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ORIGINAL ARTICLE

The faster-onset antidepressant effects of hypidone hydrochloride (YL-0919)

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Abstract

Hypidone hydrochloride (YL-0919), is a novel structural antidepressant candidate as a triple selective serotonin re-uptake inhibitor (SSRI), 5-HT_{1A} partial agonist and 5-HT_6 agonist. Here, we investigated the rapid onset antidepressant-like effects of YL-0919 and the possible mechanism in rats exposed to a chronic unpredictable stress (CUS) paradigm. In the CUS rats, it was found that fluoxetine (FLX, 10 mg/kg) treatment exerted antidepressant actions on 20-22d, while YL-0919 or vilazodone (VLZ, a dual 5-HT_{1A} partial agonist and SSRI) administrated once daily exerted faster antidepressant-like behaviors [4 days in the sucrose preference test (SPT) and 6 days in the novelty suppressed feeding test (NSF)]. Thereafter, the serum corticosterone (CORT) and adrenocorticotropic hormone (ACTH) levels were reversed by treatment with YL-0919 for 7 days. Furthermore, YL-0919 treatment for 5 days reversed the brain derived neurotrophic factor (BDNF)-mammalian target of rapamycin (mTOR) signaling and the key synaptic proteins, such as post-synaptic density (PSD95), GluR1 and presynaptic protein synapsin1. Meanwhile, the dendritic complexity of pyramidal neurons in prefrontal cortex (PFC) were also increased in the CUS rats. These data suggest that YL-0919 exerts a faster antidepressant-like effect on behaviors and this effect maybe at least partially mediated by the BDNF-mTOR signaling related dendritic complexity increase in the PFC.

Keywords Hypidone hydrochloride · Antidepressant · Faster-onset · Prefrontal cortex · Dendritic complexity

Introduction

Current antidepressant drugs typically require weeks to months before a response (defined as at least a 50% decrease from baseline in depressive symptoms) to the treatment can be observed (Montgomery 1997; Trivedi et al. 2006; Masi and Brovedani 2011) in the patients with major depressive disorders (MDD). As a clinical first-line treatment for depression, fluoxetine (FLX) is the first selective serotonin re-uptake inhibitor (SSRI) that has a recognized clinical efficacy with 3–4 weeks treatment (Perez-Caballero et al. 2014) with obvious cognitive damage (Li et al. 2017). Development of novel agents that produce a rapid antidepressant response represents a major unmet medical need for the treatment of MDD (Dwyer et al. 2013).

Hypidone hydrochloride (YL-0919) is an internationally patented antidepressant candidate with novel structure developed by our institute that has been entered into a phase II clinical trial in China. Previous studies reveal that YL-0919 is a potent triple SSRI, 5-HT_{1A} receptor partial agonist and 5-HT_6 receptor agonist (Chen et al. 2013; Chen et al. 2018), producing significant antidepressant-like and anxiolytic-like effects (Ran et al. 2018) without sexual dysfunction (Zhang et al. 2017), as well as a greater impact on extracellular 5-HT levels than a conventional SSRI (FLX) (Zhang et al. 2017; Qin et al. 2014). Further studies have found that YL-0919 has clear memory-enhancing effects on normal rodent animals (Chen et al. 2018). Interestingly, YL-0919 treatment (7 days) rapidly

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has enhanced the synaptic plasticity (LTP) of rats in the hippocampus while FLX treatment needs 3 weeks to exert this effect (Zhang et al. 2017), revealing that YL-0919 maybe exerts faster actions. Vilazodone (VLZ, a dual partial 5-HT_{1A} agonist and SSRI), approved by the FDA in 2011 for the treatment of MDD (Glazer 2011; Hopkins 2011). Recent reports have found that VLZ has a relatively rapid onset (with a response time of 1 week) action in treated depressive patients (Rickels et al. 2009; Van Amsterdam and Seyfried 2014; Shi et al. 2016). Based on these data, it is reasonable to speculate that YL-0919 may exert rapid antidepressant actions on behavioral models of depression.

Recent years, the fast-onset effect of ketamine provides some implications for the rapid-acting mechanism. A single sub-anesthetic dose of ketamine rapidly improves depressive symptoms in clinical patients within hours (Berman et al. 2000; Liebrenz et al. 2007; Hashimoto 2015), and this effect can be sustained for one week in some patients (Niciu et al. 2014; Yang et al. 2015). These unique therapeutic properties have prompted researchers to explore the mechanisms mediating the rapid antidepressant effect. It is reported that the rapid antidepressant effect of ketamine could be mediated by activation of the mechanistic target of rapamycin (mTOR) via a transient glutamate surge and brain-derived neurotrophic factor (BDNF) release (Duman 2014; Dwyer and Duman 2013). For example, ketamine rapidly activates the mTOR signaling, leading to upregulation of synaptic proteins and increased number and function of new spine synapses in the prefrontal cortex (PFC) of rat (Li et al. 2010; Li et al. 2011a, b, c; Duman and Aghajanian 2012). Thus, activation of the mTOR signaling and increased synaptogenesis in the PFC appear to be crucial in mediating the rapid antidepressant effects (Yang et al. 2013; Popoli et al. 2012).

In current study, we aimed to explore the potentially faster antidepressant-like property of YL-0919 with treatment in a well-developed chronic unpredictable stress (CUS) rodent model. Furthermore, we also investigated the key synaptic proteins related to the mTOR signaling and the dendritic complexity in the PFC to explore the possible mechanism of the faster-onset actions of YL-0919.

Materials and methods

Animals

Male Wistar rats weighing 160–180 g were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were housed in groups of five per cage and acclimatized to a sound-proof rooms with stable environmental conditions: controlled ambient temperature (24 ± 1 °C), humidity (50% ± 10%) and a 12-h light/12-h dark cycle (lights on at 8:00 am) with free access to food and water. All procedures followed the current laws of China and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86–23, revised 1996). Efforts were made to minimize suffering and to reduce the number of animals used.

Drugs and reagents

YL-0919 (white powder, purity 99.8%) was synthesized by the Department of Medicinal Chemistry at our institute. Vilazodone HCL (VLZ, purity ≥99.43%) was purchased from Zhejiang Huahai Pharmaceutical Co., Ltd. (China). Both YL-0919 (1.25, 2.50 mg/kg, i.g) and VLZ (3 mg/kg, i.g) were dissolved in 2% DMSO solution. Fluoxetine (FLX) was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in ddH_2O with the desired concentration (10 mg/kg, i.g). Doses of YL-0919 were chosen based on previous behavioral tests of the actions of antidepressants (Chen et al. 2018; Ran et al. 2018). Does of VLZ and FLX presently used in our rats can be considered comparable to those used by animals in previous studies (Chen et al. 2018; Li et al. 2018; Grippo et al. 2006). All drugs were administered via gastric in a volume of 2 ml/kg. Vehicle treated groups were also assessed simultaneously. All primary antibodies used in this study were purchased from Abcam plc.

Experimental designs

After 2 weeks of the sucrose habituation, rats were exposed to the CUS paradigm for 4 weeks and then were administrated with VLZ, YL-0919 or Vehicle by gavage (i.g) once daily in the following stress days. Rats were performed sucrose preference test (SPT) or novelty suppressed feeding test (NSF) until the drugs exerted the antidepressant-like effects. The control group was conducted as the figure shown (Fig.2a) except the CUS paradigm. Since the clinical first-line drug FLX (SSRIs) has no clear onset time in this model, we also tested the onset time of FLX (Fig.1a). All the behavioral tests were performed 1 h after the drugs or vehicle administration.

Chronic unpredictable stressor (CUS) procedure

The CUS procedure was carried out as described in (Willner et al. 1992; Papp 1998) with minor modifications (see Table 1). One or two kinds of stressors were applied per day over a period of 4 weeks. A variety of stressors were used at random to make them more unpredictable to the animals: water or food deprivation (12 h); forced swimming (10 °C, 5 min), illumination (overnight / intermittent), restraint (2 h), tail pinch (1 min, 1–2 cm from the end of the tail), damp sawdust (24 h), cage tilting

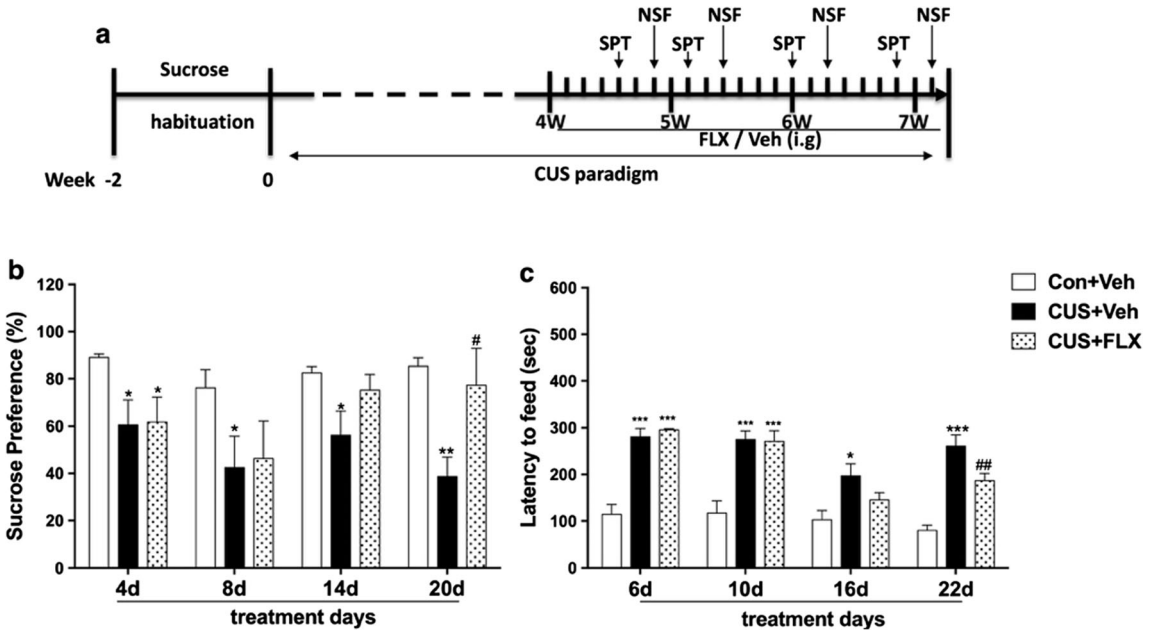

Fig. 1 Onset time-course of the antidepressant-like effect of Fluoxetine in CUS-exposed rats. a Experimental design for the behavioral tests of FLX in the CUS rats whereas the control group served as vehicle without CUS paradigm. Rats were performed the behavioral tests in the FLX treatment days (SPT, on the day 4, 8, 14, 20; NSF, on the day 6, 10, 16, 22). **b**The CUS-exposed rats treated with FLX (10 mg/kg, i.g) once daily obviously reversed the sucrose preference (%) (treatment for 20 days) in the SPT and (**c**) the latency to feed (for 22 days) in the NSF test. Data are expressed as the mean ± SEM, $N = 8$–10/group. $^{*}P < 0.05$, $^{***}P < 0.001$ vs the Con+Veh; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs the CUS + Veh (one-way ANOVA)

(45°, 12 h), and social isolation (24 h). The control rats without any stressors were placed in another quiet room and had no contact with the stressed rats.

Sucrose preference test (SPT)

A SPT (Bekris et al. 2005) performed to measure the animals' anhedonia, which is one of the core symptoms of patients with major depression (Yong et al. 2016; Zhang et al. 2017). Rats were trained for 48 h to consume two bottles of 1% (*w*/*v*) sucrose solution without a food and water supply and were returned to their home cages to receive normal treatment for 3 days. In addition, a sucrose baseline test was then performed following 14 h of food and water deprivation. The rats were allowed to choose from two bottles, one with 30 ml pure water and another one with 30 ml of the 1% sucrose solution, for 1 h. Sucrose and water intakes were weighed, and the sucrose preference was calculated (SP (%) = sucrose intake / (sucrose

Table 1 Chronic unpredictable stress procedure sequence

Week Day	Time	Week1	Week2	Week3	Week4
Wednesday	Day	Intermittent Illumination	SPT-1	SPT-2;	SPT-3
	Night	Damp Sawdust	Cage Tilting	Social Isolation	Tail Pinch
Thursday	Day	Forced Swimming	Tail Pinch;		Damp Sawdust
	Night	Water Deprivation	Water Deprivation	Intermittent Illumination	Cage Tilting
Friday	Day	Social Isolation	Forced Swimming	Water Deprivation	Food Deprivation
	Night		Restraint	Damp Sawdust	Overnight Illumination
Saturday	Day	Cage Tilting	Food Deprivation		Forced Swimming
	Night	Overnight Illumination	Overnight Illumination	Cage Tilting	Intermittent Illumination
Sunday	Day Night	Restraint Food Deprivation	Damp Sawdust	Food Deprivation Restrain	Social Isolation
Monday	Day Night	Tail Pinch Damp Sawdust	Intermittent Illumination Social Isolation	Forced Swimming Overnight Illumination	Damp Sawdust
Tuesday	Day			Tail Pinch	Restraint
	Night	Food&Water Deprivation	Food&Water Deprivation	Food&Water Deprivation	Food&Water Deprivation

+water) intake *100%). The SPT was performed twice weekly during the sucrose habituation and the CUS procedure to evaluate the CUS model and drug action.

Novelty suppressed feeding (NSF) test

Each animal was placed in one corner of an open plastic box (54 X 28 X 21 cm) with 12 pellets in the center after 24 h of food deprivation (Bodnoff et al. 1988). The experimental environment was totally new to the rats, and the light intensity was stronger than that in their home room. The latency to feed in 5 min was recorded, and eating behavior was defined as chewing and biting. Home cage consumption immediately after testing was assessed as a relative measure of hunger.

Serum corticosterone (CORT) and adrenocorticotropic hormone (ACTH) assay

Rats were sacrificed to collected blood samples 24 h after all behavioral tests. Blood samples were centrifuged (3000 x g for 20 min, 4 °C) after storage at 4 °C for 1 h. We tested the adrenocorticotropic hormone (ACTH) and corticosterone in the serum by using a rat ACTH ELISA Kit (LifeSpan BioSciences, Inc., North America) and an Enzo® Corticosterone ELISA Kit (Enzo Life Sciences, Inc., USA). The assays were performed following the manufacturer's protocol. The optical density (OD value) was measured immediately using a microplate reader (PerkinElmer EnVision™ 2104 Multilabel Reader) at 450 nm (ACTH) and 405 nm (Corticosterone).

Western blotting

Western blotting analysis was performed as previously described (Wang et al. 2016; Zhao et al. 2017) to test the expression levels of several proteins related to the mTOR signaling. On day 5 after the YL-0919 or VLZ treatments, four animals were randomly selected and sacrificed to remove their brains on ice. The prefrontal cortex (PFC) was quickly separated and the tissues were homogenized in RIPA lysis buffer mixed with a protease inhibitor and a phosphatase inhibitor cocktail. After the quantitation of total protein by using a BCA Protein Assay Kit (Applygen, China), all samples were boiled for 6 min to complete the protein denaturation progress. The density of each band on the Western blots was measured using Gel-pro Analyzer software (Media Cyber netics Ltd., USA), and the relative expression level of each target protein was calculated as the ratio of target protein band density to β-actin density.

Golgi staining

This protocol was performed according to the manufacturer's instructions in the FD Rapid GolgiStain kit. In each group, random four animals' brains were quickly removed and immersed in the impregnation solution and stored at room temperature for 2 weeks in the dark after rinsing quickly in double-distilled water. Then, the brains were transferred into solution C and stored at 4 °C for 5 days in the dark. Coronal sections (100 μm in thickness) were cut slowly on a Cryostat (Leica, Wetzlar, Germany) at −22 °C. Sections were collected on clean gelatin-coated microscope slides. Following drying, sections were rehydrated, reacted in the developing solution (an ammonium hydroxide-based solution), and dehydrated with 50, 75, 95, and 100% ethanol, respectively. Finally, cleared the sections in xylene and covered with resinous mounting media.

Neurons in the PFC were photographed under a microscope (Leica DM4000B). According to the described previously (Flores et al. 2005), only fully impregnated pyramidal neurons without obviously truncated dendrites were included in our analysis. Cells were digitally reconstructed and traced using Image-Pro Plus software (Media Cybernetics, Bethesda, MD, USA) (Wang et al. 2013). Dendritic complexity was reflected by the basal dendritic length and the number of branching points (Wang et al. 2008), which were determined by a Sholl analysis (Sholl 1953). Equidistant (10 μm apart) concentric rings were placed over the dendritic tree tracings using the center of the soma as the reference point. The basal dendritic length was calculated by multiplying the number of intersections of each ring by 10 μm and the number of dendritic branching points was counted in each successive concentric zone. For quantitative analysis, three to five neurons from each hemisphere per section, three to four sections per animal and four animals per group ($n = 96–120$ neurons per group) were needed.

Statistical analysis

The values shown are expressed as the means± standard error (SEM) and were analyzed with the GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA). Multiple comparison was used with One-way analysis of variance (ANOVA) to analyzed the effects of drugs-treatment had on the CUS rats followed by a Dunnett's t-test. For all of the tests, the level of statistical significance was set at $P < 0.05$.

Results

Onset time-course of the antidepressant-like effects of FLX in CUS rats

Animals treated with FLX (10 mg/kg, i.g) once daily for 20 days showed a significantly increased in sucrose preference (%) (Fig. 1b. $F_{2,24} = 7.386$, $P < 0.05$) which returned to level of control group ($P = 0.7717$). Similar results were found in

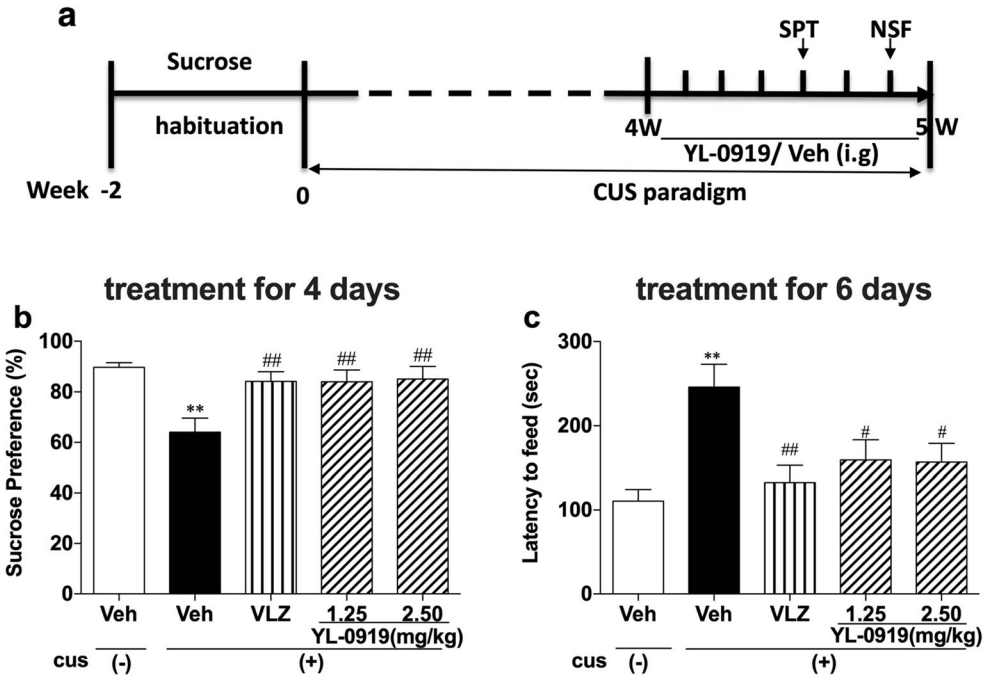

Fig. 2 The faster-onset antidepressant-like effects of YL-0919 in CUS-exposed rats. a Experimental design for the behavioral tests of YL-0919 or VLZ in the CUS rats while the CUS (−)-Veh group was performed as the design except for the CUS paradigm. In the drugs treatment days, rats were performed the SPT and the NSF test respectively on the day 4 and 6. **b** Treatment with YL-0919 (1.25 or 2.50 mg/kg) or VLZ (3.0 mg/kg) for 4 days significantly increased the SP % and (**c**) for 6 days reversed the increased latency to feed in the NSF test induced by CUS. Data are presented as the mean ± SEM, N = 8–10/group. $^{**}P < 0.01$

the NSF test. FLX treatment for 22 days remarkably reduced the latency to feed (Fig. 1c. $F_{2,24} = 6.505$, $P < 0.01$) which returned to level of control group ($P = 0.1864$). These data indicated that the onset time of FLX was approximately 3 weeks in CUS-exposed rats.

Antidepressant-like effects of YL-0919 treatment for 4–7 days in CUS-exposed rats

Treatment with YL-0919 (1.25 or 2.50 mg/kg) once daily for 4 days significantly reversed the sucrose preference (%) of CUS rats (Fig. 2b. $F_{3,48} = 6.232$, $P < 0.01$, one-way ANOVA), and a similar effect was produced by VLZ (3.0 mg/kg, i.g) ($F_{2,36} = 12.29$, $P < 0.01$). In the NSF test, YL-0919 treatment for 6 days obviously reduced the latency to feed (s) (Fig. 2c. $F_{3,48} = 6.516$, $P < 0.05$), and VLZ also had a similar effect ($F_{2,36} = 12.34$, $P < 0.01$). VLZ (3.0 mg/kg, i.g) and YL-0919 (1.25 or 2.50 mg/kg) treatment could make sucrose preference/latency to feeding of CUS(+)-Veh group return to CUS(−)-Veh group (Fig. 2b, $P = 0.5367$, $P = 0.7752$, $P = 0.8624$; Fig. 2c. $P = 0.7148$, $P = 0.3686$, $P = 0.4140$).All of the results indicated that YL-0919 and VLZ exerted a faster onset of antidepressant-like effects on behaviors in CUS rats.

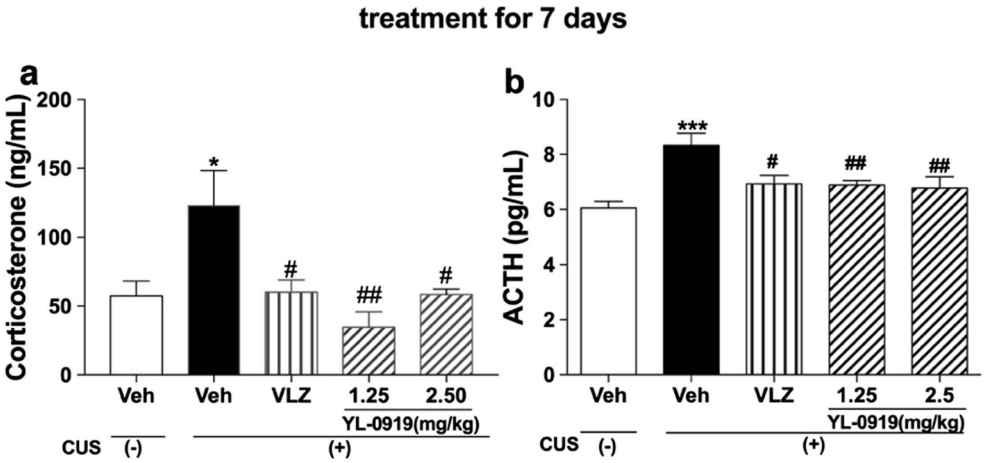

Fig. 3 The effects of YL-0919 treatment for 7 days on serum corticosterone and ACTH levels in CUS-exposed rats. a YL-0919 or VLZ treatment for 7 days reversed the increased the corticosterone concentration and (**b**) the ACTH concentration induced by CUS procedure. Data are presented as the mean ± SEM, N = 8–10/group. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with the CUS (−)-Veh group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the CUS (+)-Veh group

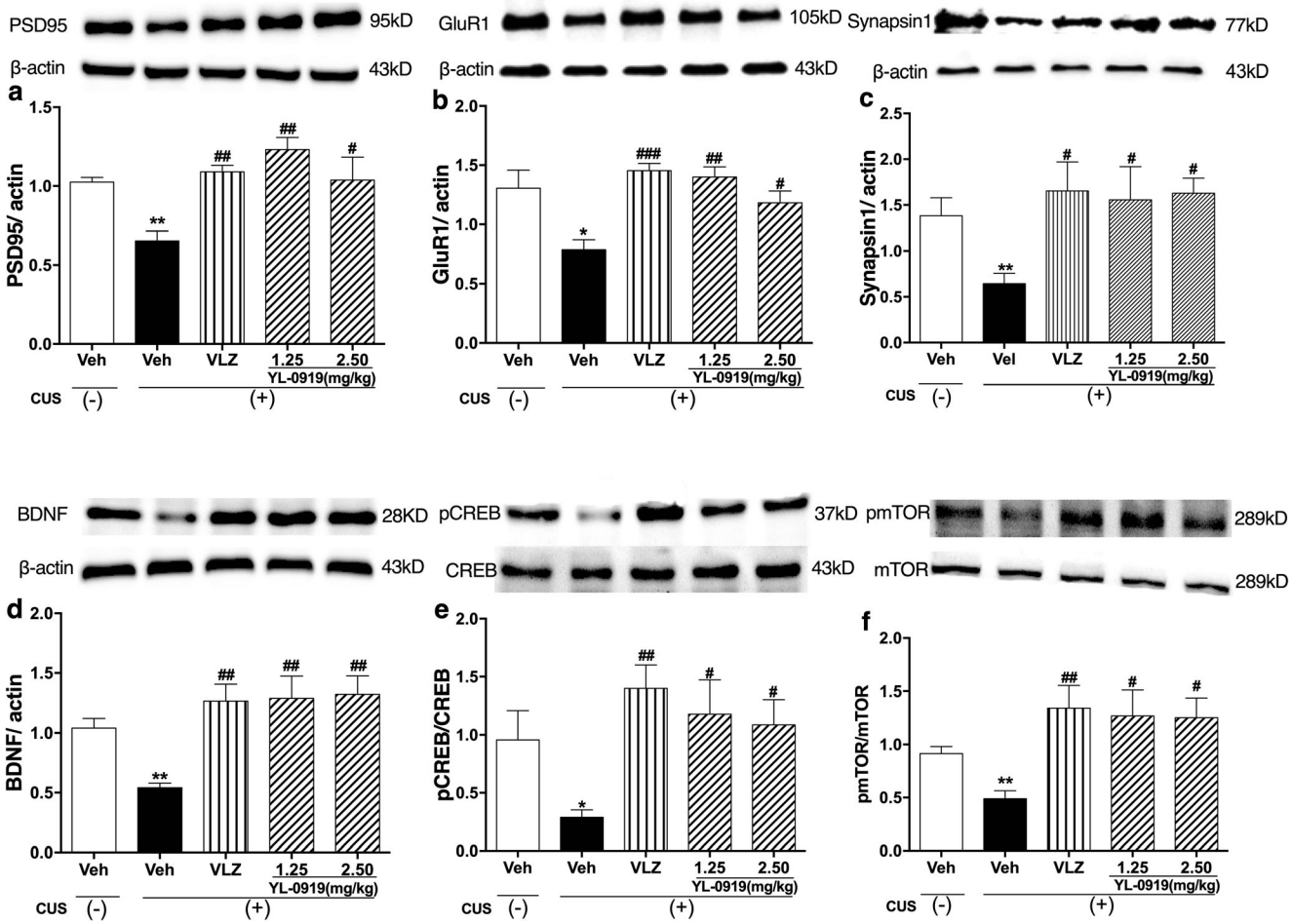

Fig. 4 YL-0919 treatment for 5 days increased synaptic proteins, BDNF, pCREB and pmTOR in the PFC. Both YL-0919 and VLZ all reversed the low expression levels of these proteins (**a**, PSD95; **b**, GluR1; **c**, Synapsin1; **d**, BDNF; **e**, pCREB; **f**, pmTOR) induced by the CUS. Each value represents the mean ± SEM ($N = 4$/group). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the CUS (−)-Veh group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#}P < 0.01$ compared with the CUS (+) -Veh group

The effects of YL-0919 treatment for 7 days on serum corticosterone and ACTH in CUS-exposed rats

The effects of YL-0919 on serum corticosterone and ACTH in CUS-exposed rats are depicted in Fig. 3. The CUS-exposed group exhibited high levels of serum CORT (Fig. 3a) and ACTH (Fig. 3b). YL-0919 (1.25 or 2.50 mg/kg, i.g) treatment for 7 days significantly reduced the concentrations of serum CORT (Fig. 3a. $F_{3,33} = 5.521$, $P < 0.01$, vs the CUS (+)-Veh group; $P = 0.7696$, $P = 0.9999$, vs the CUS (−)-Veh group) and ACTH (Fig. 3b. $F_{3,33} = 9.234$, $P < 0.01$, vs the CUS (+)-Veh group; $P = 0.2262$, $P = 0.4462$, vs the CUS (−)-Veh group). Similar effects were also found in VLZ (3.0 mg/kg, i.g) treatment group which decreased the concentrations of serum CORT (Fig. 3a. $F_{2,24} = 4.716$, $P < 0.05$, vs the CUS (+)-Veh group; $P = 0.9939$, vs the CUS (−)-Veh group) and ACTH (Fig. 3b. $F_{2,24} = 5.145$, $P < 0.05$, vs the CUS (+)-Veh group; $P = 0.1895$, vs the CUS (−)-Veh group) in the serum of rats.

Effects of YL-0919 treatment for 5 days on the BDNF-mTOR signaling in the PFC

The results of the Western blotting assay are shown in Fig.4. After exposed to CUS for 4 weeks, the BDNF-mTOR signaling including synaptic proteins (PSD95, GluR1 and Synapsin), BDNF, pCREB/CREB and pmTOR/mTOR were significantly decreased in the PFC ($P < 0.05$). Treatment with YL-0919 (1.25 or 2.50 mg/kg) for 5 days significantly reversed the reduction of the expression of these proteins induced by CUS (Fig. 4a. $F_{3,12} = 7.315$, $P < 0.01$; $P = 0.3949$, $P = 0.9996$; 4b. $F_{3,12} = 5.99$, $P < 0.05$; $P = 0.9253$, $P = 0.8572$; 4c. $F_{3,12} = 3.989$, $P < 0.05$; $P = 0.9494$, $P = 0.8613$; 4d. $F_{3,12} = 8.042$, $P < 0.05$; $P = 0.5321$, $P = 0.4275$; 4e. $F_{3,12} = 3.214$, $P < 0.05$; $P = 0.8978$, $P = 0.9760$; 4 f. $F_{3,12} = 5.229$, $P < 0.05$; $P = 0.4310$, $P = 0.4706$; one-way ANOVA). Similar to YL-0919, CUS-exposed rats administrated with VLZ for 5 days also increased the expression levels of these proteins (4a. $F_{2,9} = 25.79$, $P < 0.01$; $P = 0.5950$; 4b. $F_{2,9} = 10.55$, $P < 0.01$; $P = 0.6073$;

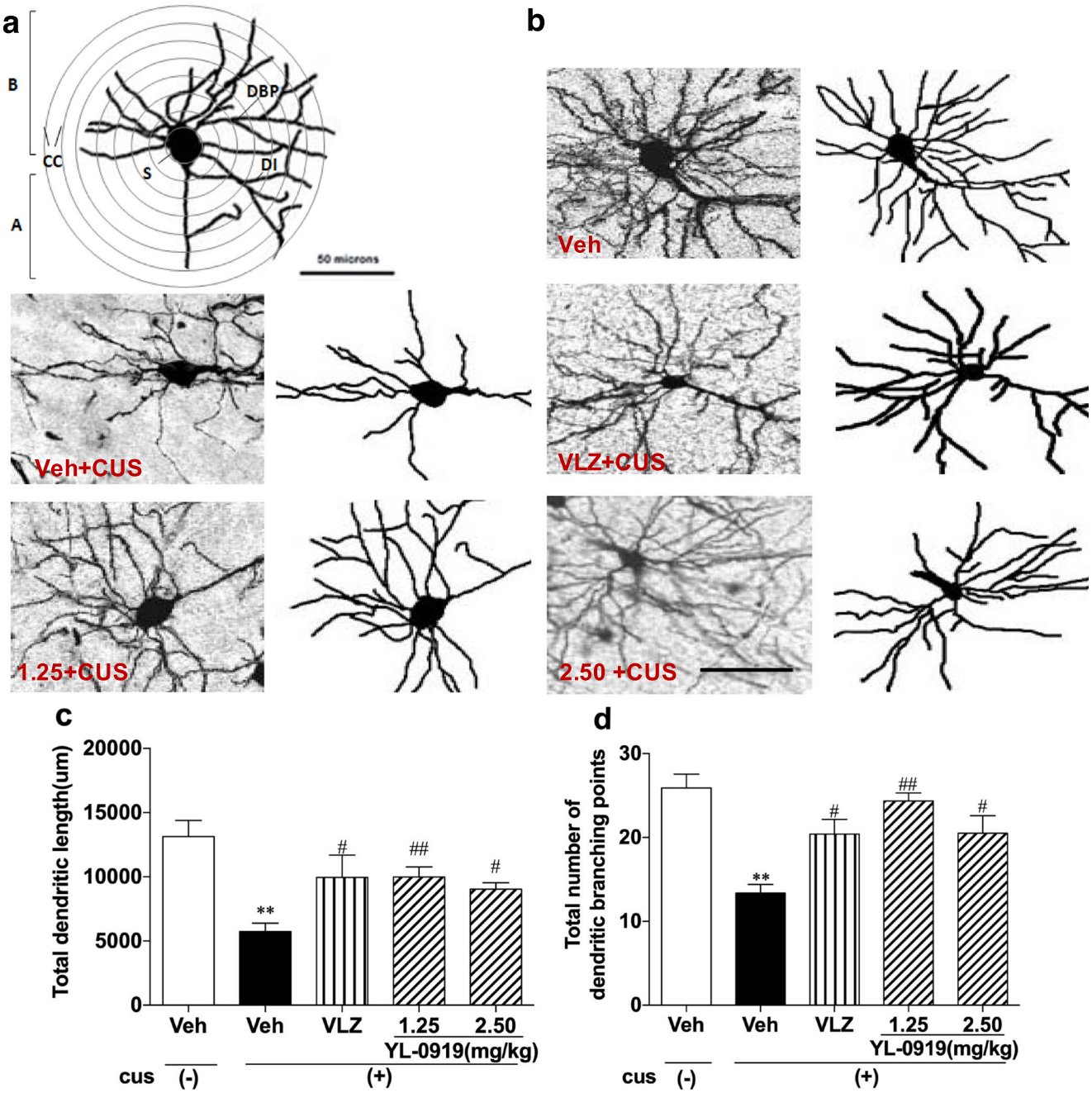

Fig. 5 YL-0919 treatment for 5 days increased the dendritic complexity of cortical neurons. a Diagram of a reconstructed dendritic tree and equidistant (10 μm apart) concentric circles that were used for quantitative analysis. A, apical dendrites; B, basal dendrites; DBP, dendritic branching points; DI, dendritic intersections; CC, concentric circles; S, soma. Scale bar = 50 μm. (**b**) Magnified (20 □objective) images of Golgi-impregnated cortical pyramidal neurons. (**c**) Similar to the sub-chronic treatment with VLZ, YL-0919 treatment for 5 days increased the total dendritic length of the pyramidal neurons. **d** YL-0919 and VLZ significantly increased the total dendritic branching points. Data are presented as the means ±SEM (N = 4 /group). $^{**}P < 0.01$ compared with the CUS (−)-Veh group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the CUS(+)-Veh group

4c. $F_{2,9} = 4.898$, $P < 0.001$; $P = 0.6965$; 4d. $F_{2,9} = 15.09$, $P < 0.01$; $P = 0.2649$; 4e. $F_{2,9} = 8.593$, $P < 0.01$; $P = 0.2790$, 4 f. $F_{2,9} = 9.869$, $P < 0.01$; $P = 0.1190$; one-way ANOVA). All of the results strongly suggested that fast-onset activity of YL-0919 was associated with the upregulation of protein expression related to the BDNF-mTOR pathway in the PFC.

Effects of YL-0919 treatment for 5 days on dendritic complexity in the PFC

By using Golgi staining, we further measured the effect of YL-0919 treatment on the dendritic complexity of pyramidal neurons in the PFC of rats (Fig. 5). It was found

that YL-0919 (1.25 or 2.50 mg/kg, i.g) administrated for 5 days significantly reversed the total dendritic length (Fig. 5a. $F_{3,12} = 10.5$, $P < 0.05$; $P = 0.1113$, $P = 0.1022$) and the total branching points (Fig. 5b. $F_{3,12} = 13.66$, $P < 0.01$; $P = 0.8544$, $P = 0.1083$) of dendrites decreased by the CUS procedure. Similar results were found in VLZ-treated rats, which increased the dendritic length and branching points (Fig. 5a. $F_{2,9} = 8.339$, $P < 0.05$, $P = 0.2526$; Fig. 5b. $F_{2,9} = 16.44$, $P < 0.05$; $P = 0.0905$; one-way ANOVA). What we found in this assay indicated that the faster-onset action of YL-0919 on depressive-like behaviors was possibly mediated by the enhancement of dendritic complexity in the PFC (Table 1).

Discussion

The present study found that, compared to the slow antidepressant effect of FLX (20-22d), both YL-0919 and VLZ treatment for 4–7 days reversed the depressive-like behaviors and high levels of serum CORT and ACTH in CUS-exposed rats. Meanwhile, treatment with YL-0919 or VLZ for 5 days increased the activation of BDNF-mTOR signaling and the dendritic complexity in the prefrontal cortex (PFC). These data indicate that YL-0919 may be a promising faster-onset antidepressant candidate which maybe at least partially mediated by activation of BDNF-mTOR signaling and subsequent increases in synaptic proteins and dendritic complexity in the PFC.

In this study, we pay more attention to the onset-time courses of our test drug (YL-0919) and the first-line clinical drug (fluoxetine) in CUS rats. Once the antidepressant-like behaviors of each drug occur in this study, we should stop the experiment procedure. YL-0919 has been entered into the phase II clinical trials and the antidepressant-like effects of YL-0919 has been reported in our previous studies. Chronic YL-0919 treatment (4 weeks) reversed the depressive-like behaviors in CUS rats (Chen et al. 2013) so we have not provided the data on SPT and NSF test after chronic treatment with YL-0919. Besides that, YL-0919 and vilazodone had shown the faster-onset time than fluoxetine in the current study, so we only explored the possible mechanism of Yl-0919 related to their rapid antidepressant effects in the followed experimental designs.

Current studies have shown the onset times of YL-0919 (triple 5-$HT_{1A/6}$ agonist and SSRI) and VLZ (dual 5-HT_{1A} partial agonist and SSRI) are faster than that of FLX (SSRI) against depression in rats exposed to CUS. It is reported that VLZ treatment for one week is found to yield a significant improvement in clinical patients (Garcia-Garcia et al. 2016; Lee et al. 2005; Warner-Schmidt and Duman 2007), which is consistent with the faster antidepressant effects of VLZ in current studies. In fact, pindolol (5-HT_{1A} partial agonist and β-adrenergic antagonist) (Partar et al. 2018; Cassani et al. 2014) or buspirone (partial 5-HT_{1A} receptor agonist) accelerate the clinical effects of antidepressant drugs (SSRIs) (Artigas et al. 2001). The 5-HT_{1A} receptor is considered that maybe have a significant impact on the onset time of SSRI antidepressants (Dawson and Watson 2009; Savitz et al. 2009; Pham et al. 2017). Hence, the findings suggest that the partial activation of the 5-HT_{1A} receptor at least in part mediated the faster antidepressant action of YL-0919. It's reported that, the lower dose of 5-HT_{1A} receptor agonist could activated the glutamic pyramidal neurons similar to a single dose of ketamine (Celada et al. 2013). YL-0919 in a low dose 2.5 mg/kg (in 10 mg/kg has no antidepressant-like behaviors) rapidly reverses the depressive-like behaviors and further experiments will be necessary to identify the detailed mechanism.

Disturbances in the HPA axis is arguably the best characterized endocrine markers of depression (Mahmoud et al. 2016). YL-0919 treatment for 7 days reverses the levels of serum corticosterone and ACTH indicated that the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis induced by CUS was rapidly attenuated by YL-0919. These findings suggest a possible mechanism for the immune alterations found in depressive disorders and for the faster-onset effect of YL-0919 treatment on immune response.

To explore the possible molecular mechanism underlying the rapid onset action of YL-0919, BDNF-mTOR signaling was measured. The results have shown that YL-0919 treatment for 5 days significantly activates the BDNF-mTOR signaling, increased the synthesis of synaptic proteins and caused the formation of dendritic complexity in the PFC. This is consistent with our previous findings that YL-0919 treatment for 7 days enhances the LTP in the hippocampus (Zhang et al. 2017). CUS paradigm exposure leads to impairments of synaptic plasticity (Kraus et al. 2017), and this conclusion corresponds to the results in current study. Recent studies have demonstrated that rapid-acting antidepressants may share the ability of ketamine to increase BDNF-mTOR signaling and rapidly reverse the effects of stress (Dwyer and Duman 2013). mTOR signaling, plays an important role in long-term synaptic plasticity by ketamine (Dwyer and Duman 2013). The activation of mTOR signaling can regulate the rapid translation of both synapsin1, PSD-95 (Lee et al. 2005) and GluR1 (Schratt et al. 2004). 5-HT receptor activities increase plasticity-related gene expression (mTOR signaling) in the frontal cortex (du Jardin et al. 2016). Thus, the activation of BDNF-mTOR signaling and the increase in dendritic complexity may play an important role in faster-onset action of YL-0919.

In conclusion, YL-0919 is a patented novel structural antidepressant reagent. Treatment with YL-0919 rapidly reverses the depressive-like behaviors induced by the CUS paradigm in rats. Increased mTOR-related dendritic complexity in the PFC

may at least partially underlie the faster antidepressant action of YL-0919. This study provides a potentially promising future of faster onset and memory enhancing antidepressant.

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Compliance with ethical standards

Conflict of interest None of the authors had conflict of interest to declare.

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