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THE EFFECT OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ON FEAR EXTINCTION IN RATS

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Abstract—Facilitating fear extinction is clinically important to improve the efficacy of current exposure therapies for the treatment of anxiety disorders, such as post-traumatic stress disorder (PTSD). The aim of this study was to determine if repeated transcranial magnetic stimulation (rTMS) facilitates fear extinction in rats, especially when paired with exposure to a conditioned stimulus (CS). Thirty-five rats were conditioned to a tone CS by pairing the tone with an electric foot shock as an aversive unconditioned stimulus (US). We assessed the effects of 10 Hz rTMS before fear extinction (experiment 1) and rTMS paired with CS during extinction (experiment 2) on the following day. Fear responses of the rats were estimated using the level of freezing upon tone stimulus and were compared between the rTMS and corresponding sham groups. The rats treated with rTMS before fear extinction showed no difference in freezing time when compared with the sham group. However, the rats treated with rTMS paired with CS during extinction showed significantly less freezing behavior than the sham group, and this enhancement of fear extinction remained after 24 h without further stimulation. This finding suggests that high-frequency rTMS paired with trauma-reminding stimuli enhances fear extinction and that rTMS in conjunction with exposure therapy is potentially useful for facilitating extinction memory in the treatment of PTSD. © 2011 Published by Elsevier Ltd on behalf of IBRO.

Key words: classical fear conditioning, exposure therapy, fear extinction, post-traumatic stress disorder, therapeutic neuromodulation, transcranial magnetic stimulation.

Fear extinction is defined as the decrease in learned fear response that normally occurs when a conditioned stimulus (CS) is repeatedly presented in the absence of the aversive unconditioned stimulus (US) (Milad and Quirk, 2002; Milad et al., 2006). An organism can learn that the CS no longer signals an aversive outcome through extinction learning and can thus adapt properly to changeable environments in which the meaning of a stimulus varies over time. Conditioned fear is an essential adaptation mechanism for survival that enables an organism to react to various stimuli signaling threats.

The extinction of conditioned fear responses has been of considerable interest because abnormal fear conditioning is implicated in the pathogenesis of anxiety disorders, such as specific phobias and post-traumatic stress disorder (PTSD) (Lissek et al., 2005). Moreover, fear extinction is an analogous model of exposure therapy, one of the most effective psychiatric treatments for anxiety disorders (Davis, 2002; Richardson et al., 2004). Fear extinction, however, appears to occur more slowly and is more fragile than fear conditioning, and a fear response, once extinguished, often returns spontaneously. Patients with anxiety disorders have shown strong acquisition and impaired extinction of fear (Lissek et al., 2005; Milad et al., 2006), which results in dropouts or relapses in a substantial portion of patients treated with conventional exposure therapy (Richardson et al., 2004). Therefore, facilitation of fear extinction is clinically crucial for the effective treatment of anxiety disorders.

One pharmacological intervention used to facilitate fear extinction is d-cycloserine (DCS), a partial agonist of the *N*-methyl-d-aspartate (NMDA) receptor, which is known to enhance the formation of fear extinction memory (Davis, 2002; Walker et al., 2002). Both systemic administration and infusion of DCS to the amygdala were reported to facilitate fear extinction in rodents in a dose-dependent manner (Davis, 2002; Walker et al., 2002; Richardson et al., 2004). Clinical studies using DCS with exposure therapy for anxiety disorders, including specific phobias, social phobias, panic disorder, and obsessive-compulsive disorder, showed increased efficiency of exposure therapy (Ressler et al., 2004; Richardson et al., 2004; Norberg et al., 2008). However, DCS treatment in one preclinical study facilitated reconsolidation of fear memory depending on the parameters of CS exposure, resulting in increased fear response (Lee et al., 2006). The memory-enhancing effect of DCS is not specific to fear extinction; thus, there is a hypothetical risk that DCS may augment fear memory or affect other components of learning and memory in patients, although there has been no report of adverse effects of DCS at low doses so far in clinical studies.

Electrical stimulation of the infralimbic region, a part of the ventromedial prefrontal cortex (vmPFC), was found to facilitate fear extinction in rats until the next day (Milad and Quirk, 2002). Neuronal responses in this region were correlated with the level of success in fear extinction, suggesting long-term fear extinction. Recent functional magnetic resonance imaging studies adopting a similar design found

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Abbreviations: CS, conditioned stimulus; DCS, d-cycloserine; PTSD, post-traumatic stress disorder; rTMS, repetitive TMS; TMS, transcranial magnetic stimulation; US, unconditioned stimulus; vmPFC, ventromedial prefrontal cortex.

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that the vmPFC is involved in fear extinction in humans (Phelps et al., 2004; Milad et al., 2007), and the thickness of the vmPFC in humans was shown to be correlated with extinction memory via structural neuroimaging (Milad et al., 2005). Furthermore, functional neuroimaging studies using symptom-provocation methods showed that the vmPFC activity was attenuated in PTSD patients, compared with that of healthy controls (Milad et al., 2006; Francati et al., 2007). These parallel findings suggest that activation of the vmPFC can be a possible treatment for exaggerated fear memory, such as that characterized in PTSD.

However, electrical stimulation of the brain is invasive and costly for clinical use in treating anxiety disorder patients. Hence, transcranial magnetic stimulation (TMS) has been suggested as a potential candidate for the treatment of dysregulated fear memory (Milad and Quirk, 2002; Milad et al., 2006). TMS is a noninvasive brain stimulation technique that induces electric currents in the brain tissue using a brief, rapidly time-varying magnetic field pulse (magnitude up to 1.0∼4.0 Tesla, rise time ∼100 μs), based on Faraday's electromagnetic induction principle (George and Belmaker, 2000; Hallett, 2000). TMS can be delivered to an awake person without any surgery or anesthesia and has minimal side effects; thus, it has been widely utilized in assessments of cortical physiology, in the functional mapping of the cerebral cortex, and in the treatment of neuropsychiatric diseases such as depression (George and Belmaker, 2000; George et al., 2000; Post and Keck, 2001; O'Reardon et al., 2007). TMS is known to modulate the brain activity of the targeted region and of remote inter-connected areas (George and Belmaker, 2000; Post and Keck, 2001). Repetitive TMS (rTMS) has been found to exert excitatory or inhibitory modulation on brain activity, depending on the stimulation frequency. High-frequency rTMS (>10 Hz) has been posited to increase regional brain activity and facilitate synaptic potentiation, whereas low-frequency rTMS (<1 Hz) downregulates and inhibits brain activity (Kimbrell et al., 1999; George and Belmaker, 2000; Maeda et al., 2000; Speer et al., 2000; Fitzgerald et al., 2006).

rTMS has been investigated as a potential treatment for PTSD in several clinical trials (Grisaru et al., 1998; McCann et al., 1998; Rosenberg et al., 2002; Cohen et al., 2004). Although the use of rTMS has shown clinical improvement compared with the sham group, its clinical effect on PTSD patients was only mild to modest, and findings related to core PTSD symptom improvement are still controversial. One crucial limitation of the previous studies is that they adopted conventional rTMS treatment and did not attempt to combine rTMS with the exposure therapy. Notably, vmPFC stimulation was effective only when paired with exposure to the CS in the rodent experiments, suggesting that combining the trauma-reminding stimuli with rTMS on the vmPFC would be beneficial in carrying out the exposure therapy (Milad and Quirk, 2002; Milad et al., 2006).

Therefore, this study aimed to examine whether rTMS paired with CS enhances fear memory extinction in rats compared with the corresponding sham group. We hypothesized that rTMS is more effective when paired with CS exposure, compared with simple rTMS treatment before fear extinction.

EXPERIMENTAL PROCEDURE

Animals

Thirty-five male Sprague–Dawley rats at 7 weeks of age and weighing 230–250 g were obtained from a commercial supplier (Koatech, Pyoungtaek, South Korea) for use in this study. The rats were housed in groups of three to four in transparent polycarbonate cages located in an environmentally controlled rearing system (22–24 °C, 50–60% humidity) and were maintained on a 12/12-h light/dark cycle with free access to food and water for a week before the experiment. All experimental procedures were conducted according to the guidelines for the care and use of laboratory animals approved by the relevant local government. All efforts were undertaken to minimize the number of animals and their suffering in the present study.

Behavioral experiment apparatus

All behavioral experiments were conducted in the same fear-conditioning chamber (TSE system, Homburg, Germany) consisting of a perspex chamber (460×460×300 mm) and a grid floor. The conditioning chamber was located in a sound-attenuating cabinet with a red ambient light (11 W). Acoustic CSs were delivered through a loud speaker attached under the cabinet ceiling and electrical foot shocks as US through the grid floor connected to a computer-controlled shocker unit (TSE system). The chamber was thoroughly cleaned with 70% aqueous ethanol between each experiment.

Transcranial magnetic stimulation

A Neopulse stimulator (Neotonus Inc., Marietta, GA, USA) and a modified figure-of-eight coil with an iron core (Epstein and Davey, 2002), which was 7 cm in diameter, were used for the rTMS. A single train of rTMS consisted of 50 pulses of stimulation at a frequency of 10 Hz with an intensity of 50% of the maximal machine output, resulting in 0.7 T, which was about 90% of the motor threshold determined by visual inspection of the forelimb movement (Hargreaves et al., 2005). The motor threshold was defined as the machine output resulting in detectable forelimb movement in five trials out of 10, but it might not be same as that measured using the motor-evoked potential.

During the stimulation, the center of the coil was placed approximately 3 mm anterior to bregma and gently touched the scalp. The orientation of the coil was set tangentially to the sagittal axis, and the handle of the coil was oriented in the rostral direction. A sham treatment was performed in the same way, but with the coil tilted at 90 degrees to shunt the magnetic field induced from the coil and mimic the acoustic artifacts of the TMS. Most of the rats exhibited little movement during the restraint, and rats showing strong resistance to the restraint and stimulation (n=3) were excluded from the study.

Experiment 1: simple rTMS

The behavioral experiments consisted of three phases: fear conditioning (day 1), extinction (day 2), and retention test (day 3). The entire scheme of the experimental procedures is depicted in Fig 1.

On day 1, rats were exposed to five tones of CS (30 s; 80 dB in sound pressure level; 4 kHz) for habituation approximately 5 min after being placed in the conditioning chamber. Following this, the same five tones as the CS were presented paired with co-

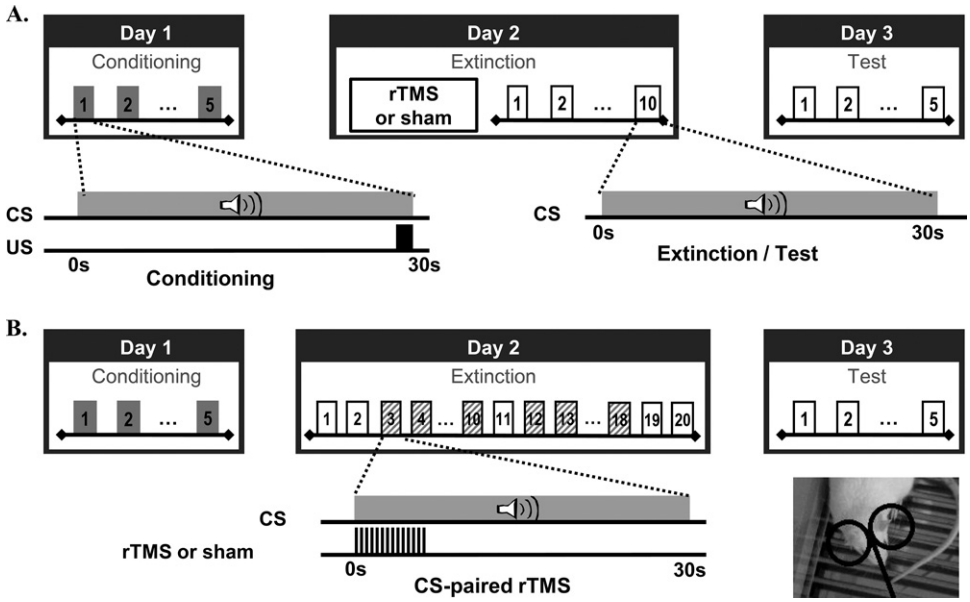

Fig. 1. A schematic illustration of the experimental procedure applied in this study. (A) Experiment 1: the effect of simple rTMS vs. sham treatment before fear extinction. (B) Experiment 2: the effect of CS-paired rTMS vs. CS-paired sham treatment during fear extinction.

terminating electric foot shocks (1 s; 1.0 mA) to generate conditioned fear to the CS. The inter-trial interval was 2~4 min with an average of 3 min throughout the entire experiment.

The following day, the rats were divided into an rTMS and a sham group and were given the rTMS or sham treatment, respectively, 5 min before the extinction process. The rats were restrained in an animal holder made of transparent acryl (Jeung Do Bio & Plant Corporation, Seoul, South Korea), and the head of each rat was aligned to the hole on the upper surface of the holder during the administration of the rTMS or sham treatment (Kim et al., 2006). Twenty trains of rTMS or sham treatments were delivered to each rat with a 25-s inter-train interval (total of 1000 pulses of stimulation over 10 min). Five minutes after the rTMS or sham treatment, the rats were moved to the same conditioning chamber and underwent fear extinction learning. Ten tone CSs were presented without the US during the extinction, and freezing responses to each CS were recorded.

On day 3, the retention test was conducted on rats 24 h after the extinction. Rats were moved to the same conditioning chamber, and five CSs were presented after approximately 5 min.

Experiment 2: CS-paired rTMS

The behavioral experiment consisted of three phases, as in experiment 1: fear conditioning (day 1), extinction (day 2), and a retention test (day 3). In experiment 2, rTMS was given paired with CS presentation during the extinction phase on day 2. The remaining parts of the experimental procedure, including fear conditioning on day 1 and the retention test on day 3, were identical to those of experiment 1.

In the extinction phase on day two, 20 CSs were presented in the same conditioning chamber approximately five minutes after placing rats in the chamber. Because it was not possible to assess the freezing behavior of the rats accurately during the rTMS or sham administration, due to the need for the restraint, 20 CS presentations were divided into five observation trials and 15 stimulation trials. Trials 1, 2, 11, 19, and 20 were the observation trials, in which only the CS was presented to the rats without any restraint for an assessment of the freezing response. The remaining 15 trials (trials 3–9 and 12–18) were the stimulation trials, in which the head of each rat was gently held by the hand of the experimenter, and a train of rTMS or sham was given paired with the onset of the CS. The rats were divided into two groups, an rTMS group and a sham group, and the corresponding treatment was given during the stimulation trials on day 2.

Data analysis

The behavior of the rats during the experiments was recorded with a digital camera, and the freezing behavior in response to the CS was scored off-line using a digital stopwatch. In this study, freezing was defined as a lack of body movement except that caused by respiratory motion and the heartbeat. The amount of freezing in each trial was quantified as the percentage of total freezing time for the duration of the CS (i.e. 30 s). The percentage of freezing response in each group was compared using a two-tailed Student's *t*-test. The overall differences in freezing during each phase between the rTMS and sham groups were also assessed using repeated measures ANOVA.

RESULTS

First, we examined the effect of 10-Hz rTMS administered before extinction learning without pairing with exposure to the CS. Rats were divided into the rTMS and sham groups and underwent fear conditioning, extinction learning, and retention tests on days 1, 2, and 3, respectively. In experiment 2, rTMS was given during the presentation of the CS to rats during extinction, paired with the onset of the tone.

On day 1 of experiment 1, rats in the rTMS ($n=8$) and sham groups ($n=7$) showed no freezing to the CS initially but reached a level of approximately 80% freezing in response to the CS by the end of the conditioning phase (Fig. 2A). The rats in both groups acquired a conditioned fear to the tone, which was initially neutral, and did not differ in their fear response, as determined by repeated measures ANOVA ($P=0.643$).

On day 2, simple rTMS delivered before the extinction did not facilitate fear extinction compared with the sham

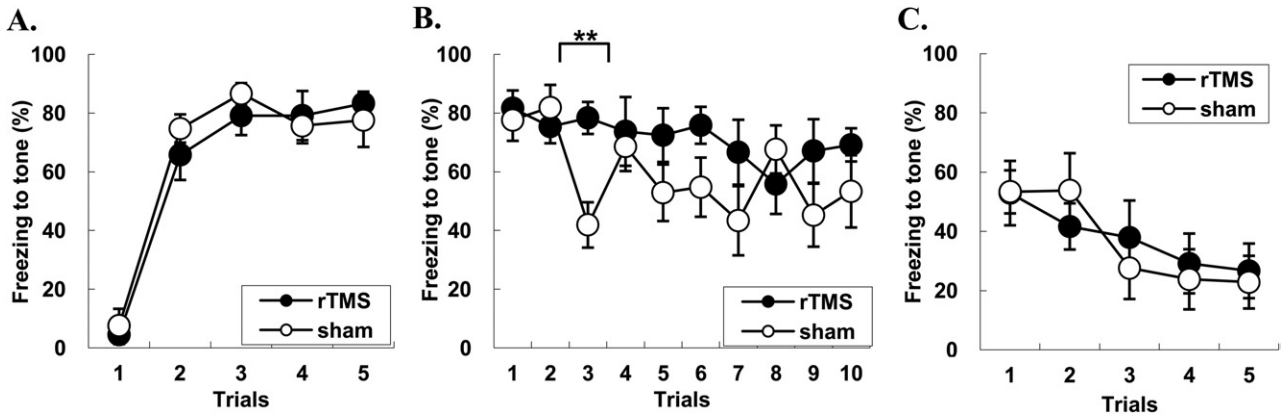

Fig. 2. Experiment 1: the freezing responses of rats treated with rTMS (n=8) vs. sham (n=7) before extinction (mean value±SE). (A) Conditioning phase (day 1). The two groups were not different in the level of freezing responses during fear conditioning. (B) Extinction phase (day 2). rTMS administered before extinction did not enhance fear extinction compared with the sham treatment. Overall freezing behavior was not different (P>0.05, repeated-measure ANOVA). (C) Test phase (day 3). The levels of freezing in response to the CS of both groups were quite similar during the test phase (P>0.50, repeated measure ANOVA). ** represents P<0.01.

treatment. Freezing responses to the CS during the extinction phase were not different between the rTMS and sham groups during the extinction phase, except for trial 3, in which rats in the sham group exhibited significantly less freezing compared with those in the rTMS group (P<0.01) (Fig. 2B). However, the freezing responses of the two groups before and after trial 3 were very similar (repeated measure ANOVA, P=0.077). Simple rTMS did not produce any enhancement in subsequent fear extinction on day 2.

On day 3, two groups were also not significantly different in the freezing response during the test phase (Fig. 2C), indicating no long-term effect on fear extinction (repeated measures ANOVA, P=0.841). Taken together, simple rTMS before extinction learning did not facilitate fear extinction.

In experiment 2, rats in the rTMS (n=9) and sham (n=8) groups acquired an approximate 80% freezing response to the originally neutral tone after fear conditioning on day 1 (Fig. 3A), as in experiment 1. Thus, rats in both groups in experiment 2 had approximately the same level of fear response to the CS during the conditioning phase (repeated measures ANOVA, P=0.991).

On the following day, freezing responses to the CS before the rTMS or sham administration (i.e. trials 1 and 2) were not significantly different between the two groups (trials 1 and 2 in Fig. 3B; P=0.148 and P=0.114, respectively). The CSs were then paired with a single train of rTMS or sham treatment during the stimulation trials (trials 3–10 and 12–18). Rats treated with CS-paired rTMS demonstrated significantly less freezing compared with the CS-paired sham group after 15 extinction trials paired with rTMS (trials 19 and 20 in Fig. 2B; P=0.045 and P=0.046, respectively). This result indicates that 15 trains of rTMS paired with CS enhanced fear extinction compared with the sham treatment.

The rats that received CS-paired rTMS also exhibited a lower level of freezing to the tone 24 h after extinction without further rTMS administration (Fig. 3C). On day 3,

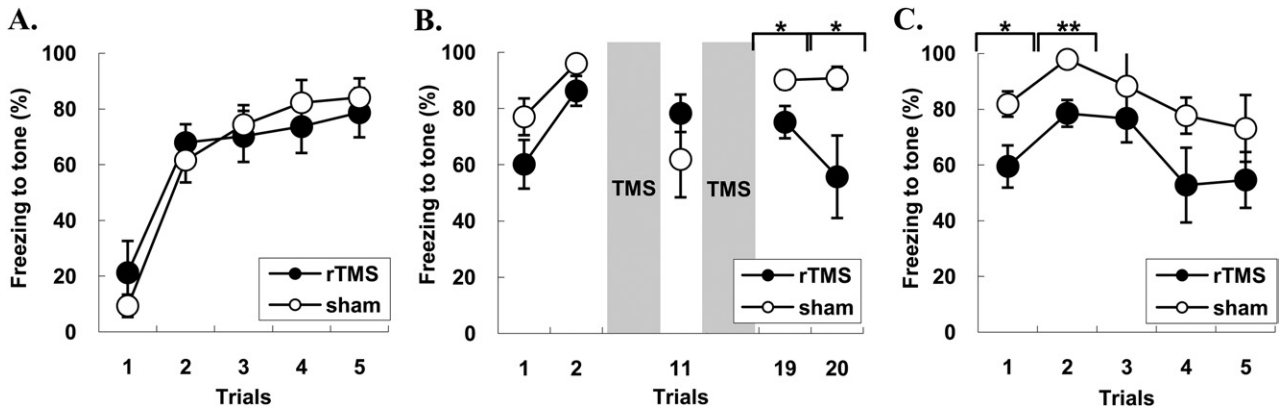

Fig. 3. Experiment 2: the freezing responses of rats treated with CS-paired rTMS (n=9) vs. CS-paired sham treatment (n=8) during extinction. (A) Conditioning phase (day 1). Both groups exhibited approximately the same level of freezing at the end of conditioning. (B) Extinction phase (day 2). Fifteen trains of CS-paired rTMS significantly reduced the freezing to CS compared with the sham treatment. (C) Test phase (day 3). The CS-paired rTMS group showed significantly less freezing than the CS-paired sham group (P<0.05, repeated-measure ANOVA). * represents P<0.05, ** represents P<0.01.

the freezing responses of the CS-paired rTMS group in trials 1 and 2 were significantly lower than those of the CS-paired sham group ($P=0.026$ and $P=0.004$, respectively). The difference in the freezing responses between the CS-paired rTMS and the sham group was not significant in trials 3–5. This result may be due to the relatively large inter-subject variability of freezing within each group during the latter part of the test phase. Rats in the rTMS group exhibited less freezing compared with those in the sham group during all of the testing trials on day 3; additionally, the overall effect was significant (repeated measures ANOVA, $P=0.035$). Taken together, these findings indicate that CS-paired rTMS during extinction learning exhibits the long-term effect of fear extinction.

DISCUSSION

To investigate the efficacy of rTMS as a potential treatment for exaggerated fear memory, this study assessed the effect of 10 Hz rTMS paired with a CS on fear extinction in rats. While simple rTMS given before fear extinction did not enhance extinction learning, rTMS paired with CS significantly facilitated fear extinction. This enhancement in extinction memory continued until the next day without further stimulation, implying the formation of long-term extinction memory. These findings suggest that the mild effect of conventional rTMS as a treatment for PTSD can become considerably effective when paired with a traumatic reminder. To our knowledge, this is the first report on the effect of rTMS on fear extinction with and without CS exposure in rats.

The finding of this study agrees with the results of a previous electrical stimulation study showing that only a stimulation paired with CS was effective in facilitating fear extinction (Milad and Quirk, 2002). CS-paired rTMS significantly accelerated fear extinction, indicating a distinctive effect of rTMS paired with CS presentation. rTMS before extinction learning (i.e. without CS presentation) failed to enhance fear extinction, excluding the possibility that simple rTMS nonspecifically affected the animal brain and the subsequent behavior in this study. This result suggests that simply combining an rTMS treatment session with conventional exposure therapy is likely not greatly effective and that rTMS paired with the CS is required to facilitate extinction learning.

What is the possible neurophysiological mechanism of facilitated fear extinction induced by CS-paired rTMS? We speculate that this mechanism may be the modulation of neuronal activity or synaptic modification in the region involved in fear extinction, possibly the vmPFC and the amygdala. High-frequency rTMS has been reported to upregulate brain activity and increase cortical excitability, as measured by motor-evoked potentials (Maeda et al., 2000; Huang et al., 2005; Fitzgerald et al., 2006) and EEG recordings (Esser et al., 2006). Furthermore, neural activity and corresponding hemodynamic responses elicited by rTMS have been directly measured in the cat visual cortex (Allen et al., 2007). We should note that rTMS modulated spontaneous activity during the resting state and during sensory stimulus-evoked activity differently, and these modulations lasted up to several minutes. Thus, the effect of rTMS on neuronal activity is dependent on the ongoing activity evoked by sensory inputs, and this dependence can account for the distinct effect of the CS-paired extinction found in this study. Pairing rTMS and sensory stimuli is essential, as evidenced by this and a previous study demonstrating the short duration of the modulation effect of rTMS (Allen et al., 2007).

However, we should note that the rTMS procedure requires repeated restraint of the rats during the extinction session, which may induce stress. Consequently, this stress may be associated with the result that the control rats showed very little extinction in experiment 2, compared with experiment 1. Given this poor extinction, Gregory Quirk suggested that we should not exclude the possibility that the rTMS may be interfering with the effects of stress (personal communication). To address this issue, rats could potentially be acclimatized to the restrainer for a certain period of time (e.g. several days) before training to reduce stress. Still, removing the rats during the extinction session will likely interfere with extinction.

One of the limitations we should address in this report is the degree of localization of the rTMS. In recent animal TMS studies, a stereotaxic frame and sedation were also used to control the precise location of the coil (Luft et al., 2002; Rotenberg et al., 2010), but this method was not feasible in the present study due to the requirement of an observable behavioral response. We also modified the commercial TMS apparatus for use on smaller rats, but the coil size was still too large to focus on the vmPFC, which is possibly implicated in consolidation, retention, and expression of extinction memory (Quirk et al., 2000; Milad and Quirk, 2002; Morgan et al., 2003; Milad et al., 2004; Santini et al., 2004; Burgos-Robles et al., 2007). Given that the stimulation of the more dorsal prelimbic areas exhibited an effect of stimulation opposite that of the infralimbic subregion of the vmPFC (Vidal-Gonzalez et al., 2006), the relatively broad application of TMS on the frontal part of rat brains may produce minimal effects or even cancellation effects between the dorsal prelimbic and infralimbic regions. Thus, it is not certain that the facilitation of fear extinction results from the activation of the vmPFC by high-frequency rTMS paired with CS.

In addition, we assessed the effect of rTMS at only one frequency, 10 Hz, in this study. We need to apply rTMS with a wide range of frequencies to find the most effective stimulation for the facilitation of fear extinction in a future investigation. For rTMS with low frequencies, longer durations of the stimulation and restraint are required to apply the same number of pulses, and the short application of rTMS with a low frequency paired with CS exposure may prevent formulating an experimental procedure comparable to the high-frequency rTMS experiment. Thus, we should develop a novel paradigm to overcome this problem in the future.

Finally, freezing responses to the tone in both groups in experiment 2 were relatively high because of the intense foot shocks used during the fear conditioning phase and

the stress from the restraints. Three rats showing high mobility were excluded from this study at the extinction phase because of the difficulty restraining these rats. The remaining rats used in experiment 2 tended to exhibit a high level of freezing relative to the original group. Thus, issues arising from this tendency toward a high level of freezing, such as a ceiling effect, should be carefully taken into account in interpreting the findings.

It is conceivable that high-frequency rTMS paired with trauma-reminding stimuli may serve as potential therapeutic intervention for patients suffering from exaggerated fear memory, such as PTSD. As shown in this study, rTMS not paired with the CS (i.e. a traumatic reminder) does not effectively enhance extinction; thus, the simple rTMS treatment currently used alone might not be suitable. This explanation can account for the slight effect reported in previous clinical studies of rTMS in treating PTSD (Grisaru et al., 1998; McCann et al., 1998; Rosenberg et al., 2002; Cohen et al., 2004), all of which adopted conventional simple rTMS protocols. Therefore, a preferable rTMS protocol combined with a cognitive intervention, such as trauma-reminding stimuli, is likely required to maximize the clinical efficacy of rTMS in treating anxiety disorders. Recently, a cognitive strategy to regulate conditioned fear, similar to cognitive-behavioral therapy (CBT) for anxiety disorders, was found to be mediated by the vmPFC connections implicated in fear extinction (Delgado et al., 2008). In conclusion, we suggest that a more refined application of rTMS in combination with exposure therapy or CBT is required to treat pathological fear, and the temporal pairing of brain stimulation and cognitive activation is crucial toward that end.

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REFERENCES

Allen EA, Pasley BN, Duong T, Freeman RD (2007) Transcranial magnetic stimulation elicits coupled neural and hemodynamic consequences. Science 317:1918.

Burgos-Robles A, Vidal-Gonzalez I, Santini E, Quirk GJ (2007) Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. Neuron 53:871–880.

Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, Grisaru N (2004) Repetitive transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in posttraumatic stress disorder: a double-blind, placebo-controlled study. Am J Psychiatry 161:515–524.

Davis M (2002) Role of NMDA receptors and MAP kinase in the amygdala in extinction of fear: clinical implications for exposure therapy. Eur J Neurosci 16:395–398.

Delgado MR, Nearing KI, LeDoux JE, Phelps EA (2008) Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. Neuron 59:829–838.

Epstein CM, Davey KR (2002) Iron-core coils for transcranial magnetic stimulation. J Clin Neurophysiol 19:376–381.

Esser SK, Huber R, Massimini M, Peterson MJ, Ferrarelli F, Tononi G (2006) A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. Brain Res Bull 69:86–94.

Fitzgerald PB, Fountain S, Daskalakis ZJ (2006) A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol 117:2584–2596.

Francati V, Vermetten E, Bremner JD (2007) Functional neuroimaging studies in posttraumatic stress disorder: review of current methods and findings. Depress Anxiety 24:202–218.

George MS, Belmaker RH (2000) Trancranial magnetic stimulation in neuropsychiatry, pp 13–185. Washington, DC: American Psychiatric Press, Inc.

George MS, Nahas Z, Molloy M, Speer AM, Oliver NC, Li XB, Arana GW, Risch SC, Ballenger JC (2000) A controlled trial of daily left prefrontal cortex TMS for treating depression. Biol Psychiatry 48:962–970.

Grisaru N, Amir M, Cohen H, Kaplan Z (1998) Effect of transcranial magnetic stimulation in posttraumatic stress disorder: a preliminary study. Biol Psychiatry 44:52–55.

Hallett M (2000) Transcranial magnetic stimulation and the human brain. Nature 406:147–150.

Hargreaves GA, McGregor IS, Sachdev PS (2005) Chronic repetitive transcranial magnetic stimulation is antidepressant but not anxiolytic in rat models of anxiety and depression. Psychiatry Res 137:113–121.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC (2005) Theta burst stimulation of the human motor cortex. Neuron 45:201–206.

Kim EJ, Kim WR, Chi SE, Lee KH, Park EH, Chae JH, Park SK, Kim HT, Choi JS (2006) Repetitive transcranial magnetic stimulation protects hippocampal plasticity in an animal model of depression. Neurosci Lett 405:79–83.

Kimbrell TA, Little JT, Dunn RT, Frye MA, Greenberg BD, Wassermann EM, Repella JD, Danielson AL, Willis MW, Benson BE (1999) Frequency dependence of antidepressant response to left prefrontal repetitive transcranial magnetic stimulation (rTMS) as a function of baseline cerebral glucose metabolism. Biol Psychiatry 46:1603–1613.

Lee JL, Milton AL, Everitt BJ (2006) Reconsolidation and extinction of conditioned fear: inhibition and potentiation. J Neurosci 26(39):10051–10056.

Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS (2005) Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav Res Ther 43:1391–1424.

Luft AR, Kaelin-Lang A, Hauser TK, Buitrago MM, Thakor NV, Hanley DF, Cohen LG (2002) Modulation of rodent cortical motor excitability by somatosensory input. Exp Brain Res 142:562–569.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A (2000) Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. Clin Neurophysiol 111:800–805.

McCann UD, Kimbrell TA, Morgan CM, Anderson T, Geraci M, Benson BE, Wassermann EM, Willis MW, Post RM (1998) Repetitive transcranial magnetic stimulation for posttraumatic stress disorder. Arch Gen Psychiatry 55:276–279.

Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL (2005) Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. Proc Natl Acad Sci U S A 102:10706–10711.

Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70–74.

Milad MR, Rauch SL, Pitman RK, Quirk GJ (2006) Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychol 73:61–71.

Milad MR, Vidal-Gonzalez I, Quirk GJ (2004) Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. Behav Neurosci 118:389–394.

Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL (2007) Recall of fear extinction in humans activates the ventromedial

prefrontal cortex and hippocampus in concert. Biol Psychiatry 62:446–454.

Morgan MA, Schulkin J, LeDoux JE (2003) Ventral medial prefrontal cortex and emotional perseveration: the memory for prior extinction training. Behav Brain Res 146:121–130.

Norberg MM, Krystal JH, Tolin DF (2008) A meta-analysis of d-cycloserine and the facilitation of fear extinction and exposure therapy. Biol Psychiatry 63(12):1118–1126.

O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, McDonald WM, Avery D, Fitzgerald PB, Loo C (2007) Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. Biol Psychiatry 62:1208–1216.

Phelps EA, Delgado MR, Nearing KI, LeDoux JE (2004) Extinction learning in humans: role of the amygdala and vmPFC. Neuron 43:897–905.

Post A, Keck ME (2001) Transcranial magnetic stimulation as a therapeutic tool in psychiatry: what do we know about the neurobiological mechanisms? J Psychiatr Res 35:193–215.

Quirk GJ, Russo GK, Barron JL, Lebron K (2000) The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J Neurosci 20(16):6225–6231.

Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M (2004) Cognitive enhancers as adjuncts to psychotherapy: use of d-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry 61:1136.

Richardson R, Ledgerwood L, Cranney J (2004) Facilitation of fear extinction by d-cycloserine: theoretical and clinical implications. Learn Mem 11:510.

Rosenberg PB, Mehndiratta RB, Mehndiratta YP, Wamer A, Rosse RB, Balish M (2002) Repetitive transcranial magnetic stimulation treatment of comorbid posttraumatic stress disorder and major depression. Am Neuropsych Assoc 14:270–276.

Rotenberg A, Muller PA, Vahabzadeh-Hagh AM, Navarro X, López-Vales R, Pascual-Leone A, Jensen F (2010) Lateralization of forelimb motor evoked potentials by transcranial magnetic stimulation in rats. Clin Neurophysiol 121:104–108.

Santini E, Ge H, Ren K, Peña de Ortiz SP, Quirk GJ (2004) Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. J Neurosci 25:5704–5710.

Speer AM, Kimbrell TA, Wassermann EM, D Repella J, Willis MW, Herscovitch P, Post RM (2000) Opposite effects of high and low frequency rTMS on regional brain activity in depressed patients. Biological Psychiatry 48:1133–1141.

Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ (2006) Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learn Mem 13:728–733.

Walker DL, Ressler KJ, Lu KT, Davis M (2002) Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of d-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci 22(6):2343–2351.

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