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ORIGINAL ARTICLE

Virus-Mediated Overexpression of ETS-1 in the Ventral Hippocampus Counteracts Depression-Like Behaviors in Rats

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Abstract ETS-1 is a transcription factor that is a member of the E26 transformation-specific (ETS) family. Galanin receptor 2 (GalR2), a subtype of receptors of the neuropeptide galanin, has been shown to have an antidepressant-like effect after activation in rodents. Our previous study has shown that overexpression of ETS-1 increases the expression of GalR2 in PC12 phaeochromocytoma cells. However, whether ETS-1 has an antidepressant-like effect is still unclear. In this study, we found that chronic mild stress (CMS) decreased the expression of both ETS-1 and GalR2 in the ventral hippocampus of rats. Meanwhile, we demonstrated that overexpression of ETS-1 increased the expression of GalR2 in primary hippocampal neurons. Importantly, we showed that overexpression of ETS-1 in the ventral hippocampus counteracted the depression-like behaviors of CMS rats. Furthermore, we found that overexpression of ETS-1 increased the level of downstream phosphorylated extracellular signal-regulated protein kinases 1 and 2 (p-ERK1/2) of GalR2 in the ventral hippocampus of CMS rats. Taken together, our findings suggest that ETS-1 has an antidepressant-like effect in rats, which might be mediated by increasing the level of GalR2 and its downstream p-ERK1/2 in the ventral hippocampus.

Keywords GalR2 · ETS-1 · Depression · Chronic mild stress

Introduction

Depression is a psychiatric disorder with an estimated lifetime prevalence of 16.2% and a 12-month prevalence of 6.6% worldwide [1]. This high prevalence results in a large social and economic burden [2]. Although many theories of depression have been established, the exact etiological mechanisms remain unknown. Therefore, identification of new regulators involved in the pathophysiology of depression will expand the understanding of depression.

Galanin, a neuropeptide, is widely distributed in the central nervous system [3, 4]. It functions *via* three G protein-coupled receptors, GalR1, GalR2, and GalR3. Previous studies have suggested that galanin and its receptors are involved in the pathology of depression. The activation of GalR1 and GalR3 results in depression-like behaviors, whereas stimulation of GalR2 leads to antidepressant-like effects [5]. Meanwhile, GalR2-knockout mice show depression-like behaviors, while GalR2-overexpressing mice exhibit antidepressant-like behaviors [6, 7].

It has been shown that stimulation of GalR2 activates phospholipase C and protein kinase C *via* $G_{\alpha q/11}$ protein [8, 9]. Subsequent reports showed that stimulation of GalR2 activates the ERK pathway and elevates the level of

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p-ERK1/2 [10, 11]. The ERK pathway has been shown to have protective effects in the hippocampus after excitotoxic damage [12]. In the rat model of depression induced by chronic mild stress (CMS), the depression-like behaviors were correlated with decreased p-ERK1/2 levels in the hippocampus [13].

ETS-1 is a member of the Ets family of transcription factors. It has an ETS domain and an evolutionarily-conserved pointed domain. The ETS domain mainly mediates DNA recognition and binding, while the pointed domain is responsible for protein-protein interactions [14]. ETS-1 is broadly expressed in many tissues, including the central nervous system, lymphoid and hematopoietic tissues, and the vascular system [15–17]. Studies have shown that ETS-1 is involved in the development of natural killer cells, angiogenesis, and radial glia formation [16, 18, 19]. In the central nervous system, ETS-1 also influences neuronal injury following ischemic stroke [20]. However, so far, there are no reports on the relationship between ETS-1 and depression.

Given that GalR2 plays an antidepressant-like role, we previously investigated the potential transcription factors that regulate its expression. We found that the region from −320 to −300 of the *GalR2* promoter has two ETS-1 binding elements and plays a crucial role in regulating *GalR2* promoter activity. Moreover, we also showed that ETS-1 binds to the ETS-1 element of *GalR2* promoter and increases the expression of GalR2 in PC12 cells [21]. The ventral hippocampus is involved in emotional responses and has been associated with several psychiatric disorders, including depression [22]. Since overexpression of ETS-1 efficiently increases the expression of GalR2 in PC12 cells [21], we speculated that alteration of GalR2 levels by manipulating ETS-1 expression using a virus-mediated gene expression pattern in the ventral hippocampus might influence depression-like behaviors. In this study, we set out to answer the above questions in CMS rat model of depression.

Materials and Methods

Animals

Male Sprague–Dawley rats (200 g–250 g) were housed at 25 °C with access to food and water *ad libitum* and under a 12/12 h light/dark cycle (lights on: 07:00–19:00). All rats were handled for 5 min daily in the first week. Then, basal behaviors were evaluated and normal rats were randomly assigned to start the experiments. The general design of this study is displayed in Fig. 1. All experiments with animals were conducted in strict accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care Committee at Capital Medical University (Permit Number: AEEI-2015-035). We used the minimum number of animals to meet the statistical analysis requirements.

Chronic Mild Stress (CMS) Procedure

The CMS procedure was carried out as previously described [23]. Briefly, rats were exposed to various stressors for 4 weeks in a random order: swimming in cold water, water deprivation, food deprivation, alteration of the day/night light cycle, tail pinching, and cage tilt. Meanwhile, the control rats remained undisturbed except for necessary procedures such as handling and routine cleaning. At the end of the CMS protocol, two classic behavioral tests, the forced swimming test (FST) and the sucrose preference test (SPT), were applied to evaluate the effects of CMS on rats.

Animal Treatment

To determine whether CMS influences the expression of GalR2 and ETS-1, rats were assigned to two groups: Control group (n = 8), CMS group (n = 8). After 4 weeks of exposure to CMS, the behaviors were evaluated. Then, the rats were sacrificed for analysis of the expression of GalR2 and ETS-1. To investigate the role of ETS-1 in the depression-like behaviors induced by CMS, rats were divided into three groups: Control group (n = 8), CMS + Lenti-GFP group (n = 8), and CMS + Lenti-ETS-1 group (n = 8). Before the injection of Lentivirus, the CMS + Lenti-GFP group and CMS + Lenti-ETS-1 group were exposed to CMS for 4 weeks. After injection of Lentivirus, the CMS + Lenti-GFP and CMS + Lenti-ETS-1 groups were exposed to CMS for another 4 weeks. The control group was left undisturbed in their home cages without any stressors. After the behavioral tests, the rats were sacrificed for further biochemical analyses.

Forced Swimming Test (FST)

The FST was carried out as described in our previous study with minor modifications [24]. Briefly, rats were placed individually into a transparent glass beaker (50 cm in height and 30 cm in diameter) filled with tap water (25 ± 2 °C) to a depth of 35 cm and left for 5 min. Immobility was defined as floating passively with only small movements to maintain the animal's head above the water. The total duration of immobility was recorded during the last 4 min of the 5-min session.

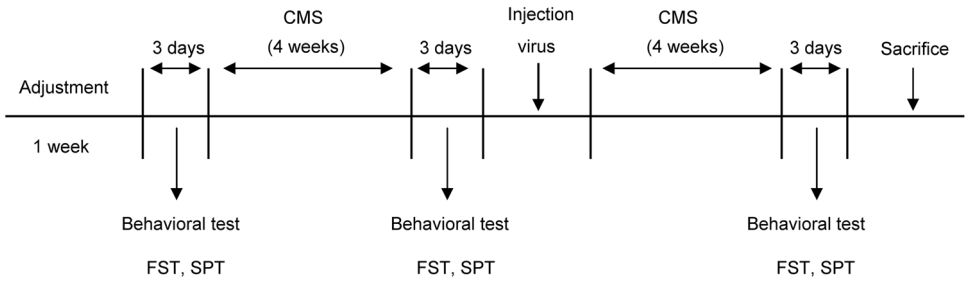

Fig. 1 Schedule for experiments. Depression-like behaviors were evaluated by the forced swimming test (FST) and sucrose preference test (SPT).

Sucrose preference Test (SPT)

The SPT was performed as described in our earlier study [24]. The evaluation of sucrose preference comprises training and testing portions. Before the test, all rats were trained to consume a sucrose solution (1%) or tap water in their cage for 24 h. After the training period, rats were deprived of food and water for 24 h. Then, they were offered a bottle of sucrose solution and a bottle of tap water for 1 h. The sucrose preference was calculated using the formula: Sucrose preference (%) = sucrose consumption (g)/[water consumption (g) + sucrose consumption (g)] × 100%.

Real-Time PCR Assay

Total RNAs were extracted with the RNeasy Lipid Mini Kit (Qiagen, Hilden, Germany). Then, cDNA from each sample was prepared with a first-strand cDNA synthesis kit (Roche, West Sussex, UK). Real-time PCR was performed using SYBR Green Master Mix (Life Technologies, CA, USA). GAPDH served as the internal reference for each sample. The primers used to detect the expression of ETS-1, GalR2, and GAPDH were as described previously [21].

Western Blot Analysis

Western blot was carried out as described previously with slight modifications [25]. Ventral hippocampus samples were dissected and total proteins were extracted with ice-cold lysis buffer. Protein samples were ran on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% BSA for 2 h at room temperature (RT) and incubated with primary antibodies: p-ERK1/2 (1:1000, Santa Cruz, CA, USA), ERK1/2-antibody (1:1000, Millipore, Billerica, MA, USA), ETS-1 (1:1000, Santa Cruz), or α-tubulin (1:5000, Millipore) for 3 h at RT. Then, the membrane was washed three times and probed with anti-mouse or rabbit secondary antibody (1: 10000, LI-COR, Lincoln, NE, USA) for 1 h at RT. After washing 3 times, the signals were detected using an Odyssey Infrared Imaging system (LI-COR) and quantified with Image J software (National Institutes of Health, Bethesda, MD, USA).

Preparation of Primary Hippocampal Neurons

Primary hippocampal neurons were cultured as described previously with minor modifications [26]. Pregnant Sprague-Dawley rats on fetal days 17–18 were anaesthetized with 6% chloral hydrate (0.5 mL/100 g). Then, fetuses were removed and their hippocampal tissues were cut into small chunks. After washing twice with Hanks' balanced salt solution (Gibco, Carlsbad, CA, USA), the hippocampal tissue was dissociated with 0.125% trypsin (Sigma-Aldrich, St. Louis, MO, USA) for 40 min at 37 °C. Then, cells were collected and suspended in Neurobasal medium (Gibco) with 2% B27 supplement (Gibco), 500 μmol/L L-glutamine (Gibco), and 100 U/mL penicillin-streptomycin (Sigma-Aldrich). Finally, the cells were plated in six-well plates coated with 0.05 mg/mL poly-d-lysine (Sigma-Aldrich) and incubated at 37 °C with 95% air and 5% CO_2.

Construction of Lentiviral Vector Expressing ETS-1 and Virus Preparation

Full-length ETS-1 was generated by PCR amplification using Flag-ETS-1 plasmid [21] as a template. Then, the amplified fragments were cloned into plasmid GV358 (Genechem, Shanghai, China), a lentiviral vector expressing the enhanced green fluorescent protein (EGFP) gene. Lentivirus-mediated overexpression of ETS-1 virus (Lenti-ETS-1) and its negative control virus only expressing the EGFP gene (Lenti-GFP) were prepared by Genechem. The EGFP expression was assessed to evaluate the infection efficiency.

Stereotaxic Surgery and Virus Injection

Each rat was anesthetized with chloral hydrate and mounted in a stereotaxic apparatus (Stoelting, Illinois, USA). Injections were made with a 10-μL Hamilton syringe (29-gauge) at 4×10^5 transducing units per rat. Each injection lasted for 5 min (0.1 μL/min). The needle

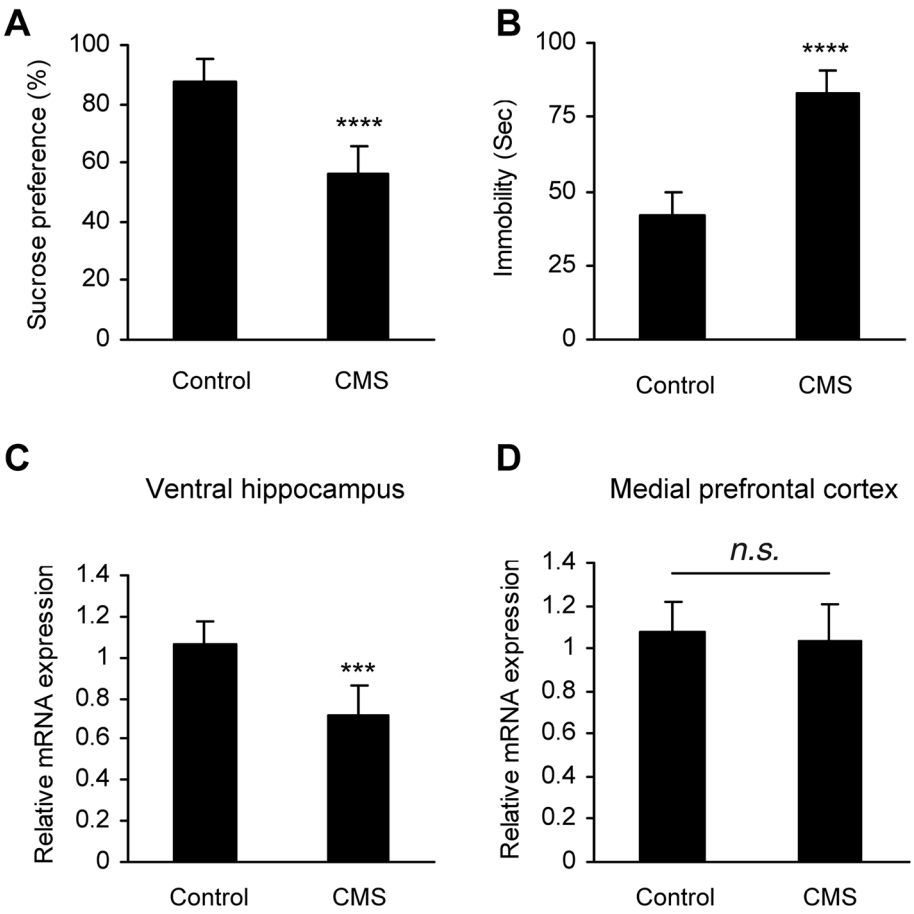

Fig. 2 CMS decreases the mRNA level of GalR2 in the rat ventral hippocampus. **A**, **B** Sucrose intake (**A**) and immobility time (**B**) after 4 weeks of CMS exposure (n = 8/group). **C**, **D** mRNA levels of GalR2 in the ventral hippocampus (**C**) and medial prefrontal cortex (**D**) after CMS exposure (n = 8/group). Mean ± SD; ****$P < 0.0001$, ***$P < 0.001$. *n.s.*, not significant.

was left in place for another 10 min to avoid reflux of the viral solution. The coordinates for the ventral hippocampus were as follows: AP −5.0 mm, L ± 4.8 mm, and V −7.2 mm from bregma [27].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad, CA, USA). Student's t test was used to evaluate the significance of differences between two sets of data. ANOVA followed by *post-hoc* Tukey's multiple comparison tests was carried out for group comparisons. All data are expressed as the mean ± SD. Differences with $P < 0.05$ were considered statistically significant.

Results

CMS Decreases the Expression of GalR2 in the Ventral Hippocampus

It has been shown that GalR2-knockout mice display depression-like behaviors [6], whereas GalR2-overexpressing mice show antidepressant-like behaviors [7]. We therefore speculated that CMS might result in a reduction of GalR2 expression in the ventral hippocampus. To test this hypothesis, we assessed the expression of GalR2 in the ventral hippocampus of CMS rats and found a decrease in sucrose intake [$t_{14} = 6.764$, $P < 0.0001$] and an increase of immobility time [$t_{14} = 10.34$, $P < 0.0001$] compared with the control after 4 weeks of CMS exposure (Fig. 2A, B). Meanwhile, CMS also efficiently decreased the expression of GalR2 in the ventral hippocampus [$t_{14} = 5.27$, $P < 0.001$] (Fig. 2C). However, the expression of GalR2 in the medial prefrontal cortex did not differ in CMS rats [$t_4 = 0.48$, $P > 0.05$] (Fig. 2D).

CMS Decreases the Expression of ETS-1 in the Ventral Hippocampus

Because there are no reports on the relationship between ETS-1 and depression, we set out to determine whether CMS influenced the expression of ETS-1 in the ventral hippocampus. Our results showed that CMS efficiently decreased the expression of ETS-1 both at the mRNA [$t_{14} = 5.05$, $P < 0.001$] and protein levels [$t_4 = 3.16$, $P < 0.05$] in the ventral hippocampus (Fig. 3A, C, D). However, CMS did not influence the expression of ETS-1

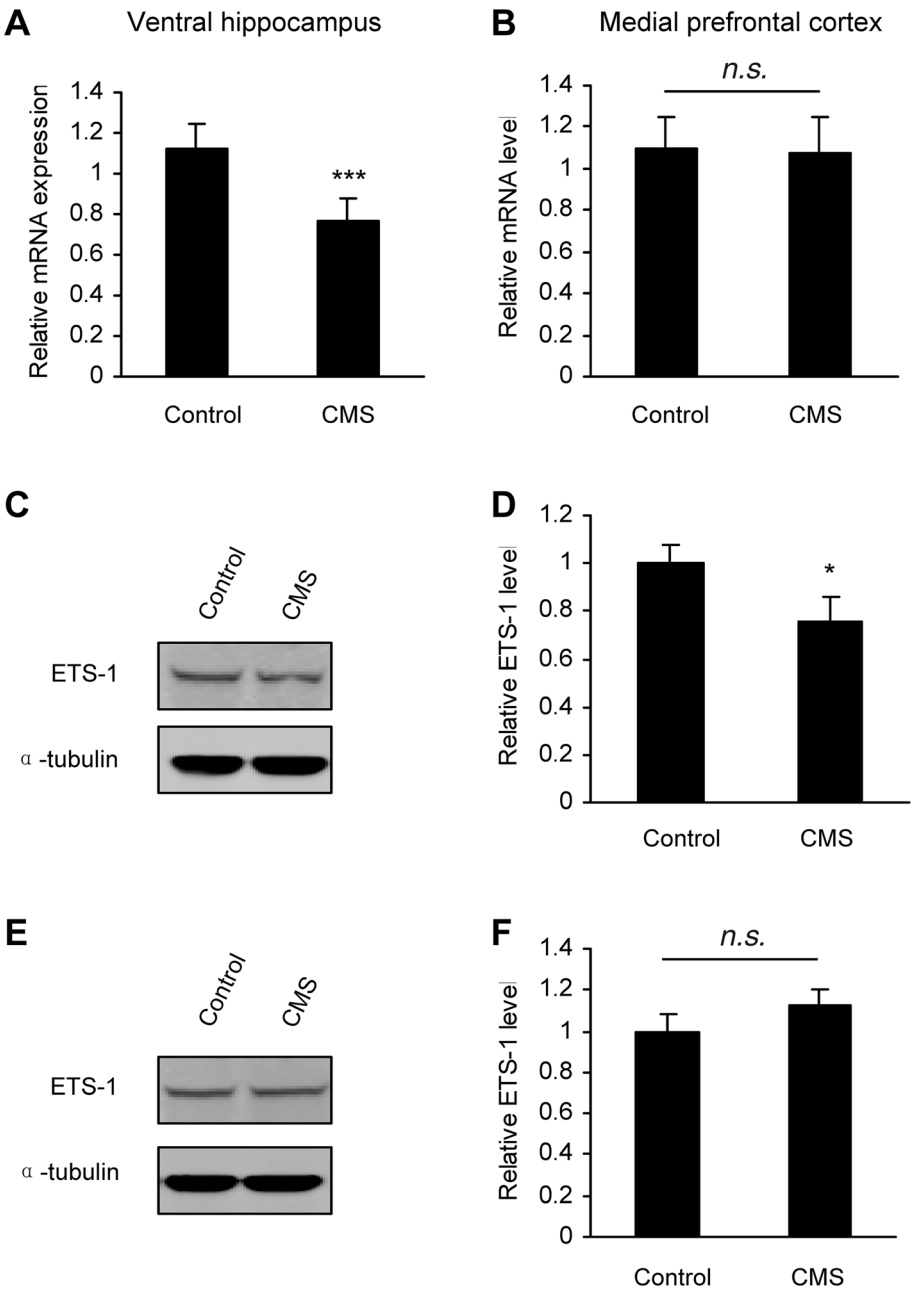

Fig. 3 CMS decreases the expression of ETS-1 in the ventral hippocampus. **A**, **B** mRNA level of ETS-1 in the ventral hippocampus (**A**) and medial prefrontal cortex (**B**) after CMS exposure (n = 8/group). **C**, **E** Western blots showing the protein levels of ETS-1 in the ventral hippocampus (**C**) and medial prefrontal cortex (**E**) after CMS exposure (n = 8/group). **D**, **F** Relative quantitative analysis of ETS-1 protein levels in the ventral hippocampus (**D**) and medial prefrontal cortex (**F**) after the CMS exposure. ***$P < 0.001$, *$P < 0.05$. *n.s.*, not significant.

at both the mRNA [$t_{14} = 0.25$, $P > 0.05$] and protein levels [$t_4 = 2.08$, $P > 0.05$] in the medial prefrontal cortex (Fig. 3B, E, F).

Overexpression of ETS-1 Increases the Expression of GalR2 in Primary Hippocampal Neurons

Our previous study has shown that overexpression of ETS-1 increases the expression GalR2 in PC12 cells [21]. We therefore speculated that ETS-1 might also up-regulate the expression of GalR2 in primary hippocampal neurons. To determine this possibility, rat primary hippocampal neurons were cultured and then infected with Lenti-ETS-1 or Lenti-GFP virus (Fig. 4A). Because we lacked GalR2-specific antibodies for rodents [28], the expression level of GalR2 was assessed at mRNA level. We found that infection with Lenti-ETS-1 virus increased the expression of ETS-1 compared with Lenti-GFP virus [$t_4 = 6.14$, $P < 0.01$] (Fig. 4B). Meanwhile, infection with Lenti-ETS-1 virus also efficiently increased the expression of GalR2 [$t_4 = 6.10$, $P < 0.01$] (Fig. 4C). These data further confirmed that GalR2 is a target gene of the transcription factor ETS-1.

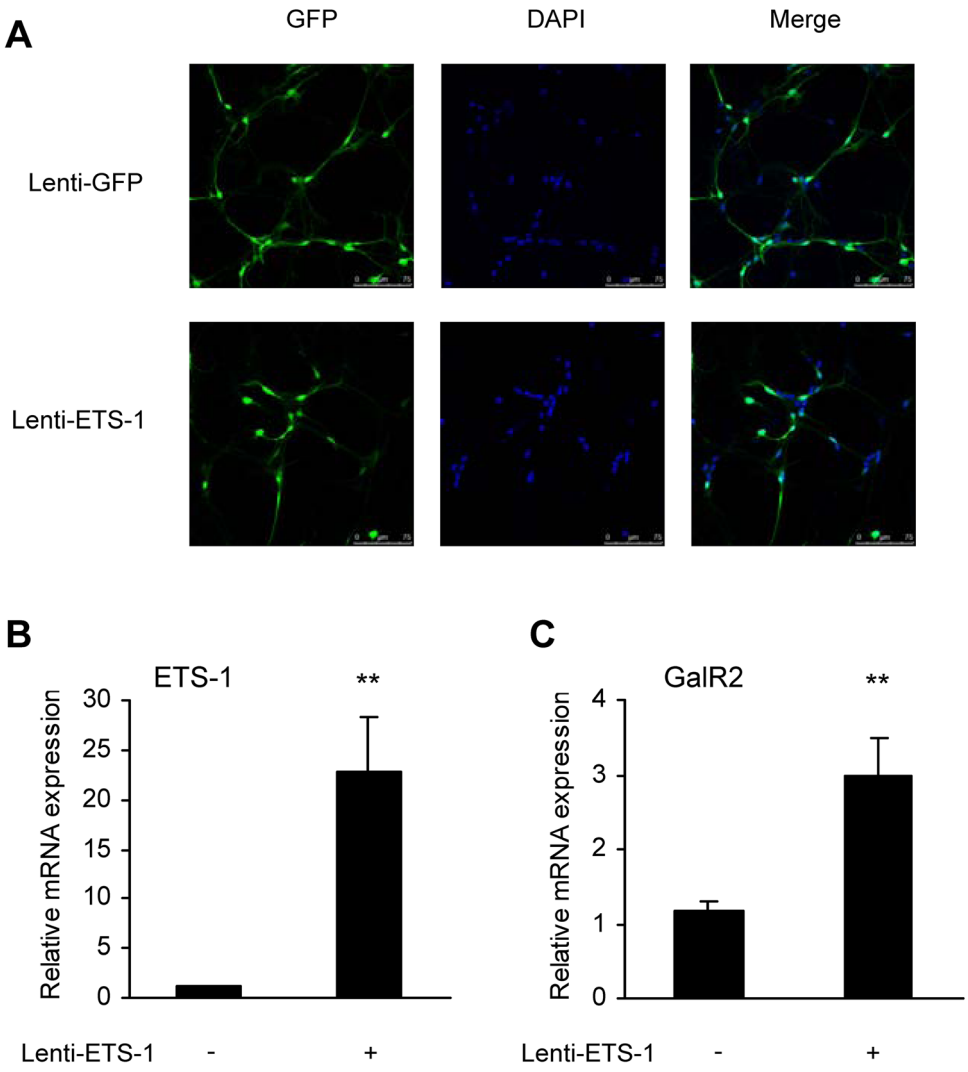

Fig. 4 Overexpression of ETS-1 increases the expression of GalR2 in primary hippocampal neurons. **A** Primary hippocampal neurons infected with Lenti-GFP or Lenti-ETS-1 virus, as indicated by GFP (green) (scale bars, 75 μm). **B**, **C** mRNA levels of ETS-1 (**B**) and GalR2 (**C**) in primary hippocampal neurons infected with Lenti-GFP or Lenti-ETS-1 virus, respectively (n = 3/group) (**$P < 0.01$)

Overexpression of ETS-1 in the Ventral Hippocampus Counteracts Depression-Like Behaviors in Rats Exposed to CMS

To investigate the role of ETS-1 in the depression-like behaviors induced by CMS, CMS rats were microinjected Lenti-ETS-1 or Lenti-GFP virus into the ventral hippocampus (Fig. S1). One-way ANOVA showed a significant difference in the levels of GalR2 mRNA between groups [$F(2,6) = 10.47$, $P < 0.05$] (Fig. 5A). Further Tukey's multiple comparison tests revealed that the expression of GalR2 mRNA was higher after injection of Lenti-ETS-1 than Lenti-GFP virus in the CMS groups ($P < 0.05$). Meanwhile, one-way ANOVA also showed a significant difference in the level of ETS-1 protein between groups [$F(2,6) = 12.83$, $P < 0.01$], and Tukey's tests revealed higher ETS-1 protein expression after injection of Lenti-ETS-1 virus than Lenti-GFP virus in the CMS groups ($P < 0.01$) (Fig. 5C). In the SPT, two-way ANOVA showed significant interactions and differences in sucrose intake between groups [$F(2,36) = 10.75$, $P < 0.001$; $F(1,36) = 7.63$, $P < 0.001$; $F(2,36) = 93.45$, $P < 0.0001$]. Further Tukey's tests revealed that CMS rats receiving Lenti-ETS-1 virus had higher sucrose intake than those with Lenti-GFP virus ($P < 0.0001$) (Fig. 5D). In the FST, we also found significant interactions and differences in immobility time between groups [$F(2,36) = 7.92$, $P < 0.001$; $F(1, 6) = 4.59$, $P < 0.05$; $F(2,36) = 153.30$, $P < 0.0001$] and Tukey's tests showed that CMS rats receiving Lenti-ETS-1 virus exhibited a decreased immobility time ($P < 0.0001$) compared with Lenti-GFP virus (Fig. 5E).

Overexpression of ETS-1 in the Ventral Hippocampus Increases the Level of p-ERK1/2

Previous studies have shown that stimulation of GalR2 activates the ERK pathway in rodent hippocampal neurons

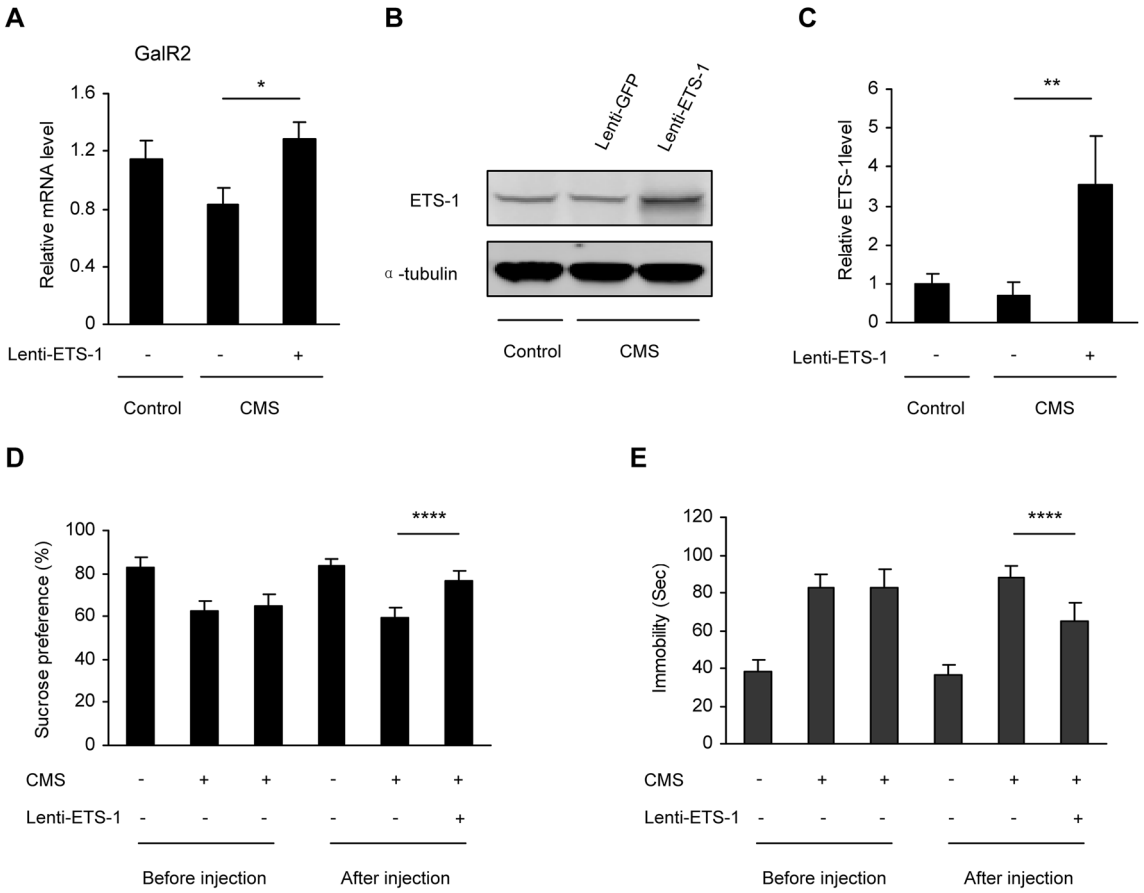

Fig. 5 Overexpression of ETS-1 in the ventral hippocampus counteracts the depression-like behaviors of CMS rats. **A** mRNA levels of GalR2 in the ventral hippocampus after injection of Lenti-GFP or Lenti-ETS-1 virus (n = 3/group). **B** Western blots of protein levels of ETS-1 in the ventral hippocampus after injection of Lenti-GFP or Lenti-ETS-1 virus (n = 3/group). **C** Relative quantitative analysis of ETS-1 protein levels. **D**, **E** Sucrose intake (**D**) and immobility time (**E**) after injection of Lenti-GFP or Lenti-ETS-1 virus in CMS rats (n = 7/group). ****$P < 0.0001$, **$P < 0.01$, *$P < 0.05$.

[10, 11]. Meanwhile, increasing evidence has shown that the ERK pathway is also involved in the pathology of depression [29, 30], and inhibiting the ERK pathway can block the activity of antidepressants [31]. Therefore, we speculated that overexpression of ETS-1 might increase the level of p-ERK1/2 in the ventral hippocampus. So, we assessed the levels of p-ERK1/2 and total ERK1/2 (t-ERK1/2) in the ventral hippocampus of CMS rats after injection of Lenti-ETS-1 virus. One-way ANOVA showed differences between groups in the levels of p-ERK1/ERK1 [$F(2,6) = 22.21$, $P < 0.01$] and p-ERK2/ERK2[$F(2,6) = 9.91$, $P < 0.05$]. However, the levels of ERK1 [$F(2,6) = 0.01$, $P > 0.05$] and ERK2 [$F(2,6) = 0.03$, $P > 0.05$] did not differ between groups. Further Tukey's multiple comparison tests showed that CMS rats receiving Lenti-ETS-1 virus had higher levels of p-ERK1/ERK1 ($P < 0.01$) and p-ERK2/ERK2 ($P < 0.05$) than those with Lenti-GFP virus. However, the levels of ERK1 ($P > 0.05$) and ERK2 ($P > 0.05$) did not differ (Fig. 6B–E).

Discussion

Our previous study has shown that ETS-1 is a positive regulator of GalR2 in PC12 cells [21]. In the present study, we found that overexpression of ETS-1 also increased the expression of GalR2 in primary hippocampal neurons. Meanwhile, both the expression of ETS-1 and GalR2 were decreased in the ventral hippocampus of CMS rats. Importantly, overexpression of ETS-1 in the ventral hippocampus counteracted the depression-like behaviors of CMS rats. Finally, overexpression of ETS-1 increased the level of GalR2 and its downstream p-ERK1/2 in the ventral hippocampus.

GalR2, a subtype of galanin receptor, is involved in many important functions. Studies have shown that GalR2 has a neuroprotective effect in the hippocampus and it regulates neurite outgrowth in adult sensory neurons [32, 33]. In the dentate gyrus, GalR2 modulates neuronal survival and seizure-induced neurogenesis [34]. In the periaqueductal grey, GalR2 is involved in galanin-induced

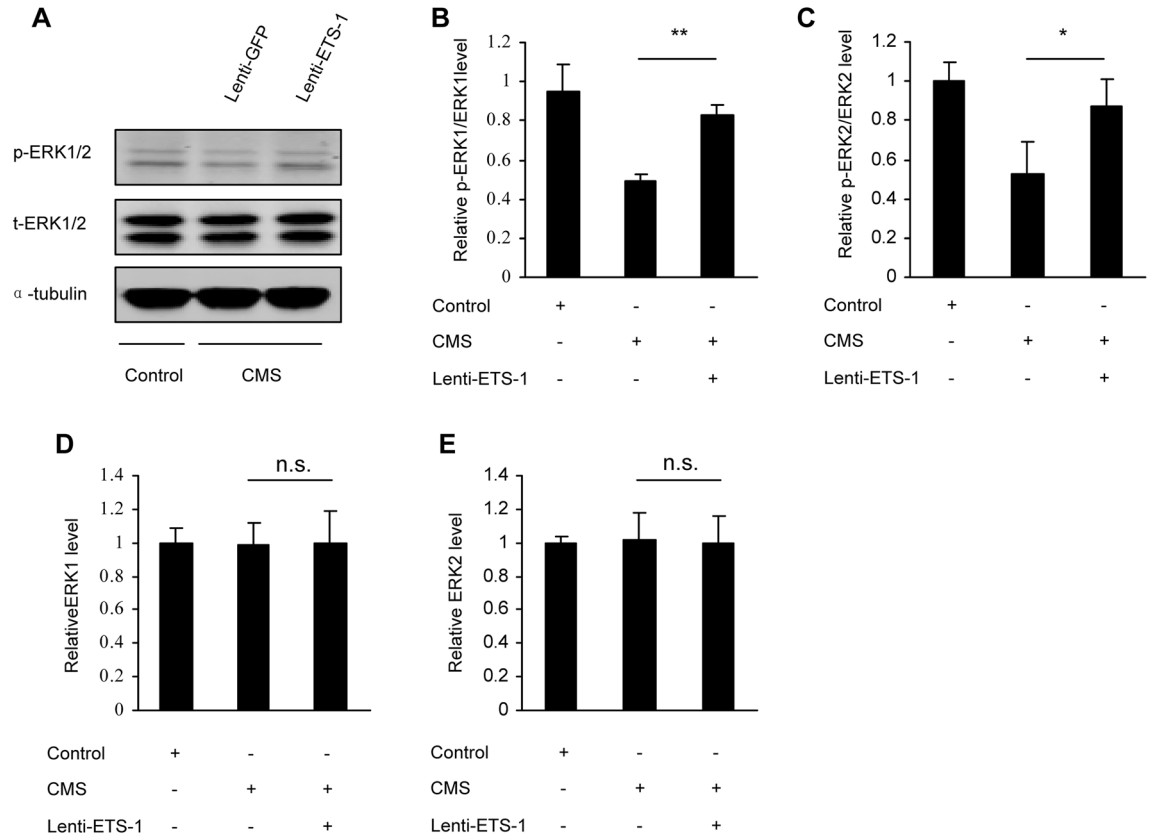

Fig. 6 Overexpression of ETS-1 in the ventral hippocampus increases the level of p-ERK1/2. **A** Western blots showing the protein levels of p-ERK1/2 and t-ERK1/2 after injection of Lenti-GFP or Lenti-ETS-1 virus in CMS rats (n = 7/group). **B**–**E** Relative quantitative analysis of the protein levels of p-ERK1/2 (**B**, **C**) and t-ERK1/2 (**D**, **E**). $^{**}P < 0.01$, $^{*}P < 0.05$. *n.s.*, not significant.

anti-nociception [35]. In addition, GalR2 also has antidepressant-like effects by increasing serotonergic transmission [36]. It has been reported that GalR2 is present in the central nervous system and many peripheral tissues [37]. In the brain, GalR2 is expressed in the hippocampus, hypothalamus, amygdala, locus coeruleus, and dorsal raphe nucleus [38]. Meanwhile, a galanin terminal network has also been demonstrated in the rat hippocampus [39]. In this study, we found that GalR2 is also present in the hippocampus, in agreement with previous findings.

Chronic mild stress is a valid and reliable method for creating an animal model of depression [40, 41]. FST and SPT are classical tests to evaluate depressive behavior in animal. In the present study, we showed that CMS exposure induced a clear decrease in sucrose intake and a remarkable increase in immobility time, indicating that CMS rats exhibited depression-like behaviors. Increasing evidence has shown that GalR2 modulates mood disorders. In GalR2-knockout mice, transgenic mice show an increased persistent depressive-like behavior in the learned helplessness test and longer immobility time in the tail suspension test [6]. However, mice overexpressing GalR2 had lower immobility times than wild-type mice [7]. Here, we examined the expression of GalR2 after CMS exposure and found that it was lower in the ventral hippocampus of CMS rats. The medial prefrontal cortex is involved in the regulation of emotion [42]. Interestingly, there was no significant change in the expression of GalR2 in the medial prefrontal cortex, suggesting the complexity of emotion regulation.

ETS-1 regulates target gene expression by binding to an ETS-binding site (GGAA/T or A/TTCC motif) [14]. Our previous study has shown that ETS-1 increases the expression of GalR2 by binding to the ETS-binding site and recruits the co-activator p300 to the *GalR2* promoter in PC12 cells [21]. To further confirm that GalR2 is a target gene of ETS-1, we infected primary hippocampal neurons and the ventral hippocampus with Lenti-ETS-1 virus. Our data demonstrated that ETS-1 increased the expression of GalR2 both *in vitro* and *in vivo*. However, we did not exclude the possibility that ETS-1 also regulates other antidepressant genes, such as brain-derived neurotrophic factor (BDNF) and p11. In future, we plan to explore whether ETS-1 regulates the expression of these antidepressant genes.

Increasing evidence has shown that ETS-1 is expressed in the central nervous system. Fleischman *et al*. have shown that ETS-1 is present in astrocytes and astrocytoma cells, and is rapidly phosphorylated during Ca^{2+}-mediated signal transduction [43]. Subsequent studies have reported that ETS-1 is co-localized with NeuN and is primarily expressed in neurons [20]. Here, we further confirmed that ETS-1 is expressed in the rat brain. It has been reported that the expression of ETS-1 is almost abolished in injured neurons of the ischemic brain but is not changed in peri-infarct areas [20]. Meanwhile, a recent study has shown that ETS-1 is involved in the regulation of α5 integrin and displays an angiogenic vascular protective response following cerebral ischemia [44]. In this study, we showed that the expression of ETS-1 was decreased in the ventral hippocampus of CMS rats and overexpression of ETS-1 in the ventral hippocampus attenuated their depression-like behaviors, further suggesting a protective role of ETS-1 in nervous system diseases.

Previous studies have shown that the ERK pathway is involved in neural proliferation, differentiation, and neurogenesis and plays an important role in learning and memory [45, 46]. Subsequent studies have reported that the ERK pathway is also involved in mood modulation [47]. In the CMS rat model of depression, the level of p-ERK1/2 was decreased in hippocampus compared with untreated rats [13]. However, antidepressants can efficiently alleviate the symptoms of depression by increasing the level of p-ERK1/2 [48]. Given the fact that p-ERK1/2 is an important downstream signaling molecule of GalR2 [10, 11], we investigated whether overexpression of ETS-1 increased the level of p-ERK1/2 in the ventral hippocampus. As expected, overexpression of ETS-1 in the ventral hippocampus increased the level of p-ERK1/2 compared with controls. All above data indicate that the antidepressant-like role of ETS-1 might be mediated by increasing the level of GalR2 and its downstream p-ERK1/2. In addition to activating the ERK pathway, GalR2 has been shown to increase serotonergic transmission, which underlies the antidepressant-like effect [36]. Here, we did not exclude the possibility that overexpression of ETS-1 might also influence serotonergic transmission. This deserves further study.

In summary, our study is the first to report that overexpression of ETS-1 in the ventral hippocampus leads to increased levels of GalR2 and its downstream p-ERK 1/2, which in turn counteracts the depression-like behaviors of CMS rats. This study expands the understanding of the pathophysiology of depression.

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Conflict of interest The authors declare that they have no conflict of interest.

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