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Repetitive transcranial magnetic stimulation effectively facilitates spatial cognition and synaptic plasticity associated with increasing the levels of BDNF and synaptic proteins in Wistar rats

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ABSTRACT

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique, by which cognitive deficits can be alleviated. Furthermore, rTMS may facilitate learning and memory. However, its underlying mechanism is still little known. The aim of this study was to investigate if the facilitation of spatial cognition and synaptic plasticity, induced by rTMS, is regulated by enhancing pre- and postsynaptic proteins in normal rats. Morris water maze (MWM) test was performed to examine the spatial cognition. The synaptic plasticity, including long-term potentiation (LTP) and depotentiation (DEP), presynaptic plasticity paired-pulse facilitation (PPF), from the hippocampal *Schaffer collaterals* to CA1 region was subsequently measured using *in vivo* electrophysiological techniques. The expressions of brain-derived neurotrophic factor (BDNF), presynaptic protein synaptophysin (SYP) and postsynaptic protein NR2B were measured by Western blot. Our data show that the spatial learning/memory and reversal learning/memory in rTMS rats were remarkably enhanced compared to that in the Sham group. Furthermore, LTP and DEP as well as PPF were effectively facilitated by 5 Hz-rTMS. Additionally, the expressions of BDNF, SYP and NR2B were significantly increased via magnetic stimulation. The results suggest that rTMS considerably increases the expressions of BDNF, postsynaptic protein NR2B and presynaptic protein SYP, and thereby significantly enhances the synaptic plasticity and spatial cognition in normal animals.

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a novel neurological technique, by which a regular magnetic field is produced to induce a secondary current in the brain (Parthoens, Verhaeghe, Wyckhuys, Stroobants, & Staelens, 2014). Long-term effects can be generated in a specific area, such as cortex and hippocampus via the magnetic field after temporary excitation is caused (Kemp & Manahan-Vaughan, 2007; Platz & Rothwell, 2010). Because it is non-invasive while stimulating brain regions, rTMS has been generally acknowledged to affect attention, memory, and other brain functions (Rossi et al., 2009). Furthermore, rTMS has been employed as an effective therapeutic approach in the clinical, such as depression (Garcia-Toro et al., 2006), schizophrenia (Wolwer et al., 2014), and Alzheimer's dementia (Ahmed, Darwish, Khedr, El Serogy, & Ali, 2012). One of the previous studies showed that multiple-session stimulation increased functional connectivity among distributed cortical hippocampal network regions and concomitantly improved associative memory performance in healthy adults (Wang et al., 2014). However, it is still little know about why rTMS is able to impact the healthy brain and result in the lasting influences.

Abbreviations: BDNF, brain derived neurotrophic factor; DEP, depotentiation; fEPSP, field excitatory postsynaptic potentials; IT, initial training; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; MWM, Morris water maze; NMDAR, N-methyl-D-aspartate receptor; PPF, paired-pulse facilitation; PTP, post tetanic potentiation; RET, reversal exploring test; RT, reversal training; rTMS, repetitive transcranial magnetic stimulation; SET, space exploring test; STP, short-time potentiation; SYP, synaptophysin; TBS, theta burst stimulation; TrkB, tyrosine kinase receptor B.

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Synaptic plasticity, in which if two neurons are active at the same time the synaptic efficiency of the appropriate synapse will be strengthened (Hebb, 1949), is first proposed as a mechanism for learning and memory on the basis of theoretical analysis. It is well established that synaptic plasticity is a critical component of the neural mechanisms underlying learning and memory (Lynch, 2004). The hippocampus is a part of the brain that is involved in learning and memory and exhibits several forms of short- and long-term synaptic plasticity. Both long-term potentiation (LTP) and long-term depression (LTD) are the outstanding model bridging memory with synaptic function (Holscher, 1999). The expressions of them are associated with pre- and/or postsynaptic changes (Maruki, Izaki, Nomura, & Yamauchi, 2001). Moreover, as we know that paired-pulse facilitation (PPF) is also involved in memory function. Importantly, a previous study clearly demonstrated that PPF was associated with enhanced presynaptic transmitter release during the second paired-pulse (Schulz, Cook, & Johnston, 1995). Although several *in vitro* studies show that high frequency rTMS can induce LTP and PPF (Ahmed & Wieraszko, 2008; Tokay, Holl, Kirschstein, Zschorlich, & Kohling, 2009), there is still lack of adequate *in vivo* data especially obtained from animal study.

Brain-derived neurotrophic factor (BDNF) is well known to be important for the survival, differentiation, growth and regeneration of neurons (Huang & Reichardt, 2001), as well as in synaptic transmission and plasticity (Waterhouse & Xu, 2009). Previous studies showed that rTMS could enhance the protein expression of BDNF in both brain and lymphocyte (Wang et al., 2011). From another point of view, NR2B plays a critical role in determining the direction of postsynaptic changes, particularly essential for LTP and LTD (Liu et al., 2004; Wong, Liu, Sheng, & Wang, 2004). Furthermore, it is well known that synaptophysin (SYP) is a presynaptic vesicle protein and a molecular marker of presynaptic density, which also affects the release of neurotransmitters (Wiedenmann & Franke, 1985). Overall, the regulation of presynaptic and postsynaptic activity strength is closely associated with the alteration of SYP and NR2B. rTMS could reverse the synaptic marker proteins expression in pathology (Zhang, Xing, Wang, Tao, & Cheng, 2015), however, it is still unclear whether or not rTMS can induce the protein expression alterations of both SYP and NR2B.

Therefore, the present study was aimed to investigate the potential positive effect of high frequency rTMS (5 Hz) on normal Wistar rats in spatial cognition and synaptic plasticity, and explore a potential molecular mechanism. We hypothesized that high frequency rTMS could play an important role in significantly enhancing spatial cognition and facilitating the hippocampal synaptic plasticity through increasing the levels of BDNF and synapse-associated proteins. This was done by establishing a rat model of rTMS treatment and performing Morris water maze (MWM) for measuring the ability of spatial learning and memory. Afterwards, STP, LTP, DEP and PPF from the hippocampal *Schaffer collaterals* to CA1 region were recorded. In order to explore the underlying mechanism, Western blot assay was employed to identify if the levels of BDNF and synapse-associated proteins were significantly changed by 5 Hz-rTMS.

2. Material and methods

2.1. Animals

Eighteen adult male Wistar rats weighting 200–250 g were purchased from the Laboratory Animal Center, Academy of Military Medical Science of People's Liberation Army. Before performing experiments, rats were allowed three days of habituation and kept in groups of 4–5 under standard laboratory conditions (24 ± 2 °C room temperature, 12 h light/dark cycle with lights on at 7:00 a.m., and freedom to food and drink) in the Medical School of Nankai University (Xu, Liu, Li, & Zhang, 2015; Zheng & Zhang, 2015). All experiments were performed according to the guidelines approved by the Committee for Animal Care at the Nankai University and in accordance with the practices outlined in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Every effort has been made to minimize animal suffering and the number of animals.

2.2. Repetitive transcranial magnetic stimulation procedure

In order to assess and thereby eliminate the interference with behavioral tasks, rats were arbitrarily divided into three groups: Sham group (n = 6), rTMS group (n = 6) and rTMS without behavioral tasks group (rTMS-WBT, n = 6). During establishing an rTMS rat model, Rapid2 Magstim magnetic stimulation device (UK) was used, which is the commercially available stimulator. In the meantime, the better focusing figure-of-eight coil was chosen, which was composed of two circular coils. The intersection point of two circular coils is at the center point of the figure-of-eight coil. For each circular coil, the internal diameter is 8 mm and the outer diameter is 30 mm. The figure-of-eight coil was placed over the scalp surface (above 2 mm) and parallel to the parietal bone of the rat (Yang, Liu, Xie, Liu, & Tian, 2015). The center point of the figure-of-eight coil was just above the central point of sagittal suture. The connecting line of two circular coil centers was perpendicular to the sagittal axis of the brain. The figure-of-eight coil handle was perpendicular to the parietal bone of rats. The induced electric field of the figure-of-eight coil in the cortex was oriented in anterior-posterior axis. Restriction of the rats during stimulation was performed by hand force (Gersner, Kravetz, Feil, Pell, & Zangen, 2011). One session of rTMS was applied daily (between 9:00 and 12:00 a.m.) for 14 consecutive days. Every session consisted of 20 burst trains, and each train contained 20 pulses at 5 Hz with 20-s inter-train intervals, in total 400 stimuli. The intensity of stimulation represented 120% of the average resting motor threshold as determined by visual inspection of bilateral forelimb movement in a preliminary experiment (28% of the maximum output) (Gersner et al., 2011; Loffler et al., 2012). The rats in the Sham group were handled in a manner similar to that in the rTMS group, while the coil was lifted to 80 mm above the rat's head (Trippe, Mix, Aydin-Abidin, Funke, & Benali, 2009).

2.3. Morris water maze experiment

Twenty-four hours after the last magnetic stimulation, animals in the Sham and rTMS groups were trained and tested in the MWM to assess their spatial cognition. The MWM consists of a metal pool with a diameter of 150 cm and a height of 60 cm, filled with tap water in which black nontoxic ink is dissolved (22 ± 2 °C, 45 cm deep). As described in our previous papers (An, Fu, & Zhang, 2015; Fu et al., 2016), it was divided into four quadrants with two imaginary perpendicular lines crossing in the center of the tank. The end of each line demarcates four cardinal points: North (N), South (S), East (E) and West (W). A 10-cm-diameter platform that could be moved is positioned in the middle of one quadrant, and its top is submerged 2 cm below the water surface. Make sure that the site of the platform and the objects around the pool are fixed and kept the moderate light and quiet environment. The four start locations of S, E, NE and SW were chosen, as these were the distal start locations that were closer to being equal in length with regard to distance from the platform (Vorhees & Williams, 2006).

The MWM test consisted of four consecutive stages: initial training (IT), space exploring test (SET), reversal training (RT) and