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Baicalin reverse AMPA receptor expression and neuron apoptosis in chronic unpredictable mild stress rats

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ABSTRACT

Changes of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expression could impacts the viability of neurons and brain levels of brain-derived neurotrophic factor (BDNF) expression in the key brain structures in the pathophysiology of depressive disorder. In the present study, chronic unpredictable mild stress (CUMS) degraded performance decreased AMPA receptor expression and increased neuron apoptosis. Treatment with baicalin (20, 40 mg/kg) significantly reversed these changes. This study demonstrates that baicalin has potent antidepressant effect, likely through up-regulated the expression of AMPA receptor and suppression neuron apoptosis in CUMS-treated rats.

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1. Introduction

Depression and stress-related mental disorders result in significant personal, economic, and social burdens [1–3]. The mechanism underlying depression remains unclear, but likely involves molecular and cellular abnormalities that interact with genetic and environmental factors [4]. Experimental studies indicated that the extent of apoptosis was increased in the hippocampus in the depression-like model, and this condition could be ameliorated by antidepressant treatment [5,6].

Recently, a growing body evidence confirmed that AMPA receptors is crucial in synaptic plasticity and cell death associated with neurological diseases and depression [7–11]. AMPA receptor are expressed throughout the central nervous system, containing GluR1, GluR2, GluR3 and GluR4 subunits [12]. The predominantly expressed subunits are GluR1 and GluR2 in hippocampus, and GluR2 subunit was the important of AMPA receptor in hippocampus [12]. AMPA receptors are largely Ca^{2+} impermeable due to the presence of the GluR2 subunit, which prevents Ca^{2+} permeability and only allows Na^{+} influx [11]. A nascent literature suggests that exposure of neurons to excitotoxicity levels of glutamate leads to decrease or loss of the GluR2 subunit of AMPA receptors, which will further result in high Ca^{2+} influx and excitotoxic neuronal death. Under physiological conditions upon high frequency stimulation, the Ca^{2+} influx through Ca^{2+} permeable AMPA receptors triggers a change in the composition of AMPA receptors by inducing the synthesis of GluR2, thereby decreasing the Ca^{2+} influx and preventing excitotoxicity [12,13]. Thus, in these cells, the loss of GluR2 lead to selective vulnerability to neuronal insults [14].

Furthermore, accumulating evidence has indicated that excessive glucocorticoid hormones resulted in the degradation of AMPA receptor [15]. Otherwise, Previous studies showing that AMPA receptor activation induce BDNF expression and against neonatal apoptosis [16–18]. The changes of AMPA receptor might therefore be one of the pathogenetic factors involved in the development of experimental and clinical depression [14].

Baicalin, a major polyphenol components isolated from *Scutellaria* roots, was found to be the main active ingredient responsible for the CNS-related activities of *Scutellariae radix*, including promoting neural differentiation [19] and protecting against traumatic seizures [20–22]. Studies in the chronic mild stress rat model demonstrated that these effects of baicalin might be dependent on the activation of Erk1/2, or on inhibition of cyclooxygenase-2 [23,24]. Based on this foundation, the study has been designed to testify the relationship apoptosis and the antidepressant mechanism of baicalin.

2. Materials and methods

2.1. Animals

Male SD rats weighing 180–220 g were purchased from Experimental Animal Center in Jiangsu Province (Nanjing, China). Ani-

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mals were allowed to adapt one week before the experiment started. The animals were randomly housed in cages under experimental conditions of room temperature (25 ± 2 °C), light (12-h light/dark cycle, lights on at 7:00 a.m.) and with free access to food and water. All animal experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.2. Drugs and reagents

Baicalin (purity > 98%) was purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, PR China), and Fluoxetine hydrochloride was from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, PR China). All primers used in this study were synthesised by Biosky Biotech Co., Ltd. (Nanjing, PR China), and all reagents used in RT-qPCR were from Roche (Nanjing, PR China). All other chemicals and reagents were of analytical grade.

2.3. Unpredictable chronic mild stress procedure

The CUMS procedure was slightly modified from that previously described by Willner et al. [44]. Briefly, the stressed groups were subjected to the following stressors for 6 weeks: food and water deprivation; stroboscopic illumination (120 flashes/min, 1 h); white noise, overnight illumination, group housing; 45° cage tilt, soiled cage overnight (300 mL of water into the bedding). The control rats had no contact with the stressed animals. All stressors were applied random and individually. These rats were deprived of food and water for 12 h preceding each sucrose test. The CUMS procedure was last for 6 weeks. Drugs were given orally once a day between 8:00 and 9:00 a.m.

2.4. Sucrose preference

All rats were given a 1% (w/v) sucrose solution for 24 h. Then, both sucrose solution and fresh water were made accessible to the rats for another 24 h. All animals were then food- and water-deprived for 23 h, then given access to both water and 1% sucrose solution for 1 h. Sucrose preference was calculated as sucrose preference (%) = sucrose intake (g)/[sucrose intake (g) + water intake (g)] × 100.

2.5. Blood and brain tissues sample collection

After the last behavioral tests, eight rats from each group were decapitated. Blood samples were collected immediately. The whole hippocampus was rapidly dissected on an ice-plate and weighed. The hippocampus was identified as described in Paxinos and Watson's atlas [45].

2.6. Serum corticosterone level determination

According to Frankel's method [25], 0.2 mL serum was taken from each sample and 2 mL of 0.1 mol/L NaOH added, then mixed for 30 s. Dichloromethane (2 mL) was added to the mixture, swirled for 3 min, and then incubated for 5 min at room temperature. After centrifuging at 2500 r/min for 20 min, 1.5 mL of the organic phase was drawn into another graduated centrifuge tube. Sulphuric acid–alcohol (concentrated sulphuric acid: 98% ethanol at a 7:3 ratio) was then added for colorimetry. The solution was vortexed for 3 min, centrifuged at 2500 r/min for 20 min, and the dichloromethane phase discarded. The aqueous phase was allowed to stand for 30 min, and fluorescence intensity was measured using these parameters: excitation wavelength, 472 nm; emission wavelength, 519 nm.

2.7. Brain slice collection and immunohistochemistry of proteins

Three rats from each group were utilized for histology. Brains were removed and quickly steeped in 4% paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4). After 24 h, the brains were dehydrated and embedded in paraffin blocks. Sections of 4 μm were cut and rehydrated. Endogenous peroxidase was quenched, and tissue blocked with normal goat serum. Sections were then incubated with anti-bax (1:200, Cell Singnaling Technology), anti-bcl-2 (1:200, Cell Singnaling Technology), anti-caspase-3 (1:200, Cell Singnaling Technology), anti-BDNF (1:200, Cell Singnaling Technology) antibodies overnight at 4 °C. After washing with PBS, the sections were incubated with the appropriate secondary antibodies biotinylated goat anti-rabbit IgG (Cell Singnaling Technology), respectively, for 2 h at 37 °C. Finally, sections were visualized using 3,3'-diaminobenzidine solution (DAB). The staining of caspase-3 proteins was measured densitometrically using Image Pro Plus software (IPP 6.0, Media Cybernetics). Brown DAB staining was considered positive staining, and the integrated optical density (IOD) of the brown label represented the expression level of bax, bcl-2, caspase-3 and BDNF proteins.

2.8. Hippocampus GluR2 mRNA levels determination

Briefly, Total RNA was isolated from tissue using Trizol reagent according to the manufacturer's instructions. All reaction was amplified by quantitative real-time PCR (Bio-Rad, USA). Cycling conditions were as follows: step 1, 1 cycles 180 s at 94 °C; step 2, 15 s at 94 °C; step 3, 40 cycles at 62 °CC for 40 s; step 4, dissociation stage. Data were collected and analyzed by the computer software. Relative quantification of gene expression was calculated using $2^{-\Delta\Delta Ct}$ data analysis method. The sequences of primers used in this experiment are summarized as follow: GluR2, F 5'-ATCTGGGATTCACTGATGGGGAC-3', and R 5'-TTTAGACACCAGGGAATCGTCGTAG-3'; GAPDH, F 5'-ATTCAACGGCACAGTCAAGG-3' and R 5'-GCAGAAGGGGCGGAGATGA-3'.

2.9. Statistical analyses

Data were expressed as means ± S.D. Statistical evaluation of the differences among the groups was performed using ANOVA. Significant differences among experimental groups were accepted at $p < 0.05$.

3. Results

3.1. Effect of baicalin on sucrose preference

Sucrose preference was a measure of anhedonia. As shown in Fig. S1, model rats had significantly reduced sucrose consumption in comparison with the sham group ($p < 0.01$). Fluoxetine (10 mg/kg) and baicalin (20 and 40 mg/kg) dramatically reversed the lower sucrose consumption in the model group ($p < 0.01$).

3.2. Effects of baicalin on serum corticosterone levels

Hypothalamic–pituitary–adrenal (HPA) axis dysfunction was assessed through determination of serum corticosterone levels. As shown in Fig. 1, serum corticosterone levels in the model group were significantly increased versus the sham group ($p < 0.01$). Chronic administration with fluoxetine (10 mg/kg, $p < 0.01$) and baicalin (20 and 40 mg/kg) significantly attenuated serum corticosterone levels ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively).

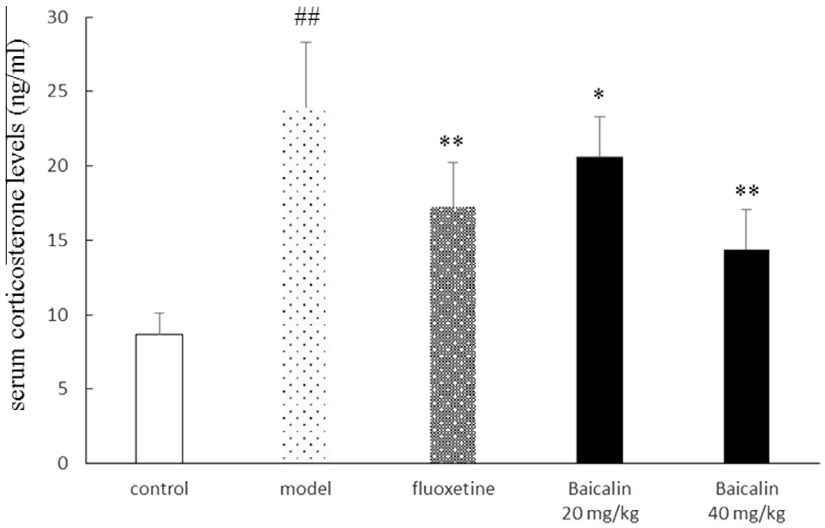

Fig. 1. Effect of baicalin on serum corticosterone levels. $^{\#\#}p < 0.01$ compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with model group.

3.3. Effect of baicalin on the expression of bcl-2, bax and caspase-3 proteins in hippocampus

Fig. 2 summarizes the putative anti-apoptosis effect of baicalin in the CUMS rat. Fluoxetine (10 mg/kg, $p < 0.01$) and baicalin (20 mg/kg and 40 mg/kg, $p < 0.05$) treatment significantly increased the expression of caspase-3 in the CUMS rat. In the hippocampus of the model rat, bax expression were obviously increased compared to the control group. This increase was reduced by administration of fluoxetine at 10 mg/kg ($p < 0.01$),

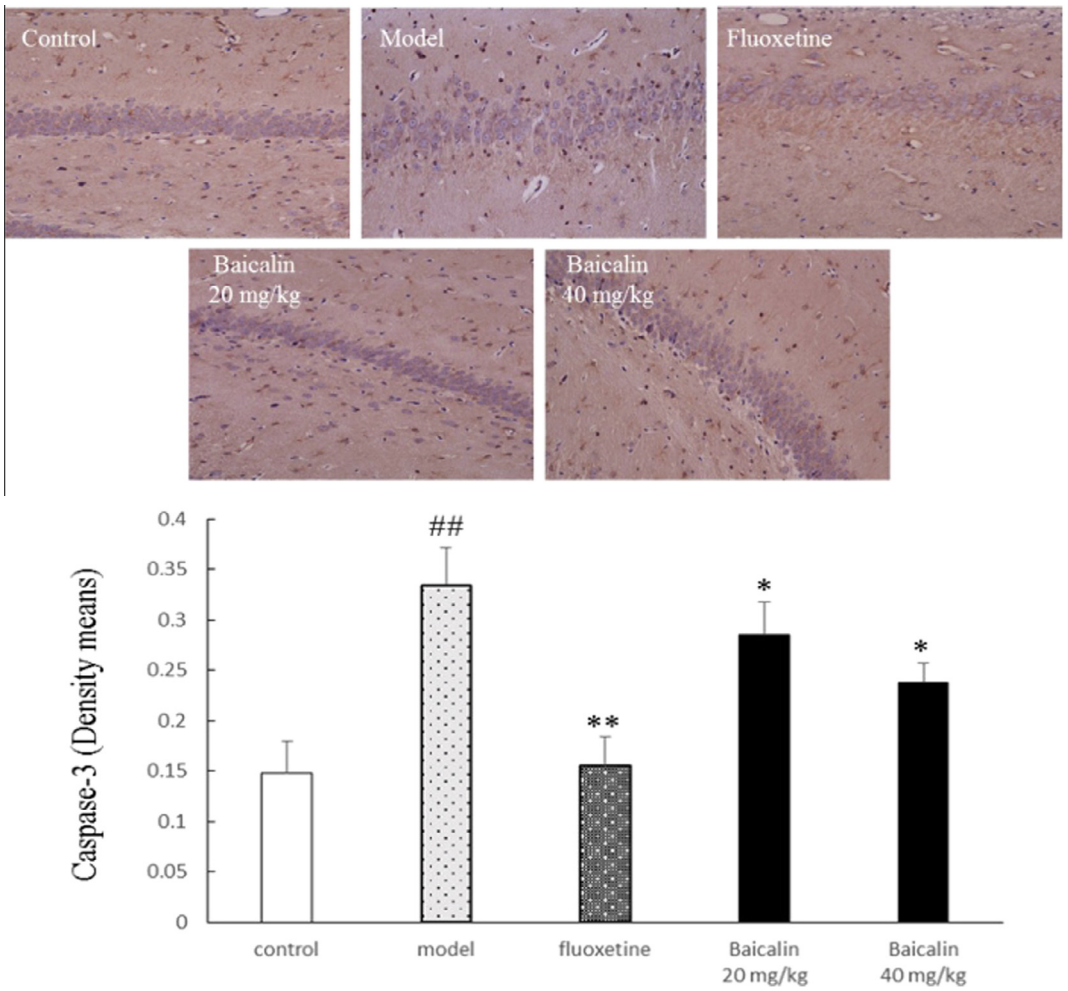

Fig. 2. Effect of baicalin on caspase-3 expression in hippocampus. $^{\#\#}p < 0.01$ compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with model group.

and baicalin at doses of 20 mg/kg and 40 mg/kg ($p < 0.05$, Fig. S2A). Similarly, the levels of bcl-2 were significantly higher in model rats compared to the sham group, and they were significantly attenuated by fluoxetine 10 mg/kg ($p < 0.01$) and baicalin 20 mg/kg and 40 mg/kg treatment ($p < 0.01$, Fig. S2B).

3.4. Effect of baicalin on the expression of BDNF proteins in hippocampus

In the hippocampus of the model rat, BDNF expression were obviously decreased compared to the control group. This increase

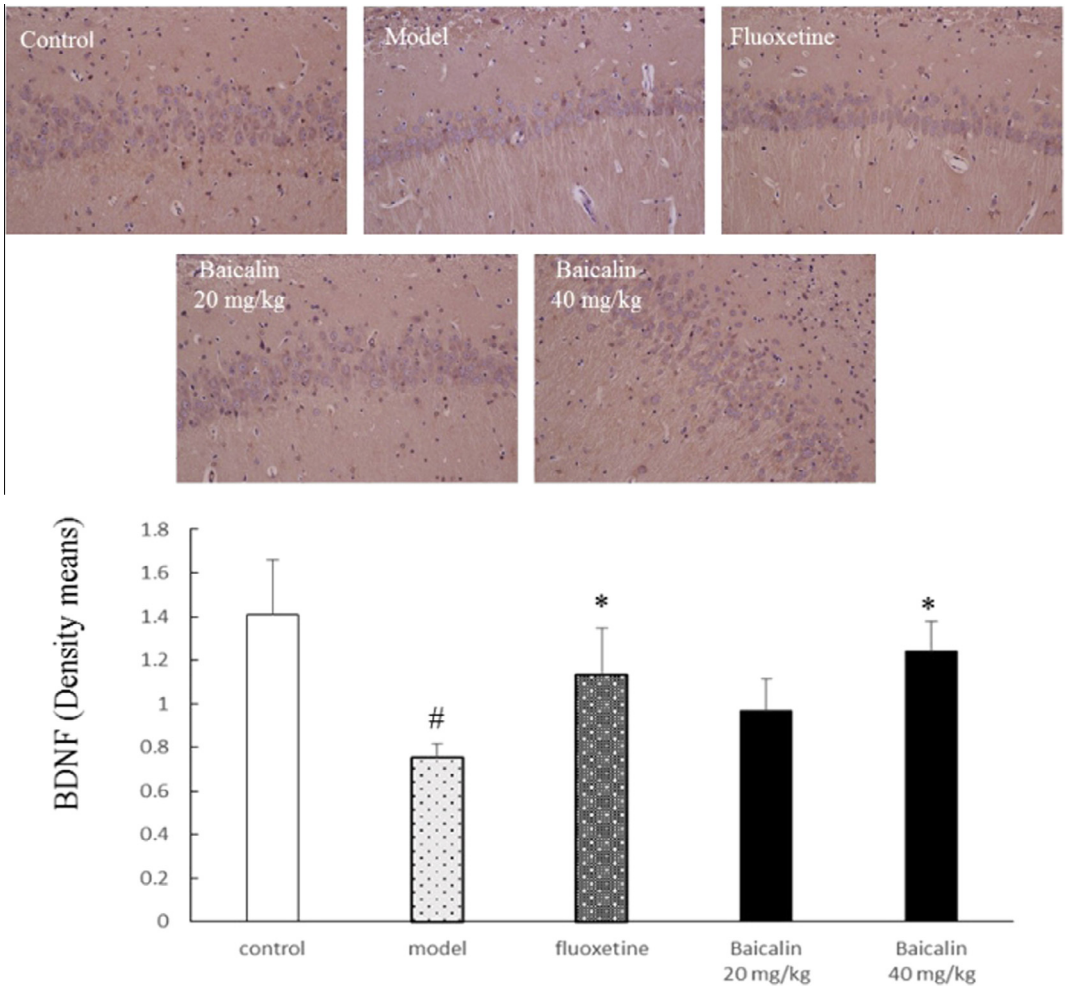

Fig. 3. Effect of baicalin on BDNF expression in hippocampus. $^{\#\#}p < 0.01$ compared with control group; $^{**}p < 0.05$ compared with model group.

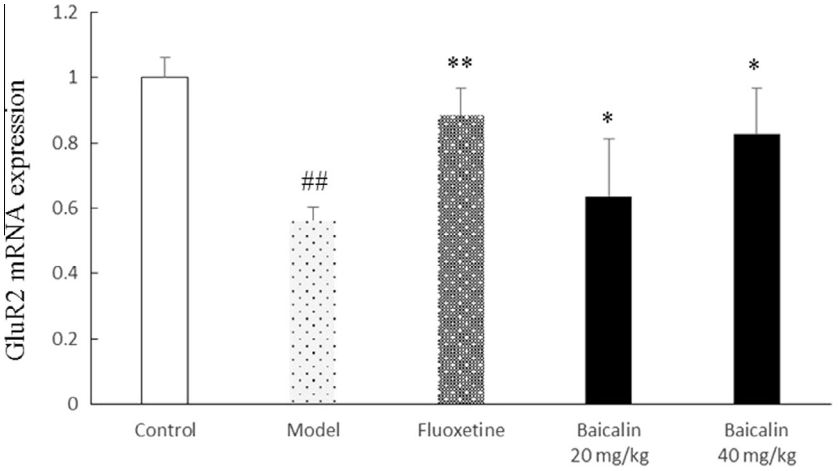

Fig. 4. Effect of baicalin on GluR2 mRNA in hippocampus. $^{\#\#}p < 0.01$ compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with model group.

was reduced by administration of fluoxetine at 10 mg/kg ($p < 0.05$), and baicalin at doses of 40 mg/kg ($p < 0.05$) (Fig. 3).

3.5. Effect of baicalin on the expression of GluR2 mRNA

As seen in Fig. 4, GluR2 expression was decreased about 1-fold in the model rats versus the control group ($p < 0.01$), but was induced by fluoxetine (10 mg/kg; $p < 0.01$) and baicalin (20 and 40 mg/kg; $p < 0.05$).

4. Discussion

Preclinical and clinical studies demonstrate that depression is associated with decreased hippocampal volume and increased neuronal deficits [26]. Previous studies have indicated that AMPA receptors mediated critical pathophysiological processes including neurotransmission, memory, neuron apoptosis and stress resistance [7]. This study evaluated whether baicalin can ameliorate CUMS-induced hippocampal neuron apoptosis and tested one possible mechanism, AMPA receptor related apoptosis, in the CUMS rat model.

CUMS rats model, a well validated animal model of depression. CUMS results in complex alterations in behavior, and biochemical and cellular cascades (e.g. immune, endocrine) whose feature is similar with those in patients with major depression [27]. Our model rats exhibited decreased sucrose preference index, and increased immobility time during the FST. These findings conform to previous CUMS rat studies [28]. Notably, our data demonstrated that CUMS-related changes could be restored by chronic baicalin treatment, confirming its antidepressant-like effect in this model.

Well-known, glucocorticoids could regulate neuronal excitability, memory acquisition, and neurogenesis [29]. Conversely, excessive glucocorticoids are associated with impairments in the depressed brain [30–32]. Several studies found that chronic stress stimulated HPA axis activity and raised blood corticosterone (analogous to human cortisol) levels in rats [33,34]. Here, CUMS significantly elevated serum corticosterone, indicative of HPA axis hyperactivity [35]. However, chronic administration of baicalin reduced serum corticosterone levels in the CUMS rats. These results conform to previous results showing that chronic treatment with baicalin attenuated stress-associated serum corticosterone level increases [36].

Excessive glucocorticoid hormones also resulted in AMPA receptor degradation [15]. Experimental studies indicated that the decreased level of GluR2 was found in patients with major depressive disorder and bipolar disorder [11,37]. Chronic administration of baicalin could up-regulated the expression of GluR2 subunit in hippocampus in CUMS-treated mice, which is consistent with the previous reports on the changes of AMPA receptor after treatment with antidepressant [38].

Alteration in the level of expression of GluR2 is expected to have profound implications for neuronal survival. Indeed, previous studies have demonstrated that positive modulators of AMPA receptors have received increasing attention as potential neuroprotective agents [39]. For example, activation AMPA receptor could modulates stress-induced transcription of BDNF and suppression neuronal apoptosis in rat hippocampus [39,40]. Otherwise, chronic antidepressant treatments could up-regulation AMPA receptor expression, and the anti-apoptotic effect of antidepressants was also noted in a rat depression model [41,42]. Similarly, we noted that CUMS contributed to hippocampal apoptosis in a way which could be alleviated by baicalin and fluoxetine. In the present research, CUMS results in increased the expression of bax and caspase-3 and decreased the expression of bcl-2. With chronic administration of baicalin were significant diminished the expression of bax and casapse-3 and up-regulated bcl-2 expression. Additionally, previous studies have demonstrated that the decrease expression of BDNF is responsible for apoptosis. Chronic administration of baicalin induced the expression of BDNF in CUMS rats, similarly to other antidepressants [43]. This suggests that the mechanism of baicalin's antidepressant effect is through up-regulate AMPA receptor expression and opposing apoptosis.

In conclusion, the present study demonstrates that: Baicalin elicits an antidepressant effect through (1) re-regulating abnormal glucocorticoid levels; (2) reversal of hippocampal AMPA receptor and BDNF expression induced by CUMS; (3) inhibiting neuroapoptosis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.041.

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