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Research Article
Fluoxetine Ameliorates Depression via Activation of CREB/BDNF/TrkB Pathway in Triple Transgenic Alzheimer's Disease Model Mice

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Abstract

Background and Objective: Alzheimer's Disease (AD) is a neuro degenerative disease associated with neuronal degeneration. Fluoxetine (FLX) is a classical drug used in the treatment of depression in a clinic. However, whether FLX has a therapeutic effect on depression and cognitive deficits in patients with early AD is unknown. This study aimed to study the therapeutic effect and possible mechanism of FLX on depression and cognitive deficits in the early AD model. **Materials and Methods:** FLX (10 mg kg^{-1}/day) was administered intragastrically once daily for 6 months to the 3-months-old male APPSwe/PS1M146V/tauP301L (3xTg-AD) mice. Behaviour tests were performed in 3 months and 6 months since drug administration. **Results:** The results showed that FLX significantly improved both recognition and spatial memory, alleviated the anxiety-like behaviour and promoted neuronal survival in 3xTg-AD mice. In addition, FLX could activate cAMP-response Element-binding protein (CREB)/brain-derived Neurotrophic Factor (BDNF)/Tropomyosin-related kinase B (TrkB) signaling. **Conclusion:** Activation of the CREB/BDNF/TrkB pathway of FLX might be considered a promising therapeutic approach for alleviating the cognitive deficits associated with early AD.

Key words: Alzheimer's disease, fluoxetine, APPSwe/PS1M146V/tauP301L mice, CREB/BDNF/TrkB pathway, neuro degeneration, spatial memory, cognitive deficits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Depression is one kind of mental disorder. Most depression patients present with slow thinking, persistent anxiety and cognitive impairment, which severely affects normal living conditions[1]. World Health Organization (WHO) recently reported children also suffer from depression and the pediatric cases increase as the age increases. As predicted, depression will become the "No. one killer" in 2030[2].

Alzheimer's disease (AD) is usually induced by progressive neuronal degeneration. Patients with AD exist poor life quality. AD can also increase the economic burden to patients. Depression is one of the most frequent neuropsychiatric complications of AD[3]. About 20-30% of AD patients are always carrying with depression. Epidemiological studies have shown that there may be a pathological link between depression and AD. It indicates that depression may be a prodromal symptom and a risk factor for AD at the same time[4]. The existence of depressive symptoms will push mild cognitive impairment development to AD[5]. Another study showed depression is an early symptom of dementia[6]. Therefore, illuminating the relationship between AD and depression is very important to AD clinical research and therapy.

Brain-derived Neurotrophic Factor (BDNF) plays critical roles in synaptic plasticity as well as the pathology and treatment of a variety of psychiatric disorders. Several findings suggest that BDNF exit survival-promoting action on a variety of CNS neurons including hippocampal and 5-hydroxytryptamine (5-HT) neurons[7]. BDNF level and function could be impaired by depression and stress-related affective disorders. BDNF is also involved in the aetiology of these illnesses[8]. Another study demonstrated that BDNF produces antidepressant-like activity in learned helplessness paradigms and the forced swim test[9]. BDNF expression is upregulated by the transcription factor c-AMP response-element binding protein (CREB). Meanwhile, BDNF activates the high-affinity tropomyosin-related kinase B receptor (TrkB). Plenty of research evidence points out targeting TrkB receptors might be a rational strategy to develop novel antidepressant drugs[10].

Fluoxetine (FLX) is a selective serotonin reuptake inhibitor, which was approved to applying on depression therapeutic by FDA. Due to the excellent therapeutic effect and fewer adverse reactions, FLX is one of the very common anti-depressants. FLX could restore the level of dopamine and norepinephrine by inhibiting the 5-HT receptor. Meanwhile, FLX can also relieve the inhibitory effect of GABA neurons releasing dopamine and norepinephrine through the 5-HT receptor[4]. A recent study showed that FLX improved behavioural performance by inhibiting the phosphorylation of Amyloid Precursor Protein (APP) at T668 in APP/PS1 mice[11]. However, there is still no evidence showed that FLX has a positive effect on mild AD patients with depression and anxiety.

In our present study, we investigated the neuroprotective effect of FLX and its potential working mechanisms through *in vivo* and *in vitro* models.

MATERIALS AND METHODS

Study area: The study was carried out at Pharmacological Laboratory, Wuhan Mental Health Center, China from January, 2019-October, 2020.

Materials: FLX hydrochloride was purchased from Eli Lilly (Wuhan Mental Health Center, Wuhan, China). Polyclonal rabbit anti-phoERK1/2, ERK1/2, CamK and NeuroN antibodies were purchased from Abcam. Monoclonal rabbit anti-BDNF, CREB, pho-CREB, pho-TrkB, TrkB, $5HT_{2A}R$ and GAPDH antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). RIPA lysate, BCA protein quantification kit and ECL luminescence kit were purchased from Hangzhou Ford Biotechnology Co., Ltd (Hangzhou, China). RNeasy kit was purchased from Qiagen (Dusseldorf, German). Corticosterone was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animal studies: 3-months-old male APPSwe/PS1M146V/tauP301L triple-transformed AD (3xTg-AD) mice and littermate wild type (WT) C57BL/6J mice were purchased from Jackson Laboratory (USA). Animals were fed *ad libitum* and maintained in a specific pathogen-free facility with constant ambient temperature and 12 hrs light cycle. All animal experimental procedures were approved by the Animal Research Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

Experimental grouping and administration: The 3xTg-AD mice were randomly divided into 2 groups: model group (3xTg-AD+Saline) and treatment group (3xTg-AD+FLX 10 mg kg^{-1}). Aged-matched WT mice were used as negative control (WT+Saline Group and WT+FLX 10 mg kg^{-1} Group), 8 mice per group. Mice from the FLX-treatment group were treated with FLX (i.g.), once daily,

administrated treatment for 6 months continually. Mice from the saline-treatment group were given saline. Behaviour tests were performed in 3 and 6 months since drug administration.

Sucrose preference test[12]: Before the experiment, all mice were trained to drink sugar water ad libitum. On the first day, 2 bottles of 1% sugar water were placed in the cage. After 24 hrs free drinking, one bottle of sugar water was replaced with pure water. Then let mice drink pure water freely for another 24 hrs. After 48 hrs, all mice fasted without water for 12 hrs. A weighed bottle of sugar water and a bottle of pure water were housed in the mouse cage. The volume of sugar water and pure water were recorded after 6 hrs. Calculate the data following the Eq.:

$$\text{Sugar water preference (\%)} = \frac{\text{Sugar water consumption}}{\text{Sugar water consumption}+\text{Pure water consumption}} \times 100$$

Forced swim test: Depressive-like behaviour was estimated by the forced-swim test[13]. Depressed mice swim in a confined space and become immobile after a period of strenuous exercise. The mice were put into transparent plexiglass (diameter: 20 cm, height: 30 cm, depth: 15 cm, water temperature: 25±2) for 6 min. The last 4 min immobility time of mice in each group was counted.

Tail suspension test[14]: The tail hanging test is often used for the evaluation of antidepressant drug through measuring the stress of rodents. Mice were suspended in a horizontal rod. 8 min were recorded and analyzed for this test.

Morris test[15]: In the beginning, the water level in the pool is about 1 cm lower than the platform. Randomly drop the mice into the sink from the water entry from different quadrants and train mice finding the visual platform. Next, the platform is placed on the centre of the first quadrant of the water maze for positioning and navigation experiments. Then change the condition of the experiment that the water level in the pool is 1 cm higher than the platform and mice were placed in the water randomly from four different quadrants. The time for the mice to find the platform is recorded. The positioning navigation experiment was continuously performed for 7 days to evaluate the spatial learning ability of these mice. The space exploration experiment was carried out by removing the platform. Mice were placed from the opposite quadrant. Lastly, we also test the spatial memory function of mice by recording the activity time that mice stay in the first quadrant (ie target quadrant) of the original platform and times of mice passing through the original platform position.

Tissue extraction and processing: After the behavioural test, we choose 4 mice from each group randomly for evaluating the morphology and pathology of neural tissues. During the experiment, the whole brain tissue was taken out and sliced for Nissl staining after cardiac perfusion. The other mice from each group were used to evaluated other biochemical markers level. During that experiment, the hippocampus, cortex and striatum were collected. The left hippocampus tissue was used to detect the level of 5-HT and its metabolite 5-HIAA. The remaining hippocampal tissue was kept in liquid nitrogen for qRT-PCR and Western Blot determination.

HPLC-ECD test[16]: Homogenized brain tissue on ice for 5 min with lysis buffer containing 0.1 M perchloric acid, 1.34 mM EDTA-2Na and 0.05% $Na_2S_2O_5$ (v/w10 µL:1 mg), then centrifuged at 20,000 rpm for 10 min, at 4°C. The supernatant was aspirated and neutralized with 2 M KH_2PO_4/K_2HPO_4 solution (pH 7.4) containing 0.01 mg mL^{-1} ascorbate oxidase. The supernatant was further centrifuged at 30,000×g for 10 min, 4°C. 10 µL supernatant was used for sample analysis through Agilent Eclipse Plus C18 reversed-phase chromatography column (25'0.46 cm, 5 µm). The mobile phase is composed by methanol-buffer (16:84, 70 mM KH_2PO_4, 3.1 mM Triethylamine, 0.1 mM EDTA-2Na and 1.05 mM octane sulfonate, pH = 3.02). The flow rate is 1 mL min^{-1} and the column temperature is 35°C. The content of 5-HT and 5-HIAA (expressed in ng mg^{-1} tissue) was collected and calculated.

Immunoblots: Hippocampus tissue (v/w 10 mL:1 mg) for western blot were harvested in RIPA buffer with protease inhibitor (Cell Signaling Technology, USA). The protein samples were separated by 10-15% SDS-PAGE and then transferred to PVDF membranes (Millipore, USA). After blocking with 5% BSA, membranes were incubated with different primary antibody solutions overnight at 4°C. Membranes were then incubated with secondary antibodies for 1 hr at room temperature. Target bands were detected with ECL luminescent solution and quantified with the gel imaging system.

Gene expression analysis: RNA in the hippocampus was extracted by Qiagen RNeasy extraction kit and RNA concentration and quality were determined by a Nanodrop spectrophotometer (Thermo Fisher Scientific, Grand Island, New York, USA). Then RNA was reversed transcription by Applied Biosciences kit (PE Applied Biosystems, Foster City, CA, USA). Gene's expression was quantitated with real-time PCR (ABI Prism 7500 Light Cycler system, PE Applied Biosystems). The primer sequence is as following: BDNF F: CCCTAGCTTGACAAGGCGAA, R: GAAGCAGCTTTCTCAACGCC; 18S RNA F: GTAACCCGTTGAACCCCATT, R: CCATCCAATC GGTAGTAGCG.

Transfection: The PC12 rat pheochromocytoma cells were cultured in F12-K medium containing 2.5% fetal bovine serum and 15% horse serum. Cells were seeded in a 96-well plate (1.0'10^4 cells/100 µL) for 24 hrs, then knocked down BDNF with siRNA for 48 hrs. Either corticosterone (24 hrs incubation) or 10 µM FLX or 10 nM BDNF (2 hrs incubation) was added into these cells. Cell viability was measured by MTT assay.

Statistical analysis: The experimental data are presented as mean±standard deviation (Mean±SD). Two-way analysis of variance (two-way ANOVA) or Tukey's test was performed and considered significant if $p<0.05$.

RESULTS

FLX improves depressive-like behaviours in 3xTg-AD mice during sugar water preference experiment: The reduction preference for sucrose in rodents is closely related to anhedonia and behavioural despair. As shown in Fig. 1a, there is no significant change in sugar water preference between Saline or FLX administration in WT mice. After 3 months and 6 months of the administration,

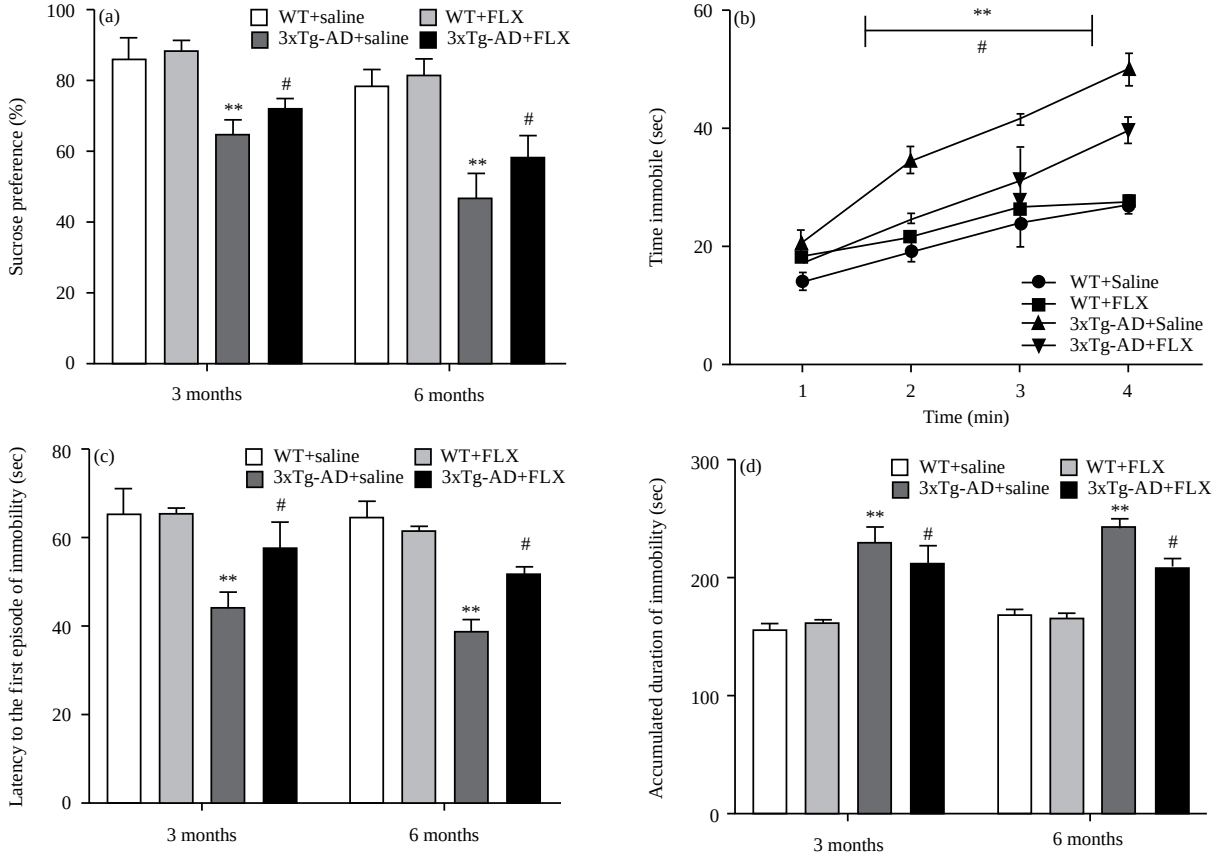

Fig. 1(a-d): FLX improves the depressive-like behavior of 3xTg-AD mice

(a) Percentage of sucrose preference in the sugar water preference experiment, (b) Immobility time of mice in 1 min during forced swimming experiment, (c) Incubation period when the mice appear immobile for the first time in the tail suspension experiment and (d) Immobility time of the mice within 5 min in the tail suspension experiment. **p<0.01 versus WT+Saline group, #p<0.05 versus model group

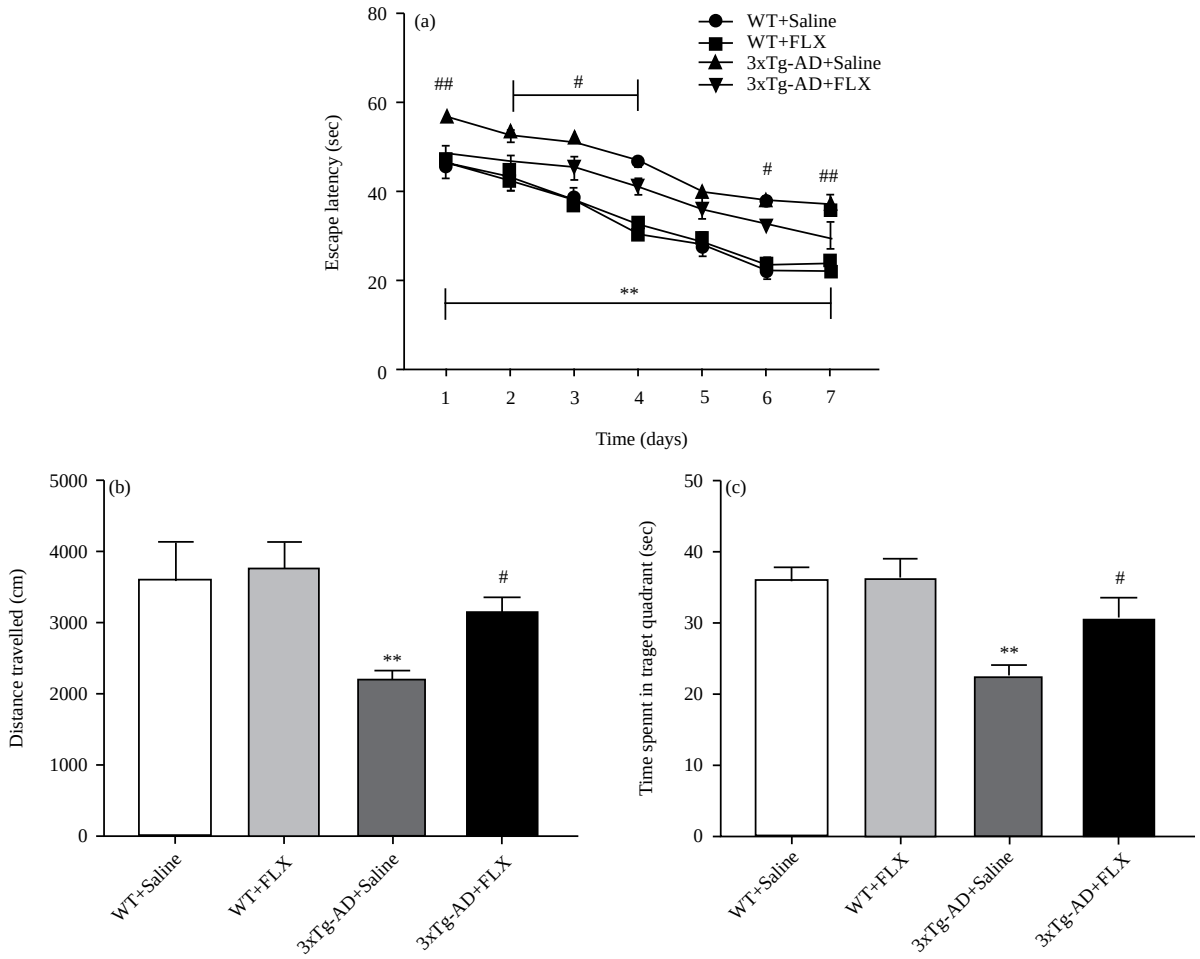

Fig. 2(a-c): FLX improves learning and memory behaviour in 3xTg-AD mice in a water maze experiment

(a) Escape latency period of mice, (b) Movement distance of the mice and (c) Time of the mice in the target quadrant. **p<0.01, versus the WT+Saline group, #p<0.05 and ##p<0.01 versus the model group

the sugar water preference rates in the model group was 65.12 and 47%, respectively, which were significantly lower than those in the WT+Saline group mice (85.75 and 78.63%, p<0.01). After 3 and 6 months of FLX administration, the sugar water preference rate in the FLX treatment group significantly increased to 72.5 and 58.88%, respectively, compared with the model treatment group, which indicates that FLX can alleviate depressive-like behaviour in 3xTg-AD mice.

FLX improves depressive-like behaviour in 3xTg-AD mice during forced swimming experiment: After 6 months of the administration, the immobility time of all mice in the last 4 min of the forced swimming experiment was counted. The results of this experiment are shown in Fig. 1b, during the experiment, the immobility time of mice in the model group is 41.5 sec, 17.5 sec longer than mice in the WT+Saline group (p<0.01). After 6 months' administration, FLX can significantly decline the immobility time in 3xTg-AD mice to 31 sec, (p<0.05), 10.5 sec lower than the immobility time of mice in the model group, which indicates that FLX can alleviate the depression-like behaviour in 3xTg-AD mice. But it didn't show a significant difference in WT mice after FLX administration.

FLX improves depressive-like behaviour in 3xTg-AD mice in tail suspension experiment: The incubation period and cumulative immobility time of mice in each group were shown in Fig. 1c-d. The incubation period of mice in the model group was 11 sec shorted significantly (p<0.01) and the cumulative immobility time was 69 sec increased significantly, compared with mice in the WT+Saline group (p<0.01). After 3 and 6 months of FLX treatment, the immobility latency of

3xTg-AD mice significantly increased to 58 and 52.3 sec ($p<0.05$) respectively. Compared with the 3xTg-AD model group, the cumulative immobility time significantly declined to 214 and 210 sec ($p<0.05$) respectively, which indicates that FLX can alleviate the depressive-like behaviour in the tail suspension experiment.

FLX improves learning and memory behaviour in 3xTg-AD mice in a water maze experiment: The results showed that the time for mice to find a transparent and invisible platform within 60 sec was recorded each day on the positioning and navigation experiment. As shown in Fig. 2a, with the increases of training times, the escape latency of each group of mice gradually decreases. Compared with the WT+Saline group, the incubation period of mice in the model group increased 10 sec and exhibited significantly ($p<0.01$) at the first days. After 6 months of FLX administration, except for the 5th day, the incubation period of 3xTg-AD mice in the FLX treatment group significantly reduced ($p<0.05$). The results of the movement distance of mice in the water maze and the movement time in the first quadrant are shown in Fig. 2b-c. Compared with mice in the WT+Saline group, the movement distance and time in the target quadrant of mice in the model group decreased significantly, was 2200 cm and 22.7 sec separately ($p<0.01$). However, treatment with FLX can significantly increase the movement distance and time of 3x Tg-AD mice (3226 cm and 31 sec separately), compared with mice in the model group ($p<0.05$). But FLX administration didn't show any modification in WT mice.

Effects of FLX on hippocampal neurons in 3xTg-AD mice: The Damage of hippocampal neurons formation and function may be the main cause of depression. The experimental results were shown in Fig. 3(a-b). Compared with mice in the WT+Saline group, the number of hippocampal neurons of mice in the model group declined approximate 50% (102 neurons of the WT+Saline group, 45 neurons of the model group), exhibited significantly ($p<0.01$). After 6 months of FLX treatment, the number of hippocampal neurons in the FLX treatment group increased significantly to 88 in 3xTg-AD mice ($p<0.05$). There is no difference in WT mice after FLX administration.

FLX increases 5-HT and its metabolites in different tissues of 3xTg-AD mice: The content of 5-HT and its metabolites in different tissues was detected by HPLC-ECD. The results are shown in Fig. 4a-b, compared with mice in the WT+Saline group, the content of 5-HT and 5-HT metabolite 5-HIAA in the hippocampus of mice in the model group was 432 and 362 ng g^{-1} respectively ($p<0.01$). As shown in Fig. 4c-d, the content of 5-HT and 5-HIAA in the cortex of mice in the model group was 307 and 124 ng g^{-1}, respectively ($p<0.01$). As shown in Fig. 4e-f, the content of 5-HT and 5-HIAA in the striatum of mice in the model group was 265 and 128 ng g^{-1} respectively ($p<0.01$). After 6 months of FLX treatment in 3xTg-AD, the content of 5-HT in different tissues increased to 495, 369 and 321 ng g^{-1} separately. The content of 5 HIAA similarly increased to 431, 186 and 159 ng g^{-1} separately, compared with a sample of mice in the model group, exhibited significantly difference ($p<0.05$). There is no difference before and after FLX treatment in WT mice.

FLX increases the expression of BDNF, p-TrkB and p-CREB in the hippocampus of 3xTg-AD mice: As shown in Fig. 5a, western Blot analysis revealed that the expression of BDNF, p-TrkB and p-CREB in the hippocampus of mice in the model group reduced significantly compared with that of the WT+Saline group. FLX can approximate 37% increase the expression of 5-HT_{2A}R in hippocampus (Fig. 5b). As shown in Fig. 5c-g, FLX also can promote the expression of p-ERK1/2, Camk, p-CREB, BDNF and p-TrkB significantly in 3xTg-AD mice by approximately 29, 25, 295, 120 and 25 separately. FLX exhibited no effect on the expression of p-ERK1/2, Camk, p-CREB, BDNF and p-TrkB in the hippocampus in WT mice compared with Saline administration. Meanwhile, FLX can increase BDNF and 5-HT_{2A}R mRNA content in hippocampus by 47.5 and 59% (Fig. 5h-i).

FLX protects PC12 cells from apoptosis by increasing BDNF: To further verify whether FLX protects neurons by increasing BDNF, a corticosterone (Cort)-induced PC12 model was used. In Fig. 6a, after transfection with BDNF siRNA, the protein expression level of BDNF decreased almost 98%. In Fig. 6b, Cort can significantly reduce cell survival rate 47% ($p<0.01$) at 200 mM, as compared with Ctrl. Compared with the Cort group, FLX and BDNF can significantly increase the cell survival rate by approximately 27 and 25%, respectively ($p<0.05$). More importantly, after knocked down BDNF, the cytoprotective effect of FLX was significantly reduced by 19% than FLX treated group ($p<0.05$).

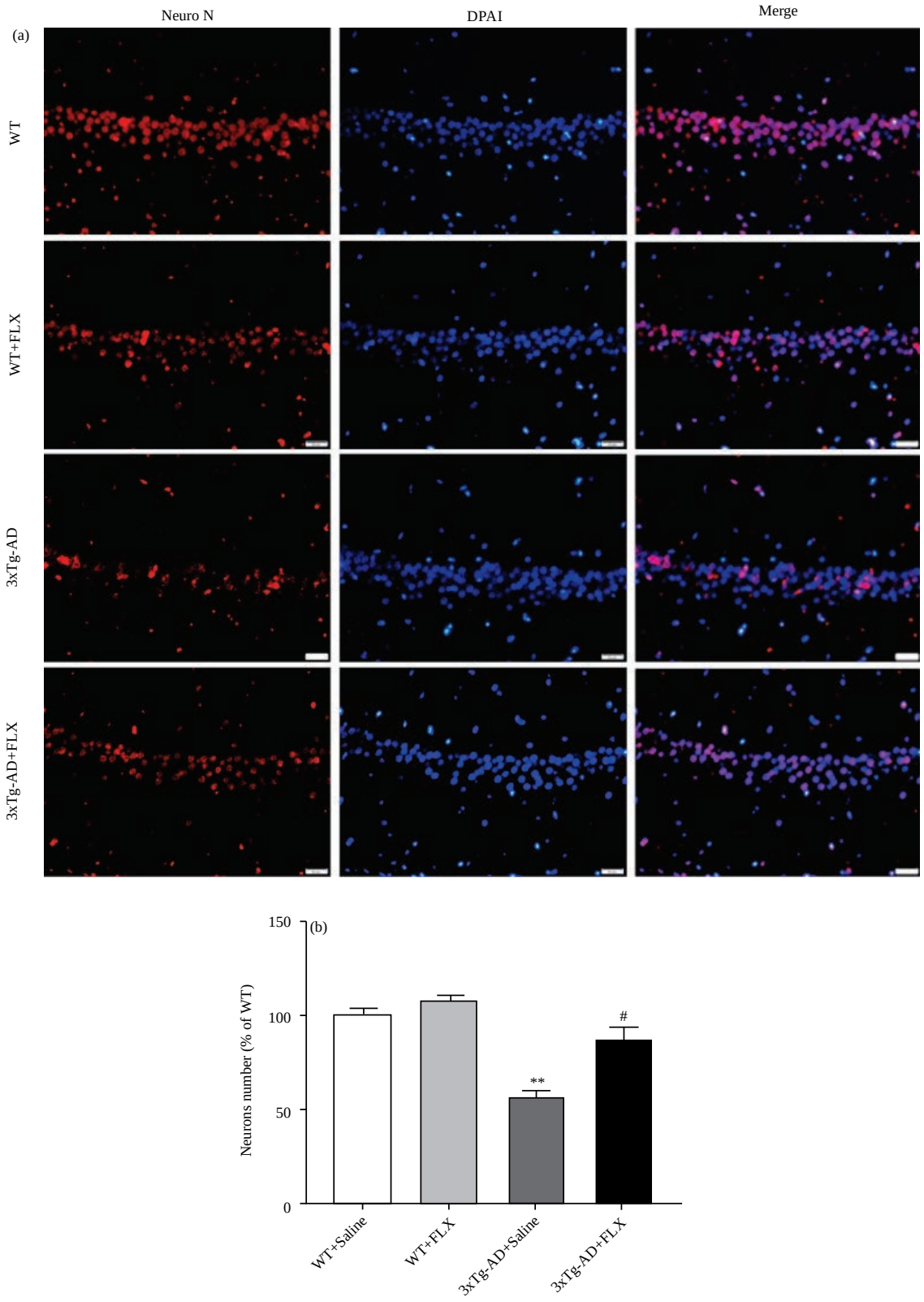

Fig. 3(a-b): FLX increases the number of hippocampal neurons in 3xTg-AD mice

(a) NeuroN staining of hippocampal neurons and (b) Hippocampal neuron number. **p<0.01 versus WT+Saline group, #p<0.05 versus the model group

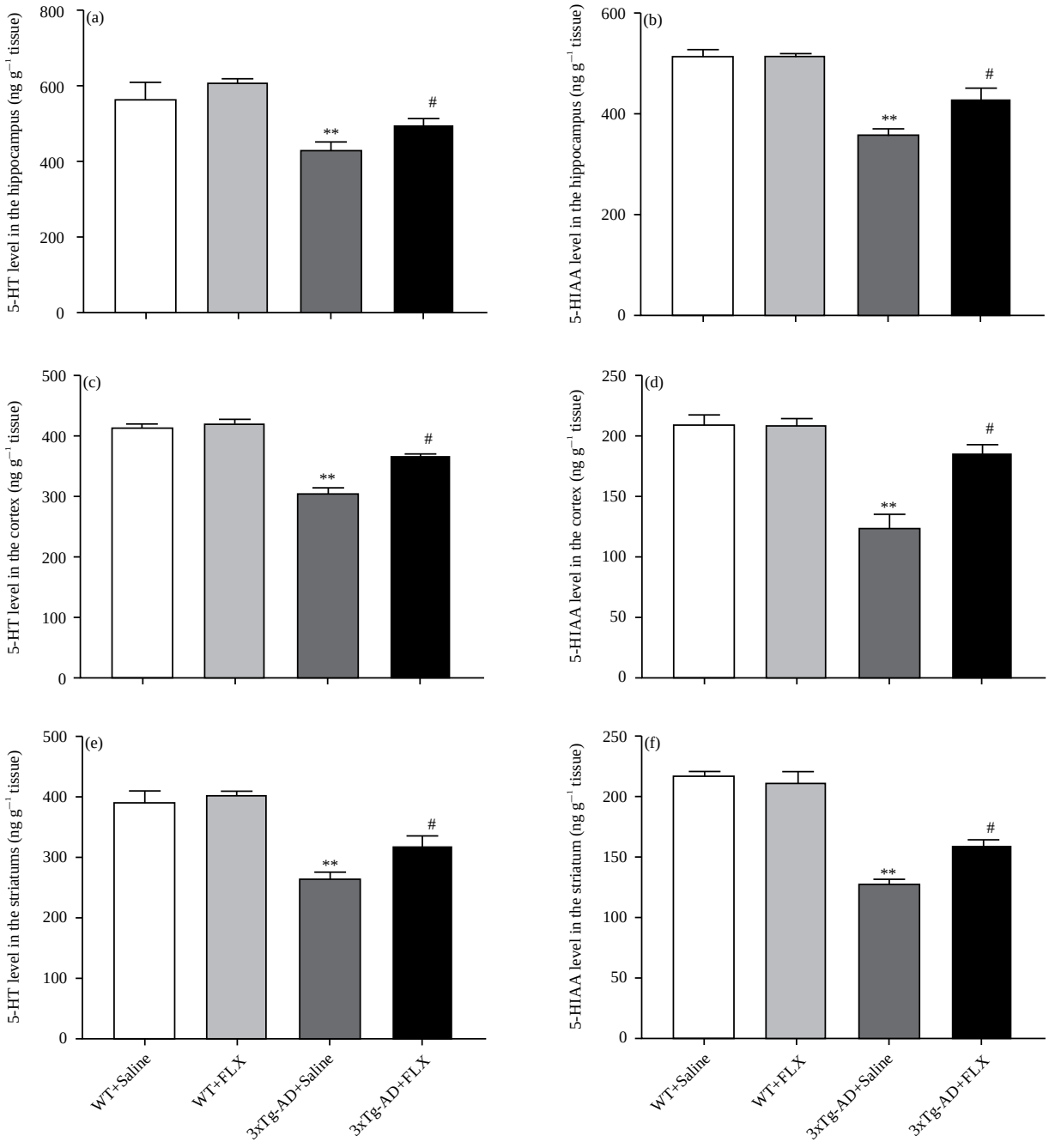

Fig. 4(a-f): FLX increases the content of 5-HT and 5-HIAA in the hippocampus, cortex and striatum of 3xTg-AD mice

(a) 5-HT content in the hippocampus, (b) 5-HIAA content in the hippocampus, (c) 5-HT content in the cortex, (d) 5-HIAA content in the cortex, (e) 5-HT content in the striatum and (f) 5-HIAA content in the striatum. **p<0.01 versus WT+Saline group, #p<0.05 versus model group

DISCUSSION

In this study, FLX can improve the depression behaviour and cognitive deficits of mice in the AD model by activating CREB/BDNF/TrkB pathway. Our research also indicates that FLX reduced the damage of hippocampal neurons.

Depression is one of the major diseases threatening human health with a high risk of suicide[17]. Depression is a prodromal symptom of AD and an independent high risk of early-stage AD and late-stage AD. Depression may also accelerate brain function degeneration, including DNA methylation, high cortisol/DHEA(S) ratio[18].

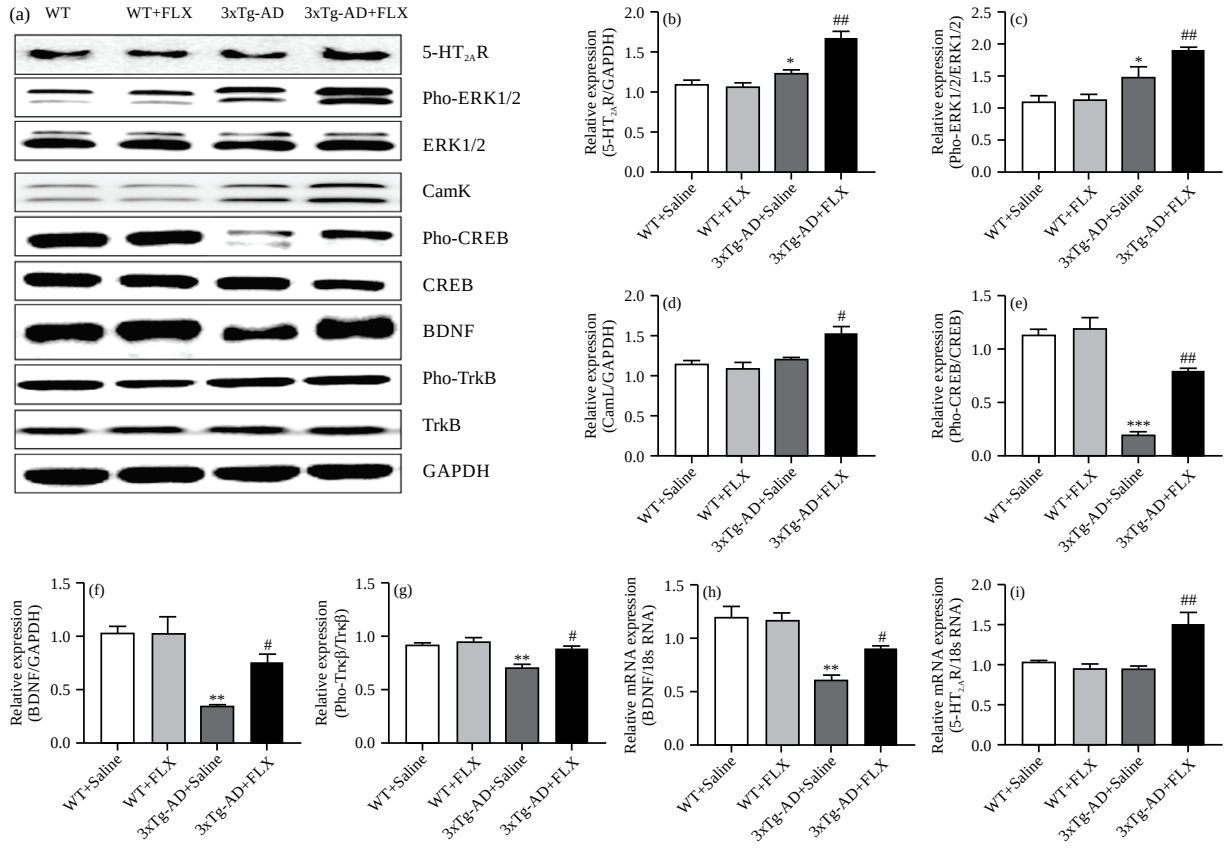

Fig. 5(a-i): FLX activates the Pho-CREB/BDNF/Pho-TrkB pathway in the hippocampus of 3xTg-AD mice

(a) Representative protein bands, (b-g) Protein gray value statistical graph, (h) BDNF mRNA content and (i) 5-HT_{2A}R mRNA content. *p<0.05, **p<0.01, ***p<0.001 versus WT+Saline group, #p<0.05 and ##p<0.01 versus the model group

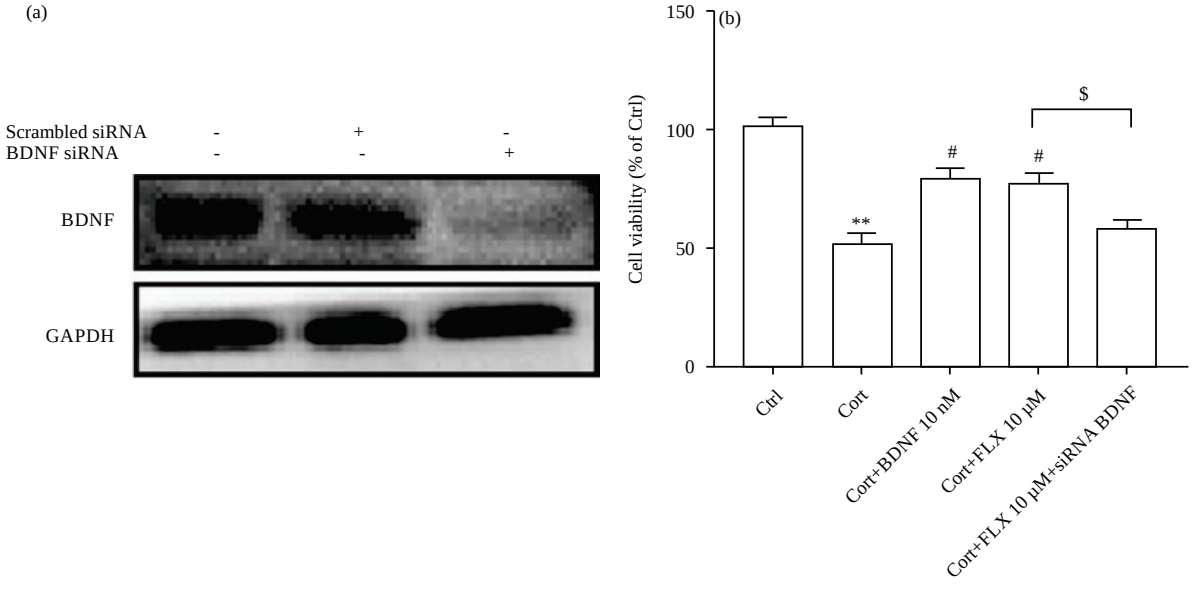

Fig. 6(a-b): FLX protects PC12 cells from apoptosis by increasing BDNF

(a) BDNF knock-down protein band and (b) Cell viability statistics. **p<0.01 versus Ctrl group, #p<0.05 versus Cort group; $p<0.05 versus Cort+FLX group

FLX is used to relieve depression and anxiety in patients as a selective 5-HT reuptake inhibitor. Studies have shown that symptom in AD patients always together with depression and anxiety. But there is still a lack of sufficient evidence for the relationship between FLX treatment for depression and improvement of AD. Studies have shown that FLX treatment in late-stage AD patients has not achieved a positive effect. At age 6.5 months, 3xTg mice displayed learning and memory deficits in the Barnes maze[19]. Another study showed cognitive impairment manifests at 4 months as a deficit in long-term retention and correlates with the accumulation of intraneuronal Aβ in the hippocampus and amygdala, but plaques and tangles are not yet apparent[20]. Therefore, we choose 3 months old 3×Tg-AD mice to evaluate the neuroprotective potential of FLX. FLX can significantly improve depressive, learning and memory behaviour. A decreased level of brain 5-HT has been theorized to be a core pathogenic factor in depression. The theory arose from clinical observations that drugs enhancing extracellular levels of 5-HT have antidepressant effects in many AD patients. In this study, we found that FLX increased 5-HT and its metabolites in 3xTg-AD mice brain.

The damage of neurons in the hippocampus under stimulation by a variety of factors will affect the plasticity of hippocampal synapses and cause depression[21,22]. BDNF is one of the important members of brain-derived neurotrophic factors which can bind to the specific receptor TrkB, then activates the MEK/ERK signalling pathway to promote the phosphorylation of CREB. CREB plays an important role in the survival and differentiation of neurons, which binds to the promoter of the BDNF gene, thus increase BDNF expression and plays a neuroprotective effect[23,24]. Relevant studies have shown that the content of BDNF in the hippocampus of patients with depression is much lower than that of normal people[25]. This study illustrates that FLX can better treat the cognitive impairment of AD and can treat the depression of early AD at the same time. It provides a research direction for the follow-up development of drugs for the treatment of AD. However, it is difficult to evaluate the behaviour improvement of AD and more suitable models *in vivo* are needed to evaluate the neuroprotective effect of fluoxetine.

CONCLUSION

In this experiment, we showed that FLX effectively reduced the damage of hippocampal neurons. Western Blot and qRT-PCR results showed that FLX significantly increased p-CREB, BDNF and p-TrkB level in the hippocampus. The protein and mRNA expression increase significantly indicated that FLX activated CREB/BDNF/TrkB pathway in the hippocampus of 3×Tg-AD mice. More importantly, FLX neuroprotection decreased after knocking down BDNF in Cort induced PC12 cells. These findings indicated that FLX may inhibit depression and anxiety in early-stage AD patients through activation of CREB/BDNF/TrkB.

SIGNIFICANCE STATEMENT

This study discovers that FLX can not only treat depressive behaviour in early AD but also protect hippocampal neurons to treat cognitive impairment in AD. The research will help researchers develop drugs to treat early AD, which is a difficult problem in the treatment of neurological diseases.

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REFERENCES

1. Stringaris, A., 2017. Editorial: What is depression? J. Child Psychol. Psychiatry, 58: 1287-1289.
2. Malhi, G.S. and J.J. Mann, 2018. Depression. Lancet, 392: 2299-2312.
3. Lyketsos, C.G. and J. Olin, 2002. Depression in Alzheimer's disease: Overview and treatment. Biol. Psychiatry, 52: 243-252.
4. Tsuno, N. and A. Homma, 2009. What is the association between depression and Alzheimer's disease? Expert Rev. Neurotherapeutics, 9: 1667-1676.
5. Caraci, F., A. Copani, F. Nicoletti and F. Drago, 2010. Depression and Alzheimer's disease: Neurobiological links and common pharmacological targets. Eur. J. Pharmacol., 626: 64-71.
6. Gualtieri, C.T. and L.G. Johnson, 2008. Age-related cognitive decline in patients with mood disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, 32: 962-967.
7. Jiang, H., H. Yin, L. Wang, C. Feng and Y. Bai *et al*., 2020. Memory impairment of chewing-side preference mice is associated with 5-HT-BDNF signal pathway. Mol. Cell. Biochem., 476: 303-310.
8. Angelucci, F., S. Brenè and A.A. Mathé, 2005. BDNF in schizophrenia, depression and corresponding animal models. Mol. Psychiatry, 10: 345-352.
9. Duman, C.H., L. Schlesinger, M. Kodama, D.S. Russell and R.S. Duman, 2007. A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. Biol. Psychiatry, 61: 661-670.

10. Rantamäki, T. and E. Castrén, 2008. Targeting TrkB neurotrophin receptor to treat depression. Expert Opin. Ther. Targets, 12: 705-715.
11. Wang, J., Y. Zhang, H. Xu, S. Zhu and H. Wang *et al*., 2014. Fluoxetine improves behavioral performance by suppressing the production of soluble β-amyloid in APP/PS1 mice. Curr. Alzheimer Res., 11: 672-680.
12. Liu, M.Y., C.Y. Yin, L.J. Zhu, X.H. Zhu and C. Xu *et al*., 2018. Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat. Protoc., 13: 1686-1698.
13. Yankelevitch-Yahav, R., M. Franko, A. Huly and R. Doron, 2015. The forced swim test as a model of depressive-like behavior. J. Visualized Exp., Vol. 2. 10.3791/52587.
14. Cryan, J.F., C. Mombereau and A. Vassout, 2005. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. Neurosci. Biobehav. Rev., 29: 571-625.
15. Vorhees, C.V. and M.T. Williams, 2006. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nature Protocols, 1: 848-858.
16. Renoir, T., M.S. Zajac, X. Du, T.Y. Pang and L. Leang *et al*., 2011. Sexually dimorphic serotonergic dysfunction in a mouse model of Huntington's disease and depression. PLoS ONE, Vol. 6. 10.1371/journal.pone.0022133.
17. Vilagut, G., C.G. Forero, G. Barbaglia and J. Alonso, 2016. Screening for depression in the general population with the center for epidemiologic studies depression (CES-D): A systematic review with meta-analysis. PLoS ONE, Vol. 11. 10.1371/journal.pone.0155431.
18. Herbert, J. and P.J. Lucassen, 2016. Depression as a risk factor for Alzheimer's disease: Genes, steroids, cytokines and neurogenesis-what do we need to know? Front. Neuroendocrinol., 41: 153-171.
19. Stover, K.R., M.A. Campbell, C.M.V. Winssen and R.E. Brown, 2015. Early detection of cognitive deficits in the 3xTg-AD mouse model of Alzheimer's disease. Behav. Brain Res., 289: 29-38.
20. Billings, L.M., S. Oddo, K.N. Green, J.L. McGaugh and F.M. LaFerla, 2005. Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron, 45: 675-688.
21. Kesner, R.P., I. Lee and P. Gilbert, 2004. A behavioral assessment of hippocampal function based on a subregional analysis. Rev. Neurosci., 15: 333-351.
22. Micheli, L., M. Ceccarelli, G. D'Andrea and F. Tirone, 2018. Depression and adult neurogenesis: Positive effects of the antidepressant fluoxetine and of physical exercise. Brain Res. Bull., 143: 181-193.
23. Lin, P.Y., E.T. Kavalali and L.M. Monteggia, 2018. Genetic dissection of presynaptic and postsynaptic BDNF-TrkB signaling in synaptic efficacy of CA3-CA1 synapses. Cell Rep., 24: 1550-1561.
24. Zhang, S.S., Y.H. Tian, S.J. Jin, W.C. Wang and J.X. Zhao *et al*., 2019. Isoflurane produces antidepressant effects inducing BDNF-TrkB signaling in CUMS mice. Psychopharmacology, 236: 3301-3315.
25. Jiang, H., S. Chen, C. Li, N. Lu and Y. Yue *et al*., 2017. The serum protein levels of the tPA-BDNF pathway are implicated in depression and antidepressant treatment. Transl. Psychiatry, Vol. 7. 10.1038/tp.2017.43.