Loss of Phenotype of Parvalbumin Interneurons in Rat Prefrontal Cortex Is Involved in Antidepressant- and Propsychotic-Like Behaviors Following Acute and Repeated Ketamine Administration

**ZhiQiang Zhou · GuangFen Zhang · XiaoMin Li ·
XiaoYu Liu · Nan Wang · LiLi Qiu · WenXue Liu ·
ZhiYi Zuo · JianJun Yang**

Abstract Accumulating evidence has demonstrated that single subanesthetic dose of ketamine exerts rapid, robust, and lasting antidepressant-like effects. Nevertheless, repeated subanesthetic doses of ketamine produce psychosis-like effects with dysfunction of parvalbumin (PV) interneurons. We hypothesized that PV interneurons play an important role in the antidepressant-like actions of ketamine, and different changes in PV interneurons occur with the antidepressant-like and propsychotic-like effects of ketamine. To test this hypothesis, ketamine's antidepressant-like effects were evaluated by the forced swimming test. Ketamine-induced stereotyped behaviors and hyperactivity actions and the function of PV interneurons were also assessed. We demonstrated that an acute dose of 10 mg/kg ketamine induced significant antidepressant-like effects and reduced the levels of PV and the gamma-aminobutyric acid (GABA)-producing enzyme GAD67 in the rat prefrontal cortex. Moreover, inhibition of ketamine-induced loss of PV by apocynin blocked these antidepressant-like effects. Repeated administration of 30 mg/kg ketamine elicited stereotyped behaviors and hyperactivity actions as well as a longer duration of PV and GAD67 loss, higher brain glutamate levels, and lower brain GABA levels than acute single dose of ketamine. Our results reveal that the loss of phenotype of PV interneurons in the prefrontal cortex contributes to the antidepressant-like actions and is also involved in the propsychotic-like behaviors following acute and repeated ketamine administration, which may be partially mediated by the disinhibition of glutamate signaling. The different degrees and durations of the actions on PV interneurons produced by the two regimens of ketamine may partly underline the behavioral variance between the antidepressant- and propsychotic-like effects.

Keywords Ketamine · Antidepressant-like behaviors · Propsychotic-like behaviors · Parvalbumin · GAD67

Introduction

Depression is a common, chronic, recurrent, and severe disease that affects millions of people worldwide [1]. Unfortunately, routinely prescribed antidepressants show a delayed onset time of action with a low remission rate [2]. Several lines of evidence have shown that the nonselective N-methyl-D-aspartate (NMDA) receptor antagonist ketamine, a commonly used general anesthetic, can produce robust, rapid, and sustained antidepressant effects following acute intravenous infusion of a single subanesthetic dose in depressed patients [3–6]. Preclinical studies have further demonstrated that ketamine at subanesthetic doses exerts antidepressant-like effects within hours in rodent models of depression [7–9]. Nonetheless, the mechanisms underlying ketamine's antidepressant-like effects are still not fully understood.

Available data have indicated that the antidepressant-like effects of ketamine are mediated by the stimulation of the α-

ZhiQiang Zhou and GuangFen Zhang contributed equally to this work.

Z. Zhou · G. Zhang · X. Li · X. Liu · N. Wang · L. Qiu · W. Liu ·
J. Yang (✉)
Department of Anesthesiology, Jinling Hospital, School of Medicine,
Nanjing University, Nanjing, China
e-mail: yjyangjj@126.com

Z. Zuo (✉)
Department of Anesthesiology, University of Virginia,
Charlottesville, VA, USA
e-mail: zz3c@virginia.edu

amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors because pretreatment with an AMPA receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)-quinoxaline (NBQX), can abolish ketamine-induced antidepressant-like behaviors [7, 10]. Previous studies also have presented that different subanesthetic doses of ketamine increase the release of glutamate in the animal brain rapidly [11–14]. Notably, recent studies have found that the time course and dose response of the ketamine-induced increase in extracellular glutamate are similar to the rapid activation of mammalian target of rapamycin (mTOR) signaling, occurring at 30–60 min and returning to the baseline within 120 min after ketamine administration. Importantly, mTOR signaling contributes to the fast antidepressant-like effects of ketamine [15, 16]. Therefore, these results suggest the stimulation of glutamate neurotransmission as a necessary step for ketamine-induced antidepressant-like properties.

The interneurons containing calcium-binding protein parvalbumin (PV) are the main class of gamma-aminobutyric acid (GABA)-ergic interneurons [17–19]. These interneurons provide the inhibitory postsynaptic potential to regulate the activity of cortical pyramidal neurons [20, 21]. A previous study shows that inhibition of NMDA receptors reduces the activity of GABAergic interneurons and consequently increases the firing rate of the majority of pyramidal neurons, i.e., resulting in the disinhibition of glutamate signaling [22]. However, presently, there is no direct evidence to support the role of PV interneurons in ketamine's antidepressant-like effects. We hypothesized that ketamine reduces the activity of PV interneurons and then leads to the disinhibition of glutamate neurotransmission to produce its antidepressant-like actions. Testing this hypothesis was the primary goal of this study.

Moreover, ketamine's psychical adverse reaction largely limits its clinical utility not only as an anesthetic but also as a potent antidepressant for refractory depressed patients with or without suicide idea [23–25]. A number of studies indicate that ketamine exposures produce propsychotic-like effects associated with reduced expression of PV and glutamic acid decarboxylase 67 (GAD67, 67 kDa, a key enzyme for GABA synthesis) in the GABAergic interneurons in rodents [26–28]. It is generally accepted that ketamine-induced propsychotic-like behaviors are attributed to the reduction in GABAergic neuronal activities producing an enhanced excitability of pyramidal cells. Thereby, the secondary purpose of this study was to differentiate the changes in PV interneurons and brain glutamate and GABA levels between the antidepressant- and propsychotic-like effects of ketamine. Understanding this difference may improve the safety profile of ketamine in clinical use, especially as a potential antidepressant.

Material and Methods

Animals

The present study was approved by the Ethics Committee of Jinling Hospital, Nanjing, China, and was performed in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health, USA. Male adult Wistar rats, weighing 200–250 g, were purchased from the Shanghai Animal Centre, Shanghai, China. The rats were housed five per cage with food and water available ad libitum and were maintained on a 12-h light/dark cycle (lights on at 7:00 am).

Drug Interventions

To evaluate the ketamine-induced antidepressant-like effects, rats subjected to the forced swimming test (FST) received a single intraperitoneal administration of 10 mg/kg ketamine (Gutian Pharmaceutical Company, Fujian, China) in a volume of 1 ml and were subjected to behavioral tests at 0.5 and 2 h after the administration. The dose of 10 mg/kg to elicit ketamine's antidepressant-like effects was selected on the basis of previous studies [15, 16].

To investigate the role of PV interneurons and the underlying mechanism during the procedure of ketamine exerting antidepressant-like effects, a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor apocynin was given at 5 mg/kg/day in the drinking water for 7 days in order to reduce the superoxide production. The dose of apocynin was selected according to a previous study showing that this dose can prevent ketamine-induced loss of phenotype of PV interneurons in the brain through reducing superoxide production [26]. On the eighth day, 10 mg/kg ketamine was given and the behavioral tests were performed at 0.5 h after ketamine administration.

To elicit propsychotic-like effects, 30 mg/kg ketamine was intraperitoneally applied for five consecutive days according to previous studies [28–33]. Locomotor activities and stereotyped behaviors were evaluated at 0.5 and 2 h after the last ketamine administration.

Additionally, to make sure that the dose of 10 mg/kg ketamine was suitable for eliciting the antidepressant-like actions in rats, we also evaluated the effects of a single dose of 30 mg/kg ketamine and then assessed the rats by the FST and open field test (OFT) in the present study.

OFT

Locomotor activities of rats were evaluated with the OFT at 0.5 and 2 h after the administration of saline or ketamine using the standard method with some modifications [34]. The OFT apparatus consists of a 75×75 cm square floor surrounded by

40 cm high wall. The floor of the arena was divided into 25 equal squares. Rats were placed in the arena center, and the numbers of crossings and rearings in 5 min were then recorded by two expert observers who were blinded to the group assignment. The open field arena was thoroughly cleaned after the test of every rat.

FST

To evaluate depression-like behaviors, FST was carried out according to previous studies [15, 35]. The FST included two separate exposures to a cylindrical tank. The tank is 65 cm tall and 30 cm in diameter and was filled with water (22–23 °C) to a depth of 40 cm so that rats could not touch the bottom of the tank. All procedures were conducted between 9:00 am–3:00 pm. For the first exposure, rats were placed in the water for 15 min (pretest session). Twenty-four hours later (i.e., immediately after the OFT, rats were placed in the water again for a 6-min session (test session). The immobility time during the last 5 min of the 6-min test was recorded in seconds by the same two observers in a blinded manner. Immobility time was defined as the duration in which rats remained floating in the water without struggling and made only those movements necessary to keep its head above water. Water in the tank was changed after the test of every rat.

Stereotype Rating

Stereotype rating was performed at 0.5 and 2 h after the administration of saline and ketamine, respectively, as described in the previous studies [36, 37]. This activity was observed for 5 min every time. For the test, the same two observers rated the stereotyped behavior every 1 min in this procedure in a blinded manner: score 1, lying down and eyes closed (asleep); score 2, lying down and eyes opened (inactive); score 3, normal grooming or chewing cage litter (in place activities); score 4, moving about in the cage, sniffing, and rearings (normal, alert, and active); score 5, running movement (hyperactive); score 6, repetitive exploration of the cage at normal activity (slow patterned); score 7, repetitive exploration of the cage with hyperactivity (fast patterned); score 8, remaining in the same place in the cage with fast repetitive head and/or foreleg movement (restricted); score 9, backing up, jumping, seizures, abnormally maintained postures, and dyskinetic movements (dyskinetic reactive).

Immunofluorescent Staining

Animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused with phosphate-buffered saline (PBS), followed by 4 % paraformaldehyde in PBS (4 % PFA). Brains were fixed in 4 % PFA at 4 °C for 4 h and equilibrated

in 10, 20, and 30 % sucrose-PBS (3 days). The brains were then mounted in optimal cutting temperature embedding medium, frozen, and cut coronally at 10-μm thickness in a cryostat. Coronal sequential sections comprising the prefrontal region (bregma +3.0 to +2.0) were used in immunofluorescent staining to detect the expression of PV, GAD67, and dihydroethidium (DHE).

To determine the expression of PV and GAD67, we first incubated brain sections to a primary antibody, rabbit polyclonal anti-GAD67 (1:300, GeneTex), and then goat antirabbit secondary antibody (1:300; Santa Cruz). After being washed for several times in PBS, sections were exposed to a mouse monoclonal anti-PV primary antibody (1:600; Millipore) and then goat antimouse secondary antibody (1:500, Bioworld). Exposure to primary antibodies was for 24 h at 4 °C and to secondary antibodies was for 1 h at room temperature. After being washed for several times in PBS, sections were air-dried and coverslipped. Specific staining for PV and GAD67 was consistent with the instruction manual and the previous documents [38–40].

To determine the generation of reactive oxygen species in the prefrontal cortex (PFC), DHE staining was performed as previously described [41, 42]. The sections were incubated with 0.05 mmol/L DHE in phosphate-buffered saline in a dark, humidified chamber at 37 °C for 30 min. DHE is oxidized by superoxide to ethidium bromide, which binds to the DNA in the nucleus and emits red fluorescence.

Sections were evaluated for fluorescence intensity at 568 and 488-nm emissions under an LSM510 Meta multiphoton laser confocal microscope using a × 40 objective. Each section was imaged within layer V and cingulate cortex across the PFC (three regions per section). Six sections were analyzed per animal. Images were then analyzed for their somatic median green and red fluorescence content using MetaMorph as described [26]. The median fluorescence/cell was then averaged across all imaged sections of the same animal, and the mean fluorescence intensity/cell was then expressed as percent of saline conditions.

Western Blotting

The levels of PV and GAD67 in the PFC were assessed by the Western blotting. The normalized protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5 % skim milk in Tris-buffered saline tween (TBST) for 1 h and then incubated with rabbit anti-GAPDH (1:1,000; Cell Signaling), mouse anti-PV (1:1,000; Millipore), and rabbit anti-GAD67 (1:500; GeneTex) overnight in a room at 4 °C temperature. After thorough washing, membranes were incubated in TBST with the secondary antibody (goat antimouse and goat antirabbit; Santa Cruz) diluted

1:1,000 for 1 h at room temperature. Bands were visualized by the enhanced chemiluminescence and quantitated with the ImageQuant Software (Syngene).

NADPH Oxidase Activity

NADPH oxidase activity was measured using a NADPH oxidase activity assay kit (Jiancheng Biologic Project Company, Nanjing, China) according to the manufacturer's instructions, as described in the previous study [43]. Briefly, rat PFC was homogenized in PBS and centrifuged at $2,500 \times g$ for 10 min. The supernatant was collected and incubated with NADPH. NADPH oxidase activity was measured by monitoring the rate of consumption of NADPH that was inhibited by the addition of diphenyliodonium and was determined by spectrophotometry at 340 nm. The unit of measure was $\mu mol \cdot min^{-1} \cdot mg\ prot^{-1}$, and the results were expressed as percent of enzyme activity compared to that of control.

Brain Glutamate and GABA Levels

Rats were sacrificed by decapitation, and the PFC was rapidly dissected out on an ice-cold plate, frozen, and stored at $-80\ ^\circ C$ for use. The levels of glutamate were determined as described previously [44, 45]. Glutamate measurement was performed by measuring the production of $NADPH^+$ in the presence of glutamate dehydrogenase and $NADP^+$. $NADP^+$ (1.0 mM) and glutamate dehydrogenase (50 units) were added to the samples, and then, the emitted fluorescence was measured using a spectrofluorimeter. The excitation wavelength was 360 nm and the emission wavelength at 450 nm. The unit of measure of glutamate was millimole per gram of protein (mmol/gprot), and the results were expressed as percent of saline conditions.

The GABA levels were determined by an enzyme-linked immunoassay (ELISA) system (Jiancheng Biologic Project Company, Nanjing, China) according to the manufacturer's instructions. In this procedure, flat-bottom 96-well plates were coated with anti-GABA monoclonal antibody to bind GABA, and the plates were incubated overnight at 4 °C. The plates were washed, and the second specific anti-GABA polyclonal antibody was added and incubated for 60 min at 37 °C so that the captured GABA bound the polyclonal antibody. After washing, the amount of specifically bound polyclonal antibody was then detected using species-specific antibody conjugated to horseradish peroxidase as a tertiary reactant. Unbound conjugate was removed by washing. The conjugates were revealed by incubating with a chromogenic substrate. The reaction was stopped by 1 N hydrochloric acid. The absorbency was measured at 450 nm using an automatic ELISA microplate reader. The unit of measure of GABA was nanogram per milligram of protein (ng/mgprot), and the results were expressed as percent of saline conditions.

Statistical Analysis

Data are expressed as the mean±S.E.M. and were analyzed with the Statistical Package for Social Sciences (SPSS version 17.0, IL, USA). Comparisons were made with one-way analysis of variance (ANOVA) followed by post hoc Bonferroni tests. Difference was considered significant at $p < 0.05$.

Results

Effects of Acute Administration of Ketamine on the Rat Behaviors Assessed by the FST and OFT

Acute administration of ketamine at 10 and 30 mg/kg significantly reduced the immobility time of rats in the FST at 0.5 and 2 h after the administration when compared with the saline administration ($F_{(5, 42)} = 49.873$; $p < 0.001$) (Fig. 1a).

Acute administration of ketamine at 30 mg/kg induced a significant increase in the crossings at 0.5 h after the administration ($F_{(5, 42)} = 13.388$; $p < 0.001$) and had no significant influence at 2 h when compared with the saline administration (Fig. 1b). Interestingly, acute administration of ketamine at 30 mg/kg decreased the rearings significantly at 0.5 h ($F_{(5, 42)} = 2.651$; $p = 0.047$) but did not affect it at 2 h when compared with the saline administration (Fig. 1b). Ketamine (10 mg/kg)-treated rats did not have any significant changes in the crossings and rearings at 0.5 and 2 h (Fig. 1b). Ketamine at 30 mg/kg increased stereotyped behaviors significantly at 0.5 h after the administration ($F_{(5, 42)} = 6.501$; $p = 0.001$) and had no significant influence at 2 h when compared with the saline administration (Fig. 1c). There was no significant difference in the stereotyped behaviors between the saline and ketamine (10 mg/kg) interventions at 0.5 and 2 h after the administration (Fig. 1c).

Effects of Apocynin Pretreatment on the Ketamine-Induced Antidepressant-Like Actions in the FST

Acute administration of ketamine at 10 mg/kg was employed to investigate the role of PV interneurons in its antidepressant-like effects as this dose did not induce hyperactivity of rats in the OFT. Pretreatment with apocynin prevented the decrease of immobility time induced by 10 mg/kg ketamine ($F_{(3, 28)} = 9.674$; $p < 0.001$). Meanwhile, apocynin alone did not elicit a significant change in the immobility time (Fig. 2a).

No significant difference was observed in the crossings ($F_{(3, 28)} = 0.370$; $p = 1.000$) and rearings ($F_{(3, 28)} = 0.754$; $p = 1.000$) after apocynin pretreatment (Fig. 2b).
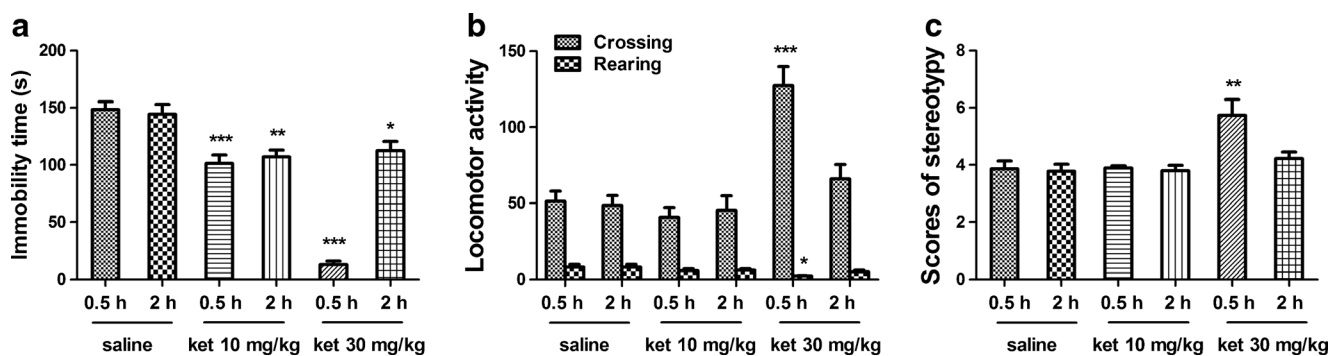
Fig. 1 Effects of acute administration of ketamine at 10 and 30 mg/kg on the immobility time, locomotor activities, and stereotyped behaviors in rats subjected to the FST and OFT. Acute doses of ketamine at 10 and 30 mg/kg both reduce the immobility time in the FST at 0.5 and 2 h after administration (**a**). Ketamine at 10 mg/kg has no significant influence on the locomotor activities (**b**) and stereotyped behaviors (**c**). Nevertheless, ketamine at 30 mg/kg increases the crossings (**b**) and stereotyped behavior scores (**c**) at 0.5 h after administration but has no significant effect at 2 h after administration. Ketamine at 30 mg/kg also decreases rearings at 0.5 h after administration (**b**). Data are expressed as the mean±standard error of the mean (S.E.M.) of eight rats per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus saline. *Ket* ketamine

Effects of Repeated Administration of Ketamine on the Rat Behaviors Assessed by the OFT

Repeated administration with 30 mg/kg ketamine increased crossings significantly at 0.5 h after the last administration ($F_{(3, 28)}=5.853$; $p=0.008$) but did not have the effects at 2 h compared with the saline administration (Fig. 3a). Interestingly, the rearings were decreased significantly at 2 h after the last administration ($F_{(3, 28)}=4.771$; $p=0.044$) (Fig. 3a). Repeated administration with 30 mg/kg ketamine increased stereotyped behaviors significantly at 0.5 h after the last administration ($F_{(3, 28)}=15.507$; $p<0.001$) but did not have the effects at 2 h compared with the saline administration (Fig. 3b).

Effects of Ketamine on the Expression of PV and GAD67 in the PFC

Ketamine administration significantly affected PV ($F_{(7, 40)}=10.082$; $p<0.001$) and GAD67 ($F_{(7, 40)}=8.493$; $p<0.001$) fluorescence intensity in the cell bodies of PV interneurons in rat PFC. Acute administration of ketamine at 10 mg/kg significantly decreased the PV ($p<0.001$) and GAD67 ($p=0.005$)

fluorescence intensity at 0.5 h after the administration. However, this change disappeared at 2 h after the administration (Fig. 4a, c). Compared with the saline administration, the repeated administration with 30 mg/kg ketamine presented a significant decrease in PV and GAD67 fluorescence intensity at 0.5 h ($p=0.040$; $p=0.046$) and 2 h ($p<0.001$; $p=0.001$) after the last administration. There was no difference in the PV fluorescence intensity between the two time points (Fig. 4c). Compared with the acute administration of ketamine at 10 mg/kg, repeated administration of 30 mg/kg ketamine reduced PV ($p=0.032$) and GAD67 ($p=0.010$) fluorescence intensity at 2 h after administration, but no difference was found between the two regimens at 0.5 h (Fig. 4). These data were consistent with the results of the Western blotting (all $p<0.05$; Fig. 5).

Effects of Apocynin Pretreatment on the NADPH Oxidase Activity and DHE Immunoreactivity in the PFC

Acute administration of ketamine at 10 mg/kg increased NADPH oxidase activity at 0.5 h after the administration, whereas pretreatment with apocynin prevented this increase
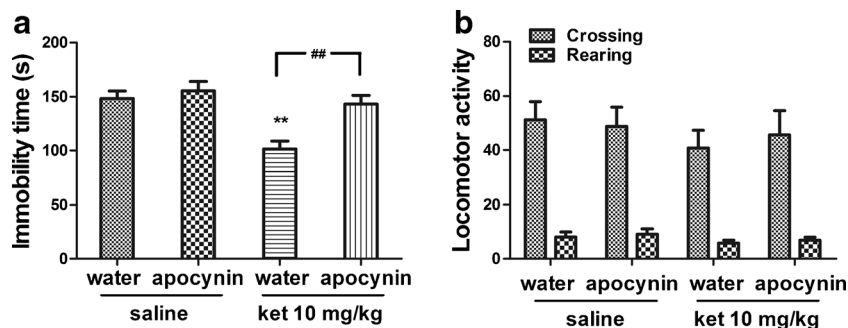

Fig. 2 Effects of pretreatment with apocynin on the immobility time and locomotor activities in rats subjected to the FST and OFT. Pretreatment with apocynin reverses ketamine-induced reduction in the immobility time in the FST (**a**) but does not affect the crossings and rearings in the OFT (**b**). Data are expressed as the mean±S.E.M. of eight rats per group. **$p<0.01$ versus saline; ##$p<0.01$. *Ket* ketamine
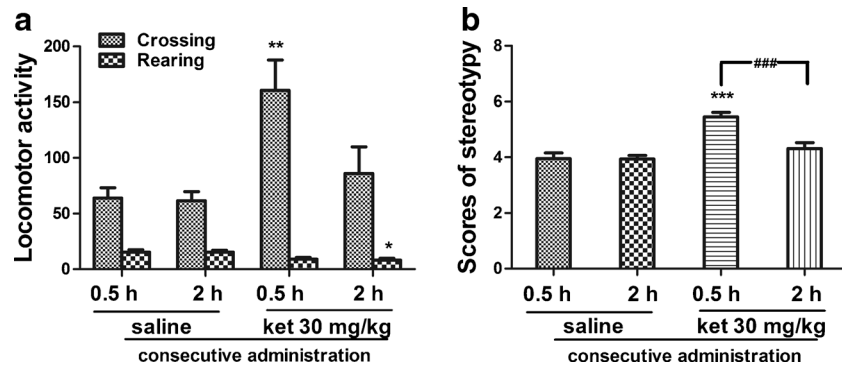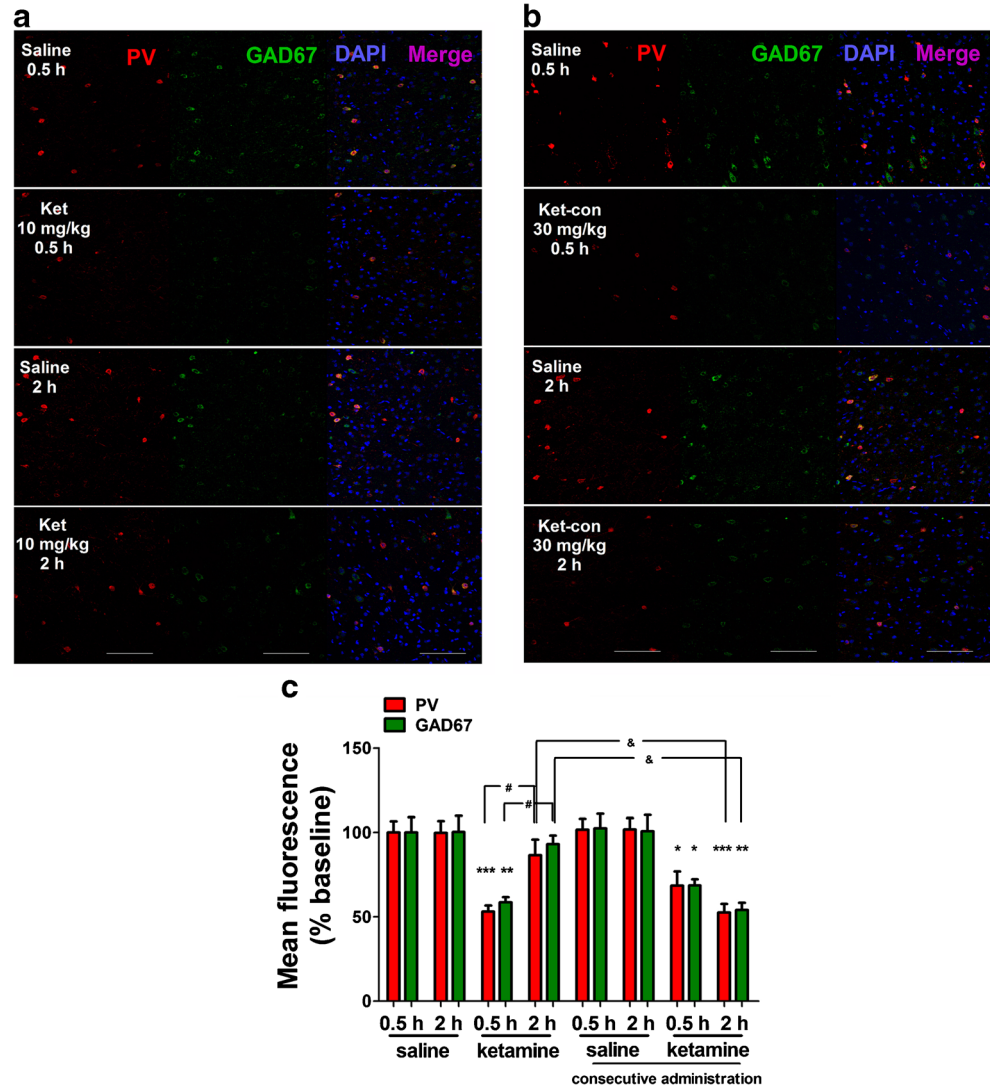
Fig. 3 Effects of repeated administration of ketamine at 30 mg/kg on the locomotor activities and stereotyped behaviors in rats subjected to the OFT. Repeated ketamine administration at 30 mg/kg increases the crossings (**a**) and stereotyped behaviors (**b**) at 0.5 h after the last administration but does not at 2 h. Repeated application of 30 mg/kg ketamine also decreases the rearings at 0.5 and 2 h (**a**). Data are expressed as the mean± S.E.M. of eight rats per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus saline; ###$p<0.001$. *Ket* ketamine

in the NADPH oxidase activity ($F(3, 20)=16.955$; $p<0.001$) (Fig. 6a). Moreover, ketamine at 10 mg/kg decreased the PV

fluorescence intensity and increased the DHE fluorescence intensity at 0.5 h after the administration, whereas

Fig. 4 Effects of acute and repeated administration of ketamine on PV and GAD67 immunoreactivity in the rat PFC. Confocal images showing the expression of PV (*left*), GAD67 (*middle*), and colocalization of markers (*right*) in the PFC after acute administration of ketamine at 10 mg/kg (**a**) and repeated doses of ketamine at 30 mg/kg (**b**). Acute dose of ketamine at 10 mg/kg decreases the PV and GAD67 fluorescence intensity in the PFC of rats at 0.5 h after administration but does not at 2 h. Repeated doses of ketamine at 30 mg/kg reduces the PV and GAD67 fluorescence intensity at 0.5 and 2 h after administration. Compared with acute administration of ketamine at 10 mg/kg, repeated doses of ketamine at 30 mg/kg decrease the PV and GAD67 fluorescence at 2 h after administration but do not at 0.5 h (**c**). Data are expressed as the mean±S.E.M. of six rats per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus the corresponding time points of the saline group; #$p<0.05$; &$p<0.05$. *Scale bar*=100 μm; *Ket* ketamine, *ket-con* ketamine consecutive administration
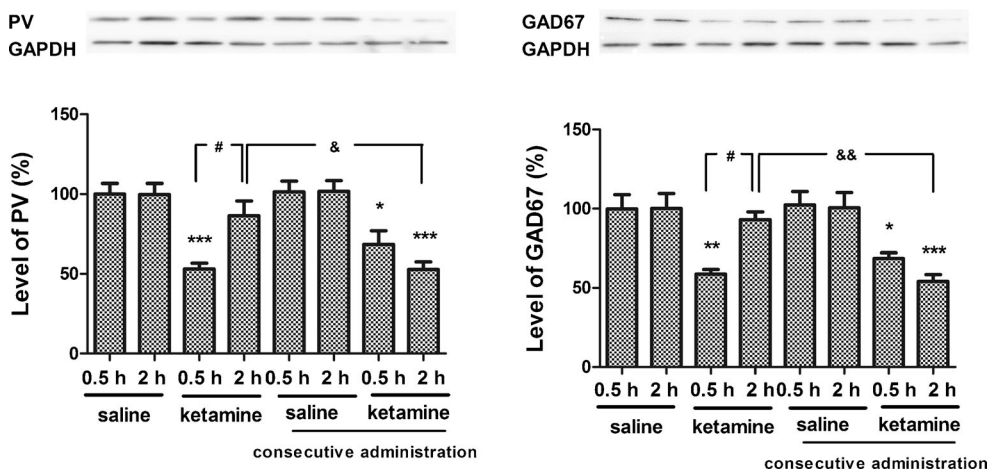
Fig. 5 Effects of acute and repeated administration of ketamine on the PV and GAD67 levels in the rat PFC determined by the Western blotting. Acute administration of ketamine at 10 mg/kg decreases the levels of PV and GAD67 in the rat PFC at 0.5 h after administration but does not at 2 h. Repeated doses of ketamine at 30 mg/kg reduce the levels of PV and GAD67 at 0.5 and 2 h after administration. Compared with acute administration of ketamine at 10 mg/kg, repeated doses of ketamine at 30 mg/kg decrease the levels of PV and GAD67 at 2 h after administration but do not at 0.5 h. Data are expressed as the mean±S.E.M. of six rats per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the corresponding time points of the saline group; #$p < 0.05$; &$p < 0.05$; &&$p < 0.01$. *Ket* ketamine, *ket-con* ketamine consecutive administration

pretreatment with apocynin prevented the decrease in the PV fluorescence intensity ($F_{(3, 20)} = 4.263$; $p = 0.018$) and the increase in the DHE fluorescence intensity ($F_{(3, 20)} = 11.732$; $p < 0.001$) in the neural cells (Fig. 6b, c).

Effects of Ketamine on the Glutamate and GABA Levels in the PFC

Acute administration of ketamine at 10 mg/kg significantly increased the glutamate levels ($F_{(3, 28)} = 20.622$; $p < 0.001$)

and decreased the GABA levels ($F_{(3, 28)} = 8.024$; $p = 0.003$) in the PFC of rats at 0.5 h after the administration. However, these changes disappeared at 2 h after the administration (Fig. 7a, b). Compared with the saline administration, the repeated administration with 30 mg/kg ketamine presented an increase in the glutamate levels ($F_{(3, 28)} = 86.640$; $p < 0.001$) and a decrease in the GABA levels ($F_{(3, 28)} = 31.036$; $p < 0.001$) at 0.5 and 2 h after the last administration. Interestingly, there was a significant difference in the glutamate levels and no significant difference in the GABA levels

Fig. 6 Effects of pretreatment with apocynin on the NADPH oxidase activity and DHE immunoreactivity in the rat PFC. Pretreatment with apocynin prevents the increase in the NADPH oxidase activity induced by 10 mg/kg ketamine at 0.5 h after the administration (**a**). Pretreatment with apocynin prevents the decrease in the PV fluorescence intensity and the increase in the DHE fluorescence intensity at 0.5 h after the ketamine administration. Confocal images show the expression of PV (*left*), DHE (*middle*), and colocalization of markers (*right*) in the PFC (**c**). Data are expressed as the mean± S.E.M. of six rats per group. *$p < 0.05$, **$p < 0.01$ versus saline. *Scale bar* = 100 μm; *Ket* ketamine
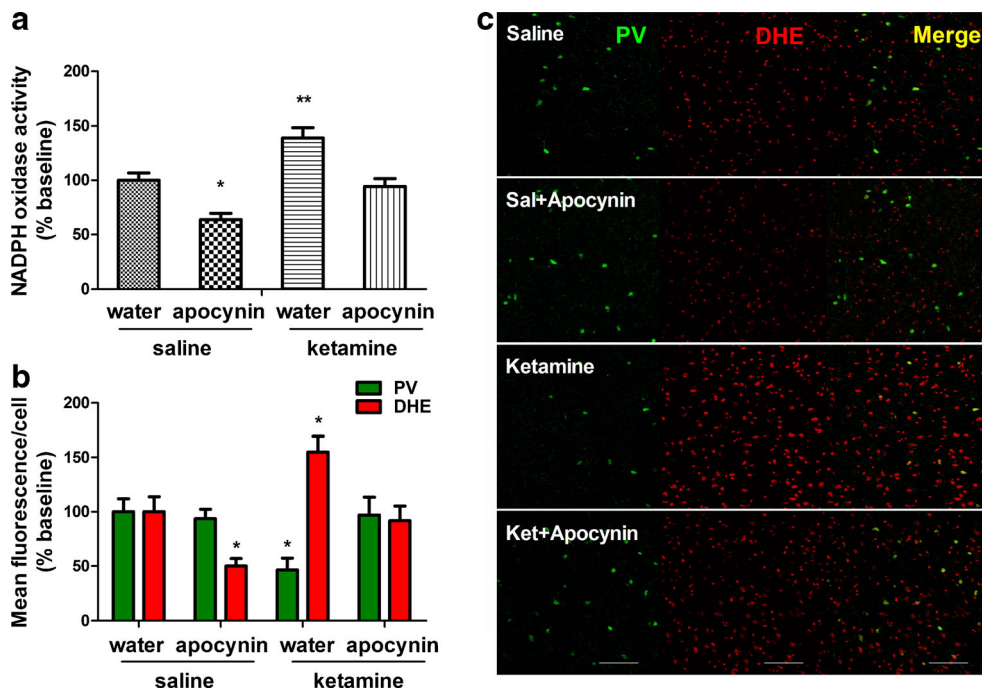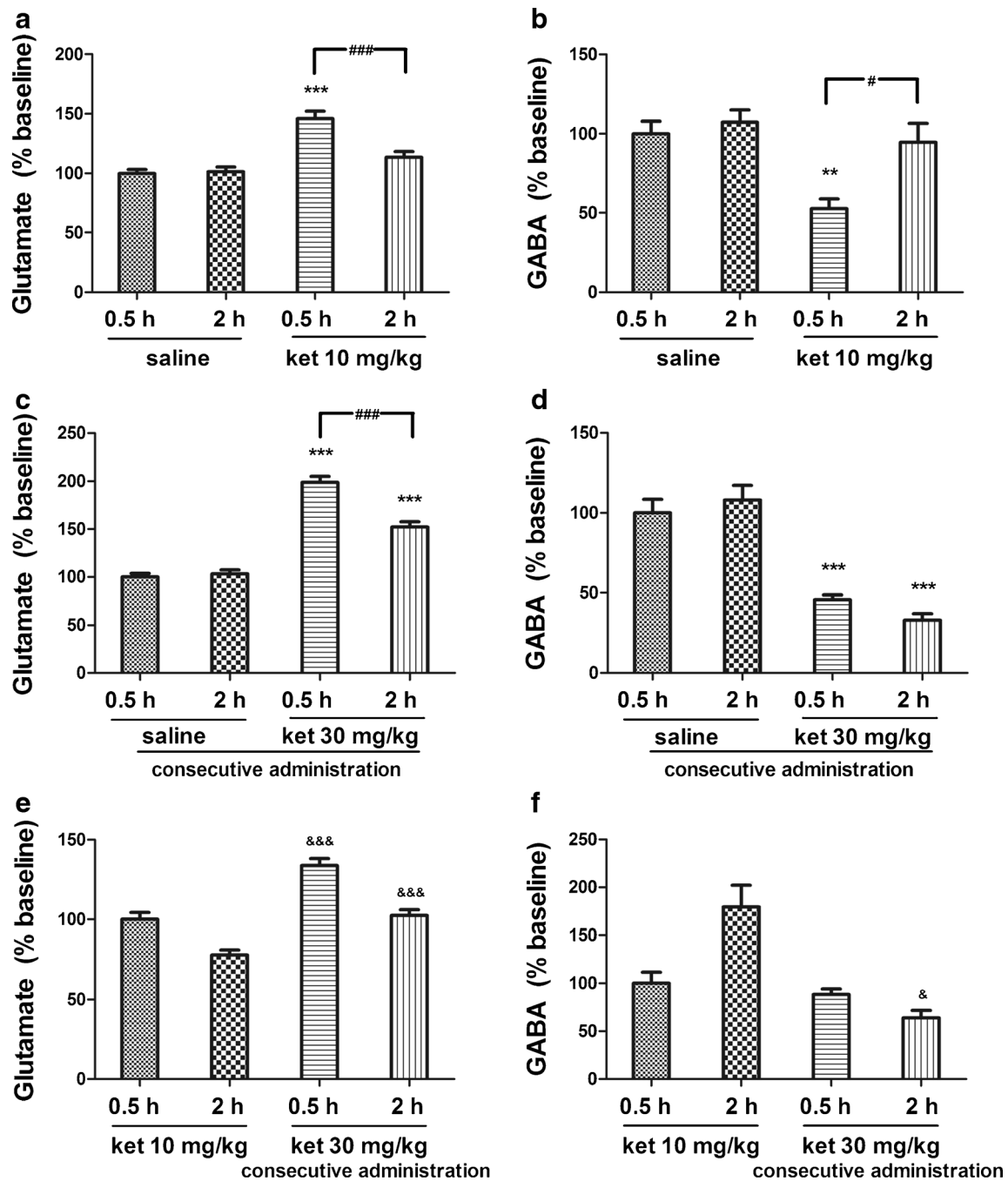
Fig. 7 Effects of ketamine on the glutamate and GABA levels in the rat PFC. Acute administration of ketamine at 10 mg/kg increases the glutamate levels and decreases the GABA levels in the rat PFC at 0.5 h, which disappears at 2 h after administration (**a**, **b**). Repeated doses of ketamine at 30 mg/kg increase the glutamate levels and decrease the GABA levels at 0.5 and 2 h after administration (**c**, **d**). Compared with acute administration of ketamine at 10 mg/kg, repeated doses of ketamine at 30 mg/kg increase the glutamate levels at 0.5 and 2 h after administration (**e**) and decrease the GABA levels at 2 h after administration but do not at 0.5 h (**f**). Data are expressed as the mean±S.E.M. of eight rats per group. **$p<0.01$, ***$p<0.001$ versus saline; #$p<0.05$, ###$p<0.001$; &$p<0.05$, &&&$p<0.001$ versus ketamine 10 mg/kg. *Ket* ketamine

between the two time points after the repeated administration of ketamine (Fig. 7c, d). The responses to repeated administration of 30 mg/kg ketamine were more robust and maintained longer than those caused by 10 mg/kg ketamine. Compared with 10 mg/kg ketamine, repeated doses of 30 mg/kg

ketamine increased glutamate levels at 0.5 and 2 h after administration ($F(3, 28)=34.339$; $p<0.001$) (Fig. 7e), decreased GABA levels at 2 h after administration ($F(3, 28)=13.597$; $p<0.001$), but did not affect the GABA levels significantly at 0.5 h (Fig. 7f).

Discussion

The results of the present study showed for the first time that the downregulation of PV and GAD67 in the PV interneurons and the subsequent disinhibition of glutamate neurotransmission were associated with both the antidepressant- and propsychotic-like actions of ketamine in rats. The two applied regimens of ketamine both reduced the levels of PV and GAD67 at 0.5 h after drug administration. However, 2 h after drug administration, this reduction disappeared after acute single administration of 10 mg/kg ketamine that induced antidepressant-like actions but still existed after repeated administration of 30 mg/kg ketamine that elicited propsychotic-like effects. Furthermore, repeated administration of ketamine led to higher glutamate levels at 0.5 and 2 h and lower GABA levels at 2 h after administration than acute dose of ketamine at 10 mg/kg.

The symptoms of human depression are complex and diversiform, and only a part of psychiatric syndromes can be recapitulated in rodents [46–48]. The animal models of the FST, tail suspension test (TST), learned helplessness (LH), and chronic unpredicted mild stress (CUMS) are widely used to explore the antidepressant-like properties of ketamine and its underlying mechanisms [8, 10, 15, 16]. A major weakness of the FST is, different from human depression, that the rodents involve only a short term of 15-min prestress [46]. Nonetheless, the FST is a more favorable behavioral test for screening new antidepressant agents and exploring the underlying mechanism of depression due to its quick and reliable characteristics across experiments. Therefore, we chose the FST to assess the antidepressant-like behaviors in the present study on the basis of the referred studies [15, 35].

Previous studies have shown that acute administration of ketamine at 10 mg/kg produces rapid, robust, and lasting antidepressant-like effects without affecting spontaneous locomotion in the FST and other depression tests in rodents [8, 15, 16]. In the present study, although acute administration of 30 mg/kg ketamine reduced the immobility time to a greater degree in the FST, the antidepressant-like effects were not identified as locomotor activity increased significantly, suggesting that the acute dose of 30 mg/kg ketamine is unsuitable for treating depression in rats. Ketamine is well known to have the ability to induce acute propsychotic-like effects, and repeated administration of 30 mg/kg has been usually used to induce propsychotic-like behaviors in previous studies [28–31, 33]. Therefore, we used the acute dose of 10 mg/kg ketamine for studying antidepressant-like effects and repeated doses of 30 mg/kg to elicit propsychotic-like actions in the present study.

The study from Li et al. [15] has shown that ketamine's antidepressant-like effects are mainly attributed to the fast stimulation of the mTOR pathway that leads to the rapid and sustained elevation of synapse-associated proteins and spine number in rat PFC. However, the mechanisms underlying the induction of mTOR signaling remain unclear. The upregulation of glutamate and phosphorylated mTOR occurs at 0.5 h and returns to the baseline within 2 h after the administration of ketamine, and the requirement for glutamate-AMPA receptor activation has been previously confirmed [15, 49]. According to the time-course of glutamate and mTOR, we chose 0.5 and 2 h to examine the changes of PV interneurons and neurotransmission. The present study suggests that an acute dose of 10 mg/kg ketamine rapidly reduces the activity of PV interneurons in rat PFC. The inhibition of these GABAergic interneurons consequently decreased the GABA release and increased the glutamate levels at 0.5 h after ketamine administration. Therefore, AMPA receptors may be stimulated by this burst of glutamate to produce a fast excitatory synaptic signal. Although the changes of PV and glutamate induced by ketamine are transient, returning to baseline by 2 h, this procedure can be considered as a "switch" to activate the intracellular signaling and increase synapse-associated proteins and spine numbers. Just like the results shown in the study by Li et al. [15], that is, the ketamine-induced increases in synapse-associated proteins were delayed relative to mTOR, peaking at 2 to 6 h and being increased for 72 h.

In addition, the inhibition of glycogen synthase kinase-3 (GSK-3) and eukaryotic elongation factor 2 (eEF2) kinase has been reported to be involved in the antidepressant-like actions of ketamine in different studies [50–52]. These various alterations induced by ketamine may enhance synaptic deconsolidation or increase synaptic proteins and ultimately lead to the antidepressant-like effects of ketamine.

The study by Behrens et al. [26] has shown that ketamine-induced loss of PV is mediated by the activation of NADPH oxidase. In the same study, apocynin, a NADPH oxidase inhibitor, reduces the superoxide production and prevents ketamine-induced loss of interneurons with PV phenotype both in vitro and in vivo [26]. Our results indicated that apocynin pretreatment blocked the antidepressant-like effects induced by 10 mg/kg ketamine in the FST, reduced the NADPH oxidase activity and superoxide production, and prevented the loss of PV immunoreactivity, suggesting the loss of PV phenotype contributing to the antidepressant-like effects of ketamine.

PV-containing interneuron synapses on the cell body or axon initial segment of glutamatergic neurons potently regulate pyramidal cell output [18]. A decrease in inhibitory GABAergic signaling from PV interneurons induced by ketamine leads to an increase in glutamate release from pyramidal neurons [53]. In the present study, an acute single dose of 10 mg/kg ketamine rapidly increased the glutamate levels and decreased the GABA levels in the PFC at 0.5 h after administration, and these changes disappeared at 2 h after ketamine administration. Nevertheless, repeated use of 30 mg/kg ketamine increased the glutamate levels and decreased the GABA

levels at both 0.5 and 2 h after the last administration. In the present study, the time course of alterations in the PV immunoreactivity was consistent with those changes in the glutamate and GABA levels in the PFC.

A previous study has indicated that the activity of cortical GABA interneurons is highly sensitive to the activation of NMDA receptors [22]. In contrast, the activity of pyramidal neurons is not directly regulated by the NMDA receptors but is susceptible to the blockade of GABA release that subsequently induces the disinhibition of glutamate signaling [22]. Xi et al. [54] have demonstrated that a low dose of NMDA receptor antagonist MK801 directly blocks the NMDA receptors on GABAergic interneurons rather than pyramidal neurons; whereas a high dose affects both GABAergic interneurons and glutamatergic pyramidal neurons. A previous study reveals that a single subanesthetic dose of ketamine (10, 20, or 30 mg/kg) dose-dependently increases glutamate levels in the PFC and a higher dose produces longer durations of response, while an anesthetic dose of ketamine (200 mg/kg) downregulates glutamate levels, and an intermediate dose (50 mg/kg) does not affect the glutamate levels [11]. Consistent with this finding, ketamine at a low dose (10 mg/kg) but not a high dose (80 mg/kg) presents antidepressant-like actions in the FST [15]. In the present study, we observed that 10 mg/kg ketamine produced antidepressant-like effects without hyperactivity, whereas repeated doses of 30 mg/kg ketamine elicited noticeable stereotyped behaviors and hyperactivity actions in rats. This difference may be attributed to the different degrees and durations of the effects of the two regimens of ketamine on PV interneurons and the subsequent glutamate neurotransmission.

In addition, in most studies [55, 56], high-performance liquid chromatography (HPLC) or capillary electrophoresis was usually used to determine brain neurotransmitter contents in samples collected through microdialysis. However, the methods for measuring glutamate and GABA used in the present study are indirect and inaccurate; further studies therefore should be performed to validate our results. Furthermore, a postmortem study quantifying the densities of GABAergic interneurons displayed no change of PV interneurons in depressed patients [57]. Moreover, chronic stress did not alter PV interneurons and PV-immunoreactive neuropil in rats [58]. Although we showed PV interneurons being involved in the antidepressant-like effects of ketamine, the previous studies did not suggest the involvement of PV interneurons in the pathogenesis of depression itself [57, 58]. Thus, the exact role of PV interneurons in the pathogenesis and treatment of depression needs further investigation.

In conclusion, our results show that the phenotype loss of PV interneurons in rat PFC contributes to the antidepressant-like actions following acute ketamine administration and is also involved in the propsychotic-like behaviors after repeated ketamine use. The downstream events may include the

disinhibition of glutamate neurotransmission. The different degrees and durations of the effects on PV interneurons produced by the two regimens of ketamine may partly underline their behavioral variance. Understanding the molecular mechanisms and the different neurobiological changes between the antidepressant- and propsychotic-like effects of ketamine can improve the safety profile of using ketamine as a novel antidepressant agent and an old general anesthetic in clinical procedures.

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