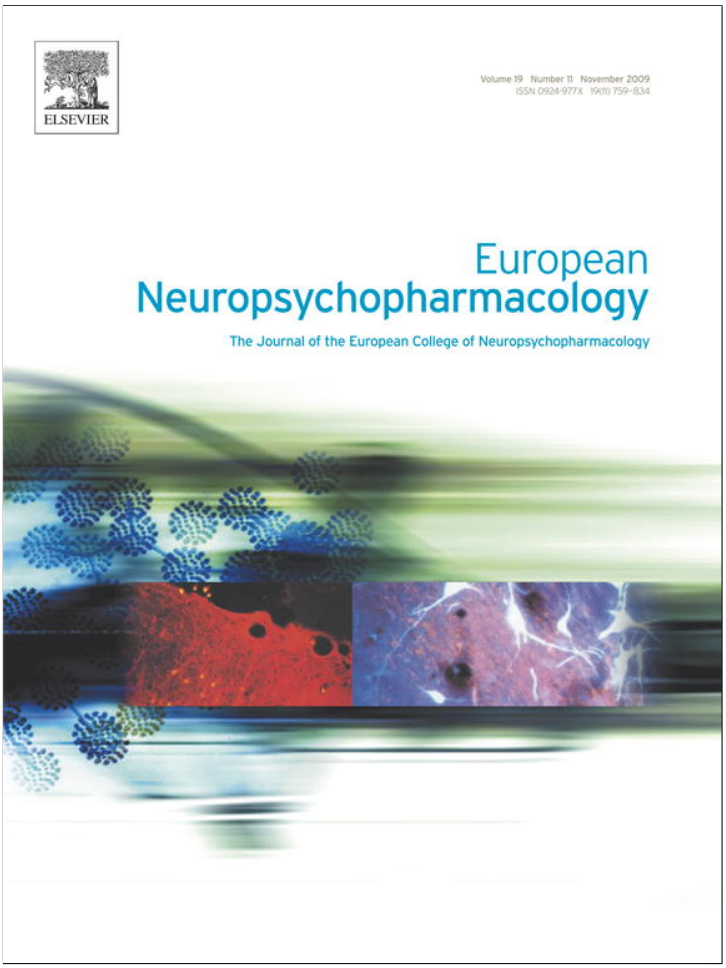

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Clomipramine treatment reversed the glial pathology in a chronic unpredictable stress-induced rat model of depression

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Abstract

Growing evidence indicates that glia pathology contributes to the pathophysiology and possibly the etiology of depression. The study investigates changes in behaviors and glial fibrillary associated protein (GFAP) in the rat hippocampus after chronic unpredictable stress (CUS), a rat model of depression. Furthermore, we studied the effects of clomipramine, one of tricyclic antidepressants (TCAs), known to modulate serotonin and norepinephrine uptake, on CUS-induced depressive-like behaviors and GFAP levels. Rats exposed to CUS showed behavioral deficits in physical state, open field test and forced swimming test and exhibited a significant decrease in GFAP expression in the hippocampus. Interestingly, the behavioral and GFAP expression changes induced by CUS were reversed by chronic treatment with the antidepressant clomipramine. The beneficial effects of clomipramine treatment on CUS-induced depressive-like behavior and GFAP expression provide further validation of our hypothesis that glial dysfunction contributes to the pathophysiology of depression and that glial elements may represent viable targets for new antidepressant drug development.

1. Introduction

Depression is a devastating disorder with high prevalence and mortality, resulting in massive socioeconomic burden (Crawford, 2004). It is characterized by chronic depressed mood, the inability to experience pleasure, withdrawal of interest, feelings of worthlessness, and suicidal tendencies (Saletu-Zyhlarz et al., 2003; Percaccio et al., 2005;

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Strauman et al., 2006). To date, available chemical antidepressants including monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and selective serotonin or norepinephrine reuptake inhibitors (SSRIs, SNRIs, respectively) have been developed out of the monoaminergic deficit hypothesis of depression that arose in the mid of 1960s (Wong and Licinio, 2004). However, the biological basis of depression and the precise mechanism of antidepressant efficacy remain unclear. Since 20–30% of patients are resistant to current drug therapies and there is a 2–3 week lag of onset to therapeutic efficacy, elucidating new mechanisms for depression is critical to the development of more effective antidepressant drugs (Nelson, 1999; Geddes et al., 2004; Montgomery, 2006).

Growing evidence indicates that glial elements are involved in the neuropathology of several neuropsychiatric illnesses including depression. Now, it is reported that cortical glial cell density is increased by neuroleptic medication in primates (Selemon et al., 1999) and that glial cell density is reduced in the prefrontal cortex in depression (Ongur et al., 1998; Rajkowska et al., 1999). Post-mortem studies of tissues from patients with depression showed a reduced number and an altered morphology of glial cells in several brain regions (Bowley et al., 2002; Rajkowska, 2002). Additionally, other post-mortem studies demonstrated altered expression of glial fibrillary associated protein (GFAP) in tissues from patients with depression (Muller et al., 2001). The results of current animal study also indicated that astroglia were significantly reduced when tree shrews were subjected to the chronic social defeat model of depression, but concomitant treatment with fluoxetine could block this effect (Phillips et al., 2004; Czéh et al., 2006). Another recent animal study showed that glial ablation in the prefrontal cortex is sufficient to induce depressive-like behaviors similar to chronic stress (Banasr and Duman, 2008). Interestingly, the other current study investigated the expression of the GFAP in various brain regions in Wistar–Kyoto (WKY) rat strain, which has been proposed as a model of depression and stress susceptibility, in comparison to Sprague–Dawley rats. They found a significant deficit in GFAP-immunoreactive cells in the hippocampus (CA3 and dentate gyrus) in WKY rat brain (Gosselin et al., 2009). These findings suggest that glial cell dysfunction is involved in the pathophysiology of major depressive disorders and support the hypothesis that the loss of glia contributes to the core symptoms of depression.

The hippocampus, the brain region playing pivotal roles in learning and memory, has been the subject of numerous studies addressing the pathophysiology of depression. A recent study of depressed patients assessing changes in total cell numbers in the pyramidal layer of the CA1 region of hippocampus reported a reduced ratio of glia per pyramidal neuron, and suggested a slight glial reduction in depression (Cobb et al., 2006). Another alteration related to glia was a reduced GFAP staining of astrocyte cell bodies in the hippocampus of both steroid-treated or depressed patients (Muller et al., 2001) suggest that the glial cell abnormalities include changes in astrocyte cell function. Recent studies provide evidence that stress exposure may be related to some of the reported glial cell pathology by demonstrating that animals exposed to chronic stress have a decreased glial density in the hippocampus and a reduced production of glial cells in the adult hippocampus. The goals of this study in the chronic unpredictable stress induced depression rat model are to show that stress decreases GFAP levels in the hippocampus and that one of the tricyclic antidepressants, clomipramine, has strong antidepressant efficacy by restoring GFAP levels.

2. Experimental procedures

2.1. Animals

Male adult Sprague–Dawley (Experimental Animal Center, Shanghai Medical College of Fudan University, China) rats were housed under a 12-h light/12-h dark cycle at constant temperature (25 °C) and humidity with free access to food and water except when animals were subjected to deprivation stressors during the chronic unpredictable stress (CUS) procedure. Experiments began after at least 1 week of habituation to the housing conditions. All rats were used strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals used and their suffering.

2.2. Experimental design

To observe the behavioral effects of antidepressants clomipramine (Clo, 5 mg/kg, intraperitoneal, i.p.) on depression model rats, 32 rats (8 in each group) were randomly divided into four groups: the normal group, the model group, the saline group and the clomipramin group. For analysis of the GFAP mRNA and protein levels in the hippocampus, an additional 32 rats (8 in each group) were divided into the four groups. The experimental design was displayed in Fig. 1. Stress groups including the model group, the saline group and the clomipramine group, were subjected to a

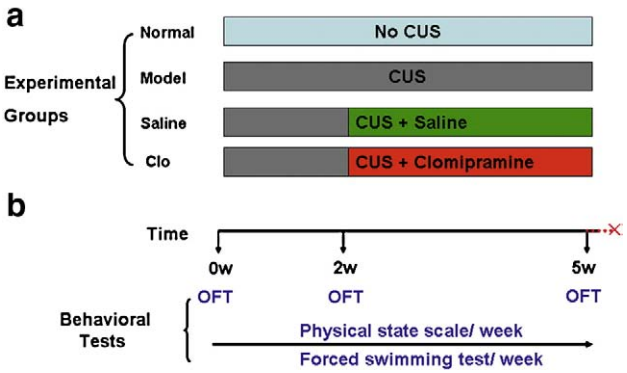

Figure 1 Animal groups, schematic representation of the experimental procedure and behavioral test. Rats were randomly divided into four groups: Normal, Model, Saline, Clo ($n=8$ each group). Stress groups were subjected to a variety of chronic stressors (CUS) during 5 weeks, whereas animals of the normal group (Normal) remained undisturbed. Animals received saline or clomipramine treatment 2 weeks after the experiment started. For analysis of GFAP expression, groups of rats ($n=4$) were sacrificed on the final day of the 5-week period. Parallel groups ($n=8$ each group) of animals were prepared for behavioral tests. Open field test (OFT) and forced swimming test (FST) was measured before stress, drug administration, and at the end of the experiment. Physical state score was measured respectively at the end of every week.

variety of chronic stressors during 5 weeks, whereas animals of the normal group remained undisturbed. Clomipramine or saline was administered every day for 3 weeks starting 2 weeks after the beginning of the experiment. Physical state was measured about once a week over the entire experimental period. Open field test was measured before stress, clomipramine or saline treatment, and at the end of the experiment.

2.3. CUS procedure

CUS is an experimental procedure in which animals are exposed to variable unpredictable stressors to induce depression in an animal model. Our CUS procedure was successfully used in the laboratory to produce behavioral changes as well as alteration of adult hippocampal neurogenesis (Liu et al., 2008). The depression model was designed to maximize the unpredictable nature of the stressors and consisted of the following stressors in random order: 30-min cage rotation, 5-min forced swimming, reversal of the light/dark cycle, 40-h food deprivation, 40-h water deprivation, 5-min hot environment (40 °C). The CUS procedure was carried out for 5 weeks in animals in the untreated model group, saline-treated group, and clomipramine-treated (Clo) group. Non-stressed animals in the normal group were left undisturbed in their home cages except for necessary procedures such as regular cage cleaning.

2.4. Physical state score

This procedure was performed as described previously (Alonso et al., 2004). Physical state was measured about once a week over the entire experimental period using a scale from 1 to 3: a health state was noted as 3 and damaged state with piloerection and/or dirty fur was noted as 1. Intermediate state was noted as 2. Each measure was scored by an experimenter unaware of the treatment group.

2.5. Open field test

The open field test was performed as previously described (Redmond et al., 1997), and was carried out before stress (0 week), 2 weeks after stress (2 weeks) and 5 weeks after stress (5 weeks). In the open field test, rats were placed at the center rectangular arena side walls. The open field apparatus consisted of a four-sided $100 \times 100 \times 40\ cm^3$ wooden box, which was covered inside with folium to increase the reflectivity of the walls. The floor of the box was divided into 16 squares. A 60 W light bulb was positioned 90 cm above the base of the apparatus, and was the only source of illumination in the room. Each animal was placed in the center of the apparatus and was allowed to explore freely for 3 min. During the test time the number of crossings (defined as at least three paws in a quadrant) and the number of rearings (defined as the animal standing upright on its hind legs) were measured. After each animal, the test apparatus was cleaned with a 10% ethanol solution and water to remove any olfactory cues.

2.6. Forced swimming test

The technique first characterized by Porsolt and colleagues (Panconi et al., 1993) was used. Briefly, rats were forced to swim individually in a cylindrical glass container (40 cm height, 18 cm diameter), which contained tap water (25 ± 1 °C) to a depth adjusted for the weight of the individual animal, so that its hind paws could just touch the bottom of the container. At first, rats were placed in the water for 15 min, and retested for another 5 min after 24 h. After the rest, rats were dried with a towel and returned to their home cages. After 1 week, the animals were re-exposed to the forced swimming for a further 5 min. The test sessions were recorded and scored by an observer who was blind to the groups of animals. The total duration of immobility during the first 5 min of the swimming session was recorded. The rat was judged to be immobile when it made only the necessary movements to keep its head above water level.

2.7. Immunohistochemistry

Rats were given an overdose of urethane (1.5 g/kg, i.p.) and perfused through the ascending aorta with 200 ml of normal saline followed by 300 ml 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brains were then removed, postfixed in the fixative solution for 4 h at 4 °C, and immersed in 30% sucrose in PB for 24–48 h at 4 °C for cryoprotection. Frozen sections (35 μm) were cut and collected in cryoprotectant solution (0.05 M PB, 30% sucrose, 30% ethylene glycol) and then stored at −20 °C until use. Free-floating sections were washed in 0.1 M PBS and then treated with 1% H_2O_2 for 20 min. After washing, nonspecific binding of antibodies was prevented by incubating the sections for 1 h with 3% normal goat serum (NGS; Vector Laboratories, Burlingame, CA, USA) in 0.1 M PBS containing 0.5% Triton X-100. The sections were subsequently incubated overnight with rabbit anti-GFAP polyclonal antibody (1:1000, DAKO, USA) diluted in 1% NGST at 4 °C for 24 h. The incubated sections were washed three times in 1% NGST and incubated in biotinylated goat anti-rabbit immunoglobulin G (IgG) (1:200, Vector Laboratories, Burlingame, CA) for 1 h at room temperature. The sections were then washed three times in 1% NGST and incubated for 1 h in avidin-biotin-peroxidase complex (1:200, Vector Laboratories) at room temperature. Finally, the sections were washed three times in 0.01 M PBS, and developed for 5 min in diaminobenzidine (1 : 200; DAB Peroxidase Substrate Kit, Vector) and then thoroughly rinsed. The sections were then mounted, dehydrated and covered. To test the specificity of the primary antibody, controls were performed, including the substitution of normal rabbit sera for the primary antibody and omission of the primary antibody. None of these controls showed any sign of immunohistochemical reaction. Images of positive staining in the hippocampus region of brains were captured.

2.8. Tissue collection and preparation of whole-cell extracts for Western blot analysis

For determination of GFAP protein level, protein extracts were obtained from the hippocampus of brain according to the following protocol. Given an overdose of urethane (1.5 g/kg, i.p.), rats were sacrificed and the hippocampus of every brain was collected on dry ice and stored at −70 °C until assayed. Each assay sample consisted of the hippocampus from one rat. Each sample was weighed and homogenized in 1.5 ml of sample buffer (0.01 M Tris–HCl buffer (pH 7.6) containing 0.25 M sucrose, 0.1 M NaCl, 1 mM EDTA, and 1 mM phenylmethylsulfonylfluoride) at 4 °C. Supernatant after 12,000 rpm centrifugation for 10 min was used for Western blotting. Samples (30 μg of total protein) were dissolved with equal volume of loading buffer (0.1 M Tris–HCl buffer (pH 6.8) containing 0.2 M DTT, 4% SDS, 20% glycerol and 0.1% bromophenol blue), separated on 10% SDS-PAGE and then electrotransferred at 100 V to Immun-Blot PVDF membrane for 1 h at 4 °C. Membranes were blocked in TBST containing 5% non-fat dried milk overnight at 4 °C before incubation for 2 h at room temperature with anti-GFAP polyclonal antibody (1:5000, DAKO, USA) diluted in TBST containing 5% BSA. Blots were washed extensively in TBST and incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase (Amersham) in TBST/1.25% BSA for 1 h at room temperature. The signal was detected by an enhanced chemiluminescence method (ECL kit, Amersham), and exposed to Kodak X-OMAT film (Eastman Kodak, Rochester, NY, U.S.A.). The intensity of the selected bands

was captured and analyzed using GeneSnap Image Analysis Software (Syngene, U.K.).

2.9. Reverse transcription-polymerase chain reaction (RT-PCR)

For determination of GFAP mRNA level, protein extracts were obtained from the hippocampus of brain according to the following protocol. Rats were sacrificed with an overdose of urethane (1.5 g/kg, i.p.) and the hippocampus of every brain was collected in dry ice. Total RNA extraction was performed using the Trizol reagent, following the instructions of the manufacturer. RNA was further purified using the RNeasy kit according to the RNA clean-up protocol, and eluted in 20 μl of RNase-free distilled H_2O. The amount of RNA was measured spectrophotometrically. Total RNA (1 μg) was used for the synthesis of the first strand of cDNA using the SuperScript reverse transcriptase. Briefly, RNA, oligo (dT) 18 primers (0.5 μg/μl) were first denatured for 5 min at 65 °C, chilled on ice for 1 min, and then incubated for 50 min at 42 °C, 15 min at 70 °C in 20 μl of a reaction mixture containing 10× first-strand buffer, 10 mM dNTP mix, 0.1 M DTT and 50 units of SuperScript II reverse transcriptase. The sequences of primers for GFAP were as follows: forward: 5′-TGAGGCAGAAGCTCCAAGATGAAA-3 1178–1201, reverse: 5′-CTGGTTTCTCGGATCTGG-3′ 1599–1622 (U59486); β-actin forward: 5-CACCATGTACCCTGGCATTG-3 reverse: 5-TAACGCAACTAAGTCATAGT-3. The primers were synthesized and purified by Shanghai Institute of Biochemistry, Chinese Academy of Science. 1 μl of cDNA was added to 49 μl of PCR mix containing 5× PCR buffer, 18 pmol/l concentrations of each primer, 2.5 mM of dNTP, and three units of Taq DNA polymerase. PCR reaction was performed as follows: 12 min at 94 °C to activate the Taq polymerase, followed by 28 cycles of 45 s at 94 °C, 1 min at 58 °C, and 1 min at 72 °C. A final elongation step at 72 °C for 10 min completed the PCR reaction. Each PCR production (10 μl) was electrophoresed in 1% agarose gel, visualized by ethidium bromide staining and scanned with ultraviolet transilluminator (GDS 8000, Gene Tools from Syngene software, U.K.). The PCR quantitative method takes advantage of the fact that β-actin was employed as internal standard in the same condition. All the results were expressed as ratios of the intensity of the GFAP bands to that of β-actin band.

2.10. Data analysis

Data are presented as mean ± S.E.M. and analyzed by SPSS 11.0. Repeated measures analysis of variance (ANOVA) followed by S–N–K test was used for post-hoc analysis for differences between groups. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of CUS and clomipramine treatment on behavior

Chronic unpredictable stress has been successfully used in our laboratory to induce a depressive-like behavior. At the beginning of the experimental procedure, there was no significant difference among the groups according to the physical state score ($F3,20=1.224$, $P>0.05$), the number of crossings and rearings in the open field test ($F3,20=0.205$, $P>0.05$; $F3,20=0.535$, $P>0.05$) and the immobility time in the forced swimming (F (4, 35) = 0.243, $P>0.05$). After CUS for 2 weeks, stressed rats in the model group showed a significant degradation of the physical state of their coats due to stress ($F3$, 20 = 33.177, $P<0.01$) compared to the non-stressed, normal group. Then stressed animals in the saline group were started on daily saline injections and animals in the clomipramine groups were started on daily clomipramine injections while CUS was continued for an additional 3 weeks. At the end of the experimental procedure after a total of 5 weeks of CUS, the model animals without treatment and saline-treated animals showed a significant decrease in scores of physical state ($F3$, 20 = 86.787, $P<0.01$). In contrast, the degradation of the physical state of the animal's coat was significantly improved by clomipramine following 3 weeks of treatment, an effect that lasted until the stress period was completed (Fig. 2).

Animals exposed to stressors also showed a significant decrease in the number of crossings in the 2nd week, which continued to the last week when the experiment ended ($F3,20=51.592$, $P<0.05$). In the clomipramine group, the number of crossings decreased significantly 2 weeks after being exposed to the stressors ($F3,20=12.033$, $P<0.05$) but improved after clomipramine treatment for 3 weeks compared to the saline group ($F3,20=76.118$, $P<0.05$) (Fig. 3a). Similar changes were seen in the vertical activity (number of rearings) for all groups of rats (Fig. 3b). These results demonstrated that clomipramine reversed the stress induced change in open field activity.

In the forced swimming test, the immobility time was measured during the first 5 min of swimming. After CUS for 2 weeks, animals exposed to stressors showed a significant increase in immobility time (F (4, 35) = 18.261, $P<0.01$). At the end of the last week, the model and saline group animals had a significantly greater increase in immobility time (F (4, 35) = 64.233, $P<0.01$). However, the clomipramine group animals revealed a significant decrease in immobility time compared

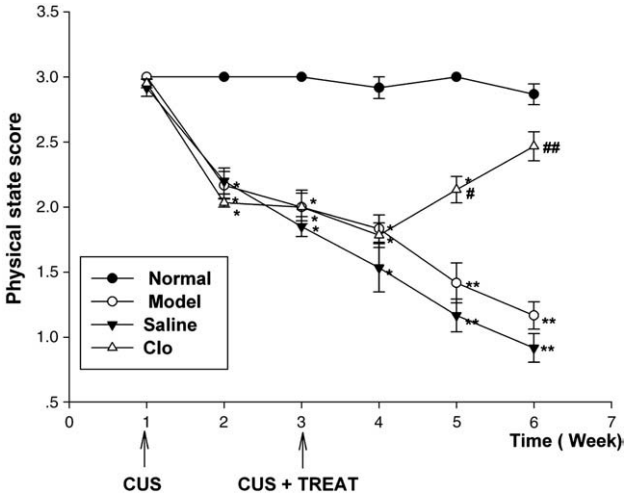

Figure 2 Physical state scores during the experimental procedure. Rats were treated with either saline or clomipramine 2 weeks after CUS was initiated for a further 3 weeks. Physical states were scored at the beginning of every week. Results are expressed as mean ± SEM ($n=8$ per group). $^{*}p<0.05$, $^{**}p<0.01$, the animals exposed to stress compared to the normal group animals; $^{\#}p<0.05$, $^{\#\#}p<0.01$, the Clo group compared to the saline group.

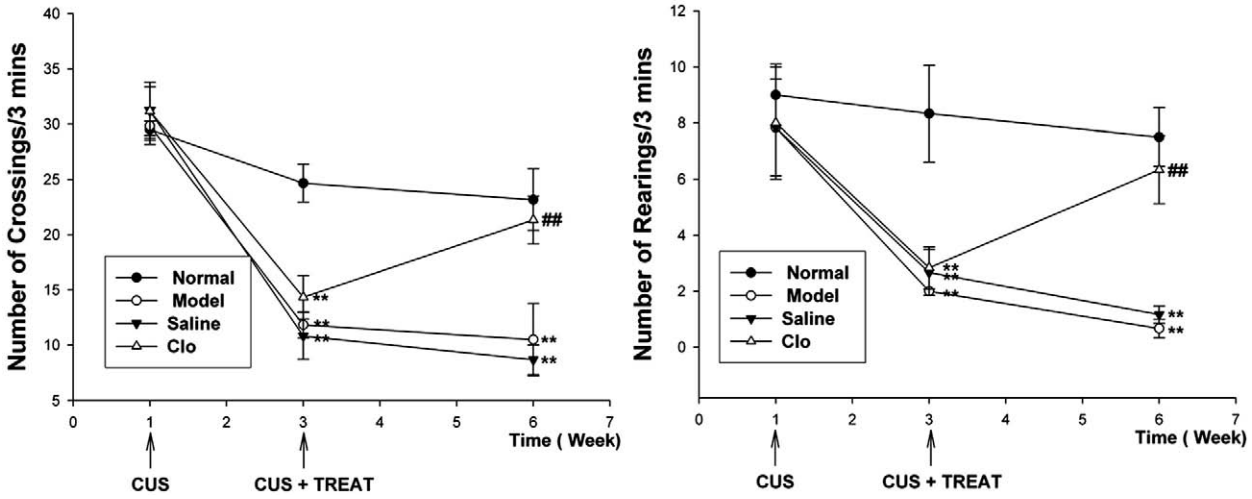

Figure 3 Open field behavior in rats after CUS procedure and clomipramine treatment. Rats were treated with either saline or clomipramine 2 weeks after the stress procedure started. Animals performed the open field test before CUS, 2 weeks and 5 weeks after CUS. (a) Number of crossings during the 3 min session. (b) Number of rearings during the 3 min session. Results are given as mean ± SEM ($n = 8$ per group). *$p < 0.05$, **$p < 0.01$, the stress groups compared to the normal group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, the Clo group compared to the saline group.

with the saline group animals (F (4, 35) = 28.674, $P < 0.05$), showing that clomipramine blocked the stress induced change of behavioral activity (Fig. 4).

3.2. Effects of CUS and clomipramine treatment on expression of GFAP in the hippocampus

By immunohistochemistry, normal rat samples in the DG or CA3 of hippocampus showed a moderate immunoreactivity for GFAP (Fig. 5a, e), whereas samples from the model or saline-treated rats revealed weak immunostaining patterns (Fig. 5b, f, c, g). However, samples from clomipramine-treated rats displayed a moderate immunoreaction for GFAP like the samples from normal rats (Fig. 5d, h).

These results were further validated by Western blot analysis. The statistical results showed that GFAP protein level in the hippocampus was significantly decreased following 3 weeks of saline treatment. However, clomipramine treatment reversed the GFAP protein level (Fig. 6a; $P < 0.05$). A single protein band of the expected size (~50 kDa) for GFAP was detected with the GFAP-specific primary antibody (Fig. 6b). In addition, no band was detected when the primary antibody was omitted (data not shown).

Semi-quantification showed that GFAP mRNA level in the hippocampus was markedly decreased in the model rats and the saline-treated rats. However, clomipramine treatment almost blocked the GFAP mRNA level in the hippocampus compared to the saline treatment group (Fig. 6c; $P < 0.01$). As shown in

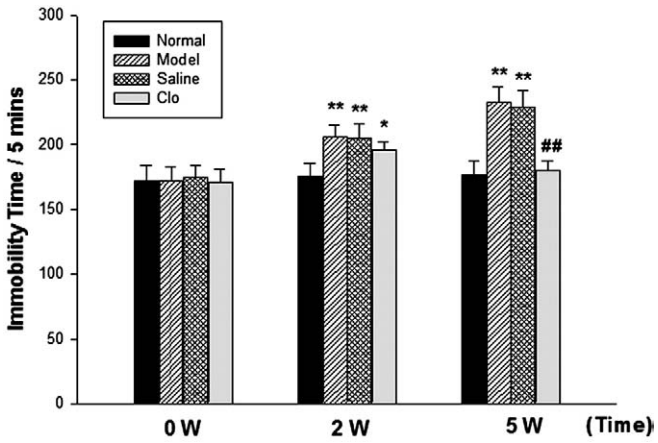

Figure 4 Immobility time in the forced swimming test during the experimental procedure. Rats were injected with either clomipramine (5 mg/kg/day) or saline 2 weeks after CUS was initiated for a further 3 weeks. Immobility time was measured during the first 5 min of forced swimming on the beginning of every week. Results are expressed as mean ± SEM ($n = 8$ per group). *$p < 0.05$, **$p < 0.01$, the stress groups compared to the normal group; $^{\#\#}p < 0.01$, the Clo group compared to the saline group.

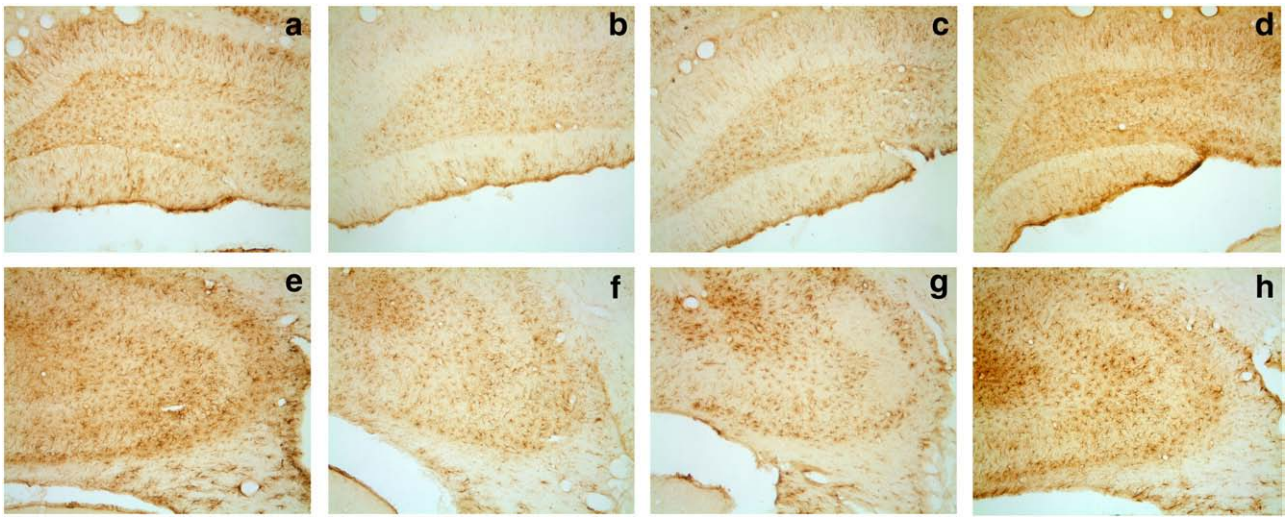

Figure 5 Change of GFAP expression in the hippocampus after CUS and clomipramine treatment in rats. Images were shown for GFAP immunostaining in the DG and CA3 subregion of the normal group (a and e), model group (b and f), saline group (c and g) and Clo group (d and h). Under a 40× zoom magnification, individual cells are clearly visualized.

Fig. 6d, an expected 726-bp PCR product was obtained in RT-PCR analysis.

4. Discussion

In the present study, we used chronic unpredictable stress-induced depression model as a well-documented animal model of depression that results in depressive-like behavioral effects similar to symptoms observed clinically. We clearly demonstrate the beneficial effects of clomipramine at reversing CUS-induced depressive-like behaviors in this model. GFAP, a marker of astroglia in the hippocampus of rats, showed significant decreases in mRNA and protein levels after exposure to unpredictable stressors. Chronic clomipramine treatment was also able to reverse the CUS-induced decrease in GFAP expression. Together, the present study provides another evidence for glial pathology in major depression, indicating that the level of astroglial marker GFAP is reduced in the hippocampus, while repeated clomipramine treatment reversed the glia pathology in the CUS-induced depression model.

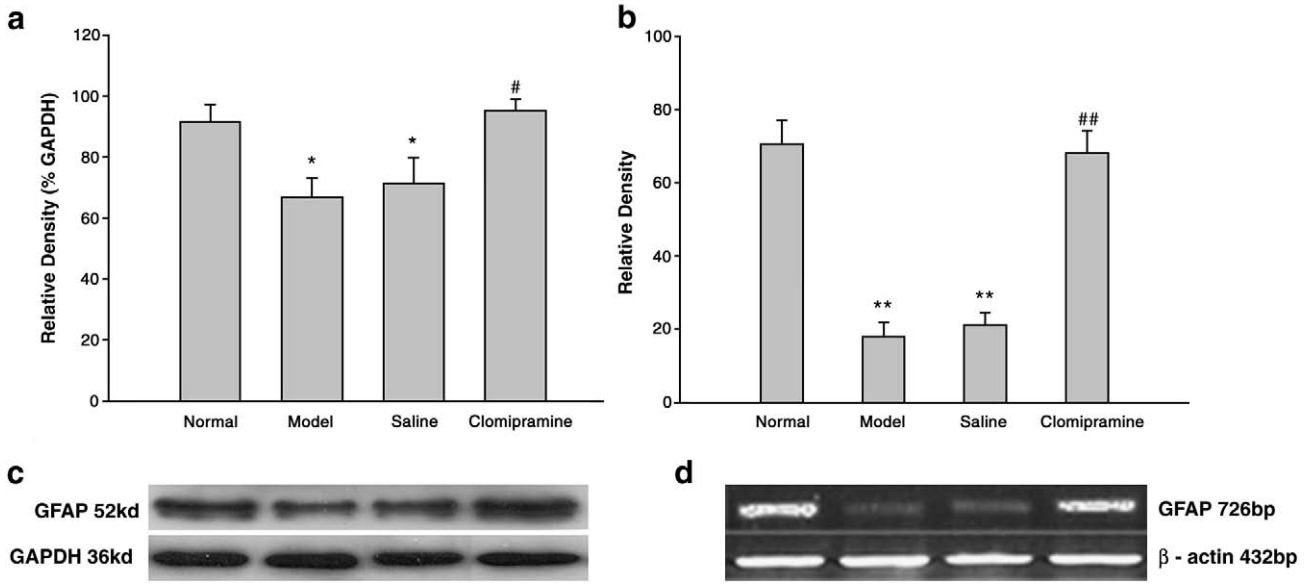

Figure 6 Changes of GFAP protein level detected by Western blot and GFAP mRNA level detected by RT-PCR in the hippocampus after CUS and clomipramine treatment in rats. The GFAP protein levels in different groups were expressed as a ratio to that of corresponding GAPDH (a). Western blot analysis detected expected size protein band of GFAP (b). The mRNA level was expressed as a ratio to that of corresponding β-actin (c) and expected size PCR products of GFAP were acquired (d). Data were represented as mean ± S.E.M. ($n=4$ in each group). $^{*}p<0.05$, $^{**}p<0.01$, the stress groups compared to the normal group; $^{\#}p<0.05$, $^{\#\#}p<0.01$, the Clo group compared to the saline group.

The CUS procedure used in this study was the same procedure reported by our previous research, which consisted of 6 different randomized stressors, 1 per day for 5 weeks (Liu et al., 2007, 2008; Yu et al., 2007). CUS is a dependable depression model with high face, predictive and construct validity (Forbes et al., 1996; Moreau, 1997). It is also one of the animal depression models for analyzing cellular and molecular mechanisms underlying the pathophysiology of depression and for exploring the mechanism of antidepressants (Sikiric et al., 2000; Zhou et al., 2005; Banasr et al., 2007; Bachis et al., 2008; Bondi et al., 2008). The number of crossings and rearings in the open field test represents the explorative behavior. The immobility time during the forced swimming test was analyzed to evaluate the active fleeing behavior. Our previous experiments had exhibited that repeated administration of clomipramine could amend the depressive-like behaviors detected by open field test and forced swimming test (Liu et al., 2008). The present study further displayed that this antidepressant also improve the physical states of depression model rats. The present data, which are in line with the previous findings on behavioral change of antidepressants, provide further evidence for the efficacy of clomipramine in CUS induced rat depression model.

Although less is known about the role of glia in depression related brain remodeling, there is growing evidence that indeed both neuronal and glial changes constitute the neuropathology of neuropsychological disorders, including depression (Harrison, 2002). On the other hand, preclinical and neuroimaging including magnetic resonance imaging (MRI) studies have implicated the hippocampus involved in the pathophysiology of depression (Sheline et al., 2002; Campbell and Macqueen, 2004; Campbell et al., 2004; Fuchs et al., 2004; Stockmeier et al., 2004). In individuals with depression, MRI studies demonstrate reduced volume of the hippocampus (Shah et al., 1998; Sheline et al., 1999; Bremner et al., 2000; Mervaala et al., 2000; Frodl et al., 2002; MacQueen et al., 2003). Also in one animal study, Czéh et al showed that chronic psychosocial stress resulted in a decrease (7%) of the hippocampal volume compared with unstressed controls which was prevented by tianeptine treatment (Czéh et al., 2001). Even though in vivo imaging studies document significant reductions of hippocampal volume in depressed patients or depression model animals, the exact underlying cellular mechanisms are unclear. Many evidences showed reductions in glial number in related limbic structures like the amygdala and prefrontal, orbitofrontal and cingulate cortices of depressed patients (Coyle and Schwarcz, 2000; Cotter et al., 2001, 2002). A recent study assessing changes in total cell numbers in the pyramidal layer of the CA1 region reported a reduced ratio of glia per pyramidal neuron, which suggests a slight glial reduction in depression patients (Cobb et al., 2006). Did the reduction of glia number in hippocampus contributed to the shrinkage of hippocampal volume in depression? Recently, Czéh and Lucassen discussed what causes the hippocampal volume decrease in depression and speculate on putative glial changes are most likely to explain the hippocampal shrinkage in depression (Czéh and Lucassen, 2007). Interestingly, using GFAP as a selective immunohistochemical marker of astroglia, few researches showed a reduction in GFAP-stained cell count in the prefrontal cortex of depression patients. A decrease in astrocytic immunoreactivity for cellular GFAP was also detected in CA1 and CA2 region of postmortem human hippocampus in depression (Muller et al., 2001). Furthermore, a significant deficit in GFAP-immunoreactive cells in the brain was demonstrated in tree shrews subjected to the chronic social defeat model of depression and the WKY strain rats (Czéh et al., 2006; Gosselin et al., 2009). Our results showed the decreased GFAP expression in the hippocampus of rats exposed to CUS. As GFAP is a key cytoskeletal protein of astroglia, the change of GFAP could be related to observed morphological alteration of hippocampal astroglia. The level of GFAP expression is also linked to the reactivity of astroglia, and GFAP plays a role in maintaining the morphology of astroglia (Karschin et al., 1986; Casanovas et al., 2000). In this study, we used GFAP immunohistochemistry, Western blotting and RT-PCR analyses to visualize and detect the levels of rat hippocampal astroglia. Our study showed that the behavioral changes are consistent with the decreased level of GFAP in the hippocampus of depression model rats. The present study now offers novel evidence that impaired glias in the hippocampus induced by stressors could be implicated in depressive-like behaviors resulted from CUS in this rat model.

It is well-known that there are three major types of neuroglia existed in the central nervous system, microglia, oligodendroglia, and astroglia, all of which respond to variations in the neural environment (Muller and Kettenmann, 1995; Coyle and Schwarcz, 2000). Although microglia are mainly involved in immune responses within the nervous system and in the elimination of cellular debris and oligodendroglia are crucial for the conduction of action potentials along axons, their roles in depression have been proceeded by our research team (data not shown). Among glial cells, astroglia provide a structural framework for the brain, maintain pH and ion homeostasis in the extracellular space, supply energy and nutrients to the neurons, clear neuronal waste, and through their end feet on blood vessels are involved in monitoring of peripheral changes in blood composition (DeWitt et al., 1998; Hirst et al., 1998; Cohen et al., 1999). Moreover, recent studies have revealed that besides these "housekeeping" functions, astroglia are in fact dynamic regulators of synaptic strength, synaptogenesis and neuronal production in the adult dentate gyrus (Garcia-Segura et al., 1999; Okugawa et al., 1999; Song et al., 2002). Thus, the changes of astroglia are likely to have an important functional significance for the etiology of depression and mechanism of antidepressants. Our previous results showed that the decrease in adult hippocampal neurogenesis was detected in rats subjected to CUS procedure. Combined with these reports, our present study implied that astroglia could play an important role in the etiology of depression. Maybe we can speculate on the possibility of the regulatory effect of astroglia on the adult hippocampal neurogenesis during depression and the antidepressant clomipramine treatment.

Clomipramine belongs to the family of tricyclic antidepressants and exerts an antidepressant effect by inhibiting the neuronal reuptake of the neurotransmitters norepinephrine and serotonin (Wille et al., 2008). In our study, daily intraperitoneal saline or clomipramine was administered after the stress-induced behavioral alterations had been established for 2 weeks. Then the action of clomipramine was followed across a clinically relevant time period of 3 weeks while the stress continued during the whole treatment period. We found that this antidepressant reversed the decreased levels of GFAP of the hippocampus in depression model rats. A recent study

showed that the number of GFAP-positive astroglia was significantly reduced in tree shrews subjected to the chronic social defeat model of depression, reversal by concomitant treatment with fluoxetine (Czéh et al., 2006). Our present study showed similar results using antidepressant clomipramine. To the best of our knowledge, it is the first time that the antidepressant clomipramine has demonstrated in depression model animals a regulatory effect on hippocampal astroglia. In our previous study, we demonstrated that clomipramine could regulate adult hippocampal neurogenesis, implying a possible mechanism underlying the antidepressant clomipramine. A lot of studies demonstrated that neurogenesis correlates with the time-to-effect (Alonso et al., 2004; Czéh et al., 2001; Malberg et al., 2000; Warner-Schmidt and Duman, 2006). Antidepressants require at least 2–4 weeks of administration before achieving therapeutic benefits, as antidepressants also need 2–4 weeks of treatment to prevent the decreased neurogenesis. Although recent study showed that shorten time, 4 days, treatment with mifepristone also reversed the decreased neurogenesis induced by chronic stress or chronic corticosterone treatment, 4 days of treatment was also reported to relieve symptoms of psychotic depression (Mayer et al., 2006; Oomen et al., 2007). Whether the glia change is affected by the duration of antidepressant treatment, it could be detected in the future study. In astroglia, GFAP immunoreactivity is regulated by activation of serotonergic and adrenergic receptors (Griffith and Sutin, 1996), which are considered to play a crucial role in the pathophysiology of mood disorders (Potter, 1996). Whether clomipramine affected the changes of GFAP through activating the serotonergic and adrenergic receptors on astroglia also merits further investigation.

In conclusion, the present study demonstrates that CUS produces a depressive-like behavior and causes changes in the expression of GFAP, the major intermediate filament protein in astroglia. These findings in rats are consistent with a growing number of studies showing glial cell pathology and in patients with depression. Furthermore, the present study also shows that clomipramine, one of the TCAs, reverses depressive-like behaviors and restores GFAP levels in the hippocampus of animals exposed to CUS synchronously. Our studies in an animal model of depression offer more promise to the general hypothesis that glial cell pathology in an animal model of depression abnormalities contributes the pathophysiology of depression and this pathological process may be targeted in the development of novel antidepressant medications.

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Contributors

Authors Jin Yu, Gen-Cheng Wu, Yan-Qing Wang and Qiong Liu designed the study and wrote the protocol. Author Hai-Yan Zhu and Bing Li managed the literature searches and analyses. Authors Qiong Liu and Hai-Yan Zhu undertook the statistical analysis, and author Qiong Liu wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

We declare that we have no duality or conflict of interest.

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