Impact of Repetitive Transcranial Magnetic Stimulation on Post-Stroke Dysmnesia and the Role of BDNF Val66Met SNP

Authors' Contribution:
Study Design **A**
Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
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Background: Little is known about the effects of low-frequency repetitive transcranial magnetic stimulation (rTMS) on dysmnesia and the impact of brain nucleotide neurotrophic factor (BDNF) Val66Met single-nucleotide polymorphism (SNP). This study investigated the impact of low-frequency rTMS on post-stroke dysmnesia and the impact of BDNF Val66Met SNP.

Material/Methods: Forty patients with post-stroke dysmnesia were prospectively randomized into the rTMS and sham groups. BDNF Val66Met SNP was determined using restriction fragment length polymorphism. Montreal Cognitive Assessment (MoCA), Loewenstein Occupational Therapy of Cognitive Assessment (LOTCA), and Rivermead Behavior Memory Test (RBMT) scores, as well as plasma BDNF concentrations, were measured at baseline and at 3 days and 2 months post-treatment.

Results: MoCA, LOTCA, and RBMT scores were higher after rTMS. Three days after treatment, BDNF decreased in the rTMS group but it increased in the sham group (P<0.05). Two months after treatment, RMBT scores in the rTMS group were higher than in the sham group, but not MoCA and LOTCA scores.

Conclusions: Low-frequency rTMS may improve after-stoke memory through various pathways, which may involve polymorphisms and several neural genes, but not through an increase in BDNF levels.

MeSH Keywords: Brain-Derived Neurotrophic Factor • Neuropsychological Tests • Polymorphism, Genetic • Polymorphism, Single Nucleotide

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Background

In the United States, about 795 000 new or recurrent cases of stroke occur each year, and the prevalence of stroke in individuals >20 years old is estimated at 6.5 million [1]. Stroke results in neuromuscular dysfunction that causes disabilities, including apraxia, pain syndrome, limb spasticity, and urinary incontinence [2]. In addition, patients with stroke suffer from cognition-related disabilities, including memory loss, speech impairment, poor problem-solving skills, and disorganized thoughts. Psychiatric diseases, including anxiety, depression, emotional instability, and fatigue, are also commonly reported [3].

Vascular cognitive impairment (VCI), varying from mild recognition impairment to dementia, may be caused by various cerebrovascular diseases such as cerebral infarction, encephalorrhagia, leukoaraiosis, and chronic cerebral ischemia [4]. Most patients with cerebrovascular disease have dysmnesia, which severely impairs memory [5,6]. Ingles et al. [7], through a 5-year follow-up study, revealed that memory impairment during intervention is an important indicator of VCI for patients with non-dementia developing into dementia. Another study reported that memory is closely associated with overall recognition function [8].

A variety of interventions are used to manage, treat, and limit the neurological complications of stroke. Movement rehabilitation techniques are used to improve neural plasticity and neuromuscular dysfunction. Some task-specific rehabilitation techniques are used to improve the movement patterns of the whole body, such as robotic ortheses for upper and lower limb movement, virtual reality technologies, and functional programmed electromyostimulation [9]. Other rehabilitation techniques, including behavioral management for urinary incontinence [10,11] and speech and language therapy for aphasia [12,13], usually result in improvements. Constraint-induced therapy and reduced nonverbal communication methods also result in promising effects [14]. At later stages of the rehabilitation process, restorative therapies such as cell-based therapies, electromagnetic stimulation, device-based strategies, and task-oriented approach have been proved beneficial in stroke patients [9]. Emotional rehabilitation, including psychotherapy with motivational interviews and cognitive behavioral therapies, are beneficial in stroke patients [9]. Besides various rehabilitation techniques, certain pharmacological agents such as antidepressants are used to improve stroke complications [15]. Language recovery may occur spontaneously or may be helped by establishing successful reperfusion in the ischemic penumbra region of the affected brain. Additionally, pharmacological agents such as donepezil can improve language disorders [16].

Rehabilitation of memory impairment consists of memory retraining, external memory aids, and special learning strategies. However, rehabilitation is a time-consuming process impaired by inconsistent outcomes and poor patient compliance [17]. In recent years, a novel noninvasive brain stimulation technology, repetitive transcranial magnetic stimulation (rTMS), offered a novel approach in rehabilitation of cognition and memory. rTMS is the process of giving repetitive stimulation on a specific area of the surface of the brain, activating neurons in the horizontal axis. rTMS treatment can improve learning and memory through the brain nucleotide neurotrophic factor (BDNF) by modulating the plasticity of neural synapses [18]. Low-frequency rTMS (1 Hz) can regulate the plasticity of hippocampal neuron synapses through the BDNF-TrkB pathway [19]. As an important neurotrophic factor, BDNF is involved in controlling neuron connections, regulating synapse development, and strengthening the plasticity of synapses [20]. Moreover, the Val66Met single-nucleotide polymorphism (SNP) in the BDNF gene is believed to correlate with prefrontal cortex, hippocampal volume, and memory function [21]. A meta-analysis revealed that the Val66Met BDNF genotype has great impacts on memory performance and structure and physiological function of the hippocampus [22]. Plasma levels of BDNF are positively associated with brain levels of BDNF [23].

The use of low- or high-frequency rTMS has yielded inconsistent results [24–28], but low-frequency rTMS has been deemed safer in epilepsy [29]. Therefore, we hypothesized that low-frequency rTMS stimulation induces brain BDNF expression at different levels in patients with different BDNF Val66Met genotypes, consequently causing distinctive modulations of cognitive and memory behaviors. The aim of the present study was to investigate the impact of low-frequency rTMS on post-stroke dysmnesia, the impact of BDNF Val66Met SNP in patients with stroke, and in the recovery phase with a disease course longer than 1 month.

Material and Methods

Subjects

Forty-four patients with stroke admitted in the Department of Neurological Rehabilitation, China Rehabilitation Research Center between June 2012 and December 2013 were enrolled in this prospective study. Stroke was diagnosed based on the cerebral apoplexy diagnostic criteria established by the 4th National Cerebrovascular Disease Conference.

Inclusion criteria were: 1) first-ever stroke confirmed by a brain computed tomography (CT) or magnetic resonance imaging (MRI); 2) disease course longer than one month with supratentorial nidus; 3) stable vital signs, no progression of neurological symptoms; 4) no severe aphasia or cognitive disorder and able of accomplishing cognitive and memory tests; 5) normal cognitive and memory functions before stroke; 6) dysmnesia

confirmed by memory tests; 7) age <60 years; and 8) voluntary participation and signed the informed consent.

Exclusion criteria were: 1) non-first stroke; 2) subtentorial nidus; 3) transcranial surgery; 4) metal or electronic device implants; 5) history of seizures; 6) cognitive or memory function recession before stroke; 7) any neuropsychiatric comorbidity that could influence the test outcomes; 8) obvious emotional disorders; or 9) any other factors that could affect clinical examination.

Patients were randomly divided into the rTMS and sham treatment groups using random number table. The study protocol was approved by the Ethics Committee of the China Rehabilitation Research Center. All patients provided a written informed consent. The study was registered at chictr.org (ChiCTR-OCH-12002238).

Ten healthy volunteers with no drug intake in the last 3 months and who had routine check-ups at the hospital were enrolled as healthy controls. After written informed consent was obtained, blood samples were collected from to measure BDNF levels. These controls did not undergo rTMS.

Assessment of cognitive functions

The Montreal Cognitive Assessment (MoCA) is a 30-point test assessing short-term memory recall (5 points), visuospatial abilities (4 points), executive functions (4 points), attention (1 point), concentration (3 points), working memory (2 points), language (5 points), and orientation to time and space (6 points) [30].

The Loewenstein Occupational Therapy of Cognitive Assessment (LOTCA) is a 91-point test assessing cognitive functions including orientation (8 points), perception (24 points), visual movement organization (28 points), thought operation (27 points), attention and concentration (4 points) in older adults with neurological impairment [31].

The Rivermead Behavior Memory Test (RBMT) is designed to predict everyday memory problems in people with acquired, non-progressive brain injury and to monitor their change in time [32].

Repetitive Transcranial Magnetic Stimulation

Patients underwent rTMS treatment (rTMS group; n=22) or sham rTMS treatment (named sham group; n=22). Magstim Super Rapid (dual power supplies, Magstim Company Limited, Whitland, UK) was used for treatment. The motor threshold (MT) for each patient was determined by the operator. rTMS parameters were set as follows: 1 Hz stimulation frequency,

100% MT, and 30 sequences of 20 pulses at the right side of the dorsolateral prefrontal cortex (DLPFC). Stimulating coils were tangent to the surface of the skull, inducing a magnetic field passing through the brain. Patients received rTMS treatment once a day, 5 days per week for 4 weeks. For sham rTMS, all parameters were the same as for the real treatment, except that the stimulating coils were placed perpendicular to the surface of the skull to mimic the treatment procedure, but inducing no magnetic field in the brain, as previously shown [33].

Aside from the real or sham rTMS treatment, all patients received regular computer-assisted cognitive training for 30 min every day. According to the severity of the cognitive disorder, the training included graphical, verbal and spatial memory. Therapists were blinded to rTMS treatment assignments. In addition, during and after rTMS treatments, patients received secondary stroke prevention drugs as recommended by the 2008 AHA/ASA recommendation for the prevention of stroke in patients with stroke and transient ischemic attacks [34].

Cognition and memory functions assessment

MoCA, LOTCA and RBMT scores were assessed for all patients at baseline and 3 days after the end of treatments. The assessment was done by an associate chief physician from the rehabilitation division and the accuracy of the results was verified by the chief physician.

BDNF measurement

Fasting blood samples were collected at morning before treatment, immediately after treatment and 2 months later. Plasma BDNF levels were measured by enzyme-linked immunosorbent assay using the RayBio Human BDNF ELISA kit (RayBiotech, Inc., Norcross, GA, USA), according to the manufacturer's instructions.

BDNF Val66Met SNP detection

Genomic DNA was isolated from blood samples and was used for BDNF Val66Met SNP detection using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primer design and sequence detection were performed by Omega Bio-Tek, Inc., Beijing, China. The forward and reverse sequences were 5'-AAA GAA GCA AAC ATC CGA GGA CAA G-3' and 5'-TTC CTC CAG CAG AAA GAG AAG AGG-3', respectively.

Statistical analysis

All data were analyzed using SPSS 18.0 (IBM, Armonk, NY, USA). Data with normal distribution are expressed as mean ± standard deviation, while non-normally distributed data are expressed as median and range. Intergroup comparisons

Table 1. Baseline characteristics of the patients.

	Patients with post-stroke dysmnesia	Healthy control	P	rTMS group	Sham group	P
N	40	10	–	19	21	–
Gender (male, %)	25 (62.5%)	13 (65%)		12 (63.2%)	13 (61.9%)	
Age (years)	44.9±11.1	42.4± 9.9	0.30	42.5±12.3	47.3±11.8	0.61
Type of stroke						
Hemorrhagic (%)	22 (55.0%)	–	–	11 (57.9%)	11 (52.4%)	0.73
Ischemic (%)	18 (45.0%)	–	–	8 (42.1%)	10 (47.6%)	
Side of stroke						
Left (%)	22 (55.0%)	–	–	11 (57.9%)	11 (52.4%)	0.73
Right (%)	18 (45.0%)	–	–	8 (42.1%)	10 (47.6%)	
Education level (years)	12.2±3.8	12.9±3.5	0.45	12.8±3.8	11.5±4.5	0.46
Disease duration (days), median (range)	61 (30, 365)	–	–	67 (30, 365)	56 (30, 296)	0.10
MoCA score		–	–	17.95±4.67	19.67±3.18	0.13
LOTCA score		–	–	67.16±9.26	67.86±8.69	0.62
RBMT score				13.11±3.14	14.19±2.62	0.31
Plasma BDNF (ng/mL)	0.71±0.39	1.31±0.45	<0.001	0.72±0.40	0.71±0.35	0.88
BDNF SNP (Alt/Met/heterozygote)	14/11/15	–	–	7/6/6	7/5/9	0.74

BDNF – brain nucleotide neurotrophic factor; LOTCA – Loewenstein Occupational Therapy of Cognitive Assessment; MoCA – Montreal Cognitive Assessment; RBMT – Rivermead Behaviour Memory Test; SNP – single nucleotide polymorphism; rTMS – repetitive transcranial magnetic stimulation.

were performed using independent t-tests or non-parametric tests; before/after comparisons were performed using paired t-tests. Categorical data were analyzed using the chi-square test. Analysis of BDNF Val66Met SNP with memory test scores and of plasma BDNF levels between the rTMS and sham groups were performed using Fisher tests. Two-sided P-values <0.05 were considered significant. For multiple comparisons, P-values <0.025 were considered statistically significant, considering the Bonferroni correction.

Results

Baseline characteristics of the patients

Four patients withdrew from the study (2 patients with respiratory tract infection and 2 patients for personal reasons). Nineteen patients in the rTMS group and 21 patients in the sham group completed the treatment, and data from 12 patients in the rTMS group and 14 in the sham group were available 2 months after treatment.

There was no difference between healthy controls and patients with stroke for age, gender, and education. No difference was

observed between the rTMS and sham groups for demographic data, recognition memory scores before treatment, plasma BDNF levels, and BDNF Val66Met SNP genotypes (Table 1).

Impact of rTMS on cognitive and memory function immediately after treatment

No difference was observed between the rTMS and sham groups for MoCA, LOTCA, and RBMT scores. After treatment, MoCA and LOTCA scores remained similar between the 2 groups, but the RBMT score was better in the rTMS group (data not shown, P=0.034). The changes in MoCA, LOTCA, and RBMT scores between baseline and 3 days post-treatment in both groups were all more important in the rTMS group compared with the sham group (all P<0.001, Table 2).

BDNF levels

Plasma BDNF levels in patients with stroke were lower compared with those of healthy controls (0.71±0.39 vs. 1.31±0.45 ng/mL, Table 1). No difference was observed in BDNF levels between the rTMS and sham groups immediately after treatment. As shown in Table 2, changes in plasma BDNF levels between baseline and 3 days post-treatment were different between

Table 2. Changes in cognition and memory function scores and plasma BDNF levels between baseline and 3 days post-treatment in the rTMS and sham groups.

	rTMS group	Sham group	P
Change in MoCA score	4.21±2.46*	1.90±1.41*	<0.001
Change in LOTCA score	8.74±5.56*	3.67±4.21*	<0.001
Change in RBMT score	4.05±2.76*	1.24±1.17*	<0.001
Change in BDNF level (ng/ml)	−0.08±0.28*	0.09±0.19*	0.03

BDNF – brain nucleotide neurotrophic factor; MoCA – Montreal Cognitive Assessment; rTMS – repetitive transcranial magnetic stimulation. * P<0.025 *vs.* baseline of the same group.

Table 3. Changes in cognition and memory function scores and plasma BDNF levels between baseline and 3 days post-treatment accordgin to BDNF SNP.

	Alt/Alt	Met/Met	Alt/Met	P
Change in MoCA score	5.57±2.37*	3.17±2.32*	3.67±2.34*	0.99
Change in LOTCA score	10.14±7.20*	5.33±3.27*	10.50±4.23*	0.13
Change in RBMT score	3.71±2.93*	4.33±3.20*	4.17±2.56*	0.74
Change in BDNF level (ng/ml)	−0.07±0.45	−0.17±0.16	−0.01±0.02	0.61

BDNF – brain nucleotide neurotrophic factor; MoCA – Montreal Cognitive Assessment; SNP – single nucleotide polymorphism.
* P<0.025 *vs.* baseline of the same group.

Table 4. Changes in cognition and memory function scores and plasma BDNF levels between baseline and 2 months post-treatment in the sTMS and sham groups.

	rTMS group	Sham group	P
Change in MoCA score	6.17±2.55*	4.14±0.95*	0.002
Change in LOTCA score	12.58±6.20*	6.20±1.71*	<0.001
Change in RBMT score	6.00±2.52*	3.00±0.96*	<0.001
Change in BDNF level	0.01±0.25	0.25±0.16*	<0.001

BDNF – brain nucleotide neurotrophic factor; MoCA – Montreal Cognitive Assessment; rTMS – repetitive transcranial magnetic stimulation. * P<0.025 *vs.* baseline of the same group.

the rTMS (decreased BDNF levels) and sham (increased BDNF levels) groups (-0.08±0.28 *vs.* 0.09±0.19, P=0.03).

Impact of BDNF Val66Met SNP

The overall genotype frequency of BDNF Val66Met was 14 ALT/ALT, 11 MET/MET, and 15 ALT/MET. The distribution was concordant with the Hardy-Weinberg law. The BDNF genotypes were 7 ALT/ALT, 6 MET/MET, and 6 ALT/MET in the rTMS group and 7 ALT/ALT, 5 MET/MET, and 9 ALT/MET in the sham group. There was no difference between the rTMS and sham groups in genotype

distribution. The Val66Met SNP had no impact on the changes on cognition, memory, and plasma BDNF concentration (Table 3).

Two-month follow-up of cognitive and memory function and plasma BDNF levels

Two months after treatment, changes in MoCA, LOTCA, and RBMT scores in the rTMS group were all higher than in the sham group (MoCA: 6.17±2.55 *vs.* 4.14±0.95, P=0.002); LOTCA: 12.58±6.20 *vs.* 6.20±1.71, P<0.001); RBMT: 6.00±2.52 *vs.* 3.00±0.96, P<0.001), while changes in plasma BDNF levels in

the rTMS group were significantly lower than in the sham group (0.01±0.25 *vs.* 0.25±0.16 ng/mL, P<0.001) (Table 4).

Adverse effects

One patient experienced transient headache and another experienced dizziness in the rTMS group, and 1 patient experienced headache in the sham group. Patients recovered from these events without any specific treatments and no patient dropped-out of the study because of adverse effects.

Discussion

This study investigated the impact of low-frequency rTMS on post-stroke dysmnesia and the impact of BDNF Val66Met SNP. The present study revealed that low-frequency rTMS at the right side of DLPFC could improve both cognitive and memory functions in patients with stroke. Moreover, the effect could last for 2 months after treatment.

Memory is supported by multiple cognitive nerve systems. Different nerve systems support distinctive aspects of memory, which is dependent on the means of memory message type, coding, and extraction [35]. The prefrontal lobe is the key brain region for memory [36], especially DLPFC, which is of great importance for memory coding and extraction [37–39]. Previous studies reported that stimulation of brain region, especially in high frequency, at the prefrontal lobe could improve cognition and memory functions. The greatest safety concern in rTMS treatment is the possible induction of epilepsy. However, stimulation at a low frequency such as ≤1 Hz could reduce the epilepsy occurrence and is considered to be safe [29]. For patients with brain injury, especially those who are in the early stage of recovery, it remains unknown whether high-frequency stimulation could cause adverse effects like brain function disorder and epilepsy. Hence, it is important to determine whether the safe low-frequency stimulation could improve cognitive and memory function, which was 1 of the purposes of the present study. Previous studies showed that the excitability of the motor cortex was decreased after low-frequency rTMS, but increased using high-frequency rTMS [24]. In depressed patients, high-frequency rTMS increased cerebral blood flow, while low-frequency rTMS induced some circumscribed decreases [25]; however, some patients had improved moods with high-frequency rTMS, while other had improved moods with low-frequency rTMS [25]. A study of post-traumatic stress syndrome showed that high-frequency rTMS achieved better outcomes than low-frequency [26]. In Alzheimer's disease, better cognitive functions were achieved using high-frequency rTMS compared with low-frequency rTMS [27]. However, a previous study showed that low-frequency rTMS reversed $A\beta_{1-42}$-mediated memory deficits in rats [28]. Therefore, data

from previous studies are controversial regarding the best frequency, although high-frequency rTMS seems to achieve better outcomes in some patients or models. Low-frequency rTMS (1 Hz) could regulate the plasticity of hippocampal neuron synapses through the BDNF-TrkB pathway [19]. In a previous study, healthy individuals and patients with mild cognitive disorder had considerable improvement in verbal and non-verbal recognition after 1 Hz rTMS stimulation at the right side of DLPFC [40]. This cognitive function improvement possibly relies on the modulation of the excitability as well as adjunctive structure of the right side of DLPFC [41], because stimulation could activate the hippocampus through an impact on subcortical structure and posterior cortex, and consequently regulate the memory extraction process [38,39]. In the present study, even if we used low-frequency rTMS, we showed that this treatment improved the condition of the cognitive functions of patients with stroke. Further studies are necessary to fully compare low- and high-frequency rTMS.

The improvement of memory function could allow individuals to gain new experiences in their environments, and consequently improve the cognitive function of the whole brain. Because every rTMS stimulation might have been stored in the stimulation region as "memory", when a new stimulation is applied, the new effect would be generated based on the former stimulations (lasting memory hypothesis) [42]. Besides, the memory-improving effect of rTMS may also be due to repeated stimulating, activation of subcortical neural network structure, and the changed synaptic plasticity [43]. Multiple cumulative biological reactions achieved by stimulation could remain for a certain time after the end of stimulation, indicating that a possible biological amplification reaction results in longer follow-up effect generated by rTMS. This cumulative effect could explain the improvement in cognitive and memory functions 2 months after rTMS treatment in the present study.

A number of studies have focused on the impact of rTMS on peripheral BDNF levels, but results are inconsistent. Few authors believe that peripheral BDNF levels in depressed patients could be changed by rTMS [44–46], while others believe that peripheral changes in BDNF levels were not related to either electric or magnetic stimulation [47]. Two studies on healthy subjects revealed that high- or low-frequency magnetic stimulation on DLPFC or motor cortex resulted in peripheral BDNF decrease [48,49], which was believed to be associated with rTMS-mediated inhibition of the glutamatergic neurotransmitter, or that rTMS mediated moderate depolarized activation of inhibitors that synthesize and release BDNF. However, a randomized, controlled, double-blind study on patients with stroke aphasia treated with low-frequency rTMS revealed that the aphasia symptoms were improved and that plasma BDNF levels were decreased, while BNDF levels were increased in the sham group [50]. In accordance with these

findings, the present study also showed that plasma BDNF concentration decreased in the rTMS group, but not in the sham group, and that plasma BDNF levels were not associated with cognitive and memory function impairment, or with improvement. Moreover, 2 months after treatment, plasma BDNF levels were slightly increased in the rTMS group, but were not higher than in the sham group. These results, although unexpected, provided more evidence for the effect of rTMS on BDNF, and this effect might not be associated with stimulation region or frequency, but merely with a series of stimulations. Notably, rTMS did not change BDNF levels, suggesting that improvements in cognitive functions was not mediated by BDNF or, at least, not by BNDF alone. The possible reason for this observation remains unclear, and should be the focus of further studies. The disease or the animal model being studied may be responsible in part for this observation.

According to available data, rTMS does not improve the central nervous system through BDNF. Many neurotransmitters and neurotrophic factors affect learning, memory, behavior, and mental abilities. Hence, memory improvements by rTMS could be achieved through multiple pathways with the involvement of many neurotransmitters. The brain expression of factors like precursor of BDNF, SYN aptophysin, glutamic acid, Y-aminobutyric acid, dopamine, acetylcholine, and estrogens can affect the plasticity of synapses, which is also regulated by rTMS [51–53]. However, the present study was not designed to examine these pathways. More studies are necessary to determine the exact mechanisms through which rTMS achieves its effects.

The present study failed to observe any correlation between BDNF Val66Met SNP and either plasma BDNF levels or cognitive function. Plasma BDNF levels and memory function were altered after rTMS treatment, but these changes were not associated with BDNF Val66Met SNP. These results are supported by similar studies [50,54]. Aside from BDNF polymorphisms, the polymorphisms of other neurotrophic factors or metabolic enzymes such as catechol-O-methyltransferase [55], methylenetetrahydrofolate reductase [56], dopamine β-hydroxylase, monoamine oxidase, dopamine receptor D, tryptophan hydroxylase 2, and tumor necrosis factor-α could also have impacts on cognitive and memory function [57,58]. However, due to the small sample size of the present study, it was impossible to test all these polymorphisms and to group patients accordingly, since it would have resulted in subgroups that were too small. Another limitation is that, following guidelines [34], these patients were treated with drugs for the prevention of secondary strokes, and the effect of these drugs on the efficacy of rTMS is currently unknown. Finally, all patients underwent cognitive training as part of the standard treatment for stroke, and we cannot exclude the possibility than this training might have influenced the results. Fundamental studies or large-scale clinical research involving multiple centers are necessary to address these issues.

Conclusions

Low-frequency rTMS at the right side of DLPFC could improve cognition and memory functions in stroke patients, and the improvement effect could be sustained for some time. Low-frequency rTMS could affect plasma BDNF levels in stroke patients. Plasma BDNF levels were not associated with patients' cognitive and memory function status. BDNF Val66Met SNP might not be associated with cognitive and memory functions and plasma BDNF levels. Other polymorphisms and neurotrophic factors could be involved in the response to rTMS.

Conflict of interest

The authors declare that they have no conflict of interest.

References:

1. Lloyd-Jones D, Adams R, Carnethon M et al: Heart disease and stroke statistics – 2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation, 2009; 119: e21–181

2. Young JA, Tolentino M: Stroke evaluation and treatment. Top Stroke Rehabil, 2009; 16: 389–410

3. Annoni JM, Staub F, Bruggimann L et al: Emotional disturbances after stroke. Clin Exp Hypertens, 2006; 28: 243–49

4. Erkinjuntti T: Diagnosis and management of vascular cognitive impairment and dementia. J Neural Transm Suppl, 2002; (63): 91–109

5. Loewenstein DA, Acevedo A, Agron J et al: Cognitive profiles in Alzheimer's disease and in mild cognitive impairment of different etiologies. Dement Geriatr Cogn Disord, 2006; 21: 309–15

6. Stephens S, Kenny RA, Rowan E et al: Neuropsychological characteristics of mild vascular cognitive impairment and dementia after stroke. Int J Geriatr Psychiatry, 2004; 19: 1053–57

7. Ingles JL, Boulton DC, Fisk JD, Rockwood K: Preclinical vascular cognitive impairment and Alzheimer disease: neuropsychological test performance 5 years before diagnosis. Stroke, 2007; 38: 1148–53

8. Reed BR, Eberling JL, Mungas D et al: Effects of white matter lesions and lacunes on cortical function. Arch Neurol, 2004; 61: 1545–50

9. Cramer SC: Repairing the human brain after stroke. II. Restorative therapies. Ann Neurol, 2008; 63: 549–60

10. Jordan LA, Mackey E, Coughlan K et al: Continence management in acute stroke: a survey of current practices in Australia. J Adv Nurs, 2011;67 : 94–104

11. Patel M, Coshall C, Rudd AG, Wolfe CD: Natural history and effects on 2-year outcomes of urinary incontinence after stroke. Stroke, 2001; 32: 122–27

12. Harnish SM, Neils-Strunjas J, Lamy M, Eliassen JC: Use of fMRI in the study of chronic aphasia recovery after therapy: a case study. Top Stroke Rehabil, 2008; 15: 468–83

13. Pulvermuller F, Berthier ML: Aphasia therapy on a neuroscience basis. Aphasiology, 2008; 22: 563–99

14. Meinzer M, Djundja D, Barthel G et al: Long-term stability of improved language functions in chronic aphasia after constraint-induced aphasia therapy. Stroke, 2005; 36: 1462–66

15. Barrett AM, Crucian GP, Schwartz RL, Heilman KM: Adverse effect of dopamine agonist therapy in a patient with motor-intentional neglect. Arch Phys Med Rehabil, 1999; 80: 600–3

16. Hillis AE: Rehabilitation of unilateral spatial neglect: new insights from magnetic resonance perfusion imaging. Arch Phys Med Rehabil, 2006; 87: S43–49

17. Piras F, Borella E, Incoccia C, Carlesimo GA: Evidence-based practice recommendations for memory rehabilitation. Eur J Phys Rehabil Med, 2011; 47: 149–75

18. Miniussi C, Rossini PM: Transcranial magnetic stimulation in cognitive rehabilitation. Neuropsychol Rehabil, 2011; 21: 579–601

19. Ma J, Zhang Z, Su Y et al: Magnetic stimulation modulates structural synaptic plasticity and regulates BDNF-TrkB signal pathway in cultured hippocampal neurons. Neurochem Int, 2013; 62: 84–91

20. Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S: Brain-derived neurotrophic factor and the development of structural neuronal connectivity. Dev Neurobiol, 2010; 70: 271–88

21. Pezawas L, Verchinski BA, Mattay VS et al: The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci, 2004; 24: 10099–102

22. Kambeitz JP, Bhattacharyya S, Kambeitz-Ilankovic LM et al: Effect of BDNF val(66)met polymorphism on declarative memory and its neural substrate: a meta-analysis. Neurosci Biobehav Rev, 2012; 36: 2165–77

23. Munno D, Sterpone S, Fania S et al: Plasma brain derived neurotrophic factor levels and neuropsychological aspects of depressed patients treated with paroxetine. Panminerva Med, 2013; 55: 377–84

24. Gangitano M, Valero-Cabre A, Tormos JM et al: Modulation of input-output curves by low and high frequency repetitive transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol, 2002; 113: 1249–57

25. Speer AM, Kimbrell TA, Wassermann EM et al: Opposite effects of high and low frequency rTMS on regional brain activity in depressed patients. Biol Psychiatry, 2000; 48: 1133–41

26. Boggio PS, Rocha M, Oliveira MO et al: Noninvasive brain stimulation with high-frequency and low-intensity repetitive transcranial magnetic stimulation treatment for posttraumatic stress disorder. J Clin Psychiatry, 2010; 71: 992–99

27. Ahmed MA, Darwish ES, Khedr EM et al: Effects of low versus high frequencies of repetitive transcranial magnetic stimulation on cognitive function and cortical excitability in Alzheimer's dementia. J Neurol, 2012; 259: 83–92

28. Tan T, Xie J, Liu T et al: Low-frequency (1 Hz) repetitive transcranial magnetic stimulation (rTMS) reverses Abeta(1-42)-mediated memory deficits in rats. Exp Gerontol, 2013; 48: 786–94

29. Rossi S, Hallett M, Rossini PM, Pascual-Leone A: Safety of TMSCG Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol, 2009; 120: 2008–39

30. Nasreddine ZS, Phillips NA, Bedirian V et al: The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc, 2005; 53: 695–99

31. Zwecker M, Levenkrohn S, Fleisig Y et al: Mini-Mental State Examination, cognitive FIM instrument, and the Loewenstein Occupational Therapy Cognitive Assessment: relation to functional outcome of stroke patients. Arch Phys Med Rehabil, 2002; 83: 342–45

32. Wilson BA: The Rivermead behavioural memory test. Thames Valley Test Company, 1991

33. Ruffini C, Locatelli M, Lucca A, Benedetti F, Insacco C et al: Augmentation effect of repetitive transcranial magnetic stimulation over the orbitofrontal cortex in drug-resistant obsessive-compulsive disorder patients: a controlled investigation. Prim Care Companion J Clin Psychiatry, 2009; 11: 226–30

34. Adams RJ, Albers G, Alberts MJ et al: Update to the AHA/ASA recommendations for the prevention of stroke in patients with stroke and transient ischemic attack. Stroke, 2008; 39: 1647–52

35. Gazzaniga MS, Ivry RB, Mangun GR: Neurosciences cognitives: La biologie de l'esprit. De Boeck Supérieur, 2000

36. Balconi M: Dorsolateral prefrontal cortex, working memory and episodic memory processes: insight through transcranial magnetic stimulation techniques. Neurosci Bull, 2013; 29: 381–89

37. Blumenfeld RS, Lee TG, D'Esposito M: The effects of lateral prefrontal transcranial magnetic stimulation on item memory encoding. Neuropsychologia, 2014; 53: 197–202

38. Rossi S, Cappa SF, Babiloni C et al: Prefrontal [correction of Prefonatl] cortex in long-term memory: an "interference" approach using magnetic stimulation. Nat Neurosci, 2001; 4: 948–52

39. Sandrini M, Cappa SF, Rossi S et al: The role of prefrontal cortex in verbal episodic memory: rTMS evidence. J Cogn Neurosci, 2003; 15: 855–61

40. Turriziani P, Smirni D, Zappala G et al: Enhancing memory performance with rTMS in healthy subjects and individuals with Mild Cognitive Impairment: the role of the right dorsolateral prefrontal cortex. Front Hum Neurosci, 2012; 6: 62

41. Vilberg KL, Rugg MD: Memory retrieval and the parietal cortex: a review of evidence from a dual-process perspective. Neuropsychologia, 2008; 46: 1787–99

42. Valero-Cabre A, Pascual-Leone A, Rushmore RJ: Cumulative sessions of repetitive transcranial magnetic stimulation (rTMS) build up facilitation to subsequent TMS-mediated behavioural disruptions. Eur J Neurosci, 2008; 27: 765–74

43. Selimbeyoglu A, Parvizi J: Electrical stimulation of the human brain: perceptual and behavioral phenomena reported in the old and new literature. Front Hum Neurosci, 2010; 4: 46

44. Zanardini R, Gazzoli A, Ventriglia M et al: Effect of repetitive transcranial magnetic stimulation on serum brain derived neurotrophic factor in drug resistant depressed patients. J Affect Disord, 2006; 91: 83–86

45. Yukimasa T, Yoshimura R, Tamagawa A et al: High-frequency repetitive transcranial magnetic stimulation improves refractory depression by influencing catecholamine and brain-derived neurotrophic factors. Pharmacopsychiatry, 2006; 39: 52–59

46. Yoshimura R, Ikenouchi-Sugita A, Hori H et al: [Blood levels of brain-derived neurotrophic factor (BDNF) in major depressive disorder]. Seishin Shinkeigaku Zasshi, 2010; 112: 982–85 [in Japanese]

47. Lang UE, Bajbouj M, Gallinat J, Hellweg R: Brain-derived neurotrophic factor serum concentrations in depressive patients during vagus nerve stimulation and repetitive transcranial magnetic stimulation. Psychopharmacology (Berl), 2006; 187: 56–59

48. Schaller G, Sperling W, Richter-Schmidinger T et al: Serial repetitive transcranial magnetic stimulation (rTMS) decreases BDNF serum levels in healthy male volunteers. J Neural Transm, 2014; 121: 307–13

49. Angelucci F, Oliviero A, Pilato F et al: Transcranial magnetic stimulation and BDNF plasma levels in amyotrophic lateral sclerosis. Neuroreport, 2004; 15: 717–20

50. Mirowska-Guzel D, Gromadzka G, Seniow J et al: Association between BDNF-196 G>A and BDNF-270 C>T polymorphisms, BDNF concentration, and rTMS-supported long-term rehabilitation outcome after ischemic stroke. NeuroRehabilitation, 2013; 32: 573–82

51. Nagappan G, Zaitsev E, Senatorov VV Jr et al: Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci USA, 2009; 106: 1267–72

52. Wang F, Geng X, Tao HY, Cheng Y: The restoration after repetitive transcranial magnetic stimulation treatment on cognitive ability of vascular dementia rats and its impacts on synaptic plasticity in hippocampal CA1 area. J Mol Neurosci, 2010; 41: 145–55

53. Yue L, Xiao-lin H, Tao S: The effects of chronic repetitive transcranial magnetic stimulation on glutamate and gamma-aminobutyric acid in rat brain. Brain Res, 2009; 1260: 94–99

54. McAllister TW, Tyler AL, Flashman LA et al: Polymorphisms in the brain-derived neurotrophic factor gene influence memory and processing speed one month after brain injury. J Neurotrauma, 2012; 29: 1111–18

55. Witte AV, Kurten J, Jansen S et al: Interaction of BDNF and COMT polymorphisms on paired-associative stimulation-induced cortical plasticity. J Neurosci, 2012; 32: 4553–61

56. Yeh TK, Hu CY, Yeh TC et al: Association of polymorphisms in BDNF, MTHFR, and genes involved in the dopaminergic pathway with memory in a healthy Chinese population. Brain Cogn, 2012; 80: 282–89

57. Gong P, Zheng Z, Chi W et al: An association study of the genetic polymorphisms in 13 neural plasticity-related genes with semantic and episodic memories. J Mol Neurosci, 2012; 46: 352–61

58. Yogeetha BS, Haupt LM, McKenzie K et al: BDNF and TNF-alpha polymorphisms in memory. Mol Biol Rep, 2013; 40: 5483–90