Repetitive Transcranial Magnetic Stimulation Ameliorates Anxiety-Like Behavior and Impaired Sensorimotor Gating in a Rat Model of Post-Traumatic Stress Disorder

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Abstract

Background
Repetitive transcranial magnetic stimulation (rTMS) has been employed for decades as a non-pharmacologic treatment for post-traumatic stress disorder (PTSD). Although a link has been suggested between PTSD and impaired sensorimotor gating (SG), studies assessing the effects of rTMS against PTSD or PTSD with impaired SG are scarce.

Aim
To assess the benefit of rTMS in a rat model of PTSD.

Methods
Using a modified single prolonged stress (SPS&S) rat model of PTSD, behavioral parameters were acquired using open field test (OFT), elevated plus maze test (EPMT), and pre-pulse inhibition trial (PPI), with or without 7 days of high frequency (10Hz) rTMS treatment of SPS&S rats.

Results
Anxiety-like behavior, impaired SG and increased plasma level of cortisol were observed in SPS&S animals after stress for a prolonged time. Interestingly, rTMS administered immediately after stress prevented those impairment.

Conclusion
Stress-induced anxiety-like behavior, increased plasma level of cortisol and impaired PPI occur after stress and high-frequency rTMS has the potential to ameliorate this behavior.
suggesting that high frequency rTMS should be further evaluated for its use as a method for preventing PTSD.

Introduction

Post-traumatic stress disorder (PTSD) refers to a condition in which exposure to life-threatening trauma results in a characteristic set of features including intrusive memory, where patients persistently experience stress-associated events [1]. The prevalence of a history of post-traumatic stress disorder is estimated at 1 percent in the general population, about 3.5 percent in civilians exposed to physical attack and more than 20 percent in veterans wounded in war [2]. PTSD pathogenesis is still unclear. It is believed that impaired sensorimotor gating (SG) is a contributing factor. SG is a pre-attentive filtering process that allows brain to avoid information overload [3] and properly encode information [4]. Indeed, a number of studies have described a relationship between PTSD and SG dysfunction [5,6]. Although a strong stimulus results in a startle reflex across species a relatively weak sensory stimulus presented 30–500 ms before the strong stimulus will reduce the startle response [7]. This phenomenon, known as the prepulse inhibition (PPI) effect, provides a simple explanation of how dysfunctional SG might contribute to PTSD. By regulating motor and pre-motor systems, PPI reduces responses toward irrelevant information, which is recognized as an operational measurement of SG [8]. Increasing evidence indicates that PPI dysfunction positively correlates with PTSD [9,10,11]. Recently, a clinical study also suggested disrupted sensory filtering in PTSD [12]. However, it remains unclear when precisely impaired SG occurs after stress and how it can be effectively attenuated.

Single prolonged stress (SPS) is a well-established animal model of PTSD [13] which mimics a portion of the neuroendocrine and behavior changes [14] associated with PTSD. For example, in this model the hypothalamus-pituitary-adrenal axis provides rapid negative feedback after administration of glucocorticoid [14] to increase anxiety behavior in the elevated plus maze [15], increase contextual freezing [16], and produces an exaggerated acoustic startle response (ASR) [17]. These changes support the validity of SPS as an animal model of PTSD. Our recent work demonstrated that an inescapable foot electric shock added to a classic SPS model (SPS & electric foot shock, SPS&S) significantly enhances fear responses [18] offering a novel PTSD model. This improved model offers significant changes in open field, elevated plus-maze, and Morris water maze performance reflecting increased anxiety-like behaviors [19] and intrusive memory [20] which are characteristic features of PTSD.

Some pharmaceuticals provide therapeutic benefit for PTSD in humans [21,22] and some of these drugs have been shown to prevent anxiety-like behaviors and cognitive impairments in stressed rats [23]. However, not all patients respond to the currently available pharmacological treatment options for PTSD [24,25]. For example, no significant differences between sertraline (a known PTSD drug) and placebo on any of the efficacy measures at endpoint in a military population [25]. Therefore, non-pharmacologic treatment approaches have been developed. Repetitive transcranial magnetic stimulation (rTMS) is a safe and non-invasive method, and studies have indicated that administration of high-frequency rTMS to the right dorsolateral prefrontal cortex is beneficial for ameliorating PTSD symptoms [26,27]. Although it was primarily utilized for treatment, we hypothesized that rTMS might also provide benefits as an early prevention measure. In this study, we demonstrated that stress-induced anxiety-like
behavior and impaired PPI occur after stress and demonstrated that rTMS could attenuate stress-associated behaviors, anxiety-like behaviors and impaired SG, in the SPS&S paradigm.

**Materials and Methods**

1. **Animals**

Eighty-eight adult male Sprague-Dawley (SD) rats (nearly 8 weeks old, 180–220 g) were purchased from the Experimental Animals Center of Fourth Military Medical University (Xi’an, China). Four rats were housed per cage in an air-conditioned room (at 22 ± 1°C) with 50–55% relative humidity under a 12-h light/dark cycle and provided with food and water *ad libitum* for 7 days before experimentation. The study protocol was approved by the Committee of Animal Care and Use for Research and Education (CACURE) of the Fourth military Medical University and complied with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

2. **Study design**

Two experiments were carried out in this study. All animals were allowed one week adaptation before experiment start. In the first experiment, 48 animals were randomly divided into 4 groups (n = 12/group) including control, SPS&S 1 d, SPS&S 7 d, and SPS&S 14 d. The rats in control group (no SPS&S) were submitted to a behavioral test 1 day after stress induction in other groups, together with the SPS&S 1d group. Behavioral test were carried out with SPS&S 7 d and SPS&S 14 d, 7 and 14 days after SPS&S stress induction (Fig. 1A).

In the second experiment, 80 rats were randomly divided into 4 groups (n = 20/group): control, SPS&S, rTMS, rTMS + SPS&S. Rats in control group were neither submitted to stress nor treated with rTMS. In the rTMS group, animals were not submitted to stress but treated with rTMS. The SPS&S group included animals submitted to SPS&S stress but without rTMS treatment while the rTMS + SPS&S animals were treated with rTMS after SPS&S stress induction (Fig. 1B). Part of animals (n = 10 for each group) were exposed to behavioral tests at SPS&S 7 d and then they were sacrificed and the expression of c-Fos and glucocorticoid receptor of medial prefrontal cortex (mPFC) were detected by Western blot and the plasma levels of corticosterone (CORT) were detected by Elisa. The remained animals were exposed to behavioral tests at SPS&S 14 d and then they were sacrificed and the expression of c-Fos and glucocorticoid receptor of mPFC were detected, as well as the plasma levels of corticosterone (CORT). All rats were naïve to the experimental apparatus.

3. **SPS&S animal model**

The detailed procedures for the SPS&S procedure were described in previous report [18]. Briefly, rats were restrained for 2 h immediately followed by 20 min forced swimming in 24°C water. A transparent acrylic cylinder (24 cm diameter, 50 cm height) was used for forced swimming, filled to about two-thirds with water. Rat activity during swimming was recorded with an attached CCD camera. After recuperating for 15 min, rats were exposed to diethyl ether until they lost consciousness. Afterwards, the animals were kept in the shock chamber until recovery (about 30 min) and the electric foot shock was applied through the metal grid installed at the bottom of the chamber.

4. **rTMS treatment**

The YRD CCY-1 magnetic stimulation apparatus with a matched circular coil (5-cm inner coil, 6.5-cm outer winding) purchased from Yiruide Co., Ltd. (Wuhan, China) was employed. In
accordance with our previous study [28] and another report [29], the rTMS parameters were modified as follows: the stimulating frequency was 10Hz and the stimulating pulse intensity 30% of the rTMS device maximum power (0.7 Tesla). Each train consisted of 51 pulses and was repeated 20 times daily (1020 pulses per day) with an inter-train interval of 15s. These were administered for 7 consecutive days (7140 pulses in total). Rats were administered the magnetic stimulation during inhalation anesthesia (1L/min oxygen with 5% isoflurane for initial anesthesia followed by a reduction to 1–2% isoflurane during the stimulation procedure). Our preliminary data showed that this isoflurane regimen do not affect the assessed behaviors (OFT, EPMT, PPI) in agreement with previous study [30]. Nevertheless, all rats, including control animals, were administered isoflurane to rule out any anesthesia related effects. During stimulation, the center of the coil was placed on the vertex, gently touching the scalp. For sham stimulation of the control group, a powerless coil was placed perpendicular to the scalp.

5. Open field test (OFT)

OF was used to measure spontaneous locomotor activity of the animals [31]. The apparatus was composed of four black acrylic plastic boxes (47×47×47 cm) (DigBehav, Jiliang Co., Ltd., Shanghai, China) placed in a soundproof box. Recordings were performed in the soundproof box illuminated by a red light (30 W). Each rat was placed in the center zone at the beginning of testing and horizontal distance traveled was automatically recorded for 10 min by an automatic analyzing system.
6. Elevated plus maze test (EPMT)

EPMT was used to determine the behavior of approach-avoidance conflict in rats [16]. The apparatus consisted of two opposing arms (50 cm×10 cm) enclosed by 40 cm high side and end walls (closed arms), the other two arms were not installed with walls (open arms) with the entire maze (DigBehav, Jiliang Co. Ltd., Shanghai, China) elevated 50 cm above the floor. During testing, rats were first placed in the central area (10×10 cm) of the maze pointed towards an open arm. Afterwards, activity was recorded for 5 min with an infrared camera in a dark room.

7. Prepulse inhibition trial (PPI)

An animal acoustic startle system (Coulbourn Instruments, USA) was used for testing [3]. The apparatus consisted of a sound controller and four chambers. Each chamber consisted of a Plexiglas box resting on a platform which recorded reflex values. Rats were placed in each Plexiglas box and were exposed to four different types of trials after 5 minute acclimation period. The first was a pulse alone trial in which 50 ms of a 108 dB white noise burst without a sound rise/fall was administered. The remaining three were varying prepulse (70 dB, 76 dB, 82 dB) + pulse trials in which 50 ms, 5 KHz sounds were presented before the onset of a 108 dB pulse without a sound rise/fall, with 500 ms between prepulse and pulse. The inter-trial interval ranged from 30 to 45 seconds. The ventilation fans were set to be intermittent and used a minimum/maximum range of 30 to 70%. Each trial was repeated 10 times with a total of 40 trials during approximately 30 minutes. The acoustic startle reflex for each rat was calculated as the average amplitude on trials in which the pulse was presented alone. Percent Prepulse inhibition (PPI) was calculated for each rat using the following equation: %PPI = (1-PP/P) × 100%, where PP represented the average response for prepulse + pulse trial, and P the mean average response with pulse alone.

8. CORT measurement by ELISA assay

Blood serums were collected via tail incision centrifuged at 4°C and the supernatants of brain homogenates from each treatment group were collected and the levels of CORT were measured by a Rat Elisa Kit according to the manufacturer’s instructions (Westang, F3704, China). The sensitivity of the measurements for CORT was 0.15ng/ml.

9. Western blotting

Every rat of the normal control and SPS groups was decapitated. The whole brain was quickly removed and placed in an ice-cold dish; the mPFC was dissected according to the atlas by cutting perpendicularly. The mPFC were lysed with SDS-PAGE sample buffer composed of 62.5 mM Tris–HCl, 2% w/v SDS, 10% glycerol, 50 mM DTT, and 0.1% w/v bromphenol blue. After homogenization, whole cell protein was obtained by centrifugation at 12,000g for 10 min and the supernatant was collected. Protein extracts were separated by SDS polyacrylamide gel electrophoresis and then transferred onto PVDF membranes (Millipore). Membrane was blocked with 5% skim milk in Tris buffered saline (TBS) and then incubated overnight at 4°C with mouse anti- Glucocorticoid receptor antibody (1:2000, Abcam, ab2768), rabbit polyclonal to c-Fos (1:2500, Abcam, ab190289) and mouse anti-β-actin antibody. After washing, membranes were incubated with a horseradish peroxidase (HRP)-conjugated IgG secondary antibody in accordance with the origin of the primary antibody for 1 h at room temperature. After wash, the antibody-reactive bands were visualized on X-ray film using super ECL plus detection reagent (Supersignal west pico chemiluminescent substrate, Thermo, USA, 34077).
10. Statistical analysis

SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the data, expressed as means ± standard error of the mean (SEM). OF, EPM and PPI indices were analyzed using one-way or two-way analysis of variance (ANOVA) across groups. PPI data among sound pressure levels in the same group were analyzed using repeated measures ANOVA. The least significant difference (LSD)–t method was further used as a post hoc test to detect group differences with homogeneity of variance, or Dunnett T3 was used. All tests were two-sided and statistical significance was defined as $P < 0.05$.

Results

1. Spontaneous locomotor activity in a rat model of PTSD

As shown in Fig. 2, there was no statistically significant difference among the four groups for the total distance of movement ($F_{3, 44} = 2.620, P = 0.063$) and the total time of movement ($F_{3, 44} = 1.046, P = 0.384$). However, significant differences were found among the four groups in distance of central movement ($F_{3, 44} = 3.363, P = 0.027$), time of central movement ($F_{3, 44} = 4.015, P = 0.013$), percent distance of central movement ($F_{3, 44} = 3.686, P = 0.019$) and percent time of central movement ($F_{3, 44} = 3.325, P = 0.031$). Compared to the control group, the SPS&S 1 day group had a significantly reduced distance of central movement, less time of central movement and less percent distance of central movement (all $P < 0.05$). On the other hand, the SPS&S 7 day group demonstrated a reduction (all $P < 0.05$) of all four indices, distance of central movement, percent distance of central movement, time of central movement, and percent time of central movement. However, only time of central movement and percent time of central movement were reduced in the SPS&S 14 day group (both $P < 0.05$).

Fig 2. Spontaneous locomotor activity in a rat model of PTSD. Total distance of movement (A), time of central movement (B), distance and time of central movement relative to overall levels (%) values (C, D). Data are expressed as mean ± standard error of the mean (SEM) ($n = 12$ per group). *$P < 0.05$ vs. control.

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2. Behavior of approach-avoidance conflict in a rat model of PTSD

As shown in Fig. 3, significant differences were found among the four groups in time spent in open arms ($F_3, 44 = 3.204, P = 0.032$), with $77.93 \pm 13.44, 36.62 \pm 13.42, 26.43 \pm 9.59,$ and $46.33 \pm 14.66$ s obtained for controls, and rats evaluated at 1, 7, and 14 days after SPS&S, respectively. Similar results were obtained for percent time spent in open arms, with $25.98 \pm 4.48, 12.21 \pm 4.47, 8.81 \pm 3.2,$ and $15.44 \pm 4.89\%$ ($F_3, 44 = 3.204, P = 0.032$) and percent numbers of entry into the open arms, with $32.19 \pm 4.63, 16.51 \pm 5.23, 12.55 \pm 5.46,$ and $16.38 \pm 4.88\%$ ($F_3, 44 = 2.968, P = 0.042$) for control animals and rats evaluated at 1, 7, and 14 days after SPS&S, respectively. However no significant difference was obtained for absolute numbers of entry into the open arms ($F_3, 44 = 2.392, P = 0.081$). These data indicated that rats submitted to SPS&S and evaluated 1 and 7 and 14 days showed a reduction in time spent in the open arms and percent time spent in the open arms compared to the control group (all $P < 0.05$), although the SPS&S 14 day group showed a slight increase compared with other SPS&S 7 animals. Each SPS&S group also showed a reduction in percent number of entries into the open arms (all $P < 0.05$).

3. Sensorimotor gating in a rat model of PTSD

A statistically significant difference was observed in average %PPI (70–82 dB) among the four groups ($F_3, 44 = 2.869, P = 0.047$) as shown in Fig. 4. Indeed, average %PPI (70–82 dB) of control animals ($40.03 \pm 2.50\%$) was higher than those found in the three SPS&S groups (all $P < 0.05$), including rats evaluated at 1 ($19.29 \pm 4.72\%$), 7 ($20.58 \pm 5.01\%$), and 14 ($17.26 \pm 5.24\%$) days.

4. Effect of rTMS on spontaneous locomotor activity in a rat model of PTSD

Significant differences were observed among the four groups in distance of central movement ($F_3, 36 = 7.360, P = 0.001$) (Fig. 5A), time of central movement ($F_3, 36 = 5.319, P = 0.004$)
percent distance of central movement ($F_3, 36 = 6.705, P = 0.001$) (Fig. 5C) and percent time of central movement ($F_3, 44 = 3.707, P = 0.020$) (Fig. 5D) at SPS&S 7 d. In addition, compared with the SPS&S group, rats treated with rTMS + SPS&S significantly increased the distance of central movement ($978.03 \pm 73.91$ vs $421.16 \pm 65.73$), percent distance of central movement ($11.67 \pm 0.88$ vs $5.40 \pm 0.93$), time of central movement ($47.93 \pm 5.28$ vs $19.40 \pm 2.81$), and percent time of central movement ($17.60 \pm 2.26$ vs $8.14 \pm 1.55$) (all $P < 0.05$).

There were also significant differences among the four groups in distance of central movement ($F_3, 36 = 12.169, P < 0.01$) (Fig. 5E), time of central movement ($F_3, 36 = 4.892, P = 0.006$) (Fig. 5F), percent distance of central movement ($F_3, 36 = 6.583, P = 0.001$) (Fig. 5G) and

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**Fig 4. Sensorimotor gating in a rat model of PTSD.** %PPI (Prepulse inhibition (%)) among four groups ($n = 12$ per group). Mean ± SEM of %PPI by prepulses with different intensities (72, 76, and 82 dB) and calculated all over the three prepulse intensities in each group (Av.PPI is average of total 70–82 dB %PPI in each group). *$P < 0.05$.

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**Fig 5. Effect of rTMS on spontaneous locomotor activity in a rat model of PTSD.** Distance of central movement and Time of central movement in absolute after SPS&S treatment for 7 day (A, B) or 14 day (E, F) and relative to overall levels (%) values after SPS&S treatment for 7 day (C, D) or 14 day (G, H) tested in open field. Data are expressed as mean ± SEM ($n = 10$ per group). *$P < 0.05$ vs. control or SPS&S + rTMS group.

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percent time of central movement ($F_{3, 44} = 3.269, P = 0.334$) (Fig. 5H) at SPS&S 14 d. In addition, compared with the SPS&S group, rats treated with rTMS + SPS&S significantly increased the distance of central movement ($1027 \pm 191.28 \text{ vs.} 693.31 \pm 133.68$), percent distance of central movement ($12.68 \pm 3.74 \text{ vs.} 6.22 \pm 3.62$), time of central movement ($47.86 \pm 25.17 \text{ vs.} 28.69 \pm 14.63$), and percent time of central movement ($14.08 \pm 3.08 \text{ vs.} 9.81 \pm 2.74$) (all $P < 0.05$).

5. Effect of rTMS on behavior of approach-avoidance conflict in a rat model of PTSD

At SPS&S 7 d, significant differences were found among the four groups in time spent in open arms ($F_{3, 36} = 6.368, P = 0.001$) and percent time spent in open arms ($F_{3, 36} = 6.441, P = 0.001$) (Fig. 6A and 6C). However, no significant difference was obtained for percent and number of entries into open arms ($F_{3, 36} = 0.544, P = 0.656, F_{3, 36} = 2.017, P = 0.129$) as shown in Fig. 6B and 6D. Here also, compared with the SPS&S group, rats treated with rTMS + SPS&S significantly increased the time spent in open arms ($62.15 \pm 7.01 \text{ vs.} 26.84 \pm 5.28$), and percent time spent in open arms ($24.01 \pm 0.03 \text{ vs.} 8.42 \pm 2.27$) (both $P < 0.05$).

There were also significant differences among the four groups in time spent in open arms ($F_{3, 36} = 18.221, P < 0.01$) and percent time spent in open arms ($F_{3, 36} = 8.207, P < 0.01$) (Fig. 6E and 6G). However, no significant difference was obtained for percent and number of entries into open arms ($F_{3, 36} = 0.081, P = 0.970, F_{3, 36} = 0.860, P = 0.469$) as shown in Fig. 6F and 6H at SPS&S 14 d. Here also, compared with the SPS&S group, rats treated with rTMS + SPS&S significantly increased the time spent in open arms ($54.35 \pm 9.61 \text{ vs.} 30.98 \pm 8.01$), and percent time spent in open arms ($20.09 \pm 4.59 \text{ vs.} 10.39 \pm 4.02$) (both $P < 0.05$).

6. Effect of rTMS on sensorimotor gating in a rat model of PTSD

We observed a statistically significant difference in average %PPI (70–82dB) between the SPS&S group (22.30 ± 2.82) and the remaining three groups at SPS&S 7 d, including control animals (46.25 ± 3.63) and rats treated with rTMS (37.53 ± 4.27) and SPS&S + rTMS (37.58 ± 2.08) ($F_{3, 36} = 8.456, P < 0.001$) as shown in Fig. 7A. Furthermore, %PPI values obtained for the SPS&S group were significantly lower compared with the other groups at all intensities (all $P < 0.05$).

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Fig 6. Effect of rTMS on behavior of approach-avoidance conflict in a rat model of PTSD. Time spent in open arms and number of entries into open arms in absolute after SPS&S treatment for 7 day (A, B) or 14 day (E, F) and relative to overall levels (%) values after SPS&S treatment for 7 day (C, D) or 14 day (G, H) tested in the elevated plus maze. Data are expressed as mean ± SEM (n = 10 per group). *$P < 0.05$ vs. control or SPS&S + rTMS group.

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There were also statistically significant differences in average %PPI (70–82dB) between the SPS&S group and the remaining three groups at SPS&S 14d ($F_3$, $36 = 14.35$, $P < 0.01$) as shown in Fig. 7B. Furthermore, %PPI values obtained for the SPS&S group were also significantly lower than that of the other groups at all intensities (all $P < 0.05$).

7. Effect of rTMS on the expression of c-fos and glucocorticoid receptor (GR) in the mPFC and the plasma level of cortisol

At SPS&S 7 d, significant differences were observed among the four groups in the expression of c-fos ($F_3$, $17 = 12.058$, $P < 0.01$) (Fig. 8B) and GR ($F_3$, $17 = 11.370$, $P < 0.01$) (Fig. 8C) in the mPFC. Meanwhile, compared with the control or rTMS group, rats treated with SPS&S or rTMS + SPS&S significantly increased the expression of c-fos and GR (all $P < 0.01$); There was no significant difference among the four groups in the expression of c-fos ($F_3$, $20 = 0.740$, $P = 0.540$) (Fig. 8B) and GR ($F_3$, $17 = 0.969$, $P = 0.432$) (Fig. 8C) in the mPFC at SPS&S 14 d. Two-way ANOVA further revealed significant differences of stress factor (control vs. SPS&S vs.

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**Fig 7. Effect of rTMS on sensorimotor gating in a rat model of PTSD.** %PPI among four groups after SPS&S treatment for 7 day (A) or 14 day (B). Mean ± SEM of %PPI by prepulses with different intensities (72, 76, and 82 dB) and calculated over all three prepulse intensities in each group (Av. PPI is the average of total 70–82dB %PPI in each group). *$P < 0.05$. doi:10.1371/journal.pone.0117189.g007
**Discussion**

Several studies have suggested that decreased central movements in the OFT [32] and decreased open-arm parameters in the EPMT paradigm represent surrogates for anxiety-like behavior [33]. Our data described here suggest that SPS&S is an effective model to mimic stress-related anxiety behavior as a component of the PTSD characteristics. Indeed, we found that stress reduced central movements in the OFT and open-arm movements in the EPMT without causing over all locomotor impairment, indicating that the stressed rats exhibited persistent anxiety-like behaviors. These findings further validated the SPS&S model for PTSD. Impairment of PPI represents SG dysfunction which could be explained by the theory of attentive processing. PPI refers to a situation in which the subject notices the weaker or secondary stimulus thus transferring attention from the stronger stimulus, and results in a reduced startle response [34]. Previous studies using human subjects have demonstrated that selectively paying attention to the prepulse stimulus enhances the magnitude of PPI [34,35], indicating that PPI is a reflection of general attention processing ability and therefore relevant to provide protection for the pre-attention stimulus [36].

The failure of patients with PTSD to inhibit negative emotion and memories of traumatic experiences may imply a defect in PPI. They may suffer impairment of intra-attention and be unable to restrain the onset or recall of negative information, suggesting that PPI dysfunction is a component of the pathophysiological mechanism of PTSD [9,10,11,12]. This idea is further supported by animal studies. For example, stressed alpha-2C adrenergic receptor knockout mice have impaired PPI [37]. In addition, a study comparing predator stress versus footshock stress in rats demonstrated that changes in c-fos expression in different regions correlated with effects on PPI [38]. In the present work, we provide evidence for the first time that impaired PPI and anxiety behavior occur in SPS&S rats with a reduction in PPI arising immediately and persisting for some time: traumatic events can induce impairment immediately after the stress and early intervention is therefore needed after the traumatic exposure.

Two approaches have been proposed as early interventions for PTSD. In the first, action precedes the stress with the intention of enhancing resistance to stress while in the second...
approach, treatment is introduced after in order to prevent establishment of a stress related syndrome [27]. Since many traumatic incidents are unpredictable, the latter approach is likely the one that is feasible. Accordingly, we determined whether rTMS, a well-established therapeutic method PTSD treatment, offered benefits as early intervention after stress. Prior studies have indicated that administration of high-frequency rTMS to the right dorsolateral prefrontal cortex is beneficial for ameliorating PTSD symptoms [26,27]. Our data presented herein demonstrated for the first time that chronic administration of rTMS has anxiolytic effect on stress-related anxiety-like behaviors without affecting control rats.

Several studies have suggested that DAergic and 5-HTergic systems are associated with anti-PTSD treatments [39,40]. Kanno et al. [41] found that chronic rTMS, but not acute rTMS improves behavior of rats on the plus-maze, which might involve the serotonergic system. In addition, there are several other biological aspects that should be considered to understand the mechanism of rTMS, including various aspects of stress biology, immune function disruptions, neural structure, and function, as well as circadian rhythms [42]. Some animal studies also suggest that rTMS affects neurotransmitter systems of 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) [43,44,45]. High-frequency rTMS has been reported to regulate brain activity and increase cortical excitability [46]. In addition, neural activity and corresponding hemodynamic responses induced by rTMS have been shown in the cat visual cortex [47].

Our previously studies showed that high frequency (15 Hz) rTMS could elevate the expression of BDNF in the hippocampus and decrease the level of plasma ACTH and CORT in chronic unpredictable mild stress (CUMS) rats [28,48]. In the present study, we found the CORT level was increased after SPS&S which was in accordance with previously study [49], and high frequency rTMS treatment also alleviated this situation, the underlying mechanisms still need further studied.

It is well known that the main pathway of PPI is cortico-striato-pallido-thalamic (CSPT) [50] the inferior colliculus [51], superior colliculus [52] and pallidotegmental area [53] playing key roles. The medial prefrontal cortex (mPFC) is a higher order structure that controls stress and fear responses of the hippocampus and amygdala [54], and it plays an important role in the regulation of fear, anxiety and aggression [55]. In particularly, PPI is normally regulated by the mPFC [56,57], and studies have showed that mPFC is closely involved in the pathogenesis of PTSD [58,59]. We speculate that stress affects mPFC or other brain regions that are sensitive to magnetic stimulation at early stages after stress and rTMS stimulates those circuits to protect against PPI impairments. In the present study, we found that chronic rTMS could prevent PPI impairment with little effect on control rats; the expression of c-fos and GR in the mPFC was increased 7 d after SPS&S, and was recovered 14 d after SPS&S, and chronic rTMS also elevated the expression of c-fos but did not affect the expression of GR in the mPFC. It is well known that the expression of c-fos and GR was increased in the animal model of PTSD [60,61], and the expression of c-fos and GR was changed immediately after stress, it is hard to speculate the involvement of c-fos and GR expression of mPFC on the effect of rTMS. Further studies are required to optimize the conditions of rTMS for maximizing its preventative potential.

In summary, stress rapidly causes stress-induced anxiety-like behavior and altered PPI, which could be prevented by high-frequency rTMS. Our findings suggest that high frequency rTMS should be further evaluated for its use as a method for preventing PTSD.

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Author Contributions
Conceived and designed the experiments: Q-RT H-NW Y-HB Z-WP. Performed the experiments: H-NW Y-HB Y-CC R-GZ. Analyzed the data: J-LG. Contributed reagents/materials/analysis tools: Y-HZ. Wrote the paper: H-NW H-HW Y-HZ.

References
1. Nemeroff CB, Brenner JD, Foa EB, Mayberg HS, North CS, et al. (2006) Posttraumatic stress disorder: a state-of-the-science review. J Psychiatr Res 40: 1–21. PMID:16242154
2. Jackson G (1991) The rise of post-traumatic stress disorders. BMJ 303: 533–534. PMID:1912880
3. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156: 117–154. PMID:11549216
4. Yeomans JS, Frankland PW (1995) The acoustic startle reflex: neurons and connections. Brain Res Brain Res Rev 21: 301–314. PMID:8806018
5. Neylan TC, Fletcher DJ, Lenoci M, McCallin K, Weiss DS, et al. (1999) Sensory gating in chronic post-traumatic stress disorder: reduced auditory P50 suppression in combat veterans. Biol Psychiatry 46: 1656–1664. PMID:10624547
6. Ghisolfi ES, Margis R, Becker J, Zanardo AP, Strimitzer IM, et al. (2004) Impaired P50 sensory gating in post-traumatic stress disorder secondary to urban violence. Int J Psychophysiol 51: 209–214. PMID:14962572
7. Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156: 234–258. PMID:11549226
8. Sperling NJ, Morgan CA, Southwick SM, Davis M, Charney DS (1996) Baseline startle amplitude and prepulse inhibition in Vietnam veterans with posttraumatic stress disorder. Psychiatry Res 64: 169–178. PMID:8944395
9. Gillette GM, Skinner RD, Rasco LM, Fielstein EM, Davis DH, et al. (1997) Combat veterans with post-traumatic stress disorder exhibit decreased habituation of the P1 midlatency auditory evoked potential. Life Sci 61: 1421–1434. PMID:935232
10. Vestslering JJ, Brailey K, Constats JI, Sutker PB (1998) Attention and memory dysfunction in posttraumatic stress disorder. Neuropsychology 12: 125–133. PMID:9460704
11. Stewart LP, White PM (2008) Sensory filtering phenomenology in PTSD. Depress Anxiety 25: 38–45. PMID:17203460
12. Liberzon I, Krislov M, Young EA (1997) Stress-resist: effects on ACTH and fast feedback. Psycho- neuroendocrinology 22: 443–453. PMID:9364622
13. Liberzon I, Lopez JF, Flagel SB, Vazquez DM, Young EA (1999) Differential regulation of hippocampal glucosecorticoid receptors mRNA and fast feedback: relevance to post-traumatic stress disorder. J Neuroendocrinol 11: 11–17. PMID:9918224
14. Imanaka A, Morinobu S, Toki S, Yamawaki S (2006) Importance of early environment in the development of post-traumatic stress disorder-like behaviors. Behav Brain Res 173: 129–137. PMID:16860405
15. Iwamoto Y, Morinobu S, Takahashi T, Yamawaki S (2007) Single prolonged stress increases contextual freezing and the expression of glycine transporter 1 and vesicle-associated membrane protein 2 mRNA in the hippocampus of rats. Prog Neuropsychopharmacol Biol Psychiatry 31: 642–651. PMID:17267088
16. Khan S, Liberzon I (2004) Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. Psychopharmacology (Berl) 172: 225–229. PMID:14586539
17. Wang W, Liu Y, Zheng H, Wang HN, Jin X, et al. (2008) A modified single-prolonged stress model for post-traumatic stress disorder. Neurosci Lett 441: 237–241. doi:10.1016/j.neulet.2008.06.031 PMID:18577419
18. Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, et al. (2009) Single prolonged stress: toward an animal model of posttraumatic stress disorder. Depress Anxiety 26: 1110–1117. doi: 10.1002/di.20629 PMID:19918929
20. Wang HN, Peng Y, Tan QR, Wang HH, Chen YC, et al. (2009) Free and Easy Wanderer Plus (FEWP), a polyherbal preparation, ameliorates PTSD-like behavior and cognitive impairments in stressed rats. Prog Neuropsychopharmacol Biol Psychiatry 33: 1458–1463. doi: 10.1016/j.pnpbp.2009.07.031 PMID: 19665511

21. Marshall RD, Beebe KL, Oldham M, Zaninelli R (2001) Efficacy and safety of paroxetine treatment for chronic PTSD: a fixed-dose, placebo-controlled study. Am J Psychiatry 158: 1982–1988. PMID: 11775045

22. Tucker P, Zaninelli R, Yehuda R, Ruggiero L, Dillingham K, et al. (2001) Paroxetine in the treatment of chronic posttraumatic stress disorder: results of a placebo-controlled, flexible-dose-parallel trial. J Clin Psychiatry 62: 860–868. PMID: 11775045

23. Wang HN, Peng Y, Tan QR, Chen YC, Zhang RG, et al. (2010) Quetiapine ameliorates anxiety-like behavior and cognitive impairments in stressed rats: implications for the treatment of posttraumatic stress disorder. Physiol Res 59: 263–271. PMID: 19537923

24. Carlson KF, Kehle SM, Meis LA, Greer N, Macdonald R, et al. (2011) Prevalence, assessment, and treatment of mild traumatic brain injury and posttraumatic stress disorder: a systematic review of the evidence. J Head Trauma Rehabil 26: 103–115. doi: 10.1097/HTR.0b013e3181b5e50f PMID: 20631631

25. Friedman MJ, Marmar CR, Baker DG, Sikes CR, Farfel GM (2007) Randomized, double-blind comparison of sertraline and placebo for posttraumatic stress disorder in a Department of Veterans Affairs setting. J Clin Psychiatry 68: 711–720. PMID: 17503980

26. Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, et al. (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. PMID: 14992978

27. Boggio PS, Rocha M, Oliveira MO, Fecteau S, Cohen RB, et al. (2010) Noninvasive brain stimulation with high-frequency and low-intensity repetitive transcranial magnetic stimulation treatment for post-traumatic stress disorder. J Clin Psychiatry 71: 992–999. doi: 10.4088/JCP.08m04638blu PMID: 20051219

28. Feng SF, Shi TY, Fan Y, Wang WN, Chen YC, et al. (2012) Long-lasting effects of chronic rTMS to treat chronic rodent model of depression. Behav Brain Res 232: 245–251. doi: 10.1016/j.bbr.2012.04.019 PMID: 22537774

29. Baek K, Chae JH, Jeong J (2012) The effect of repetitive transcranial magnetic stimulation on fear extinction in rats. Neuroscience 200: 159–165. doi: 10.1016/j.neuroscience.2011.09.050 PMID: 21989475

30. McAuliffe JJ, Loepke AW, Miles L, Joseph B, Hughes E, et al. (2009) Desflurane, isoflurane, and sevoflurane provide limited neuroprotection against neonatal hypoxia-ischemia in a delayed preconditioning paradigm. Anesthesiology 111: 533–546. doi: 10.1097/ALN.0b013e3181b86003 PMID: 19672176

31. Ferguson SA, Paule MG, Holson RR (2001) Neonatal dexamethasone on day 7 in rats causes behavioral alterations reflective of hippocampal, but not cerebellar, deficits. Neurotoxicol Teratol 23: 57–69. PMID: 11274876

32. Conrad KL, Louderback KM, Gessner CP, Winder DG (2011) Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. Physiol Behav 104: 248–256. doi: 10.1016/j.physbeh.2011.03.001 PMID: 21936387

33. Ramos A (2008) Animal models of anxiety: do I need multiple tests? Trends Pharmacol Sci 29: 493–498. doi: 10.1016/j.tips.2008.07.005 PMID: 18755516

34. Filion DL, Poje AB (2003) Selective and nonselective attention effects on prepulse inhibition of startle: a comparison of task and no-task protocols. Biol Psychol 64: 283–296. PMID: 14630408

35. Thorne GL, Dawson ME, Schell AM (2005) Attention and prepulse inhibition: the effects of task-relevant, irrelevant, and no-task conditions. Int J Psychophysiol 56: 121–128. PMID: 15804447

36. Bitsios P, Giakoumaki SG (2005) Relationship of prepulse inhibition of the startle reflex to attentional and executive mechanisms in man. Int J Psychophysiol 55: 229–241. PMID: 15649554

37. Sallinen J, Haapalinna A, Viitamaa T, Kobilka BK, Scheinin M (1998) Adrenergic alpha2C-receptors modulate the acoustic startle reflex, prepulse inhibition, and aggression in mice. J Neurosci 18: 3035–3042. PMID: 9526020

38. Baisley SK, Cloninger CL, Bakshi VP (2011) Fos expression following regimens of predator stress versus footshock that differentially affect prepulse inhibition in rats. Physiol Behav 104: 796–803. doi: 10.1016/j.physbeh.2011.08.001 PMID: 21843541

39. Horger BA, Roth RH (1996) The role of mesoprefrontal dopamine neurons in stress. Crit Rev Neurobiol 10: 395–418. PMID: 8978988

40. Weiss SJ (2007) Neurobiological alterations associated with traumatic stress. Perspect Psychiatr Care 43: 114–122. PMID: 17576304
41. Kanno M, Matsumoto M, Togashi H, Yoshioka M, Mano Y (2003) Effects of repetitive transcranial magnetic stimulation on behavioral and neurochemical changes in rats during an elevated plus-maze test. J Neurosci 21: 5–14. PMID: 12767491

42. George MS, Wassermann EM, Post RM (1996) Transcranial magnetic stimulation: a neuropsychiatric tool for the 21st century. J Neuropsychiatry Clin Neurosci 8: 373–382. PMID: 9116472

43. Keck ME, Sillaber I, Eber K, Welt T, Toschi N, et al. (2000) Acute transcranial magnetic stimulation of frontal brain regions selectively modulates the release of vasopressin, biogenic amines and amino acids in the rat brain. Eur J Neurol : 12: 3713–3720. PMID: 11092641

44. Yue L, Xiao-lin H, Tao S (2009) The effects of chronic repetitive transcranial magnetic stimulation on glutamate and gamma-amino butyric acid in rat brain. Brain Res 1260: 94–99. PMID: 19401169

45. Wang HY, Crupi D, Liu J, Stucky A, Crucianti G, et al. (2011) Repetitive transcranial magnetic stimulation enhances BDNF-TrkB signaling in both brain and lymphocyte. J Neurosci 31: 11044–11054. doi: 10.1523/JNEUROSCI.2125-11.2011 PMID: 21795553

46. 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. PMID: 16890483

47. Allen EA, Pasley BN, Duong T, Freeman RD (2007) Transcranial magnetic stimulation elicits coupled neural and hemodynamic consequences. Science 317: 1918–1921. PMID: 17901333

48. Wang HN, Wang L, Zhang RG, Chen YC, Liu L, et al. (2014) Anti-depressive mechanism of repetitive transcranial magnetic stimulation in rat: the role of the endocannabinoid system. J Psychiatr Res 51: 79–87. doi: 10.1016/j.jpsychires.2014.01.004 PMID: 24479995

49. Jia M, Meng F, Smerin SE, Xing G, Zhang L, et al. (2012) Biomarkers in an animal model for revealing neural, hematologic, and behavioral correlates of PTSD. J Vis Exp.

50. Kumari V, Gray JA, Geyer MA, ffytche D, Soni W, et al. (2003) Neural correlates of tactile prepulse inhibition: a functional MRI study in normal and schizophrenic subjects. Psychiatry Res 122: 99–113. PMID: 12714174

51. Herbert H, Aschoff A, Ostwald J (1991) Topography of projections from the auditory cortex to the inferior colliculus in the rat. J Comp Neurol 304: 103–122. PMID: 2016407

52. Meloni EG, Davis M (2000) GABA in the deep layers of the superior Colliculus/Mesencephalic reticular formation mediates the enhancement of startle by the dopamine D1 receptor agonist SKF 82958 in rats. J Neurosci 20: 5374–5381. PMID: 10984322

53. Takahashi K, Nagai T, Kamei H, Maeda K, Matsuya T, et al. (2007) Neural circuits containing pallidotegmental GABAergic neurons are involved in the prepulse inhibition of the startle reflex in mice. Biol Psychiatry 62: 148–157. PMID: 17027927

54. Zelikowsky M, Hersman S, Chawla MK, Barnes CA, Fanselow MS (2014) Neuronal ensembles in amygdala, hippocampus, and prefrontal cortex track differential components of contextual fear. J Neurosci 34: 8462–8466. doi: 10.1523/JNEUROSCI.3624-13.2014 PMID: 24948801

55. Sailer U, Robinson S, Fischmeister FP, Konig D, Oppenauer C, et al. (2008) Altered reward processing in the nucleus accumbens and mesial prefrontal cortex of patients with posttraumatic stress disorder. Neuropsychopharmacology 46: 2836–2844. doi: 10.1016/j.neuropsychologia.2008.05.022 PMID: 18597797

56. Swerdlow NR, Light GA, Trim RS, Breier MR, Hines SR, et al. (2013) Forebrain gene expression predicts deficits in sensorimotor gating after isolation rearing in male rats. Behav Brain Res 257: 118–128. doi: 10.1016/j.bbr.2013.09.005 PMID: 24076151

57. Swerdlow NR, Powell SB, Breier MR, Hines SR, Light GA (2013) Coupling of gene expression in medial prefrontal cortex and nucleus accumbens after neonatal ventral hippocampal lesions accompanies deficits in sensorimotor gating and auditory processing in rats. Neupharmacology 75: 38–46. doi: 10.1016/j.neuropharm.2013.06.003 PMID: 23810830

58. Li Y, Han F, Shi Y (2013) Increased neuronal apoptosis in medial prefrontal cortex is accompanied with changes of Bcl-2 and Bax in a rat model of post-traumatic stress disorder. J Mol Neurosci 51: 127–137. doi: 10.1007/s12031-013-9965-z PMID: 23381833

59. Zhao D, Han F, Shi Y (2014) Effect of glucose-regulated protein 94 and endoplasmic reticulum modulator caspase-12 in medial prefrontal cortex in a rat model of posttraumatic stress disorder. J Mol Neurosci 54: 147–155. doi: 10.1007/s12031-014-0263-1 PMID: 24610447

60. Wen L, Han F, Shi Y (2014) Changes in the Glucocorticoid Receptor and Ca/Calreticulin-Dependent Signalling Pathway in the Medial Prefrontal Cortex of Rats with Post-traumatic Stress Disorder. J Mol Neurosci.

61. Seo JH, Kim TW, Kim CJ, Sung YH, Lee SJ (2013) Treadmill exercise during pregnancy ameliorates posttraumatic stress disorder-induced anxietylike responses in maternal rats. Mol Med Rep 7: 389–395. doi: 10.3892/mmr.2012.1197 PMID: 23174863