www.nature.com/aps

Acta Pharmacologica Sinica

ARTICLE

Nicotine pretreatment alleviates MK-801-induced behavioral and cognitive deficits in mice by regulating Pdlim5/CRTC1 in the PFC

Qian Wang[1], Meng-wei Wang[2], Yan-yun Sun[2], Xiao-yan Hu[1], Pan-pan Geng[1], Hui Shu[2], Xiao-na Wang[2], Hao Wang[1], Jun-fang Zhang[3,4], Hong-qiang Cheng[5], Wei Wang[6] and Xin-chun Jin[1,2]

Increasing evidence shows that smoking-obtained nicotine is indicated to improve cognition and mitigate certain symptoms of schizophrenia. In this study, we investigated whether chronic nicotine treatment alleviated MK-801-induced schizophrenia-like symptoms and cognitive impairment in mice. Mice were injected with MK-801 (0.2 mg/kg, i.p.), and the behavioral deficits were assessed using prepulse inhibition (PPI) and T-maze tests. We showed that MK-801 caused cognitive impairment accompanied by increased expression of PDZ and LIM domain 5 (Pdlim5), an adaptor protein that is critically associated with schizophrenia, in the prefrontal cortex (PFC). Pretreatment with nicotine (0.2 $mg \cdot kg^{-1} \cdot d^{-1}$, s.c., for 2 weeks) significantly ameliorated MK-801-induced schizophrenia-like symptoms and cognitive impairment by reversing the increased Pdlim5 expression levels in the PFC. In addition, pretreatment with nicotine prevented the MK-801-induced decrease in CREB-regulated transcription coactivator 1 (CRTC1), a coactivator of CREB that plays an important role in cognition. Furthermore, MK-801 neither induced schizophrenia-like behaviors nor decreased CRTC1 levels in the PFC of Pdlim5$^{-/-}$ mice. Overexpression of Pdlim5 in the PFC through intra-PFC infusion of an adreno-associated virus AAV-Pdlim5 induced significant schizophrenia-like symptoms and cognitive impairment. In conclusion, chronic nicotine treatment alleviates schizophrenia-induced memory deficits in mice by regulating Pdlim5 and CRTC1 expression in the PFC.

Keywords: schizophrenia; nicotine; working memory; Pdlim5; CRTC1; prefrontal cortex

Acta Pharmacologica Sinica (2023) 44:780–790; https://doi.org/10.1038/s41401-022-00974-8

INTRODUCTION

Approximately 50%–90% of schizophrenia patients have been observed to have smoking habits [1]. This is significantly higher than the ~25%–30% rate found among the general population [2]. The rate of smoking may be higher in schizophrenia patients because patients consume tobacco to relieve schizophrenia-induced cognitive impairment [3], which is the core symptom of schizophrenia [4] and is linked with brain function impairment [5]. Nicotine is the critical psychoactive ingredient that promotes the consumption of tobacco [6]. Thus, smoking-obtained nicotine is indicated to improve cognition [7] and mitigate certain schizophrenic symptoms [1, 3, 6, 8, 9]. It has been reported that by activating the nicotine α5-acetylcholine receptor (α5-nAChRs), nicotine reverses cognitive impairment in a schizophrenia mouse model caused by a CHRNA5 gene mutation [10]. However, how nicotine alleviates schizophrenia-induced cognitive impairment remains largely unexplored.

Human genetic studies have reported that PDZ and LIM domain 5 (Pdlim5) is associated with gene expression in schizophrenia [11], as well as the occurrence of both bipolar disorder and schizophrenia [12]. In addition, Pdlim5 is associated with both schizophrenia in the Chinese Han population [13] and paranoid schizophrenia in Emirati patients [14]. Notably, Iwamoto et al. used DNA chip analysis to explore the levels of Pdlim5 in the postmortem brain tissue of several neuropsychiatric patients, such as bipolar disorder, schizophrenia or depression, and found that Pdlim5 in the prefrontal cortex (PFC) area was upregulated [15]. In addition, the levels of Pdlim5 mRNA expression in the peripheral leukocytes of schizophrenia patients without medication were significantly upregulated compared to the control [16]. Although Pdlim5 in neurons plays a critical role in dendritic spine shrinkage [17], there is no direct evidence that Pdlim5 plays a role in schizophrenia-induced cognitive deficit, and the mechanism needs to be further investigated.

[1]Beijing Key Laboratory of Cancer Invasion and Metastasis Research, Department of Anatomy, Histology and Embryology, School of Basic Medical Sciences, Advanced Innovation Center for Human Brain Protection, Capital Medical University, Beijing 100069, China; [2]Institute of Neuroscience, The Second Affiliated Hospital of Soochow University, Suzhou 215004, China; [3]School of Medicine, Ningbo University, Ningbo 315211, China; [4]Zhejiang Provincial Key Laboratory of Pathophysiology, Ningbo 315211, China; [5]Department of Pathology and Pathophysiology, Zhejiang University School of Medicine, Hangzhou 310058, China and [6]Department of Physiology and Pathophysiology, Capital Medical University, Beijing 100069, China
Correspondence: Wei Wang (wangwei@ccmu.edu.cn) or Xin-chun Jin (xinchunjin@gmail.com)
These authors contributed equally: Qian Wang, Meng-wei Wang, Yan-yun Sun

Received: 8 April 2022 Accepted: 2 August 2022
Published online: 29 August 2022

As a transcriptional coactivator of cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB), CRTC1 (the CREB-regulated transcription coactivator) has been shown to modulate learning and/or memory [18]. For example, CRTC1 has been reported to play key roles not only in memory consolidation but also in memory reconsolidation [8, 19]. Emerging evidence suggests that CRTC1 dysregulation may be implicated in the etiopathogenesis of psychiatric disorders, such as addiction, anxiety, and mood disorders [18]; for example, CRTC1 knockout mice were shown to exhibit depression-like symptoms and altered expression of neuroplasticity-related genes [20]. Therefore, we postulated that dysregulation of Pdlim5-CRTC1 signaling may underlie the molecular mechanism for schizophrenia-like behavior and/or cognitive impairment that is also linked with PFC dysfunctions.

Acute MK-801 treatment has been used to induce schizophrenia-like behavior and cognitive impairment [21, 22], and working memory (WM) impairment in patients has often been referred to as a core sign of schizophrenia [23]. The delayed alteration task (DAT) in the T-maze, which can detect WM, is a widely used model to detect schizophrenia-induced cognitive deficits [24], and we have previously shown that acute nicotine treatment significantly increased the spatial WM of rats detected by the T-maze task [9]. In the current study, we propose our hypothesis that chronic nicotine treatment alleviates schizophrenia-induced cognitive deficits by regulating Pdlim5 and CRTC1 in mice.

MATERIALS AND METHODS

Animals

Male 8- to 10-week-old male C57BL/6 mice (Shanghai SLAC Company, China) were housed 4–5 per cage at a constant temperature (23 ± 1 °C) with a 12:12 h light-dark cycle. One week before the start of the task, all the mice were allowed to drink freely and have a restricted diet to maintain ~85% of their body weight. The procedures in these experiments were approved by the Soochow University Animal Care Committee and were performed in accordance with the NIH Laboratory Animal Care and Use Guidelines. The efforts are aimed at minimizing animal suffering and reducing the number of animals used.

Pdlim5$^{-/-}$mice:

Pdlim5$^{-/-}$ mice were a gift from Professor Hong-qiang Cheng's lab and were generated by targeting the third exon of the murine ENH gene [25].

Drugs and drug administration

At 0.5 h before WM testing in the T-maze, MK-801 (Sigma, St. Louis, MO, USA) or vehicle (sterile saline) was administered intraperitoneally (i.p.) at a dose of 0.2 mg/kg, which is an established method for animal models of schizophrenia [26, 27]. Nicotine (Sigma, St. Louis, MO, USA) or vehicle (sterile saline) was given subcutaneously once daily at a dose of 0.2 mg/kg [9] for 2 weeks.

Surgery and pdlim5 AAV-virus delivery

AAV vectors were designed and produced by Shanghai GeneChem Co., Ltd., China. The coding sequence of the Pdlim5 (NM_001190852) gene was amplified by PCR and integrated into the GV467 vector (CMV-betaGlobin-MCS-EGFP-3Flag-SV40 PolyA) by *Bam*HI. Viral particles (CMV-betaGlobin-Pdlim5-EGFP-3Flag-SV40 PolyA) were generated by transient transfection of AAV-293T cells with a transfer plasmid, and infection was performed according to the manual. The empty vector (AAV-GFP) and recombinant vectors (AAV9-Pdlim5) contained enhanced green fluorescence protein (EGFP) as a marker to track AAV-mediated target gene expression using fluorescence microscopy. A total of 1.37×10^{12} vg/mL virus was used to transfect the mouse PFC. Pentobarbital sodium (2%, 5 mL/kg, i.p.) was used to anesthetize mice, and then, the mice were mounted onto a stereotaxic frame. AAV vectors were bilaterally (0.5 μL/side) infused into the PFC (AP: +1.7, ML: 0.3, DV: −1.5 mm to bregma) at a speed of 0.1 μL/min, and after the infusion was completed, the injection needle stayed in place for another 5 min. After 3 weeks, immunofluorescence combined with Western blotting was used to detect successful viral infection and whether Pdlim5 was overexpressed.

Behavioral training and testing

PPI. To assess prepulse inhibition (PPI), mice were put into acoustically isolated startle chambers (MED associates Inc. USA). Mice received training according to the PPI procedure similar to those reported previously [28]. PPI was calculated as the percent inhibition of the startle amplitude caused by a single pulse: %PPI = (amplitude in the single pulse test-amplitude in the prepulse plus pulse test)/(amplitude on pulse alone trial) × 100.

Delayed alternation T-maze task. Mice received delayed alternation task (DAT) training in the T-maze as we have reported [29]. At first, mice were trained to get used to a T-maze followed by a training to learn to visit the two arms alternatively for reward. When the mice completed one initial test and nine formal tests, the daily session ended. Regardless of which arm they chose in the initial experiment, the mice could be rewarded because both arms were decoyed. During the formal experiment, the mice were required to learn to choose the opposite arm to be rewarded. If the WM or the ability to correct mistakes was destroyed, they would make mistakes and choose the arm they visited without being rewarded. After each test, the mice were returned to the starting box and separated by a closed sliding door. The mice could not explore the maze if the sliding door was not removed and after an interval of 10 s (delay), and the next experiment began after the sliding door was removed. A 75% alcohol solution was used to wipe the maze to clean the odors between trials.

It took ~7 days for mice to reach an 80% success rate (the percentage of correct selections among all selections and the correct selections are always 9). Once the mouse performance stabilized at a success rate of ~80% in the test for three continuous days [29], mice were systemically injected with MK-801 followed by a WM test 4 h later. The mice must choose the opposite arm to be rewarded during the test session. If the mice made an error, they would receive a correction procedure by keeping the bait in the baited arm. The counted errors are divided into two types: Lose-shift failure is defined as the continuous wrong choice that the mice incorrectly selected in the prior trial, and Win-shift failure means that the mice entered the wrong arm that was a correct choice in the prior trial [29].

Experimental procedures

Experiment 1. The experiment determined nicotine's effect on MK-801-induced schizophrenia-like behavior and WM impairment. Mice were randomly assigned to four groups: (1) saline, (2) MK-801, (3) MK-801+nicotine, and (4) nicotine. The experimental design is presented in Fig. 1a.

Experiment 2. The experiment aimed to explore the mechanism of nicotine on MK-801-induced schizophrenia-like behavior and WM impairment.

Experiment 3. This experiment examined the effect of MK-801 on schizophrenia-like behaviors and WM in Pdlim5$^{-/-}$mice. Mice were randomly divided into two groups: (1) saline and (2) MK-801.

Experiment 4. This experiment examined the role of Pdlim5 upregulation in schizophrenia-like behavior and WM. The Pdlim5 virus was injected into the bilateral PFC.

Cell culture

Primary neuron culture. Cervical dislocation was used to sacrifice pregnant rats (pregnancy 17–18 days), the fetal rat brains were removed, and the cortex was obtained. The cortex was crushed, and 0.25% trypsin (HyClone) containing 0.02% DNase I (Solarbio) was supplied and incubated at 37 °C for 10 min. Digestion was terminated after culture medium containing 10% FBS was provided. The cell suspension was filtered through a 70 μm sieve (Biologix) followed by a 5 min centrifuge at 1000 rpm. The pellet was resuspended in DMEM/F12 + 10% FBS after the supernatant was discarded. After counting, the cells were seeded in a 24-well plate precoated with 20 μg/mL poly-*L*-lysine (PLL, Sigma) at a density of 250,000 cells per well. Neurobasal medium (Gibco) containing 2% B27 (Stemcell) and 1% GlutaMax (Thermo) + 0.5% p/s (Beyotime) was used to replace the medium 4 h later.

Primary cortical neurons on day 4 of culture were treated with 20 μM MK-801 [30], 100 ng/mL nicotine [31] or both MK-801 and nicotine for 72 h.

SH-SY5Y cell culture. SH-SY5Y cells (American Type Culture Collection) were cultured as we described [32].

Western blot

After the behavioral test was completed, the mice were immediately perfused with ice-cold phosphate-buffered saline (PBS). The hippocampus and PFC were collected for the protein expression analysis of Pdlim5 and CRTC1 as we described [33]. In brief, tissues were homogenized in lysis buffer, and a BCA protein assay kit (Beyotime, Haimen, Jiangsu, China) was used to determine the protein concentrations in the homogenates. Following electrophoresis and membrane transfer, the membranes were incubated with primary antibodies against Pdlim5 (1:1000, Abcam), CRTC1 (1:1000, Abcam), and β-actin (1:10000, Huabio, Hangzhou, Zhejiang, China) overnight at 4 °C. After washing with PBS-T, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies (Boster, Wuhan, Hubei, China) for 2 h at room temperature. A SuperSignal West Pico HRP substrate kit (Thermo Fisher, Rockford, IL, USA) was used to develop the membranes, and images were captured. The intensities of the protein bands were quantified after normalization to β-actin or tubulin.

Immunofluorescence staining for Pdlim5 and CRTC1

After the behavioral test was completed, the mice were perfused with PBS followed by 4% PFA. Cryosections with a 20 μm thickness were fixed with 4% PFA to analyze Pdlim5 and CRTC1 expression, as described previously [34]. In brief, nonspecific antibody binding was blocked using blocking buffer containing 0.3% Triton X-100, 1% BSA, and 5% goat serum for 2 h. Cryosections containing the PFC were incubated with primary antibodies against Pdlim5 (1:200, Abcam) and CRTC1 (1:200, Abcam) overnight at 4 °C, followed by an incubation with Alexa Fluor 488-conjugated secondary antibody (1:800) or Cy3-conjugated secondary antibodies (1:800) for 2 h at room temperature. An LSM 700 microscope (Zeiss) was applied to visualize the immunofluorescence, and images were captured to show the positive signal in the PFC.

To evaluate the expression of Pdlim5, CRTC1 and p-CRTC1 in MK-801-treated SH-SY5Y cells, cells were fixed with 4% PFA for 30 min followed by blocking nonspecific binding by incubating the cells with a 10% goat serum and 0.03% Triton X-100 solution for 2 h at room temperature. Cells were incubated with rabbit anti-Pdlim5 (1:200, Abcam), rabbit anti-CRTC1 (1:200, CST) or rabbit anti-p-CRTC1 (1:200, CST) in blocking solution at 4 °C overnight. Next, the cells were incubated with an Alexa Fluor 488-conjugated secondary antibody (anti-rabbit, 1:800) for 2 h at room temperature. Immunostaining was visualized under an LSM 700 confocal laser scanning microscope (Zeiss).

Real-time RT–PCR

According to the manufacturer's procedure and our previous study [8], total RNA was isolated using TRIzol reagent (Invitrogen). In brief, TaqMan® Reverse Transcription Kits (Applied Biosystems) were used to reverse transcribe (RT) RNA (0.5 μg) with random primers in a 20 μL final reaction volume. SYBR® Green PCR Master Mix (Applied Biosystems) was used to amplify the RT products (0.5 μL) in a 10 μL final reaction volume by the 7900HT Fast Real-Time PCR System (Applied Biosystems). The conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at two temperatures (95 °C for 15 s and 60 °C for 1 min). Primers (Integrated DNA Technologies) for miRNA-21 and U6 were designed based on known sequences of mouse; miRNA21 (M)-forward: 5′-TGTCGGGTAGCTTATCAGAC-3′; miRNA21 (M)-reverse: 5′-TTCAGACAGCCCATCGACTG-3′; U6(M) - forward: 5′-CTCGCTTC GGCAGCACA-3′: U6(M)-reverse: 5′-AACGCTTCACGAATTTGCGT-3′; miRNA21 (H)- forward: 5′-GCCGCTAGCTTATCAGACTGA-3′; miRNA 21(H)- reverse: 5′-GTGCAGGGTCCGAGGT-3′. SDS Enterprise Database software (Applied Biosystems) was used to calculate the fluorescence threshold value (Ct value). The comparative $\Delta\Delta C_t$ method was used to calculate the relative mRNA expression as described previously [35]. Briefly, mean C_t values were normalized to the internal control GAPDH, and the difference was defined as ΔC_t. The difference between the mean ΔC_t values of treated and untreated cells was calculated and defined as $\Delta\Delta C_t$. The level of comparative mRNA expression was calculated as $2^{-\Delta\Delta Ct}$.

Statistical analysis

One-way or two-way ANOVA was used to analyze the data, and the source of significance was detected using Bonferroni post hoc tests. Statistical significance was defined as a probability level of <0.05. SPSS 18.0 statistical programs (SPSS, Chicago, IL, USA) were used to perform the statistical analysis. Data in the text and figures are expressed as the mean ± SEM.

RESULTS

Chronic nicotine treatment alleviates MK-801-induced schizophrenia-like behaviors and cognitive impairment

In rodent models, MK-801 has often been applied to induce schizophrenia-like behavior [36] and cognitive deficits [37]. Here, the experimental procedure with a detailed description is provided in Fig. 1a. The PPI test was used to verify the successful establishment of MK-801-induced schizophrenia-like behavior [36]. As shown in Fig. 1b, systemic administration of MK-801 significantly decreased mouse performance in the PPI task, and nicotine treatment significantly inhibited this effect. Three-way ANOVA results showed a main effect of MK-801 ($F(1,121) = 5.373$, $P = 0.022$) and nicotine ($F(1,121) = 31.479$, $P < 0.001$) and an interaction of MK-801×nicotine ($F(1,121) = 18.588$, $P < 0.001$) but no main effect of dB ($F(2,121) = 1.669$, $P = 0.19$).

To detect schizophrenia-induced cognitive deficits, a delayed alteration T-maze (DAT) task was used to test WM performance in rodents [23, 24]. Administration of MK-801 significantly impaired mouse WM, and pretreatment with nicotine significantly alleviated this effect. For correct choice (Fig. 1c), a two-way ANOVA revealed a main effect of MK-801 $F(1,47) = 9.571$, $P = 0.003$) and nicotine ($F(1,47) = 2.656$, $P = 0.110$) and an interaction of MK-801×nicotine ($F(1,47) = 4.501$, $P = 0.039$). For Lose-shift failure (Fig. 1d), two-way ANOVA revealed a main effect of MK-801 ($F(1,48) = 13.172$, $P = 0.001$) and nicotine ($F(1,48) = 3.724$, $P = 0.060$) and an interaction of MK-801×nicotine ($F(1,48) = 10.134$, $P = 0.003$). For Win-shift failure (Fig. 1e), two-way ANOVA revealed no main effect of MK-801 ($F(1,47) = 2.454$, $P = 0.124$), nicotine ($F(1,47) = 0.572$, $P = 0.453$), or MK-801×nicotine interaction ($F(1,47) = 1.153$, $P = 0.288$).

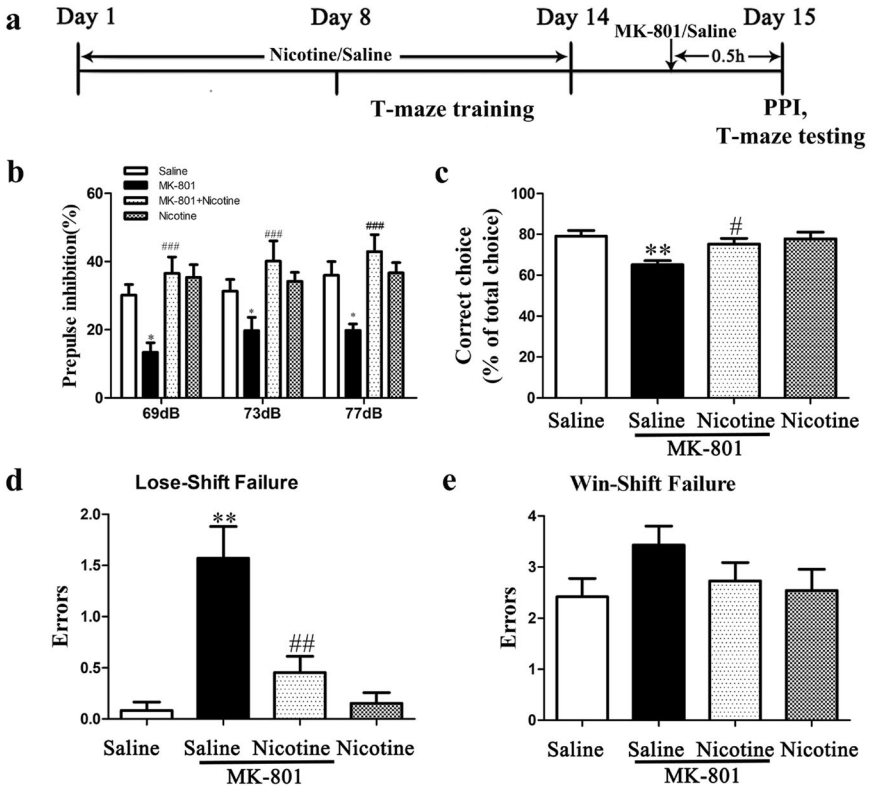

Fig. 1 Nicotine pretreatment significantly reduced MK-801-produced schizophrenia-like behaviors and WM impairment. a The experimental procedure. **b** MK-801 treatment significantly impaired PPI in wild-type mice (*$P < 0.05$ vs. Saline group), and chronic nicotine treatment significantly reduced this inhibition ($^{###}P < 0.001$ vs. MK-801 group). **c** MK-801 treatment significantly reduced the percentage of correct choices (**$P < 0.01$ vs. Saline group), and nicotine treatment reduced this inhibition ($^{#}P < 0.05$ vs. MK-801 group). **d** MK-801-treated mice had a significant upregulation of Lose-shift failure (**$P < 0.01$ vs. Saline group), and nicotine treatment reduced this change ($^{##}P < 0.01$ vs. MK-801 group). **e** MK-801 and nicotine induced no significant change in Win-shift failure ($P > 0.05$ vs. MK-801 group, $n = 8$/group).

Nicotine treatment inhibited MK-801-induced Pdlim5 upregulation and CRTC1 downregulation

The expression of Pdlim5 in the PFC and hippocampus was explored. MK-801 treatment significantly increased the expression of Pdlim5 in the PFC, and nicotine treatment significantly alleviated this change (Fig. 2a, b). Two-way ANOVA revealed a main effect of MK-801 ($F(1,21) = 4.992$, $P = 0.037$, Fig. 2b) and nicotine ($F(1,21) = 9.154$, $P = 0.007$, Fig. 2b) and an interaction of MK-801×nicotine ($F(1,21) = 5.971$, $P = 0.024$). MK-801 treatment did not significantly change Pdlim5 expression in the hippocampus. Two-way ANOVA revealed no main effect of MK-801 ($F(1,17) = 0.076$, $P = 0.786$, Fig. 2c) or nicotine ($F(1,17) = 0.224$, $P = 0.643$, Fig. 2c) or an interaction of MK-801×nicotine ($F(1, 17) = 0.001$, $P = 0.978$). Since only significant changes in Pdlim5 expression were detected in the PFC, double staining for Pdlim5 and NeuN was performed to check the distribution of Pdlim5 expression in the PFC. As shown in Fig. 2a, Pdlim5 was observed in both the cell bodies and terminals of neurons in the PFC. MK-801 significantly increased the expression of Pdlim5 in the PFC, and pretreatment with nicotine decreased its expression, indicating that chronic nicotine treatment improved MK-801-induced memory deficits by modulating the expression of Pdlim5 in the PFC.

CREB-regulated transcription coactivator 1 (CRTC1), which has been shown to be distributed in the brain [18], has been reported to be critically involved in memory processes [38]. Next, the expression of CRTC1 in the PFC and hippocampus was determined. Figure 2d shows that MK-801 treatment caused a significant decrease in the expression of CRTC1 in the PFC, and nicotine pretreatment prevented this reduction. Two-way ANOVA showed a main effect of MK-801 ($F(1,21) = 4.899$, $P = 0.039$) and nicotine ($F(1, 21) = 13.040$, $P = 0.002$) and an interaction of MK-801×nicotine ($F(1,21) = 5.802$, $P = 0.026$). MK-801 treatment did not significantly alter CRTC1 expression in the hippocampus (Fig. 2e). Two-way ANOVA revealed no main effect of MK-801 ($F(1,17) = 0.761$, $P = 0.396$) or nicotine ($F(1,17) = 0.857$, $P = 0.368$) and an interaction of MK-801×nicotine ($F(1,17) = 0.006$, $P = 0.941$). Together, we found that both upregulated Pdlim5 and downregulated CRTC1 specifically in the PFC, not the hippocampus, were involved in MK-801-induced behavior deficits, which could be alleviated by chronic nicotine treatment.

In Pdlim5$^{-/-}$ mice, MK-801 did not impair PPI, working memory or CRTC1 expression

To test the essential role of Pdlim5 in MK-801-induced behavioral impairment, we next applied Pdlim5 knockout mice in the study (Fig. 3a). Importantly, we found that MK-801 treatment did not induce PPI impairment in Pdlim5$^{-/-}$ mice (Fig. 3b). Two-way ANOVA showed no main effect of MK-801 ($F(1,64) = 2.252$, $P = 0.138$), dB ($F(2,64) = 0.560$, $P = 0.574$), or MK-801×dB ($F(2,64) = 0.096$, $P = 0.909$). Moreover, MK-801 treatment did not produce WM impairment in Pdlim5$^{-/-}$ mice (Fig. 3c, d, e). One-way ANOVA showed no significant difference in correct choices ($F(1,21) = 1.177$, $P = 0.678$), Lose-shift failure ($F(1,21) = 0.072$, $P = 0.791$) and Win-shift failure ($F(1,21) = 0.361$, $P = 0.55$) between the MK-801 and saline groups.

We examined CRTC1 expression in the PFC and hippocampus of Pdlim5$^{-/-}$ mice to further determine whether schizophrenia-induced impairment of WM was mediated by Pdlim5-CRTC1 signaling. Figure 3f, g shows that MK-801 treatment did not significantly alter the expression of CRTC1 in the PFC

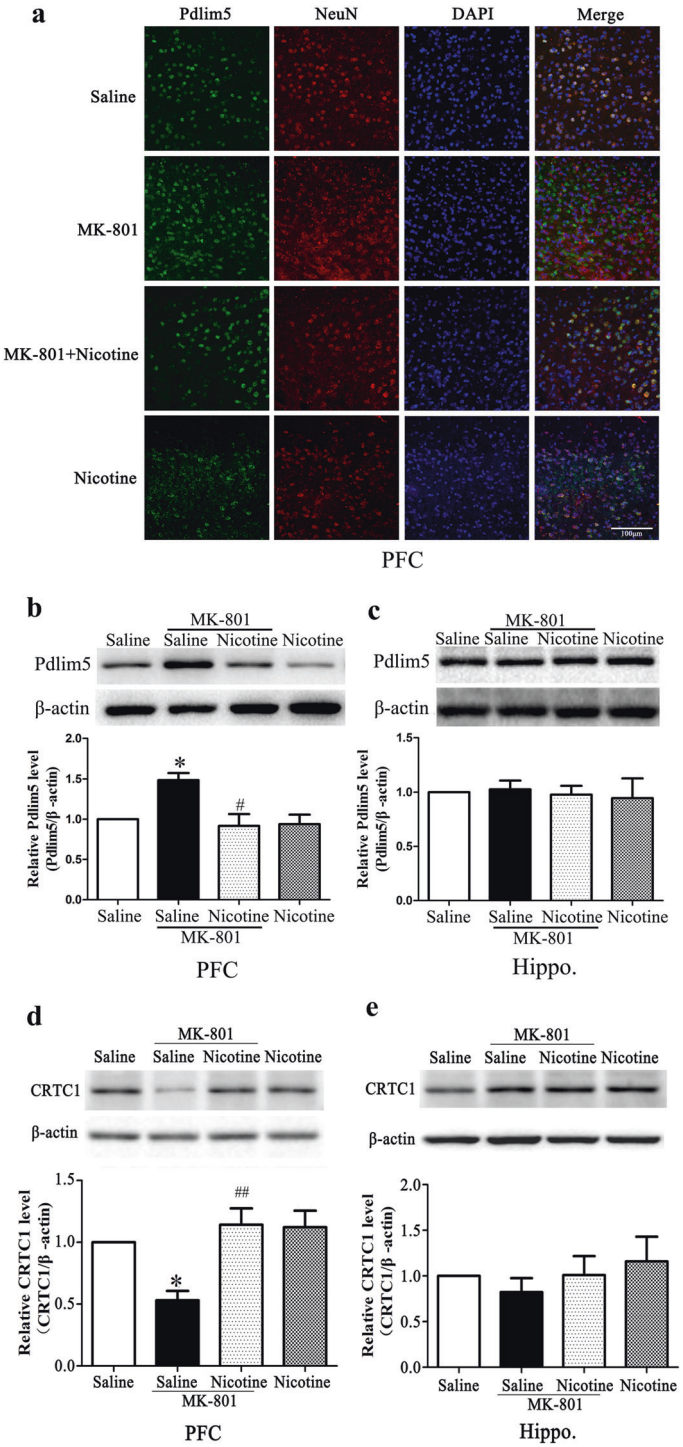

Fig. 2 Chronic nicotine treatment alleviated MK-801-induced Pdlim5 upregulation and CRTC1 decrease. a Representative photomicrographs of immunofluorescence staining for Pdlim5 in the PFC. MK-801 treatment significantly upregulated Pdlim5 expression in the PFC, and chronic nicotine treatment prevented this upregulation, $n = 3$/group, scale bar = 100 μm. **b** A representative immunoblot demonstrated that MK-801 treatment significantly increased Pdlim5 expression in the PFC. Pdlim5 expression was quantified by detecting the relative band intensities, and nicotine treatment significantly alleviated this change ($^{*}P < 0.05$ vs. saline group, $^{\#}P < 0.05$ vs. MK-801 group, $n = 6$). **c** A representative immunoblot showed that MK-801 did not significantly affect Pdlim5 expression in the hippocampus. Pdlim5 expression was quantitated by detecting the relative band intensities ($P > 0.05$, $n = 5$). **d** A representative immunoblot demonstrated that treatment with MK-801 significantly decreased CRTC1 expression in the mouse PFC ($^{*}P < 0.05$ vs. Saline group), and chronic nicotine treatment prevented this decrease ($^{\#\#}P < 0.01$ vs. MK-801 group, $n = 6$). **e** MK-801 did not significantly affect CRTC1 expression in the hippocampus ($P > 0.05$, $n = 5$).

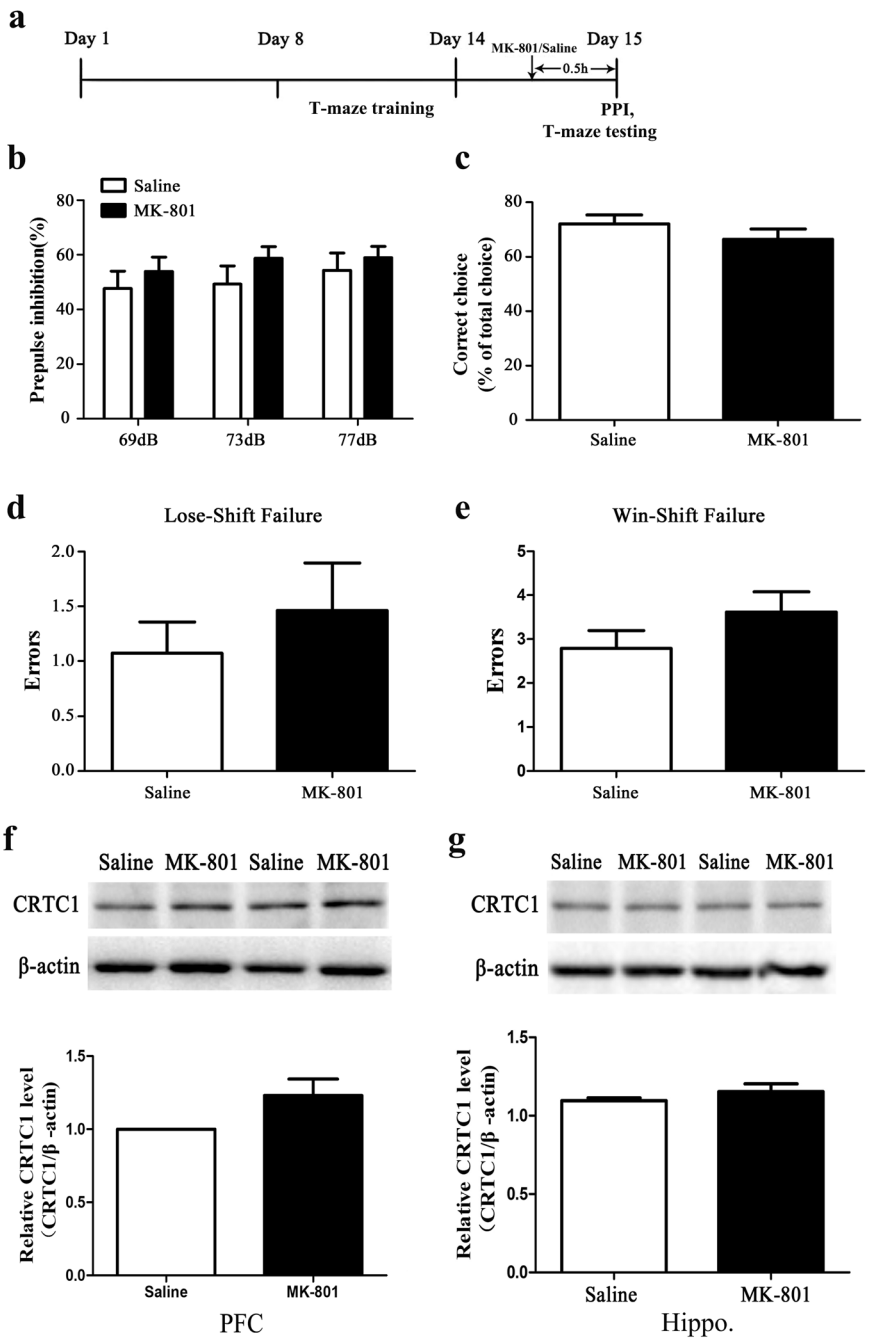

Fig. 3 Effect of MK-801 treatment on cognitive performance and CRTC1 expression in Pdlim5$^{-/-}$ mice. a The experimental protocol. **b** MK-801 did not induce schizophrenia-like behaviors in Pdlim5$^{-/-}$ mice ($P > 0.05$). MK-801 did not affect the correct choices (**c**), or Lose-shift failure (**d**), Win-shift failure (**e**) of Pdlim5$^{-/-}$ mice in the DAT task. $n = 11–12$. **f** A representative immunoblot revealed that MK-801 treatment did not alter the CRTC1 level significantly in the PFC of Pdlim5$^{-/-}$ mice. CRTC1 expression was quantified by detecting the relative band intensities. $P > 0.05$ vs. saline group, $n = 8$. **g** MK-801 had no significant effect on CRTC1 levels in the hippocampus of Pdlim5$^{-/-}$ mice ($P > 0.05$). $n = 8$/group.

($F(1,14) = 4.290$, $P = 0.057$, Fig. 3f) or the hippocampus ($F(1,14) = 1.130$, $P = 0.306$, Fig. 3g) of Pdlim5$^{-/-}$ mice.

Pdlim5 overexpression in the PFC mediated by AAV-Pdlim5 induced schizophrenia-like behavior and cognitive deficits and decreased CRTC1 levels in the PFC of WT mice

AAV-Pdlim5 was injected into the PFC of WT mice to determine the association between Pdlim5 upregulation and schizophrenia-induced memory impairment. We first confirmed that AAV-Pdlim5 induced Pdlim5 overexpression in the PFC using immunofluorescence staining (Fig. 4a) and Western blotting (Fig. 4b). Pdlim5-AAV induced overexpression of Pdlim5 in the PFC but not in the hippocampus of the mice (Fig. 4c) and decreased CRTC1 expression in the PFC (Fig. 4d) but not in the hippocampus (Fig. 4e). The PPI and DAT T-maze were used to detect schizophrenia-like behaviors and WM, respectively. Based on our results, overexpression of Pdlim5 in the PFC significantly impaired performance on the PPI task (Fig. 4f) and DAT task (Fig. 4g–i). Thus, our results indicated an important role of Pdlim5-CRTC1 signaling in MK-801-induced schizophrenia-like behavior and cognitive impairment.

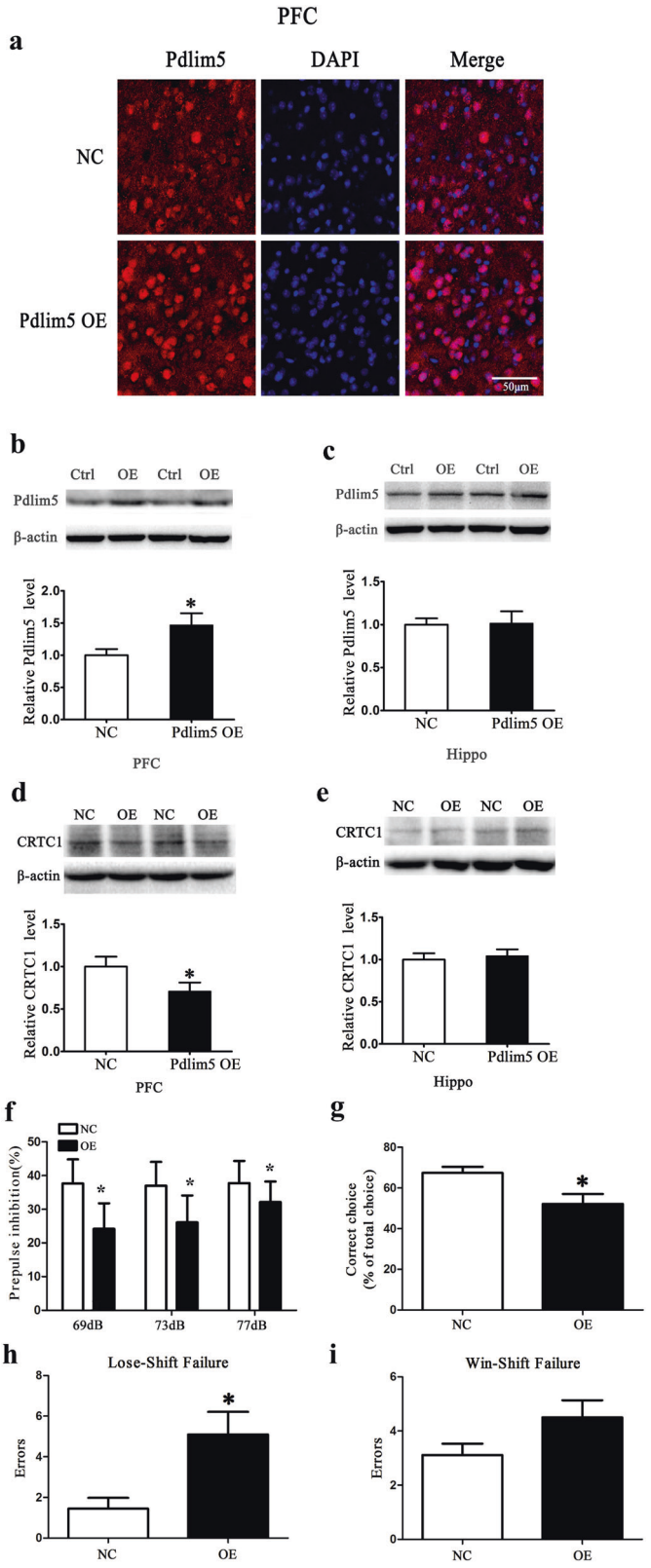

Fig. 4 Effect of Pdlim5 overexpression (OE) by intra-PFC infusion of AAV-Pdlim5 on CRTC1 expression, schizophrenia-like behaviors and cognitive performance in WT mice. a Representative photomicrographs of immunofluorescence staining of Pdlim5 expression after intra-PFC infusion of AAV-Pdlim5. A representative immunoblot demonstrated that AAV-Pdlim5 significantly increased Pdlim5 expression in the PFC, scale bar = 50 μm (**b**) but not in the hippocampus (**c**). Pdlim5 expression was quantified by detecting the relative band intensities. Pdlim5-AAV decreased CRTC1 expression in the PFC (**d**) but not in the hippocampus (**e**) of the mice. $^*P < 0.05$ vs. NC group. **f** Pdlim5 OE decreased the performance on the PPI task. $^*P < 0.05$ vs. the NC group. **g** Pdlim5 OE decreased correct choices. $^*P < 0.05$ vs. the NC group. **h** Pdlim5 OE caused significantly more Lose-shift failure than the NC group. $^*P < 0.05$ vs. the NC group. **i** Pdlim5 OE did not affect Win-shift failure compared to mice in the NC group, $n = 9$ for the NC group, $n = 10$ for the OE group.

Nicotine treatment reduced MK-801-induced Pdlim5 upregulation and CRTC1 downregulation in neurons

To further confirm the effect of MK-801 and nicotine treatment on Pdlim5 and CRTC1 expression in neurons, primary cortical neurons on day 4 of culture were exposed to 20 μM MK-801 for 72 h [30], and immunostaining was used to detect the expression of Pdlim5, CRTC1 and p-CRTC1 levels in neurons. MK-801 increased Pdlim5 and p-CRTC1 levels and decreased CRTC1 expression in cortical neurons (Fig. 5a). Nicotine treatment attenuated the MK-801-induced downregulation of CRTC1 expression in the nucleus of primary cortical neurons (Fig. 5b).

Nicotine restored MK-801-induced upregulation of Pdlim5-related miRNA-21 expression

Notably, miRNA-21 (miR-21) has been shown to downregulate Pdlim5 [39]. To determine the role of miRNA-21 in MK-801-induced upregulation of Pdlim5, the expression of miRNA-21 was detected in vivo and in vitro after nicotine and MK-801 treatment using real-time PCR. Our data demonstrated that MK-801 significantly decreased the levels of miRNA-21 in the PFC ($F(1,8) = 71.906$, $P < 0.001$, Fig. 6a) and SH-SY5Y cells ($F(1,11) = 18.028$, $P = 0.002$, Fig. 6b), and nicotine treatment significantly inhibited these changes.

DISCUSSION

In the present study, our results demonstrated that MK-801 produced schizophrenia-like behavior and WM deficits by upregulating Pdlim5 and downregulating CRTC1 in the PFC but not the hippocampus, and chronic nicotine treatment significantly alleviated these changes. Notably, overexpression of Pdlim5 in the PFC by Pdlim5-AAV significantly induced schizophrenia-like symptoms and cognitive impairment, whereas MK-801 induced neither schizophrenia-like behavior nor WM impairment in Pdlim5$^{-/-}$ mice (Fig. 7).

To our knowledge, the current treatments for positive symptoms (hallucinations and delusions) of schizophrenia have been quite successful, but strategies for treating negative symptoms (blunting affect, poverty of speech and thought, loss of motivation) and cognitive impairment remain to be explored. Moreover, cognitive deficit is considered the key injury of schizophrenia [4] and is closely related to brain functional impairment [5]. As these impairments induce severe life difficulties and result in chronic mental disability [40], the underlying mechanism is not well known; therefore, there is no therapeutic strategy to alleviate the symptoms and cure the disease.

Patients with schizophrenia are highly linked with the smoking population. In other words, compared to the general population, patients with schizophrenia are more prone to severe smoking and nicotine dependence [1, 41]. Tobacco consumption has been reported to improve spatial WM and attentional deficits in smokers with schizophrenia [42]. In addition to tar and nitric oxide, which are toxic to cells, another important component of tobacco is nicotine. Previous studies have shown that long-term smokers have a plasma nicotine content of ~100 ng/mL, while

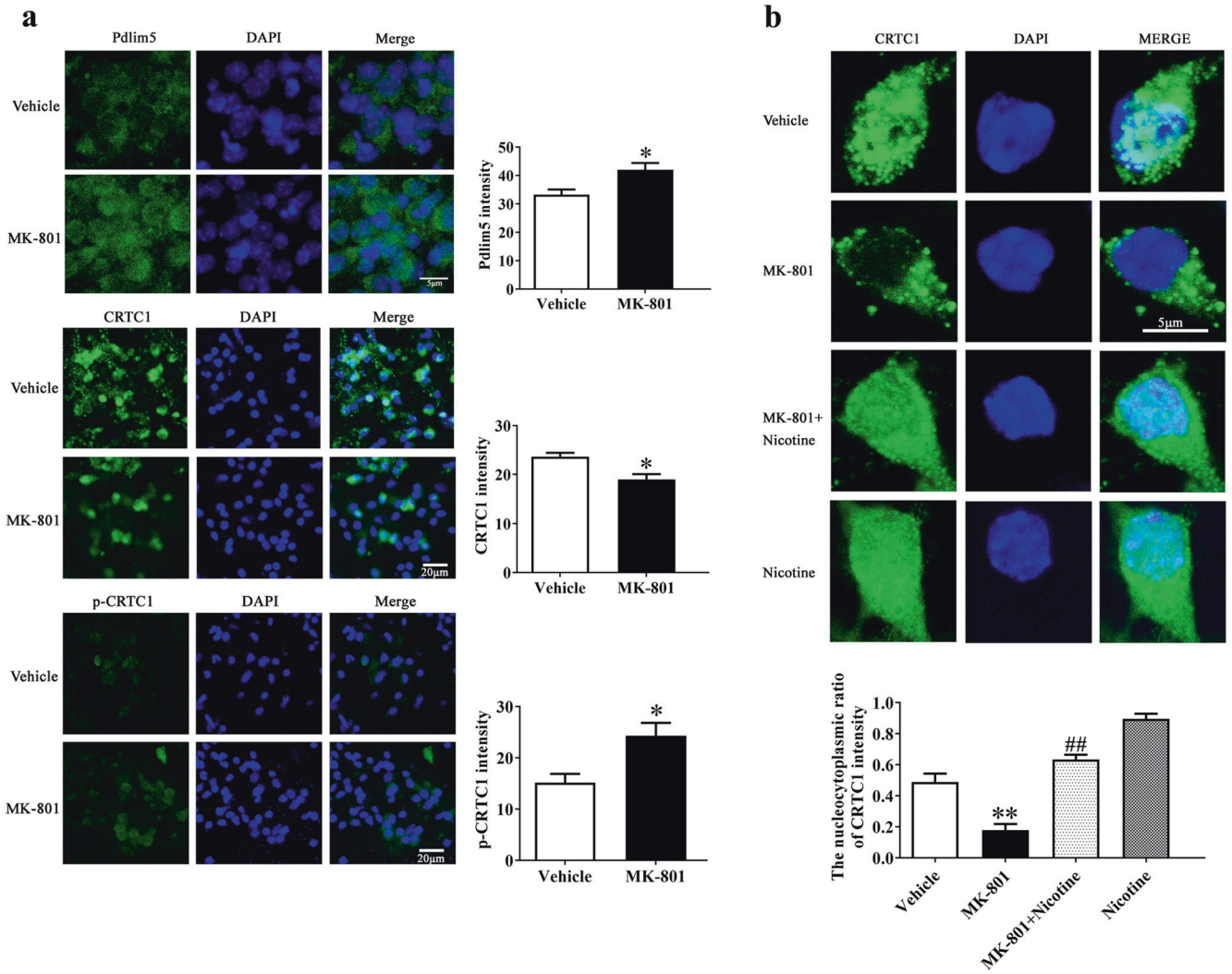

Fig. 5 MK-801 treatment induced Pdlim5 upregulation and CRTC1 downregulation in neurons. a Representative immunofluorescence staining showed that MK-801 increased Pdlim5 (upper panel), decreased CRTC1 levels (middle panel) and increased p-CRTC1 levels (bottom panel) in primary cortical neurons. Pdlim5 expression was quantified by detecting the relative immunofluorescence intensities (*$P < 0.05$ vs. Vehicle group). **b** MK-801 downregulated CRTC1 expression in the nucleus of primary cortical neurons. Nicotine treatment attenuated this change. **$P < 0.01$ vs vehicle. ##$P < 0.01$ vs MK-801.

nicotine affects cell viability only when its concentration exceeds 1 μg/mL [31]. Numerous studies have demonstrated that nicotine significantly improves cognitive function [43]. For example, nicotine has been shown to attenuate maternal lipopolysaccharide exposure-induced schizophrenia-like memory impairment [44, 45]. In addition, administration of nicotine through subcutaneous injection increased the alertness of individuals with schizophrenia in continuous performance tests [46]. Furthermore, Hambsch et al. showed that chronic nicotine selectively improved short-term memory in a G72 mouse model of schizophrenia [47]. Our current study provided evidence that chronic nicotine treatment alleviated MK-801-induced schizophrenia-like behavior and WM deficits, further supporting that schizophrenia patients smoke to relieve their cognitive deficits via nicotine intake.

We have previously reported that acute nicotine treatment improves spatial WM by reducing the Win-Shift Failure in the T-maze task rather than the Lose-Shift Failure [9]. In the control group of the current study, chronic nicotine treatment did not improve WM because the correct choice rate was already 80%, while in our nicotine-enhanced WM study, the correct choice rate was 70%. In the current study, the result was probably due to ceiling effects, and nicotine could no longer enhance WM.

Although the hippocampal-prefrontal neural circuit has been shown to play an important role in WM in the T-maze task [48], PFC dysfunction may underlie the mechanism for debilitating consequences that lead to schizophrenia and other neuropsychiatric diseases [49, 50]. In addition, functional neuroimaging and molecular studies mainly point to PFC abnormalities in schizophrenia patients [51]. In this study, MK-801 increased PFC-dependent Lose-shift failure without affecting hippocampus-dependent Win-Shift Failure. In addition, we showed that Pdlim5 upregulation and CRTC1 downregulation in the PFC but not in the hippocampus are responsible for MK-801-induced behavioral deficits, further supporting that the PFC plays a more critical role in schizophrenia-induced cognitive deficits.

Pdlim5 has been reported to be associated with schizophrenia and bipolar disorder [52], Alzheimer's disease [53], and depression [54]. For example, a previous postmortem study showed that the Pdlim5 gene was upregulated in the PFC of patients with schizophrenia (Iwamoto et al., 2004). In addition, chronic but not acute methamphetamine injection could upregulate Pdlim5 expression in the PFC of mice [55], suggesting that increased Pdlim5 levels in the PFC may cause neuropsychiatric behavioral phenotypes [52]. Our results showed that MK-801 upregulated

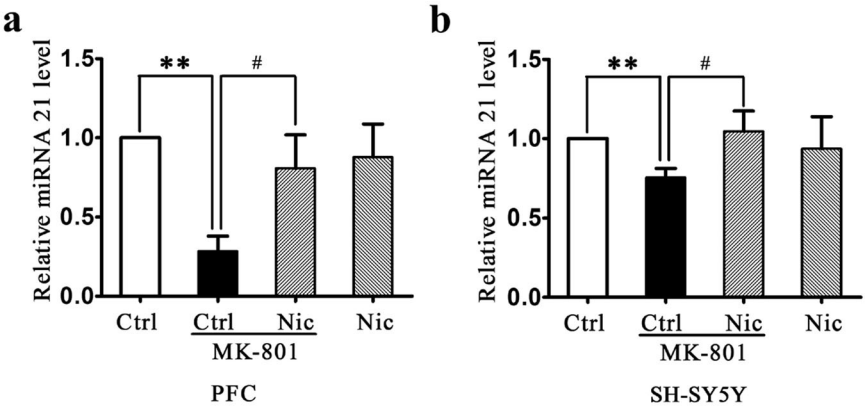

Fig. 6 Effect of nicotine treatment on MK-801-induced miR-21 expression in vivo and in vitro. The real-time PCR results showed that nicotine reduced the MK-801-induced decrease in miRNA-21 expression in the mouse PFC (**a**) and SH-SY5Y cells (**b**). $^{**}P < 0.01$ vs. Ctrl group; $^{\#}P < 0.05$ vs. MK-801 group.

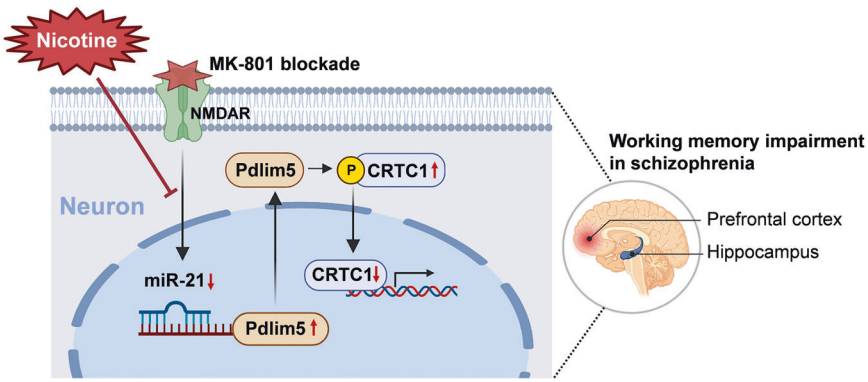

Fig. 7 Summary. MK-801-induced behavioral and cognitive impairment is characterized by upregulated Pdlim5 expression and downregulated CRTC1 levels specifically in the PFC region. Chronic nicotine pretreatment restores the MK-801-induced deficits by downregulating Pdlim5 expression and upregulating CRTC1 expression.

Pdlim5 in the PFC and that overexpression of Pdlim5 in the PFC induced PPI and WM deficits, indicating that Pdlim5 played an important role in schizophrenia-induced memory deficits. Notably, MK-801 did not induce PPI and WM impairment in Pdlim5$^{-/-}$ mice, which is consistent with a previous study showing that Pdlim5 deficiency in heterozygous animals showed a protective effect against methamphetamine-induced PPI deficits and locomotor hyperactivity in the open field and that Pdlim5 inhibition with PKCE-TIP also showed a protective effect on PPI in wild-type mice [55]. These results suggest that suppression of Pdlim5 levels may be a possible therapeutic direction for treating schizophrenia or other neuropsychiatric disorders.

The subcellular distribution of CRTC1 is regulated by phosphorylation status. Under basal conditions, they are phosphorylated and sequestered in the cytoplasm by scaffolding the proteins of 14-3-3, and nuclear translocation requires the activation of both calcium and cAMP signaling pathways [18]. Notably, CRTC1 nuclear translocation is essential for memory consolidation [18]. CRTC1 not only plays an important role in memory disorder in Alzheimer's disease [56] and fear memory [38] but also contributes to ischemic stroke-induced memory impairment [34]. In this study, we found that MK-801 treatment induced significant downregulation of CRTC1 in WT mice but not Pdlim5$^{-/-}$ mice. In addition, Pdlim5 overexpression suppressed CRTC1 expression, suggesting that CRTC1 is involved in MK-801-induced memory deficits and Pdlim5 signaling.

It has been reported that the posttranscriptional regulator miR-21, which has been reported to play an important role in neuroplasticity and schizophrenia pathology [39, 57–59], can be induced in neurons by prolonged *N*-methyl-*D*-aspartic acid receptor (NMDAR) stimulation [60]. Here, we showed that the NMDAR antagonist MK-801 produced a significant decrease in miR-21 and that MK-801 may decrease miR-21 by blocking the interaction of NMDA with the receptor. In addition, our results showed that nicotine alleviated MK-801-induced miR-21, which is consistent with a previous study showing that nicotine upregulates miR-21 [61]. Nuclear factor kappa-B (NF-κB) binding sites were located in miR-21 gene transcriptional elements, and nicotine enhanced the binding of NF-κB to the promoters of miR-21. Knockdown of NF-κB markedly suppressed nicotine-induced cell proliferation and upregulation of miR-21 [62]. Therefore, nicotine may alleviate MK-801-induced miR-21 through regulating NF-κB.

Overall, our results indicate that MK-801 induced WM impairment in mice with schizophrenia and elevated Pdlim5 expression and decreased CRTC1 expression in the PFC but not in the hippocampus. Chronic nicotine pretreatment reduced Lose-Shift failure to attenuate WM impairment in mice with MK-801-induced schizophrenia, which was mediated by downregulating Pdlim5 expression and upregulating CRTC1 expression.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (81671145, 81870973, 81701316). We thank Dr Chun-lei Zhang for advice and

comments on the paper. We thank Prof Xue-chu Zhen for providing the PPI equipment.

AUTHOR CONTRIBUTIONS

This work was performed and accomplished by all authors. QW, MWW, YYS, XYH, HS, XNW, and HW contributed to the execution of the entire research project and the statistical analyses. QW, YS, PPG, JFZ, HQC, WW, and XCJ wrote the paper. All authors have read and approved the final paper.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. de Leon J, Diaz FJ. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. Schizophr Res. 2005;76:135–57.
2. Hughes JR, Hatsukami DK, Mitchell JE, Dahlgren LA. Prevalence of smoking among psychiatric outpatients. Am J Psychiatry. 1986;143:993–7.
3. D'Souza MS, Markou A. Schizophrenia and tobacco smoking comorbidity: nAChR agonists in the treatment of schizophrenia-associated cognitive deficits. Neuropharmacology. 2011;62:1564–73.
4. Elvevag B, Goldberg TE. Cognitive impairment in schizophrenia is the core of the disorder. Crit Rev Neurobiol. 2000;14:1–21.
5. McGurk SR, Meltzer HY. The role of cognition in vocational functioning in schizophrenia. Schizophr Res. 2000;45:175–84.
6. Semenova S, Jin X, McClure-Begley TD, Tadman MP, Marks MJ, Markou A. Differential effects of withdrawal from intermittent and continuous nicotine exposure on reward deficit and somatic aspects of nicotine withdrawal and expression of alpha4beta2* nAChRs in Wistar male rats. Pharmacol Biochem Behav. 2018;171:54–65.
7. Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. Psychopharmacology (Berl). 2006;184:523–39.
8. Shu H, Wang M, Song M, Sun Y, Shen X, Zhang J, et al. Acute nicotine treatment alleviates LPS-induced impairment of fear memory reconsolidation through AMPK activation and CRTC1 upregulation in hippocampus. Int J Neuropsychopharmacol. 2020;23:687–99.
9. Shu H, Zheng GQ, Wang X, Sun Y, Liu Y, Weaver JM, et al. Activation of matrix metalloproteinase in dorsal hippocampus drives improvement in spatial working memory after intra-VTA nicotine infusion in rats. J Neurochem. 2015;135:357–67.
10. Koukouli F, Rooy M, Tziotis D, Sailor KA, O'Neill HC, Levenga J, et al. Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. Nat Med. 2017;23:347–54.
11. Horiuchi Y, Arai M, Niizato K, Iritani S, Noguchi E, Ohtsuki T, et al. A polymorphism in the PDLIM5 gene associated with gene expression and schizophrenia. Biol Psychiatry. 2006;59:434–9.
12. Zain MA, Roffeei SN, Zainal NZ, Kanagasundram S, Mohamed Z. Nonsynonymous polymorphisms of the PDLIM5 gene association with the occurrence of both bipolar disorder and schizophrenia. Psychiatr Genet. 2013;23:258–61.
13. Li C, Tao R, Qin W, Zheng Y, He G, Shi Y, et al. Positive association between PDLIM5 and schizophrenia in the Chinese Han population. Int J Neuropsychopharmacol. 2008;11:27–34.
14. Moselhy H, Eapen V, Akawi NA, Younis A, Salih B, Othman AR, et al. Secondary association of PDLIM5 with paranoid schizophrenia in Emirati patients. Meta Gene. 2015;5:135–9.
15. Iwamoto K, Kakiuchi C, Bundo M, Ikeda K, Kato T. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. Mol Psychiatry. 2004;9:406–16.
16. Numata S, Ueno S, Iga J, Yamauchi K, Hongwei S, Hashimoto R, et al. Gene expression in the peripheral leukocytes and association analysis of PDLIM5 gene in schizophrenia. Neurosci Lett. 2007;415:28–33.
17. Herrick S, Evers DM, Lee JY, Udagawa N, Pak DT. Postsynaptic PDLIM5/Enigma Homolog binds SPAR and causes dendritic spine shrinkage. Mol Cell Neurosci. 2010;43:188–200.
18. Saura CA, Cardinaux JR. Emerging roles of CREB-regulated transcription coactivators in brain physiology and pathology. Trends Neurosci. 2017;40:720–33.
19. Xue ZC, Wang C, Wang QW, Zhang JF. CREB-regulated transcription coactivator 1: important roles in neurodegenerative disorders. Sheng Li Xue Bao. 2015;67:155–62.
20. Breuillaud L, Rossetti C, Meylan EM, Merinat C, Halfon O, Magistretti PJ, et al. Deletion of CREB-regulated transcription coactivator 1 induces pathological aggression, depression-related behaviors, and neuroplasticity genes dysregulation in mice. Biol Psychiatry. 2012;72:528–36.
21. Csernansky JG, Martin M, Shah R, Bertchume A, Colvin J, Dong H. Cholinesterase inhibitors ameliorate behavioral deficits induced by MK-801 in mice. Neuropsychopharmacology. 2005;30:2135–43.
22. van der Staay FJ, Rutten K, Erb C, Blokland A. Effects of the cognition impairer MK-801 on learning and memory in mice and rats. Behav Brain Res. 2011;220:215–29.
23. Castner SA, Goldman-Rakic PS, Williams GV. Animal models of working memory: insights for targeting cognitive dysfunction in schizophrenia. Psychopharmacology (Berl). 2004;174:111–25.
24. Jentsch JD, Tran A, Le D, Youngren KD, Roth RH. Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology. 1997;17:92–9.
25. Cheng H, Kimura K, Peter AK, Cui L, Ouyang K, Shen T, et al. Loss of enigma homolog protein results in dilated cardiomyopathy. Circ Res. 2010;107:348–56.
26. Park SJ, Lee Y, Oh HK, Lee HE, Ko SY, Kim B, et al. Oleanolic acid attenuates MK-801-induced schizophrenia-like behaviors in mice. Neuropharmacology. 2014;86:49–56.
27. Shao S, Li M, Du W, Shao F, Wang W. Galanthamine, an acetylcholine inhibitor, prevents prepulse inhibition deficits induced by adolescent social isolation or MK-801 treatment. Brain Res. 2014;1589:105–11.
28. Chen HH, Stoker A, Markou A. The glutamatergic compounds sarcosine and N-acetylcysteine ameliorate prepulse inhibition deficits in metabotropic glutamate 5 receptor knockout mice. Psychopharmacology (Berl). 2010;209:343–50.
29. Shen X, Sun Y, Wang M, Shu H, Zhu LJ, Yan PY, et al. Chronic N-acetylcysteine treatment alleviates acute lipopolysaccharide-induced working memory deficit through upregulating caveolin-1 and synaptophysin in mice. Psychopharmacology (Berl). 2018;235:179–91.
30. Li C, Tang Y, Yang J, Zhang X, Liu Y, Tang A. Sub-chronic antipsychotic drug administration reverses the expression of neuregulin 1 and ErbB4 in a cultured MK801-induced mouse primary hippocampal neuron or a neurodevelopmental schizophrenia model. Neurochem Res. 2016;41:2049–64.
31. Naik P, Fofaria N, Prasad S, Sajja RK, Weksler B, Couraud PO, et al. Oxidative and pro-inflammatory impact of regular and denicotinized cigarettes on blood brain barrier endothelial cells: is smoking reduced or nicotine-free products really safe? BMC Neurosci. 2014;15:51.
32. Liu J, Jin X, Liu KJ, Liu W. Matrix metalloproteinase-2-mediated occludin degradation and caveolin-1-mediated claudin-5 redistribution contribute to blood-brain barrier damage in early ischemic stroke stage. J Neurosci. 2012;32:3044–57.
33. Chen S, Sun Y, Li F, Zhang X, Hu X, Zhao X, et al. Modulation of alpha7nAchR by melatonin alleviates ischemia and reperfusion-compromised integrity of blood-brain barrier through inhibiting HMGB1-mediated microglia activation and CRTC1-mediated neuronal loss. Cell Mol Neurobiol. 2021. https://doi.org/10.1007/s10571-021-01122-2. online ahead of Print.
34. Zhang X, Shen X, Dong J, Liu WC, Song M, Sun Y, et al. Inhibition of reactive astrocytes with fluorocitrate ameliorates learning and memory impairment through upregulating CRTC1 and synaptophysin in ischemic stroke rats. Cell Mol Neurobiol. 2019;39:1151–63.
35. Sun Y, Chen X, Zhang X, Shen X, Wang M, Wang X, et al. β2-Adrenergic receptor-mediated HIF-1alpha upregulation mediates blood brain barrier damage in acute cerebral ischemia. Front Mol Neurosci. 2017;10:257.
36. Suryavanshi PS, Ugale RR, Yilmazer-Hanke D, Stairs DJ, Dravid SM. GluN2C/GluN2D subunit-selective NMDA receptor potentiator CIQ reverses MK-801-induced impairment in prepulse inhibition and working memory in Y-maze test in mice. Br J Pharmacol. 2014;171:799–809.
37. Lainiola M, Procaccini C, Linden AM. mGluR3 knockout mice show a working memory defect and an enhanced response to MK-801 in the T- and Y-maze cognitive tests. Behav Brain Res. 2014;266:94–103.
38. Nonaka M, Kim R, Fukushima H, Sasaki K, Suzuki K, Okamura M, et al. Region-specific activation of CRTC1-CREB signaling mediates long-term fear memory. Neuron. 2014;84:92–106.
39. Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, et al. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest. 2014;124:2136–46.
40. Hahn B, Harvey AN, Concheiro-Guisan M, Huestis MA, Holcomb HH, Gold JM. A test of the cognitive self-medication hypothesis of tobacco smoking in schizophrenia. Biol Psychiatry. 2013;74:436–43.
41. Ziedonis D, Hitsman B, Beckham JC, Zvolensky M, Adler LE, Audrain-McGovern J, et al. Tobacco use and cessation in psychiatric disorders: National Institute of Mental Health report. Nicotine Tob Res. 2008;10:1691–715.
42. Sacco KA, Termine A, Seyal A, Dudas MM, Vessicchio JC, Krishnan-Sarin S, et al. Effects of cigarette smoking on spatial working memory and attentional deficits in schizophrenia: involvement of nicotinic receptor mechanisms. Arch Gen Psychiatry. 2005;62:649–59.

43. Gould TJ, Leach PT. Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. Neurobiol Learn Mem. 2013;107:108–32.
44. Waterhouse U, Brennan KA, Ellenbroek BA. Nicotine self-administration reverses cognitive deficits in a rat model for schizophrenia. Addict Biol. 2017.23:620–30.
45. Waterhouse U, Roper VE, Brennan KA, Ellenbroek BA. Nicotine ameliorates schizophrenia-like cognitive deficits induced by maternal LPS exposure: a study in rats. Dis Model Mech. 2016;9:1159–67.
46. Young JW, Meves JM, Geyer MA. Nicotinic agonist-induced improvement of vigilance in mice in the 5-choice continuous performance test. Behav Brain Res. 2013;240:119–33.
47. Hambsch B, Keyworth H, Lind J, Otte DM, Racz I, Kitchen I, et al. Chronic nicotine improves short-term memory selectively in a G72 mouse model of schizophrenia. Br J Pharmacol. 2014;171:1758–71.
48. Cunningham C, Campion S, Teeling J, Felton L, Perry VH. The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain Behav Immun. 2007;21:490–502.
49. Wallace TL, Bertrand D. Importance of the nicotinic acetylcholine receptor system in the prefrontal cortex. Biochem Pharmacol. 2013;85:1713–20.
50. Zhang CL, Aime M, Laheranne E, Houbaert X, El Oussini H, Martin C, et al. Protein kinase A deregulation in the medial prefrontal cortex impairs working memory in murine oligophrenin-1 deficiency. J Neurosci. 2017;37:11114–26.
51. Senkowski D, Gallinat J. Dysfunctional prefrontal gamma-band oscillations reflect working memory and other cognitive deficits in schizophrenia. Biol Psychiatry. 2015;77:1010–9.
52. Samsom JN, Wong AH. Schizophrenia and depression co-morbidity: what we have learned from animal models. Front Psychiatry. 2015;6:13.
53. Miao X, Liu W, Fan B, Lin H. Transcriptomic heterogeneity of Alzheimer's disease associated with lipid genetic risk. Neuromol Med. 2020;22:534–41.
54. Lu Y, Jiang J, Si J, Wu Q, Tian F, Jiao K, et al. PDLIM5 improves depression-like behavior of prenatal stress offspring rats via methylation in male, but not female. Psychoneuroendocrinology. 2020;115:104629.
55. Horiuchi Y, Ishikawa M, Kaito N, Iijima Y, Tanabe Y, Ishiguro H, et al. Experimental evidence for the involvement of PDLIM5 in mood disorders in hetero knockout mice. PLoS One. 2013;8:e59320.
56. Parra-Damas A, Chen M, Enriquez-Barreto L, Ortega L, Acosta S, Perna JC, et al. CRTC1 function during memory encoding is disrupted in neurodegeneration. Biol Psychiatry. 2016;81:111–23.
57. Zheutlin AB, Jeffries CD, Perkins DO, Chung Y, Chekroud AM, Addington J, et al. The role of microRNA expression in cortical development during conversion to psychosis. Neuropsychopharmacology. 2017;42:2188–95.
58. John J, Bhatia T, Kukshal P, Chandna P, Nimgaonkar VL, Deshpande SN, et al. Association study of MiRSNPs with schizophrenia, tardive dyskinesia and cognition. Schizophrenia Res. 2016;174:29–34.
59. Strickland ER, Hook MA, Balaraman S, Huie JR, Grau JW, Miranda RC. MicroRNA dysregulation following spinal cord contusion: implications for neural plasticity and repair. Neuroscience. 2011;186:146–60.
60. Yelamanchili SV, Chaudhuri AD, Chen LN, Xiong H, Fox HS. MicroRNA-21 dysregulates the expression of MEF2C in neurons in monkey and human SIV/HIV neurological disease. Cell Death Dis. 2010;1:e77.
61. Zhang Y, Pan T, Zhong X, Cheng C. Nicotine upregulates microRNA-21 and promotes TGF-beta-dependent epithelial-mesenchymal transition of esophageal cancer cells. Tumour Biol. 2014;35:7063–72.
62. Shin VY, Jin H, Ng EK, Cheng AS, Chong WW, Wong CY, et al. NF-kappaB targets miR-16 and miR-21 in gastric cancer: involvement of prostaglandin E receptors. Carcinogenesis. 2011;32:240–5.