Research Paper

Augmentation of fear extinction by theta-burst transcranial magnetic stimulation of the prefrontal cortex in humans

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Background: Fear extinction alone does not erase the original fear memory. Interventions that enhance extinction can be beneficial for the treatment of fear-related disorders. Repetitive transcranial magnetic stimulation has been shown to improve memory performance. The present study examined the effects of intermittent theta-burst stimulation (iTBS) on fear extinction and the return of fear memory in humans. **Methods:** Ninety-one young healthy volunteers underwent 3 experiments using a randomized controlled experimental design. Participants first acquired fear conditioning, after which they received 30 Hz iTBS before and after extinction training. The iTBS was applied to 1 of 2 targets: the left dorsolateral prefrontal cortex (dlPFC) and the vertex (control). Fear responses were measured 24 hours later and 1 month later. **Results:** During the spontaneous recovery and reinstatement tests, iTBS of the left dlPFC before and after extinction significantly reduced fear response, whereas iTBS of the vertex had no effect on fear memory performance. This combined approach had a relatively long-lasting effect (i.e., at least 1 month). **Limitations:** We did not explore the effect of iTBS of the dlPFC on the expression of fear without extinction training. The neural mechanisms of iTBS with fear extinction to inhibit the fear response are unclear. Our results are preliminary and should be interpreted with caution. **Conclusion:** The present results showed that 30 Hz iTBS of the left dlPFC enhanced retention of fear extinction. Our study introduces a new intervention for fear memory and suggests that the left dlPFC may be a treatment target for fear-related disorders.

Introduction

More than 70% of people experience a traumatic event in their lifetime.[1] However, excessive traumatic experiences may disrupt the normal neural basis of memory, resulting in the formation of maladaptive emotional memories that underlie anxiety and fear-related disorders (e.g., posttraumatic stress disorder [PTSD]).[2–4] Fear memory has been successfully modelled in humans and animals using Pavlovian fear conditioning, in which an initially neutral conditioned stimulus (e.g., a picture, tone or context) is paired with a noxious unconditioned stimulus (e.g., an electric shock) that elicits an unconditioned fear response.[5] Pavlovian fear conditioning models have been used widely to investigate the pathogenesis of fear-based disorders and novel interventions.[5] Currently, one of the primary treatments for PTSD is exposure therapy. Exposure therapy relies on extinction theory, which involves exposure to the original conditioned stimulus without pairing it with an unconditioned stimulus.[6] However, extinction training alone does not erase the original fear memory, and the fear response returns under some conditions, such as during reinstatement,[7] renewal[8] and spontaneous recovery.[9] Recent studies have shown that extinction can be enhanced by pharmacological or nonpharmacological treatments.[10,11] However, pharmacological treatments often have side effects and limited efficacy, reducing their widespread clinical application and necessitating the search for new therapeutic approaches.

A body of literature supports the efficacy of noninvasive neuromodulation in facilitating the extinction process, such as transcranial magnetic stimulation,[12] vagus nerve stimulation[13] and transcranial direct current stimulation.[14] Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive technology that uses magnetic fields to regulate the electrical activity of nerve cells in the brain. It has been approved by the United

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States Food and Drug Administration as a treatment for major depression and other mental disorders. Existing data have indicated the effectiveness of rTMS for the treatment of PTSD,[15–17] and a combination of exposure therapy and rTMS may be more effective than either treatment modality alone.[18,19]

One of the key anatomic regions of interest for rTMS is the dorsolateral prefrontal cortex (dlPFC), which participates in the encoding of emotional memory.[20] Inhibiting left prefrontal cortex (PFC) activity can disrupt memory performance,[21] indicating that the left dlPFC is vital for memory expression. A functional imaging study indicated that activation of the left dlPFC is associated with the cognitive regulation of fear responses.[22] Moreover, during fear extinction, the dlPFC exhibited functional coupling with the ventromedial PFC to ultimately modulate amygdala activity.[23] High-frequency rTMS of the left lateral PFC, paired with a cue during extinction training, enhanced fear extinction.[12] Previous studies have found that rTMS of the medial PFC enhances the retention of fear extinction.[24] Remaining to be investigated are the effects on fear memory of transcranial magnetic stimulation of the dlPFC before and after extinction training.

In addition to traditional rTMS, theta-burst stimulation (TBS) is a novel transcranial magnetic stimulation protocol that induces changes in corticospinal excitability through long-term potentiation and long-term depression.[25] During TBS, short bursts of 50 Hz stimulation are repeated at intervals of 200 ms (5 Hz). Intermittent TBS (iTBS) leads to excitatory effects related to Ca^{2+} influx through postsynaptic *N*-methyl-D-aspartate receptors, which then trigger cascades that lead to long-term potentiation and higher levels of excitation of the stimulated cortex.[25–27] Fear extinction requires synaptic plasticity in the PFC mediated by *N*-methyl-D-aspartate receptors.[28] A previous study showed that iTBS applied for 190 seconds significantly increased cortical excitability for up to 1 hour.[29] Furthermore, iTBS can improve social and occupational functioning and alleviate depressive symptoms in PTSD patients,[30] with a shorter duration of action than conventional rTMS[31] and without apparent adverse effects.[32] Compared with conventional rTMS, the rapid and effective regulation of TBS makes it an attractive treatment option for mental disorders. One study found that iTBS of the left dlPFC improved performance on a working-memory task.[33] However, the effects of iTBS of the left dlPFC on fear memory are still unknown.

Overall, previous findings have provided a theoretical rationale for using iTBS to influence fear memory. However, we do not know whether synergistic effects of iTBS of the left dlPFC and extinction training can disrupt fear memory. We investigated the effects of iTBS of the left dlPFC combined with extinction training on the expression and reinstatement of fear memory in humans.

Methods

Participants

We recruited 91 participants from universities and businesses through posters and advertisements. Inclusion criteria were as follows: 18 to 40 years of age and generally good health as determined by a physician. Exclusion criteria were as follows: current or previous DSM-IV Axis I disorder, the use of any medications, self-reported pregnancy or menstrual period, lifetime history of head injury, and current or previous neurologic disorder (e.g., seizure disorder, brain tumour, stroke or cerebral aneurysm). All participants were scheduled for a screening interview, during which they received further details about the experimental protocol and signed an informed consent form. The study was approved by the Institutional Review Board of Peking University Sixth Hospital. Participants were paid 400 RMB (equivalent to US$56.60). During the baseline session, all participants completed questionnaires asking about basic demographic information, including sex, age, education, height, weight and body mass index (BMI). We used the Self-Rating Depression Scale (SDS)[34] and Self-Rating Anxiety Scale (SAS)[35] to measure depression and anxiety at baseline. We assessed baseline cognitive function using the Montreal Cognitive Assessment (MoCA)[36] and the digit span test.[37]

Fear conditioning

The fear conditioning protocol was based on our previous studies.[10,38] Before fear learning, participants determined the intensity of the unconditioned stimulus shock for themselves. Beginning at a very mild level (20 V), the shock intensity was gradually increased until it reached a level that the individual felt was uncomfortable but not painful (the highest level was 66.6 V). All shocks were given for 200 ms, at a current of 50 pulses per second.

To establish conditioning, participants were instructed to pay attention to the computer screen and try to learn the relationship between different conditioned stimuli (coloured square pictures) and the unconditioned stimulus (a mild electric shock to the wrist). Some conditioned stimuli were not paired with an electric shock (CS^-), and some conditioned stimuli were paired with an electric shock (CS^+) on a partial reinforcement schedule (50% reinforced). To counteract the effect of coloured squares on memory, we used 2 different orders of presentation to counterbalance for the designation of the coloured squares (blue or red) as CS^+ or CS^-; in one, blue was the CS^+ and red was the CS^-, and in the other, red was the CS^+ and blue was the CS^-. We assigned the application of counterbalancing combinations using random numbers. All conditioned stimuli were presented for 4 seconds, with an intertrial interval of 8 to 12 seconds. Fear acquisition consisted of 12 nonreinforced presentations of each conditioned stimulus, intermixed with 12 reinforced CS^+ presentations. To assess expectation of the reinforcer and avoid the influence of the electric shock on skin conductance response (SCR), we included only nonreinforced CS^+ when calculating the acquired fear response. We divided nonreinforced conditioned stimuli into 4 blocks, with 3 CS^+ and 3 CS^- presentations each. We found a greater SCR to the CS^+ than to the CS^- (mean differential SCR $\geq$ 0.1), suggesting that fear conditioning was established.[39] After fear conditioning, 21 participants were excluded from the study because their difference in SCR

values between the CS^+ and CS^- was less than 0.1. Seventy participants completed the study.

Fear extinction

Fear extinction training consisted of 15 nonreinforced presentations of each conditioned stimulus (CS^+ and CS^-). More presentations of the conditioned stimulus during fear extinction could ensure that the response to the conditioned stimulus was thoroughly extinguished in all groups. Extinction training was divided into 5 blocks, with 3 CS^+ and 3 CS^- presentations in each block. The extinction score was calculated as the average of the 3 CS^+ and 3 CS^- presentations in each block. No time interval, rest period or signalled transitions occurred between blocks.

Spontaneous recovery and reinstatement tests

In the tests, none of the conditioned stimuli were reinforced. Twenty-four hours after fear extinction, we performed the spontaneous recovery test, with 15 nonreinforced presentations of each conditioned stimulus (CS^+ and CS^-). Participants then underwent a reinstatement test that consisted of 3 unsignalled electric shocks and 15 nonreinforced presentations of each conditioned stimulus (CS^+ and CS^-). The interval between the last spontaneous recovery trial and the reinstating electric shock was 60 seconds. During the tests, the conditioned stimuli were presented for 4 seconds, followed by an interstimulus interval of 8 to 12 seconds, during which participants looked at a fixation point on a computer screen.

iTBS intervention and target selection

We applied brain stimulation noninvasively using iTBS before and after extinction training (day 2) using the Rapid 2 system (Magstim). Before stimulation, resting motor thresholds were determined as the lowest stimulation intensity applied over the primary motor cortex that evoked a visible contraction of the relaxed right first dorsal interosseous muscle in response to at least 5 of 10 consecutive stimulations. We delivered iTBS to the left dlPFC (80% active motor threshold, 1800 pulses, and triplet bursts with a pulse frequency of 30 Hz and burst frequency of 5 Hz), based on previous iTBS studies.[40,41] Other than the difference in burst frequency, the 30 Hz iTBS protocol was identical to the original iTBS protocol.[25] Previous studies found that 30 Hz iTBS induced neurophysiological effects similar to conventional 50 Hz iTBS.[32,40]

The location of the left dlPFC was determined by the standard F3 location using international electroencephalogram 10/20 system measurements. In the control group, we chose the vertex as the stimulation target, and the coil was placed over Cz using the 10/20 electroencephalogram system, oriented in line with the longitudinal fissure and with the coil handle pointed posteriorly.[42] In cognitive neuroscience, rTMS over the vertex is the most common control condition used because noise, twitches and some cortical activity caused by vertex transcranial magnetic stimulation can be equivalent to dlPFC stimulation.[43,44]

Experimental design

In experiment 1, we first investigated the effect of left dlPFC iTBS before extinction on fear expression. Thirty-five participants (vertex iTBS group, $n = 19$; left dlPFC iTBS group, $n = 16$) were recruited and randomly allocated to 2 groups. Both groups provided basic demographic information (sex, age, education, height, weight and BMI); completed the SAS, SDS and MoCA; and performed the digit span test on day 1. On day 2, participants received iTBS of the vertex or left dlPFC. Extinction training was performed immediately after stimulation. The spontaneous recovery and reinstatement tests occurred 24 hours after extinction training.

In experiment 2, to assess whether the blockade of fear memory persisted, 31 participants (vertex iTBS group, $n = 17$; left dlPFC iTBS group, $n = 14$) from experiment 1 returned to the laboratory and completed a 1-month follow-up study. Four participants did not have time to participate in follow-up testing. Experiment 2 consisted of a spontaneous recovery test and a reinstatement test.

In experiment 3, we explored the effect of left dlPFC iTBS after extinction training on fear expression. Thirty-five participants (vertex iTBS group, $n = 17$; left dlPFC iTBS group, $n = 18$) were recruited and randomly allocated to 2 groups. Both groups provided basic demographic information (sex, age, education, height, weight and BMI); completed the SAS, SDS and MoCA; and performed the digit span test on day 1. On day 2, participants underwent extinction training. Then, iTBS stimulation was performed immediately after extinction. The spontaneous recovery and reinstatement tests were conducted 24 hours later.

Participants who experienced side effects within 60 minutes of iTBS were asked to report to the experimenters. Three participants in the vertex iTBS group reported a headache, and 2 participants in the left dlPFC iTBS group reported pain in the stimulated area of the forebrain.

Psychophysiological stimulation and assessment

Electric shocks were delivered using a constant-current STM200 stimulator (BIOPAC Systems). A stimulating electrode was attached to the right inner wrist. Stimulus presentation was controlled by a computer using E-Prime 2.0 (Psychology Software Tools). Fear response was assessed by SCR, which was recorded using shielded silver/silver chloride electrodes attached to the second and third fingers of the left hand. We measured SCR waveforms using a BIOPAC MP150 system with AcqKnowledge 4.0 software (BIOPAC Systems). We assessed the greatest base-to-peak change in SCR in a 0- to 6-second window after the onset of each conditioned stimulus onset; these values were then square-root transformed to normalize distribution.

Statistical analysis

Quantitative data are expressed as mean ± standard error of the mean. We used independent-sample *t* tests to analyze differences in demographic data, SDS scores, SAS scores, MoCA

scores, digit span test scores and shock intensity between the 2 groups. We used the χ^2 test to analyze differences in sex frequencies between the 2 groups. During fear acquisition, we did not include reinforced CS^+ in the analysis to avoid the direct effect of unconditioned shock stimulation. We assessed the differential SCR by subtracting responses to the CS^- from responses to the CS^+ in corresponding trials. Differential scores were averaged across participants. We extracted mean differential SCRs during fear acquisition (4 blocks), extinction training (5 blocks), the spontaneous recovery test (first block and last block) and the reinstatement test (first block). We analyzed fear conditioning using repeated-measures analysis of variance (ANOVA), with group (vertex iTBS group and left dlPFC iTBS group) as the between-subjects factor and fear conditioning block (blocks 1–4) as the within-subjects factor. During fear extinction, we used repeated-measures ANOVA, with group (vertex iTBS group and left dlPFC iTBS group) as the between-subjects factor and fear extinction block (blocks 1–5) as the within-subjects factor. We analyzed the spontaneous recovery and reinstatement tests using repeated-measures ANOVA, with group (vertex iTBS group and left dlPFC iTBS group) as the between-subjects factor and test (spontaneous recovery: first block of the spontaneous recovery test and last block of extinction; reinstatement: first block of the reinstatement test and last block of the spontaneous recovery test) as the within-subjects factor. All of the analyses performed are shown in Appendix 1, Table S1, available at jpn.ca/200053-a1. We performed all statistical analyses using SPSS 24.0 (SPSS). Significant effects in the ANOVAs were followed by a Bonferroni post hoc test. Two-tailed values of $p < 0.05$ were considered statistically significant.

Results

iTBS of the left dlPFC before extinction training enhanced extinction retention and prevented the return of fear

In experiment 1, we first analyzed whether iTBS of the left dlPFC influenced fear extinction and fear expression (Figure 1A). We found no differences in sex, age, education, height, weight, BMI, SDS score, SAS score, MoCA score, digit span test score (forward and backward) or shock intensity between the vertex iTBS group and the left dlPFC iTBS group (Table 1). Both groups exhibited comparable fear learning, indicated by a significant main effect of the fear conditioning block ($F_{3,99} = 7.404$, $p < 0.001$) but no main effect of group ($F_{1,33} = 0.212$, $p = 0.65$) and no group × block interaction ($F_{3,99} = 0.001$, $p = 0.98$). These results indicated that all participants in both groups achieved successful and comparable fear acquisition (mean differential SCR > 0.1; Figure 2A).

To investigate the effect of left dlPFC iTBS on fear extinction and fear expression, participants received iTBS of the left dlPFC or vertex 24 hours after fear conditioning and then underwent extinction training immediately afterward. Repeated-measures ANOVA revealed a main effect of the extinction block ($F_{4,132} = 6.284$, $p < 0.001$) but no main effect of group ($F_{1,33} = 0.477$, $p = 0.50$) and no group × block interaction ($F_{4,132} = 0.403$, $p = 0.81$; Fig. 2B). These results showed that iTBS of the left dlPFC had no effect on the fear extinction process.

To assess the spontaneous recovery of fear memory, participants were tested 24 hours after extinction training. This analysis showed main effects of test ($F_{1,33} = 6.277$, $p = 0.017$) and a significant group × test interaction ($F_{1,33} = 4.920$, $p = 0.034$), but no main effect of group ($F_{1,33} = 1.218$, $p = 0.28$; Figure 2C). The post hoc test showed that spontaneous recovery of the fear response to the conditioned stimulus occurred in the vertex iTBS group ($p = 0.001$) but not in the left dlPFC group. Fear responses in the last block of the spontaneous recovery were similar in both groups. During the reinstatement test, the analysis revealed main effects of test ($F_{1,33} = 6.405$, $p = 0.016$), group ($F_{1,33} = 5.268$, $p = 0.028$) and a significant group × test interaction ($F_{1,33} = 4.223$, $p = 0.048$; Figure 2C). No reinstatement occurred in the left dlPFC iTBS group. These findings showed that iTBS of the left dlPFC inhibited the expression of fear and prevented the return of fear.

Blockade of conditioned fear was maintained for at least 1 month

In experiment 2, 31 participants from experiment 1 were invited to return to the laboratory 1 month later to assess the lasting effect of the combination of extinction and iTBS. In the spontaneous recovery test, the mean differential SCR in the first block in the left dlPFC iTBS group was lower than in the vertex iTBS group ($F_{1,29} = 2.395$, $p = 0.023$; Figure 3). Fear responses in the last block of the spontaneous recovery test were similar in both groups. During the reinstatement test, repeated-measures ANOVA showed a main effect of group ($F_{1,29} = 4.388$, $p = 0.045$) and a significant group × test interaction ($F_{1,29} = 5.842$, $p = 0.022$), but no main effect of test ($F_{1,29} = 3.338$, $p = 0.08$). The post hoc test showed significant reinstatement of conditioned fear in the vertex iTBS group ($p = 0.004$) but not in the left dlPFC iTBS group (Figure 3). These results indicated that blockade of the return of fear induced by the combination of left dlPFC iTBS and fear extinction training was long-lasting.

iTBS of the left dlPFC after extinction training inhibited fear memory

In experiment 3, we further investigated whether iTBS of the left dlPFC after fear extinction training decreased the expression of fear (Figure 1B). We found no differences in sex, age, education, height, weight, BMI, SDS score, SAS score, MoCA score, digit span test score (forward and backward) or shock intensity between the vertex iTBS group and the left dlPFC iTBS group (Table 2). Both groups exhibited comparable fear learning, indicated by a significant main effect of the fear conditioning block ($F_{3,99} = 5.092$, $p = 0.003$) but no main effect of group ($F_{1,33} = 0.059$, $p = 0.81$) or group × block interaction ($F_{3,99} = 0.046$, $p = 0.99$). These results indicated that all participants in both groups achieved successful and comparable fear acquisition (mean differential SCR > 0.1; Figure 4A). During extinction training, repeated-measures ANOVA revealed a main effect of the extinction block ($F_{4,132} = 4.973$, $p = 0.001$) but

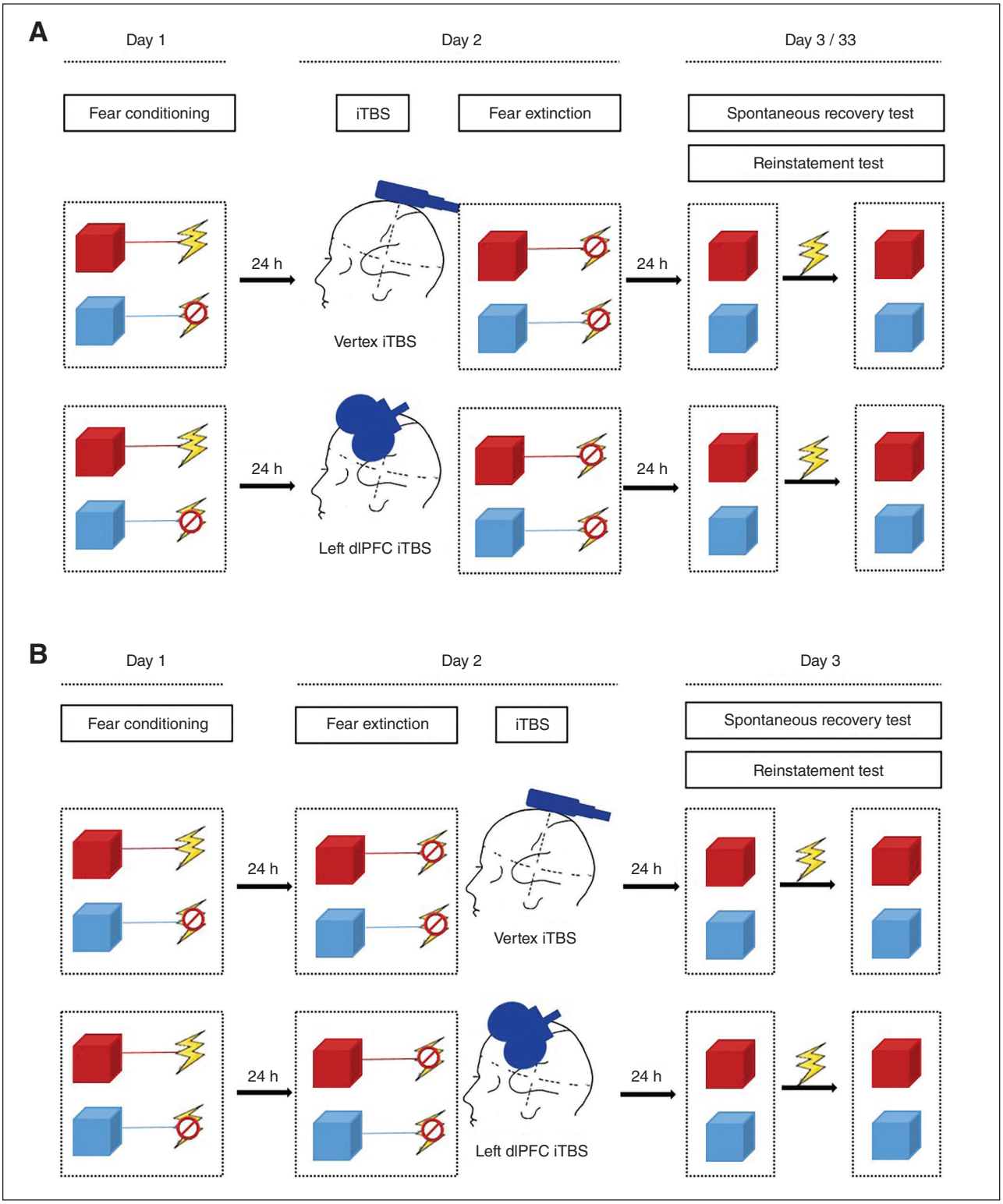

Fig. 1: Procedure and timeline of the experiments. (A) Experimental design for experiments 1 and 2. Participants were trained in fear conditioning with visual cues on day 1. They received iTBS of the left dlPFC 24 hours later (day 2), and extinction training immediately after iTBS. On day 3, 24 hours after extinction training, participants were tested for fear expression. In experiment 2, participants from experiment 1 completed a follow-up study on day 33. (B) Experimental design for experiment 3. Participants were trained in fear conditioning with visual cues on day 1. They received iTBS of the dlPFC 24 hours later (day 2), after extinction training. On day 3, participants were tested for fear expression. dlPFC = dorsolateral prefrontal cortex; iTBS = intermittent theta-burst stimulation.

Table 1: Demographic data and shock intensity, experiment 1

Group	iTBS*		t or χ^2	p value
	Vertex (n = 19)	Left dlPFC (n = 16)		
Sex, % female	36.84	25.00	0.57	0.45
Age, yr	23.05 ± 1.04	23.31 ± 1.21	–0.16	0.87
Education, yr	15.58 ± 0.48	15.56 ± 0.41	0.03	0.98
Height, cm	171.74 ± 2.26	173.38 ± 1.80	–0.55	0.59
Weight, kg	65.05 ± 2.86	64.66 ± 2.69	1.00	0.92
Body mass index, kg/m^2	21.91 ± 0.64	21.43 ± 0.68	0.51	0.61
Self-Rating Depression Scale				
Score	29.79 ± 1.57	28.81 ± 1.36	0.46	0.65
Standard score	37.23 ± 1.97	32.60 ± 1.70	0.46	0.65
Self-Rating Anxiety Scale				
Score	26.00 ± 1.10	26.13 ± 1.13	–0.08	0.94
Standard score	32.50 ± 1.38	32.73 ± 1.40	–0.08	0.94
Montreal Cognitive Assessment score	27.53 ± 0.28	27.68 ± 0.32	–0.38	0.71
Digit span test score				
Forward	9.26 ± 0.18	9.06 ± 0.37	0.51	0.61
Backward	7.21 ± 0.37	6.25 ± 0.31	1.94	0.06
Shock intensity, V	41.91 ± 1.49	45.96 ± 2.28	–1.53	0.14

dlPFC = dorsolateral prefrontal cortex; iTBS = intermittent theta-burst stimulation.
*Unless otherwise indicated, results are expressed as mean ± standard error of the mean.

no main effect of group ($F_{1,33}$ = 0.005, p = 0.95) and no group × block interaction ($F_{4,132}$ = 0.071, p = 0.79; Figure 4B), suggesting that the extinction process was comparable between groups.

Next, we investigated the effect of iTBS of the left dlPFC on fear expression after fear extinction training. Spontaneous recovery and reinstatement of fear memory were tested 1 day after extinction. During spontaneous recovery, we found a significant main effect of group ($F_{1,33}$ = 4.871, p = 0.034), but only a trend toward a group × test interaction ($F_{1,33}$ = 3.985, p = 0.054). The mean differential SCR in the vertex iTBS group was significantly higher than in the left dlPFC iTBS group (p < 0.001; Figure 4C). Fear responses in the last 3 trials of the spontaneous recovery test were similar in both groups. During the reinstatement test, repeated-measures ANOVA showed main effects of test ($F_{1,33}$ = 5.208, p = 0.029) and group ($F_{1,33}$ = 4.823, p = 0.035) and a significant group × test interaction ($F_{1,33}$ = 7.777, p = 0.009). Follow-up t tests indicated the significant reinstatement of conditioned fear in the vertex iTBS group ($F_{1,33}$ = 28.482, p < 0.001; Figure 4C), but not in the left dlPFC iTBS group. These findings indicated that iTBS of the left dlPFC after extinction also inhibited fear response.

Discussion

We tested the effect of 30 Hz iTBS of the left dlPFC on fear extinction in humans. Our results showed that iTBS of the left dlPFC before and after extinction training inhibited fear memory compared to iTBS of the vertex. Moreover, the synergistic effects of left dlPFC iTBS combined with extinction training induced long-lasting inhibition of the return of fear. These findings suggest that iTBS can prompt extinction retention, and the combination of exposure therapy and iTBS may be useful for treating fear-related disorders in humans.

We found that iTBS strengthened the retention of extinction memory, making it potentially more resistant to the return of fear. We stimulated the left dlPFC, which is a traditional target for treating depression and PTSD.[45] A previous study found that decreasing activity in the left PFC before or during memory encoding disrupted memory performance,[21] supporting the hypothesis that the left dlPFC is necessary for memory expression. High-frequency rTMS and iTBS increases the level of excitation of the stimulated cortex.[25,29] Our findings were consistent with a recent study showing that high-frequency rTMS of the left lateral PFC, paired with a cue during extinction training, enhanced fear extinction.[12] High-frequency stimulation of the left dlPFC regulates the expression of fear through projections to the ventromedial PFC, which in turn inhibits amygdala activity.[23] Stimulation of the medial PFC has been shown to modulate the processing of conditioned fear.[24] However, some studies did not observe effects of high-frequency rTMS of the left dlPFC in response to negative stimuli.[46] Based on memory theory, this indicates that high-frequency rTMS of the left dlPFC may enhance extinction memory and suppress original fear memory. Moreover, iTBS of the vertex in the present study did not enhance extinction memory or decrease fear response. Applying stimulation of the vertex is a common control condition for transcranial magnetic stimulation studies. A previous study found that vertex stimulation did not evoke changes in blood-oxygen-level-dependent activation at the site of stimulation.[47] We conclude that the left dlPFC may be a critical target that mediates the expression of fear in humans.

We found that iTBS before and after extinction training effectively disrupted the expression of fear. We found that iTBS did not affect the extinction process, indicating that it is critical for the consolidation of extinction memory. One possibility is

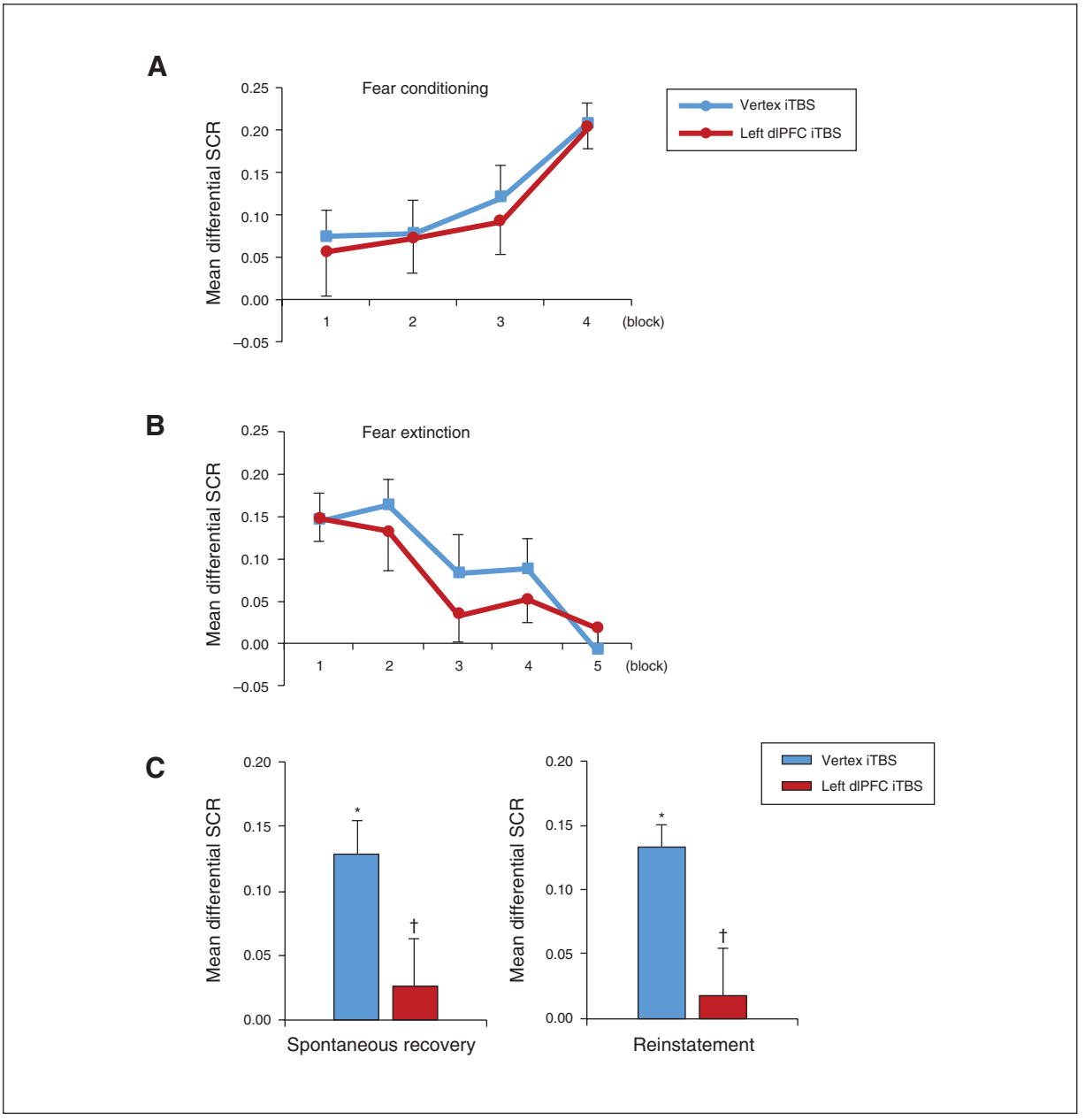

Fig. 2: Intermittent theta-burst stimulation of the left dlPFC before extinction reduced fear expression and fear reinstatement. (A) Mean differential SCR (CS^+ minus CS^-) during fear conditioning. (B) Mean differential SCR during fear extinction. (C) Mean differential SCR during spontaneous recovery and reinstatement tests. Data are expressed as mean ± standard error of the mean (n = 16 to 19 per group). *$p < 0.05$, comparison between the last 3 trials of fear extinction and the first 3 trials of the spontaneous recovery test, and between the first 3 trials of the spontaneous recovery test and the first 3 trials of the reinstatement test (all within-group); †$p < 0.05$, comparison with the mean differential SCR in the vertex group. CS^+ = conditioned stimulus with electric shock; CS^- = conditioned stimulus without electric shock; dlPFC = dorsolateral prefrontal cortex; iTBS = intermittent theta-burst stimulation; SCR = skin conductance response.

that iTBS increases intracellular Ca^{2+} concentration via the concomitant activation of *N*-methyl-D-aspartate receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, inducing long-term potentiation at presynaptic neurons and enhancing synaptic strength — effects that persist for several days, weeks or months.[48,49] As well, iTBS enhanced neurogenesis in the dentate gyrus and induced the differentiation and growth of neural stem cells.[50] High-frequency stimulation or TBS also increased dopamine release, increased the affinity of brain-derived neurotrophic factor for tropomyosin receptor kinase B receptors, and prolonged serum brain-derived neurotrophic factor secretion,[51,52]

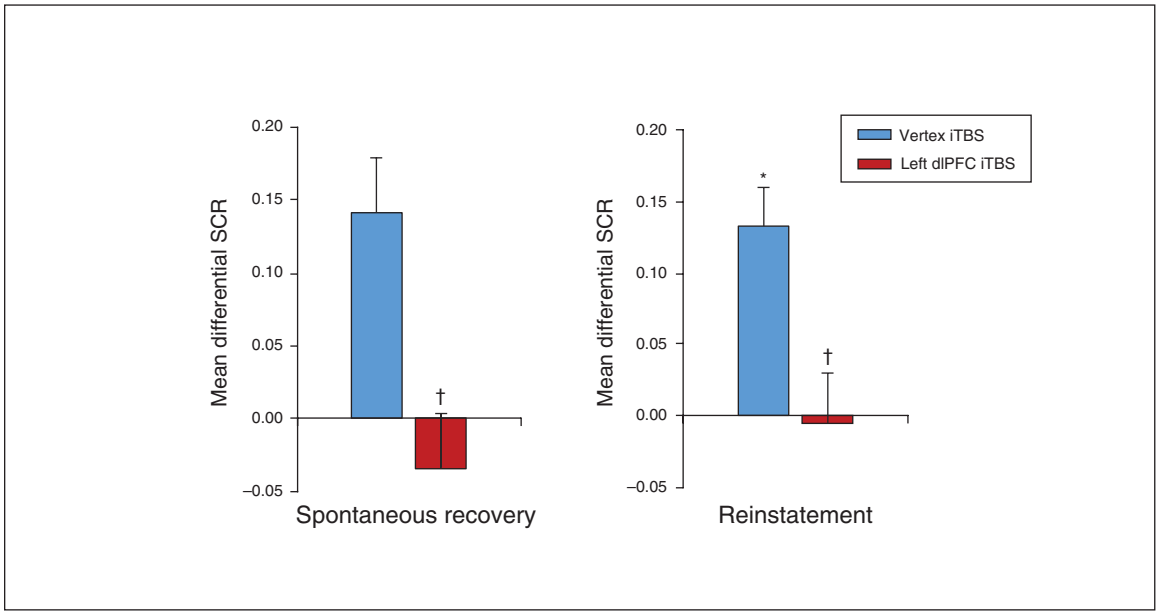

Fig. 3: Persistence of the blockade of fear responses by iTBS combined with extinction. Mean differential SCR (CS^+ minus CS^-) during the spontaneous recovery and reinstatement tests 1 month later. Data are expressed as mean ± standard error of the mean (n = 14 to 17 per group). *$p < 0.05$, comparison between the first 3 trials of the spontaneous recovery test and the first 3 trials of the reinstatement test; †$p < 0.05$, comparison with the mean differential SCR in the vertex group. CS^+ = conditioned stimulus with electric shock; CS^- = conditioned stimulus without electric shock; dlPFC = dorsolateral prefrontal cortex; iTBS = intermittent theta-burst stimulation; SCR = skin conductance response.

Table 2: Demographic data and shock intensity, experiment 3

	iTBS*			
Group	Vertex (n = 17)	Left dlPFC (n = 18)	t or χ^2	p value
Sex, % female	64.71	50.00	0.77	0.38
Age, yr	21.59 ± 0.59	22.00 ± 0.52	–0.52	0.61
Education, yr	15.35 ± 0.56	15.50 ± 0.49	–0.20	0.85
Height, cm	167.29 ± 3.81	171.17 ± 1.73	–1.48	0.15
Weight, kg	62.29 ± 3.81	60.53 ± 2.52	0.39	0.70
Body mass index, kg/m^2	22.04 ± 0.97	20.49 ± 0.49	1.45	0.16
Self-Rating Depression Scale				
Score	32.76 ± 1.63	31.28 ± 1.20	0.74	0.46
Standard score	40.96 ± 2.04	39.10 ± 1.50	0.74	0.46
Self-Rating Anxiety Scale				
Score	28.35 ± 1.20	28.17 ± 0.90	0.13	0.90
Standard score	35.44 ± 1.50	35.21 ± 1.13	0.13	0.90
Montreal Cognitive Assessment score	27.82 ± 0.31	27.39 ± 0.22	1.16	0.26
Digit span test score				
Forward	10.12 ± 0.31	10.11 ± 0.28	0.02	0.99
Backward	7.82 ± 0.43	7.44 ± 0.22	0.80	0.43
Shock intensity, V	45.40 ± 1.82	45.74 ± 1.62	–0.14	0.89

dlPFC = dorsolateral prefrontal cortex; iTBS = intermittent theta-burst stimulation.
*Unless otherwise indicated, results are expressed as mean ± standard error of the mean.

all vital for the extinction of fear.[53–57] Furthermore, iTBS-induced changes of imaging and electroencephalograms may have contributed to promoting extinction retention. A previous study showed that iTBS of the left dlPFC reduced functional connectivity between the default mode network and the dorsal anterior cingulate cortex, an important brain region for fear extinction.[58] The pattern of iTBS resembles theta oscillations seen in memory systems.[59,60] Rodent studies

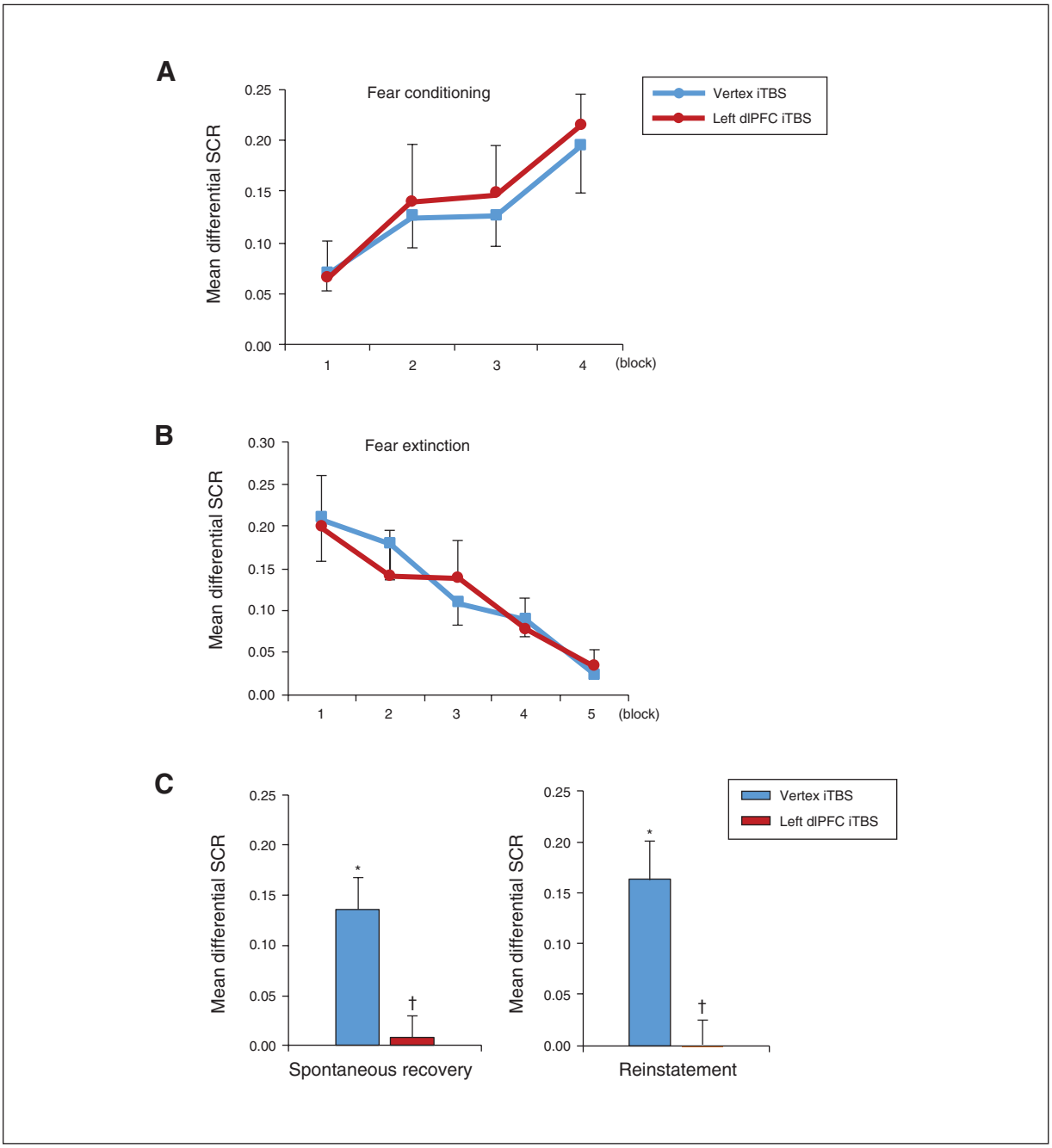

Fig. 4: Intermittent theta-burst stimulation of the left dlPFC after extinction reduced fear expression and fear reinstatement. (A) Mean differential SCR (CS^+ minus CS^-) during fear conditioning. (B) Mean differential SCR during fear extinction. (C) Mean differential SCR during the spontaneous recovery and reinstatement tests. Data are expressed as mean ± standard error of the mean (n = 17 to 18 per group). *$p < 0.05$, comparison between the last 3 trials of fear extinction and the first 3 trials of the spontaneous recovery test, and between the first 3 trials of the spontaneous recovery test and the first 3 trials of the reinstatement test (all within-group); †$p < 0.05$, comparison with the mean differential SCR in the vertex group. CS^+ = conditioned stimulus with electric shock; CS^- = conditioned stimulus without electric shock; dlPFC = dorsolateral prefrontal cortex; iTBS = intermittent theta-burst stimulation; SCR = skin conductance response.

showed that synchronized theta activity between the amygdala, medial PFC and hippocampus enhanced fear memory consolidation.[61] These mechanisms are nonexclusive and likely all contributed to the effectiveness of iTBS. Future studies should use imaging techniques to investigate the mechanisms of action of iTBS before and after extinction.

In the present study, 3 transcranial magnetic stimulation pulses per burst were given at 30 Hz. Several previous studies

have reported that 30 Hz iTBS induced neurophysiological effects over the primary motor cortex that were similar to 50 Hz iTBS,[32,40] which probably has more side effects than 30 Hz iTBS. The motor cortex and frontal eye fields, which are in close proximity to the PFC, were affected in the long term after 30 Hz continuous TBS.[62] The decrease in fear response we found in the spontaneous recovery test and reinstatement test in the present study showed that 30 Hz iTBS was sufficient to facilitate electrophysiological and behavioural changes. No other study has used 30 Hz iTBS to treat mental disorders. Future studies should investigate and compare the prolonged effects of 30 Hz iTBS of the dlPFC compared with other frequencies.

Limitations

The present study had limitations. First, we did not explore the effect of iTBS of the dlPFC without extinction training on the expression of fear. Simple TBS alone may affect the fear response in spontaneous recovery and reinstatement tests. The present study was focused primarily on investigating the effects of iTBS on extinction retention. Second, we focused on iTBS of the left dlPFC and did not test the effect of iTBS of the right dlPFC or continuous TBS of the left dlPFC on the expression of fear memory. Third, a previous study reported sex differences in fear extinction.[63] However, because of the sample size in the present study, we did not analyze the sexes separately. Moreover, women during their menstrual cycles exhibited less fear extinction recall than males, with similar fear acquisition and extinction.[64,65] Considering the effect of hormone levels on fear memory, we excluded menstruating women from the study. Future studies should explore possible sex differences in treatment outcomes for iTBS combined with extinction to inhibit fear responses. Fourth, our results were behavioural findings. We did not explore the neural mechanisms of 30 Hz iTBS combined with extinction to inhibit fear response; therefore, the results can be viewed only as preliminary.

Conclusion

We found that 30 Hz iTBS combined with extinction eliminated and inhibited fear responses. The beneficial effects of this combination procedure persisted for at least 1 month. These findings highlight the potential of noninvasive neuromodulation techniques for regulating fear memory, raising the possibility of potential therapeutic applications for fear-related disorders. Future studies should investigate the specific neural mechanisms involved in the combined effects of 30 Hz iTBS and extinction and clinical applications.

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