Pharmacology, Biochemistry and Behavior 184 (2019) 172738

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Repetitive high-frequency transcranial magnetic stimulation reverses depressive-like behaviors and protein expression at hippocampal synapses in chronic unpredictable stress-treated rats by enhancing endocannabinoid signaling

Shan-shan Xue[a,1], Fen Xue[a,1], Quan-rui Ma[b], Shi-quan Wang[c], Ying Wang[a], Qing-rong Tan[a], Hua-ning Wang[a], Cui-hong Zhou[a,*], Zheng-wu Peng[a,*]

[a] Department of Psychiatry, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China
[b] Department of Human Anatomy and Histology and Embryology, Basic Medical College, Ningxia Medical University, 750004, China
[c] Department of Anesthesiology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China

ARTICLE INFO

Keywords:
rTMS
Depression
Hippocampus
Endocannabinoids

ABSTRACT

The anti-depressant effect of repetitive transcranial magnetic stimulation (rTMS), a clinically-useful treatment for depression, is associated with changes to the endocannabinoid system (ECS). However, it is currently unknown whether different frequencies of rTMS alter the ECS differently. To test this, rats exposed to chronic unpredictable stress (CUS) were treated with rTMS at two different frequencies (5 (high) or 1 Hz (low), 1.26 Tesla) for 7 consecutive days. Twenty-four hours after the final rTMS treatment, we evaluated depressive-like behaviors and the expression of several synaptic proteins and ECS-related proteins in the hippocampus. In addition, we knocked-down diacylglycerol lipase alpha (DAGLα) and cannabinoid type 1 receptor (CB1R), two important components of the ECS, and measured depressive-like behaviors and synaptic protein expression following rTMS. Furthermore, we measured the expression levels of several components of the ECS system in hippocampal-derived astrocytes and neurons exposed to repetitive magnetic stimulation (rMS) with different parameters (5 or 1 Hz, 0.84 or 1.26 Tesla). Interestingly, we found that only high-frequency rTMS ameliorated depressive-like behaviors and normalized the expression of hippocampal synaptic proteins in CUS-treated rats; this effect was eliminated by knockdown of DAGLα or CB1R. Moreover, we found that rMS at 5 Hz increased the expression of DAGLα and CB1R in hippocampal astrocytes and neurons. Collectively, our results suggest that high-frequency rTMS exerts its anti-depressant effect by up-regulating DAGLα and CB1R.

1. Introduction

Depression is a common and debilitating illness associated with significant morbidity and family burden. Currently, depression is treated with antidepressants and psychological interventions. However, the antidepressants currently available, such as selective serotonin reuptake inhibitors (SSRIs), must be taken daily for at least a year and are often associated with side effects (Mello et al. 2013). Furthermore, low remission rates and delayed onset of efficacy are commonly observed after multiple courses of pharmacotherapy (Daly et al. 2018). In addition, psychological interventions produce only partial benefits, and the requirement for expensive professional psychotherapists has limited their application (Karyotaki et al. 2017; March et al. 2004). There is thus an urgent need to identify better and cheaper therapeutic options for depression.

Repetitive transcranial magnetic stimulation (rTMS) is widely used

Abbreviations: SSRIs, selective serotonin reuptake inhibitors; rTMS, repetitive transcranial magnetic stimulation; rMS, repetitive magnetic stimulation; CUS, chronic unpredictable stress; ECS, endocannabinoid system; PSD95, post-synaptic density protein 95; SYN, synaptophysin; DAGLα, diacylglycerol lipase alpha; CB1R, cannabinoid type 1 receptor; CB2R, cannabinoid type 2 receptor; shRNA, short hairpin RNA; AEA, N-arachidonoyl ethanolamine; MAGL, monoacylglycerol lipase; FAAH, fatty acid amide hydrolase; NAPE-PLD, N-acyl phosphatidyl ethanolamine-phospholipase D; SPT, sucrose preference test; OFT, open-field test; FST, forced swimming test

* Corresponding authors.
E-mail addresses: zhoucuihong@sibs.ac.cn (C.-h. Zhou), pengzw@fmmu.edu.cn (Z.-w. Peng).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.pbb.2019.172738
Received 21 March 2019; Received in revised form 19 June 2019; Accepted 19 June 2019
Available online 20 June 2019
0091-3057/

to treat psychiatric disorders noninvasively. In 2008, the U.S. Food and Drug Administration approved rTMS as a treatment for antidepressant-resistant depression. Recently, the acute and short-term antidepressant efficacy of rTMS has been reported in clinical trials (Brunoni et al. 2017; Fitzgerald et al. 2018; Kedzior et al. 2017). Notably, rTMS was successfully used as an alternative treatment of depression during pregnancy, which is complex due to risks associated with medication use (Eryilmaz et al. 2015; Felipe and Ferrao 2016; Ferrao and da Silva 2018). Moreover, rTMS is superior to antidepressants in preventing the recurrence of depressive episodes, particularly in first-episode depressed patients (Haesebaert et al. 2018; Richieri et al. 2013). As such, the functional mechanisms of rTMS may be different to those of antidepressants. It is therefore critical to investigate the molecular mechanisms through which rTMS improves depression.

The endocannabinoid system (ECS) comprises the cannabinoid type 1 receptor (CB1R), type 2 receptor (CB2R), and two endogenous ligands, 2-arachidonoyl glycerol (2-AG), and N-arachidonoyl ethanolamine (AEA). The ECS is a widespread neuromodulatory system that responds to endogenous and environmental insults, regulates synaptic plasticity, and modulates central nervous system development (Lu and Mackie 2016). The synthesis of 2-AG and AEA occurs through hydrolysis of their precursors by diacylglycerol lipase alpha (DAGLα) and N-acyl phosphatidyl ethanolamine-phospholipase D (NAPE-PLD), respectively; while their catabolism occurs through monoacylglycerol lipase (MAGL) and fatty acid amidehydrolase (FAAH), respectively.

The ECS has been shown to be perturbed in animal models of depression. For example, CB1R deficiency was correlated with depressive-like behaviors in mice (Lomazzo et al. 2017; Valverde and Torrens 2012), and ECS dysregulation occurred in a rat model of depression (Smaga et al. 2017a). Additionally, EC signaling in the brain was compromised after chronic stress (Jennings et al. 2016; Morena et al. 2016), and augmenting EC signaling mitigated chronic stress-induced depressive-like behaviors (Griebel et al. 2018; Zhong et al. 2014). Furthermore, circulating levels of 2-AG were found to be reduced in patients with major depression (Coccaro et al. 2018). Consistent with this, we previously demonstrated that administering a low dose of MAGL inhibitors produced antidepressant effects in acute stress-exposed mice through long-term depression at glutamatergic synapses, whereas a high dose produced pro- and antidepressant effects in acute stress- and chronic corticosterone-exposed mice, respectively, through GABAergic synaptic disinhibition in the hippocampus (Wang, Y. et al., 2017). Indeed, our previous work confirmed the antidepressant effect of rTMS and showed that it increased hippocampal CB1R expression in chronically-stressed rats (Wang et al. 2014). Recently, we demonstrated that rTMS up-regulated 2-AG levels in the hippocampus and ameliorated depressive-like behaviors in adolescent rats (Fang and Wang 2018). Thus, rTMS appears to exert its antidepressant effect through the regulation of ECS.

The effects of antidepressants on the ECS are inconsistent. Previous studies indicated that chronic treatment with desipramine increased CB1R density in the hippocampus and hypothalamus of rats without altering EC levels (Hill et al. 2006). Conversely, citalopram was reported to reduce CB1R transcription and signal transduction in the hippocampus and hypothalamus (Hesketh et al. 2008). Moreover, chronic administration of imipramine, escitalopram, and tianeptine increased AEA levels and elevated CB1R density in the hippocampus (Smaga et al. 2014; Smaga et al. 2017b). Collectively, these findings suggest that the dysregulation of EC in the hippocampus contributes to the pathogenesis of depression and indicate a potential link between rTMS and antidepressant treatment and ECS signaling.

Studies suggest that high-frequency (≥ 1 Hz) rTMS increases local cortical excitability after stimulation, whereas low-frequency stimulation (0.1–1 Hz) decreases local cortical excitability (Fitzgerald et al. 2006). Recently, we reported that high-frequency rTMS (5/10 Hz) rather than low-frequency rTMS (1 Hz) ameliorated depressive-like behaviors in chronic unpredictable stress (CUS)-treated rats (Peng et al. 2018). In the present study, we used CUS to establish an animal model of depression and investigated the effects of different frequencies of rTMS (1 and 5 Hz) on depressive-like behaviors and hippocampal synaptic proteins. We also determined whether knockdown of diacylglycerol lipase alpha (DAGLα) and cannabinoid type 1 receptor (CB1R), two important components of the ECS, would block the effects of rTMS. Furthermore, we investigated the effects of repetitive magnetic stimulation (rMS) with different parameters (5 or 1 Hz, 0.84 or 1.26 Tesla) on the expression of ECS protein components in hippocampal astrocytes and neurons.

2. Materials and methods

2.1. Animals

The experimental procedures on animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Use and Protection Committee of the Fourth Military Medical University. Adult Sprague-Dawley (SD) rats (weighing 280–320 g), pregnant rats, and newborn rats were purchased from the Fourth Military Medical University Animal Center (Xi'an, China). Rats were group-housed (four per cage) in wire bottom cages at 20–25 °C and maintained on a daily 12 h light/dark cycle (lights on from 8:00 a.m. to 8:00 p.m.). Food and water were available *ad libitum* except during the CUS experiment (see section 2.3).

2.2. Experimental design

2.2.1. Experiment I

As shown in Fig. 1A, after 7 days of acclimatization, 48 rats were randomly assigned to one of the following four groups ($n = 12$ per group): sham, CUS, CUS + rTMS-1 Hz and CUS + rTMS-5 Hz. Rats in the sham group were kept in their home cages for 4 weeks and then subjected to sham rTMS treatments for 7 days. Rats in the CUS, CUS + rTMS-1 Hz and CUS + rTMS-5 Hz groups were subjected to CUS for 4 weeks and then either sham rTMS treatment, rTMS at 1 Hz/1.26 Tesla (T) or rTMS at 5 Hz/1.26 T for 7 days, respectively. Behavioral tests were conducted 24 h after the final intervention. Animals were subsequently sacrificed and expression levels of CB1R, DAGLα, post-synaptic density protein 95 (PSD95), synaptophysin (SYN), MAGL, FAAH, and NAPE-PLD in the hippocampus were determined by western blot.

2.2.2. Experiment II

As shown in Fig. 4A, 48 rats were randomly divided into four groups: scramble + CUS, scramble + CUS + rTMS, shCB1R + CUS + rTMS, shDAGLα + CUS + rTMS. Rats in the scramble + CUS and scramble + CUS + rTMS groups were injected with scramble shRNA lentivirus. Rats in the shCB1R + CUS + rTMS and shDAGLα + CUS + rTMS groups were injected with CB1R-shRNA or DAGLα-shRNA lentivirus in the hippocampus. One week after lentivirus injection, all rats were subjected to CUS for 4 weeks followed by sham or 5 Hz/1.26 T rTMS treatments for 7 days. Behavioral tests were conducted and protein expression was analyzed 24 h after the final intervention.

2.2.3. Experiment III

To investigate whether rMS directly influenced the expression of EC related genes in hippocampal cells, hippocampus-derived astrocytes and neurons were cultured. Purified astrocytes and neurons were plated in 6-cm^2 dishes coated with poly-L-lysine (Corning, New York, NY, USA) and incubated at 37 °C with 5% CO_2 for 3 days. Then, cells were treated with rMS. The stimulation parameters were as follows: 1 Hz/0.84 T, 1 Hz/1.26 T, 5 Hz/0.84 T and 5 Hz/1.26 T. After stimulation, total mRNA and protein were extracted from cells immediately. The

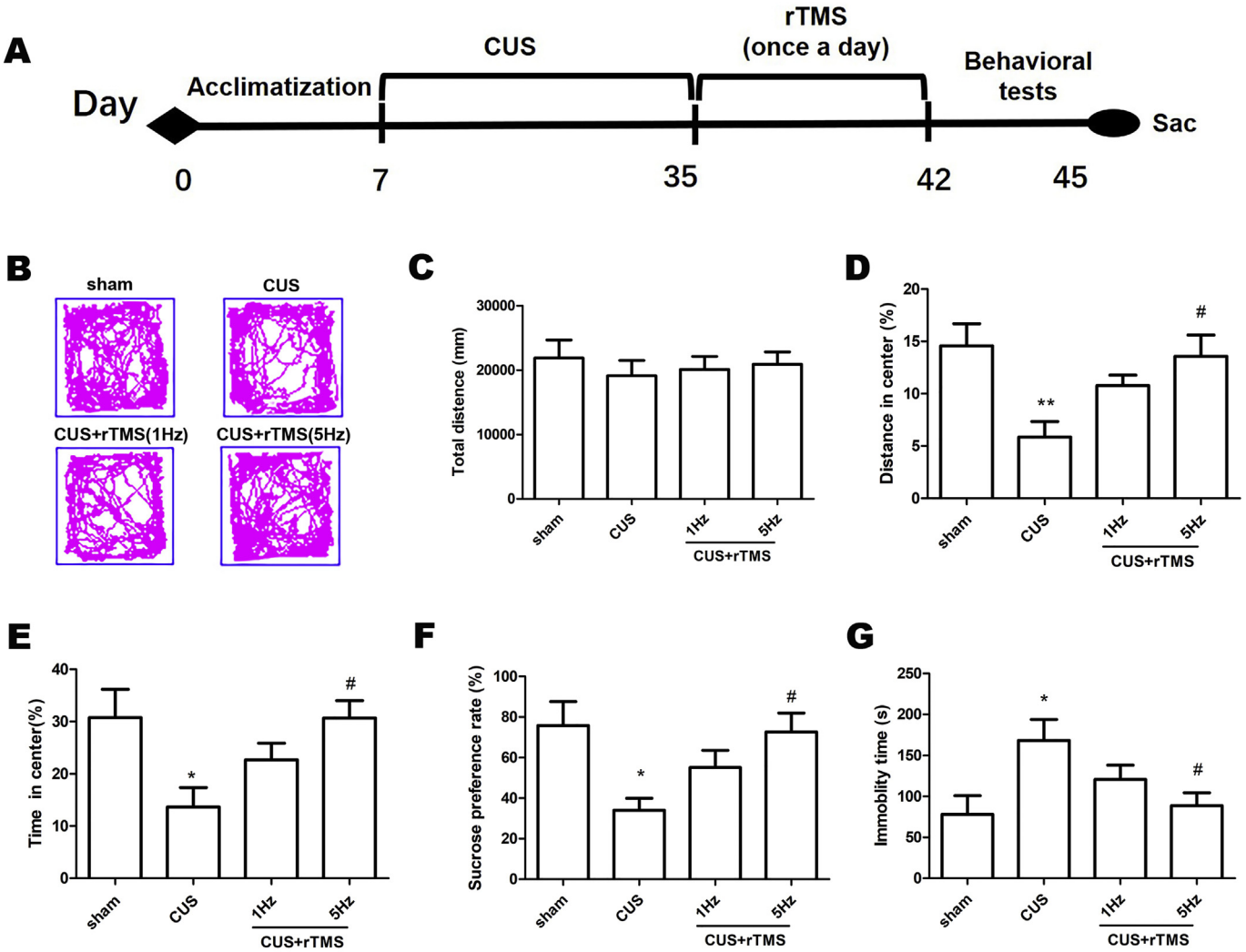

Fig. 1. Treatment with rTMS at 5 Hz ameliorates depressive-like behavior in CUS-treated rats. (A) Timeline for Experiment I. (B) Open field movement traces. (C) Quantification of the total distance traveled in the open field test. (D) The percentages of distance traveled in the center of the open field test. (E) The percentages of time spent in the center of the open field test. (F) The percentages of sucrose consumption in the sucrose preference test. (G) Immobility in the forced swimming test (FST). $^{*}P < 0.05$ *vs.* sham; $^{**}P < 0.01$ *vs.* sham; $^{\#}P < 0.05$ *vs.* CUS; $^{\#\#}P < 0.01$ *vs.* CUS.

expression of CB1R, DAGLα, FAAH, NAPE-PLD, and MAGL mRNA and protein were detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and western blot analysis, respectively.

2.3. CUS

CUS was performed as described previously (Chen et al. 2015). Briefly, rats were subjected to varied and repeated unpredictable stressors for 4 weeks as follows: (a) inversion of the dark/light cycle; (b) low-intensity stroboscopic illumination (in the dark for 12 h, 10 Hz); (c) tube restraint (2 h); (d) tail pinch (1 min); (e) cage rotation (20 min); (f) tilting the cage by 45 degrees (12*h*); (g) ice-cold water swim (4 °C for 5 min); (h) wet bedding (250 mL of water added to the cage); (i) food and water deprivation (24 h); (j) foot shocks (1.5 mA intensity, 2 s duration with 1 s intervals). One or two randomly selected types of stimulation were applied daily. Each stressor was administered equally two or three times during the treatment.

3. 2.4 rTMS treatment

rTMS was administered with a circular coil (inner diameter, 2.5 cm; outer diameter, 5 cm; custom-made YIRD, China) as described previously (Liu et al. 2018). The stimulation comprised six trains of 10 pulses delivered with 8-s intertrain interval at 5 Hz or 900 pulses at 1 Hz. The intensity of stimulation was 1.26 T. Stimulation was performed for 7 consecutive days. In the rTMS-treated groups, the center of the coil was placed over the vertex of the skull, and the handle of the coil was parallel to the line of the rat's vertebral column. In the sham groups, stimuli were delivered with the coil held 10 cm above the head to ensure that the animal felt the vibrations produced by the click of the rTMS coil but did not receive brain stimulation (Esser et al. 2006). Movement was restricted by hand during the stimulation. To exclude putative effects of nonspecific stress, each animal was allowed to adapt to the rTMS artifact noise and was subjected to a daily sham stimulation procedure for 15 min every day for 1 week. Real and sham rTMS treatments did not produce notable seizures or any behavioral changes during the entire treatment period. For the *in vitro* cultured cells experiments, 500 pulses of rMS were administered. The center of the coil was placed over the center of the culture plate. In the sham groups, stimuli were delivered with the coil held 10 cm above the culture plate to ensure that the cells were not affected by stimulation.

3.1. 2.5 Behavioral tests

3.1.1. 2.5.1 Sucrose preference test (SPT)

During the training phase, rats were provided access and allowed to

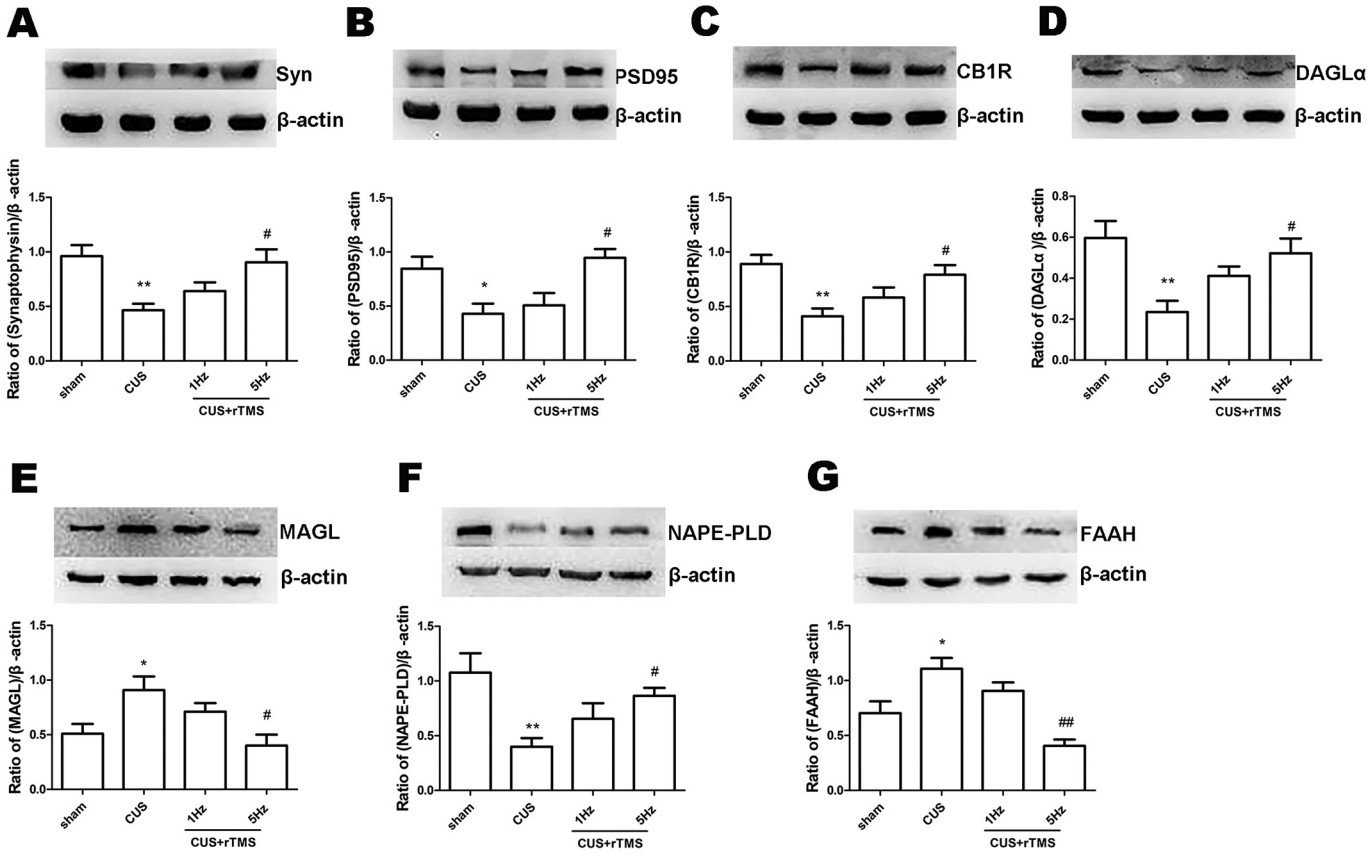

Fig. 2. rTMS stimulation at 5 Hz influences PSD95, SYN, CB1R, DAGLα, MAGL, NAPE-PLD, and FAAH expression in the hippocampus of CUS-treated rats. Representative immunoblots and densitometry analysis of (A) SYN, (B) PSD95, (C) CB1R, (D) DAGLα, (E) MAGL, (F) NAPE-PLD, and (G) FAAH expression in the total hippocampus of sham, CUS, CUS + rTMS-1 Hz and CUS + rTMS-5 Hz groups. $^{*}P < 0.05$ *vs.* sham; $^{**}P < 0.01$ *vs.* sham; $^{\#}P < 0.05$ *vs.* CUS; $^{\#\#}P < 0.01$ *vs.* CUS.

freely consume 1% sucrose solution for 24 h. Subsequently, water and 1% sucrose were provided simultaneously, and animals were allowed to consume fluid freely for 24 h. Rats were then deprived of water and food for 24 h before testing. In the testing phase, water and 1% sucrose were placed in pre-weighed bottles, and rats were allowed to consume the fluid freely for 1 h. Sucrose preference was calculated as sucrose preference (%) = sucrose consumption / (sucrose consumption + water consumption) according to previous studies (Liu et al. 2018; Ota et al. 2014).

3.1.2. 2.5.2 Open-field test (OFT)

The open-field test was performed according to previous methods (Liu et al. 2016). Briefly, animals were placed in the center of an open-field box. Activity was recorded for 5 min using an open-field activity system (Dig Behav, Ji liang Co. Ltd., Shanghai, China) and activity software (Top Scan, Clever Sys Inc., USA).

3.1.3. 2.5.3 Forced swimming test (FST)

Rats were subjected to a forced swimming test 12 h after the open-field test. In brief, rats were placed individually in a cylinder (height: 45 cm, diameter: 20 cm) containing water 30 cm high (25 °C) for 6 min. An additional pre-test was conducted 24 h before the test, during which rats were individually placed in a cylinder of water with conditions described above for 15 min. The time that rats remained floating in the water with an upright position and stopped struggling was recorded. The immobility duration indicating a state of helplessness was determined and analyzed for the last 4 min of the 6 min test.

4. 2.6 shRNA and lentivirus construction

Lentivirus vector construction and packaging were performed by Shanghai Genechem Co. Ltd. (Shanghai, China). Based on rat *CB1R* and *DAGLα* mRNA sequence (accession number: NM_012784.4; NM_001005886.1), three shRNAs targeting different regions of *CB1R* mRNA (shCB1R-a, b, c) or *DAGLα* mRNA (shDAGLα-a, b, c) and a control shRNA that did not correspond to any mammalian mRNA (scramble) were generated. The targeting sequences were as follows: shCB1R-a, 5′-AGA UGA CGG CAG GAG ACA A-3′; shCB1R-b, 5′-GGA GAC ACA ACA AAC AUU A-3′; shCB1R-c, 5′-CCA ACA TTA CAG AGT TCT A-3′; shDAGLα-a, 5′-GGG TGC TGG AGA ATT ACA AC-3′; shDAGLα-b, 5′-GGC TTT CAC GAC AAG GTG TA-3′; shDAGLα-1c, 5′-TGC CCG GCA TCA CTG CCC TGA AAC A-3′; scramble, 5′-TTC TCC GAA CGT GTC ACG T-3′. shRNA sequences were inserted into the LV-pGLV-H1-GFP lentiviral vector labeled with green fluorescent protein (GFP) (Genechem Co. Ltd., Shanghai, China) and packaged in HEK 293 T cells. Subsequently, lentiviral particles expressing either one of shCB1R, shDAGLα, or scramble sequences were cloned. Finally, the silencing efficiency of shCB1R and shDAGLα was assessed in astrocyte cultures by RT-qPCR. shCB1R-b and shDAGLα-c constructs were selected for subsequent *in vivo* experiments (data not shown).

4.1. 2.7 Primary cultures of astrocytes and neurons

Astrocytes were prepared and cultured from brains of SD newborn rats according to previous work (Xue et al. 2019). Briefly, the hippocampus was dissected from newborn rats under a stereomicroscope. Single cell suspensions obtained by mechanical dissociation filtered with a 200-molybdenum wire mesh screen were cultured in Dulbecco's

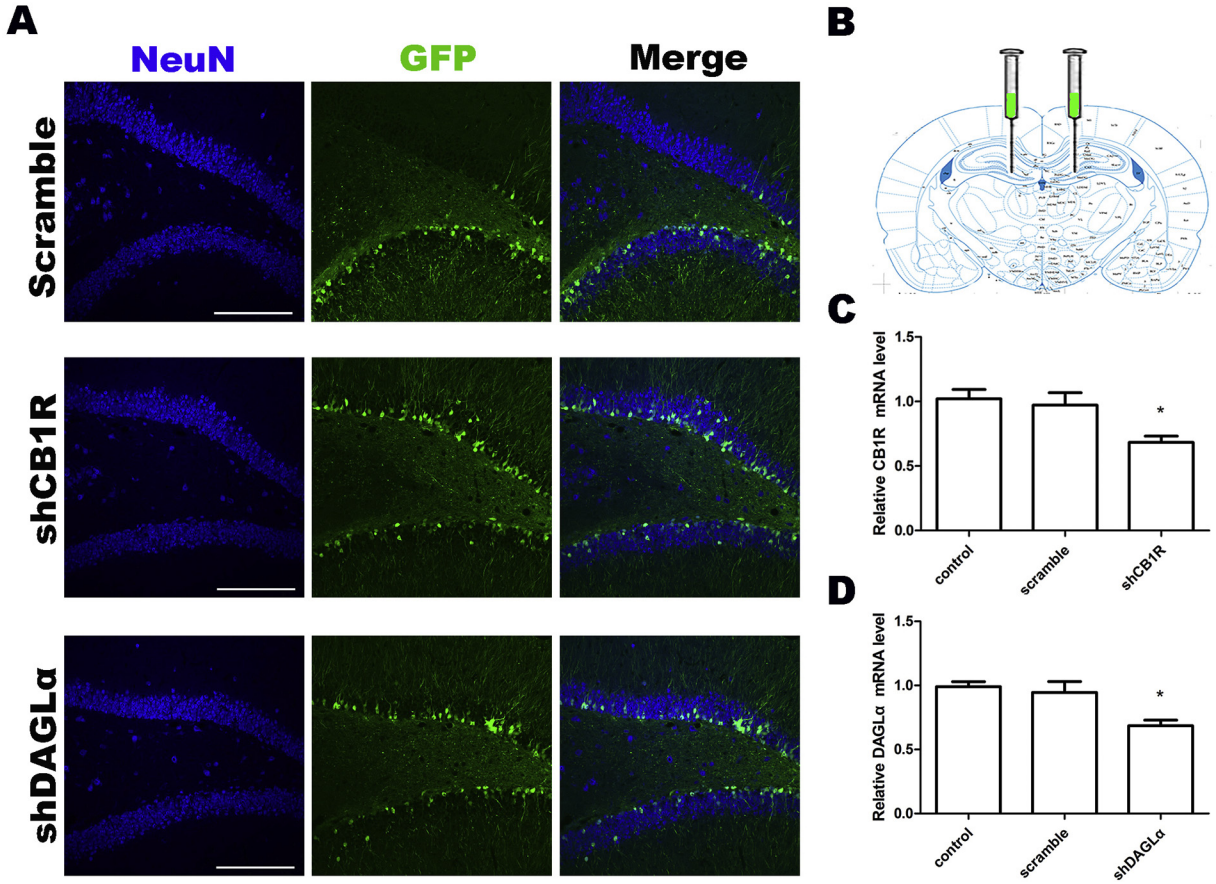

Fig. 3. *CB1R* and *DAGLα* mRNAs were down-regulated in the dentate gyrus of rats. (A) Photomicrographs of NeuN (blue) and lentivirus infection (green) signals in the dentate gyrus. (B) Illustration of bilateral injections of lentivirus in the dentate gyrus of rats. (C) Histograms showing the levels of *CB1R* and *DAGLα* mRNAs in the hippocampus of rats after lentivirus infection. Bar: 200 μm. *$P < 0.05$ *vs.* scramble. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and incubated at 37 °C with 5% CO_2 until confluency was achieved. After 10 days of culture, astrocyte-enriched cultures were obtained by shaking mixed glial cultures at a speed of 240 rpm to remove impurities. Astrocyte identity was confirmed by positive immunostaining for glial fibrillary acidic protein (GFAP) (Fig. 6A).

Primary hippocampal neurons were cultured as described previously (Nan et al. 2019). Briefly, the hippocampus was obtained from SD rat embryos (embryonic day 18) and dissociated in Hank's Balanced Salt Solution containing 10 mg/mL DNase I (Sigma-Aldrich, St. Louis, MO, USA) and incubated in 0.025% trypsin-EDTA for 20 min at 37 °C. The mixture was inactivated with DMEM/F12 supplemented with 10% FBS and centrifuged at 1000 *g* for 5 min. The cells were re-suspended in serum-free neurobasal medium supplemented with 2% B27 supplement and 2 mM GlutaMAX. Cells were plated directly onto 6-well plates pretreated with 0.5 mg/mL poly-L-lysine (Sigma-Aldrich) and incubated at 37 °C and 5% CO_2. Half the volume of the medium was exchanged with fresh medium twice weekly. Neuronal identity was confirmed by positive immunostaining for beta III tubulin (Tuj1) (Fig. 7A).

4.2. 2.8 Stereotaxic surgery and microinjections

Rats were anesthetized and submitted to stereotaxic surgery for microinjection of lentivirus according to previously published protocols (Uzakov et al. 2015). The coordinates of the injection sites were determined, and holes were made in the skull manually using a hand-drill (RWD, China). Subsequently, lentiviruses targeting *CB1R* or *DAGLα* mRNAs were stereotaxically microinjected (1×10^8 TU/mL, 2.5 μL per side) into the dentate gyrus (AP –3.0 mm; L ± 1.8 mm; H 3.6 mm from dura) by an automatic nanoinjector at a rate of 0.25 μL/min. The non-targeting shRNA lentiviral vector (scramble) was used as a control. The syringe needle was left in position for 5 min after delivery to prevent reflux. After 2 weeks, *CB1R* and *DAGLα* mRNA were assessed by RT-qPCR.

4.3. 2.9 RT qPCR analysis

Total RNA was isolated from cells or tissues using the RNAiso Plus kit (Takara, Shiga, Japan) and then reverse transcribed using the PrimeScript RT Reagent Kit (Takara). cDNA was quantified using qPCR with SYBR Premix ExTaq (Takara). The primers used for qPCR were as follows: GAPDH, forward and reverse, 5′- CCA ATG TGT CCG TCG TGG ATC T-3′ and 5′-GTT GAA GTC GCA GGA GAC AAC C-3′, respectively; CB1R, forward and reverse, 5'-CTG AGG GTT CCC TCC CGG CA-3′ and 5'-TGC TGG GAC CAA CGG GGA GT-3′, respectively; DAGLα, forward and reverse, 5'-CAC GAG ATG CTA CGC TAC AAA GA-3′ and 5′- GGC AGA GAC AAC ACG AGC A-3′, respectively; MAGL, forward and reverse, 5'-CGG AAC AAG TCG GAG GTT GA-3′ and 5′-TGT CCT GAC TCG GGG ATG AT-3′, respectively; FAAH, forward and reverse, 5'-GCC CTT CAG AGA GCA GCT CT-3′ and 5′-CTT TTC AGC TGA CCG AGG AC-3′, respectively; NAPE-PLD, forward and reverse, 5'-CGG CTA TTC CCA TTG GAG CTT A-3′ and 5′-GCT TGA ACG TCA ATG TGA ATC CTT A-3′, respectively.

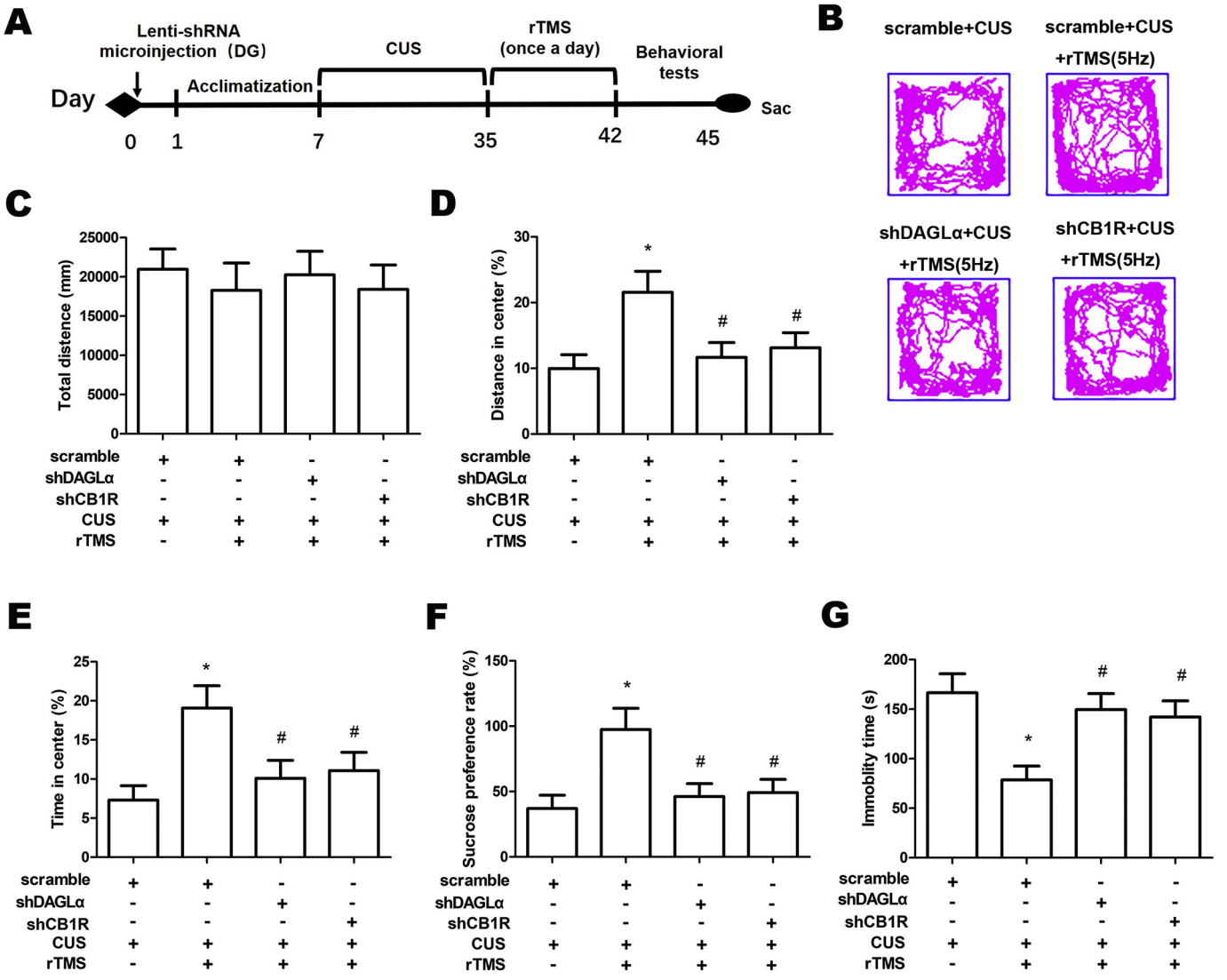

Fig. 4. *CB1R* or *DAGLα* knockdown in the adult hippocampus inhibits the protective effects of 5 Hz rTMS treatment on CUS-treated rats. (A) Timeline for Experiment II. (B) Real-time movement traces in the open field. (C) Quantification of total distance traveled in the open field. (D) Percentages of distance traveled in the center of the open field test. (E) Percentages of time spent in the center of the open field test. (F) Percentages of sucrose consumption in the sucrose preference test. (G) Immobility in the forced swimming test (FST). $^{*}P < 0.05$ *vs.* scramble + CUS; $^{**}P < 0.01$ *vs.* scramble + CUS; $^{\#}P < 0.05$ *vs.* scramble + CUS + rTMS; $^{\#\#}P < 0.01$ *vs.* scramble + CUS + rTMS.

4.4. 2.10 immunohistochemistry

Three weeks after virus injection, rats were deeply anesthetized with chloralhydrate solution (40 mg/kg) and then perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4). Brains were removed and further fixed in 4% paraformaldehyde overnight and then transferred to 30% sucrose in PBS. Coronal sections of the entire hippocampus (16 μm) were prepared using a cryostat. For immunofluorescence detection of GFP, brain coronal sections (16 μm) were incubated with primary antibody against NeuN (a neuronal marker) (ab177487, 1:500, Abcam, Cambridge, UK) at 4 °C overnight after blocking with 5% (*w/v*) bovine serum albumin (Sigma-Aldrich; Merck KGaA), followed by incubation with the corresponding Alexa Fluor 405 goat anti-rabbit IgG (A-31556, 1:1000, Invitrogen, Thermo Fisher Scientific) for 2 h at room temperature (23 °C). Astrocytes and neurons were fixed in 4% paraformaldehyde at 4 °C for 0.5 h. After washes in PBS, the cells were incubated with mouse anti-GFAP (ab7260, 1:1000, Abcam) or mouse anti-Tuj1 (ab78078, 1:500, Abcam) diluted in buffer (1% w/v bovine serum albumin and 0.3% Triton in PBS), overnight at 4 °C. The cells were washed and incubated with fluorescent secondary antibodies (Alexa Fluor 594 donkey anti-mouse IgG, R37119, 1:1000, Invitrogen) for 2 h and then incubated with DAPI for 20 min to stain cellular nuclei. All images were captured using an Olympus FV1200 confocal laser-scanning microscope (Olympus, Tokyo, Japan).

4.5. 2.11 Western blot analysis

Western blot analysis was performed according to the standard protocol (Molecular Cloning, Edition II). Tissues were lysed in a buffer composed of 62.5 mM Tris-HCl, 2% w/v SDS, 10% glycerol, 50 mM dithiothreitol, and 0.1% w/v bromophenol blue. Protein concentrations were determined using BCA Protein Assay Kit (Invitrogen; Thermo Fisher Scientific, Inc.). Then, samples were separated by SDS-PAGE and transferred onto a PVDF membrane (40 μg of total protein per lane). After blocking with 5% non-fat dried milk, the membrane was incubated with following primary antibodies: anti-CB1R (ab23703, 1:300, Abcam, Cambridge, UK), anti-DAGLα (ab81984, 1:500, Abcam, Cambridge, UK), anti-PSD95 (ab18258, 1:1000, Abcam, Cambridge, UK), anti-SYN (ab6326, 1:1000, Abcam, Cambridge, UK), anti-NAPE-PLD (ab95397, 1:200, Abcam, Cambridge, UK), anti-MAGL (ab24701,

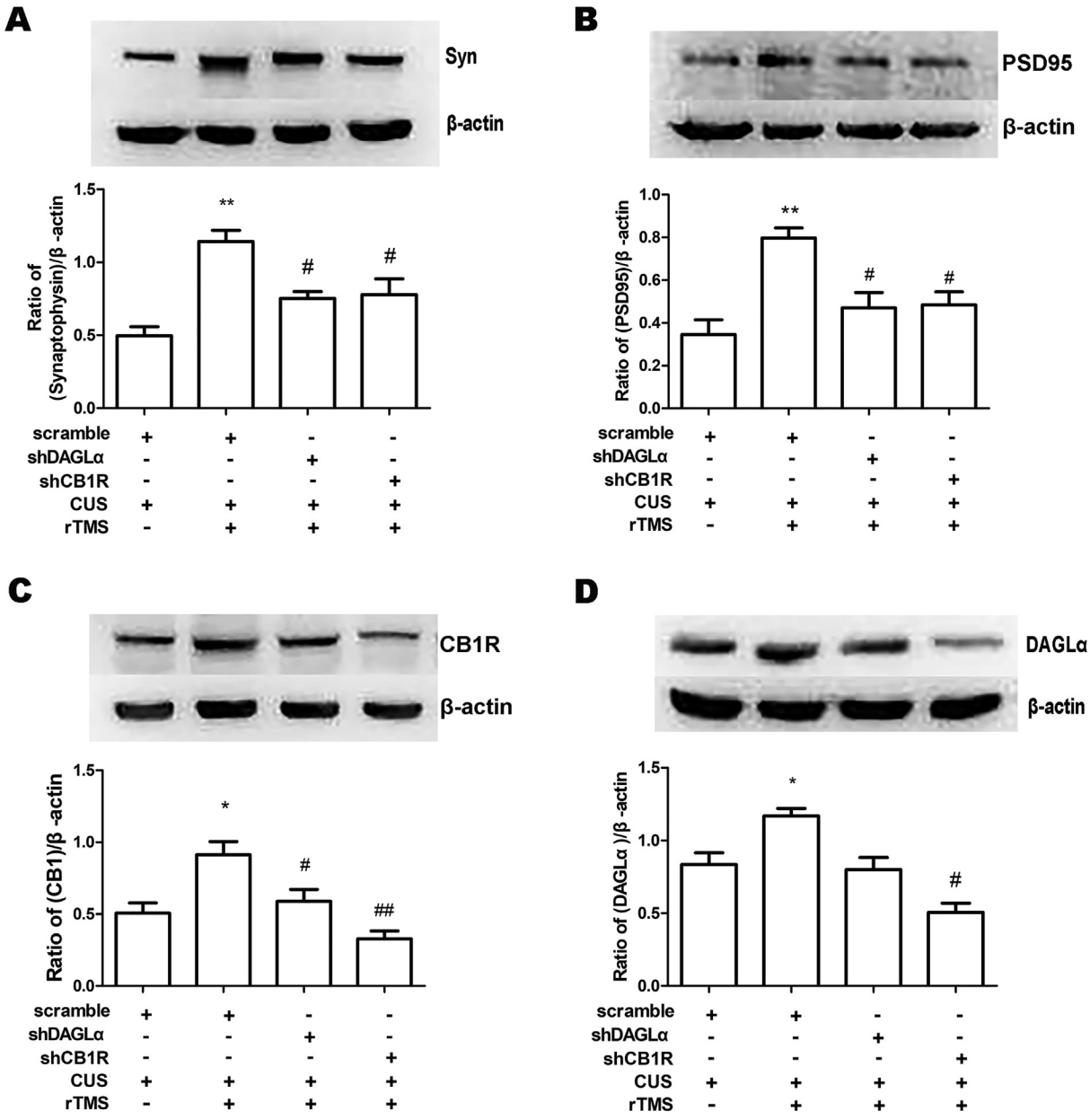

Fig. 5. Knockdown of *CB1R* or *DAGLα* with short hairpin RNA lentivirus in the hippocampus inhibits the effect of 5 Hz rTMS stimulation on protein expression in CUS-treated rats. Representative immunoblots and densitometry analysis histograms represent the expression level of (A) SYN, (B) PSD95, (C) CB1R, (D) DAGLα in the total hippocampus of scramble + CUS, scramble + CUS + rTMS, shCB1R + CUS + rTMS and shDAGLα + CUS + rTMS groups. $^{*}P < 0.05$ *vs.* scramble + CUS; $^{**}P < 0.01$ *vs.* scramble + CUS; $^{\#}P < 0.05$ *vs.* scramble + CUS + rTMS; $^{\#\#}P < 0.01$ *vs.* scramble + CUS + rTMS.

1:2000, Abcam, Cambridge, UK), anti-FAAH (ab54615, 1:500, Abcam, Cambridge, UK) and anti-β-actin antibodies (ab8227, 1:5000, Abcam, Cambridge, UK). Subsequently, the membranes were incubated with secondary antibodies (donkey anti-rabbit IgG, 1:10,000, Abcam; donkey anti-mouse IgG, 1:10,000, Abcam, Cambridge, UK) for 1 h at room temperature. The signals were visualized on Super Signal West Pico Chemiluminescent Substrate (34,077; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and analyzed with Bio-Rad QuantityOne1-D Analysis Software (1,709,600; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

4.6. 2.12 statistical analyses

All data are presented as mean ± standard deviation and were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. $P < 0.05$ was considered statistically significant.

5. 3. Results

5.1. 3.1 5 Hz rTMS treatment ameliorated depressive-like behaviors

As shown in Fig. 1, we observed no differences in the total distance traveled in the OFT among the groups ($F_{3,\ 44} = 0.2615$, $P = 0.8527$; Fig. 1B, C), demonstrating that CUS and rTMS treatment did not impair motor ability in rats. However, significant differences were observed in distance traveled in the center ($F_{3,\ 44} = 5.737$, $P < 0.01$; Fig. 1B, D) and time spent in the center of the OFT ($F_{3,\ 44} = 4.098$, $P < 0.05$; Fig. 1B, E). Significant differences were also observed in sucrose preference in the SPT ($F_{3,\ 44} = 4.426$, $P < 0.05$; Fig. 1F) and immobility times in the FST ($F_{3,\ 44} = 3.819$, $P < 0.05$; Fig. 1G). CUS significantly reduced mean sucrose preference in the SPT and significantly increased mean immobility time in the FST (CUS *vs.* sham, $P < 0.05$). In contrast, 5 Hz rTMS treatment ameliorated depressive-like behaviors in CUS-

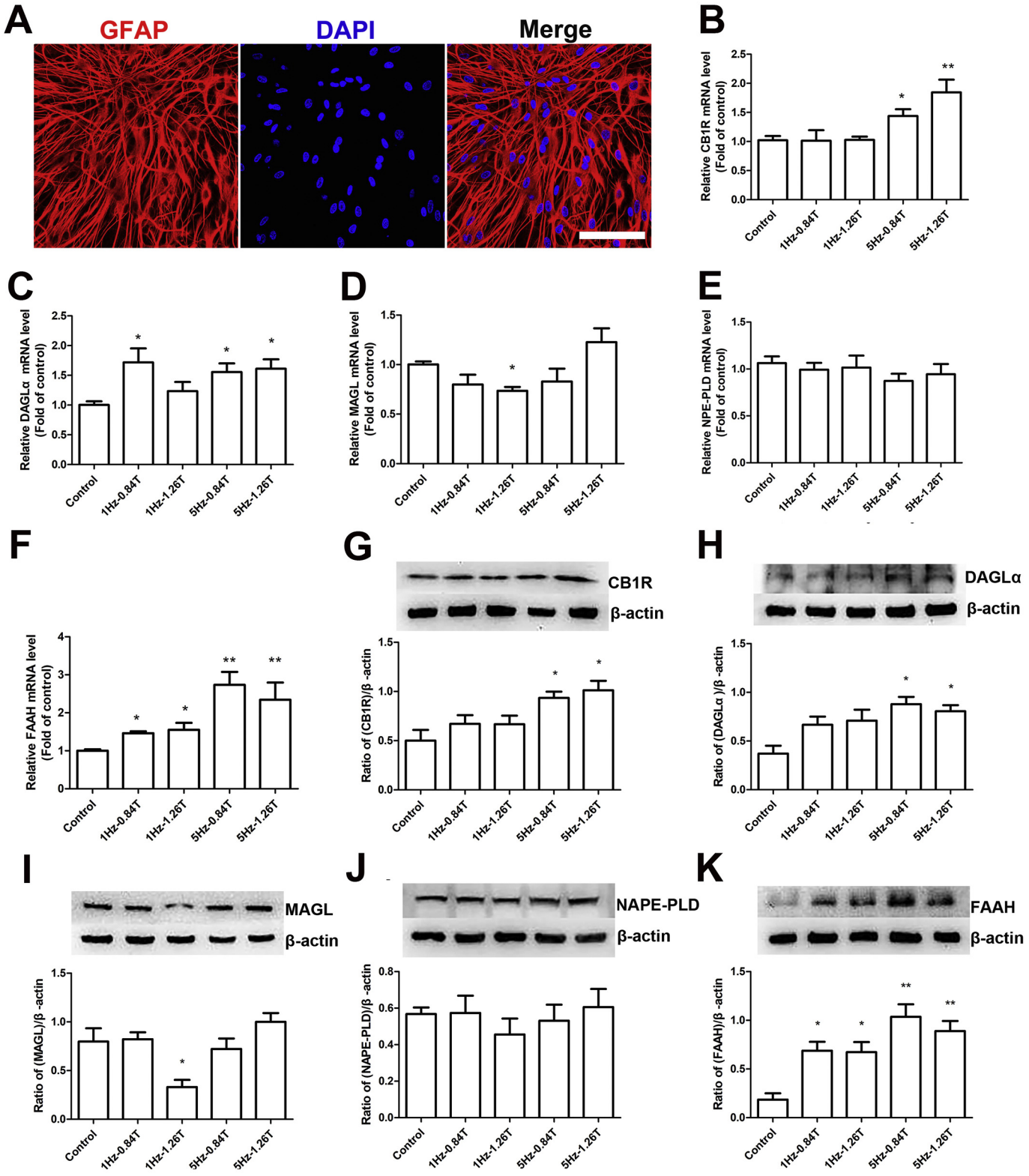

Fig. 6. Repetitive magnetic stimulation modulates endocannabinoid-related gene expression in hippocampal-derived astrocytes *in vitro*. (A) Representative micrographs of GFAP immunocytochemical staining for astrocyte identity. (B—F) The mRNA levels of *CB1R, DAGLα, MAGL, NAPE-PLD* and *FAAH* after repetitive magnetic stimulation. (G-K) Representative immunoblots bands and densitometry analysis of protein levels of CB1R, DAGLα, MAGL, NAPE-PLD and FAAH proteins. Bar: 100 μm.$^{*}P < 0.05$ *vs.* Control; $^{**}P < 0.01$ *vs.* Control.

treated rats, as evidenced by increased sucrose preference in the SPT (CUS *vs.* CUS + rTMS-5 Hz, $P < 0.05$) and decreased immobility time in the FST (CUS *vs.* CUS + rTMS-5 Hz, $P < 0.05$). There were no significant differences between CUS + rTMS-1 Hz and CUS groups in sucrose preference and immobility time in the FST ($P > 0.05$).

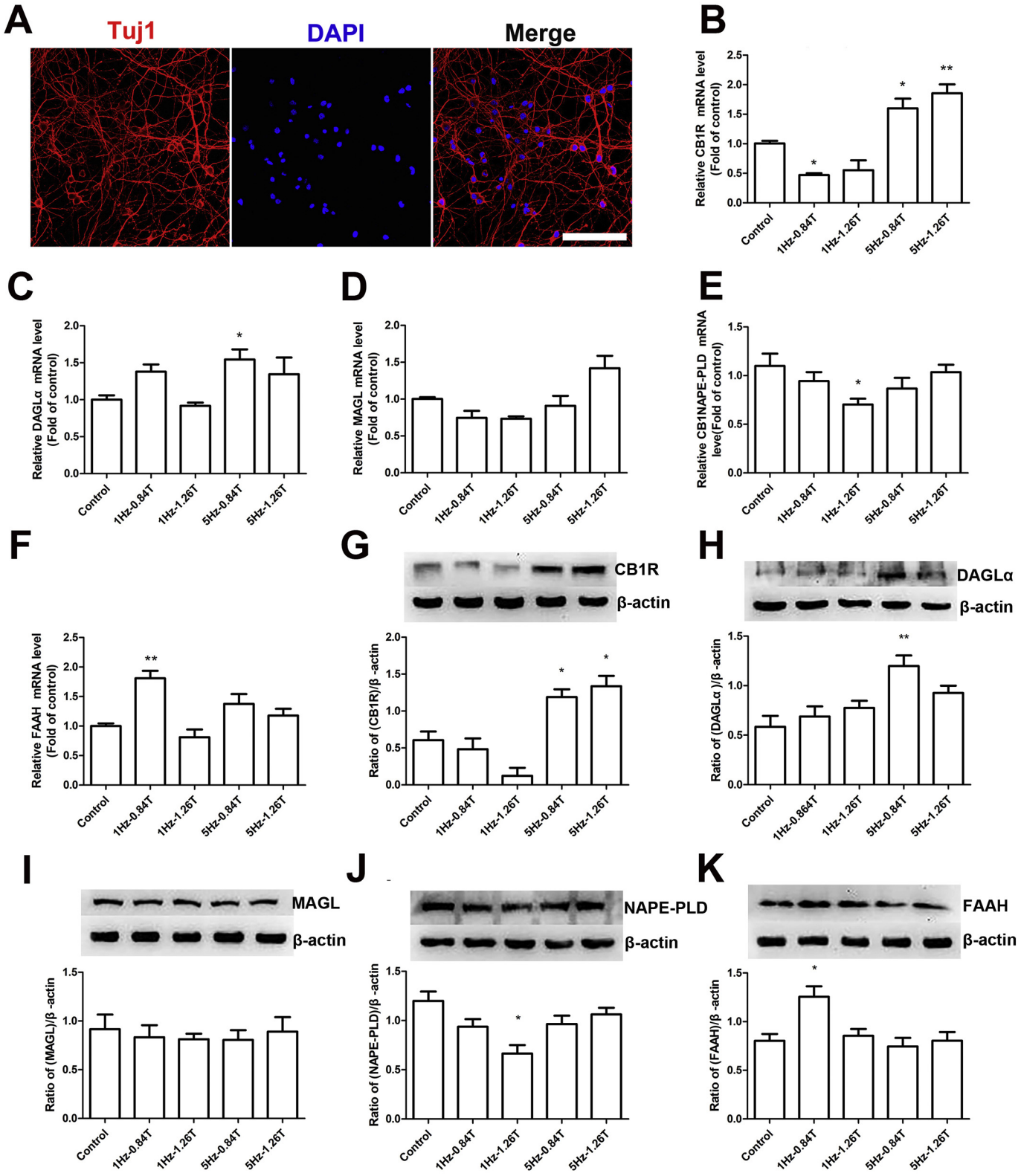

Fig. 7. Repetitive magnetic stimulation modulates endocannabinoid-related gene expression in hippocampal-derived neurons *in vitro*. (A) Representative micrographs of Tuj1 immunocytochemical staining for neuron identity. (B–F) The mRNA levels of *CB1R, DAGLα, MAGL, NAPE-PLD* and *FAAH* after repetitive magnetic stimulation. (G-K) Representative immunoblot bands and densitometry analysis of protein levels of CB1R, DAGLα, MAGL, NAPE-PLD and FAAH proteins. Bar: 100 μm. $^{*}P < 0.05$ *vs.* Control; $^{**}P < 0.01$ *vs.* Control.

5.2. 3.2 5 Hz rTMS reversed CUS-induced changes to synaptic proteins and regulates CB1R, DAGLα, MAGL, NAPE-PLD, and FAAH expression in the hippocampus

As shown in Fig. 2, there were significant differences between the four groups in the expression levels of SYN ($F_{3,44} = 6.299$, $P < 0.01$, Fig. 2A) and PSD95 ($F_{3,44} = 6.309$, $P < 0.01$, Fig. 2B) proteins in the hippocampus. Compared with rats in the sham group, rats receiving CUS stimulation had significantly decreased PSD95 ($P < 0.05$) and SYN ($P < 0.01$) protein levels, while 5 Hz rTMS treatment effectively reversed these changes (CUS + rTMS-5 Hz *vs.* CUS, $P < 0.05$). There were significant differences among the four groups in the expression levels of CB1R ($F_{3,44} = 6.362$, $P < 0.01$, Fig. 2C), DAGLα ($F_{3,44} = 5.719$, $P < 0.01$, Fig. 2D), MAGL ($F_{3,44} = 5.037$, $P < 0.05$, Fig. 2E), NAPE-PLD ($F_{3,44} = 5.295$, $P < 0.01$, Fig. 2F) and FAAH ($F_{3,44} = 11.72$, $P < 0.01$, Fig. 2G) in the hippocampus. CUS significantly decreased the protein levels of CB1R, DAGLα, and NAPE-PLD (sham *vs.* CUS, $P < 0.01$), while 5 Hz rTMS treatment significantly increased levels of these proteins in CUS-treated rats (CUS + rTMS-5 Hz *vs.* CUS, $P < 0.05$). Moreover, CUS significantly increased MAGL and FAAH (sham *vs.* CUS, $P < 0.05$) and 5 Hz rTMS significantly corrected these expression changes (CUS + rTMS-5 Hz *vs.* CUS, $P < 0.05$). No significant differences were observed between CUS + rTMS-1 Hz and CUS group in the expression levels of the above mentioned proteins.

5.3. 3.3 Hippocampal CB1R or DAGLα knockdown inhibited the antidepressant effects of rTMS

As shown in Fig. 3, to elucidate the contribution of ECs to the effects of rTMS treatment in CUS-treated rats, we knocked-down the mRNAs of CB1R and 2-AG synthetase DAGLα in the dentate gyrus (DG) of the hippocampus using bilateral injections of LV-GFP shRNA (shCB1R, shDAGLα or scramble, Fig. 3A and B). Lentivirus carrying shRNA and GFP successfully infected the dentate gyrus and *CB1R* (scramble *vs.* shCB1R, Fig. 3C) and *DAGLα* (scramble *vs.* shDAGLα, Fig. 3D) mRNA levels were effectively down-regulated.

As shown in Fig. 4, no significant differences in the total distance traveled in the open-field arena were observed among groups ($F_{3,44} = 0.1710$, $P = 0.9151$, Fig. 4B and C), suggesting that the lentivirus injection did not affect motor function. However, significant differences in distance traveled in the center ($F_{3,44} = 4.095$, $P < 0.05$, Fig. 4D) and time spent in the center ($F_{3,44} = 4.614$, $P < 0.01$, Fig. 4E) of the OFT, sucrose preference in the SPT ($F_{3,44} = 3.694$, $P < 0.05$, Fig. 4F), and immobility times in the FST ($F_{3,44} = 5.131$, $P < 0.01$, Fig. 4G) were observed. Meanwhile, 5 Hz rTMS treatment (scramble + CUS + rTMS group) successfully reversed all the changes associated with CUS. The anti-depressant effects of rTMS were inhibited by *CB1R* or *DAGLα* mRNA knockdown.

5.4. 3.4 Protective effects of rTMS on hippocampal synaptic proteins are inhibited by downregulation of CB1R or DAGLα

As shown in Fig. 5, shCB1R effectively down-regulated CB1R protein ($F_{3,44} = 10.27$, $P < 0.01$, Fig. 5C) and shDAGLα effectively down-regulated DAGLα protein ($F_{3,44} = 14.37$, $P < 0.01$, Fig. 5D) levels in the hippocampus and eliminated the effects of 5 Hz rTMS treatment on the expression of SYN ($F_{3,44} = 11.74$, $P < 0.01$, Fig. 5A) and PSD95 ($F_{3,44} = 9.342$, $P < 0.01$, Fig. 5B) in CUS-treated rats. Compared with those in the scramble + CUS + rTMS group, protein levels of PSD95 and SYN were down-regulated in the shCB1R + CUS + rTMS group (shCB1R + CUS + rTMS *vs.* scramble + CUS + rTMS, $P < 0.05$) and shDAGLα + CUS + rTMS group (shDAGLα + CUS + rTMS *vs.* scramble + CUS + rTMS, $P < 0.05$).

6. 3.5 rMS modulates ECS-related gene expression in hippocampal cells *in vitro*

To investigate the effects of rMS on cells, primary cultured astrocytes and neurons were treated with rMS using different stimulus parameters *in vitro*. As shown in Fig. 6, there were significant differences between the groups with different stimulus parameters in the expression of CB1R ($F_{4,35} = 6.117$, $P < 0.01$, Fig. 6B; $F_{4,35} = 5.477$, $P < 0.05$, Fig. 6G), DAGLα ($F_{6,49} = 3.393$, $P < 0.05$, Fig. 6C; $F_{4,35} = 5.255$, $P < 0.05$, Fig. 6H), MAGL ($F_{4,35} = 4.068$, $P < 0.05$, Fig. 6D; $F_{4,35} = 6.305$, $P < 0.01$, Fig. 6I) and FAAH ($F_{4,35} = 8.669$, $P < 0.01$, Fig. 6F; $F_{4,35} = 10.20$, $P < 0.01$, Fig. 6K). However, there were no significant differences in the expression of NAPE-PLD ($F_{4,35} = 0.5884$, $P = 0.6761$, Fig. 6E; $F_{6,49} = 0.4649$, $P = 0.7604$, Fig. 6J). Compared with that in the control group, the expression level of CB1R increased in astrocytes stimulated with 5 Hz/0.84 T and 5 Hz/1.26 T ($P < 0.05$); the expression level of DAGLα increased markedly in astrocytes stimulated with 1 Hz/0.84 T, 5 Hz/0.84 T and 5 Hz/1.26 T ($P < 0.05$) and the expression level of MAGL decreased in astrocytes stimulated with 1 Hz/1.26 T ($P < 0.05$). Moreover, the expression level of FAAH increased markedly in astrocytes stimulated with 1 Hz/0.84 T, 1 Hz/1.26 T, 5 Hz/0.84 T and 5 Hz/1.26 T ($P < 0.05$).

Furthermore, we evaluated the effects of magnetic stimulation on ECS-related gene expression in primary cultured neurons. As shown in Fig. 7, there were significant differences between groups in expression of CB1R ($F_{4,35} = 23.53$, $P < 0.01$, Fig. 7B; $F_{4,35} = 16.59$, $P < 0.01$, Fig. 7G), DAGLα ($F_{4,35} = 4.193$, $P < 0.01$, Fig. 7C; $F_{4,35} = 6.397$, $P < 0.01$, Fig. 7H), MAGL ($F_{4,35} = 6.837$, $P < 0.01$, Fig. 7D; $F_{4,35} = 0.1626$, $P = 0.9526$, Fig. 7I), NAPE-PLD ($F_{4,35} = 4.615$, $P < 0.05$, Fig. 7E; $F_{4,35} = 5.686$, $P < 0.05$, Fig. 7J), and FAAH ($F_{4,35} = 9.688$, $P < 0.01$, Fig. 7F; $F_{4,35} = 5.78$, $P < 0.01$, Fig. 7K). Compared with that in the control group, the expression level of CB1R decreased in neurons stimulated with 1 Hz/0.84 T ($P < 0.05$) but increased in neurons stimulated with 5 Hz/0.84 T and 5 Hz/1.26 T ($P < 0.05$); that of DAGLα increased in neurons stimulated with 5 Hz/0.84 T ($P < 0.05$) and that of MAGL was not affected by magnetic stimulation. Moreover, the expression level of FAAH increased after stimulated with 1 Hz/0.84 T ($P < 0.05$) and that of NAPE-PLD decreased after stimulated with 1 Hz/1.26 T ($P < 0.05$).

7. 4. Discussion

The present study investigated the effects of 5 Hz or 1 Hz rTMS on depressive-like behaviors and expression of hippocampal synapse-related proteins in CUS-treated rats, as well as the influence of rMS (5 or 1 Hz, 0.84 or 1.26 T) on the expression of DAGLα, MAGL, NAPE-PLD, FAAH, and CB1R in hippocampal-derived astrocytes and neurons. We also investigated whether the activation of the ECS, specifically CB1R and DAGLα, was involved in the antidepressant effects of rTMS. The major findings of our study are as follows: (1) 5 Hz but not 1 Hz rTMS ameliorated depressive-like behaviors induced by CUS; (2) the expression of PSD95, SYN, NAPE-PLD, CB1R, and DAGLα increased in the hippocampus of CUS-treated rats following 5 Hz rTMS treatment; conversely, the expression of MAGL and FAAH decreased after rTMS treatment; (3) CB1R and DAGLα knockdown by shRNA in the hippocampus eliminated the benefits of 5 Hz rTMS; (4) 5 Hz rMS elevated the expression of DAGLα and CB1R in hippocampal-derived astrocytes and neurons. These data indicate that the activation of the ECS in the hippocampus is involved in the antidepressant effects of 5 Hz rTMS treatment.

The hippocampus regulates emotion and susceptibility to chronic stress through its connections with the amygdala and limbic hypothalamic-pituitary-adrenal axis (Joshi et al. 2016). Previous studies suggest variations in structural and synaptic plasticity of the hippocampus reflect clinical state and are related to treatment responses in depression (Bannerman et al. 2014; Duman et al. 2016). For instance, a decrease in

hippocampal volume has been reported in first-episode depression, especially in chronically-depressed patients (MacQueen et al. 2003; McKinnon et al. 2009). Further, these effects are influenced by the number of prior depressive episodes (Videbech and Ravnkilde 2004). Existing data suggest that pharmacotherapy protects against disease-related hippocampal volume reductions. Restoration of hippocampal synaptic plasticity may thus be a cellular mechanism underlying the beneficial effects of antidepressants and enriched environments on depression-associated cognitive deficits (Betry et al. 2015; Mahati et al. 2016). The regulation of plasticity in the hippocampus by rTMS at different frequencies has been reported previously (Lenz et al. 2016). A recent study suggests that high frequency rTMS (5 Hz) facilitates spatial cognition and synaptic plasticity in the hippocampus of Wistar rats (Shang et al. 2016), and lateralized hippocampal volume was increased following high frequency left prefrontal rTMS in patients with major depression (Hayasaka et al. 2017). In addition, high frequency rTMS ameliorated depressive-like behaviors and inhibited hippocampal neuronal apoptosis in a rat model of depression (Zhao et al. 2018). Furthermore, low frequency rTMS enhanced spatial learning and synaptic plasticity in a rat model of vascular dementia (Yang et al. 2015) and modulated hippocampal structural synaptic plasticity including SYN, Growth associated protein-43, and PSD95 in aging mice (Ma et al. 2014). Low frequency rTMS (0.5 Hz, 15 days) also increased hippocampal expression of SYN relative to that in normal rats (Li et al. 2019). Considering these observations, we selected CUS as a model of depression and evaluated the anti-depressant effects and protein expression of PSD95 and SYN in the hippocampus—which are two synaptic markers involved in modulation of synaptic strength and plasticity (Schnell et al. 2002) following different frequencies of rTMS treatment. Our data revealed that 5 Hz rTMS ameliorated CUS-induced decreases in distance traveled in the central area and time spent in the central area in the OFT, sucrose preference in the SPT, and increased immobility time in the FST. In addition, the expression of PSD95 and SYN was decreased in CUS-treated rats, and these changes were reversed by 5 Hz rTMS. In contrast, depressive-like behaviors and decreased expression of PSD95 and SYN were unchanged following 1 Hz rTMS treatment, suggesting that 5 Hz rTMS is an effective therapy for depression, and its beneficial effects are related to protecting hippocampal plasticity.

Growing evidence suggests that hippocampal synaptic plasticity is regulated by the ECS (Wilson and Nicoll 2001). 2-AG is produced almost exclusively by DAGLα and mediates retrograde synaptic suppression in the central nervous system (Tanimura et al. 2010). Moreover, 2-AG plays an important role in the stress response and negative feedback (Di et al. 2016). Importantly, blocking 2-AG hydrolysis with the MAGL inhibitor JZL184 produced antidepressant-like effects and enhanced adult hippocampal neurogenesis and synaptic plasticity in CUS-treated mice (Zhang et al. 2015). AEA, another endogenous ligand, is also involved in hippocampal synaptic plasticity (Kim and Alger 2010). Consistent with these results, our data revealed that 5 Hz rTMS but not 1 Hz reversed CUS-induced decrease in expression of PSD95, Syn, NAPE-PLD, DAGLα, and CB1R, as well as CUS-induced increase in expression of MAGL and FAAH in the hippocampus. Furthermore, microinjections of DAGLα/CB1R-shRNA in the DG before rTMS inhibited its protective effects on hippocampal plasticity and abolished its antidepressant-like effects, suggesting that 2-AG generation and CB1R activation play a crucial role in the antidepressant effects of 5 Hz rTMS. In contrast, recent work indicates that CB2Rs mediate cell type-specific self-inhibitory plasticity in CA3/CA2 which acts complementary to presynaptic CB1R (Stempel et al. 2016). However, the present study did not investigate the effects of rTMS on the activation of CB2R or the involvement of AEA in the anti-depressant effects of 5 Hz rTMS, which would provide greater insight into the validity of our hypothesis.

The biological effects of rTMS are dependent on the parameters, duration of treatment, and experimental design (Galletly et al. 2012; Herrmann and Ebmeier 2006). A previous study reported that high-frequency rTMS was more effective than low-frequency rTMS for treating depression (Speer et al. 2009) and increased *SYN* mRNA transcription when compared with low-frequency rTMS in the rat brain (Lee et al. 2014). However, it was observed that both high-frequency and low-frequency rTMS yielded significant antidepressant effects in a genetic rat model of depression (Hesselberg et al. 2016). In contrast, another study reported that low-frequency rTMS enhanced structural synaptic plasticity, but high-frequency stimulation decreased the survival rate of hippocampal neurons (Grehl et al. 2015). Nevertheless, our data indicated that the antidepressant effects of 5 Hz rTMS superseded those of 1 Hz rTMS and were related to the regulation of DAGLα/CB1R expression in the hippocampus. The biological effects of other parameters of rTMS, such as intensity and duration of treatment, require further investigation. Furthermore, the present study revealed that with the same treatment duration and stimulus times, rMS at 5 Hz elevated the expression of DAGLα and CB1R in hippocampal-derived astrocytes and neurons. Thus, we speculate that different frequencies and intensities may induce different biological effects on ECS activation, and 5 Hz rMS may be more suitable than 1 Hz rMS for the activation of the ECS. Nevertheless, the precise mechanism underpinning this is elusive and necessitates further investigation.

In conclusion, we demonstrated that 5 Hz but not 1 Hz rTMS treatment ameliorated depressive-like behaviors and impairments in expression of hippocampal synapse-related proteins after exposure to CUS in rats. In addition, 5 Hz rTMS increased the expression of NAPE-PLD, CB1R, and DAGLα; and decreased the expression of MAGL and FAAH in the hippocampus of CUS-treated rats. Moreover, 5 Hz rMS, especially at the intensity of 0.84 T, elevated the expression of DAGLα and CB1R in hippocampal-derived astrocytes and neurons. Notably, microinjections of shDAGLα or shCB1R in the hippocampus before 5 Hz rTMS abolished its therapeutic effects. However, the effects of rTMS on the activation of CB2R and the direct influence of DAGLα or CB1R knockout on depressive-like behaviors remain unclear. Further studies are required to explore the detailed signaling cascades underlying the regulation of the ECS after rTMS treatment.

Conflicts of interest

The authors declare no biomedical conflict of interest.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (grant numbers 81630032 and 81571309), National Science and Technology Support Program of China (grant number 2015BAI13B00).

References

Bannerman, D.M., Sprengel, R., Sanderson, D.J., McHugh, S.B., Rawlins, J.N., Monyer, H., Seeburg, P.H., 2014. Hippocampal synaptic plasticity, spatial memory and anxiety. Nat. Rev. Neurosci. 15 (3), 181–192.

Betry, C., Etievant, A., Pehrson, A., Sanchez, C., Haddjeri, N., 2015. Effect of the multimodal acting antidepressant vortioxetine on rat hippocampal plasticity and recognition memory. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 58, 38–46.

Brunoni, A.R., Chaimani, A., Moffa, A.H., Razza, L.B., Gattaz, W.F., Daskalakis, Z.J., Carvalho, A.F., 2017. Repetitive transcranial magnetic stimulation for the acute treatment of major depressive episodes: a systematic review with network meta-analysis. JAMA Psychiatry 74 (2), 143–152.

Chen, Y.-h., Zhang, R.-g., Xue, F., Wang, H.-n., Chen, Y.-c., Hu, G.-t., Peng, Y., Peng, Z.-w., Tan, Q.-r., 2015. Quetiapine and repetitive transcranial magnetic stimulation ameliorate depression-like behaviors and up-regulate the proliferation of hippocampal-derived neural stem cells in a rat model of depression: the involvement of the BDNF/ERK signal pathway. Pharmacol. Biochem. Behav. 136, 39–46.

Coccaro, E.F., Hill, M.N., Robinson, L., Lee, R.J., 2018. Circulating endocannabinoids and affect regulation in human subjects. Psychoneuroendocrinology 92, 66–71.

Daly, E.J., Singh, J.B., Fedgchin, M., Cooper, K., Lim, P., Shelton, R.C., Thase, M.E., Winokur, A., Van Nueten, L., Manji, H., Drevets, W.C., 2018. Efficacy and safety of intranasal Esketamine adjunctive to Oral antidepressant therapy in treatment-resistant depression: a randomized clinical trial. JAMA Psychiatry 75 (2), 139–148.

Di, S., Itoga, C.A., Fisher, M.O., Solomonow, J., Roltsch, E.A., Gilpin, N.W., Tasker, J.G.,

2016. Acute stress suppresses synaptic inhibition and increases anxiety via endocannabinoid release in the basolateral amygdala. J. Neurosci. 36 (32), 8461–8470.
Duman, R.S., Aghajanian, G.K., Sanacora, G., Krystal, J.H., 2016. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat. Med. 22 (3), 238–249.
Eryilmaz, G., Sayar, G.H., Ozten, E., Gul, I.G., Yorbik, O., Isiten, N., Bagci, E., 2015. Follow-up study of children whose mothers were treated with transcranial magnetic stimulation during pregnancy: preliminary results. Neuromodulation 18 (4), 255–260.
Esser, S.K., Huber, R., Massimini, M., Peterson, M.J., Ferrarelli, F., Tononi, G., 2006. A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. Brain Res. Bull. 69 (1), 86–94.
Fang, G., Wang, Y., 2018. Effects of rTMS on hippocampal endocannabinoids and depressive-like behaviors in adolescent rats. Neurochem. Res. 43 (9), 1756–1765.
Felipe, R.M., Ferrao, Y.A., 2016. Transcranial magnetic stimulation for treatment of major depression during pregnancy: a review. Trends Psychiatry Psychother 38 (4), 190–197.
Ferrao, Y.A., da Silva, R.M.F., 2018. Repetitive transcranial magnetic stimulation for the treatment of major depression during pregnancy. Braz J Psychiatr 40 (2), 227–228.
Fitzgerald, P.B., Fountain, S., Daskalakis, Z.J., 2006. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin. Neurophysiol. 117 (12), 2584–2596.
Fitzgerald, P.B., Hoy, K.E., Elliot, D., McQueen, S., Wambeek, L.E., Chen, L., Clinton, A.M., Downey, G., Daskalakis, Z.J., 2018. A pilot study of the comparative efficacy of 100 Hz magnetic seizure therapy and electroconvulsive therapy in persistent depression. Depress Anxiety 35 (5), 393–401.
Galletly, C., Gill, S., Clarke, P., Burton, C., Fitzgerald, P.B., 2012. A randomized trial comparing repetitive transcranial magnetic stimulation given 3 days/week and 5 days/week for the treatment of major depression: is efficacy related to the duration of treatment or the number of treatments? Psychol. Med. 42 (5), 981–988.
Grehl, S., Viola, H.M., Fuller-Carter, P.I., Carter, K.W., Dunlop, S.A., Hool, L.C., Sherrard, R.M., Rodger, J., 2015. Cellular and molecular changes to cortical neurons following low intensity repetitive magnetic stimulation at different frequencies. Brain stimulation 8 (1), 114–123.
Griebel, G., Stemmelin, J., Lopez-Grancha, M., Fauchey, V., Slowinski, F., Pichat, P., Dargazanli, G., Abouabdellah, A., Cohen, C., Bergis, O.E., 2018. The selective reversible FAAH inhibitor, SSR411298, restores the development of maladaptive behaviors to acute and chronic stress in rodents. Sci. Rep. 8 (1), 2416.
Haesebaert, F., Moirand, R., Schott-Pethelaz, A.M., Brunelin, J., Poulet, E., 2018. Usefulness of repetitive transcranial magnetic stimulation as a maintenance treatment in patients with major depression. World J Biol Psychiatry 19 (1), 74–78.
Hayasaka, S., Nakamura, M., Noda, Y., Izuno, T., Saeki, T., Iwanari, H., Hirayasu, Y., 2017. Lateralized hippocampal volume increase following high-frequency left prefrontal repetitive transcranial magnetic stimulation in patients with major depression. Psychiatry Clin. Neurosci. 71 (11), 747–758.
Herrmann, L.L., Ebmeier, K.P., 2006. Factors modifying the efficacy of transcranial magnetic stimulation in the treatment of depression: a review. J Clin Psychiatry 67 (12), 1870–1876.
Hesketh, S.A., Brennan, A.K., Jessop, D.S., Finn, D.P., 2008. Effects of chronic treatment with citalopram on cannabinoid and opioid receptor-mediated G-protein coupling in discrete rat brain regions. Psychopharmacology 198 (1), 29–36.
Hesselberg, M.L., Wegener, G., Buchholtz, P.E., 2016. Antidepressant efficacy of high and low frequency transcranial magnetic stimulation in the FSL/FRL genetic rat model of depression. Behav. Brain Res. 314, 45–51.
Hill, M.N., Ho, W.S., Sinopoli, K.J., Viau, V., Hillard, C.J., Gorzalka, B.B., 2006. Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology 31 (12), 2591–2599.
Jennings, E.M., Okine, B.N., Olango, W.M., Roche, M., Finn, D.P., 2016. Repeated forced swim stress differentially affects formalin-evoked nociceptive behaviour and the endocannabinoid system in stress normo-responsive and stress hyper-responsive rat strains. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 64, 181–189.
Joshi, S.H., Espinoza, R.T., Pirnia, T., Shi, J., Wang, Y., Ayers, B., Leaver, A., Woods, R.P., Narr, K.L., 2016. Structural plasticity of the Hippocampus and amygdala induced by electroconvulsive therapy in major depression. Biol. Psychiatry 79 (4), 282–292.
Karyotaki, E., Riper, H., Twisk, J., Hoogendoorn, A., Kleiboer, A., Mira, A., Mackinnon, A., Meyer, B., Botella, C., Littlewood, E., Andersson, G., Christensen, H., Klein, J.P., Schroder, J., Breton-Lopez, J., Scheider, J., Griffiths, K., Farrer, L., Huibers, M.J., Phillips, R., Gilbody, S., Moritz, S., Berger, T., Pop, V., Spek, V., Cuijpers, P., 2017. Efficacy of self-guided internet-based cognitive behavioral therapy in the treatment of depressive symptoms: a meta-analysis of individual participant data. JAMA Psychiatry 74 (4), 351–359.
Kedzior, K.K., Schuchinsky, M., Gerkensmeier, I., Loo, C., 2017. Challenges in comparing the acute cognitive outcomes of high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) vs. electroconvulsive therapy (ECT) in major depression: a systematic review. J. Psychiatr. Res. 91, 14–17.
Kim, J., Alger, B.E., 2010. Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. Nat. Neurosci. 13 (5), 592–600.
Lee, S.A., Oh, B.M., Kim, S.J., Paik, N.J., 2014. The molecular evidence of neural plasticity induced by cerebellar repetitive transcranial magnetic stimulation in the rat brain: a preliminary report. Neurosci. Lett. 575, 47–52.
Lenz, M., Galanis, C., Muller-Dahlhaus, F., Opitz, A., Wierenga, C.J., Szabo, G., Ziemann, U., Deller, T., Funke, K., Vlachos, A., 2016. Repetitive magnetic stimulation induces plasticity of inhibitory synapses. Nat. Commun. 7, 10020.
Li, Y., Li, L., Pan, W., 2019. Repetitive transcranial magnetic stimulation (rTMS) modulates hippocampal structural synaptic plasticity in rats. Physiol. Res. 68 (1), 99–105.
Liu, L., Zhang, Q., Cai, Y., Sun, D., He, X., Wang, L., Yu, D., Li, X., Xiong, X., Xu, H., Yang, Q., Fan, X., 2016. Resveratrol counteracts lipopolysaccharide-induced depressive-like behaviors via enhanced hippocampal neurogenesis. Oncotarget 7 (35), 56045–56059.
Liu, G., Feng, D., Wang, J., Zhang, H., Peng, Z., Cai, M., Yang, J., Zhang, R., Wang, H., Wu, S., Tan, Q., 2018. rTMS ameliorates PTSD symptoms in rats by enhancing glutamate transmission and synaptic plasticity in the ACC via the PTEN/Akt Signalling pathway. Mol. Neurobiol. 55 (5), 3946–3958.
Liu, M.Y., Yin, C.Y., Zhu, L.J., Zhu, X.H., Xu, C., Luo, C.X., Chen, H., Zhu, D.Y., Zhou, Q.G., 2018. Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat. Protoc. 13 (7), 1686–1698.
Lomazzo, E., Konig, F., Abassi, L., Jelinek, R., Lutz, B., 2017. Chronic stress leads to epigenetic dysregulation in the neuropeptide-Y and cannabinoid CB1 receptor genes in the mouse cingulate cortex. Neuropharmacology 113 (Pt A), 301–313.
Lu, H.C., Mackie, K., 2016. An introduction to the endogenous cannabinoid system. Biol. Psychiatry 79 (7), 516–525.
Ma, J., Zhang, Z., Kang, L., Geng, D., Wang, Y., Wang, M., Cui, H., 2014. Repetitive transcranial magnetic stimulation (rTMS) influences spatial cognition and modulates hippocampal structural synaptic plasticity in aging mice. Exp. Gerontol. 58, 256–268.
MacQueen, G.M., Campbell, S., McEwen, B.S., Macdonald, K., Amano, S., Joffe, R.T., Nahmias, C., Young, L.T., 2003. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc. Natl. Acad. Sci. U. S. A. 100 (3), 1387–1392.
Mahati, K., Bhagya, V., Christofer, T., Sneha, A., Shankaranarayana Rao, B.S., 2016. Enriched environment ameliorates depression-induced cognitive deficits and restores abnormal hippocampal synaptic plasticity. Neurobiol. Learn. Mem. 134 (Pt B), 379–391.
March, J., Silva, S., Petrycki, S., Curry, J., Wells, K., Fairbank, J., Burns, B., Domino, M., McNulty, S., Vitiello, B., Severe, J., Treatment for Adolescents With Depression Study, T, 2004. Fluoxetine, cognitive-behavioral therapy, and their combination for adolescents with depression: treatment for adolescents with depression study (TADS) randomized controlled trial. JAMA 292 (7), 807–820.
McKinnon, M.C., Yucel, K., Nazarov, A., MacQueen, G.M., 2009. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J. Psychiatry Neurosci. 34 (1), 41–54.
Mello, P.G., Silva, G.R., Donat, J.C., Kristensen, C.H., 2013. An update on the efficacy of cognitive-behavioral therapy, cognitive therapy, and exposure therapy for posttraumatic stress disorder. Int. J. Psychiatry Med. 46 (4), 339–357.
Morena, M., Patel, S., Bains, J.S., Hill, M.N., 2016. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology 41 (1), 80–102.
Nan, K., Han, Y., Fang, Q., Huang, C., Yu, L., Ge, W., Xiang, F., Tao, Y.X., Cao, H., Li, J., 2019. HMGB1 gene silencing inhibits neuroinflammation via down-regulation of NF-kappaB signaling in primary hippocampal neurons induced by Abeta25-35. Int. Immunopharmacol. 67, 294–301.
Ota, K.T., Liu, R.J., Voleti, B., Maldonado-Aviles, J.G., Duric, V., Iwata, M., Dutheil, S., Duman, C., Boikess, S., Lewis, D.A., Stockmeier, C.A., DiLeone, R.J., Rex, C., Aghajanian, G.K., Duman, R.S., 2014. REDD1 is essential for stress-induced synaptic loss and depressive behavior. Nat. Med. 20 (5), 531–535.
Peng, Z.W., Xue, F., Zhou, C.H., Zhang, R.G., Wang, Y., Liu, L., Sang, H.F., Wang, H.N., Tan, Q.R., 2018. Repetitive transcranial magnetic stimulation inhibits Sirt1/MAO-A signaling in the prefrontal cortex in a rat model of depression and cortex-derived astrocytes. Mol. Cell. Biochem. 442 (1–2), 59–72.
Richieri, R., Guedj, E., Michel, P., Loundou, A., Auquier, P., Lancon, C., Boyer, L., 2013. Maintenance transcranial magnetic stimulation reduces depression relapse: a propensity-adjusted analysis. J. Affect. Disord. 151 (1), 129–135.
Schnell, E., Sizemore, M., Karimzadegan, S., Chen, L., Bredt, D.S., Nicoll, R.A., 2002. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc. Natl. Acad. Sci. U. S. A. 99 (21), 13902–13907.
Shang, Y., Wang, X., Shang, X., Zhang, H., Liu, Z., Yin, T., Zhang, T., 2016. Repetitive transcranial magnetic stimulation effectively facilitates spatial cognition and synaptic plasticity associated with increasing the levels of BDNF and synaptic proteins in Wistar rats. Neurobiol. Learn. Mem. 134 (Pt B), 369–378.
Smaga, I., Bystrowska, B., Gawlinski, D., Pomierny, B., Stankowicz, P., Filip, M., 2014. Antidepressants and changes in concentration of endocannabinoids and N-acylethanolamines in rat brain structures. Neurotox. Res. 26 (2), 190–206.
Smaga, I., Jastrzebska, J., Zaniewska, M., Bystrowska, B., Gawlinski, D., Faron-Gorecka, A., Broniowska, Z., Miszkiel, J., Filip, M., 2017a. Changes in the brain endocannabinoid system in rat models of depression. Neurotox. Res. 31 (3), 421–435.
Smaga, I., Zaniewska, M., Gawlinski, D., Faron-Gorecka, A., Szafranski, P., Cegla, M., Filip, M., 2017b. Changes in the cannabinoids receptors in rats following treatment with antidepressants. Neurotoxicology 63, 13–20.
Speer, A.M., Benson, B.E., Kimbrell, T.K., Wassermann, E.M., Willis, M.W., Herscovitch, P., Post, R.M., 2009. Opposite effects of high and low frequency rTMS on mood in depressed patients: relationship to baseline cerebral activity on PET. J. Affect. Disord. 115 (3), 386–394.
Stempel, A.V., Stumpf, A., Zhang, H.Y., Ozdogan, T., Pannasch, U., Theis, A.K., Otte, D.M., Wojtalla, A., Racz, I., Ponomarenko, A., Xi, Z.X., Zimmer, A., Schmitz, D., 2016. Cannabinoid type 2 receptors mediate a cell type-specific plasticity in the Hippocampus. Neuron 90 (4), 795–809.
Tanimura, A., Yamazaki, M., Hashimotodani, Y., Uchigashima, M., Kawata, S., Abe, M., Kita, Y., Hashimoto, K., Shimizu, T., Watanabe, M., Sakimura, K., Kano, M., 2010. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. Neuron 65 (3), 320–327.
Uzakov, S.S., Ivanov, A.D., Salozhin, S.V., Markevich, V.A., Gulyaeva, N.V., 2015. Lentiviral-mediated overexpression of nerve growth factor (NGF) prevents beta-

amyloid [25-35]-induced long term potentiation (LTP) decline in the rat hippocampus. Brain Res. 1624, 398–404.
Valverde, O., Torrens, M., 2012. CB1 receptor-deficient mice as a model for depression. Neuroscience 204, 193–206.
Videbech, P., Ravnkilde, B., 2004. Hippocampal volume and depression: a meta-analysis of MRI studies. Am. J. Psychiatry 161 (11), 1957–1966.
Wang, H.N., Wang, L., Zhang, R.G., Chen, Y.C., Liu, L., Gao, F., Nie, H., Hou, W.G., Peng, Z.W., Tan, Q., 2014. Anti-depressive mechanism of repetitive transcranial magnetic stimulation in rat: the role of the endocannabinoid system. J. Psychiatr. Res. 51, 79–87.
Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410 (6828), 588–592.
Xue, F., Xue, S.S., Liu, L., Sang, H.F., Ma, Q.R., Tan, Q.R., Wang, H.N., Zhou, C.H., Peng, Z.W., 2019. Early intervention with electroacupuncture prevents PTSD-like behaviors in rats through enhancing hippocampal endocannabinoid signaling. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 93, 171–181.
Yang, H.Y., Liu, Y., Xie, J.C., Liu, N.N., Tian, X., 2015. Effects of repetitive transcranial magnetic stimulation on synaptic plasticity and apoptosis in vascular dementia rats. Behav. Brain Res. 281, 149–155.
Zhang, Z., Wang, W., Zhong, P., Liu, S.J., Long, J.Z., Zhao, L., Gao, H.Q., Cravatt, B.F., Liu, Q.S., 2015. Blockade of 2-arachidonoylglycerol hydrolysis produces antidepressant-like effects and enhances adult hippocampal neurogenesis and synaptic plasticity. Hippocampus 25 (1), 16–26.
Zhao, L., Ren, H., Gu, S., Li, X., Jiang, C., Li, J., Zhang, M., Mu, J., Li, W., Wang, W., Zhang, Z., Song, J., 2018. rTMS ameliorated depressive-like behaviors by restoring HPA axis balance and prohibiting hippocampal neuron apoptosis in a rat model of depression. Psychiatry Res. 269, 126–133.
Zhong, P., Wang, W., Pan, B., Liu, X., Zhang, Z., Long, J.Z., Zhang, H.T., Cravatt, B.F., Liu, Q.S., 2014. Monoacylglycerol lipase inhibition blocks chronic stress-induced depressive-like behaviors via activation of mTOR signaling. Neuropsychopharmacology 39 (7), 1763–1776.