Effects of curcumin on chronic, unpredictable, mild, stress-induced depressive-like behaviour and structural plasticity in the lateral amygdala of rats

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Abstract
Depression is a neuropsychiatric disease associated with wide ranging disruptions in neuronal plasticity throughout the brain. Curcumin, a natural polyphenolic compound of curcuma longa, has been demonstrated to be effective in the treatment of depressive-like disorders. The present study aimed to investigate the mechanisms underlying the antidepressant-like effects of curcumin in a rat model of chronic, unpredictable, mild, stress (CUMS)-induced depression. The results showed that CUMS produced depressive-like behaviours in rats, which were associated with ultra-structural changes in neurons within the lateral amygdala (LA). In addition, the expression of synapse-associated proteins such as brain-derived neurotrophic factor (BDNF), PSD-95 and synaptophysin were significantly decreased in the LA of CUMS-treated rats. Chronic administration of curcumin (40 mg/kg, i.p. 6 wk) before stress exposure significantly prevented these neuronal and biochemical alterations induced by CUMS, and suppressed depressive-like behaviours, suggesting that this neuronal dysregulation may be related to the depressive-like behaviours caused by CUMS. Together with our previous results, the current findings demonstrate that curcumin exhibits neuroprotection and antidepressant-like effects in the CUMS-induced depression model. Furthermore, this antidepressant-like action of curcumin appears to be mediated by modulating synapse-associated proteins within the LA. These findings provide new insights into the underlying mechanisms leading to neural dysfunction in depression and reveal the therapeutic potential for curcumin use in clinical trials.

Introduction
Depression has become the most prevalent psychiatric disorder and imposes a substantial societal burden (Lépine and Briley, 2011; Hidaka, 2012). Increasing evidence has indicated that depression is related to structural and functional changes in specific brain regions (Vyas et al., 2002; Stockmeier et al., 2004; Oh et al., 2012). It is generally recognised that synaptic plasticity is the basic underlying mechanism responsible for modifying brain circuitry, and the potential for application of this process in the clinical treatment of depression has received more and more attention (Duman and Aghajanian, 2012; Castrén and Hen, 2013; Russo and Nestler, 2013). Therefore, investigations directed toward this area are urgently needed to develop novel protocols to achieve more selective and safe therapeutic strategies for the treatment of depression.

In recent years, some herbal medicines, with their high safety margins, have proved to be effective pharmacotherapies in the treatment of depression (Chainani-Wu, 2003; Nemeroff, 2007; Van der Watt et al., 2008). Curcumin, the main biologically active component of Curcuma longa, exhibits a wide variety of pharmacological activities including anti-inflammatory, antioxidant, immunomodulatory and neuroprotective activities (Maheshwari et al., 2006; Aggarwal and Harikumar, 2009; Yu et al., 2013a, b). More recently, the potential antidepressant effect of curcumin in various animal models of depression has been increasingly recognised (Xu et al.,
Curcumin appears to exert its antidepressant action by modulating the monoaminergic system (Kulkarni et al., 2008) and may also inhibit glutamate release in nerve terminals from rat pre-frontal cortex, an effect, which is similar to that of the classical antidepressant fluoxetine (Lin et al., 2011). Recent studies in our laboratory have shown that chronic curcumin treatment significantly reduced the immobility time in the forced swim test via activating MAPK/ERK-dependent brain-derived neurotrophic factor expression of mice (Zhang et al., 2012). Although curcumin has been demonstrated to be a multi-target natural compound, which may modulate numerous pathways (Zhou et al., 2011), detailed characterisation of the neuronal mechanisms underlying its antidepressant effects remains largely unknown.

Mounting evidence has indicated that depression is associated with a range of changes in synaptic form and function within several brain regions (Christoffel et al., 2011; Popoli et al., 2012). For instance, stress, a related neuropsychiatric process, induces morphological changes upon dendrites and spines in hippocampal (McEwen, 2000; Stockmeier et al., 2004; Donohue et al., 2006) and pre-frontal cortex (PFC) pyramidal neurons (Cook and Wellman, 2004; Goldwater et al., 2009), which suggests the existence of a generalised change in neuronal structure and plasticity in conditions like stress and depression. However, within the amygdala, another important brain region involved in depression, opposite changes in neuronal structures were observed in response to stress protocols. Specifically, chronic restraint stress (CRS) and chronic immobilisation stress (CIS) protocols, which induced dendritic retraction in the hippocampus and PFC, induced dendritic arborisation in amygdaloid neurons (Vyas et al., 2002; Barbon et al., 2011; Eiland et al., 2012). But, data from other studies have shown that CRS decreases interneuron-related proteins and dendritic arborisation in the lateral and basolateral amygdala (Vyas et al., 2002). Interestingly, both of these studies failed to show any alteration in hippocampal parameters, results, which differed from those of other studies. These findings raise the possibility that certain forms of chronic stress could affect neuronal plasticity within specific regions of the amygdala. To date, little attention has been directed toward this prospect, which may have important implications for application in the clinical treatment of depression.

Chronic unpredictable mild stress (CUMS) serves as an animal model, which has been widely used for investigating the pathophysiological mechanisms underlying depression and evaluating the efficacy of antidepressants (Willner, 1997, 2005). This stress-induced model of depression could overcome the stress habituation that usually occurs with CRS and demonstrates consolidated long-lasting behavioural deficits. Therefore, in the present study, we utilised the CUMS model to investigate whether long-term treatment with curcumin could prevent the CUMS-induced neuronal structural changes and depressive-like behaviours observed in these rats. To further examine the underlying mechanisms of curcumin, the expression of BDNF and structural plasticity-associated proteins were also assessed in the lateral amygdala, a specific brain region associated with depression.

Method

Animals

Male Wistar rats weighing 200–220 g were obtained from the Shandong University Animal Centre. All procedures were approved by the Shandong University Animal Care and Use Committee and were performed in accordance with the guidelines of the ‘Principles of Laboratory Animal Care’ (NIH publication No. 80-23, revised 1996). Rats were housed in standard plastic cages under room temperature (22–24 °C) with a 12-h light/dark cycle (lights on 6:00 a.m.). Unless otherwise stated standard laboratory food and water were available throughout the experiments. Animals were allowed to acclimatise to the laboratory conditions for seven to eight days prior to the experimental procedures. All efforts were made to minimise the number of animals used and their suffering.

Drugs and treatment

Curcumin (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO, Sigma, USA) at a concentration of 10 mg/ml and the concentration of DMSO did not exceed 0.1% of the total volume. After one week of acclimatisation, curcumin or DMSO were administered i.p. in a constant volume thirty minutes prior to the stress exposure once a day for six weeks.

Animals were randomly allocated to one of the following four groups of sixteen rats: (a) control, (b) CUMS, (c) curcumin (40 mg/kg)+CUMS, (d) vehicle (0.1% DMSO, 10 ml/kg)+CUMS.

CUMS procedure

The CUMS procedure was performed as described previously with minor modifications (Mao et al., 2009) (Fig. 1a). Briefly, the non-stressed control animals were housed in groups of four per cage in standard plastic cages in a separate room and had no contact with the stressed groups. For another three groups, rats were assigned to four per cage accompanied with stress treatment (n=16/treatment group). Animals were exposed to the weekly stress regime consisting of 48 h food deprivation, 24 h water deprivation, 1 min tail pinch (1 cm from the end of the tail), 45° cage tilt, 5 min cold swimming (at 4 °C), 2 h physical restraint and overnight illumination. One of these stressor episodes was applied daily.
to each rat in a random order for a continuous six-week period.

**Behavioural testing**

Behavioural tests were performed from the next day after six weeks of CUMS exposure in sequence as follows.

**Open-field test**

The spontaneous exploratory behaviour was measured in the open-field test, which was performed with minor modifications as described previously (Walsh and Cummins, 1976). Briefly, the large open field was a square plywood platform (100 × 100 × 40 cm) with a black surface covering the inside walls. The floor was divided into 25 equal squares by black lines. Rats were placed individually in the centre of the open field to explore freely for a 5 min session. The horizontal locomotor activity (segments crossed with four paws) and vertical activity (number of rearings) were recorded by the observer blind to the treatment condition of the animal. The apparatus was cleaned with detergent prior to each test session to remove any olfactory cues.

**Forced swim test**

The day after the open field test, the forced swim test was conducted as described previously (Porsolt et al., 1977; Duman et al., 2007). In brief, rats were placed individually in a glass cylinder (height: 80 cm, diameter: 30 cm) filled with 40 cm of water at 25 °C for two consecutive swim sessions. In this cylinder, rats cannot touch the bottom or escape. For the first exposure, rats were placed in the water for 15 min of forced swimming (training session). Twenty-four hours later, rats were placed in the cylinder again for a 5 min period (test session). In the test session rats were scored by an observer blind to the

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**Fig. 1.** Effects of curcumin on body weight. (a) schematic figure of the treatment protocol. (b) curcumin (40 mg/kg daily) reversed the decreased body weight induced by CUMS exposure. All values are presented as means±S.E.M (n=16). *p<0.05 as compared with the control group (non-stressed). #p<0.05 as compared to the CUMS-treated group. Data were analysed with a one-way ANOVA followed by the Newman-Keuls post-hoc test.
treatment condition of the animal for immobility time (floating with only small movements necessary to keep their head above water), swimming time (pedalling or making circular movements), and struggling time (climbing walls or diving attempts to escape). Water in the tank was changed after each session.

Sucrose preference test

The sucrose preference test was performed the next day after the forced swim test, as described previously, with minor modifications (Mao et al., 2009). In the first phase, rats were placed individually in the cages for an adaptation period to the sucrose solution (1%, w/v) before the start of the test session. Two bottles with a 1% sucrose solution were placed in each cage for the first 24-h period, and then one of the bottles containing the 1% sucrose solution was replaced with tap water for the second 24-h period. After the adaptation period, rats were deprived of water and food for 24 h. The test was performed at 9:00 a.m. with the rat being placed individually in the cages and permitted free access to two bottles, one containing 100 ml of 1% sucrose solution and one containing 100 ml of tap water. After 3 h, the consumed volumes of sucrose solution and tap water were recorded. The reduced sucrose preference, which is used as an index of anhedonia, was calculated according to the following formula: sucrose consumption/[water consumption + sucrose consumption] ×100%.

Electron microscopy for structural analysis of the lateral amygdala

After behavioural testing, five rats per group were anaesthetised with sodium pentobarbital (150 mg/kg, i.p.) and processed for transmission electron microscopy (TEM) analysis. The lateral amygdala (LA) (1 mm × 1 mm × 1 mm) was dissected carefully on ice, rinsed in cold phosphate-buffered saline (PBS) and placed in 2.5% glutaraldehyde at 4 °C for 4 h. The tissue was rinsed in PBS followed by 0.1 M sodium citrate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide for 1 h followed by PBS rinses, undergoing a graded ethanol dehydration series and infiltrated with a mixture of one-half propylene oxide overnight before being embedded in resin. Ultra-thin sections (70 nm thick) were cut on an ultramicrotome (ultracut N; Leica, Germany) and collected on 300 mesh thin-bar copper grids. The sections were stained with 4% uranyl acetate for 20 min and with 0.5% lead citrate for 5 min. Then the ultrastructure of the LA was observed under the transmission electron microscope (Philips Tecnai 20 U-Twin, Holland). For the analysis of the synaptic density, at least 30 micrographs were taken randomly per rat. The estimation of synaptic density used the size-frequency method as described previously (DeFelipe et al., 1999; Merchán-Pérez et al., 2009). The number of synapses per unit volume (Nv) was calculated using the formula \( N_v = N_A/d \), where \( N_A \) is the number of synaptic profiles per unit area and \( d \) is the average cross-sectional length of the synaptic junctions. The measurement was performed by experimenters who blind to the treatment of each group and was assisted by Image J analysis software (National Institutes of Health, Scion Corporation, USA).

Western blot analysis

Western blot analysis was performed as described previously, with minor modifications (Zhang et al., 2012). Briefly, animals were decapitated 24 h after the final behavioural test. The brain of each animal was quickly removed and the LA was carefully dissected on ice. In this study, the LA of each rat (n=6/group) was analysed separately. The LA was immediately homogenized in 600 μl ice-cold lysis buffer that included a cocktail of protease inhibitors and was incubated on ice for 30 min. The homogenate was then centrifuged at 14 000 rpm at 4 °C for 10 min and supernatants were collected. The protein concentration was measured with the BCA protein assay kit (Beyotime, China). The samples were boiled in sample buffer for 5 min and equal amounts of total protein (50 μg per lane) were separated by 10% (15% for BDNF separation) SDS-PAGE gels. After electrophoresis, the separated proteins were transferred onto the PVDF membrane (Millipore) and incubated with blocking buffer PBST (5% non-fat dried milk in PBS containing 0.1% Tween-20) for 1 h at room temperature. Subsequently, the membranes were washed three times and then probed with primary antibodies overnight at 4 °C: polyclonal rabbit anti-BDNF (1:500, Santa Cruz Biotechnology, USA), monoclonal rabbit anti-PSD-95 (1:800, Santa Cruz Biotechnology, Santa Cruz, CA), monoclonal rabbit anti-synaptophysin (1:1000, Cell Signalling Technology, USA) and polyclonal rabbit anti-β-actin (1:5000, Santa Cruz Biotechnology, USA), followed by incubation with the secondary horseradish peroxidase-conjugated antibody (1:5000, Santa Cruz Biotechnology, USA). Immunoactivity was detected using the enhanced chemiluminescence kit (ECL; GE Healthcare, UK). Protein band densities were combined for each groups, normalised to β-actin and assessed using Image-J software (NIH). In this study, the LA of each rat was analysed separately. Final data were presented as a percentage of the control, which was assigned a value of 100%.

Immunofluorescence assay

After behavioural testing, five rats per group were anaesthetised with sodium pentobarbital (150 mg/kg, i.p.) and perfused with 0.1 M phosphate-buffered saline (PBS; pH 7.4; 300 ml) containing 4% paraformaldehyde. Brains were removed and post-fixed in the same solution overnight at 4 °C, then transferred to 30% sucrose (4 °C). The brains were then slowly frozen and cut into serial coronal sections (40 μm). The sections containing the LA were stored in cryoprotectant at −20 °C. For immunooactivity
assay, sections were rinsed with 50 mM potassium phosphate-buffered saline (KPBS) (pH 7.2) three times and incubated with blocking solution (50 mM KPBS, 0.1% bovine serum albumin, 0.2% Triton X-100) for 1 h at room temperature. Then, the cells were incubated overnight at 4 °C with the primary antibody: polyclonal rabbit anti-BDNF (1:500, Santa Cruz Biotechnology, USA), monoclonal rabbit anti-PSD-95 (1:800, Santa Cruz Biotechnology, USA) and monoclonal rabbit anti-synaptophysin (1:1000, Cell Signalling Technology, USA). Thereafter, sections were rinsed again and incubated in the appropriate fluorescent-conjugated secondary antibody (goat anti-mouse/rabbit IgG, 1:200; Sigma-Aldrich) for 1 h. Images were captured on a fluorescence microscope (IX71, Olympus). A consistent threshold was used to select positive staining for each image, but excluded the background staining. The intensity of staining was measured using Image-Pro Plus 6.0 software. All image quantification was performed by taking photomicrographs of at least three bilateral sections from different levels within the LA per animal, and four to five animals per group were analysed. The anatomical landmarks of LA were determined according to the rat brain atlas (Paxinos and Watson, 2005).

Data analysis

All statistical procedures were performed on SPSS version 13.0. The data were expressed as mean±S.E.M generated from at least three independent experiments. A one-way analysis of variance (ANOVA) was performed for multiple comparisons and Newman-Keuls post-hoc tests to identify subsequent pair-wise differences. A p<0.05 was required for results to be considered statistically significant.

Results

Effects of curcumin on the body weight

Body weights were assessed to confirm the efficacy of CUMS and one-way ANOVA revealed that there was a significant difference in groups [F (3, 60)=10.36, p<0.05] (Fig. 1b). Post-hoc analysis indicated that animals exposed to CUMS decreased body weight gain relative to non-stressed animals, while chronic pre-administration of curcumin (40 mg/kg daily) significantly increased the body weight compared to the CUMS group. However, there was no significant difference between the vehicle-treated CUMS group and CUMS group with regard to weight gain (p>0.05).

Effects of curcumin on activity in the open-field test

Horizontal (number of crossings) and vertical (number of rearings) activity was measured in the open-field test. One-way ANOVA revealed that there was a significant difference in the number of crossings [F (3, 60)=11.73, p<0.05] and rearings [F (3, 60)=9.27, p<0.05] in the open-field test (Fig. 2). Post-hoc analysis indicated that a 6-wk CUMS exposure significantly reduced the number of crossings and rearings in rats as compared to the control group. Chronic administration of curcumin (40 mg/kg daily) to CUMS-exposed rats significantly increased the number of crossings and rearings compared to the CUMS-exposed group. These results suggest that curcumin ameliorated the depressed locomotor activities resulting from CUMS exposure. There were no overall significant differences between the vehicle-treated CUMS group and CUMS group with regard to the horizontal and vertical activity (p>0.05).

Effects of curcumin in the forced swim test

The effects of CUMS exposure and curcumin (40 mg/kg) administration on immobility and swimming time in the forced swim test are presented in Fig. 3. One-way ANOVA revealed significant differences in the immobility times [F (3, 60)=17.18, p<0.05] and swimming times [F (3, 60)=15.37, p<0.05] in the forced swim test. Post-hoc analysis indicated that 6-wk CUMS exposure significantly increased immobility time in rats as compared to the non-stressed animals, while daily pre-administration of curcumin at 40 mg/kg daily significantly decreased immobility duration compared to the CUMS group (Fig. 3a). Such decreases in the duration of immobility suggest an antidepressant-like effect of curcumin in this forced swim test. Moreover, curcumin produced a complementary increase in swimming times compared with the CUMS group (Fig. 3b). There were no significant differences among these groups with
regard to struggling behaviour \( F(3, 43)=0.62, p>0.05 \) (Fig. 3c). These findings showed a clear antidepressant-like effect of curcumin in the forced swim test as indicated by the decreased immobility and increased swimming scores.

**Effects of curcumin on the sucrose preference test**

Figure 4 shows the effect of curcumin treatment on sucrose preference in CUMS-exposed rats. The reduced consumption of sucrose solution is an indicator of anhedonia-like behaviour. One-way ANOVA revealed a significant difference in the percentage of sucrose consumption \( F(3, 60)=18.92, p<0.05 \) in the sucrose preference test. Post-hoc analysis indicated that the 6-wk CUMS exposure significantly reduced the percentage of sucrose consumption as compared to the non-stressed group, while chronic pre-treatment with curcumin (40 mg/kg daily) significantly increased the percentage of sucrose consumption compared to the CUMS group. Such increases in the percentage of sucrose consumption suggest a potent antidepressant-like effect of curcumin in these CUMS-exposed rats. There were no significant differences between the vehicle-treated CUMS group and CUMS group with regard to sucrose preference \( p>0.05 \).

**Effects of curcumin on LA ultrastructure**

The ultrastructure of LA neuronal synapses in rats was examined using transmission electron microscopy. The quantification of synapse density in the LA indicated that CUMS-exposure rats had significantly lower numbers of synapses compared with non-stressed rats and
this synapse loss was significantly prevented by curcumin treatment \( [F (3, 16)=14.18, p<0.05] \) (Fig. 5d). As shown in Fig. 5a, the morphology of LA neurons revealed no abnormalities in the control group. In this group, the spine synapses, where post-synaptic density and pre-synaptic vesicles accumulated, were clearly recognised. However, after 6-wk of CUMS exposure, the ultra-structural abnormalities were clearly observed. Now, a decrease in synaptic number density, surface density and thickness of PSD was present in LA neurons (Fig. 5b). In contrast, chronic pre-treatment with curcumin (40 mg/kg daily) induced an increase in these synaptic-associated parameters (Fig. 5c). These ameliorations, when compared to the CUMS group, indicated a significant neuroprotective effect of curcumin, which suggests one of the potential mechanisms for its antidepressant-like effects. Frequently, mitochondrial degradation, rounded nuclei with dispersed chromatin, and vacuoles in the cytoplasm were seen in the CUMS-exposure rats, all of which were also ameliorated by curcumin treatment. There were no apparent differences between the vehicle-treated CUMS group and CUMS group with regard to the ultrastructure of these synapse parameters in the LA (Fig. 5f).

**Effects of curcumin on BDNF protein levels in the LA**

The content of mature BDNF within the LA region was investigated to assess the possible involvement of neurotrophic factors in the anti-depressant effects induced by curcumin. Immunofluorescence assay showed that, after a 6-wk exposure to CUMS, mature BDNF expression within LA regions was decreased, while pre-treatment with curcumin (40 mg/kg daily) significantly ameliorated this CUMS-induced down-regulation of mature BDNF \([F (3, 16)=14.18, p<0.05]\) (Fig. 6a). The immunofluorescence results of mature BDNF expression were further supported by Western blot analysis. One-way ANOVA revealed that LA mature BDNF protein levels differed significantly among the four groups \([F(3, 20)=17.27, p<0.05]\) (Fig. 6b). Post-hoc analysis indicated that exposure to 6-wk CUMS caused a significant decrease in LA mature BDNF protein levels as compared to the control group. In contrast, chronic pre-treatment with curcumin (40 mg/kg daily) significantly reversed this change in LA mature BDNF protein levels as compared to the CUMS-exposed group. No significant differences were observed between the vehicle-treated CUMS group and CUMS group with regard to mature BDNF protein levels in the LA \((p>0.05)\). These results suggest that an additional potential mechanism through which curcumin may produce antidepressant-like effects is by the prevention of the decrease in LA mature BDNF protein expression resulting from CUMS-exposure.

**Effects of curcumin on PSD-95 protein levels in the LA**

To determine whether curcumin treatment affected synaptic-associated proteins in the LA, the expression levels of PSD-95, a major component of post-synaptic densities, was investigated. An immunofluorescence assay was performed to observe intracellular PSD-95 levels in LA regions. The results demonstrated that PSD-95 immunofluorescence in the LA, which was decreased by CUMS-exposure was also reversed by pre-administration of curcumin for six weeks \([F (3, 16)=10.07, p<0.05]\) (Fig. 7a). To confirm the above results, Western blot assays were performed to measure the PSD-95 protein levels. PSD-95 protein levels in the LA differed significantly among the groups as revealed by one-way ANOVA \([F (3, 20)=15.96, p<0.05]\) (Fig. 7b). Post-hoc analysis indicated that 6-wk CUMS exposure caused a significant decrease of PSD-95 protein levels in the LA as compared to the control group. Chronic pre-treatment with curcumin significantly increased PSD-95 protein levels in the LA of CUMS-exposed rats as compared to the CUMS group. No significant differences were observed between the vehicle-treated CUMS group and CUMS group with regard to PSD-95 protein levels in the LA \((p>0.05)\). These findings indicate that the amelioration of PSD-95 expression by curcumin may also be involved in its antidepressant-like effects to CUMS-exposure.

**Effects of curcumin on synaptophysin protein levels in the LA**

Expression changes of LA synaptophysin, a pre-synaptic marker, were also evaluated. The results from immunofluorescence labelling showed that upon exposure to CUMS, there was a decrease in immunofluorescence of synaptophysin, which was significantly reversed by curcumin treatment \([F (3, 16)=12.63, p<0.05]\) (Fig. 8a). These results were further confirmed by Western blot assay, with the one-way ANOVA revealing a significant difference in the content of synaptophysin protein within the LA region \([F(3, 20)=19.83, p<0.05]\) (Fig. 8b). Post-hoc analysis indicated that 6-wk of CUMS-exposure induced a significant decrease of synaptophysin protein levels as compared to the control group. Chronic pre-treatment of CUMS-exposed rats with curcumin (40 mg/kg daily) produced a significant increase in the level of this protein in the LA as compared to the CUMS group. No significant differences were observed between the vehicle-treated CUMS group and CUMS group with regard to the synaptophysin protein levels in the LA \((p>0.05)\). These results indicated a potential role for synaptophysin in the possible mechanisms of antidepressant-like effects of curcumin.

**Discussion**

Despite increasing evidence revealing a relationship between depression and structural plasticity in specific brain regions, no effective therapeutic tools directed toward this possibility have been developed to promote
antidepressant effects. In the present study, we demonstrated that curcumin, a natural polyphenolic compound, can function as a potential agent that suppresses depressive-like behaviour via the prevention of structural and protein changes associated with synaptic plasticity within an area of the brain associated with depression.

Fig. 5. Representative electron micrograph of the lateral amygdala of rats from the control group (non-stressed), CUMS group, curcumin (40 mg/kg)+CUMS group and vehicle (0.1% DMSO, 10 ml/kg)+CUMS group. Arrows indicate structural synapses. Scale bar is 0.5 μM (left) or 1 μM (right). Error bars show s.e.m. *p<0.05 as compared with the control group (non-stressed). #p<0.05 as compared to the CUMS-treated group.
First, we confirmed that CUMS could induce depressive-like behaviours, similar to that of previous findings (Willner, 2005; Luo et al., 2008), and caused structural changes in synapses within the LA. In this way, a valid and reliable model of depression was established. More importantly, long-term treatment of curcumin before stress exposure significantly ameliorated these behavioural and structural changes, as well as dramatically preventing the reductions of mature BDNF, PSD-95 and synaptophysin expressions in the LA as induced by CUMS.

It is generally thought that chronic stress serves as a means to produce depressive-like behaviour in animals as evidenced by behavioural measurements of anhedonia (sucrose preference test) or despair (forced swim test) (Krishnan and Nestler, 2010; Nestler and Hyman, 2010). The reduced sucrose preference is an indicator of anhedonia-like behavioural change, the core symptom of major depressive disorders, while the forced swim test is a standard test usually used to screen compounds for an antidepressant-like effect. The CUMS-induced depression model satisfies these criteria and therefore

Fig. 6. Effects of curcumin on mature BDNF expression in the lateral amygdala of rats. (a) Immunofluorescence analysis of mature BDNF fragmentation in coronal brain sections and histograms showing the fluorescence intensities in the LA neurons of each group (n=5). Scale bar is 200 μM. Images are representative from at least three independent experiments. (B) Western blot analysis. Band 1: control group; Band 2: CUMS group; Band 3: curcumin (40 mg/kg)+CUMS group; Band 4: vehicle (0.1% DMSO, 10 ml/kg)+CUMS group. Normalised intensity bands of BDNF are presented as the means±S.E.M (n=6). *p<0.05 as compared with the control group (non-stressed). #p<0.05 as compared to the CUMS-treated group. Data were analysed with a one-way ANOVA followed by the Newman–Keuls post-hoc test.
can be used for evaluating the efficacy of chronic antidepressant treatments (Schmidt and Duman, 2010; Christiansen et al., 2011). The results of the present study showed that six weeks of CUMS, resulted in depression-like behaviour in rats, as indicated by the significant decrease in sucrose consumption and increase in immobility time in the forced swim test. Long-term treatment with curcumin significantly prevented these behavioural changes, suggesting the antidepressant-like effects of curcumin. Similarly, in the open-field test, CUMS significantly suppressed locomotor activity and rearings, suggesting a loss of exploration and
interest in a novel environment (Katz et al., 1981). This decreased locomotor activity caused by CUMS was prevented by long-term pre-treatment with curcumin. Taken together, results obtained from the behavioural tests indicated that curcumin administration could produce antidepressant-like effects in the CUMS-induced rat model of depression.

In addition, the present study showed that these behavioural antidepressant-like effects of curcumin in the CUMS model of depression were accompanied
with amelioration of structural changes in the LA. The amygdala is a critical structure considered to be associated with depression. Previous results from our laboratory demonstrated that NMDAR-dependent long-term potential (LTP) and long-term depression (LTD) can be reliably induced in the LA, (Yu et al., 2008, 2010), suggesting that the LA is a key locus for synaptic changes. In this present study, the results of ultra-structural assessment by electron microscopy showed that CUMS exposure lead to ultra-structural changes in LA neurons, such as decreases in synaptic number, surface density and thickness of PSD. These findings suggest that the underlying mechanisms of CUMS-induced depressive-like behaviours may involve structural disorders of neurons in specific brain regions. The fact that long-term treatment with curcumin significantly prevented these ultra-structural abnormalities in the LA of CUMS-exposed rats, and ameliorated depressive-like behaviours provides strong evidence that the potential antidepressant mechanisms of curcumin may be associated with its neuroprotective ability to regulate synaptic structural plasticity.

To further study the signalling pathways linked to the synaptic structural changes associated with the antidepressant-like effects of curcumin, we examined the expression of mature BDNF, a neurotrophin that plays an important role in protecting and regulating the functional integrity of neurons throughout life, and is crucial for modulating neuronal plasticity (Huang and Reichardt, 2011). There is growing evidence indicating that neurotrophic factors may function as attractive candidates for the treatment of depression (Karege et al., 2002; Castren et al., 2007) and, it has been reported that, long-term antidepressant treatment restored BDNF to normal levels in chronic stress treated animals (Xu et al., 2006). Here, we report that CUMS exposure significantly decreased BDNF protein levels in the LA, while long-term treatment of curcumin significantly prevented this change in BDNF expression in CUMS-exposed rats. Therefore, the potential neuroprotective effect of curcumin may be associated with its ability to modulate synaptic structural plasticity via BDNF proteins. More interestingly, we also found that the reduced BDNF level was accompanied by a decrease in synaptophysin and PSD-95 levels, and the deficits of these synaptic proteins induced by CUMS were also effectively prevented by curcumin. Mounting evidence has suggested that changes in synaptic function are usually coordinated with an array of structural-associated proteins within the synapse (Christoffel et al., 2011). Synaptophysin is the major pre-synaptic vesicle protein marker that participates in vesicle fusion and synaptic transmission (Greengard et al., 1993). It has been demonstrated that in the absence of synaptophysin behavioural changes and learning deficits are observed in mice (Schmitt et al., 2009). Antidepressant drugs have the capacity to up-regulate the expression of synaptic vesicle proteins (Rapp et al., 2004). In addition, the scaffolding protein PSD-95, a major post-synaptic component of synaptic plasticity, is also significantly decreased in the LA of CUMS-exposed rats. Thus, in this study, the reduction in synaptic proteins in the CUMS model may be responsible for the ultra-structural changes such as the decreased synaptic density. More importantly, long-term treatment with curcumin significantly prevented this CUMS-induced reduction in both synaptophysin and PSD-95 levels. Therefore, changes in these synaptic plasticity-associated proteins, as described above, may underlie changes in functional and structural plasticity associated with depression. Curcumin may activate the BDNF signalling pathway, and thereby regulate the expression of its downstream signalling components, the synaptic plasticity-associated structural proteins of LA neurons and hence prevent depressive-like behaviours in rats.

It should be pointed out that the present study mainly investigated the preventive activity of long-term pre-treatment of curcumin on the CUMS-induced behavioural and neurobiological changes. However, whether curcumin could alter these changes after the depression-type phenotype manifests have not been fully identified. Previous studies have been reported that curcumin influence on moderating hypothalamus-pituitary-adrenal disturbances, protecting against oxidative stress, lowering inflammation and neurodegeneration, all of which are compromised in major depressive disorder (Lopresti et al., 2012). Therefore, the potential molecular mechanism of the antidepressant effects of curcumin would be achieved by further investigation in future studies.

In conclusion, the results of the present study suggest that chronic pre-administration of curcumin is effective in preventing depressive-like behaviour and may possess neuroprotective actions in this CUMS-induced depression model. The study also provides the first in vivo demonstration that the possible mechanism of this protection of curcumin may involve regulating changes in synaptic structural plasticity within the rat amygdala. Given these results, pre-treatment with curcumin has a potential function as a novel antidepressant agent for the amelioration of depression. At the same time, whether curcumin administration has therapeutic effects on depressive-like changes in depression-type phenotype manifest, which are currently under investigation in our laboratory, will provide further insight into this issue.

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Conflict of Interest
None.

References


