Original Research

BDNF Polymorphism and Differential rTMS Effects on Motor Recovery of Stroke Patients

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ABSTRACT

Background: The brain-derived neurotrophic factor (BDNF) gene often shows a single nucleotide polymorphism that is thought to influence synaptic plasticity. It also affects the modulatory effects of repetitive transcranial magnetic stimulation (rTMS) on motor cortex excitability.
Objective: This study investigated whether BDNF polymorphism influences the effect of rTMS on the motor recovery of patients with stroke.
Methods: Forty-four patients (mean age 53.8 years) experiencing unilateral motor weakness after stroke were recruited. rTMS was applied over the primary motor cortex of the affected hemisphere at 10 Hz with 1000 pulses/day for 10 days. Each patient's motor functions were assessed using the Fugl-Meyer assessment (FMA) and the box and block test (BBT) before, immediately after and 2 months after the intervention. BDNF genotyping was performed via PCR assays of whole blood samples. The patients' data were grouped and analysed into Val/Val and Met allele groups according to the presence or absence of the BDNF polymorphism.
Results: Nine patients (20.5%) were classified into the Val/Val group, and thirty-seven patients (79.5%) were classified into the Met allele group. The patients' baseline motor functions did not differ between the two groups. The FMA and BBT scores showed significant improvement immediately after and 2 months after rTMS in both groups. In addition, the time and groups were found to interact significantly, with the Val/Val group improving to a greater extent than the Met allele group in terms of their FMA and BBT scores.
Conclusions: The findings suggest that the BDNF gene polymorphism negatively influences the effect of rTMS on the motor recovery of upper extremities in stroke patients.

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Introduction

Repeated transcranial magnetic stimulation (rTMS) reportedly has a beneficial effect on the motor functions of patients with stroke [1–3]. In a previous study, it was found that a single session of 10 Hz rTMS facilitated practice-dependent plasticity and improved motor learning in patients with chronic stroke [4]. In addition, consecutive multi-session rTMS applied during the subacute period of stroke has had positive long-term effects on motor recovery [5–7]. However, even among healthy patients, the inter-individual response to rTMS is highly variable [8], and a number of factors contribute to this variability, such as the patient's age [9], the time of day [10] and the patient's menstrual cycle [11].

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors and plays a major role in neuronal survival, synaptic plasticity and learning and memory [12]. A single nucleotide polymorphism (SNP) of the BDNF gene significantly impairs the intracellular trafficking and activity-dependent release of the BDNF [13,14]. Considering that one of the possible mechanisms of rTMS in facilitating motor function is the promotion of plastic changes in synaptic efficacy [8], BDNF polymorphism may affect the synaptic plasticity induced by rTMS in the human brain. Consistent with such notions, a previous study reported decreased or absent after-effects of theta burst stimulations in healthy patients carrying the Met allele of the BDNF gene [15]. However, no reports have considered the influence of BDNF polymorphism on the rTMS effects in stroke patients. Thus, this study investigated whether BDNF polymorphism significantly influences the beneficial effects of rTMS on the motor functions and recovery of patients with stroke.

Materials and methods

Study patients

Subacute stroke patients with unilateral motor weakness were recruited according to the following inclusion criteria: (1) had suffered their first-ever stroke, whether ischemic or hemorrhagic; (2) were within a post-stroke onset time of less than 2 weeks; and (3) had suffered moderate to severe motor impairment in their affected upper extremities (an upper limb score of less than 40 according to the Fugl-Meyer assessment (FMA-UL)) [16]. Patients were excluded if they had (1) suffered any clinically significant or unstable medical disorder, (2) experienced any neuropsychiatric comorbidity, (3) suffered direct injury to the primary motor cortex, (4) suffered complete internal carotid artery occlusion, (5) a history of seizure disorder or post-stroke seizure or (6) an intracranial metallic implant.

Forty-seven stroke patients with hemiparesis were recruited in accordance with these inclusion criteria. Three patients dropped out during the experimental procedure for various personal reasons, leaving forty-four patients in the final analysis (Fig. 1A). The study protocol was approved by the Institutional Review Board of Samsung Medical Center (CRS110051), and written informed consent was obtained from all of the patients.

Experimental design

The study was designed as a parallel-group double-blind clinical analysis. The patients' motor functions were assessed prior to (Pre-rTMS), immediately after (Post-rTMS) and 2 months after (Follow-up) rTMS intervention (Fig. 1B).

Motor cortex mapping for determining the resting motor threshold

To determine the optimal scalp location and rTMS intensity, single-pulse TMS was administered to each patient using a TMS
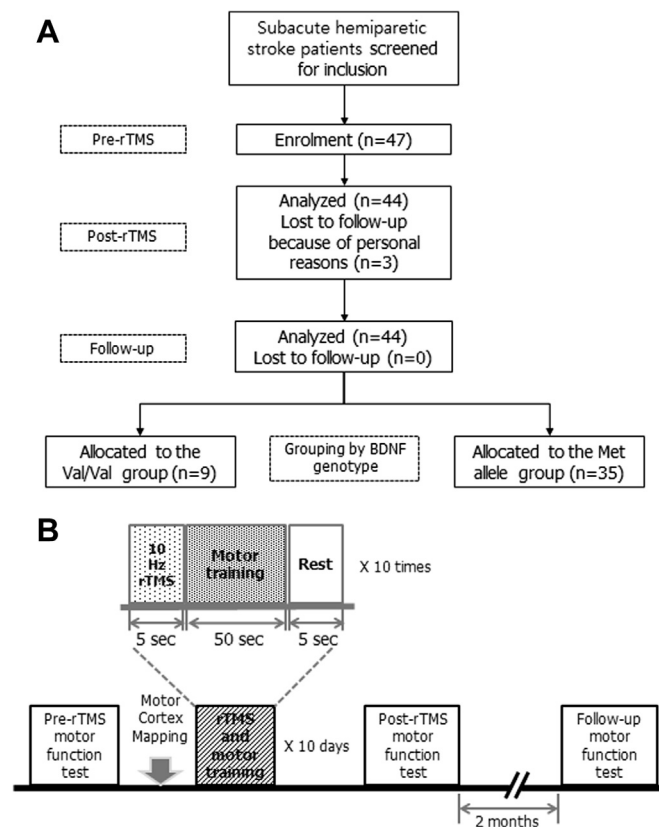

Figure 1. Experimental design. (A) Study flow chart. (B) rTMS and motor training paradigm.

system (Magstim Rapid2® stimulator, Magstim Ltd., UK) and a 70 mm figure-eight coil before the 10-day rTMS intervention and according to our previously reported protocol [7]. Once a hotspot was identified, a single-pulse stimulus was delivered to the site to determine the resting motor threshold (RMT), defined as the lowest stimulus intensity necessary to produce motor-evoked potentials (MEPs) of a peak-to-peak amplitude $\geq$ 50 µV in 5 of 10 subsequent trials.

Repetitive transcranial magnetic stimulation

Over a 2-week period, the patients underwent 10 sessions of rTMS to the primary motor cortex of the affected hemisphere. A Magstim Rapid2® stimulator with two booster modules was used to administer the therapeutic rTMS. Fifty trains were applied at 10 Hz for 5 s, and the coil over the target motor cortex area was applied at 90% RMT in correspondence with the paretic hand. For patients with no apparent MEPs on the affected hemisphere, the hotspot and intensity were determined using the mirror image of the unaffected hemisphere [7]. One thousand pulses were delivered with a 55 s inter-train interval consisting of 50 s of motor training and 5 s of rest. The motor cortex was stimulated by holding the figure-eight coil tangentially to the skull at an approximate 45° angle to the midsagittal plane with the handle pointing posteriorly. The rTMS protocols used in the study followed those used in previous reports [4,7,17] and rTMS application safety guidelines [18]. The motor practice consisted of 50 s of reaching and grasping exercises, which were conducted after each rTMS train by the same licensed physical therapist, who did not participate in the patients' function evaluations. The motor training protocol included active and active-assistive ranges of motion exercise of the affected

extremity, grasping and moving exercises and an exercise involving the release of cups and cubes. The patients were instructed to give their best effort when performing the motor tasks for the designated time. All of the patients participated in the same number of scheduled conventional physical and occupational therapy sessions, which involved gait, fitness and ADL training among other training types, for 3 h each day.

Assessment of motor function

The upper-limb score of the Fugl-Meyer assessment (FMA-UL, range: 0–66) [19] and the box and block test (BBT) were used to evaluate the motor functions of the patients' affected upper limbs and hands [20]. The lower-limb score of the FMA (FMA-LL, range: 0–34) was used to evaluate the motor functions of the patients' affected lower limbs [19]. The differences in motor function between the assessment time points were determined as follows: (Post-rTMS score − Pre-rTMS score) and (Follow-up score − Post-rTMS score). All of the assessments were performed by the same researcher, who did not know the patients' BDNF genotypes ahead of time.

BDNF genotyping

For the BDNF genotyping process, whole blood was collected into EDTA tubes. Genomic DNA was isolated from peripheral blood leukocytes according to standard proteinase-K RNase digestion procedures followed by phenol-chloroform extraction. The BDNF Val66Met polymorphism was genotyped via PCR-RFLP [15]. The genotyping was successful for all of the patients.

Data analysis

Blinding was maintained throughout the clinical trial until data entry and processing were complete, the data were verified and the database was locked. One researcher (W.H.C.) then analysed the data after unblinding was completed. According to the genotyping results, the patients were classified into two groups: a Val/Val group and a Met allele (Val/Met or Met/Met) group. The Kolmogorov–Smirnov test was used to assess whether the assessment scores were normally distributed. All of the parametric data were shown to be normally distributed. Therefore, to test the effects of rTMS across all of the time points (Pre-rTMS, Post-rTMS and Follow-up), repeated measures ANOVA with time was used as the within-patient factor and the group (Val/Val vs Met allele) was used as the between-patient factor. Post-hoc analysis was performed using Bonferroni correction [21]. An independent t-test was used to compare the values between the two groups at each time point (Pre-rTMS, Post-rTMS and Follow-up). The data were analysed using SPSS ver. 20.0 for Windows, and P-values < 0.05 were considered statistically significant.

Results

Patient grouping by BDNF genotype

Of the 44 patients who completed the experimental procedures, 9 were found to have the Val/Val genotype, 29 were found to have the Val/Met genotype and 6 were found to have the Met/Met genotype. Therefore, nine patients were classified into the Val/Val group, and thirty-five were classified into the Met allele group. There were no significant differences in general baseline characteristics between the two groups (Table 1).

Table 1
General patient characteristics and rTMS variables per study group.

	Val/Val group ($n = 9$)	Met allele group ($n = 35$)
Sex (M:F)	5:4	20:15
Age	58.4 ± 9.6	53.4 ± 13.7
Stroke type and lesion		
Ischemic:hemorrhagic	8:1	24:11
Right:left	4:5	17:18
Supratentorial:infratentoria	7:2	28:7
Cortical:subcortical	3:6	8:27
Stroke duration (days)	16.6 ± 7.6	15.5 ± 6.6
Affected side MEP (response:no response)	1:8	3:32
rTMS intensity (%)	40.8 ± 10.6	40.4 ± 8.9

Motor functions of the affected upper limb and hand

There was no significant difference in the baseline (Pre-rTMS) motor functions of the affected upper limb and hand between the two groups. Repeated measures ANOVA showed a significant interaction between time (the Pre-rTMS vs Post-rTMS vs Follow-up time points) and group (Val/Val vs Met allele groups), as measured by the FMA-UL [$F_{(1,42)} = 5.975$, $P = 0.019$] and BBT [$F_{(1,42)} = 5.021$, $P = 0.030$] (Fig. 2). The FMA-UL and BBT showed significant improvement in both groups immediately after ($P < 0.05$) and 2 months after ($P < 0.05$) rTMS intervention (Fig. 2 A-1, B-1). However, the improvements in the post-rTMS and Follow-up FMA-UL scores were significantly greater in the Val/Val group than in the Met allele group ($P < 0.05$, Fig. 2). Furthermore, the improvement in Follow-up BBT was significantly greater in the Val/Val group than in the Met allele group ($P < 0.05$, Fig. 2 A-2, B-2). Thus, the motor function of the affected upper limb improved in both groups. However, compared with the patients in the Met allele group, the patients in the Val/Val group experienced greater improvement that lasted longer (2 months) after rTMS intervention.

Motor function of the affected lower limb

The baseline motor function of the affected lower limb did not differ significantly between the groups. Repeated measures ANOVA showed no significant interaction between time (the Pre-rTMS vs Post-rTMS vs Follow-up time points) and group (Val/Val vs Met allele groups), as measured by the FMA-LL. The FMA-LL demonstrated significant improvement immediately after ($P < 0.05$) and 2 months after ($P < 0.05$) rTMS intervention in both groups. However, improvements in the FMA-LL immediately after rTMS and at the follow-up did not differ significantly between the two groups (Fig. 3 A-1, A-2). Thus, the motor function of the affected lower limb improved in both groups without a significant difference between the two groups.

Discussion

This study investigated the effects of rTMS on the motor recovery of stroke patients according to BDNF genotypes. Subacute stroke patients with no SNP in the BDNF gene (Val/Val group) showed greater improvement in motor function of the affected upper extremity than patients with the SNP in the BDNF gene (Met allele group) after consecutive multi-session high-frequency rTMS. In contrast, all of the patients showed similar motor recovery of the affected lower extremity regardless of the existence of an SNP in the BDNF gene. These findings suggest that the BDNF genotype influences the effects of rTMS on the motor function and recovery of stroke patients when rTMS is applied during the subacute period after stroke. However, the influence of BDNF polymorphism may be
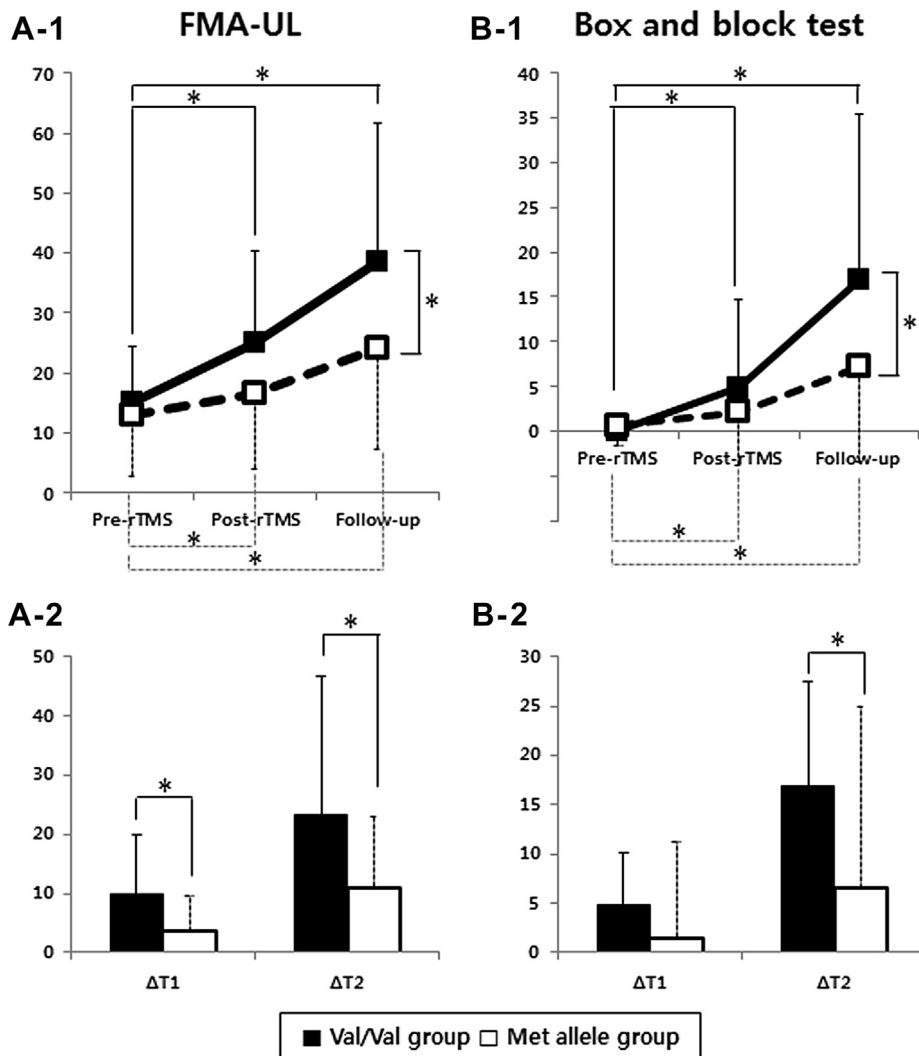
Figure 2. Changes in upper extremity motor function. (A-1) Fugl-Meyer assessment upper limb scores. (B-1) Box and block test scores. (A-2, B-2) Changes in upper extremity motor function over time. $\Delta T1 = $ (Post-rTMS score − Pre-rTMS score) and $\Delta T2 = $ Follow-up score − Pre-rTMS score. Error bars represent the standard deviation for each group at each time point. *$P < 0.05$.

greater for upper than lower limb effects, or limited to the rTMS-targeted brain region.

Recent studies suggested that the short-term effects of rTMS may involve changes in the effectiveness of synapses between cortical neurons, such as the long-term potentiation (LTP) and long-term depression (LTD) of synaptic connections [22]. In animal studies, the BDNF protein has been shown to modulate NMDAR-dependent LTP and LTD [23,24]. Considering that one of the possible mechanisms of rTMS has been reported as an NMDA-dependent LTP- and LTD-related process [8], this shared common pathway raises the possibility of interaction between rTMS treatment and the BDNF genotypes of rTMS recipients. However, such a cellular mechanism was not clearly demonstrated in this study, and further research is strongly invited to clarify the interaction response of the BDNF genotype and rTMS. Although an SNP in the BDNF gene does not affect the structure and function of the BDNF protein, it significantly impairs the intracellular trafficking and activity-dependent release of the protein [13,14]. Kleim et al. [22] showed that BDNF polymorphism is associated with modified experience-dependent short-term plasticity in the human motor cortex. In this context, patients carrying the Met allele of the BDNF gene demonstrated decreased or absent after-effects under

intermittent and continuous theta burst stimulation [15]. Inducing plasticity in neural circuits by rTMS was found to be the most difficult in non-Val/Val individuals. These reports made it clear that BDNF polymorphism appears to be a relevant factor influencing an individual's response to rTMS. Because rTMS is gaining interest as a new therapeutic tool for the neurorehabilitation of stroke patients [7,16], a greater understanding of the influence of BDNF polymorphism on rTMS effects is important to establish an optimal therapeutic strategy for individual patients.

Previous reports have implicated the SNP of the BDNF gene in clinical outcomes after stroke [24–26]. Siironen et al. [25] reported that the Met allele of the BDNF gene is associated with poor recovery at 3 months after aneurysmal subarachnoid hemorrhage. Kim el al. [24] showed BDNF polymorphism in stroke patients to be independently associated with a poor outcome at 2 weeks and at 1 year, with physical disability and cognitive function worsening over the period. However, they did not discriminate between ischemic and hemorrhagic strokes. Cramer [26] reported an association between BDNF polymorphism and relatively poor recovery only during the acute period up to 1 month but not at 3 months after ischemic stroke. They speculated that the association between stroke recovery and BDNF polymorphism may be more pronounced
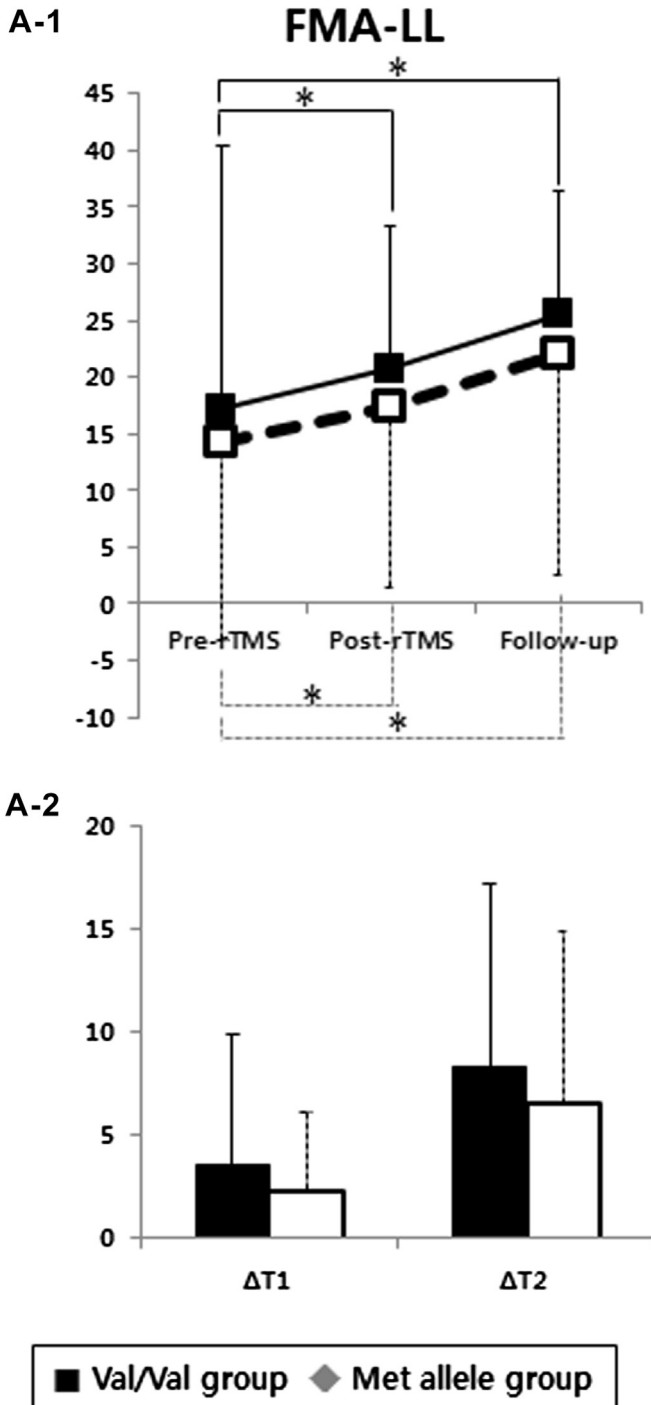
A-1

A-2

■ Val/Val group ◆ Met allele group

Figure 3. Changes in lower extremity motor function. (A-1) Fugl-Meyer assessment lower limb scores. (A-2) Changes in lower extremity motor function over time. $\Delta T1$ = (Post-rTMS score − Pre-rTMS score) and $\Delta T2$ = (Follow-up score − Pre-rTMS score). *$P < 0.05$.

in patients with hemorrhagic stroke than in patients with ischemic stroke. However, no report has addressed the specific influence of BDNF polymorphism on motor recovery after stroke. In this study, greater improvement in motor function of the affected upper limb was found in the Val/Val group than in the Met allele group after applying consecutive multi-session high-frequency rTMS. These results are consistent with the previous finding that BDNF polymorphism decreases the modulatory effects of rTMS in healthy subjects [15].

In the current study, recovery of the lower extremity function was not influenced by the presence of BDNF polymorphism. rTMS was applied over the target motor cortex area corresponding to the paretic hand. Consequently, improvements in the motor functions of the affected upper limb and hand were shown to differ significantly between the two groups, and improvement in the motor function of the affected lower limb was not shown to differ between the two groups. These findings indirectly suggest that BDNF polymorphism influences the response to rTMS but not the recovery of general motor function. Furthermore, the effect size produced by rTMS was compared with that of sham stimulation, reported in a previous study following a similar experimental design [7]. The improvement in upper extremity motor function of the Met allele group was greater than that of the sham rTMS group. These results also suggest that rTMS is meaningful in improving motor functions in stroke patients, even given the presence of BDNF polymorphism in Met allele patients. However, further study is needed to confirm this point.

The percentage of patients with the Val/Val genotype was relatively small compared with the percentages in other studies conducted in Western countries [14,15,26,27]. According to the literature, BNDF polymorphism differs significantly between ethnicities. In the Caucasian population, Val/Val is the most frequent genotype and the Met/Val genotype is most frequent among Koreans [28]. Shimizu et al. [29] reported significant differences between Japan, Italy and the USA in terms of BDNF polymorphism frequency. The genetic difference between ethnic groups may explain the ethnic-based differences in mental traits and the prevalence of psychiatric disorders such as schizophrenia and depressive disorders [30,31]. In the field of neurorehabilitation, the genetic difference is considered a cause of different therapeutic responses to rTMS between patients of different ethnic backgrounds. Therefore, further study that includes multiple ethnic groups may clarify the relation between BDNF polymorphism and rTMS-induced neuroplasticity.

This study was limited in that it did not evaluate the different characteristics of neural network modulation by rTMS for every BDNF genotype. In a previous study involving stroke patients, the consecutive multi-session high-frequency rTMS modulated engagement of the subcortical motor circuitry [16]. Further study investigating the different modulating effects of rTMS on motor network plasticity according to BDNF genotypes is required. Another limitation of this study was its rTMS methodology. It is well known that the RMT of the motor cortex in affected and unaffected hemispheres may change in an early stroke phase [32]. In terms of rTMS, two different approaches have been proposed for influencing motor function after stroke [33]. High-frequency rTMS can be used to upregulate excitability within the affected cortices, and low-frequency rTMS can be used to downregulate excitability within the unaffected cortices. Each rTMS method has been shown to have a positive effect on motor recovery in stroke patients during the subacute phase [33]. In this study, high-frequency rTMS without a navigation system was shown to be effective for subacute stroke patients [5,7]. Nevertheless, mirroring the stimulation site from the unaffected motor cortex is a much cruder method, as areas other than the affected motor cortex may be stimulated [7,34]. This is a limitation of this study. rTMS with a navigation system should provide better results in future research.

In conclusion, this study proposes that BDNF polymorphism influences the neural response to rTMS in patients with stroke. The facilitating effect of rTMS on long-term motor recovery was found to be better in patients without the BDNF SNP than in those with the SNP. BDNF polymorphism must be considered as a significant influencing factor when forming a therapeutic rTMS strategy for subacute stroke patients.

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