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Nicotine Significantly Improves Chronic Stress-Induced Impairments of Cognition and Synaptic Plasticity in Mice

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Abstract The aim of this study was to examine if nicotine was able to improve cognition deficits in a mouse model of chronic mild stress. Twenty-four male C57BL/6 mice were divided into three groups: control, stress, and stress with nicotine treatment. The animal model was established by combining chronic unpredictable mild stress (CUMS) and isolated feeding. Mice were exposed to CUMS continued for 28 days, while nicotine (0.2 mg/kg) was also administrated for 28 days. Weight and sucrose consumption were measured during model establishing period. The anxiety and behavioral despair were analyzed using the forced swim test (FST) and open-field test (OFT). Spatial cognition was evaluated using Morris water maze (MWM) test. Following behavioral assessment, both long-term potentiation (LTP) and depotentiation (DEP) were recorded in the hippocampal dentate gyrus (DG) region. Both synaptic and Notch1 proteins were measured by Western. Nicotine increased stressed mouse's sucrose consumption. The MWM test showed that spatial learning and reversal learning in stressed animals were remarkably affected relative to controls, whereas nicotine partially rescued cognitive functions. Additionally, nicotine considerably alleviated the level of anxiety and the degree of behavioral despair in stressed mice. It effectively mitigated the depression-induced impairment of hippocampal synaptic plasticity, in which both the LTP and DEP were significantly inhibited in stressed mice. Moreover, nicotine enhanced the expression of synaptic and Notch1 proteins in stressed animals. The results suggest that nicotine ameliorates the depression-like symptoms and improves the hippocampal synaptic plasticity closely associated with activating transmembrane ion channel receptors and Notch signaling components.

Keywords Nicotine · Chronic unpredictable mild stress · Synaptic plasticity · Notch1 signaling · C57 mice

Introduction

Depression is a severe psychiatric disease [1]. Genetic and environment-triggered types of the disease produced a significant decline in quality of life and might lead to life-threatening events like suicide attempts [2]. Long-term depressed mood and anhedonia are two core symptoms for depression. Additionally, cognitive deficits have been widely identified in depressed patients and are considered as a core element of this mental disease [3]. However, the explicit mechanism underlying the depression development is still unclear to some extent. The current antidepressants are principally based on monoaminergic neurotransmission, such as the tricyclic antidepressants, the selective serotonin, and noradrenalin reuptake inhibitors [4]. Nonetheless, most of the antidepressant treatments normally do not generate satisfactory effects on a therapeutic response for their long delay of activity and side effects [5]. Novel antidepressant drug strategies are needed to improve cognitive symptoms, particularly functional recovery.

Nicotine is the main psychoactive component of tobacco [6], which stimulates the central nervous system (CNS). These effects include antidepressant-like effects [7–10], alterations in cognition [11], and memory. For example, acute administration of nicotine (0.2 mg/kg) has been shown to have antidepressant-like effects in male BALB/C mice using forced swimming test (FST) [8]. In addition, it has been reported that

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chronic administration (14 day) nicotine (0.4 mg/kg) results in antidepressant-like effects on Wistar rats [10]. These findings suggest that there are actual antidepressant-like effects of nicotine, especially in the aspects of improving the anxiety and depressive emotion. And more importantly, the antidepressant-like action of nicotine is mainly mediated by the serotonergic system [8, 12] and dopaminergic transmission through nicotinic acetylcholine receptors (nAChRs) stimulation [13, 14]. Furthermore, nicotine is reported to be participated in cognitive functions such as attention, learning, and memory consolidation [15]. The stress-induced impairment of spatial memory in Wistar rats can be blocked by nicotine [16]. Acute and chronic nicotine treatments greatly improved cognitive deficits of dopamine transporter knockout (DAT KO) mice in the cued and spatial learning [17]. Importantly, previous studies showed that the synaptic plasticity in the hippocampus was improved by nicotine, which was the neurobiology basis of learning and memory [18, 19]. For instance, the long-term potentiation (LTP) in the dentate gyrus (DG) can be enhanced by nicotine [20]. In addition, the induction of long-time depression (LTD) can also be affected differentially by nicotine [21]. However, there are few reports about the ameliorative effects of nicotine to cognitive impairments of depression, which is associated with the alteration of synaptic plasticity [22].

Notch signaling pathway is a very preserved cell signaling system existing in most multicellular organisms [23]. It reports that there are four Notch receptor genes (Notch1–4) and five DSL (Delta, Serrate, Lag2) ligand genes (Dll-1,-3, and 4; Jag1 and Jag2) in mammals [24]. Notch ligands binding triggers proteolytic cleavage of the receptor, releasing the Notch intracellular domain (NICD) to enter the nucleus. And then, the NICD directly regulates the function of the transcription factor CSL (CBF1, Suppressor of Hairless, Lag1), which promotes the expression of downstream target genes [25, 26]. Previous studies suggested that Notch signaling also played an important role in neuronal plasticity at the Drosophila [27, 28]. Furthermore, several investigations suggest that the Notch signaling is associated with the hippocampal synaptic plasticity, learning, and memory in rodents. Mice with reduced Notch levels show impaired LTP at the hippocampal CA1 synapses. The impairment of synaptic plasticity in Notch antisense transgenic mice was significantly improved by Notch ligand Jagged-1 [29]. In addition, it is important to point out that Notch signaling has been altered in depression. It is very helpful for post-stroke depression therapeutically to block gamma secretase mediated Notch signaling [30]. The expression of NICD and its downstream targets Hes1 and Hes5 was decreased in chronic mild stressed animals [31]. More importantly, several studies indicated that nicotine could regulate the expression of the Notch signaling receptors and ligands [32–34]. Logically, the above results may give us more hints on which the Notch signaling is involved with the antidepressant-like effects of nicotine. Accordingly, we wonder if nicotine effectively improves spatial cognition and synaptic plasticity via enhancing the protein levels of Notch1, Jagged-1, and Hes1in chronic unpredictable mild stress (CUMS) mice.

The in vivo study was aimed to investigate the underlying mechanism of nicotine protective effect on the impairment of synaptic plasticity and cognitions induced by CUMS in mice. We hypothesized that nicotine may be able to reverse the synaptic plasticity impairment and to some extent improve cognitive impairments via increasing the expression of Notch1, Jagged-1, and Hes1 proteins in CUMS mice. This was done by establishing a CUMS mouse model, and both body weight and sucrose preference were measured. After that, several behavioral experiments including the forced swim test, the open field test, and the Morris water maze test were performed. Furthermore, both LTP and depotentiation (DEP) from the hippocampal perforant pathway (PP) to DG region were recorded. Finally, the protein expressions of NR2A, NR2B, GAD67, Notch1, Jagged-1, and Hes1 in the hippocampus were assayed by Western to explore the possible mechanism.

Materials and Methods

Animals and Drug Treatments

Twenty-four male C57BL/6 mice (25–30 g body weight) were purchased from the Laboratory Animal Center of Academy of Military Medical Science of People's Liberation Army and reared in standard rodent cages in the animal house of Medical School, Nankai University, under the condition of a constant temperature of 25 °C (±2 °C) and a 12-h light/dark cycle (lights on at 7 a.m.). Food and water were freely available during all phases of the experiment, with the exception of model establishing and sucrose consumption phases. After 4 days of habituation to the environment, the mice were randomly divided into three groups, which were control group (Con, $n = 8$), chronic mild unpredictable stressed group (CUS, $n = 8$), and stressed + nicotine group (CUS + Nic, $n = 8$). The mice in the CUS and CUS + Nic groups were housed separately with each individual in one cage, while the animals in the Con group were housed with three individuals in one cage. Every effort has been made to minimize the number of animals used and their suffering. All experiments were carried out according to protocols approved by the Ethical Commission at Nankai University and in accordance with the practices outlined in the NIH Guide for the Care and Use of Laboratory Animals.

(-)-Nicotine ditartrate was bought from Merck Millipore, Germany. In the CUS + Nic group, the mice treated with nicotine were daily administrated by intraperitoneal injection

at a dose of 0.2 mg/kg from the 1st to 28th day [35, 8]. At the same time, both Con and CUS animals received vehicles by intraperitoneal injection at the same dose.

Chronic Unpredictable Mild Stress Procedure

The stress procedure was conducted for 28 days (Fig. 1a), according to the modification method of Willner and Yun-Xia Wang [36, 37]. Briefly, CUMS-treated mice were subjected to numerous stressors in a chronic, unavoidable, and unpredictable way along with a random schedule. The stressors include reversed light/dark cycle (24 h), ice water swimming (4 °C, 1 min), oven (45 °C, 5 min), cage tilt (45°, 12 h), confinement in tube (2 h), damp bedding (150 mL water +200 g bedding, 24 h), white noise (85 dB, 5 min), tail pinch (1 min), and water and food deprivation (24 h). They were applied on purpose, at random times of both night and day, in order to be completely unpredictable (as shown in Table 1). All animals in both the CUS and CUS + Nic groups were exposed to the same single stressor simultaneously in 1 day. No single stressor was applied for 2 days consecutively.

Body Weight Measurement and Sucrose Preference Test

The body weight was measured every day at 10 a.m. and recorded on the 1st, 4th, 8th, 11th, 15th, 18th, 22nd, 25th, and 28th day (Fig. 1). The sucrose preference test (SPT) was slightly modified from the previous description [38, 39]. Four days before CUMS, all animals were habituated to drink sucrose solution (1 %, *w*/*v*) by replacing normal water for 2 days (48 h). Besides, the position of the bottles was changed several times during the period. Afterwards, all mice were deprived of food and water for 24 h, starting at 10 a.m. 24 h later. Each animal was provided with 1 % sucrose solution and normal water individually for 1 h, and the weights of sucrose solution and water consumed were recorded accordingly. The sucrose preference value was obtained from the following formula:

$$\text{Sucrose preference}\ (\%) = \text{Sucrose intake}\ (\text{g}) \Big/ [\text{sucrose intake}\ (\text{g}) + \text{water intake}\ (\text{g})] \times 100\%.$$

On the last 2 days of each week, the SPT was performed accordingly.

Behavioral Tests

All behavioral experiments were performed in the dark room of the light cycle. In order to avoid the immediate effect of stressor and pharmacological treatment, the behavioral test was carried out 12 h after the last SPT.

Forced Swim Test

During the FST experiment, mice were placed into a cylinder container (30 cm in diameter, 24 cm in deep, filled with 23–25 °C water to a depth of 12 cm). As described previously by Porsolt et al. [40], each mouse was placed into the water gently and individually for 6 min during the trial. Immediately after the test, the animal was covered by a dry towel and then placed under a heating lamp until it was dry. The water was changed between tests. During the test, the duration of immobility, defined as an absence of movement necessary to keep the head above the water or climb upward-directed against the walls, was determined over the last 4 min. Longer immobility duration shows more serious depression-like behavior.

Open Field Test

The test was performed in a bare square box (48 cm long × 48 cm wide × 36 cm high) made of compressed wood. The floor was partitioned into 16 equal squares by white-colored grids, including peripheral area (12 around squares) and central area (4 middle squares). Mice were placed in a particular middle square of the center arena and tracked by a CCD camera connected to a personal computer, through which data were collected and analyzed (Ethovision 2.0, Noldus, Wagenigen, Netherlands). Animals were allowed to explore the maze for 5 min, after which they were returned to their home age [41].

The maze was cleaned with 75 % ethanol wipes before starting testing with the next mouse. Total distance was analyzed as measures of spontaneous activity. Central area duration, and central area visits were taken as measures of anxiety and exploratory behavior [42].

Morris Water Maze Test

The day after the open-field test (OFT) experiment, mice were trained and tested in Morris water maze (MWM) to monitor the spatial cognition. The water maze tank was 150 cm in diameter and 60 cm in height, filled with water (25 ± 1 °C) to the depth of 45 cm. The water was made opaque by white dye. The tank was divided into four quadrants by two imaginary perpendicular lines crossing in the center of the tank. The four quadrants were clockwisely named as northeast (N), southeast (E), southwest (S), and northwest (W). A submerged platform (5 cm in diameter), painted white, was placed in the middle of the target quadrant of the pool, 1.5 cm below surface of water for the mice to climb on so as to escape from the water. The swimming path was recorded using a computerized video tracking system as the OFT experiment described. The room was furnished with several extra-maze cues immobile throughout the entire experiment process.

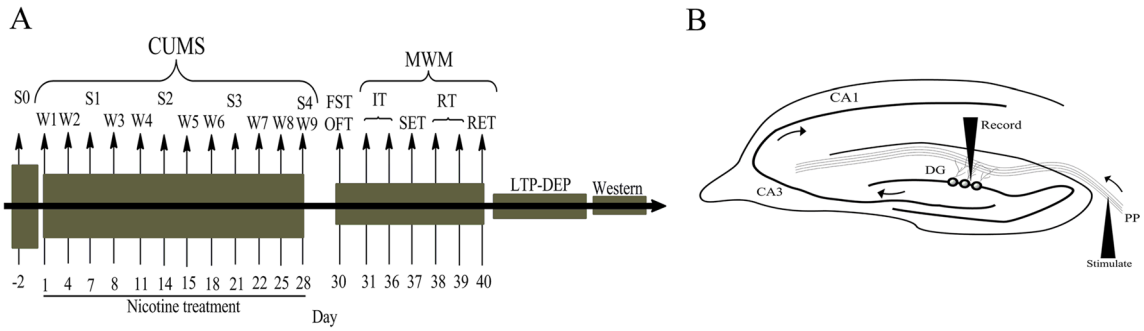

Fig. 1 Experimental procedure and schedule. *W* weight test, *S* sucrose preference test

Training and testing in the MWM comprised of four consecutive stages: initial training (IT); space exploring test (SET); reversal training (RT); reversal exploring test (RET). This protocol was adopted and modified on the basis of our previous study [39]. The skeleton of MWM experiment design was shown in Fig. 1.

In the IT stage, a mouse was given six sessions of training (one session per day). Each session consisted of four trials and each trial lasted for 1 min. The platform was placed in the center of N quadrant. The mouse was placed into the water facing the pool wall at one of the four starting points (north, south, east, and west) in a semirandom order. The animal was forced to swim and learnt to find the hidden platform. The mouse was taken to the cage if it found the platform and stayed on it for about 3 s. However, mice would be guided to the platform and stayed for at least 3 s when they failed to find the platform within 1 min. The mouse was returned to its cage afterward for a 5-min inter-trial rest, when other mice were trained. Two parameters were recorded, which were escape latency and swimming speed. The mean escape latency of four trials was noted as the daily result of learning ability for the mouse. Secondly, the SET stage was performed using one trial without the platform after the last session of the IT stage approximately 24 h later. The mice were released individually into the water from one of the starting points and allowed to swim freely for 60 s. Two parameters were recorded: platform crossings and N quadrant dwell time (the percentage of time spent in the target quadrant). Thirdly, the RT stage was conducted for 2 days from the eighth day in the same way and with the same parameters in the IT stage. The difference was that the platform was moved into the opposite quadrant in the center of the S quadrant. Finally, in the RET stage, the approach employed and the parameters collected were the same as those in the SET stage.

Table 1 CUMS protocol

Days Weeks	1	2	3	4	5	6	7
1	C	T	W	S	L	F	C
2	T	L	C	O	S	F	D
3	C	D	O	W	T	F	S
4	T	W	L	D	T	F	O

R reversed light/dark cycle (24 h), *I* ice water swimming (4 °C, 1 min), *O* oven (45 °C, 5 min), *C* cage tilt (45°, 12 h), *F* confinement in tube (2 h), *D* damp bedding (150 mL water + 200 g bedding, 24 h), *W* white noise (85 dB, 5 min), *T* tail pinch (1 min), *F* water and food deprivation (24 h)

Electrophysiological Tests

After finishing all behavioral assays, the electrophysiological test was performed in vivo. Hippocampal synaptic responses were quantified as the field excitatory postsynaptic potentials (fEPSPs) extracellularly recorded in the PP to DG region (Fig. 1b). After anesthetized by 30 % urethane anesthesia (0.4 mL/10 g, i.p.), mice were placed in a stereotaxic frame (SN-3, Narishige, Japan). At the electrode inputting region of the left side, the scalp was incised and a small hole was drilled in the skull using a dental drill. A concentric bipolar stimulating electrode was slowly implanted into the PP (3.8 mm posterior to the bregma, 3.0 mm lateral to midline, 1.5 mm ventral below the dura). A monopolar extracellular stainless steel recording electrode was positioned into DG region (2.0 mm anterior to the bregma, 1.4 mm lateral to midline, 1.5 mm ventral below the dura). During recording, the optimal depths of electrodes were determined using the electrophysiological criteria. A stable normalized baseline was recorded for 20 min after positioning the electrodes. Test stimuli were delivered to the PP every 30 s at an intensity that evoked a response of 70 % of its maximum (range 0.3–0.5 mA). Once the response stabilized, sampling was made under single-pulse stimulation (0.2 ms, 0.05 Hz) for 20 min as the baseline. After the baseline, a theta burst stimulation (TBS), which was consisted of 30 trains of 12 pulses (200 Hz) at 5 Hz, was used to make LTP. Following TBS, the single-pulse stimulation was resumed to collect the evoked response every 60 s for 60 min at the baseline intensity (Scope software, PowerLab; AD Instruments, New South Wales, Australia). Next, low-frequency stimulation (LFS) (900 pulses of 1 Hz for 15 min) was delivered to induce DEP. Following LFS, single-pulse

recording resumed every 60 s for 60 min to sample the evoked response. The evoked responses of the last stabilized 20 min of LTP were normalized and used as the baseline of DEP. Initial data was measured in Clampfit 10.2 (Molecular Devices, Sunnyvale, CA, USA). The fEPSPs slope was evaluated as the average slope from 10 to 90 % of the decreasing phase to assess the synaptic efficacy.

Western Blotting

Each hippocampus was dissected from the brain after electrophysiological test and immediately stored at −80 °C until needed. The method of Western blotting assay was modified on the base of previous studies [43]. Firstly, each hippocampus was mashed with a grinder and 200 μL lysis buffer (Beyotime Biotechnology, Haimen, China) containing Phenylmethanesulfonyl fluoride (PMSF, 1:100 dilutions). The lysates were centrifugated at 12,000 r/min for 20 min at 4 °C and the supernatant was moved to a 1.5-mL tube. The protein concentration was determined using the BCA Protein Assay Kits according to instructions (Beyotime Biotechnology, Haimen, China). Secondly, the same amount of protein (40 μg) was electrophoresed by SDS-PAGE 10–13 % gels and then electrotransferred proteins onto 0.45 μm (for NMDA2A/2B, Notch1) or 0.2 μm (for Jagged-1, Hes1, GAD67, β-actin) polyvinylidene difluoride (PVDF) membranes (Millipore Corporation). After that, the membrane was blocked in Tris-buffered saline (TBS) including 5 % skim milk for 90 min at room temperature and then incubated with primary antibody (Anti-NR2A, ab169873, 1:2000 dilution, Abcam, UK; Anti-NR2B, ab65783, 1:2000 dilution, Abcam, UK; Anti-GAD67, ab26116, 1:2000 dilution, Abcam, UK; Anti-Notch1, ab52627, 1:2000 dilution, Abcam, UK; Anti-Jagged1, ab7771, 1:1000 dilution, Abcam, UK; Anti-Hes1, ab71559, 1:1000 dilution, Abcam, UK) diluted in blocking buffer overnight 4 °C. Following the next day, the PVDF membranes were washed 4 × 10 min with Tris-buffered saline/Tween 20 (TBST) and incubated with secondary antibody (1:3000 dilution; Cell Signaling Technology, Beverly, MA, USA) subsequently. After 4 × 10 min TBST washed, the membranes were detected the protein band intensities by using chemiluminescent HRP substrate. Finally, the quantitation analysis was performed by ImageJ Launcher and compared to the loading control proteins β-actin (sc-47,778, 1:1000, Santa Cruz, USA).

Data Presentation and Statistics

All data were presented as mean ± S.E.M. A two-way repeated measures ANOVA was used with group and day as the factors to measure the performance of mice during body weight, sucrose consumption, and MWM escape latencies. Other data, obtained from the SET/RET stage, the FST, the OFT, electrophysiological experiments, and Western, were assessed by a one-way ANOVA for multiple comparisons with stress or nicotine. To detect significant differences between groups, ANOVAs were supported by LSD post hoc tests. All analyses were performed using SPSS (20.0) software and differences were considered significant when $p < 0.05$.

Results

The hypothesis has been verified by following evidences, which were obtained from physical and behavioral tests, electrophysiological recordings, and Western analysis.

Effects of Nicotine on Body Weight and Sucrose Preference in Stressed Mice

As shown in Fig. 2a, the body weights in every group were increased during the 4 weeks. However, a two-way repeated measures ANOVA showed that there were the statistical difference of day ($F_{(2.720,\ 57.118)} = 32.052$, $p < 0.001$), day × group interaction ($F_{(5.440,\ 57.118)} = 1.272$, $p = 0.287$), and group ($F_{(2,\ 21)} = 6.018$, $p < 0.01$). In addition, post hoc tests showed that the mice's weight was statistically lower in the CUS group compared to that in the Con ($p < 0.05$ on the 4th; $p < 0.001$ from the 8th ~ 11th; $p < 0.05$ on the 18th, 22nd, 25th). However, there was no significant difference of the body weight between CUS group and CUS + Nic group ($p > 0.05$).

The effects of CUMS and nicotine treatment on the sucrose preference percentage were presented in Fig. 2b. A two-way repeated measures ANOVA exhibited that there were statistical difference for day ($F_{(3.268,\ 68.619)} = 3.975$, $p < 0.01$), group ($F_{(2,\ 21)} = 20.954$, $p < 0.001$) and day × group interaction ($F_{(6.535,\ 68.619)} = 0.828$, $p = 0.560$). Post hoc tests showed that the sucrose preference was considerably lower in the CUS group than that in the Con group ($p < 0.01$ on the 14th; $p < 0.01$ on the 21st and $p < 0.001$ on the 28th). Furthermore, it was significantly increased by nicotine in the CUS + Nic group compared to that in the CUS group ($p < 0.05$, on the 28th). It suggests that nicotine effectively alleviate the development of depressive-like symptoms in a mouse model of CUMS. Furthermore, following behavior tests may provide more information related to nicotine treatment.

Effects of Nicotine on the Forced Swim Test in Stressed Mice

Figure 3a showed the effects of CUMS and nicotine treatment on the immobility time in the FST. A one-way ANOVA revealed that there was a significant effect of the nicotine treatment ($F_{(2,\ 21)} = 3.621$, $p < 0.05$). Post hoc test showed that the immobility time was longer in the CUS group than that in the Con group ($p < 0.05$). Meanwhile, there was no statistical difference of the immobility time between the CUS + Nic group and the CUS group ($p = 0.07$). The data suggested that

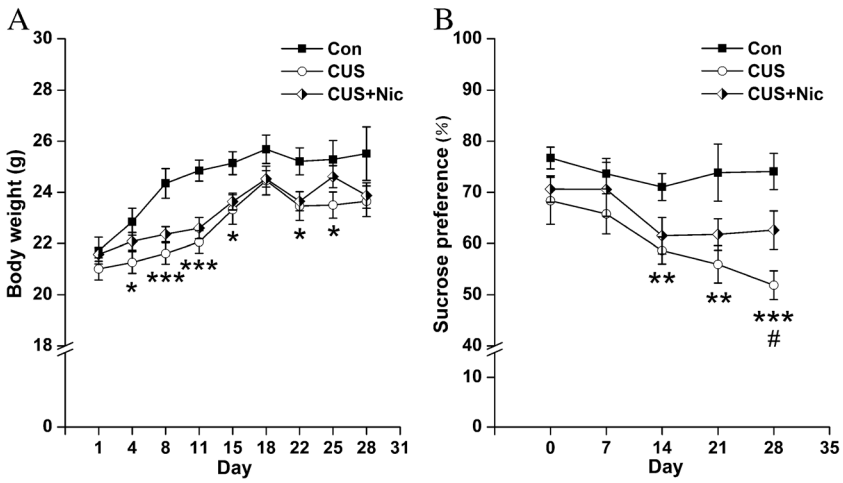

Fig. 2 Effects of CUMS and nicotine treatment on body weight (**a**) and sucrose preference (**b**). Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ significant difference between the Con group and the CUS group; $^{\#}p < 0.05$ significant difference between the CUS group and the CUS + Nic group, $n = 8$ in each group

CUMS treatment induced behavioral despair in mice, while nicotine administration could partially mitigate damages.

Effects of Nicotine on the Open Field Test in Stressed Mice

A one-way ANOVA showed that there was a significant effect of the nicotine treatment on the total distance (Fig. 3b, $F_{(2,\ 21)} = 8.598$, $p < 0.01$). Post hoc test showed that the total distance was much smaller in the CUS group compared to that in the Con group (Fig. 3b, $p < 0.001$). It was greater in the CUS + Nic group than that in the CUS group (Fig. 3b, $p < 0.05$), suggesting that there was a significant effect of nicotine on locomotor activity. In addition, a one-way ANOVA exhibited that there was a significant effect of the nicotine treatment on the center duration (Fig. 3c, $F_{(2,\ 21)} = 6.667$, $p < 0.01$). Post hoc test showed that the center duration was considerably smaller in the CUS group than that in the Con group (Fig. 3c, $p < 0.01$). Furthermore, it was significantly increased by nicotine in the CUS + Nic group compared to that in the CUS group (Fig. 3b, $p < 0.05$). There was no significant difference of the center duration between the CUS + Nic group and the Con group (Fig. 3, $p > 0.05$). However, a one-way ANOVA showed that there was no effect of nicotine treatment on the central area entries (Fig. 3d, $F_{(2,\ 21)} = 2.502$, $p > 0.05$). Our results showed that CUMS treatment induced anxiety syndrome in mice; however,

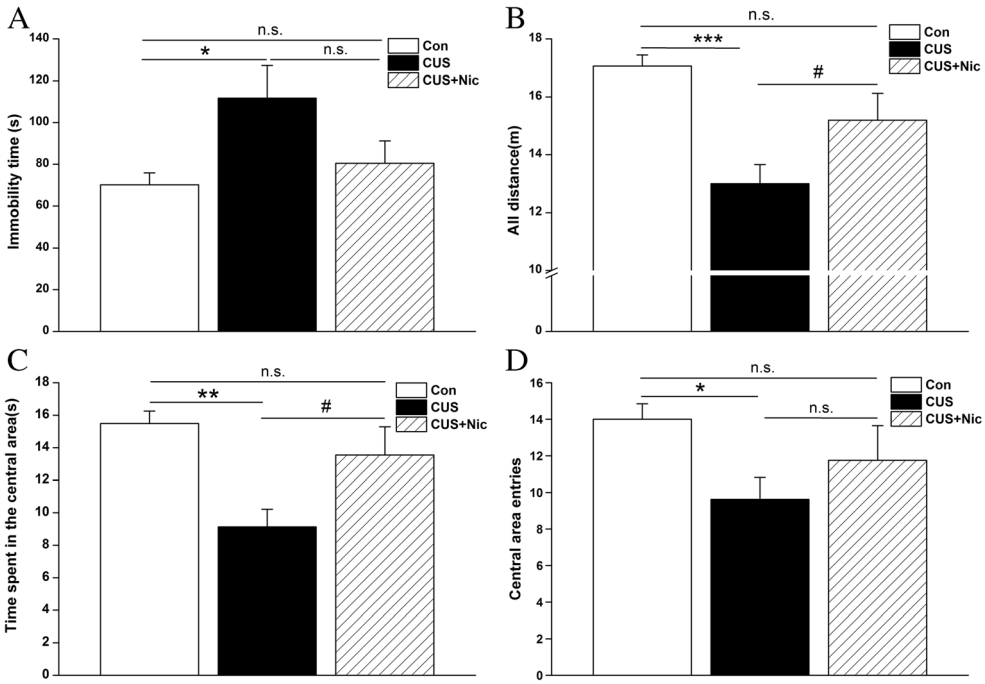

Fig. 3 The performances of mice in the FST and OFT among three groups. **a** Immobility time was determined in the FST. **b**, **c**, **d** All distances, central area duration and entries were measured in the OFT. Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ comparison between the Con and the CUS group; $^{\#}p < 0.05$ comparison between the CUS group and the CUS + Nic group, $n = 8$ in each group

nicotine administration improved exploratory and anxiolytic behavior.

Effects of Nicotine on the MWM in Stressed Mice

The data, obtained from the MWM test in three groups on each test day, were shown in Fig. 4. During the IT stage, the escape latencies were noticeably reduced with training (Fig. 4a, left). A two-way repeated measures ANOVA showed that there were statistical differences of day ($F_{(3.912,\ 82.144)} = 16.865$, $p < 0.001$), group ($F_{(2,\ 21)} = 5.656$, $p < 0.001$), except for day × group interaction ($F_{(7.823,\ 82.144)} = 23.599$, $p = 0.991$) as the three groups did improve over the 6 days of training. Following post hoc analysis showed that there were statistical differences of the escape latencies between the CUS group and the Con group (Fig. 4a, left, $p < 0.05$ on the 3rd day; $p < 0.05$ on the 4th day; and $p < 0.05$ on the 5th day), demonstrating that mice in the CUS group took more time to discover the platform compared to that in the Con group. However, the escape latencies were significantly decreased in the CUS + Nic group compared to that in the CUS (Fig. 4a, left, $p < 0.05$ on the 4th day). In addition, there were no statistical differences of swimming speed among the three groups (Fig. 4b, left, $p > 0.05$). In the SET stage, the reference memory was measured. A one-way ANOVA showed that there were statistical differences of group in the platform crossings (Fig. 4c, $F_{(2,\ 21)} = 4.398$, $p < 0.05$) and the N quadrant dwell time (Fig. 4d, $F_{(2,\ 21)} = 8.056$, $p < 0.01$). Post hoc test showed that the platform crossings (Fig. 4c, $p < 0.05$) and the N quadrant dwell time (Fig. 4d, $p < 0.01$) were significantly reduced in the CUS group compared to that in the Con group. Stimulatingly, the above indicators were remarkably enhanced with nicotine administration (Fig. 4c, d, $p < 0.05$).

The learning flexibility was then examined after the hidden platform was moved into the contralateral quadrant in the RT stage. The average escape latency was measured for each mouse on each of 2-day training (Fig. 4a, right). A two-way repeated measures ANOVA showed that there were significant differences of day ($F_{(1,\ 21)} = 23.830$, $p < 0.001$), group ($F_{(2,\ 21)} = 4.115$, $p < 0.05$), except for day × group interaction ($F_{(2,\ 21)} = 23.599$, $p > 0.05$). Subsequent post hoc test showed that the escape latencies were longer in the CUS group than that in the Con group (Fig. 4a, right, $p < 0.05$ on the 2nd day). However, there was a significant effect of nicotine treatment, in which the escape latencies were statistically shortened in the CUS + Nic group compared to that in the CUS group (Fig. 4a, right, $p < 0.05$ on the 2nd day), indicating that nicotine-treated mice were able to utilize a spatial strategy to learn the position of the platform. Additionally, there were no differences of swimming speed among these three groups throughout the test (Fig. 4b, right, $p > 0.05$). On the other hand, the reference memory was assessed, indicating that there were significant differences of group in the platform crossings (Fig. 4e, $F_{(2,\ 21)} = 4.904$, $p < 0.05$) and the S quadrant dwell time (Fig. 4f, $F_{(2,\ 21)} = 4.398$, $p < 0.05$). Post hoc test showed that the platform crossings (Fig. 4e, $p < 0.01$) and the S quadrant dwell time (Fig. 4f, $p < 0.05$) were significantly reduced in the CUS group compared to that in the Con group. In addition, the platform crossings and the S quadrant dwell time in the CUS + Nic group were considerably increased compared to that in the CUS group (Fig. 4e, f, $p < 0.05$). There were no statistical differences of the two parameters between the Con group and the CUS + Nic group (Fig. 4e, f, $p > 0.05$). The above data suggested that the impairment of spatial learning and memory induced by CUMS treatments was significantly improved by nicotine. Moreover, the underlying cellular mechanism of the spatial memory impairments may be revealed by LTP and depotentiation recordings.

Effects of Nicotine on the LTP and Depotentiation in Stressed Mice

In the LTP test, a basal fEPSPs was evoked by the stimulation of PP in the hippocampal DG region, and then theta burst stimulation (TBS) was delivered to induce LTP for 60 min. The inset shows an example of fEPSPs at the baseline—TBS, LTP, and depotentiation of a mouse in the Con group (Fig. 5a). Evidently, the slope of fEPSPs is instantly enhanced after TBS and then more or less stabilized to a level above the baseline period in these three groups (Fig. 5a, left). The last 15-min recordings were used for measuring the group data. A one-way ANOVA showed that there was a statistical difference of group ($F_{(2,\ 21)} = 10.191$, $p < 0.01$, Fig. 5b). In addition, the mean fEPSP slopes were much smaller in the CUS group than that in the Con group (Fig. 5b, $p < 0.001$); however, they were significantly increased by nicotine in the CUS + Nic group compared to that in the CUS group (Fig. 5b, $p < 0.05$). In order to examine whether or not depotentiation, a form of LTP reversal, was efficiently involved in the process, an LFS induction protocol was employed for inducing depotentiation (Fig. 5a, right).

Accordingly, LTP-evoked responses in the last 20 min were normalized and used as the baseline of depotentiation (Fig. 5a, right). A one-way AVONA confirmed the statistical difference of groups (Fig. 5c, $F_{(2,\ 21)} = 26.835$, $p < 0.001$). It can be seen that the depotentiation is considerably inhibited in the CUS group compared to that in the Con group (Fig. 5c, $p < 0.001$), while it is significantly strengthened by nicotine in the CUS + Nic group compared to that in the CUS group (Fig. 5c, $p < 0.05$). However, there is still statistical differences of the fEPSPs slopes

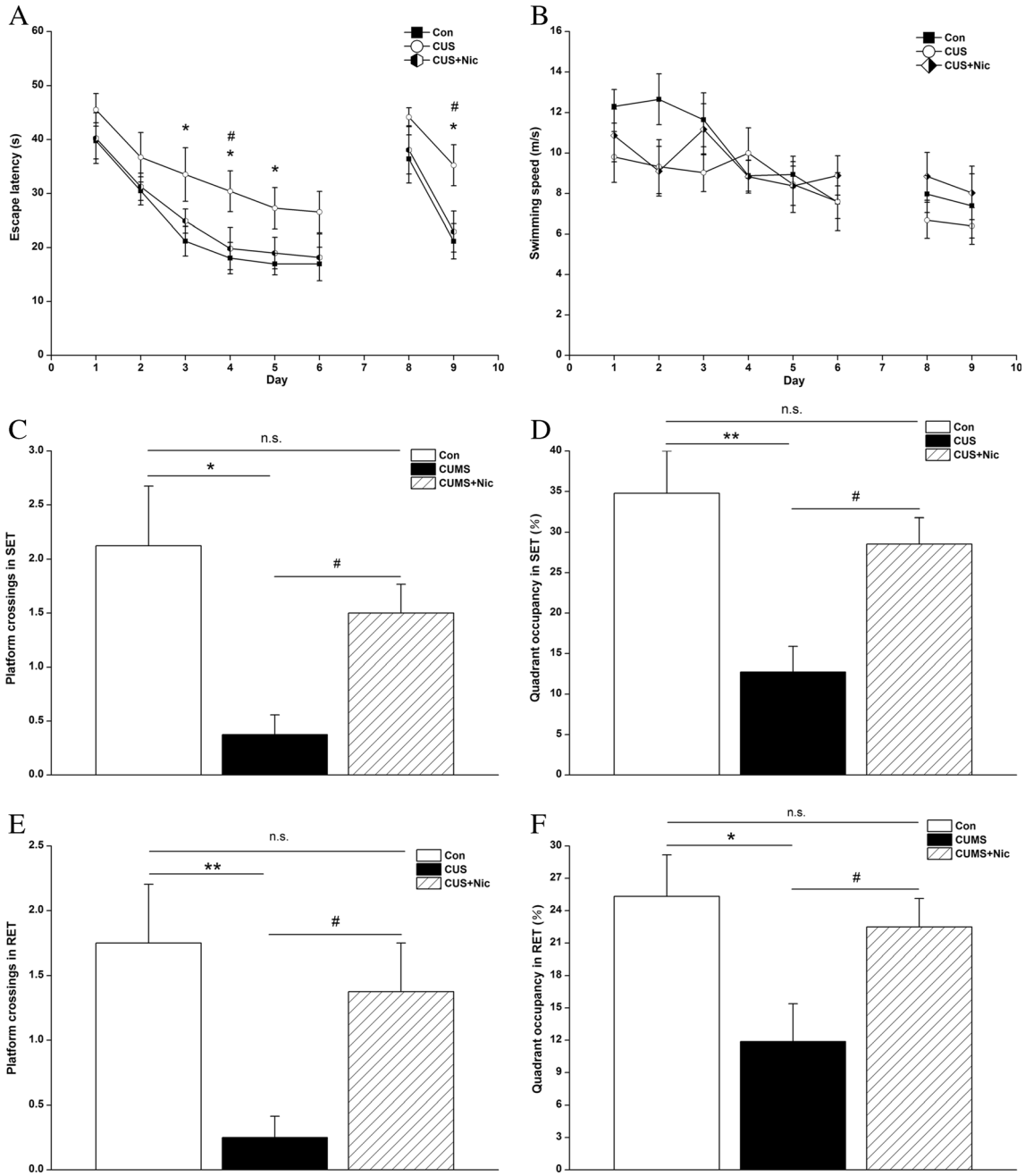

Fig. 4 Performances of mice in the MWM test. **a** Mean escape latency was determined for each day in the IT (*left*) and the RT (*right*) stages among three groups. **b** Mean swimming speed in both the IT (*left*) and RT (*right*) stages. **c** Number of platform area crossings in the SET stage. **d** N quadrant dwell time in the SET stage. **e** Number of platform area crossings in the RET stage. **f** S quadrant dwell time in the RET stage. Data are presented as mean ± SEM. $*p < 0.05$, $**p < 0.01$ comparison between the Con group and the CUS group; $^{\#}p < 0.05$ comparison between the CUS group and the CUS + Nic, $n = 8$ in each group

between the Con group and the CUS + Nic group (Fig. 5c, $p > 0.001$). Collectively, the results suggested that the impairment of synaptic plasticity induced by CUMS was significantly improved by nicotine. We suppose that either transmembrane ion channel receptors or Notch signaling components may be activated. Accordingly, Western blot tests were performed.

Effects of Nicotine on NR2A, NR2B, and GAD67 Expression in Stressed Mice

In the Western blotting assay test, three prominent bands at about 180, 163, and 67 kDa were detected by NR2A, NR2B, and GAD67 antibodies, respectively (Fig. 6). A one-way ANOVA showed that there were significant differences of

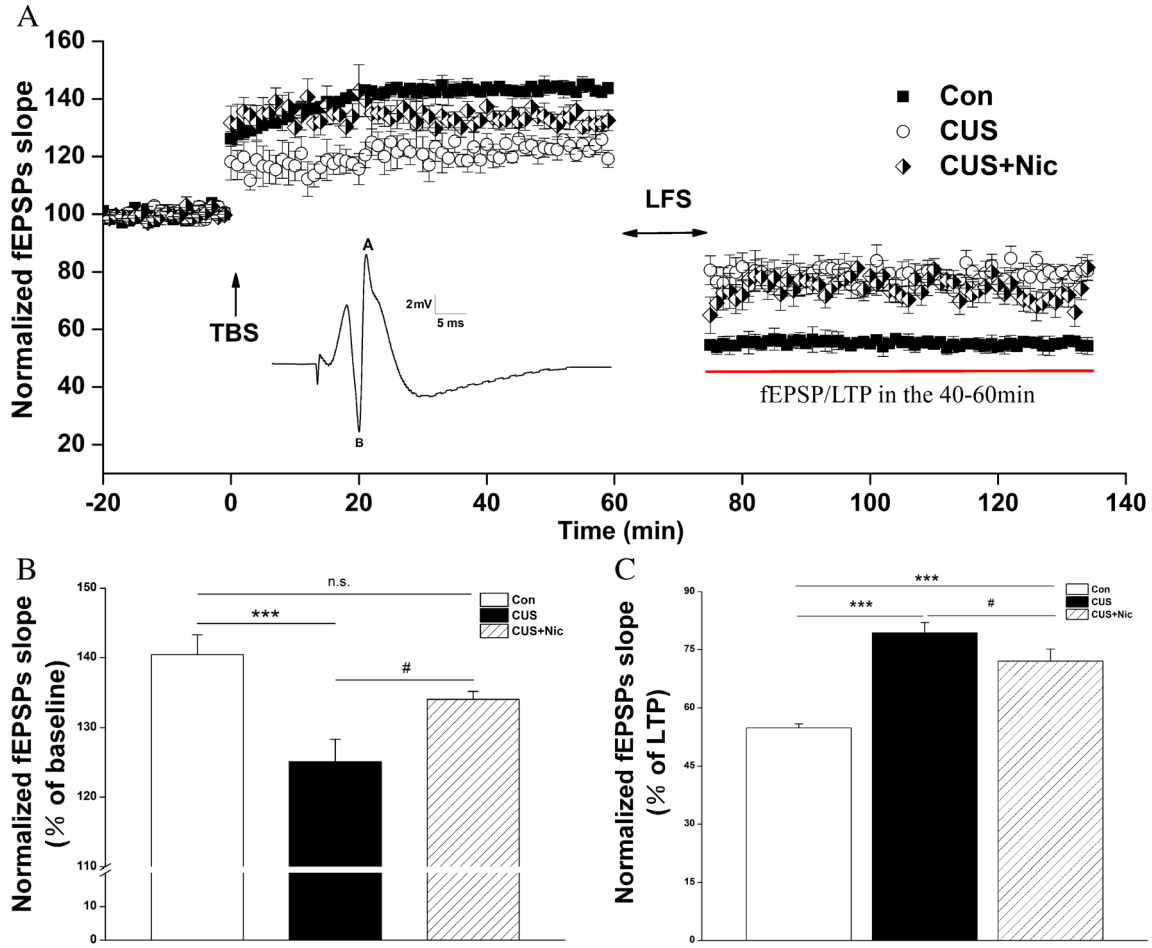

Fig. 5 The effects of CUMS and nicotine on the long-term potentiation and depotentiation from the hippocampal PP to DG. **a** The changes of time coursing in fEPSPs slopes in both LTP and depotentiation stages in the three groups. The first 20 min of evoked responses were normalized and used as the baseline responses of LTP. The last 20 min of evoked responses during LTP were normalized and used as the baseline responses of depotentiation which was induced by low frequency stimulation (LFS). The *inset* shows an example of fEPSPs at baseline—TBS, LTP, and depotentiation. **b** Magnitude of LTP was determined as responses between 45 and 60 min after the TBS. **c** Magnitude of depotentiation was determined as responses between 45 and 60 min after the LFS. Data are presented as mean ± SEM. ***$p < 0.001$ comparison between the Con group and either the CUS group or the CUS + Nic group; $^{\#}p < 0.05$ comparison between the CUS group and the CUS + Nic, $n = 8$ in each group

the protein levels of NR2A, NR2B, and GAD67 among these three groups (Fig. 6b–d, NR2A: $F_{(2, 6)} = 14.685$, $p < 0.01$; NR2B: $F_{(2, 6)} = 46.976$, $p < 0.001$; GAD67: $F_{(2, 6)} = 14.820$, $p < 0.01$). The levels of NR2A, NR2B, and GAD67 expression were significantly reduced in the CUS group compared to that in the Con group (Fig. 6b–d, NR2A: $p < 0.01$; NR2B: $p < 0.001$; GAD67: $p < 0.01$). Importantly, it could be seen that they were statistically enhanced by nicotine in the CUS + Nic group compared to that in the CUS group (Fig. 6b–d, NR2A: $p < 0.05$; NR2B: $p < 0.01$; GAD67: $p < 0.05$). Nevertheless, there were still statistical differences of them between the Con group and the CUS + Nic group (Fig. 6b–d, NR2A: $p < 0.05$; NR2B: $p < 0.01$; GAD67: $p < 0.05$). The results show that the expression of synaptic proteins has been decreased by CUMS treatment, but the level of NR2A and NR2B is upregulated by nicotine.

Effects of Nicotine on Notch1, Jagged-1, and Hes1 Expression in Stressed Mice

Based on the same approach, three prominent bands at about 125, 95, and 30 kDa were detected by Notch1, Jagged-1, and Hes1 antibodies, respectively (Fig. 7). A one-way ANOVA showed that there were significant differences of the protein levels of Notch1, Jagged-1, and Hes1 among the three groups (Fig. 7b, d, f, Notch1: $F_{(2, 6)} = 20.061$, $p < 0.01$; Jagged-1: $F_{(2, 6)} = 256.198$, $p < 0.001$; Hes1: $F_{(2, 6)} = 11.576$, $p < 0.01$). The levels of Notch1, Jagged-1, and Hes1 expression were considerably decreased in the CUS group compared to that in the Con group (Fig. 7b, d, f, Notch1: $p < 0.01$; Jagged-1: $p < 0.01$; Hes1: $p < 0.01$). Interestingly, they were significantly increased by nicotine in the CUS + Nic group compared to that in the CUS group (Fig. 7b, d, f, Notch1: $p < 0.05$; Jagged-1:

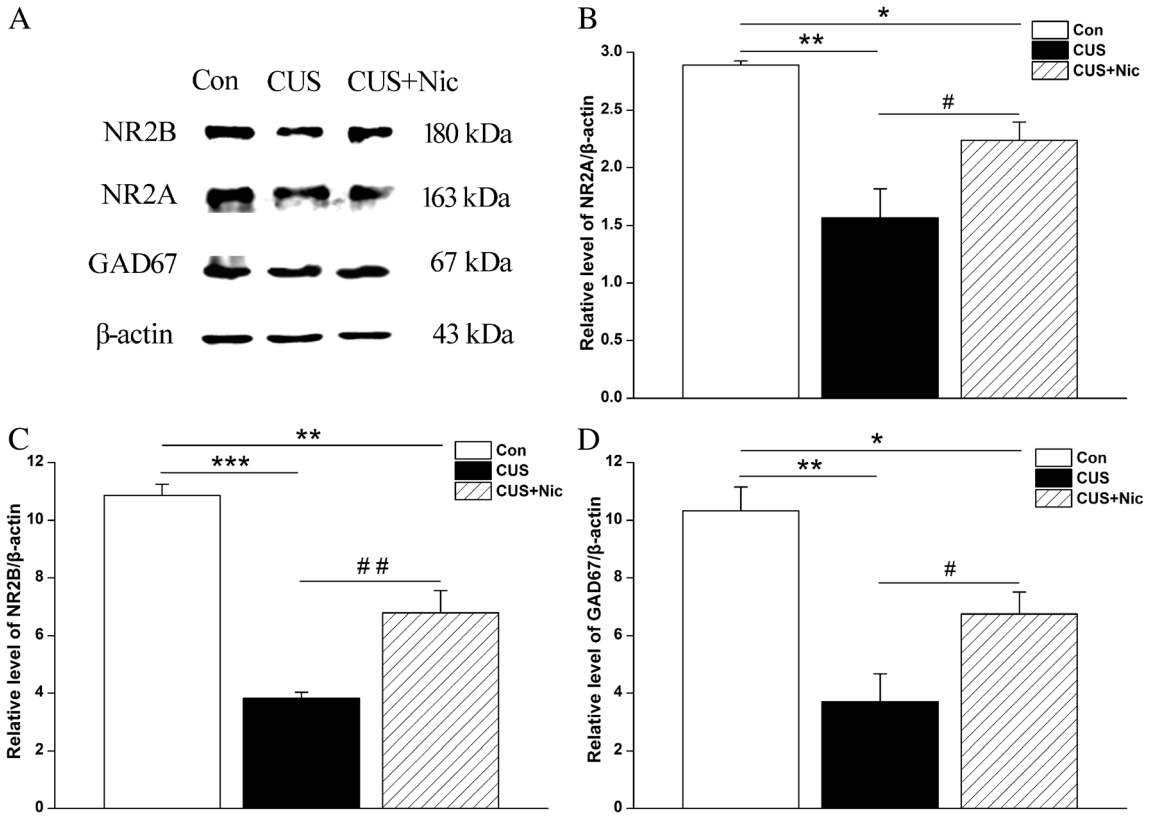

Fig. 6 Nicotine significantly enhances the expression of NR2A, NR2B, and GAD67 in the hippocampus. **a** Results are immunoblots from single representative experiments. The expression values of NR2A (**b**), NR2B (**c**), and GAD67 (**d**) were normalized with β-actin value and then compared to control. Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ comparison between the Con group and the CUS group / CUS + Nic group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ comparison between the CUS group and the CUS + Nic group; $n = 3$ in each group

$p < 0.05$; Hes1: $p < 0.01$). However, there were still statistical differences of Notch1 and Jagged-1 between the Con group and the CUS + Nic group (Fig. 7b, d, Notch1: $p < 0.05$; Jagged-1: $p < 0.05$). The data suggest that the expressions of Notch1 signaling components, including receptor (Notch1), ligand (Jagged-1) and the downstream target protein (Hes1), are down-regulated in the CUMS group; however, they are significantly up-regulated by nicotine.

Discussion

The present study revealed that nicotine could successfully mitigate the development of depressive-like symptoms in a mouse model of CUMS after chronic nicotine administration for 28 consecutive days. More importantly, the spatial memory damages including spatial learning and re-acquisition in reversal learning were effectively improved after nicotine-treatment. Moreover, the impairment of synaptic plasticity induced by CUMS was significantly impeded by nicotine. Additionally, nicotine treatment is able to upregulate the level of NR2A, NR2B, GAD67, Notch1, Jagged-1, and Hes1 expressions in the hippocampus of stressed mice.

Firstly, our study aimed to verify the validity of CUMS as an animal model to investigate depression [37]. The SPT is used to operationally determine anhedonia, which is the core symptom of the depressive disorders [3]. It was found that there were significant differences of both body weight and sucrose preference between the CUS group and the Con group. One of our previous studies showed that reduced intake of a sucrose preference and body weight were a sign of anhedonia [44]. In the present study, the results demonstrated that the CUMS mouse's model, combined with isolation rearing, well simulated the depression-like behavior. Furthermore, nicotine treatment showed a significant antidepressant-like effect on the depressive model, which was similar to the previous findings related to nicotine treatment [45, 8].

The FST is a commonly used approach for examining antidepressant effect of drugs [40, 46]. Behavioral immobility in the FST has been interpreted as an index of despair [47]. A previous study showed that the duration of immobility time was significantly decreased by acute nicotine (0.2 mg/kg) [8]. In addition, it reported that the immobility time of mice was considerably reduced by nicotine (0.2 mg/kg), and the effect of which was more or less similar to fluoxetine (20 mg/kg) [48]. Acute or chronic administration of nicotine (0.4 mg/kg)

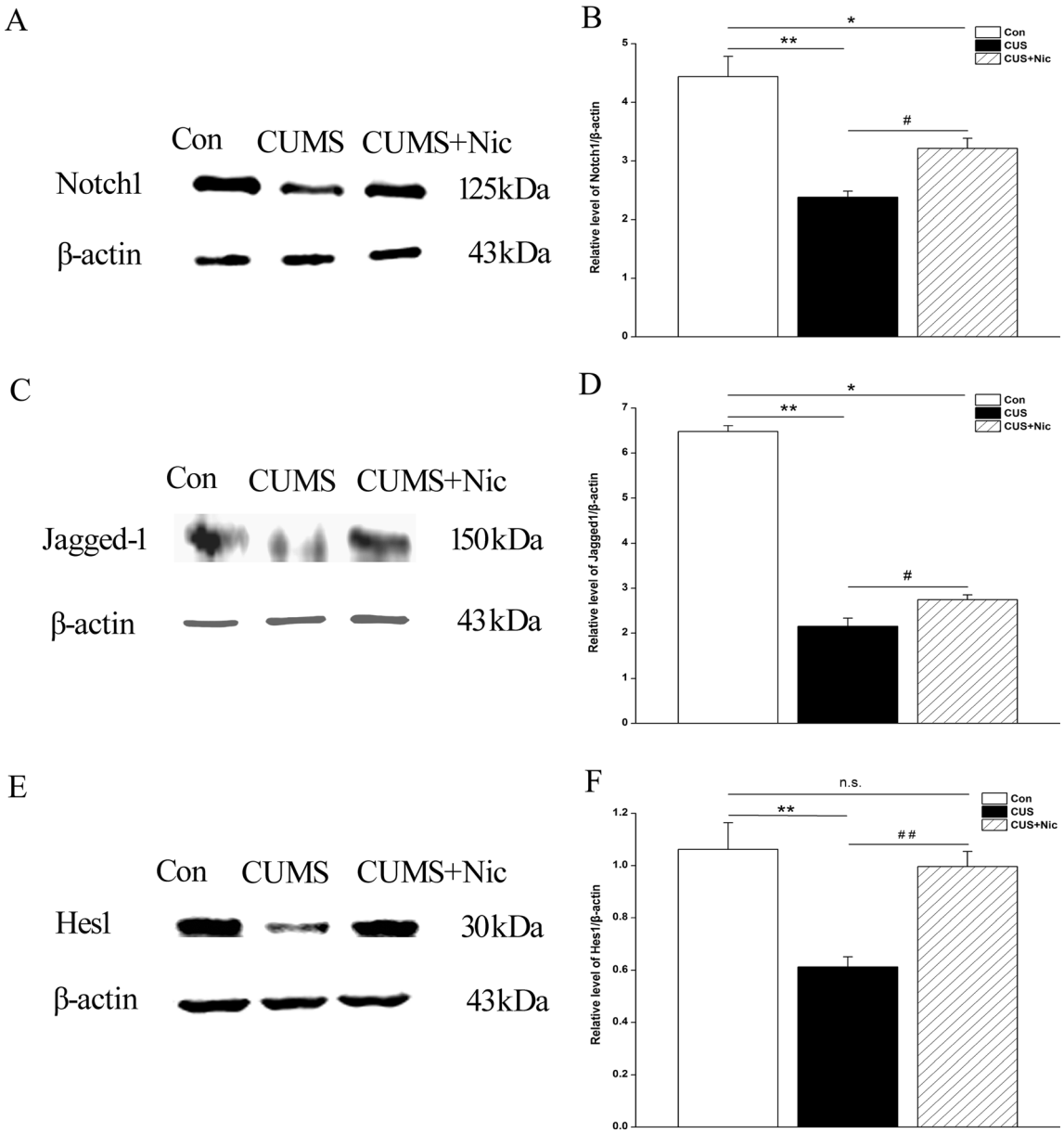

Fig. 7 Nicotine significantly enhances the expression of Notch1, Jagged-1, and Hes1 in the hippocampus. **a**, **c**, **e** Results are immunoblots from single representative experiments. The expression values of Notch1 (**b**), Jagged-1 (**d**), and Her1 (**f**) were normalized with β-actin value, and then compared to control. Data are presented as mean ± SEM. $*p < 0.05$ and $**p < 0.01$ comparison between the Con group and the CUS group / CUS + Nic group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ comparison between the CUS group and the CUS + Nic group; n.s. $p > 0.05$ comparison between the Con group and the CUS + Nic group; $n = 3$ in each group

is able to improve the immobility in the FST of Flinders Sensitive Line rats, which is regarded as a "genetic animal model of depression" [9, 49]. Moreover, after the administration of nicotine (0.4 mg/kg s.c.) acutely (1 day), subchronically (7 days), and chronically (14 days), the immobility was significantly reduced and the swim distance was considerably increased in depressive rats during the FST [12]. Our data showed that the immobility time was statistically reduced by nicotine in the CUS + Nic group compared to that in the CUS group, indicating that the animal's behavioral depression was effectively alleviated. The data obtained from the OFT experiment further showed that the spontaneous activity and exploratory behavior were significantly enhanced by nicotine, suggesting that the anxiety symptoms were efficiently relieved by nicotine, which was mostly similar to a standard antidepressant drug, fluoxetine [8, 9].

In order to investigate if the deficits in spatial learning/memory and spatial reversal learning could be alleviated by nicotine in CUMS-treated mice, the MWM experiment was carried out. In both the IT and SET stages, the results showed

that there were statistical differences of latency prolongations and the reductions of both quadrant dwell time and platform crossings in stressed mice compared to that in normal ones, indicating that spatial learning and reference memory were significantly impaired in depressive-like mice. It is consistent with that of previous studies [50, 3]. Profoundly, the mice in the CUS + Nic group spent much less time finding the platform, and the quadrant dwell time and the platform crossings were considerably increased compared to that in the CUS group, signifying that nicotine could efficiently mitigate the cognitive deficit induced by depression. This finding is consistent with previous studies, in which nicotine (0.2 mg/kg, i.p) was used for treating aged rats with impaired acquisition [51]. Moreover, the hidden platform was moved into the contralateral quadrant to test learning flexibility during the RT and RET phases, which was attributed to the ability on erasing the previously acquired information and developing a new memory. The results exhibited that the stressed animals failed to promptly modify the search strategy in response to the change of platform position and produced a worse performance in reacquisition of learned skill, indicating that the cognitive flexibility was definitely impaired. Nevertheless, the escape latencies in the CUS + Nic group were significantly decreased, suggesting that nicotine could efficiently improve the cognitive flexibility in depressive mice. This is also consistent with the outcomes of a previous study, in which nicotine (0.35 mg/kg, 8 week) was applied in the treatment of DAT KO and significantly improved animal's cognitive deficits without long-term tolerance [17].

Physiologically, a higher fEPSPs slope is normally associated with more active synaptic transmission and better learning and memory [52]. Our data show that the strength of synaptic connections is significantly weaker in stressed mice as shown by reduced fEPSPs slopes rather than in normal mice (Fig. 5), which are consistent with the performance of learning stage. Very interestingly, the negative effect of stresses on LTP was considerably mitigated by chronic nicotine administration, signifying that it was probably related to the improvements in cognitive deficits in depressive mice. In addition, DEP plays an important role in the time- and state-dependent erasure of memory and enables the storage of new information by the hippocampus [53]. It was found that the DEP was dramatically inhibited in stressed mice (Fig. 5c), suggesting that the resilience of synaptic structures was significantly impaired. Interestingly, the synaptic plasticity damage was considerably alleviated by nicotine intervention, which was closely associated with animal's performances in reversal learning of MWM. A previous investigation from our lab reported that the balance between LTP and DEP played a crucial role in cognitive stability and flexibility [54]. Therefore, potentiation and depotentiation may underlie cognitive function in learning and reversal learning, respectively.

It is well known that the NR2A and NR2B receptors, as the function subunit of NMDA receptor, are required for LTP induction. It has been formerly demonstrated that there are certain pharmacological similarities between the nAChRs and the glutamatergic NMDA receptors. For example, the NMDA antagonist MK-801 has been shown to effectively block muscle and neuronal nicotinic channels [55]. Besides, mecamylamine, as a nicotinic channel blocker, seems to antagonize NMDA responses [56]. Nevertheless, other previous investigations showed that NMDA receptors activation played a major role in mediating the effect of nicotine, especially in behavioral sensitization and synaptic plasticity in sensory neocortex [57, 58]. Moreover, the GluN2B subunit-containing NMDA receptor is necessary for low-frequency stimulation to induce DEP [53]. Taken together, the role of the glutamatergic system in mediating the antidepressant effect of nicotine has not been certainly understood. It was found that the level of the NR2A and NR2B subunits of NMDA receptor was significantly reduced in stressed mice compared to that in normal animals (Fig. 6b, c), representing that there was a lower level of NR2A and NR2B proteins in the depressive state than that in normal state. Importantly, the level of both NR2A and NR2B expression was significantly enhanced by nicotine (Fig. 6b, c), which was possibly one of the underlying mechanisms for alleviating the impairments of LTP and DEP, as well as the cognitive deficits induced by depression. In addition, it reports that chronic stress downregulates the GABA and GAD67 expressions [59, 60], suggesting that the GABAergic system is also important in the stress-related psychiatric disorders. In the study, it was found that chronic stress in experimental mice significantly reduced the level of GAD67 expression; however, it was effectively enhanced by nicotine in stressed mice (Fig. 6d). The findings are also supported by a recent study, in which nicotine restores GAD67 gene expression in the brain of heterozygous reeler mice [61]. Taken together, the above results show that nicotine produces the antidepressant-like effects partially by improving the NMDA-dependent synaptic plasticity and GABAergic system in a mouse model of depression using the CUMS paradigm.

A previous study reported that the Notch signaling was involved in chronic mild stressed related apoptosis injury and synaptic integration of newborn cells in the ischemic stroke animals [30]. On the other hand, Notch1, NICD1(Notch1 intracellular domain), and Jagged-1 are colocalized with synaptic proteins at the synapse in mature neurons in C57BL6/129 mice, and the increased neuronal activity after treatment with NMDA leads to higher Notch1, NICD1, and Jagged1 levels [62]. Besides, a recent report has shown that Notch1 can influence the expression of the NMDAR subunits, NR1 and NR2B rather than NR2A in the adult hippocampus [63]. A peptide agonist of Notch enhances both LTP and LTD in the hippocampal CA1 synapses [29]. Moreover, it reported that the learning and memory were impaired in Notch heterozygous knockout mice [64]. Some recent studies

have shown that Notch significantly affects the activation of NF-κB in several types of cells [65, 66]. NF-κB has been shown to upset whole-cell currents through NMDA receptor channels [67]. Our data showed that the level of Notch1, Jagged-1, and Hes1 expressions were significantly decreased in the CUS group compared to that in the Con group. This is in accordance with the report, in which the expression of NICD has been significantly decreased at stressed rats [31]. It suggests that the decrease of Notch1, Jagged-1, and Hes1 levels is practicably associated with the inhibition of LTP, which is consistent with the findings obtained from the previous studies [29, 62]. Most importantly, the level of Notch1, Jagged-1, and Hes1expressions was significantly enhanced by nicotine, which was obviously accompanied with the upregulation of LTP and DEP in stressed mice compared to that in normal animals.

Conclusion

In summary, our results show that nicotine at a dose of 0.2 mg/kg is effective in impeding the symptom of anhedonia in the mouse model of chronic mild stress, presenting potential antidepressant-like properties. Furthermore, it significantly improves the impairments of cognitive functions and synaptic plasticity in the depressive-like mice, which is along with enhancing the level of synaptic and Notch1 proteins. In conclusion, the results suggest that nicotine ameliorates the depressant-like symptoms through the improvement of synaptic plasticity, which may be closely associated with activating transmembrane ion channel receptors as well as Notch signaling components, Notch1, Jagged-1, and Hes1.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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