OXFORD

Regular Research Article

MAD2B Blunts Chronic Unpredictable Stress and Corticosterone Stimulation–Induced Depression-Like Behaviors in Mice

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Abstract

Background: Depression is a prevalent and recurrent psychiatric disorder. Aberrant neural structure and activity play fundamental roles in the occurrence of depression. Mitotic arrest deficient protein (MAD2B) is highly expressed in neurons and may be implicated in synaptic plasticity in the central nervous system. However, the effect of MAD2B in depression, as well as the related molecular mechanism, is uncertain.

Methods: Here, we employed mouse models of depression induced by chronic unpredictable stress exposure or corticosterone (CORT) stimulation. Depression-like behaviors in mice were evaluated by sucrose preference, forced swimming, and tail suspension tests. Hippocampal MAD2B overexpression was mediated by adeno-associated virus 8 containing enhanced green fluorescent protein. In vitro primary neuronal cells were obtained from the hippocampus of rat embryos and were treated with CORT, and MAD2B overexpression was performed using lentivirus. MAD2B and glutamate metabotropic receptor 4 (GRM4) levels were evaluated by western blots and quantitative PCR. Primary neuronal miR-29b-3p expression was detected by quantitative PCR.

Results: MAD2B expression was reduced in the hippocampus in mice exhibiting depressive-like behaviors. However, hippocampal MAD2B overexpression protected mice from developing either chronic unpredictable stress– or CORT-induced depression-like behaviors, an effect associated with reduced expression of *GRM4*, a presynaptic receptor involved in depression. Moreover, MAD2B overexpression in primary neuronal cells also decreased *GRM4* expression while enhancing the level of *miR-29b-3p*; this phenomenon was also observed under CORT stimulation.

Conclusions: Our results suggest an important role of neuronal MAD2B in the pathogenesis of depression via the miR-29b-3p/GRM4 signaling pathway. MAD2B could be a potential therapeutic target for depressive disorders.

Keywords: depression, MAD2B, miR-29b-3p, GRM4

Significance Statement

Depression is a common mental illness characterized by anhedonia and depressed mood. A comprehensive investigation of the pathophysiology of depression is crucial to developing a precise therapeutic strategy. In this study, we used 2 mouse models of depression to investigate the effect and mechanisms of MAD2B in the development of depression. This study describes a significant decrease of MAD2B in the hippocampus in mice exhibiting depressive-like behaviors. However, hippocampal MAD2B overexpression protected mice from depression-like behaviors, which may be associated with the reduced level of glutamate metabotropic receptor 4 (*GRM4*) and increased level of *miR-29b-3p*. These findings suggest that MAD2B may be a promising therapeutic target for the treatment of depression and also provide a potential link between MAD2B and depression.

INTRODUCTION

Depression is one of the most common psychiatric disorders and is mainly characterized by anhedonia and depressed mood (Bromet et al., 2011; Kessler and Bromet, 2013; Malhi and Mann, 2018). It is the major cause of disability and suicide worldwide (Beurel et al., 2020). It is known that depression is associated with structural and functional abnormalities of brain regions that regulate mood, emotion, and cognition, such as the prefrontal cortex and hippocampus (Neumeister et al., 2005; Drevets et al., 2008). Synaptic dysfunction has also been implicated in depression (Duman and Aghajanian, 2012). For example, synaptic loss, altered neurotransmitters, and decreased synaptic signaling proteins have been observed in both rodent and human studies (Sanacora et al., 2008; Duman and Aghajanian, 2012; Kang et al., 2012). Although the mechanisms underlying the pathophysiology of depression have been widely explored, a comprehensive understanding of the pathogenesis in depression still needs to be further enriched.

Mitotic arrest deficient 2 like2 (MAD2B), also called MAD2L2 or Rev7, contains a HORMA domain, which is a multifunctional protein–protein interaction module (Rosenberg and Corbett, 2015). By binding to different partner proteins, MAD2B plays a critical role in a variety of biological processes (Zhang et al., 2007; Sale, 2015; de Krijger et al., 2021a). For example, MAD2B is involved in translesion DNA synthesis and DNA repair activities, which are important for genome integrity (Murakumo, 2002; Boersma et al., 2015; de Krijger et al., 2021b). The anaphase promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase that controls cell cycle progression and neuronal survival and functions (Almeida, 2012; Huang and Bonni, 2016). Cdh1 is the main activator of APC/C in the adult brain (Almeida et al., 2005; Bobo-Jimenez et al., 2017). MAD2B inhibits APC/C function through interaction with Cdh1 (Chen and Fang, 2001; Listovsky and Sale, 2013). Our previous study showed that MAD2B is implicated in neuronal development and high glucose-induced neuronal injury (Meng et al., 2012, 2014). However, the role of MAD2B in depression and depression-related synaptic dysfunction is unknown.

Moreover, the glutamate system plays a fundamental role in synaptic plasticity. Accumulating evidence suggests that glutamate signaling is aberrant in the brains of depressed patients (Sanacora et al., 2012; Murrough et al., 2017). Glutamate metabotropic receptor 4 (GRM4), which modulates glutamatergic, dopaminergic, GABAergic, and serotonergic neurotransmission, is upregulated in the brains of depressed individuals (Pilc et al., 2008; Lopez et al., 2014). It has been suggested that GRM4 could be a potential therapeutic target for treating major depressive disorder (Podkowa et al., 2015). Additionally, the expression of *GRM4* is regulated by microRNAs (miRNAs), such as miR-1202, miR-29b-3p, and miR-335, in the brain in depression (Lopez et al., 2014; Li et al., 2015; Wan et al., 2018). MiRNAs are small noncoding RNAs that serve as endogenous fine-tuners and regulate the transcription and translation of their target genes (Bushati and Cohen, 2007; Catalanotto et al., 2016). Recent studies have shown that miRNAs are involved in the pathogenesis of neuropsychiatric diseases (Issler and Chen, 2015). Chronic stress increases the occurrence of depression, and corticosterone (CORT) is a well-validated pharmacological stressor (Dinan, 1994; Kessler, 1997; Bai et al., 2018; Dieterich et al., 2019). Here, we utilized 2 models, chronic unpredictable stress (CUS) exposure and CORT stimulation, in mice to investigate whether and how MAD2B is implicated in the pathogenesis of depressive-like behaviors.

MATERIALS AND METHODS

Animals

Wild-type C57BL/6J male mice were purchased from Charles River Experimental Animal Technology Co., Ltd. (Beijing, China). Pregnant Sprague–Dawley rats were purchased from Speifel Experimental Animal Technology (Beijing, China). Animals were housed in a temperature ($22°C\pm1°C$) and humidity-controlled room under a 12-hour-light/-dark cycle with ad libitum access to water and food. All experimental procedures were approved by the Huazhong University of Science and Technology Ethics Committee for Care and Use of Laboratory Animals. Protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Chronic Unpredictable Stress

The CUS mouse model was utilized based on previous studies (Goshen et al., 2008; Peng et al., 2012). Eleven stressors were used in this study: swimming in warm (25°C) water for 10 minutes, swimming in cold (18°C) water for 6 minutes, water and food deprivation for 12 hours, exposure to wet bedding for 4 hours, shaking on a level shaker for 30 minutes, restraint for 2 hours, a 45° tilt for 12 hours, day and night inversion, cage exchange for 2 hours, tail pinching for 5 minutes, and removal of bedding for 12 hours. In brief, adult male mice (7–8 weeks old) were exposed to 2–3 different stressors per day for 5 weeks. The stimuli were performed at random times during the day, and mice were not exposed to the same stressor on any 2 consecutive days. Behavioral tests were conducted after the CUS procedure.

Chronic CORT Injection

The CORT-induced depressive-like mouse model was utilized by following the protocol established by previous studies (Zhao et al., 2008; Kv et al., 2018). CORT was dissolved in the vehicle (0.9% saline containing 0.1% dimethyl sulfoxide and 0.1% Tween 80). Mice received subcutaneous injection of CORT (20 mg/kg) or vehicle once per day for 21 consecutive days.

Stereotactic Viral Injection

Male C57BL/6J mice at the age of 7–8 weeks were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body weight) and then placed on a stereotaxic apparatus (68,030, RWD Life Science, China) as previously described (Xie et al., 2021). Briefly, control (AAV8-enhanced green fluorescent protein [eGFP]) or MAD2B-overexpressing adeno-associated virus 8 (AAV8-MAD2B-OE-eGFP) was injected into the hippocampus (coordinates: AP −2.0 mm, ML 2.68 mm, DV 1.68 mm from the bregma). One microliter of AAV8 (vector, pAV-CMV-P2A-GFP; titer, 2×10^{13} U/mL) was delivered into each side of the hippocampus at a rate of 20 nL/min. An additional 10 minutes was maintained for diffusion and prevention of backflow. CUS or CORT stimulation was conducted 3 weeks after AAV8 injection.

Behavioral Tests

After treatment with CUS or CORT, mice were subjected to behavioral tests. All behavioral tests were performed in the same period during the 12-hour-light cycle and in a double-blind manner.

Sucrose Preference Test

—Mice were housed 1 per cage and had free access to food during the experiment. First, mice were habituated to drink from 2 bottles of plain water for 24 hours. Next, each mouse received 1 bottle of plain water and 1 bottle of 1% sucrose (wt/vol) water for 24 hours,

followed by deprivation of water for 24 hours and then a sucrose preference test for 12 hours. During the test, mice were given 1 bottle of plain water and 1 bottle of 1% sucrose water. The weights of plain water and sucrose water were measured before and after the test. Sucrose preference was calculated using the formula [sucrose water (g)/ [sucrose water (g) + plain water (g)] × 100%]. Sucrose preference <60% was defined as no preference.

Tail Suspension Test

—Mice were individually suspended by their tails on rods using adhesive tape placed on the last one-third of the tail. The heads of the mice were 15 cm above the bottom of the apparatus. The experiment lasted 6 minutes, and data were recorded using SuperMaze software (Xinruan Information Technology Co. Ltd., Shanghai, China). The duration of immobility in the last 5 minutes was used for the analysis.

Forced Swimming Test

—Mice were placed in a transparent cylinder individually (30 cm high, 15 cm in diameter) filled with 24°C water to a depth of 20 cm. The duration of the experiment was 6 minutes, and the immobility time in the last 5 minutes was recorded using SuperMaze software (Xinruan Information Technology Co. Ltd., Shanghai, China) for further analysis.

Fatigue Rotarod Test

—Mice were placed on the rotarod, which was continuously accelerated from 4 rpm/min to 40 rpm/min. The latency of each mouse to fall off the rotarod was recorded during the 5 minutes test.

Primary Neuronal Culture and Treatments

Primary neuronal cells were prepared from the hippocampus of E16-E18 Sprague Dawley rat embryo, as previously described (Wang et al., 2015). The tissue was digested with 0.25% trypsin for 8 minutes at 37°C, and cells were plated in 6-well plates coated with 0.1 mg/mL poly-D-lysine. Neurons were grown in neurobasal medium (Gibco Invitrogen) supplemented with B27 (1:50 dilution; Invitrogen, Shanghai, China), 0.5 mM glutamine, 25 μM glutamate, and 50 μg/mL penicillin-streptomycin. Three days later, one-half of the medium was replaced with fresh warm culture medium. Cells were maintained in a humidified incubator with 5% CO_2 at 37°C for 6 days before being used for the different experiments. Briefly, neuronal cells were infected with MAD2B-overexpressing lentivirus (LV-MAD2B-OE-eGFP) or control lentivirus (LV-eGFP; vector, pLent-EF1a-FH-CMV-GFP-P2A; titer, 1×10^8 U/mL) and cultured for another 3 days; neurons with or without lentiviral transduction were treated with CORT (100 μm) for 24 hours for subsequent experiments.

Fluorescence and Image Acquisition

Mice injected with AAV8 were anesthetized with 5% chloral hydrate (0.6 mL/kg) and subjected to transcardial perfusion with phosphate-buffered saline (PBS). Brains were removed and fixed with 4% paraformaldehyde for 24 hours prior to dehydration first in 20% and then in 30% sucrose solution. Brains were sliced into 30-μm-thick coronal sections on a cryostat. Brain sections containing the hippocampus were mounted on microscope slides and examined by fluorescence microscopy.

Three days after lentiviral transduction, primary neuronal cells were washed with PBS 3 times and were then fixed with 4% paraformaldehyde for 5 minutes prior to PBS washing and fluorescence microscope imaging.

Western-Blot Analysis

Western-blot analysis was conducted as previously described (Xie et al., 2021). Total protein was extracted from mouse hippocampi or cultured neurons using RIPA lysis buffer (Beyotime, Shanghai, China) with phenylmethylsulfonyl fluoride. The lysates were centrifuged at 12 000× g for 15 minutes at 4°C, and the supernatants were used for Western-blot analysis. The protein concentration was measured using a BCA Protein Assay Kit (Beyotime, Shanghai, China) according to the manufacturers' instructions. Proteins were loaded on 10% polyacrylamide gels and were then electrophoretically transferred onto a 0.22-μm polyvinylidene fluoride membrane (Merck Millipore, MA, USA). The membrane was blocked with 5% skim milk for 1 hour at room temperature and then incubated at 4°C overnight with the primary antibody (MAD2B, 1:1000; Abcam, Cambridge, MA, USA), GRM4 (1:600, ProteinTech Group, Inc., Wuhan, China), and α-tubulin (1:1000, ProteinTech Group, Inc.). Horseradish peroxidase-conjugated Ig (1:20 000, Jackson ImmunoResearch, West Grove, PA, USA) was used as the secondary antibody. Immunoreactive bands were detected with enhanced chemiluminescence reagents (Pizyme Biotech, Shanghai, China). Band intensities were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

RNA Isolation and Quantitative Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated from mouse hippocampi or cultured neurons using TRIzol following the manufacturer's recommendations. Isolated RNA was used to generate cDNA with a cDNA Synthesis Kit (Vazyme, Jiangsu, China) following the manufacturer's recommendations. Quantitative PCR (qPCR) was performed using ChamQ SYBR qPCR Master Mix (Vazyme) and an ABI StepOnePlus qPCR system. The relative mRNA transcript level was determined using the 2–ΔΔCT method with normalization to the gene expression of β-actin or U6. The primers used in the study are shown in Tables 1 and 2.

Statistics

The data are presented as the mean±SEM values and were analyzed by 2-tailed unpaired t test or 2-way ANOVA with GraphPad Prism 8.0 (San Diego, CA, USA). Values were considered statistically significant when $*P<.05$, $**P<.01$, and $***P<.001$.

RESULTS

MAD2B Expression Is Decreased in the Hippocampus in CUS- or CORT-Exposed Mice

To investigate the molecular mechanism underlying depression, we utilized CUS and CORT exposure–induced mouse models of depression based on previous studies (Goshen et al., 2008; Zhao et al., 2008; Peng et al., 2012; Kv et al., 2018). Adult wild-type C57BL/6J male mice were randomly divided into control and CUS or CORT groups. The depressive phenotypes in mice were

Table 1. Primers for Quantitative PCR (RNA Isolated From Mice)

Genes	Forward primer 5'-3'	Reverse primer 5'-3'
GRM4	AGGACCAGCGGACACTTGACC	AGGAGGCAGATGAGCGACAGG
GR	ACTCCAAAGAATCCTTAGCTCC	TATACAAGTCCATCACGCTTCC
β-actin	GTTGGAGCAAACATCCCCCA	CGCGACCATCCTCCTCTTAG

evaluated by a series of behavioral tests, including sucrose preference, forced swimming, and tail suspension tests. We observed that before CUS or CORT stimulation, all mice preferred sucrose water, and there was no difference between any 2 groups (supplementary Figure 1A and B). However, after CUS or CORT exposure, mice showed decreased sucrose preference and increased immobility in the forced swimming and tail suspension tests compared with controls (supplementary Figure 1C–H). Notably, the time spent on the rotarod was similar between the 2 groups, indicating no difference in locomotor activity (supplementary Figure 1I and J). These results demonstrate that the CUS and CORT mouse models induced depressive-like behaviors in the sucrose preference, forced swimming, and tail suspension tests.

MAD2B may be implicated in synaptic plasticity (Meng et al., 2012, 2014). Thus, we evaluated whether MAD2B is involved in the development of depression in mice. We observed marked reductions in immunoreactive MAD2B on western blots in the CUS and CORT groups compared with the control group (Figure 1A–D).

MAD2B Overexpression Protects Mice From Developing CUS Exposure-Induced Depression-Like Behaviors

To further study the function of MAD2B in depression, we injected AAV8 containing eGFP (AAV8-eGFP) or overexpressed MAD2B fused to eGFP (AAV8-MAD2B-OE-eGFP) into the hippocampus of mice (Figure 2A). The efficiency of AAV8 infection of the hippocampus was evaluated by eGFP fluorescence. eGFP positivity in the hippocampus was observed in both the AAV8-eGFP and AAV8-MAD2B-OE-eGFP groups (Figure 2A). The expression of MAD2B was checked by western blotting. We found elevated MAD2B expression

Table 2. Primers for Quantitative PCR (RNA Isolated from Cultured Neurons)

| Genes	Forward primer 5'-3'	Reverse primer 5'-3'
MAD2B	AAGTACAACGTGCCGGTTCAGATG	TCACCACCACCACCTTCTCCAC
GRM4	AGAAGGACGGCACGGAGGTC	CTTGAAGAGGCGGAGGATGTTGG
miR-29b-3p	TGCGGTAGCACCATTTGAAAT	CCAGTGCAGGGTCCGAGGT
miR-335	TGTTTTGAGCGGGGGTCAAGAGC	CTCTCATTTGCTATATTCA
miR-328-3p	AGGAGGGGCTCAGGGAGAAA	ATTTGGGGACAGGGGACGG
miR-370	GTAGGCGATATCGTCTGCTAC	TAGAAGGTAGCACCCGATG
GR	AAGGCGATACCAGGCTTCAGAAAC	ATGATCTCCAACCCAGGGCAAATG
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
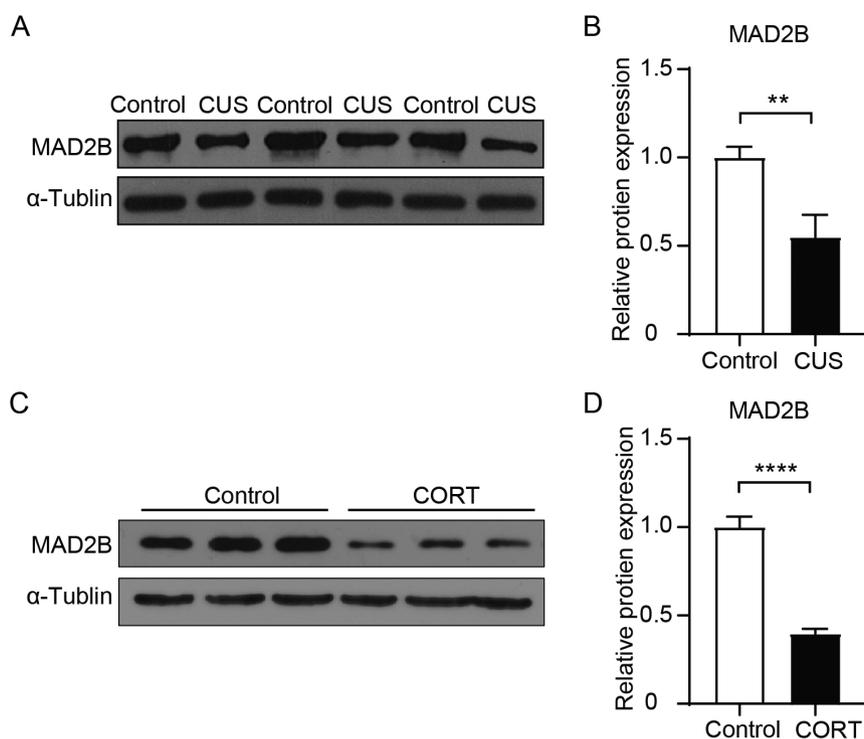
Figure 1. Hippocampal mitotic arrest deficient protein (MAD2B) expression is decreased in chronic unpredictable stress (CUS)- or corticosterone (CORT)-stimulated mice. (A-B) Representative images (A) and quantification (B) of western blots documenting immunoreactive MAD2B and α-tubulin in the hippocampus in CUS-treated mice. (C-D) Hippocampal MAD2B expression in CORT-treated mice. n = 6 mice per group in each experiment. The data are presented as the mean ± SEM. **$P < .01$, ****$P < .0001$.
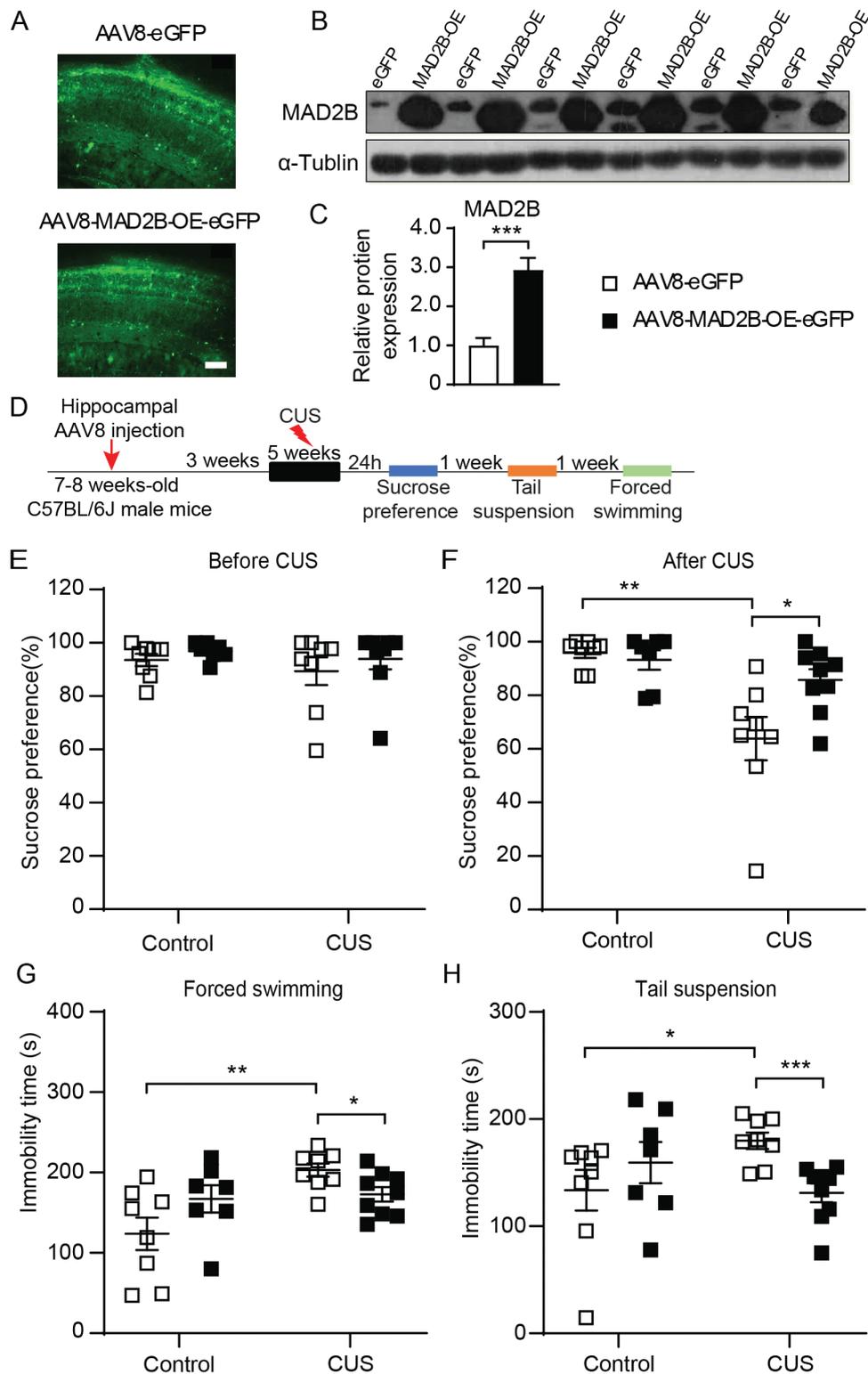
Figure 2. Hippocampal MAD2B overexpression prevents CUS-induced depression-like behaviors in mice. (A) Representative hippocampal sections showing enhanced green fluorescent protein (eGFP) expression in adeno-associated virus 8 (AAV8)-MAD2B-OE-eGFP- and AAV8-eGFP-injected mice. Scale bar, 100 μm. (B-C) Representative images (B) and quantification (C) of western blots showing immunoreactive MAD2B in the hippocampus in AAV8-MAD2B-OE-eGFP- and AAV8-eGFP-injected mice (n=6). (D) The timeline of behavioral tests after CUS. (E) Sucrose preference test in AAV8-MAD2B-OE-eGFP and AAV8-eGFP mice before exposed to CUS (n=7–8 mice). (F-H) Sucrose preference test (F), forced swimming test (G), and tail suspension test (H) in AAV8-MAD2B-OE-eGFP and AAV8-eGFP mice after exposure to CUS (n=7–8 mice). The data are presented as the mean±SEM. *P<.05, **P<.01, ***P<.001.

in the AAV8-MAD2B-OE-eGFP group compared with the AAV8-eGFP group (Figure 2B and C).

By using these mice, we examined the effect of MAD2B overexpression on CUS exposure-induced depressive-like behaviors

(Figure 2D). Three weeks after AAV8-eGFP and AAV8-MAD2B-OE-eGFP injection, mice were randomly divided into the control and CUS groups. Before CUS, there was no difference in sucrose preference between any pair of groups (Figure 2E). However,

sucrose preference was significantly reduced in AAV8-eGFP-injected mice after 5 weeks of CUS exposure compared with that in unstimulated mice (Figure 2F). In contrast, there was no difference in sucrose preference between AAV8-MAD2B-OE-eGFP mice exposed to CUS and unstimulated mice (Figure 2F). Thus, after CUS, the AAV8-MAD2B-OE-eGFP mice showed a greater preference for sucrose water than AAV8-eGFP mice (Figure 2F). Moreover, there was no difference in immobility time in the forced swimming and tail suspension tests between the AAV8-MAD2B-OE-eGFP and AAV8-eGFP groups without CUS treatment (Figure 2G and H). However, after CUS exposure, AAV8-eGFP mice exhibited increased immobility time both in the forced swimming and tail suspension tests, which was not observed in the AAV8-MAD2B-OE-eGFP groups (Figure 2G and H). AAV8-MAD2B-OE-eGFP mice showed significantly less immobility than AAV8-eGFP groups after CUS exposure in the forced swimming and

tail suspension tests (Figure 2G and H). Notably, depressive phenotypes did not differ between AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice without CUS treatment (Figure 2F–H). There was no difference in the time spent on the rotarod between any pair of groups, indicating similar locomotor activity (supplementary Figure 2A). These results indicate that MAD2B overexpression prevents CUS exposure-induced depressive-like behaviors in mice.

Overexpression of MAD2B Partially Attenuates CORT Treatment-Induced Depressive-Like Behaviors in Mice

Next, we assessed whether MAD2B overexpression affects CORT administration-induced depressive-like behaviors in mice (Figure 3A). Three weeks after AAV8-eGFP and
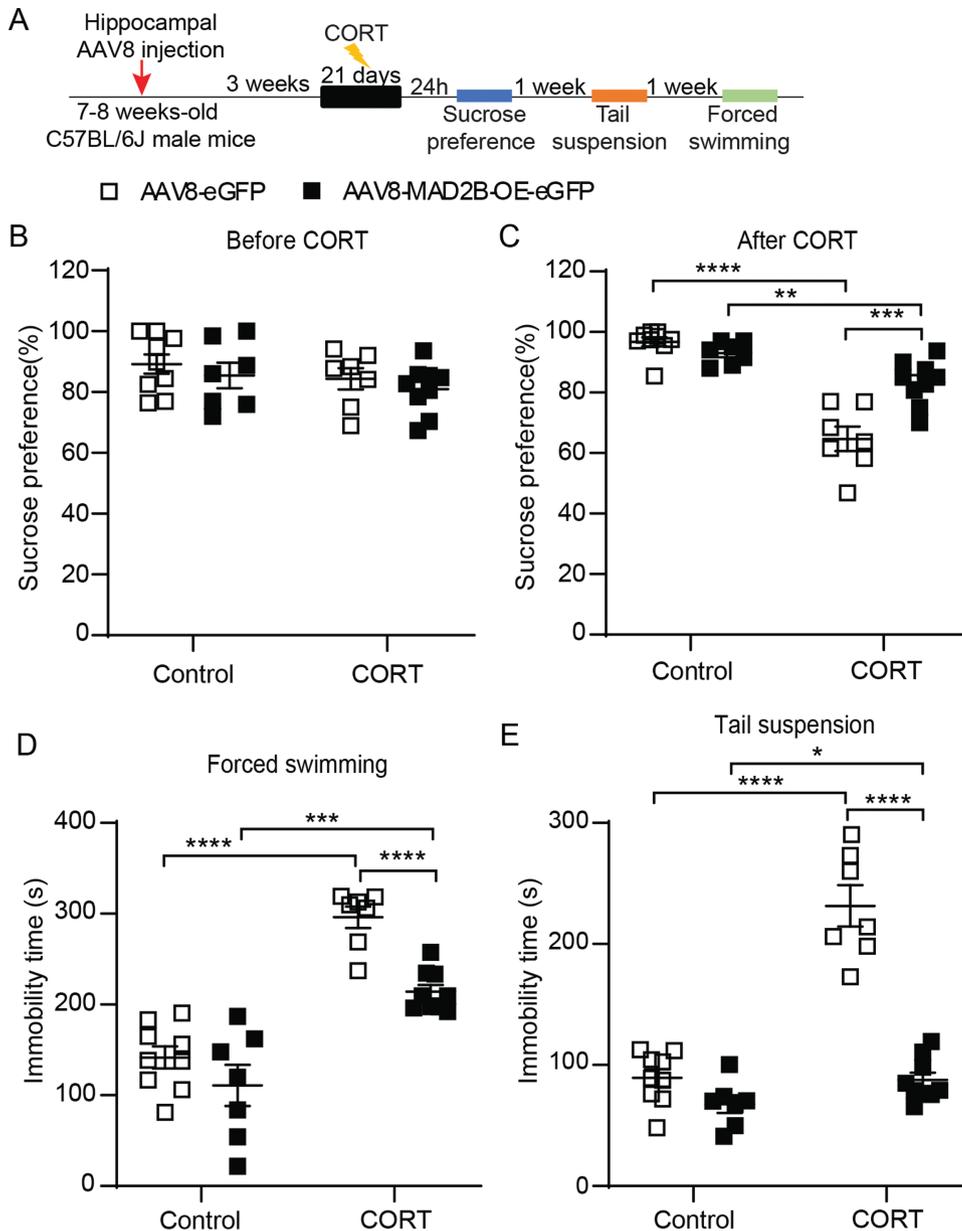

Figure 3. Hippocampal MAD2B overexpression protects mice from CORT-induced depression-like behaviors. (A) The timeline of behavioral tests after CORT stimulation. (B) Sucrose preference test before CORT injection in AAV8-MAD2B-OE-eGFP and AAV8-eGFP mice. Sucrose preference test (C), forced swimming test (D), and tail suspension test (E) in AAV8-MAD2B-OE-eGFP and AAV8-eGFP mice after CORT injection. n = 8–9 mice per group. The data are presented as the mean ± SEM. *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.
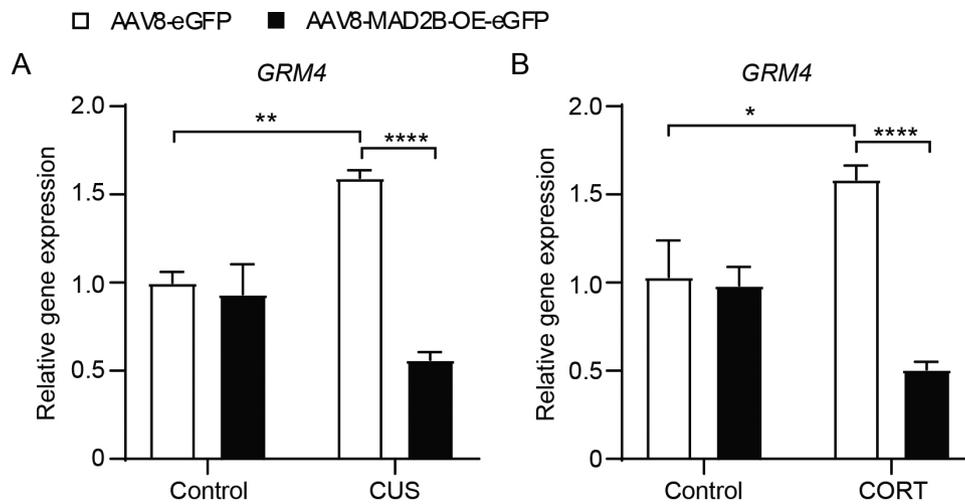
Figure 4. Overexpression of MAD2B in the hippocampus reduces the transcript of glutamate metabotropic receptor 4 (GRM4). Relative gene expression of *GRM4* in the hippocampus in AAV8-MAD2B-OE-eGFP and AAV8-eGFP mice after exposure to CUS (A) or after CORT injection (B). n = 6 mice per group. The data are presented as the mean ± SEM. *P < .05, **P < .01, ****P < .0001.

AAV8-MAD2B-OE-eGFP injection, mice were randomly divided

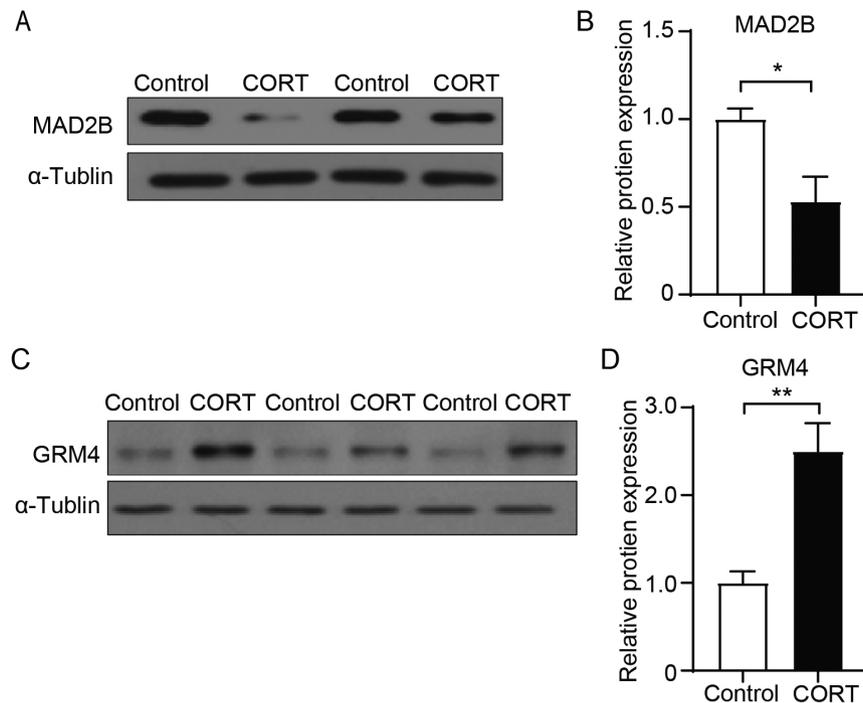time in the forced swimming and tail suspension tests after

Figure 5. CORT treatment decreases MAD2B expression and increases GRM4 expression in primary neuronal cells. Representative images (A) and quantification (B) of western blots documenting immunoreactive MAD2B and α-tubulin in primary neurons after CORT stimulation (100 μM, 24 hours; n = 6). Representative images (C) and quantification (D) of western blots documenting immunoreactive GRM4 and α-tubulin in primary neurons after CORT stimulation (n = 6). The data are presented as the mean ± SEM. *P < .05, **P < .01.

into control and CORT groups. Before CORT injection, the sucrose preference was similar between any pair of groups (Figure 3B). However, after 21 days of CORT stimulation, both AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice showed a significant reduction in sucrose preference compared with control mice (Figure 3C), while the reduction in sucrose preference was less in CORT-stimulated AAV8-MAD2B-OE-eGFP mice than in AAV8-eGFP mice (Figure 3C). Moreover, both AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice exhibited increased immobility

CORT administration (Figure 3D and E), but the immobility time was lower in AAV8-MAD2B-OE-eGFP mice than in AAV8-eGFP mice in both tests (Figure 3D and E). The fatigue rotarod test showed no significant difference in locomotor activity between any pair of groups (supplementary Figure 2B). These results suggest that while hippocampal MAD2B overexpression did not prevent CORT-induced depressive-like behaviors in mice, the behavioral effects of CORT were attenuated in mice with MAD2B overexpression.

Hippocampal MAD2B Overexpression Downregulates *GRM4* Expression Under CUS or CORT Exposure

It has been reported that increased levels of glucocorticoids reduce glucocorticoid receptor (GR) expression and glutamate release in mouse models of depression (Chiba et al., 2012; Sarawagi et al., 2021). To investigate the mechanism underlying MAD2B in the control of depression, we examined the hippocampal gene expression of GR both in AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice. We found a decreased transcription of *GR* in the hippocampus in both groups after CUS or CORT exposure (supplementary Figure 3A and B), as described by previous studies (Chiba et al., 2012). However, the gene expression of *GR* did not differ between AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice in the unstimulated or CUS/CORT-treated groups (supplementary Figure 3A and B). These data suggest that the protective effect of hippocampal MAD2B overexpression was not mediated by GR in mice. Because GRM4 is also involved in the pathogenesis of depression (Podkowa et al., 2015), we checked hippocampal GRM4 expression in both AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice. Without CUS or CORT stimulation, the gene expression of *GRM4* did not differ between AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice (Figure 4A and B). CUS or CORT exposure significantly increased the transcript of *GRM4* in AAV8-eGFP mice but not in AAV8-MAD2B-OE-eGFP mice compared with that in unstimulated mice (Figure 4A and B). Importantly, hippocampal *GRM4* gene expression was significantly different between AAV8-eGFP and AAV8-MAD2B-OE-eGFP mice after CUS or CORT exposure (Figure 4A and B). These results suggest that MAD2B overexpression in the hippocampus inhibits the level of *GRM4* after CUS or CORT stimulation.

MAD2B Overexpression Decreases the Transcript Level of GRM4 in Primary Neuronal Cells

To clarify the link between MAD2B and GRM4, we stimulated primary neuronal cells isolated from the hippocampus with 100 µM CORT for 24 hours. CORT administration decreased MAD2B expression and increased the level of GRM4 in primary neurons (Figure 5A–D), as we observed in the hippocampus in mice exhibiting depressive-like behaviors. Next, we infected primary neuronal cells with MAD2B-overexpressing lentivirus (LV-MAD2B-OE-eGFP) or control lentivirus (LV-eGFP). The efficiency of lentiviral transduction was checked by eGFP fluorescence, and the effect was evaluated by qPCR in both groups (Figure 6A and B). After transduction, *MAD2B* was significantly increased and *GRM4* was decreased in LV-MAD2B-OE-eGFP-positive neurons compared with the LV-eGFP group (Figure 6B and C).

MAD2B Overexpression Increases *miR-29b-3p* Expression in Primary Neuronal Cells

It has been reported that GRM4 expression is directly regulated by miR-29b-3p, miR-335, miR-328-3p, and miR-370-3p (Li et al., 2015; Wan et al., 2018; Xiao et al., 2019). We checked the gene expression of these miRNAs in LV-MAD2B-OE-eGFP and LV-eGFP neurons. We found that *miR-29b-3p* expression was decreased in the LV-MAD2B-OE-eGFP group, while there was no difference in *miR-335*, *miR-328-3p*, or *miR-370-3p* expression between the LV-MAD2B-OE-eGFP and LV-eGFP groups (Figure 7A–D). These results suggest that miR-29b-3p may mediate the effect of MAD2B on the control of GRM4 expression.

To evaluate the effect of miR-29b-3p in mediating the effects of CORT, neurons were treated with CORT 3 days after LV-MAD2B-OE-eGFP and LV-eGFP transduction. We found that in the LV-eGFP

groups, CORT treatment decreased the expression of *MAD2B* and *miR-29b-3p* and increased the level of *GRM4* (Figure 8A–C). However, compared with the corresponding LV-eGFP groups, MAD2B overexpression increased MAD2B and miR-29b-3p expression as well as reduced the level of GRM4 in both CORT stimulation or non-stimulation groups (Figure 8A–C). These results suggest that miR-29b-3p may be a mediator of MAD2B in the regulation of GRM4 expression in the context of CORT stimulation.

DISCUSSION

Depression, a common type of psychiatric disorder, seriously affects the quality of life and health, leading to a high suicide rate (Harwood et al., 2001; Doraiswamy et al., 2002; Hawton et al., 2013). Previous studies have shown that chronic stress leads to hippocampal atrophy, neuronal damage, and a decrease in neuronal cells in the brains of individuals with depression (Watanabe et al., 1992; Stein-Behrens et al., 1994; MacMaster and Kusumakar, 2004). Disruption of neural plasticity is a fundamental mechanism in the pathogenesis of depression (Liu et al., 2017). However, the pathogenesis of depression remains to be further elucidated. Here, we found that MAD2B exerts a protective effect that may prevent or attenuate stress-induced depressive-like behaviors by controlling glutamate receptor-GRM4 expression in the hippocampus.

In the current study, we utilized 2 different mouse models of depression induced either by CUS exposure or by CORT administration. Depression-like behaviors in mice were assessed using classical behavioral methods, such as sucrose preference, forced swimming, and tail suspension tests. Our results showed that mice exposed to chronic stress exhibited increased immobility time in the forced swimming and tail suspension tests and decreased sucrose preference compared with controls. In addition, we used
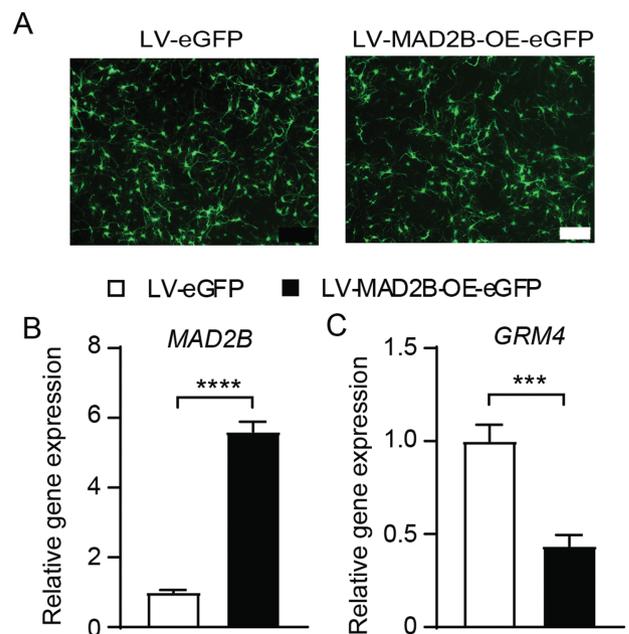
Figure 6. **Overexpression of MAD2B reduces GRM4 mRNA expression in primary neuronal cells.** (A) Representative images showing eGFP expression in lentivirus (LV)-MAD2B-overexpression (OE)-eGFP- or LV-eGFP-transduced primary neurons. Scale bar, 100 µm. Relative gene expression of *MAD2B* (B) and *GRM4* (C) in MAD2B-overexpressing neuronal cells (n = 7). The data are presented as the mean ± SEM. ***P < .001, ****P < .0001.
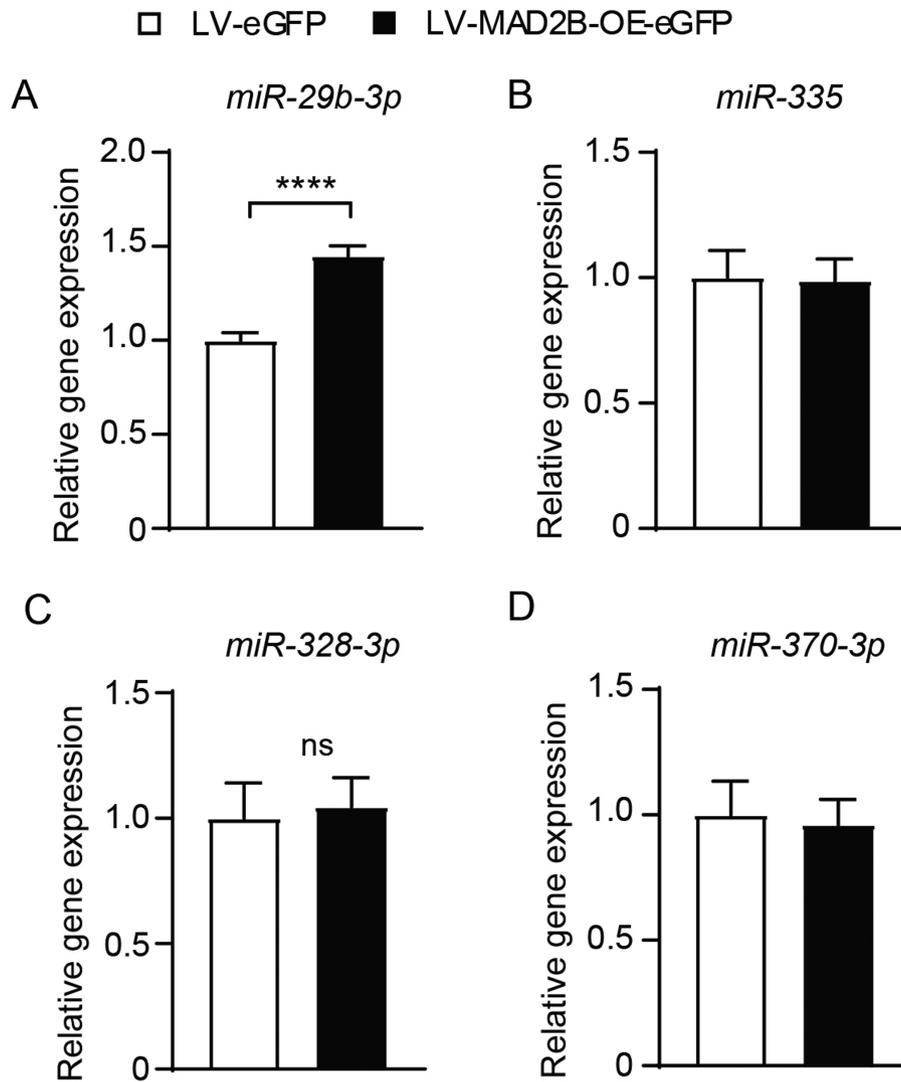
Figure 7. MAD2B overexpression leads to increased expression of miR-29-3p. Relative expression of *miR-29-3p* (A), *miR-335* (B), *miR-328-3p* (C), and *miR-370-3p* (D) in MAD2B-overexpressing neuronal cells (n=7). The data are presented as the mean±SEM. ****$P<.0001$.

the fatigue rotarod test to evaluate the locomotor activity of mice, and there was no significant difference between mice exhibiting depressive-like behaviors and controls.

Our previous studies have shown that MAD2B is highly expressed in the cortex and hippocampus, which plays a crucial role in the occurrence and development of neurological diseases (Meng et al., 2012, 2014). In diabetic rats, MAD2B is significantly enhanced in the cortex, which promotes aberrant neuronal cell cycle activation and apoptotic cell death (Meng et al., 2014). Oxygen-glucose deprivation/reoxygenation also increases MAD2B expression in neurons and leads to neuronal injury (Meng et al., 2016). Additionally, the degradation of MAD2B is regulated by adenosine 5'-monophosphate-activated protein kinase in neurons (Meng et al., 2017). It has been reported that MAD2B is implicated in numerous biological processes (de Krijger et al., 2021a). However, whether MAD2B is involved in the development of depression remains unclear. Here, we found that MAD2B was downregulated in the hippocampus in depressed mice compared with control mice, suggesting an important role of MAD2B in the development of depression. Further work could attempt to determine how depression reduces MAD2B expression in the hippocampus, especially in neurons.

To further clarify the effect of MAD2B on the development of depression, we constructed MAD2B-overexpressing AAV8, which was injected into the hippocampus. The expression of MAD2B in the hippocampus was verified by western-blot analysis. Three weeks after injection, mice were exposed to CUS or CORT and subsequently subjected to behavioral tests. The results showed that, at the basal level, overexpression of MAD2B in the hippocampus did not affect depression-like behaviors in mice. However, mice injected with the control virus exhibited a depressive phenotype after CUS or CORT treatment, while this phenomenon was prevented or attenuated by overexpression of MAD2B in the hippocampus. These results suggest that MAD2B overexpression in the hippocampus does not affect the tested behaviors under normal conditions but plays an important role in the development of depressive-like behaviors in mice exposed to stressful stimuli. Notably, MAD2B overexpression prevented the depressive-like behaviors induced by CUS but only partially prevented these behaviors in mice after CORT treatment. Depression is a complex multifactorial neuropsychiatric disorder. Stressful life events are associated with the pathogenesis of depression (Kessler, 1997; Kendler et al., 1999). The activation of the hypothalamus–pituitary–adrenal axis
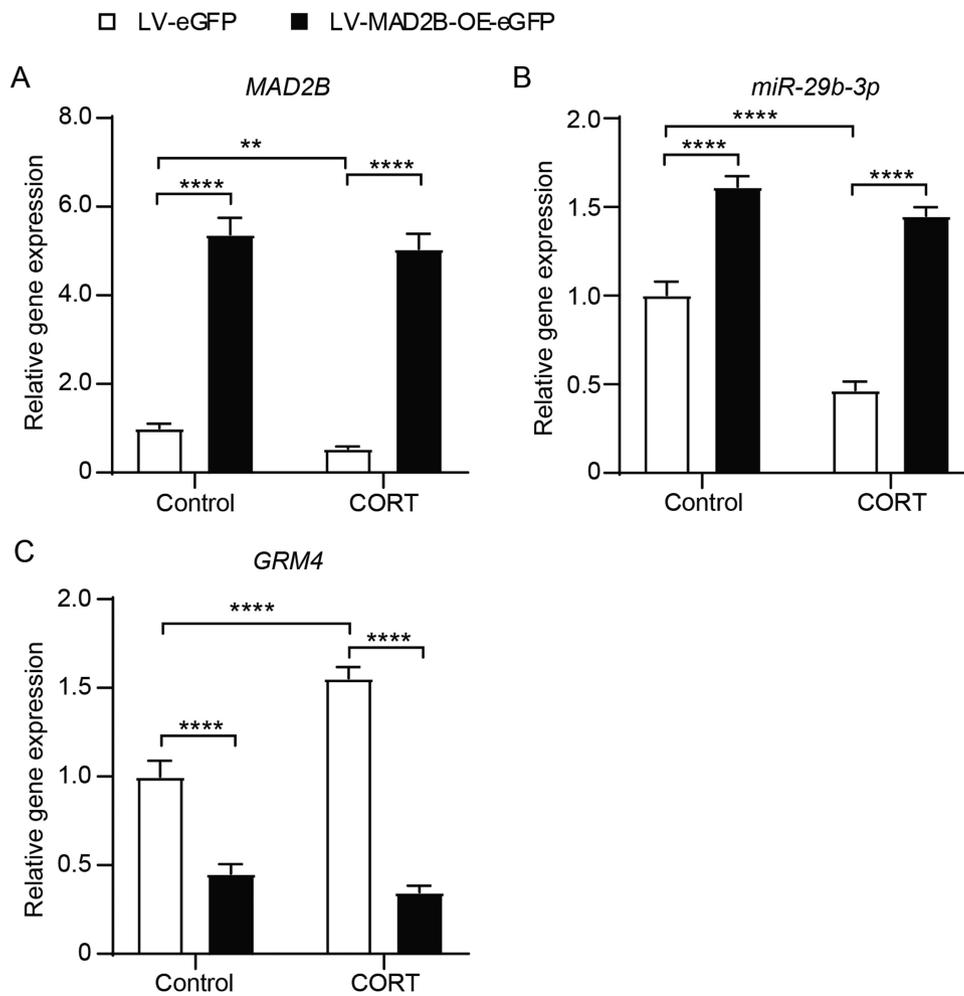
Figure 8. MAD2B overexpression affects the levels of miR-29-3p and *GRM4* in CORT-treated neuronal cells. Relative gene expression of *MAD2B* (A), *miR-29-3p* (B), and *GRM4* (C) in CORT-treated LV-MAD2B-OE-eGFP and LV-eGFP neuronal cells (n = 7). The data are presented as the mean ± SEM. **P < .01, ****P < .0001.

and elevated corticoid levels are the links between stress and depression (Reus and Miner, 1985; Dinan, 1994). Based on these clinical observations, animal models were developed to study the mechanisms of depression. However, there are still some problems that exist in each animal model. For example, the physiological parameters may display some variability in mice, which probably causes depressive-like behavioral variation under CUS or CORT treatment (Beck and Luine, 2002; Berger et al., 2019). Additionally, it has been shown that repeated corticosterone injections lead to time-related behavioral phenotypes in mice (Zhao et al., 2008, 2009). The complex mechanisms underlying corticosterone might lead to the less robust effects of MAD2B overexpression than CUS in mice, which still needs to be further investigated.

According to previous studies, long-term psychosocial stress leads to glucocorticoid resistance and downregulates the expression of GR (Anacker et al., 2011; Chiba et al., 2012). However, overexpression of MAD2B in the hippocampus had no effect on the expression of *GR* in either basal or mice exhibiting depressive-like behaviors. These results suggest that the protective role of MAD2B is independent of GR.

Emerging evidence has shown that disrupted neuroplasticity is the foundation of depression, which is associated with an altered glutamate system (Duman and Aghajanian, 2012; Y. T. Wang et al., 2021). Glutamate signaling and receptors affect synaptic plasticity (Barnes et al., 2020). It has been reported that GRM4 is involved in the pathogenesis of depression and is elevated in the brains of depressed mice (Li et al., 2015; Dadkhah et al., 2017). Our results also showed a significantly increased level of *GRM4* in the hippocampus in depressed mice compared with control mice. However, MAD2B overexpression inhibited the transcription of *GRM4* in mice under CUS or CORT stimulation. At the basal level, overexpression of MAD2B did not affect the expression of *GRM4*.

Next, we further explored the mechanism of MAD2B in the control of depression in primary neurons isolated from the hippocampus. It has been reported that CORT-induced hippocampal neuronal damage is involved in depression and aging (Woolley et al., 1990). Therefore, we treated primary neurons with CORT. The results showed that the expression of MAD2B was downregulated while GRM4 was increased in CORT-treated neurons. Moreover, we infected primary neurons with MAD2B-overexpressing lentivirus and control lentivirus. MAD2B overexpression reduced the level of *GRM4* in primary neurons. These results suggest that MAD2B may play a protective role by negatively regulating neuronal GRM4 expression.

It has been reported that the expression of GRM4 can be directly regulated by microRNAs (Lopez et al., 2014; Li et al., 2015; Wan et al., 2018). For example, miR-29b-3p negatively regulates GRM4

expression, and overexpression of miR-29b-3p leads to reduced GRM4 expression and alleviation of depressive behaviors in a rat model (Wan et al., 2018). We found that *miR-29b-3p* expression was significantly reduced in primary neurons treated with CORT. Overexpression of MAD2B increased the level of *miR-29b-3p* under basal CORT-stimulated conditions. These results suggest that the protective effect of MAD2B may be mediated by elevated miR-29b-3p. However, how MAD2B regulates the expression of miR-29b-3p should be evaluated in further work.

In conclusion, we identified MAD2B as a critical regulator of hippocampal neurons in controlling the development of depression. Overexpression of MAD2B in the hippocampus decreases the level of GRM4 and prevents depression-like behaviors in mice exposed to chronic stress through an increase in miR-29b-3p expression. Our findings improve the understanding of the pathogenesis of depression and provide a new approach to the treatment of depression.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

Author Contributions

Cheng Miao and Yanfang Su performed the experiments and collected the data. Xiao-Lan Wang analyzed the data and drafted the manuscript. Xianfang Meng and Chun Zhang designed the experiments, supervised the work, and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Ethics Approval

The study was approved by the Huazhong University of Science and Technology Ethics Committee for Care and Use of Laboratory Animals.

Interest Statement

The authors declare that there is no conflict of interest.

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