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# Anti-depressive mechanism of repetitive transcranial magnetic stimulation in rat: The role of the endocannabinoid system

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ABSTRACT

Repetitive transcranial magnetic stimulation (rTMS) to treat depression has been thoroughly investigated in recent years. However, the underlying mechanisms are not fully understood. In this study, a chronic unpredictable mild stress (CUMS) paradigm was applied to male Sprague Dawley rats. Then rTMS was performed for 7 consecutive days, and the anti-depressive effects were evaluated by the sucrose preference test (SPT), the forced swimming test (FST), and the open-field test (OFT). Hippocampal cannabinoid type I receptor (CB1) expression was measured, and the expression levels of brain-derived neurotrophic factor (BDNF), Bcl-2, and Bax and the number of bromodeoxyuridine (BrdU)-positive cells were also investigated. These parameters were also observed after the selective CB1 receptor antagonist AM251 was used as a blocking agent. The results showed that CUMS induced a significant decrease in sucrose preference, a significant increase in immobility time in the FST, and a significantly decreased horizontal distance in the OFT. In addition, reduced hippocampal CB1 receptor, BDNF, and Bcl-2/Bax protein expression levels in CUMS rats, as well as decreased cell proliferation were also observed in the dentate gyrus. Meanwhile, rTMS treatment up-regulated cell proliferation; elevated CB1 receptor, BDNF, and Bcl-2/Bax expression levels in the hippocampus; and ameliorated depressive-like behaviors. All of these beneficial effects were abolished by AM251. These results indicate that rTMS increases BDNF production and hippocampal cell proliferation to protect against CUMS-induced changes through its effect on CB1 receptors.



## 1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a promising noninvasive treatment for a variety of neuropsychiatric conditions; the number of applications continues to increase, with a large number of ongoing clinical trials in a variety of diseases (Devlin and Watkins, 2007; George et al., 2007). One of the first clinical uses of rTMS and its only Food and Drug Administration-approved therapeutic indication is high-frequency stimulation to the left dorsolateral prefrontal cortex for the treatment of medication-resistant depression (O'Reardon et al., 2007; Padberg and George, 2009). However, the underlying mechanisms regarding how rTMS can alter mood are not completely understood. Paralleling our limited understanding of the mechanism of rTMS, its therapeutic efficacy, while statistically significant, also remains limited.

The basic principle of rTMS is that magnetic pulses enter the brain unimpeded and depolarize neurons in the area under the magnetic coil, and this exerts distant effects in networks connected to the stimulation site (Berlim et al., 2012; Hallett, 2007). By inducing electric currents in brain tissue with a time-varying strong magnetic field, rTMS has the potential to modulate neuronal circuits. There is evidence that rTMS can activate both cortical neurons and more distal cells via transsynaptic connections (Post and Keck, 2001). Regarding the mechanism of depression, there are several

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biological aspects that should be considered to understand the mechanism of TMS, including various aspects of stress biology, immune function disruptions, neural structure, and function, as well as circadian rhythms (George et al., 1996). Evidence from animal studies suggests that rTMS affects neurotransmitter systems, including dopamine (DA), 5-hydroxytryptamine (5-HT), glutamate, and gamma-aminobutyric acid (GABA); stress-induced activity of the hypothalamic–pituitary–adrenal (HPA) system; and neurotrophic signaling factors, such as brain-derived neurotrophic factor (BDNF) (Keck et al., 2000; Wang et al., 2011a; Yue et al., 2009). Clinical studies have also examined the effects of rTMS on neurotransmitter systems and endocrinological responses in the HPA system in depressed patients (Baeken et al., 2009, 2011). Although many studies have explored the mechanism of action of rTMS, the details of its clinical effects remain need to be fully elucidated. In particular, a review of clinical studies indicates that rTMS has most extensively applied as an adjunctive treatment for refractory depression and pharmacoresistant depression (Benadhira et al., 2005; Demitrack and Thase, 2009; Janicak et al., 2010). This suggests that it might act via a pathway besides those altered by current antidepressant medications.

Recent pharmacological and genetic findings indicate that the endocannabinoid system may be involved in the pathophysiology of depression (Hill and Gorzalka, 2005a). This is supported by several pieces of evidence showing that endocannabinoids and CB1 receptor are widely distributed in brain areas that are often related to affective disorders (Devane et al., 1988), and the density of endocannabinoids and CB1 receptor binding site is decreased in a number of brain regions in chronic unpredictable stress (CUS) model of depression (Ballmaier et al., 2007; Hill et al., 2005, 2008a). In addition, the expression of endocannabinoids and CB1 receptor is regulated by antidepressant drugs (Hill et al., 2008b), and administration of inhibitors of anandamide uptake or metabolism, as well as CB1 receptor agonists induces antidepressant-like effects in different animal models (Adamczyk et al., 2008; Hill and Gorzalka, 2005b). On the other hand, rimonabant, a CB1 receptor antagonist, has been reported that it linked to increase risk of anxiety, depression and suicidal thoughts in the treatment of obesity disorders in humans (Le Foll et al., 2009; Moreira et al., 2009). In addition, basic research found that pretreatment with electroacupuncture induced rapid tolerance to cerebral ischemia through regulation of endocannabinoid system (Wang et al., 2011b), it suggests that endocannabinoid system might be involved in the biological effect of physical therapy. However, little is known about the effects of rTMS treatments on endocannabinoid system in the depressive animal models. Based on these findings, the present study investigated whether the CB1 receptor in the hippocampus is involved in the mediation of the antidepressant-like effect of rTMS in a rat model of depression.

## 2. Materials and methods

### 2.1. Ethics

Animal maintenance and all experimental procedures described in this study were approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University (FMMU, Xi'an, China) and fully complied with Chinese legislation on research involving animal subjects.

### 2.2. Animals

Adult male Sprague Dawley rats (180–230 g) were used. They were provided by the FMMU Experimental Animal Center and were allowed to habituate for 1 week. Animals were housed four per cage and maintained under standard laboratory conditions (23 ± 1.1 °C, 12-h light/dark cycle with lights switched on at 07:00 hours, and humidity at 40–60%). Unless otherwise specified, the animals were given free access to standard rodent diet and tap water.

### 2.3. Experimental design

#### 2.3.1. Experiment 1

To assess the effects of rTMS treatment on CB1 receptor expression in the CUMS animal model, rats were randomly divided into four groups: Sham, Sham + rTMS, CUMS, and CUMS + rTMS ($n = 9$ each group). The rats in the Sham group were kept in their home cages for the 4-week period and then subjected to Sham rTMS treatments for 7 days, the rats in Sham + rTMS group were kept in their home cages for the 4-week period and then subjected to real rTMS treatments for 7 days, the rats in the CUMS group were subjected to CUMS for 4 weeks and then to Sham rTMS treatments for 7 days, and the animals in the CUMS + rTMS group were subjected to CUMS for 4 weeks and then to real rTMS treatments for 7 days. The behavioral scores were evaluated 24 h after the last rTMS treatment, and then the CB1 receptor protein expression was determined by western blot and immunohistochemical analysis.

#### 2.3.2. Experiment 2

To further elucidate the role of the CB1 receptor in the behavioral effects of rTMS treatment and rTMS-induced hippocampal neuroprotection and neurogenesis, rats were randomly assigned to Sham, CUMS, CUMS + rTMS, CUMS + rTMS + AM251, CUMS + AM251, CUMS + rTMS + Vehicle groups ($n = 9$ each group). The rats in Sham, CUMS, and CUMS + rTMS groups received the same treatments as above; rats in CUMS + rTMS + AM251 group exposed to CUMS and then received AM251 and real rTMS treatments for 7 days; rats in the CUMS + AM251 group received CUMS and AM251 and Sham rTMS treatments for 7 days; and rats in CUMS + rTMS + Vehicle group received CUMS and 0.6 ml/kg Vehicle (DMSO:saline = 1:4) and real rTMS treatments for 7 days. The behavioral scores were evaluated 24 h after the last rTMS treatment, and then the expression levels of proteins reflecting hippocampal neuroprotection and neurogenesis were determined by western blot and immunohistochemical analysis.

### 2.4. rTMS treatment

rTMS was performed mostly as described in our previous studies (Feng et al., 2012). A commercially available stimulator (inner diameter, 2.5 cm; outer diameter, 5 cm; custom-made YIRD, China) was employed. 900 daily magnetic stimulation pulses were applied to the rTMS groups; the daily stimulation was comprised of 15 trains of 60 pulses delivered at 15 Hz (15 s/train) with a 15-s inter-train interval, and the intensity of stimulation represented 100% of the rTMS device's maximum power. The trains of rTMS were administered daily for 7 days (a total of 6, 300 pulses in the experiment). In the rTMS groups, the center of the coil was placed over the vertex of the skull; 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 without brain stimulation (Esser et al., 2006). Rat movement was restricted by hand force during stimulation. Therefore, 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, and the rats gradually adapted to the rTMS procedure. The real and sham rTMS treatments did not produce notable seizures or any behavioral changes during the entire treatment period.