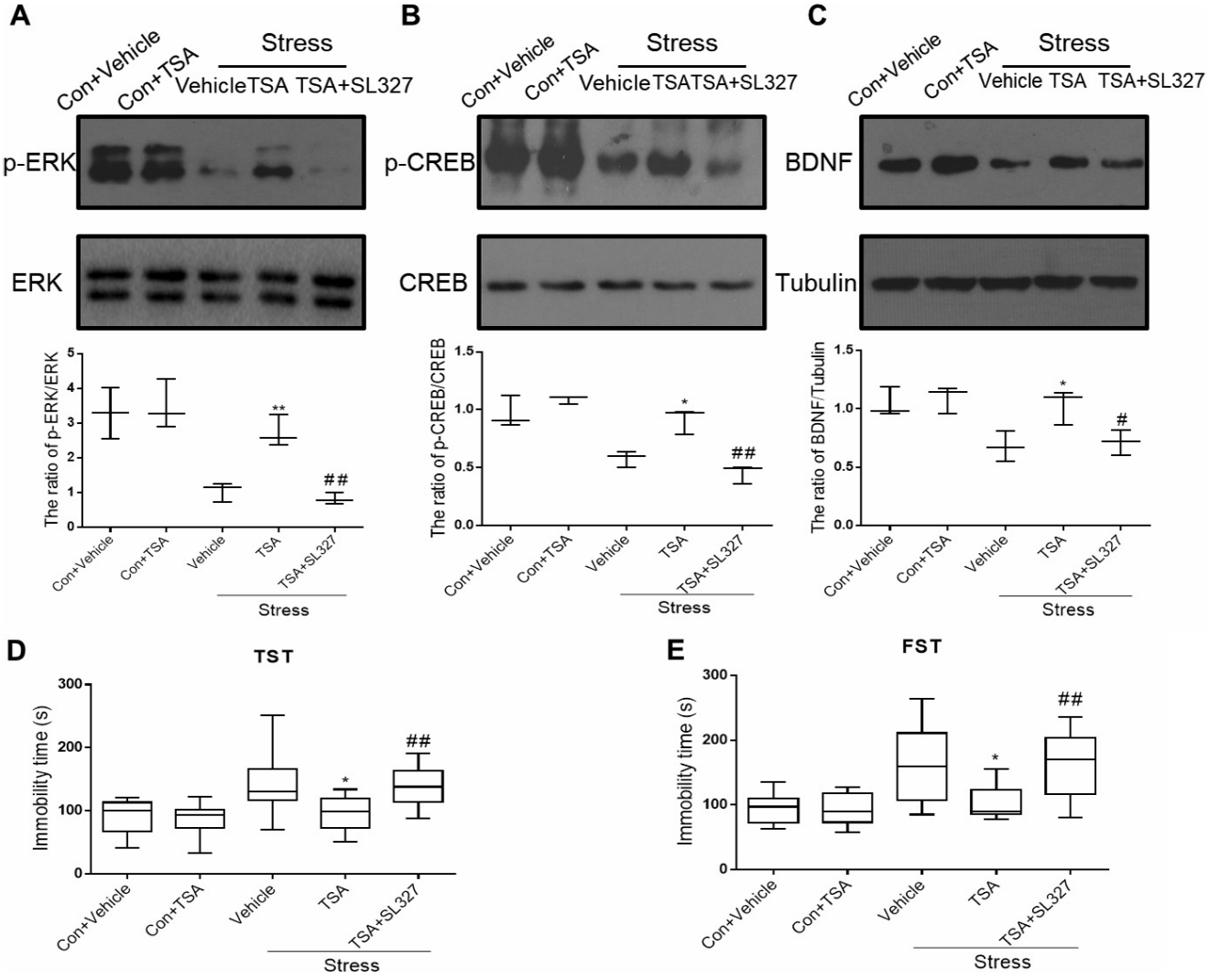

Fig. 6. The inhibitor SL327 blocked the decreased immobility duration induced by Tanshinone IIA. After 3 weeks of spatial restraint stress, control mice and stressed mice were administered vehicle, TSA (40 mg/kg), or SL327 (30 mg/kg). Hippocampal tissues were collected. **(A–C)** Western blot was used to analyze the levels of p-ERK, p-CREB, BDNF. **(D, E)** After treatment with SL327, the immobility time in the tail suspension test (TST) and in the forced swim test (FST) was examined (N = 8–11). Data were analyzed with a one-way ANOVA, all values were presented as mean ± SEM. ($^{*}P < 0.05$, $^{**}P < 0.01$ as compared with the stress + Vehicle group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ as compared to the Str + TSA group). (Con: Control, Str: Stress, Tanshinone IIA: TSA).

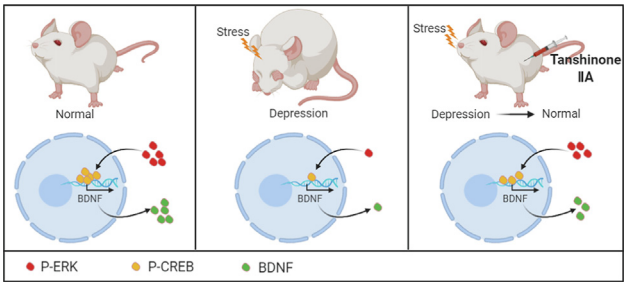

Fig. 7. The proposed mechanism is that TSA improved depression-like behavior by activating the ERK-CREB-BDNF signaling pathway.

of TSA were mediated by ERK-CREB regulated increases in BDNF expression in the hippocampus of mice.

Our behavioral results are consistent with previous studies, with stressed mice showing increased immobility time in TST and FST (Kim et al., 2015; Zhang et al., 2015). The effects of stress on body weight remain controversial with some studied indicating a decrease in body weight (Vancassel et al., 2008; Surget et al., 2009), while some other studies showed no change (Ibarguen-Vargas et al., 2008; Mutlu et al., 2009). The distinctions may be due to differences in animal strains, stress schedule and stimulus intensity. We report that the increased immobility time in TST and FST induced by spatial restraint stress was significantly reversed by chronic treatment with Tanshinone IIA. These findings suggest an antidepressant-like action of Tanshinone IIA.

There is increasing evidence which suggests that a decrease in BDNF support is a significant factor in the pathogenesis of depression. Decreased plasma levels

of BDNF were found in depressed patients (Khan et al., 2019). Moreover, a decrease in BDNF in hippocampus in chronic stress studies was found (Nibuya et al., 1999). In accordance with these views, our study revealed that chronic Tanshinone IIA treatment ameliorated the reduction of BDNF levels that were produced by stress protocol.

The gene of BDNF contains CRE, which binds to phosphorylated CREB to enhance transcription. Chronic antidepressants treatment increases the expression, phosphorylation of CREB and its downstream target gene BDNF in the hippocampus, amygdala and other limbic brain regions thought to be involved in depression (Nibuya et al., 1996; Thome et al., 2000). Consistent with these, chronic Tanshinone IIA treatment improved the reduction of BDNF and p-CREB levels in hippocampus.

ERK belongs to the MAPK family, which is involved in physiological activity, including signal transmission and recognition and cell growth, development, and proliferation (Almeida et al., 2006). Previous study has demonstrated that BDNF could improve depressive behavior by activating the ERK pathway (Shirayama et al., 2002), and preclinical studies have suggested that depression was relevant with an abnormal ERK signaling pathway (Dwivedi et al., 2001), whereas chronic antidepressants treatment increased the expression of p-ERK in the hippocampus and amygdala (First et al., 2011). These results suggest that ERK signaling pathway may be potential participate in the antidepressant therapy. And consistent with the previous study, our results demonstrated that the expression levels of p-ERK, p-CREB, BDNF were decreased in hippocampus of stressed mice, while chronic Tanshinone IIA administration ameliorated these alterations. Therefore, these findings suggest that TSA can improve depression-like behavior in mice by activating the ERK-CREB-BDNF signaling Pathway.

Tanshinone IIA is one of the main active ingredients in the Chinese herbal medicine '*Salvia miltiorrhiza*' that can promote blood circulation and counteract blood stasis. Previous studies have shown that TSA can alleviate neuronal impairment in Alzheimer's disease and stroke (Tang et al., 2010; Wang et al., 2011; Chen et al., 2012; Qian et al., 2012; Chen et al., 2018). Recently, several studies have indicated that TSA significantly activated the ERK pathway and induced the autophagy cell death (Yun et al., 2014). Moreover, TSA restored Aβ-induced spatial memory impairment and neuron damage by up-regulating p-ERK and inhibiting the autophagy (Zhu et al., 2017). Consistent with these report, the present study also found that TSA could increase p-ERK in the hippocampus of mice. These findings suggest that p-ERK play vital roles in neuroprotective of TSA.

Our studies demonstrated the antidepressant effect of TSA in a depressive mouse model mediated by activating the ERK-CREB-BDNF signaling pathway.

ACKNOWLEDGEMENTS

This study was supported by the grants from National Key R&D Program of China (2017YFE0103700), the National Natural Science Foundation of China (81601154), Jiangsu Key Laboratory of Neuropsychiatric Diseases (BM2013003) and the Priority Academic Program Development of Jiangsu Higher Education Institutions of China.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests.

REFERENCES

Almeida RC, Souza DG, Soletti RC, Lopez MG, Rodrigues AL, Gabilan NH (2006) Involvement of PKA, MAPK/ERK and CaMKII, but not PKC in the acute antidepressant-like effect of memantine in mice. Neurosci Lett 395:93–97.

Bjorkholm C, Monteggia LM (2016) BDNF - a key transducer of antidepressant effects. Neuropharmacology 102:72–79.

Bousser MG (2000) [Depression and neurology]. Encephale 26 Spec No 3:1-2.

Butler L, Pilkington K (2013) Chinese herbal medicine and depression: the research evidence. Evid Based Complement Alternat Med 2013 739716.

Castren E, Voikar V, Rantamaki T (2007) Role of neurotrophic factors in depression. Curr Opin Pharmacol 7:18–21.

Chen J, Bi Y, Chen L, Zhang Q, Xu L (2018) Tanshinone IIA exerts neuroprotective effects on hippocampus-dependent cognitive impairments in diabetic rats by attenuating ER stress-induced apoptosis. Biomed Pharmacother 104:530–536.

Chen L, Miao Z, Xu X (2017) beta-hydroxybutyrate alleviates depressive behaviors in mice possibly by increasing the histone3-lysine9-beta-hydroxybutyrylation. Biochem Biophys Res Commun 490:117–122.

Chen Y, Wu X, Yu S, Fauzee NJ, Wu J, Li L, Zhao J, Zhao Y (2012) Neuroprotective capabilities of Tanshinone IIA against cerebral ischemia/reperfusion injury via anti-apoptotic pathway in rats. Biol Pharm Bull 35:164–170.

Chen YH, Zhang RG, Xue F, Wang HN, Chen YC, Hu GT, Peng Y, Peng ZW, Tan QR (2015) Quetiapine and repetitive transcranial magnetic stimulation ameliorate depression-like behaviors and up-regulate the proliferation of hippocampal-derived neural stem cells in a rat model of depression: The involvement of the BDNF/ERK signal pathway. Pharmacol Biochem Behav 136:39–46.

Cheng L, Zhou S, Zhao Y, Sun Y, Xu Z, Yuan B, Chen X (2018) Tanshinone IIA attenuates osteoclastogenesis in ovariectomized mice by inactivating NF-kB and Akt signaling pathways. Am J Transl Res 10:1457–1468.

Dwivedi Y, Rizavi HS, Roberts RC, Conley RC, Tamminga CA, Pandey GN (2001) Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. J Neurochem 77:916–928.

Feng P, Guan Z, Yang X, Fang J (2003) Impairments of ERK signal transduction in the brain in a rat model of depression induced by neonatal exposure of clomipramine. Brain Res 991:195–205.

First M, Gil-Ad I, Taler M, Tarasenko I, Novak N, Weizman A (2011) The effects of fluoxetine treatment in a chronic mild stress rat model on depression-related behavior, brain neurotrophins and ERK expression. J Mol Neurosci 45:246–255.

Flood DG, Finn JP, Walton KM, Dionne CA, Contreras PC, Miller MS, Bhat RV (1998) Immunolocalization of the mitogen-activated protein kinases p42MAPK and JNK1, and their regulatory kinases MEK1 and MEK4, in adult rat central nervous system. J Comp Neurol 398:373–392.

Guan L, Jia N, Zhao X, Zhang X, Tang G, Yang L, Sun H, Wang D, Su Q, Song Q, Cai D, Cai Q, Li H, Zhu Z (2013) The involvement of ERK/CREB/Bcl-2 in depression-like behavior in prenatally stressed offspring rats. Brain Res Bull 99:1–8.

Guan R, Wang J, Li Z, Ding M, Li D, Xu G, Wang T, Chen Y, Yang Q, Long Z, Cai Z, Zhang C, Liang X, Dong L, Zhao L, Zhang H, Sun

D, Lu W (2018) Sodium Tanshinone IIA sulfonate decreases cigarette smoke-induced inflammation and oxidative stress via blocking the activation of MAPK/HIF-1alpha signaling pathway. Front Pharmacol 9:263.
Hamidpour M, Hamidpour R, Hamidpour S, Shahlari M (2014) Chemistry, pharmacology, and medicinal property of sage (salvia) to prevent and cure illnesses such as obesity, diabetes, depression, dementia, lupus, autism, heart disease, and cancer. J Tradit Complement Med 4:82–88.
Hashimoto K (2006) Depression and BDNF. Nihon Yakurigaku Zasshi 127:201–204.
Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677–736.
Ibarguen-Vargas Y, Surget A, Touma C, Palme R, Belzung C (2008) Multifaceted strain-specific effects in a mouse model of depression and of antidepressant reversal. Psychoneuroendocrinology 33:1357–1368.
Imanshahidi M, Hosseinzadeh H (2006) The pharmacological effects of Salvia species on the central nervous system. Phytother Res 20:427–437.
Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 109:143–148.
Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G (2005) Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol Psychiatry 57:1068–1072.
Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS, National Comorbidity Survey R (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 289:3095–3105.
Khan MS, Wu GWY, Reus VI, Hough CM, Lindqvist D, Westrin A, Nier BM, Wolkowitz OM, Mellon SH (2019) Low serum brain-derived neurotrophic factor is associated with suicidal ideation in major depressive disorder. Psychiatry Res 273:108–113.
Kim TK, Kim JE, Park JY, Lee JE, Choi J, Kim H, Lee EH, Kim SW, Lee JK, Kang HS, Han PL (2015) Antidepressant effects of exercise are produced via suppression of hypocretin/orexin and melanin-concentrating hormone in the basolateral amygdala. Neurobiol Dis 79:59–69.
Lam BY, Lo AC, Sun X, Luo HW, Chung SK, Sucher NJ (2003) Neuroprotective effects of tanshinones in transient focal cerebral ischemia in mice. Phytomedicine 10:286–291.
Lesch KP (2004) Gene-environment interaction and the genetics of depression. J Psychiatry Neurosci 29:174–184.
Li CF, Chen SM, Chen XM, Mu RH, Wang SS, Geng D, Liu Q, Yi LT (2016a) ERK-dependent brain-derived neurotrophic factor regulation by hesperidin in mice exposed to chronic mild stress. Brain Res Bull 124:40–47.
Li Y, Chen Z, Yu N, Yao K, Che Y, Xi Y, Zhai S (2016b) Chinese herbal medicine for postpartum depression: a systematic review of randomized controlled trials. Evid Based Complement Alternat Med 2016:5284234.
Liu BB, Luo L, Liu XL, Geng D, Li CF, Chen SM, Chen XM, Yi LT, Liu Q (2015) Essential oil of Syzygium aromaticum reverses the deficits of stress-induced behaviors and hippocampal p-ERK/p-CREB/brain-derived neurotrophic factor expression. Planta Med 81:185–192.
Liu C, Wu Y, Zha S, Liu M, Wang Y, Yang G, Ma K, Fei Y, Zhang Y, Hu X, Yang W, Qian Y (2016) Treatment effects of tanshinone IIA against intracerebroventricular streptozotocin induced memory deficits in mice. Brain Res 1631:137–146.
Lu M, Luo Y, Hu P, Dou L, Huang S (2018) Tanshinone IIA inhibits AGEs-induced proliferation and migration of cultured vascular smooth muscle cells by suppressing ERK1/2 MAPK signaling. Iran J Basic Med Sci 21:83–88.
Mutlu O, Ulak G, Laugeray A, Belzung C (2009) Effects of neuronal and inducible NOS inhibitor 1-[2-(trifluoromethyl) phenyl] imidazole (TRIM) in unpredictable chronic mild stress procedure in mice. Pharmacol Biochem Behav 92:82–87.
Nibuya M, Nestler EJ, Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16:2365–2372.
Nibuya M, Takahashi M, Russell DS, Duman RS (1999) Repeated stress increases catalytic TrkB mRNA in rat hippocampus. Neurosci Lett 267:81–84.
O'Leary D, Paykel E, Todd C, Vardulaki K (2001) Suicide in primary affective disorders revisited: a systematic review by treatment era. J Clin Psychiatry 62:804–811.
Ortiz J, Harris HW, Guitart X, Terwilliger RZ, Haycock JW, Nestler EJ (1995) Extracellular signal-regulated protein-kinases (Erks) and Erk kinase (Mek) in brain – regional distribution and regulation by chronic morphine. J Neurosci 15:1285–1297.
Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229:327–336.
Qian YH, Xiao Q, Xu J (2012) The protective effects of tanshinone IIA on beta-amyloid protein (1–42)-induced cytotoxicity via activation of the Bcl-xL pathway in neuron. Brain Res Bull 88:354–358.
Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, Zink M, Hortnagl H, Flor H, Henn FA, Schutz G, Gass P (2005) Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. J Neurosci 25:6243–6250.
Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22:3251–3261.
Sonnenberg CM, Beekman AT, Deeg DJ, van Tilburg W (2000) Sex differences in late-life depression. Acta Psychiatr Scand 101:286–292.
Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl) 85:367–370.
Sui H, Zhao J, Zhou L, Wen H, Deng W, Li C, Ji Q, Liu X, Feng Y, Chai N, Zhang Q, Cai J, Li Q (2017) Tanshinone IIA inhibits beta-catenin/VEGF-mediated angiogenesis by targeting TGF-beta1 in normoxic and HIF-1alpha in hypoxic microenvironments in human colorectal cancer. Cancer Lett 403:86–97.
Surget A, Wang Y, Leman S, Ibarguen-Vargas Y, Edgar N, Griebel G, Belzung C, Sibille E (2009) Corticolimbic transcriptome changes are state-dependent and region-specific in a rodent model of depression and of antidepressant reversal. Neuropsychopharmacology 34:1363–1380.
Tang C, Xue H, Bai C, Fu R, Wu A (2010) The effects of Tanshinone IIA on blood-brain barrier and brain edema after transient middle cerebral artery occlusion in rats. Phytomedicine 17:1145–1149.
Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S, Storm D, Duman RS (2000) cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. J Neurosci 20:4030–4036.
Vancassel S, Leman S, Hanonick L, Denis S, Roger J, Nollet M, Bodard S, Kousignian I, Belzung C, Chalon S (2008) n-3 polyunsaturated fatty acid supplementation reverses stress-induced modifications on brain monoamine levels in mice. J Lipid Res 49:340–348.
Wang Y, Li M, Liang Y, Yang Y, Liu Z, Yao K, Chen Z, Zhai S (2017) Chinese herbal medicine for the treatment of depression: applications, efficacies and mechanisms. Curr Pharm Des 23:5180–5190.
Wang X, Xie Y, Zhang T, Bo S, Bai X, Liu H, Li T, Liu S, Zhou Y, Cong X, Wang Z, Liu D (2016) Resveratrol reverses chronic restraint stress-induced depression-like behaviour: Involvement of BDNF level, ERK phosphorylation and expression of Bcl-2 and Bax in rats. Brain Res Bull 125:134–143.
Wang L, Zhang X, Liu L, Cui L, Yang R, Li M, Du W (2010) Tanshinone II A down-regulates HMGB1, RAGE, TLR4, NF-kappaB expression, ameliorates BBB permeability and

endothelial cell function, and protects rat brains against focal ischemia. Brain Res 1321:143–151.
Wang W, Zheng LL, Wang F, Hu ZL, Wu WN, Gu J, Chen JG (2011) Tanshinone IIA attenuates neuronal damage and the impairment of long-term potentiation induced by hydrogen peroxide. J Ethnopharmacol 134:147–155.
Wysokinski A (2016) Serum levels of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) in depressed patients with schizophrenia. Nord J Psychiatry 70:267–271.
Yang L, Yue N, Zhu XC, Han QQ, Liu Q, Yu J, Wu GC (2013) Electroacupuncture upregulates ERK signaling pathways and promotes adult hippocampal neural progenitors proliferation in a rat model of depression. BMC Complement Altern Med 13.
Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. J Neurosci 22:1532–1540.
Yun SM, Jung JH, Jeong SJ, Sohn EJ, Kim B, Kim SH (2014) Tanshinone IIA induces autophagic cell death via activation of AMPK and ERK and inhibition of mTOR and p70 S6K in KBM-5 leukemia cells. Phytother Res 28:458–464.
Zhang G, Chen L, Yang L, Hua X, Zhou B, Miao Z, Li J, Hu H, Namaka M, Kong J, Xu X (2015) Combined use of spatial restraint stress and middle cerebral artery occlusion is a novel model of post-stroke depression in mice. Sci Rep 5:16751.
Zhang WJ, Feng J, Zhou R, Ye LY, Liu HL, Peng L, Lou JN, Li CH (2010) Tanshinone IIA protects the human blood-brain barrier model from leukocyte-associated hypoxia-reoxygenation injury. Eur J Pharmacol 648:146–152.
Zhu J, Liao S, Zhou L, Wan L (2017) Tanshinone IIA attenuates Abeta25-35-induced spatial memory impairment via upregulating receptors for activated C kinase1 and inhibiting autophagy in hippocampus. J Pharm Pharmacol 69:191–201.

(Received 24 September 2019, Accepted 15 January 2020)
(Available online 31 January 2020)