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Short- and long-term alterations of FKBP5-GR and specific microRNAs in the prefrontal cortex and hippocampus of male rats induced by adolescent stress contribute to depression susceptibility

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ABSTRACT

Maladaptation of the hypothalamic-pituitary-adrenal (HPA) axis is involved in susceptibility to depression. Glucocorticoid receptors (GRs) and the co-chaperone protein, FK506 binding protein 51 (FKBP5), play crucial roles in dysfunction of the HPA axis. Further, certain microRNAs (miRNAs), such as miR-124a and miR-18a, which could reduce GR protein expression, contribute to affective disorders, while miR-511 as a regulator of FKBP5 is involved in an increased risk of depression. However, the short-term and persistent impacts of adolescent stress on miR-124a, miR-18a, and miR-511 expressions in the brain are unknown. Using depression models of chronic unpredictable mild stress (CUMS) or dexamethasone administration of adolescent rats, the authors of the present study probed the depressive-like behaviors, GR and FKBP5 expressions, and miR-124a, miR-18a, and miR-511 expressions in the prefrontal cortex and hippocampus. The GR antagonist RU486 was used as intervention. The results revealed that both CUMS and dexamethasone administration in the adolescent period resulted in anhedonia, altered locomotor behaviors, anxiety, and cognitive impairment. A remarkable decrease in GR expression, and increase in FKBP5, miR-124a, and miR-18a expressions were detected in the prefrontal cortex and hippocampus of adolescent rats. Furthermore, the similar long-term changes on behaviors and expressions of GR, FKBP5 and GR-related microRNAs were found in the adult rats following CUMS and dexamethasone treatment in adolescence. However, reduced miR-511 expression was observed only in the prefrontal cortex of adult rats exposed to adolescent CUMS or dexamethasone administration. These data suggested that the downregulation of GR, upregulation of FKBP5, miR-124a, and miR-18a in the prefrontal cortex and hippocampus, and downregulation of miR-511 in the prefrontal cortex were relevant to depressive-like behaviors.

1. Introduction

Depression is a highly prevalent mood disorder with different population risks. Exposure to early life adversities is considered a major risk factor for the onset and development of affective disorders (Heim and Binder, 2012; Heim and Nemeroff, 2001). In particular, adolescence is a critical window of brain development; during this stage, the brain is sensitive to stress reactivity, which heightens vulnerability to psychiatric disorders later in life (Lo Iacono and Carola, 2017; Yohn and Blendy, 2017). It was reported that childhood adversities accounted for 29.8% of all mental disorders (Kessler et al., 2010). Recent rodent studies demonstrated that juvenile social isolation stress increased depressive-like behaviors in adult mice (Lo Iacono et al., 2015). Likewise, a meta-analysis showed that childhood maltreatment such as child abuse or neglect elevated the risk of depression and anxiety disorders in adulthood (Li et al., 2016).

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is the most consistent and prominent neuroendocrine abnormality in depression. Glucocorticoids (corticosterone in rodents and cortisol in humans) secreted by the adrenal cortex mediate their actions on feedback regulation of HPA axis primarily through binding to glucocorticoid receptors (GRs) when levels of glucocorticoids are high. Failed negative feedback of the HPA axis via GRs induced by constant stress was found in depressive animal models and depressed patients (Pariante, 2009; Pariante and Lightman, 2008). Other studies and our previous data showed that early life stress reduced GR and GR mRNA

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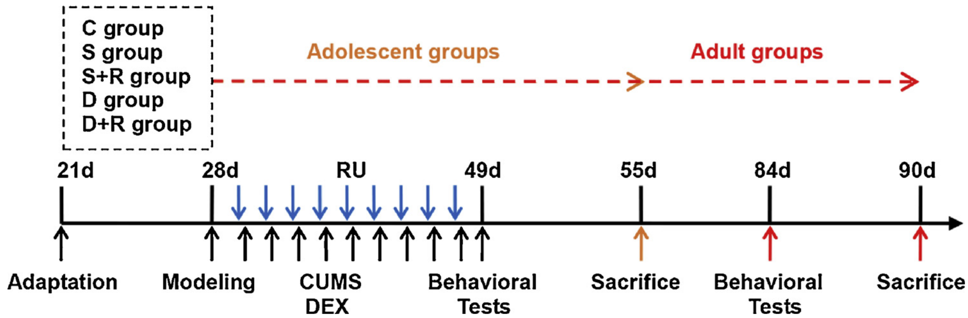

Fig. 1. Schematic diagram of the experimental procedure.

expression in the brain (Arabadzisz et al., 2010; Ladd et al., 2004; Xu et al., 2017). Further, chronic corticosterone or dexamethasone treatment in rodents induced depressive-like behaviors and reduced expression levels of GR protein and mRNA in the frontal cortex and hippocampus (Hu et al., 2016; Siopi et al., 2016; Zhao et al., 2018). Those studies indicated that GR dysfunction was the key element of HPA maladaptation. More importantly, FK506 binding protein 51 (FKBP5) acts as a co-chaperone of GR, which modulates GR sensitivity that interferes with GR-mediated feedback of the HPA axis. FKBP5 is able to bind to the GR complex (FKBP5-GR) via heat shock protein 90 (HSP90), which reduces the receptor's affinity for cortisol and restricts its nuclear translocation (Binder, 2009). Further, GR activation induces increased FKBP5 transcription, thus creating an intracellular ultra-short negative feedback loop for GR sensitivity (Vermeer et al., 2003). Research revealed that chronic mild stress increased FKBP5 levels and deceased nuclear GR levels in rodents, and the abnormities could be counteracted by antidepressant treatment (Xing et al., 2015). In addition, FKBP5 single nucleotide polymorphisms (SNPs) interacting with childhood adversity could predict a higher risk of depression (Tozzi et al., 2016). Our previous study also indicated that early adolescent stress and dexamethasone administration decreased GR expression and increased FKBP5 expression in the basolateral amygdala and persisted into adulthood (Xu et al., 2017). Collectively, these findings suggested that GR/FKBP5 was involved in HPA axis dysregulation and played crucial roles in susceptibility to depression.

Micro-RNA (miRNA) is able to bind with the 3′-untranslated regions (3′-UTRs) of the target messenger RNAs (mRNAs) to regulate their expression by either repressing translation or inducing degradation of mRNA (Huntzinger and Izaurralde, 2011). Some miRNAs such as miR-124a in the corpus callosum and miR-18a in the hypothalamus were confirmed to be involved in stress-induced HPA activities (Shimizu et al., 2015a, b). Both miR-124a and miR-18a repressed GR activity and reduced GR protein expression in the brain (Uchida et al., 2008; Vreugdenhil et al., 2009). Meanwhile, a recent study demonstrated that miR-511 mimic reduced FKBP5 mRNA level and the increased level of FKBP5 protein induced by glucocorticoids, which indicates that miR-511 was a gene-related regulator of FKBP5 (Zheng et al., 2016). Studies reported that patients with depression had higher levels of miR-511 in the basolateral amygdala (Maheu et al., 2015) and that long-term 0.1 mg/ml corticosterone treatment upregulated miR-511 expression in the cortex of mice (Zheng et al., 2016). In contrast, one study found depressed subjects had reduced miR-511 expression in the prefrontal cortex (Smalheiser et al., 2012). Therefore, whether miR-511 is associated with depression susceptibility is still unclear.

The neural circuit including the amygdala, prefrontal cortex, and hippocampus is associated with the regulation of emotion and cognition, as well as HPA-axis responses to stress (Drevets et al., 2008; Herman et al., 2005). Our previous study found that both chronic unpredictable mild stress (CUMS) and dexamethasone treatment in the adolescent period induced decreased GR expression and increased FKBP5 and miR-124a expressions in the basolateral amygdala of both adolescent and adult rats, while the increased miR-18a expression in the basolateral amygdala was found only in adolescent rats (Xu et al., 2017), which indicated that the regulation of miRNAs on GR/FKBP5 during the stress reaction had age- and region-specific brain associations. Therefore, the first aim of the study was to investigate the short-term and persistent effects of adolescent CUMS and dexamethasone administration on depressive-like behaviors, GR and FKBP5 expressions in the prefrontal cortex and hippocampus. Second, the study probed the alterations of miR-124a and miR-18a that could repress GR expression and of miR-511 that might reduce FKBP5 expression in the prefrontal cortex and hippocampus. In addition, the effect of RU486, the antagonist of GR, on the aforementioned abnormalities was explored.

2. Materials and methods

2.1. Experimental animals and grouping

A total of 100 male Wistar rats (55–65 g, 21 days old) were obtained from the Experimental Animal Center of Shandong University. Rats were housed five per cage under standard laboratory conditions (23 ± 2 °C, 12-hour light/dark cycle) with food and water available ad libitum. All experimental procedures were performed with the approval of the Ethics Committee of the School of Medicine, Shandong University and in compliance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85-23, revised 1985).

After 7 days of acclimatization, the animals were randomly divided into five groups (20 in each group): control group (C), CUMS group (S), CUMS and RU486 group (S + R), dexamethasone group (D), and dexamethasone and RU486 group (D + R). After 3-week CUMS or drug administration, half of the rats of each group were sacrificed after behavioral tests at the age of 55 days, while the others were raised into adulthood and sacrificed after behavioral tests at the age of 90 days (see Fig. 1). Therefore, the current study consisted of 10 groups: adolescent control group (AdoC), adolescent CUMS group (AdoS), adolescent CUMS and RU486 group (AdoS + R), adolescent dexamethasone group (AdoD), adolescent dexamethasone and RU486 group (AdoD + R), adult control group (AduC), adult CUMS group (AduS), adult CUMS and RU486 group (AduS + R), adult dexamethasone group (AduD), and adult dexamethasone and RU486 group (AduD + R).

2.2. Chronic unpredictable mild stress

CUMS was performed to establish an animal model of depression according to previous studies (Cai et al., 2015; Shang et al., 2017). Rats in the S and S + R groups were exposed to various chronic unpredictable mild stressors according to a random schedule for 21 consecutive days. The stressors were included as follows: food deprivation for 24 h; water deprivation for 24 h; noise (92 dB, 1500 Hz) for 2 h; pinching tail for 1 min; hot stress in oven at 45 °C for 5 min; day and night reversal for 24 h; and unpredictable foot shocks (1 mA, one shock/5 s, 10-s duration for a total of 10 min). The stressors were applied once daily at random times in order to be completely unpredictable, and each stressor was applied 3 times during the CUMS procedure.

2.3. Drug administration

Rats in the S + R group were treated with a single daily administration of RU486 at 10 mg/kg 30 min before the CUMS procedure (Wulsin et al., 2010). Rats in the D group received a single daily injection of dexamethasone at 1.5 mg/kg (Sigwalt et al., 2011). Rats in the D + R group were treated with RU486 30 min before the application of dexamethasone. All drugs (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in propylene glycol and administrated subcutaneously at a dose of 1 ml/kg body weight for consecutive 21 days. Rats in the non-drug groups and one-time drug administration groups received an equivalent volume of propylene glycol to run in parallel.

2.4. Behavioral tests

Behavioral tests were carried out 24 h after the last stressor and drug treatment. The order of behavioral tests in each animal was the same, as follows: (1) sucrose preference test (SPT), (2) open field test (OFT), (3) elevated plus maze (EPM) test, and (4) Morris water maze (MWM) test.

2.4.1. Sucrose preference test

The SPT was conducted to evaluate an anhedonia state according to a previous study (Willner et al., 1987). Before the test, all animals were trained to habituate to sucrose solution (1%, w/v). Briefly, two bottles of 1% sucrose solution were placed in each cage for 24 h; then one of the bottles was replaced with tap water for 24 h. After adaptation, animals were deprived of water and food for 23 h. On the day of test, each rat was given a free choice with two bottles for 1 h, one with 1% sucrose solution and the other with tap water. In order to avoid possible effects of place preference, positions (left and right) of the two bottles were switched every 30 min during the test. The bottles were weighed before and after the test, and the sucrose preference percentage was calculated as the sucrose preference percentage (%) = sucrose consumption / (sucrose consumption + water consumption).

2.4.2. Open field test

The OFT was used to measure exploratory and anxiety behaviors (Walsh and Cummins, 1976). Each animal was placed in the center of the apparatus and allowed to explore freely for 5 min. Behaviors of rats were monitored and recorded by use of a SMART video tracking system. The apparatus was cleaned with 75% ethanol and dried after each behavioral recording to eliminate the residual odor of the last rat. Locomotor activity was scored as the locomotor activity (n) = the number of crossings + the number of rearings.

2.4.3. Elevated plus maze

The EPM test was performed to assess anxiety-like behaviors (Pellow et al., 1985). The apparatus consisted of two opposite open arms, two opposite closed arms, and a central square area. Each rat was placed in the central square area of the maze facing an open arm and allowed free exploration in the maze for 5 min. Behaviors of rats in the maze were recorded by the SMART video tracking system. The apparatus was cleaned with 75% ethanol to remove odors after each trial. The percentage of time spent in the open arms was analyzed to assess the anxiety level.

2.4.4. Morris water maze

The MWM test was generally carried out to evaluate spatial learning and memory ability (Morris, 1981). The water maze was a cylindrical pool (120 cm in diameter, 50 cm in height) equipped with a platform 2 cm underneath the water surface. During a 5-day training session, all animals were trained 4 times daily to find the platform within 60 s. In each training session, the animal was placed into the water facing the wall of the pool from a different quadrant. If the rat failed to climb onto the platform with 60 s, it was guided to the platform and permitted to stay there for 15 s. On the sixth day, the probe test was conducted with the absence of the platform and the original platform quadrant was defined as the target quadrant. The rat was placed into the opposite quadrant of the target quadrant for a free 60-s probe. The tracking system was used to record the rats' activities. The percentage of time spent in the target quadrant was analyzed.

2.5. Immunohistochemistry

2.5.1. Tissue preparation

Four rats of each group were deeply anesthetized with pentobarbital sodium and transcardially perfused with saline solution and 4% paraformaldehyde in phosphate buffer (0.1 mol/L, pH 7.4). After perfusion, the whole brain was completely taken out and fixed in 4% paraformaldehyde at 4 °C for 24 h. Then, paraffin-embedded prefrontal cortex (from bregma 4.7 mm to 4.2 mm) and hippocampus (from bregma –2.5 mm to –3.0 mm) tissues were sliced into 4-μm coronal sections.

2.5.2. GR and FKBP5 immunohistochemistry

First, the sections were deparaffinized, dehydrated, and immersed in citrate buffer (0.01 mol/L, pH 6.0) in a microwave oven to retrieve GR and FKBP5 antigens. Then, the sections were incubated with 3% hydrogen peroxide for 10 min at room temperature. Subsequently, the sections were blocked with normal goat serum and incubated at 4 °C overnight with primary antibodies: anti-GR (1:250, Santa Cruz Biotechnology) and anti-FKBP51 (1:250, Santa Cruz Biotechnology). The next day, sections were incubated with biotinylated goat anti-rabbit secondary antibodies for 30 min at 37 °C. Sections were visualized with diaminobenzidine under a microscope. Finally, the sections were dehydrated, hyalinized, and mounted. Images were photographed and saved using a digital microscope camera (Zeiss Axiocam MRc5).

2.6. Western blotting

2.6.1. Tissue collection

Six rats of each group were decapitated, and the brains were rapidly removed from the skull and dissected on ice. The prefrontal cortex (from bregma 4.7 mm to 2.5 mm) and whole hippocampus (one side used for Western blotting, the other side used for real-time polymerase chain reaction [PCR]) were quickly dissected using a stainless steel brain matrices according to the stereotaxic atlas of the rat brain and maintained at −80 °C for further protein and RNA extraction.

2.6.2. Protein extraction

The tissue samples were weighted and homogenized in an appropriate amount of lysis buffer and phenylmethanesulfonyl fluoride (PMSF). Then, the supernatants were collected after centrifugation at 12,000 rpm for 25 min at 4 °C. Protein concentration was analyzed with use of the BCA Protein Assay Kit (Beyotime Institute of Biotechnology).

2.6.3. GR and FKBP5 Western blotting

The supernatants were adjusted to the same concentration with lysis buffer and mixed with a 5× sample loading buffer. Equal amounts of proteins were boiled at 100 °C for 5 min. Then, 20 μg protein in each lane was separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, MA). After blocked in 5% bovine serum albumin (BSA) in Tris-buffered saline Tween-20 (TBST) for 1 h at room temperature, the membranes were incubated overnight with primary antibodies at 4 °C. The following antibodies were used: anti-GR (1:200, Abcam, USA), anti-FKBP51 (1:1000, Abcam, USA), and GAPDH (1:5000, Biogot Biotechnology Co., Ltd, China). On the next day, the membranes were washed in TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000; ZSGB-BIO, China) for 1 h at room temperature. Then, the membranes were washed again and visualized on photographic films by using a chemiluminescent method (Millipore, MA). The immunoblots were analyzed with the use of Image J 14.0 software. The relative levels of GR and FKBP5 were normalized to GAPDH.

2.7. Real-time PCR assay

Total RNA extracted from homogenized prefrontal cortex and hippocampus in Trizol reagent (Invitrogen, CA, USA) was used for detecting the expression of miR-124a, miR-18a, and miR-511 by means of real-time PCR. RNA concentration and purity were measured by using the NanoDrop ND-1000 (Nanodrop Technologies, Wilmington, DE, USA). Then, RNA was reverse-transcribed to cDNA by using with the corresponding primers on the Applied Biosystems' GeneAmp PCR System 9700, and real-time PCR was carried out by using the ABI PRISM 7900 system (Applied Biosystems). PCR conditions were at 95 °C for 10 min, and then 40 amplification cycles (95 °C for 10 s, 60 °C for 60 s). The $2^{-\Delta\Delta CT}$ method was applied to analyze relative gene expression. Relative quantification of miR-124a, miR-18a, and miR-511 was normalized against their corresponding U6 as an internal standard. Real-time PCR experiments were performed at Kangchen Bio-Tech (Shanghai, China).

2.8. Statistical analysis

All the statistical analyses were performed by using SPSS 19.0 software. Data for the adolescent groups and adult groups were processed separately. Significant differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's post hoc test. All graphical results were presented as mean ± SEM, and the level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Both adolescent stress and dexamethasone administration induced depressive-like behaviors

Fig. 2A shows the anhedonia behaviors in the SPT of both adolescent and adult rats. Both adolescent stress and dexamethasone administration resulted in a reduced percentage of sucrose consumption in adolescent rats [F(4, 45) = 41.011, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [F(4, 45) = 49.871, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486 elevated the percentage of sucrose consumption in both adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively.

Fig. 2B shows the locomotor activity in the OFT of both adolescent and adult rats. Both adolescent stress and dexamethasone administration reduced the locomotor activity in adolescent rats [F(4, 45) = 13.219, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [F(4, 45) = 10.735, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with control animals. RU486-treated rats in both adolescent groups ($P < 0.01$, $P < 0.01$) and adult groups ($P < 0.01$, $P < 0.01$) exhibited increased locomotor activity compared with the corresponding S group and D group, respectively.

Fig. 2C shows the anxiety-like behaviors in the EPM test of both adolescent and adult rats. Both adolescent stress and dexamethasone administration decreased the percentage of time spent in the open arms in adolescent rats [F(4, 45) = 5.764, $P = 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.05$] and adult rats [F(4, 45) = 6.808, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with the corresponding controls. RU486 augmented the percentage of time spent in the open arms in both adolescent rats ($P < 0.01$, $P < 0.05$) and adult rats ($P < 0.01$, $P < 0.05$) compared with the corresponding S group and D group, respectively.

Fig. 2D and E show memory impairment in the MWM test of both adolescent and adult rats. Both adolescent stress and dexamethasone administration reduced the percentage of time spent in the target quadrant in adolescent rats [F(4, 45) = 4.864, $P = 0.002$; post hoc, AdoS vs AdoC, $P < 0.05$, AdoD vs AdoC, $P < 0.01$] and adult rats [F(4, 45) = 4.147, $P = 0.006$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486 improved the percentage of time spent in the target quadrant in both adolescent rats ($P < 0.05$, $P < 0.01$) and adult rats ($P < 0.05$, $P < 0.05$) compared with the corresponding S group and D group, respectively.

3.2. Both adolescent stress and dexamethasone administration decreased GR expressions in the prefrontal cortex and hippocampus

3.2.1. GR expressions in the prefrontal cortex and hippocampus in immunohistochemistry

Fig. 3A shows the GR expression and the ratio of GR-positive cells in the prefrontal cortex detected by immunohistochemistry with use of the high-power microscope (400×). Both adolescent stress and dexamethasone administration decreased the percentage of GR-positive cells in the prefrontal cortex of adolescent rats [F(4, 15) = 18.588, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [F(4, 15) = 21.986, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with control rats. Conversely, RU486 upregulated the percentage of GR-positive cells in the prefrontal cortex of both adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. Fig. 3B-D show GR expressions and the ratio of GR-positive cells in the CA1, CA3 and the dentate gyrus (DG) of hippocampus detected by immunohistochemistry with use of the high-power microscope (400×). As shown in Fig. 3B, both adolescent stress and dexamethasone administration decreased the ratio of GR-positive cells in the hippocampal CA1 subregion of adolescent rats [F(4, 15) = 3.384, $P = 0.037$; post hoc, AdoS vs AdoC, $P < 0.05$, AdoD vs AdoC, $P < 0.05$] and adult rats [F(4, 15) = 16.398, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with control groups. However, a higher ratio of GR-positive cells was observed in the hippocampal CA1 subregion of the adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. As shown in Fig. 3C, both adolescent stress and dexamethasone administration decreased the ratio of GR-positive cells in the hippocampal CA3 subregion of adolescent rats [F(4, 15) = 101.776, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [F(4, 15) = 6.252, $P = 0.004$; post hoc, AduS vs AduC, $P < 0.05$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486-treated rats demonstrated a higher ratio of GR-positive cells in the hippocampal CA3 subregion of both adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.05$, $P < 0.01$) compared with the corresponding S group and D group, respectively. As shown in Fig. 3D, both adolescent stress and dexamethasone administration decreased the ratio of GR-positive cells in the DG of hippocampus in the adolescent rats [F(4, 15) = 44.170, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [F(4, 15) = 19.530, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486 increased the ratio of GR-positive cells in the DG of hippocampus in both the adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively.

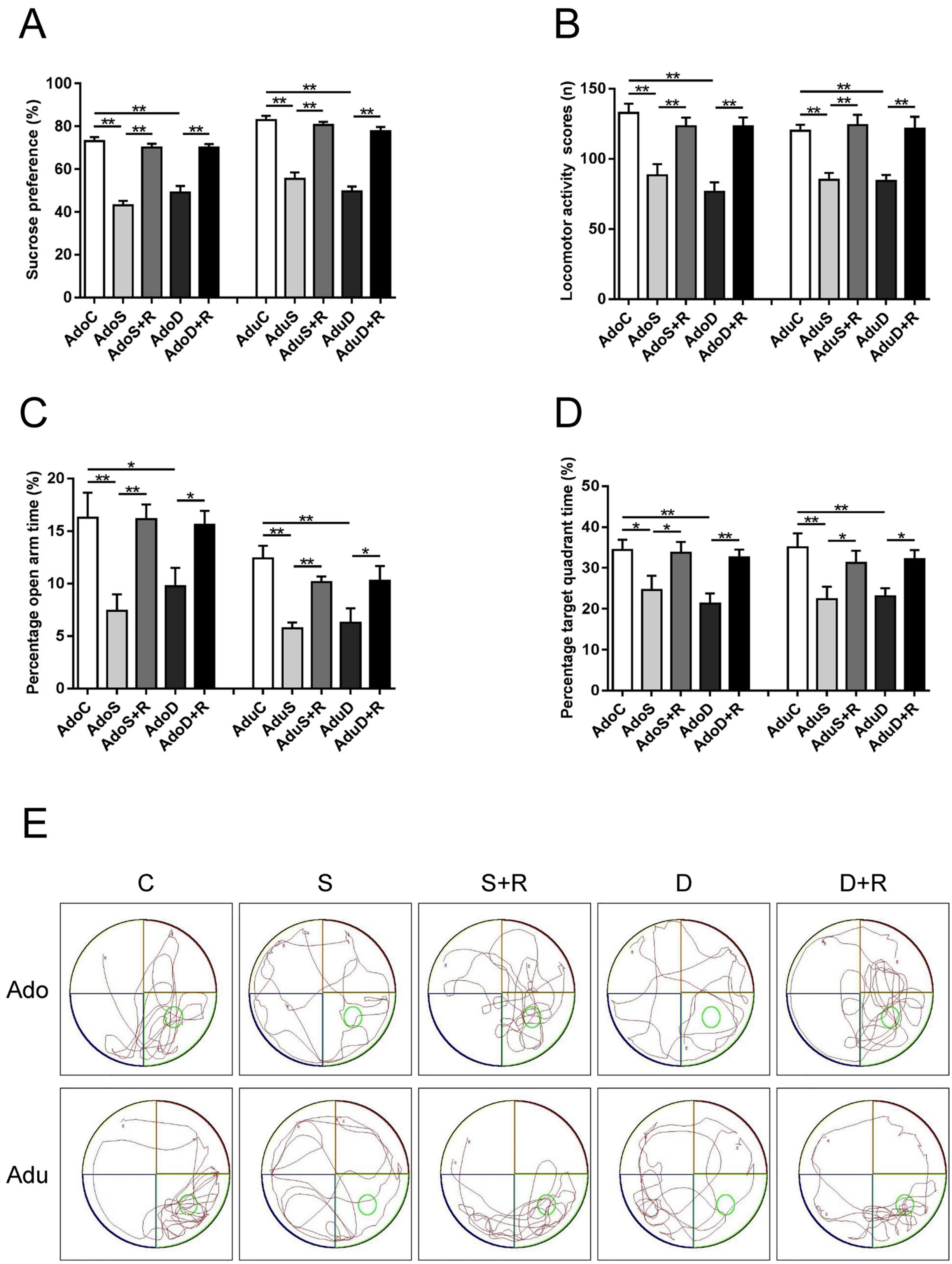

Fig. 2. Behavioral and memory changes of adolescent and adult rats. (A) Sucrose preference in the SPT; (B) Locomotor activity in the OFT; (C) The percentage of open arm time in the EPM test; (D) The percentage of time in the target quadrant in the MWM test; (E) Tracks of different groups in the MWM probe trial. Results were presented as mean ± SEM (n = 10 each group). $^*P < 0.05$, $^{**}P < 0.01$.

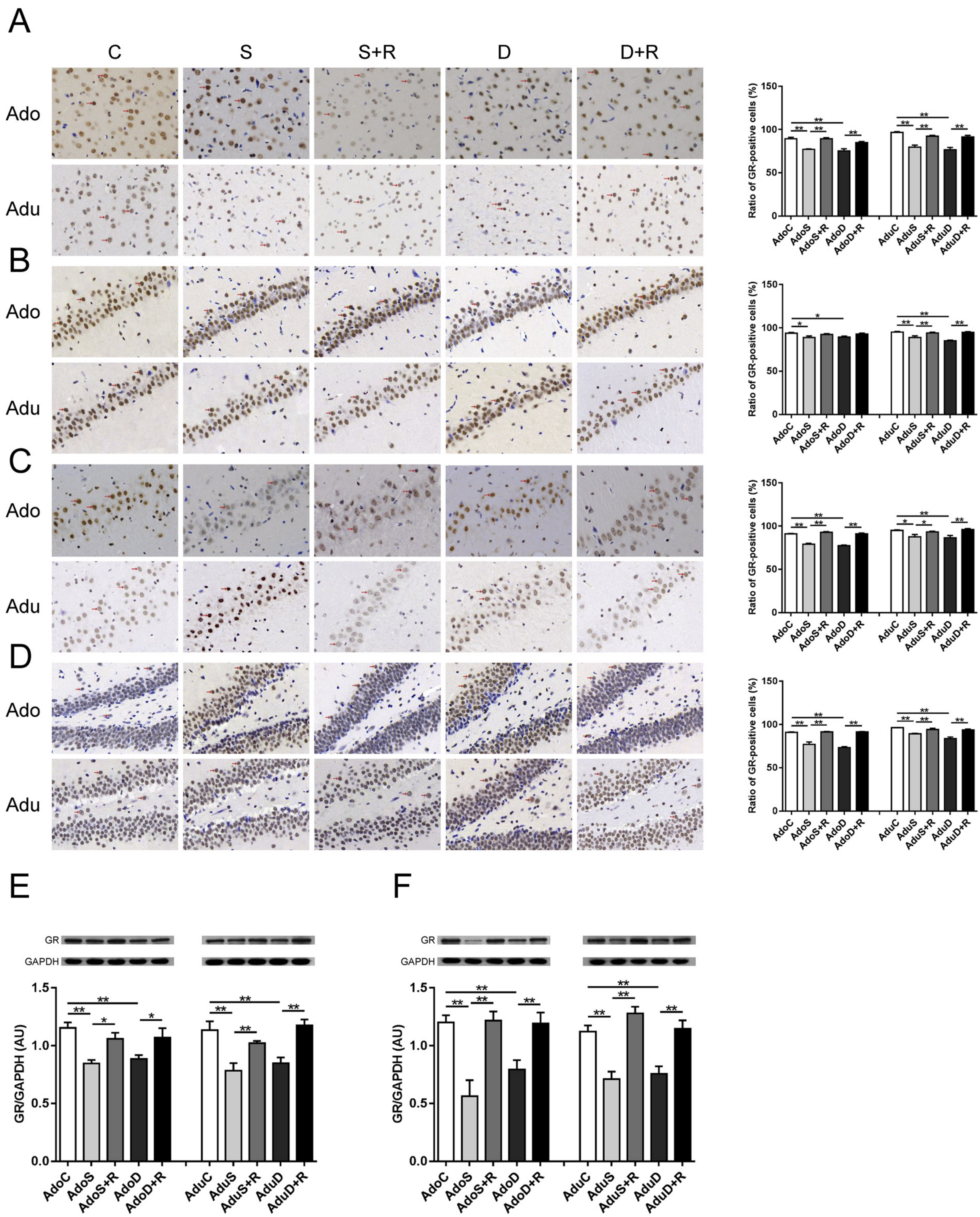

Fig. 3. GR expressions in the prefrontal cortex and hippocampus of adolescent and adult rats. (A) Representative pictures of GR expression and data analysis of GR-positive cells in the prefrontal cortex detected by immunohistochemistry; (B) Representative pictures of GR expression and data analysis of GR-positive cells in the CA1 subregion of the hippocampus detected by immunohistochemistry; (C) Representative pictures of GR expression and data analysis of GR-positive cells in the CA3 subregion of the hippocampus detected by immunohistochemistry; (D) Representative pictures of GR expression and data analysis of GR-positive cells in the DG subregion of the hippocampus detected by immunohistochemistry. Values were expressed as mean ± SEM (n = 4 each group). *$P < 0.05$, **$P < 0.01$. (E) GR expression in the prefrontal cortex detected by Western blotting; (F) GR expression in the hippocampus detected by Western blotting. Results were expressed as mean ± SEM (n = 6 each group). *$P < 0.05$, **$P < 0.01$.

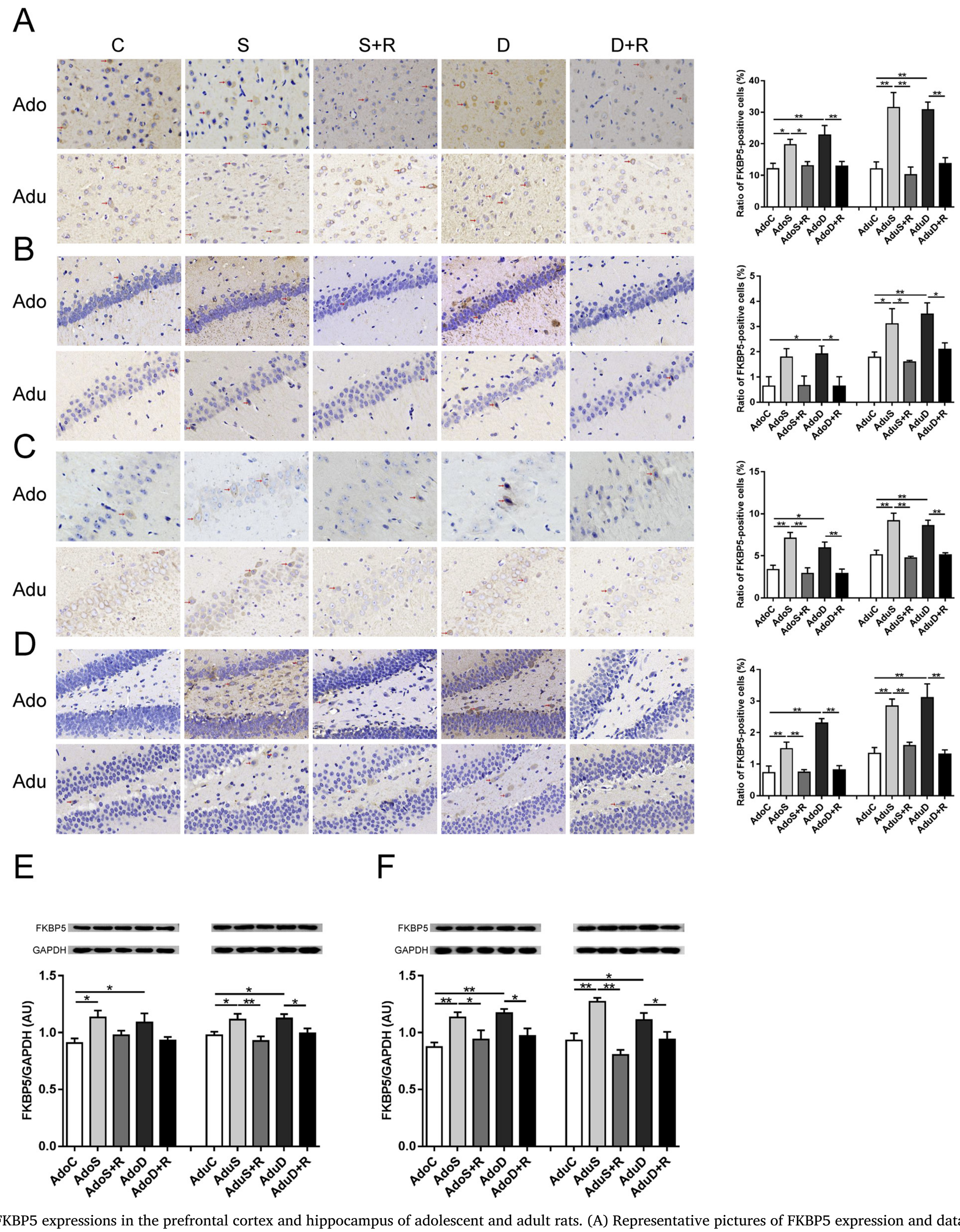

Fig. 4. FKBP5 expressions in the prefrontal cortex and hippocampus of adolescent and adult rats. (A) Representative pictures of FKBP5 expression and data analysis of FKBP5-positive cells in the prefrontal cortex detected by immunohistochemistry; (B) Representative pictures of FKBP5 expression and data analysis of FKBP5-positive cells in the CA1 subregion of the hippocampus detected by immunohistochemistry; (C) Representative pictures of FKBP5 expression and data analysis of FKBP5-positive cells in the CA3 subregion of the hippocampus detected by immunohistochemistry; (D) Representative pictures of FKBP5 expression and data analysis of FKBP5-positive cells in the DG subregion of the hippocampus detected by immunohistochemistry. Values were presented as mean ± SEM (n = 4 each group). $*P < 0.05$, $**P < 0.01$. (E) FKBP5 expression in the prefrontal cortex detected by Western blotting; (F) FKBP5 expression in the hippocampus detected by Western blotting. Results were expressed as mean ± SEM (n = 6 each group). $*P < 0.05$, $**P < 0.01$.

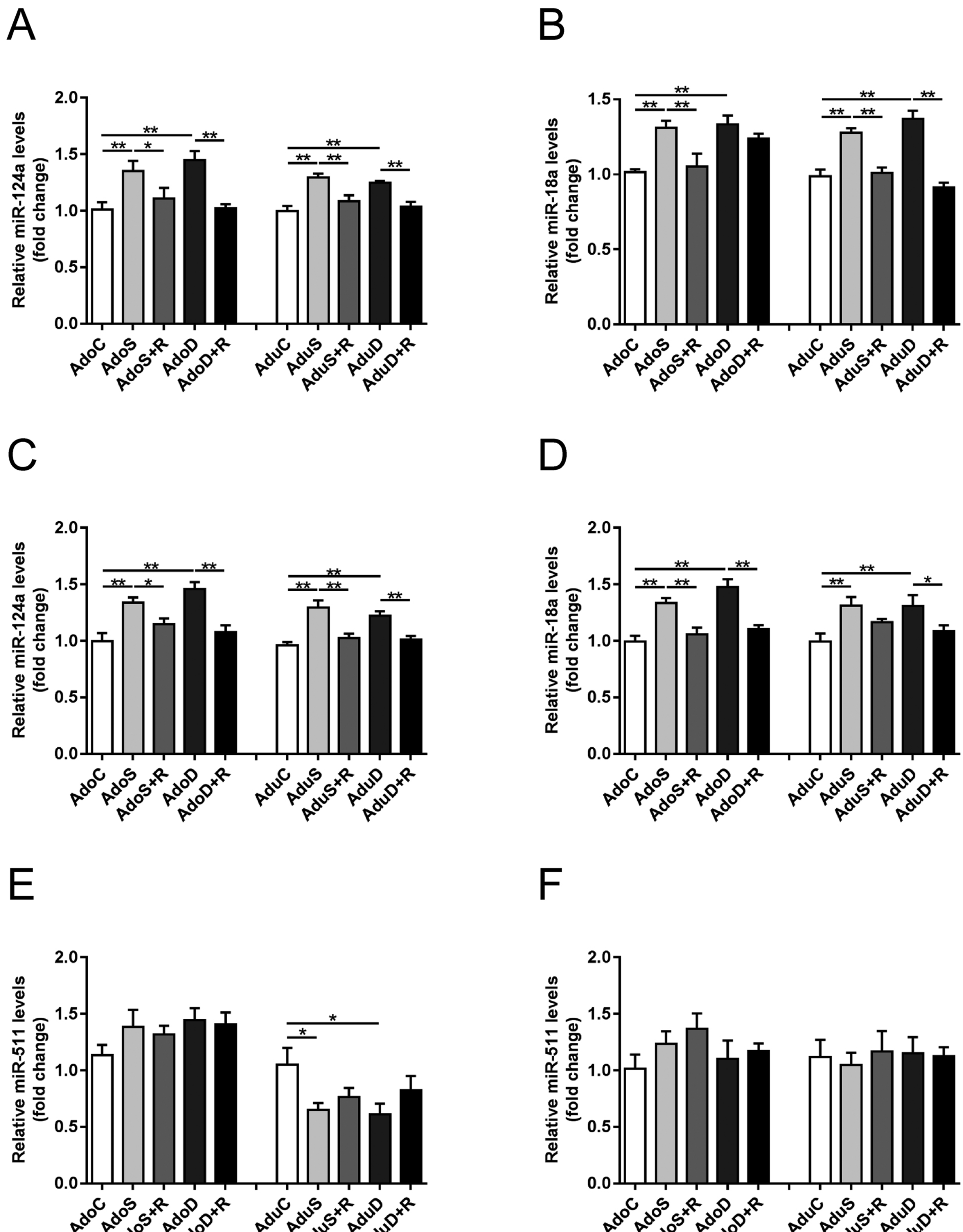

Fig. 5. Expressions of miR-124a, miR-18a, and miR-511 detected by real-time PCR. (A) Relative miR-124a levels in the prefrontal cortex; (B) Relative miR-18a levels in the prefrontal cortex; (C) Relative miR-124a levels in the hippocampus; (D) Relative miR-18a levels in the hippocampus; (E) Relative miR-511 levels in the prefrontal cortex; (F) Relative miR-511 levels in the hippocampus. Data were presented as mean ± SEM (n = 6 each group). *$P < 0.05$, **$P < 0.01$.

3.2.2. GR expressions in the prefrontal cortex and hippocampus in Western blotting

Fig. 3E and F show GR expressions in the prefrontal cortex and hippocampus detected by Western blotting. As shown in Fig. 3E, both adolescent stress and dexamethasone administration induced decreased prefrontal cortex level of GR in adolescent rats [$F(4, 25) = 5.867$, $P = 0.002$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 9.462$, $P < 0.001$; post hoc, AduS vs AduC,

$P < 0.01$, AduD vs AduC, $P < 0.01$] compared with control rats. Conversely, RU486 recovered the GR level in the prefrontal cortex of both adolescent rats ($P < 0.05$, $P < 0.05$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. As shown in Fig. 3F, both adolescent stress and dexamethasone administration decreased the hippocampus level of GR in adolescent rats [$F(4, 25) = 9.936$, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 15.978$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486-treated rats manifested a higher GR level in the hippocampus of both adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively.

3.3. Both adolescent stress and dexamethasone administration increased FKBP5 expressions in the prefrontal cortex and hippocampus

3.3.1. FKBP5 expressions in the prefrontal cortex and hippocampus in immunohistochemistry

Fig. 4A shows the expression of FKBP5 and the ratio of FKBP5-positive cells in the prefrontal cortex detected via immunohistochemistry with use of the high-power microscope (400×). Both adolescent stress and dexamethasone administration induced a higher percentage of FKBP5-positive cells in the prefrontal cortex of adolescent rats [$F(4, 15) = 6.080$, $P = 0.004$; post hoc, AdoS vs AdoC, $P < 0.05$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 15) = 12.936$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. Conversely, RU486 downregulated the percentage of FKBP5-positive cells in the prefrontal cortex of both adolescent rats ($P < 0.05$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. Fig. 4B-D show FKBP5 expressions and the ratio of FKBP5-positive cells in the CA1, CA3 and the DG of hippocampus detected by immunohistochemistry with use of the high-power microscope (400×). As shown in Fig. 4B, adolescent dexamethasone administration increased the ratio of FKBP5-positive cells in the hippocampal CA1 subregion of the AdoD group [$F(4, 15) = 3.391$, $P = 0.036$; $P < 0.05$] and adult rats [$F(4, 15) = 5.126$, $P = 0.008$; post hoc, AduS vs AduC, $P < 0.05$, AduD vs AduC, $P < 0.01$] compared with control groups. However, a lower ratio of FKBP5-positive cells was observed in the hippocampal CA1 subregion of the AdoD group ($P < 0.05$) and adult rats ($P < 0.05$, $P < 0.05$) compared with the corresponding S group and D group, respectively. As shown in Fig. 4C, both adolescent stress and dexamethasone administration upregulated the ratio of FKBP5-positive cells in the hippocampal CA3 subregion of adolescent rats [$F(4, 15) = 9.421$, $P = 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.05$] and adult rats [$F(4, 15) = 14.199$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with control rats. However, RU486-treated rats manifested a lower ratio of FKBP5-positive cells in the hippocampal CA3 subregion of both adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. As shown in Fig. 4D, both adolescent stress and dexamethasone administration induced an increased ratio of FKBP5-positive cells in the DG of hippocampus in the adolescent rats [$F(4, 15) = 17.439$, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 15) = 12.268$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486 decreased the ratio of FKBP5-positive cells in the DG of hippocampus in both the adolescent rats ($P < 0.01$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively.

3.3.2. FKBP5 expressions in the prefrontal cortex and hippocampus in Western blotting

Fig. 4E and F show FKBP5 expressions in the prefrontal cortex and hippocampus detected by Western blotting. As shown in Fig. 4E, both adolescent stress and dexamethasone administration induced an increased prefrontal cortex level of FKBP5 in adolescent rats [$F(4, 25) = 3.420$, $P = 0.023$; post hoc, AdoS vs AdoC, $P < 0.05$, AdoD vs AdoC, $P < 0.05$] and adult rats [$F(4, 25) = 4.567$, $P = 0.007$; post hoc, AduS vs AduC, $P < 0.05$, AduD vs AduC, $P < 0.05$] compared with controls. However, RU486 downregulated the FKBP5 level only in the prefrontal cortex of adult rats ($P < 0.01$, $P < 0.05$) compared with the corresponding S group and D group, respectively. As shown in Fig. 4F, both adolescent stress and dexamethasone administration induced an increased hippocampus level of FKBP5 in adolescent rats [$F(4, 25) = 5.147$, $P = 0.004$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 10.397$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.05$] compared with controls. Conversely, RU486 restored FKBP5 level in the hippocampus of both adolescent ($P < 0.05$, $P < 0.05$) and adult rats ($P < 0.01$, $P < 0.05$) compared with the corresponding S group and D group, respectively.

3.4. Effects of adolescent stress and dexamethasone administration on miR-124a, miR-18a, and miR-511 expressions in the prefrontal cortex and hippocampus

3.4.1. Expressions of miR-124a and miR-18a in the prefrontal cortex and hippocampus in real-time PCR

Fig. 5A and B show levels of miR-124a and miR-18a in the prefrontal cortex detected by real-time PCR. As shown in Fig. 5A, both adolescent stress and dexamethasone administration elevated prefrontal cortex levels of miR-124a in adolescent rats [$F(4, 25) = 6.974$, $P = 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 11.055$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486-treated rats displayed decreased miR-124a levels in the prefrontal cortex of both adolescent rats ($P < 0.05$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. As shown in Fig. 5B, both adolescent stress and dexamethasone administration induced a higher prefrontal cortex level of miR-18a in adolescent rats [$F(4, 25) = 7.272$, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 24.133$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. Treatment with RU486 reversed the prefrontal cortex level of miR-18a only in the AdoS + R group ($P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. Fig. 5C and 5D show levels of miR-124a and miR-18a in the hippocampus detected by real-time PCR. As shown in Fig. 5C, both adolescent stress and dexamethasone administration upregulated the hippocampus level of miR-124a in adolescent rats [$F(4, 25) = 10.052$, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 12.056$, $P < 0.001$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with controls. However, RU486 reduced the miR-124a level in the hippocampus of both adolescent rats ($P < 0.05$, $P < 0.01$) and adult rats ($P < 0.01$, $P < 0.01$) compared with the corresponding S group and D group, respectively. As shown in Fig. 5D, both adolescent stress and dexamethasone administration elevated the hippocampus level of miR-18a in adolescent rats [$F(4, 25) = 14.324$, $P < 0.001$; post hoc, AdoS vs AdoC, $P < 0.01$, AdoD vs AdoC, $P < 0.01$] and adult rats [$F(4, 25) = 4.124$, $P = 0.011$; post hoc, AduS vs AduC, $P < 0.01$, AduD vs AduC, $P < 0.01$] compared with the corresponding controls. RU486 treatment reduced the miR-18a level in the hippocampus only of adolescent rats ($P < 0.01$, $P < 0.01$) and the AduD + R group ($P < 0.05$) compared with the corresponding S group and D group, respectively.

3.4.2. Expressions of miR-511 in the prefrontal cortex and hippocampus in real-time PCR

Fig. 5E shows miR-511 expression in the prefrontal cortex detected by real-time PCR. Both adolescent stress and dexamethasone administration did not induce a significant elevated prefrontal cortex level of miR-511 [$F(4, 25) = 1.274$, $P = 0.307$] in adolescent rats. Interestingly, the AduS group and the AduD group exhibited decreased prefrontal cortex level of miR-511 [$F(4, 25) = 2.656$, $P = 0.056$; post hoc, AduS vs AduC, $P < 0.05$, AduD vs AduC, $P < 0.05$] compared with the AduC group. However, RU486 did not reverse the prefrontal cortex level of miR-511 in the AduS + R group ($P > 0.05$) and the AduD + R group ($P > 0.05$). Fig. 5F shows miR-511 expression in the hippocampus detected by real-time PCR. The level of miR-511 in the hippocampus had no statistically significant differences in both adolescent groups [$F(4, 25) = 1.153$, $P = 0.355$] and adult groups [$F(4, 25) = 0.117$, $P = 0.975$].

4. Discussion

In the present study, the authors probed the short-term and long-term effects of both adolescent CUMS and dexamethasone administration on depressive-like behaviors and expressions of GR, FKBP5, as well as miR-124a, miR-18a, and miR-511, in the prefrontal cortex and hippocampus. In addition, the impacts of chronic treatment with RU486 on depressive-like behaviors and biological molecules were explored. Results showed that both adolescent CUMS and dexamethasone administration induced short-term and constant depressive-like behaviors, reduced GR expression, and increased FKBP5, miR-124a, and miR-18a levels in the prefrontal cortex and hippocampus. Intriguingly, reduced miR-511 expression was observed only in the prefrontal cortex of adult rats that experienced CUMS or dexamethasone administration in the adolescent period. Along with this, we observed that the antagonist of GR, RU486, ameliorated the depressive-like behaviors and partial abnormal molecules.

CUMS has been acknowledged as a valid animal model that can emulate the behavioral and neurobiological abnormalities of depressive patients (Willner, 1997). Repeated exogenous corticosterone administration is another valid preclinical model of depression that can directly and indirectly inhibit the function of the HPA axis and allows one to investigate the effects of glucocorticoids on depressive-like behaviors and neurobiological alterations (Sterner and Kalynchuk, 2010). The current study demonstrated that both CUMS and chronic dexamethasone, a synthetic glucocorticoid, administration in adolescent rats induced a depressive state and memory impairment. Lower percentage of sucrose preference in the SPT indicated anhedonia behaviors and lower appetite. Less locomotor activity in the OFT represented lower exploratory activities. Decreased percentage of time spent in the open arms in the EPM test indicated more anxiety-like behaviors. Declined percentage of time spent in the target quadrant in the MWM test reflected memory impairment. The results were consistent with previous reports (Darcet et al., 2014; Shang et al., 2017). In addition, we observed those abnormal behaviors in the adult rats. Combined with our previous study and other studies, the results reinforced the conclusions that both adolescent chronic stress and dexamethasone/corticosterone exposure induced persistent depressive-like behaviors and memory impairment in adulthood (Barfield et al., 2017; Xu et al., 2017; Yohn and Blendy, 2017).

Hyperactivity of the HPA axis has been closely implicated in the pathogenesis of depression and dysregulation of HPA axis is attributed to impaired negative feedback inhibition, which is possibly caused by impaired GR function (Anacker et al., 2011; Holsboer, 2000). Patients with major depression manifested a diminished number of GR on lymphocytes, while increased GR levels were observed in patients receiving antidepressants (Calfa et al., 2003). CUMS-exposed mice showed a significant decrease in GR mRNA level in the hippocampus, while antidepressant treatment effectively elevated the hippocampal GR mRNA level (Mao et al., 2009). In addition, long-term treatment with dexamethasone induced depressive-like behaviors and reduction in GR protein levels in the hippocampus, which were reversed by pretreatment with melatonin (Ruksee et al., 2014). Consistent with previous studies, the current study found that both adolescent CUMS and dexamethasone administration resulted in reduced GR levels in the prefrontal cortex and hippocampus and the abnormalities were recovered with RU486 treatment. More importantly, similar results were detected in adult rats that experienced CUMS and dexamethasone administration during the adolescent period. The results confirmed the crucial role of GR in the prefrontal cortex and hippocampus on negative feedback regulation of HPA axis and provided a mechanism of adolescent stress augmenting vulnerability to depressive disorder.

FKBP5 suppresses GR sensitivity by reducing the receptor's affinity for cortisol and translocation to the nucleus (Binder, 2009). Results showed that both adolescent CUMS and dexamethasone administration elevated FKBP5 levels in the prefrontal cortex and hippocampus, which concurred with previous studies that chronic stress increased FKBP5 expressions in both prefrontal cortex and hippocampus of rats (Chen et al., 2016; Guidotti et al., 2013). Similar to the findings in adolescent rats, upregulated FKBP5 expressions were detected in the prefrontal cortex and hippocampus of adult rats that underwent adolescent CUMS or dexamethasone administration. Meanwhile, treatment with RU486 reversed most of the abnormalities in both adolescent and adult rats. These data expanded the conclusion that FKBP5 overexpression inhibiting GR activity was involved in the vulnerability to depressive disorder induced by adolescent stress.

MiR-124a is able to bind to the GR 3′-UTR and repress GR activity by reducing GR protein levels (Vreugdenhil et al., 2009). A higher level of miR-124 was detected in peripheral blood mononuclear cells of patients with major depressive disorder, and miR-124 levels were reduced after 8 weeks of antidepressant treatment (He et al., 2016). Expression of miR-124-3p exhibited upregulation in the prefrontal cortex of both chronic corticosterone-induced depressive-like rats and depressed subjects, which was accompanied by a significant downregulation of GR (Nr3c1) (Roy et al., 2017). Moreover, studies manifested significant upregulation of miR-124 and downregulation of GR (Nr3c1) in the prefrontal cortex and hippocampus of corticosterone-induced depressed animals (Dwivedi et al., 2015; Wang et al., 2017). Our results were similar in this regard that both adolescent CUMS and dexamethasone administration resulted in elevated expression levels of miR-124a accompanied by decreased GR levels in the prefrontal cortex and hippocampus of adolescent rats, and the aberrant alterations persisted into adulthood. However, RU486 normalized the alterations in the long run. The results suggested that miR-124a overexpression in the prefrontal cortex and hippocampus mediated the susceptibility to depressive disorder by reducing GR levels.

MiR-18a is identified as a regulator of GR because it can inhibit GR mRNA translation and reduce the expression of GR protein (Uchida et al., 2008). MiR-18a manifested significant upregulation in the frontal cortex of learned helplessness rats subjected to repeated inescapable shocks (Smalheiser et al., 2011). The current study showed that both adolescent CUMS and dexamethasone administration induced higher expression levels of miR-18a in the prefrontal cortex and hippocampus of both adolescent rats and adult rats. Meanwhile, this abnormality was partially improved by treatment with RU486. The result was a little different from that of our former study in which increased miR-18a expression in the basolateral amygdala was detected only in adolescent rats (Xu et al., 2017). Given that miR-18a has region-specific expression patterns, we concluded that upregulation of miR-18a in the prefrontal cortex and hippocampus was associated with the vulnerability to depressive disorder.

Study results confirmed miR-511 as a modulator of FKBP5, and there was a bidirectional positive feedback loop between miR-511 and GR activity (Zheng et al., 2016). Previous results demonstrated a significant increased miR-511 level in the basolateral amygdala of depressed subjects (Maheu et al., 2015). Further, glucocorticoids could upregulate miR-511 expression in the cortex, spleen, and liver (Puimege et al., 2015; Zheng et al., 2016). However, our results displayed a tendency toward increased miR-511 expression at a value of $P > 0.05$ in the prefrontal cortex of adolescent rats exposed to CUMS and dexamethasone administration compared with the control groups. The possible reasons accounting for this phenomenon are as follows. First, the dosages and application method of glucocorticoids used in our experiment were different from those of the previous study. Further, both CUMS and high dose of dexamethasone induced the rapid reduction of GR, which likely suppressed the upregulation of miR-511 via the positive feedback loop. Therefore, the level of miR-511 exhibited an increase without significant statistical difference. This mechanism was more evident in adulthood. In adult groups, the AduS group and AduD group had significant decreased miR-511 expressions in the prefrontal cortex, which was consistent with the report that miR-511 expression in the prefrontal cortex of depressed subjects was decreased by more than 40% compared with normal control subjects (Smalheiser et al., 2012). Our results implicated that lower miR-511 level in the prefrontal cortex of the AduS group and the AduD group failed to decrease the higher level of FKBP5 protein. Moreover, expression of miR-511 in the hippocampus had no statistically significant differences in both the adolescent and adult groups. It might be that the prefrontal cortex was more sensitive to the alterations of miR-511 expression induced by stress. Our study first revealed that CUMS-induced miR-511 expression was region-specific in the brain, and downregulation of miR-511 expression in the prefrontal cortex was more closely related with susceptibility to depressive disorder.

5. Conclusions

The present study demonstrated that both adolescent CUMS and dexamethasone administration resulted in anhedonia, altered locomotor behaviors, anxiety, and cognitive impairment. Accompanied with these symptoms, decreased GR expression, and increased FKBP5, miR-124a and miR-18a expressions were detected in the prefrontal cortex and hippocampus of adolescent rats. And that the alterations persisted into adulthood. However, decreased miR-511 expression was observed only in the prefrontal cortex of adult rats that underwent adolescent stress and dexamethasone administration. In addition, RU486 reversed the vast majority of behavioral and biochemical changes. Our findings indicated that the lower levels of GR, higher levels of FKBP5, miR-124a, and miR-18a in the prefrontal cortex and hippocampus, and the downregulation of miR-511 in the prefrontal cortex might be related to depressive disorder. It should be noted that administration of dexamethasone occupies GR and inhibits the corticosterone secretion in response to stress. In contrast, corticosterone, induced in secretion by CUMS, binds to both mineralocorticoid receptors (MRs) and GRs. Dexamethasone treatment therefore does not mimic CUMS exposure.

6. Limitations

A few limitations in the current study should be addressed. Previous studies demonstrated that mifepristone treatment in the controls did not affect the depressive-like behaviors including immobility time during the forced swimming test and the discrimination index during the novel object recognition test (Aisa et al., 2008; Iijima et al., 2010). Other study reported that although RU486 treatment can regulate 62 genes in the DG subregion of hippocampus of control rats, there was only gene of protein tyrosine phosphatase, non-receptor type 14 (PTPN14) that responded to RU486 treatment in both control and stressed rats. Further, transcriptional changes of genes induced by chronic stress were normalized in the DG by RU486 treatment, while administration of RU486 did not alter transcriptional levels of these genes in controls (Datson et al., 2012). The control + RU486 group and the CUMS + dexamethasone (+RU486) groups were not included in the current study according to ethics approval. However, we could not exclude that there was an intrinsic effect of the GR antagonist and that the effects of dexamethasone administration on stress were different. In addition, considering the heterogeneity and complexity of the hippocampal structure, we explored the expressions of protein and microRNAs only in the whole hippocampus instead of hippocampal subregions in the current study. Further study is needed.

Conflict of interests

None.

Authors' contribution

Fang Pan was involved in study design and data interpretation; Jingjing Xu performed the majority of the laboratory work and contributed to the analysis of data and writing of the paper; Rui Wang, Yuan Liu, Wei Wang, Dexiang Liu, and Hong Jiang were responsible for the animal model and behavioral tests. All authors approved the final article to be published.

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