The effects of different frequencies of repetitive transcranial magnetic stimulation (rTMS) on intracranial neurotransmitters in patients with swallowing disorders after cerebral infarction

Yupeng Du (duyupeng0701@163.com)

Department of Rehabilitation, The Third Affiliated Hospital of Zhejiang Chinese Medical University

https://orcid.org/0000-0003-2349-8959

Li Wei

State Key Laboratory of Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China;

Huangwei Jiang

Hangzhou Hospital of Traditional Chinese Medicine

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Abstract

Objective To study the improvements and mechanism of repetitive transcranial magnetic stimulation (rTMS) on swallowing disorders after cerebral infarction. Methods A total of 60 patients with swallowing disorders were randomly assigned to receive high/low-frequency rTMS treatment, another 30 patients without rTMS treatment were included in the control group. The Kubota's water-swallowing test, video fluoroscopic swallowing study (VFSS), and major intracranial neurotransmitters were analyzed before and after treatment. Results No significant difference was observed in the Kubota's water-swallowing test scores, the VFSS scores, or the levels of intracranial neurotransmitters between the three groups before treatment. The Kubota's water-swallowing test scores were significantly reduced after high-frequency rTMS treatment than in the control group; the aspiration degree was significantly increased after high-frequency rTMS treatment than in the control group; the levels of glutamate and dopamine were significantly increased after high-frequency rTMS treatment than in the control group; Moreover, the Kubota's water-swallowing test scores were significantly reduced after high-frequency rTMS treatment than after low-frequency rTMS treatment; the levels of glutamate and dopamine were significantly increased after high-frequency rTMS treatment than after low-frequency rTMS treatment. Conclusions High-frequency rTMS was effective for swallowing disorders, which may be related to increased levels of intracranial glutamate and dopamine.

Background

Swallowing disorders represent one of the most common complications after cerebral infarction. The incidence is up to 65% after acute cerebral infarction and further increased in patients with brain stem infarction. It is an independent risk factor for mortality of cerebral infarction, which greatly affects patient recovery and quality of life [1].

Repetitive transcranial magnetic stimulation (rTMS) is a new safe effective treatment for swallowing disorders after cerebral infarction[2]. Numerous studies have demonstrated its efficacy for swallowing disorders [3, 4]. However, the mechanism of rTMS for the treatment of swallowing disorders remains unknown. Some studies have suggested that rTMS treats swallowing disorders after cerebral infarction by modulating the balance of intracranial neurotransmitters via the N-methyl-D-aspartate (NMDA) receptor and dopaminergic receptor pathways [5, 6].

Information transmitted between neurons formed the brain activity, and the brain activity can be best recorded by extracellular field activity recordings, such as electroencephalogram (EEG). Neuronal networks' oscillations may serve lots of physiological functions, which contained the coordination of movement patterns and the combination of sensory information. Recently, some studies have reported ultra-slow oscillations in human EEG with frequencies that range from 0.001 to 0.01 Hz [7]. Vadim V. Nikulin et al. reported monochromatic ultra-slow oscillations in human EEG and their relation to hemodynamics[8]. S. Y. MOK first reported that network-wide ultra-slow oscillations aggregate the firing rates of cortical cultures [9]. Siow-Cheng Chan's results provided theoretical support for astrocytes as

active players in the regulation of neural activity and identified neuron–astrocyte interactions as a potential primary mechanism for the emergence of ultra-slow cortical oscillations[10].Therefore, ultra-slow oscillations are very important in the study of the brain.

Brain neurotransmitters regulate brain electrical signals through synthesis, release, uptake, absorption and metabolic activities, which affects EEG morphology [11]. Edmund G et al reported that norepinephrine and serotonin play different roles on EEG activity through the basal forebrain, the former increasing γ activity and eliciting waking and the latter decreasing γ activity and with no significant change in amounts of wake or slow wave sleep[12].EEG registration from brain areas associated with different types of neurodegenerative pathology, in combination with pharmacological testing of involved neurotransmitter systems, allows discovery of both progression and mechanisms of diseases[13]. Vorobyov V et al studied the cortical and hippocampal EEG effects of neurotransmitter agonists in spontaneously hypertensive vs. kainate-treated rats, Their results demonstrate sensitization of NMDA receptors and α2-adrenoceptors both in spontaneously hypertensive (SH), and kainate-treated (KA) rats and that of γ-aminobutyric acid (GABA) b receptors specifically in SH rats[14]. The coupling between local cortical inhibitory neurons and cortical excitatory pyramidal neurons can be reflected by EEG oscillations. The less activation of acetylcholine neurons or synaptic damage of the cortical inhibitory excitatory feedback loops may resulting a lower alpha peak frequency, a convert from alpha to theta power, and higher oscillations amplitudes in EEG[15]. Nowadays EEG approach might be considered as a potentially effective tool for analysis of the brain neurotransmitter and compensatory/adaptive mechanisms in alzheimer disease (AD) progression[16]. To date, the correlations between brain neurotransmitters and EEG in swallowing disorder patients treated with rTMS are still unclear.

Encephalofluctuograph is a non-invasive approach to brain function detection to indirectly measure the levels of brain neurotransmitters, which mainly depend on analyzing the ultra-slow cortical oscillations of EEG. It can reflect the level of neurotransmitters in the brain by scanning dominant frequency and analyzing information in consistent with neurochemical oscillation, which being hidden in the ultra-slow frequency [17, 18], which has been reported to the diagnosis of Parkinson's disease, primary insomnia, and job-related burnout[11, 19, 20].

In this study, we planned to research the effects and mechanism of rTMS on swallowing disorders after cerebral infarction, thus providing theoretical basis for the clinical application of rTMS.

Methods

rTMS Treatment site: The projection area of the swallowing center in the human skull was determined with reference to Emilia Michou's study(Michou and Hamdy, 2009).

1.1 Case description:

A total of 60 patients with swallowing disorders were enrolled in this study and randomly assigned to the low-frequency rTMS group or the high-frequency rTMS group. Moreover, 30 patients with swallowing

disorders were included in the control group. This research was approved by the Ethics Committee of The Third Affiliated Hospital of Zhejiang Chinese Medical University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All methods were performed in accordance with relevant guidelines and regulations. Written informed consent was obtained from all individual participants included in the study.

1.1.1 Inclusion criteria: (1) Patients who met the International Classification of Diseases (ICD)-10 diagnostic criteria for cerebral infarction; (2) cerebral infarction confirmed with CT or MRI; (3) Kubota's water-swallowing test and video fluoroscopic swallowing study (VFSS) screening indicated swallowing disorders, needing oral feeding modification; (4) conscious, stable vital signs, and ability to cooperate during treatment; (5) ≥ 18 and ≤ 80 years of age; (6) the duration of disease was one to six months since onset.

1.1.2 Exclusion criteria: (1) Swallowing disorders unrelated to cerebral infarction; (2) metal object and pacemaker in the body; (3) uncontrolled blood pressure (> 180/120 mm Hg); (4) vital organ failure; (5) cerebral infarction patients in critical condition; (6) brain stem infarction;

(7) epencephalon infarction.

1.2 Treatment

1.2.1 Routine Treatment

All enrolled patients received routine treatment for cerebral infarction, including amlodipine (to control blood pressure), atorvastatin (to lower blood lipids), and aspirin (anti-platelet agent), and routine treatment for swallowing disorders, including acetic acid stimulation, low-frequency electrical stimulation of pharyngeal muscles, and facial muscle function training.

1.2.2 rTMS Treatment

The Rapid2 transcranial magnetic stimulator (Magstim Company Limited, UK) with a figure-eight coil (inner diameter: 10 mm, outer diameter: 50 mm; one side) was used. During magnetic stimulation, the coil was secured in place. The standard stimulation mode was used.

(1) High-frequency rTMS group

Treatment site: The projection area of the swallowing center in the human skull was determined with reference to Emilia Michou,s study(Michou and Hamdy, 2009). The area to be stimulated was 3 cm anterior to the center of the top of head and then 8 cm lateral towards the infarction side (A model of rTMS stimulation site was showed in Figure 1, point C was the stimulation site).

Stimulation frequency: 10 Hz.

Stimulus intensity: The intensity was 80% of the movement threshold.

Total stimuli in each session and interval: rTMS was performed at 10 Hz stimulation for 1 s, interval 20 s, and then repeated next stimulation and interval.

Treatment time: rTMS was applied 20 minutes every day, for consecutive 30 days.

(2) Low-frequency rTMS group

Treatment site: Same as the high-frequency rTMS group.

Stimulation frequency: 1 Hz.

Stimulus intensity: The intensity was 80% of the movement threshold.

Total stimuli in each session and interval: rTMS was performed at 1 Hz stimulation, for 1 s, interval 20 s, and then repeated next stimulation and interval.

Treatment time: rTMS was applied 20 minutes every day, for consecutive 30 days.

(3) Control group

The control group received routine treatment but with no rTMS treatment. The total treatment time was 30 days.

1.3 Outcome measures

1.3.1 Kubota's water-swallowing test

Before and after treatment, the Kubota's water-swallowing test was performed to assess the patient's swallowing function as follows: the patient was instructed to drink 30 mL of warm water, and the time required and any choking were recorded to classify swallowing function as profile I to V. Profile I: the patient drinks 30 mL of warm water all at once within five seconds (defined as a score of 0); profile II: the patient drinks the water after swallowing twice or more within five to 10 seconds with no choking (defined as a score of 2); profile III: the patient drinks the water all at once within five to 10 seconds with choking (defined as a score of 4); profile IV: the patient drinks the water after swallowing twice or more within five to 10 seconds with choking (defined as a score of 6); profile V: the patient chokes multiple times while drinking the water and found it difficult to finish drinking the water within 10 seconds (defined as a score of 8).

1.3.2 VFSS

Before and after treatment, VFSS was performed with a digital gastrointestinal X-ray machine (VS-20D, Shimazu, Japan) to assess the patient's swallowing function as follows:

Mouth period: the food in the mouth cannot be delivered to the pharynx (defined as a score of 0); cannot move a piece of food into the pharynx or can only form a paste in the pharynx (defined as a score of 2); cannot completely move all food into the pharynx at one time, some food remains after swallowing action (defined as a score of 4).

Pharynx period: cannot cause the pharynx to lift up, swallowing reflex is insufficient (defined as a score of 0); a large amount of residual food remains in the pyriform fossa (the residual food is larger than 1/2 of the pyriform fossa) (defined as a score of 1); a small amount of residual food (less than 1/2 of the residual food) remains in the pyriform fossa (defined as a score of 2); can move food into the esophagus using a swallowing action (defined as a score of 3).

Degree of aspiration: Most of the food was inhaled by mistake, no cough was observed (defined as a score of 0); most of the food was inhaled by mistake, cough present (defined as a score of 1); a small amount of aspiration was observed, no cough (defined as a score of 2); a small amount of aspiration was observed, cough present (defined as a score of 3); no food was inhaled by mistake, no cough observed (defined as a score of 4).

1.3.3 Detection of major intracranial neurotransmitters

The patients and control subjects underwent intracranial neurotransmitter detection using the Encephalofluctuograph analytical instrument (ML2001 encephalofluctuograph analyzer, Beijing Tongren Optoelectronics Technology Co., Ltd.). According to the international 10-20 electrode system, the anterior and posterior attachment of the sutura nasofrontalis to the external occipital protuberance as one of the base line, the left and right articulation of the anterior fossa of both ears as the other base line. A total of 12 leads, which included F3(left frontal), F4(right frontal), C3(left the central), C4(right the central), P3(left top), P4(right top), O1(left occipital), 02(right occipital), F7(left anterior temporal), F8(right anterior temporal), T5(left posterior temporal), and T6(right osterior temporal) were used to record the EEG. Bilateral reference electrodes were placed on the right and left earlobes.

The electrical signals were recorded for 18 minutes via encephalofluctuograph analytical instrument and were stored for EEG analysis. Then the EEG signals were analyzed by encephalofluctuograph to finally obtain a new informative frequency hidden in the brain waves, which typically falls in the range of 1–255 MHz. The waves are correlated to a super-slow shocking S system, including the S1–S255 series, which could reflect the activity of different intracranial neurotransmitters, with the S1, S2, S4, S5, S7, and S11 series corresponding to γ-aminobutyric acid (GABA), glutamate (Glu), 5-hydroxytryptamine (5-HT), acetylcholine (Ach), norepinephrine (NE), and, dopamine (DA) respectively. The data of neurotransmitters was analyzed and converted into value form by FoxPro v6.0 database software in ML2001 encephalofluctuograph analyzer before export.

1.4 Statistical analysis

The data were analyzed using SPSS 18.0 software. The data are presented as the mean±standard error of mean (S.E.M.) or the mean±standard deviation (S.D.). Descriptive statistics were performed to determine whether the data were normally distributed. Normally distributed data were analyzed with one-way analysis of variance (ANOVA) and independent sample t test, and non-normally distributed data with non-parametric test. $P \leq 0.05$ was considered statistically significant.

Results

2.1 Patient demographics and characteristics

Patient demographics and characteristics were showed in Table 1. Descriptive statistics were performed to determine whether the data were normally distributed, and the results confirmed that the data were indeed normally distributed. Comparisons of age, mean duration of disease, Kubota's water swallow test score, and VFSS score (Oral period, Pharynx period, Aspiration Degree)among the groups were performed with one-way analysis of variance (ANOVA), and a post hoc test was used to determine the differences among the groups. Table 1 showed that there was no significant difference in age, race, sex, mean duration of disease, affection brain area, ischemic, recurrent stroke, Kubota's water swallow test score, and VFSS score among three groups.

2.2 Kubota's water-swallowing test scores in three groups after treatment

After treatment, the Kubota's water-swallowing test score was significantly lower in the high-frequency rTMS group than in the control group, meanwhile the Kubota's water-swallowing test score was significantly lower in high-frequency rTMS group than in low-frequency rTMS group ($P \leq 0.05$).However, there was no significant difference in Kubota's water-swallowing test score between the low-frequency rTMS group and the control group after treatment (Fig 2).

2.3 VFSS scores in three groups after treatment

After treatment, the aspiration degree was significantly increased in the high-frequency rTMS treatment group than in the control group ($P \leq 0.05$, Fig 3C); however, no significant difference was observed in the oral period and pharynx period among the three groups after treatment (Fig 3A, 3B).

2.3 Intracranial neurotransmitters before and after treatment

There was no significant difference in intracranial neurotransmitters before treatment in three groups (Fig 4A, 4C, 4E, 4G, and 4J). After treatment, the levels of DA and Glu were significantly increased in the high-frequency rTMS group than in the control group; meanwhile the levels of DA and Glu were significantly increased in the high-frequency rTMS group than in the low-frequency rTMS group ($P \leq 0.05$, Fig 4D, Fig 4K). There was no significant difference in the other neurotransmitters after treatment (Fig 4B, 4F, and 4H).

Discussion

At present, the anterior lateral part of the motor cortex and the motor cortex of the central sulcus are considered to be the cortical central control regions for swallowing[21]; therefore, we used which as the body surface projection area when encephalofluctuograph performing. rTMS penetrates the skull to directly act on the cerebral cortex, stimulating the local cortical neurons to directly or indirectly promote remodeling of the swallowing cortex center[22]. rTMS modulates cortical excitability by focally stimulating the cortical region[23]. Stimulation by TMS is produced by transmitting a high current pulse of short duration through the skull, via an insulated coil held over the scalp [3]. Some studies reported that high-frequency rTMS may enhance muscular control for swallowing [24].Lee JH reported that rTMS over a hot spot for the suprahyoid muscle caused more improvement in swallowing function when compared to that over the interconnected site[25]. Therefore rTMS may through stimulating the swallowing muscular control or modulating cortical excitability to treating the swallowing disorders, however the detail mechanism was still unclear.

Until now, only a few studies have examined the utility of rTMS in terms of recovery of the swallowing function, meanwhile no standard rTMS protocol for treating dysphasia has been developed. It is known that high-frequency rTMS($\geq$5 Hz) has been shown to have excitatory effects, whereas low-frequency rTMS ($\leq$ 1 Hz) has been shown to have inhibitory effects on cortical neurons[3]. Higher frequency may be more effective. The most serious side effect of high-frequency rTMS is the induction of a seizure [26]. To yield a greater therapeutic effect and minimize seizure risk, we selected 10 Hz as the high frequency in our study. To avoid the short-term effect of rTMS, we designed 30 days as the course of rTMS treatment in our study. Meanwhile to avoid the interruption effects of rTMS there was no rest day during the 30 days treatment in our study.

In our study, we found that after treatment, the Kubota's water-swallowing test scores were significantly lower, and the aspiration degree was significantly increased in high-frequency rTMS group than in the control group; however no significant difference was observed in the low-frequency rTMS group and the control group, which consistent with previous report[27]. Currently, most researchers believe that rTMS changes the excitability of the surrounding area of the lesion site [28]. Our results showed that low frequency rTMS was less effective than high-frequency rTMS; we consider the reason maybe high-frequency rTMS increase the excitability of the surrounding area of the lesion site, while the low-frequency rTMS decrease it.

Changes in synaptic reconnection are the basis for brain remodeling, and this process is closely associated with neurotransmitters[29]. In our study, we found that after treatment, the levels of glutamate and dopamine were significantly increased in the high-frequency rTMS group than in the control group. Moreover, the levels of glutamate and dopamine were significantly increased in the high-frequency rTMS group compared with those in the low-frequency rTMS group, which indicated that high-frequency rTMS had a more significant effect on neurotransmitter levels than low-frequency rTMS.

NMDA is a specific glutamate receptor which is also the theoretical material basis of synaptic plasticity. High levels of glutamate may activate more glutamate NMDA receptors, which may exert protective effects on the swallowing center [30]. NMDA can detect neuronal synergistic activity between pre-synaptic and post-synaptic connections, which enhances synaptic connections. The strengthen connection of intersynaptic is macroscopically manifested as remodeling of the swallowing center network and improvement in swallowing. In addition, the relay nucleus of the primary swallowing center of the brain stem has an excitatory amino acidergic neuron region which receives the regulation of excitatory neurotransmitters, such as glutamate, on the primary swallowing center [31]. Therefore, after high-frequency rTMS treatment, more glutamate travels along the neuronal projection to the region, thus stimulating the swallowing center of the brain stem to send excitatory or inhibitory signals to the effectors to general rhythmic swallowing movement. On the other hand, some studies have demonstrated that rTMS reduces the level of intracranial glutamate in patients with swallowing disorders after cerebral infarction [32, 33], which contradicts with our results. It may because the effects of rTMS are time-dependent and gradually decreases after treatment; therefore, the levels of neurotransmitters are related to the time and measurement method.

Previous studies have mainly reported the levels of brain neurotransmitters in rats [34, 35], which cannot really reflect the changes in the human brain. Furthermore, there have been only a few reports in patients. Dopamine has a neuroprotective effect and is closely related to ischemic neuronal damage, which promotes neuronal recovery [36]. In physiological conditions, dopamine inhibits the release of glutamate through the D2-like receptor. During cerebral ischemia, dopamine promotes the release of glutamate [37]. Studies demonstrate that high-frequency rTMS on the lesion side increases the level of dopamine in the brain, which acts on D2-like receptors to inhibit the high-affinity glutamate reuptake system, resulting the glutamate increasing[38]. High-frequency rTMS has been demonstrated to increase the level of glutamate by activating the glutamate-specific receptor NMDA to promote remodeling of the damaged center [39]. Therefore, we consider increasing glutamate and dopamine levels in the brain may be one of the therapeutic mechanisms of high-frequency rTMS on swallowing disorders after cerebral infarction.

Due to the limited sample size in this study, our study results might have some level of bias. We preliminarily reported the changes of neurotransmitters in brain after rTMS treatment, large group size and deep mechanism of the changes of neurotransmitters in brain needs further study in future.

Conclusions

In summary, we used encephalofluctuograph to accurately and non-invasively detect the levels of intracranial neurotransmitters in swallowing disorder patients after cerebral infarction following treated with different frequencies of rTMS. We found that high-frequency rTMS was effective for swallowing disorders, which may be related to increased levels of intracranial glutamate and dopamine.

Abbreviations

rTMS: repetitive transcranial magnetic stimulation

VFSS: video fluoroscopic swallowing study

EEG: electroencephalogram

NMDA: N-methyl-D-aspartate

SH: spontaneouslyhypertensive

KA: kainate-treated

GABA: γ-aminobutyric acid

AD: alzheimer disease

ICD-10: International Classification of Diseases-10

ANOVA : one-way analysis of variance

Declarations

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declared that they had no conflict of interest.

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Authors' contributions

YD designed the study and was involved throughout the entire implementation process. LW carried out the sample collection, the neurotransmitters detection and the statistical analysis. JH carried out the Kubota’s water-swallowing test, and video fluoroscopic swallowing study (VFSS) test. All the authors reviewed the final version of the manuscript and approve it for publication.

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No.

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Tables

Table 1 Patient demographics and characteristics

Variables of interest	Low-frequency rTMS group（n=30）	High-frequency rTMS group（n=30）	Control group （n=30）
Age (years) (mean±S.D.)	70.61 ± 2.63	68.47 ± 1.98	71.06 ± 3.56
Race, Han, % (n)	100	100	100
Sex (% female)	40.00	46.67	43.33
Mean duration of disease (months) (mean±S.D.)	4.51 ± 1.28	4.28 ± 1.05	3.88 ± 2.10
Affected brain area (left hemisphere) (% total)	43.33	56.67	60.00
Affected brain area (right hemisphere) (% total)	56.67	43.33	40.00
Ischemic (% total)	100	100	100
Recurrent stroke	NO	NO	NO
Kubota's water-swallowing test score (mean±S.D.)	4.63 ± 1.81	4.42 ± 1.67	4.53 ± 1.75
VFSS score (Oral period) (mean±S.D.)	1.58 ± 0.64	1.68 ± 0.62	1.66 ± 0.71
VFSS score (Pharynx period) (mean±S.D.)	1.39 ± 0.89	1.34 ± 0.85	1.34 ± 0.71
VFSS score (Aspiration degree) (mean±S.D.)	1.63±0.82	1.61 ± 0.59	1.61 ± 0.64

Figures

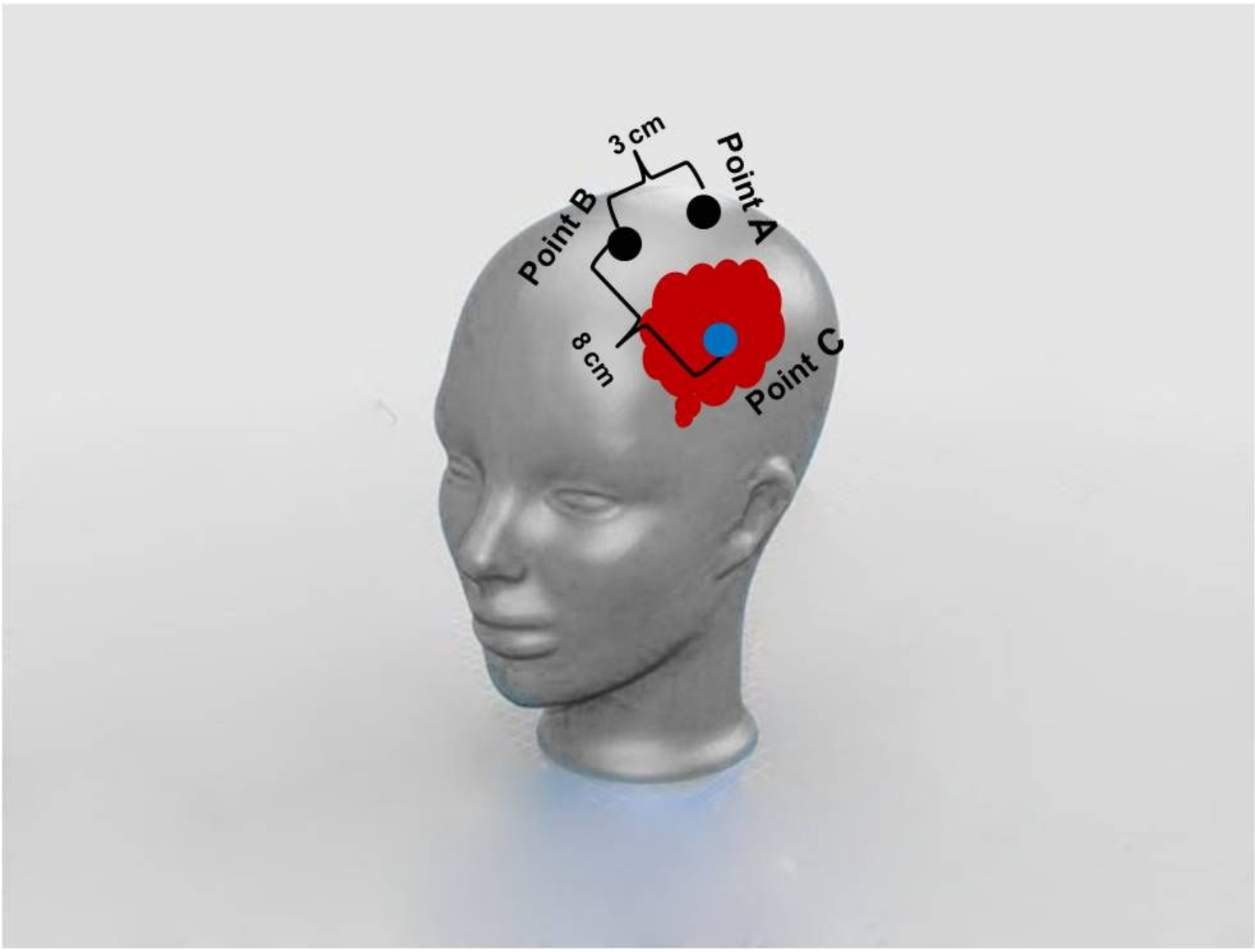

Figure 1

rTMS stimulation site on patient. (Point C is the stimulation site. Location of point A: the intersection of sagittal line with binaural tips connection; Location of point B: 3 cm forwards from point A; Location of point C: 8 cm lateral towards the infarction side from point B.)

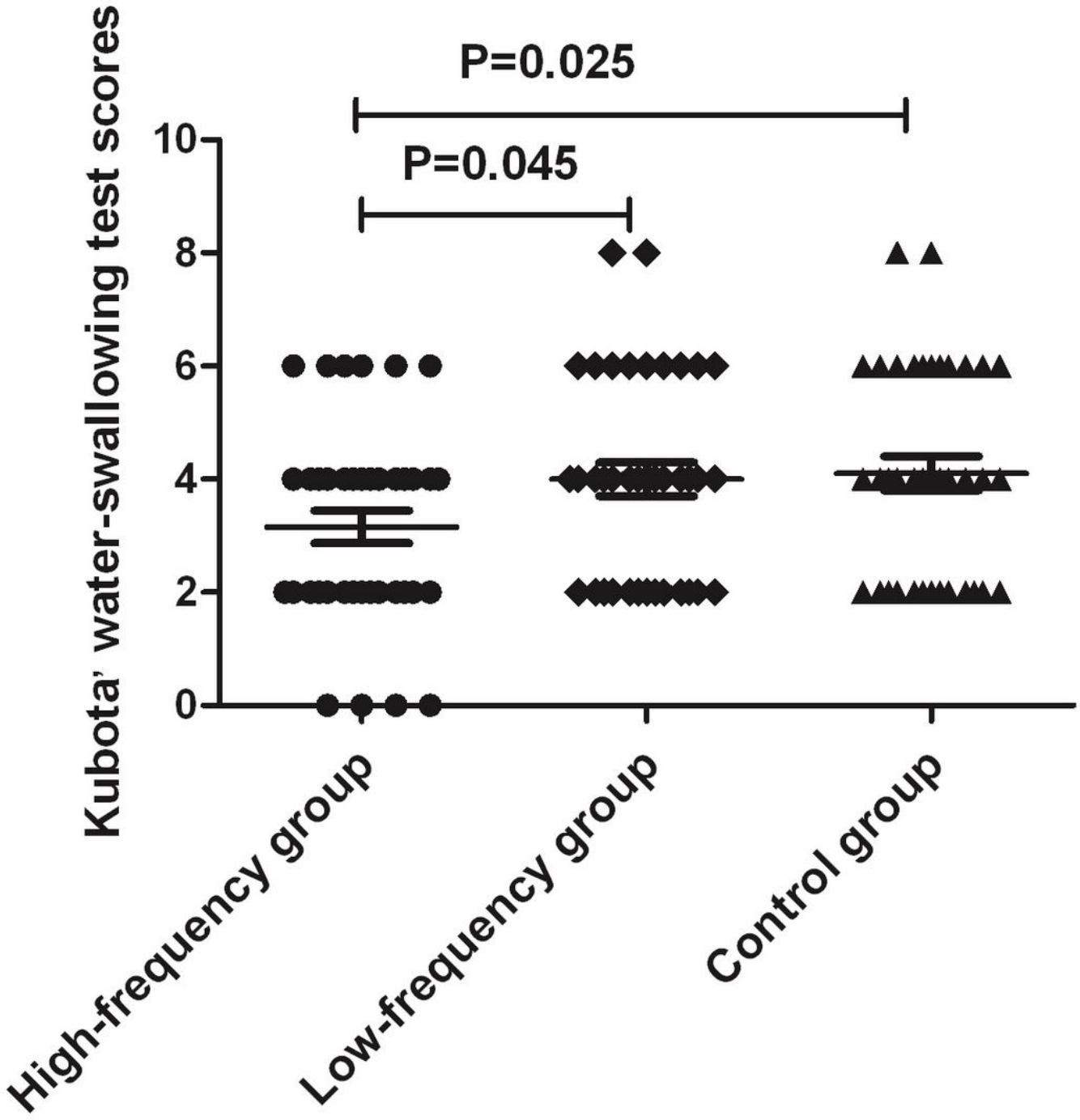

Figure 2

Kubota's water-swallowing test scores in three groups after treatment. (Descriptive statistics were performed to determine whether the data were normally distributed. The data were normally distributed. Comparisons between groups were performed with one-way analysis of variance (ANOVA), and a post hoc test was used to determine the differences among the groups.)

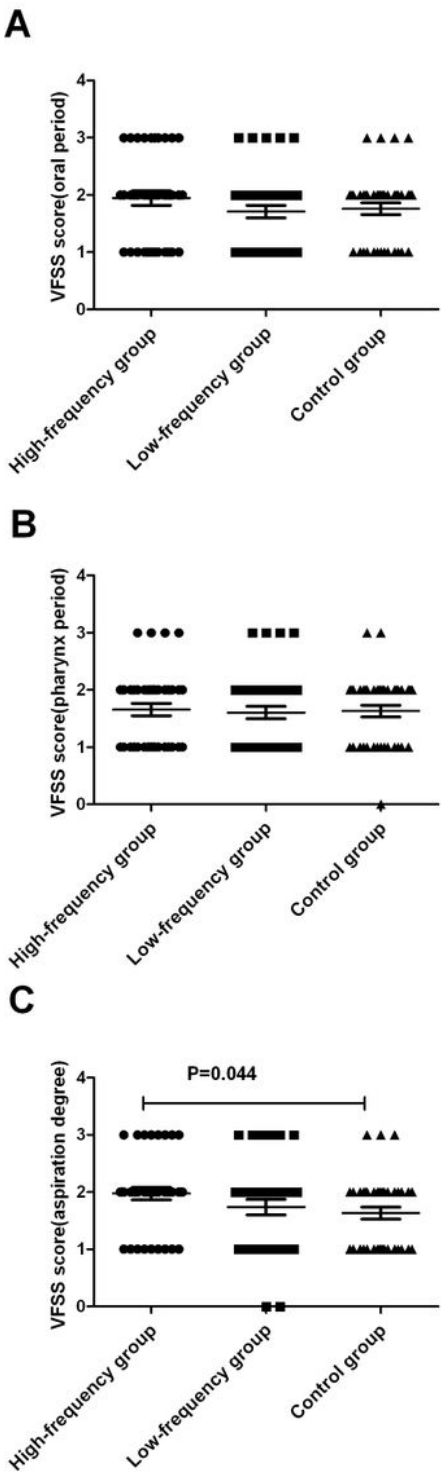

Figure 3

3A VFSS scores (oral period) in three groups after treatment; Figure 3B VFSS scores (pharynx period) in three groups after treatment; Figure 3C VFSS scores (aspiration degree) in three groups after treatment. (Descriptive statistics were performed to determine whether the data were normally distributed. The data were normally distributed. Comparisons between groups were performed with one-way analysis of variance (ANOVA), and a post hoc test was used to determine the differences among the groups.)

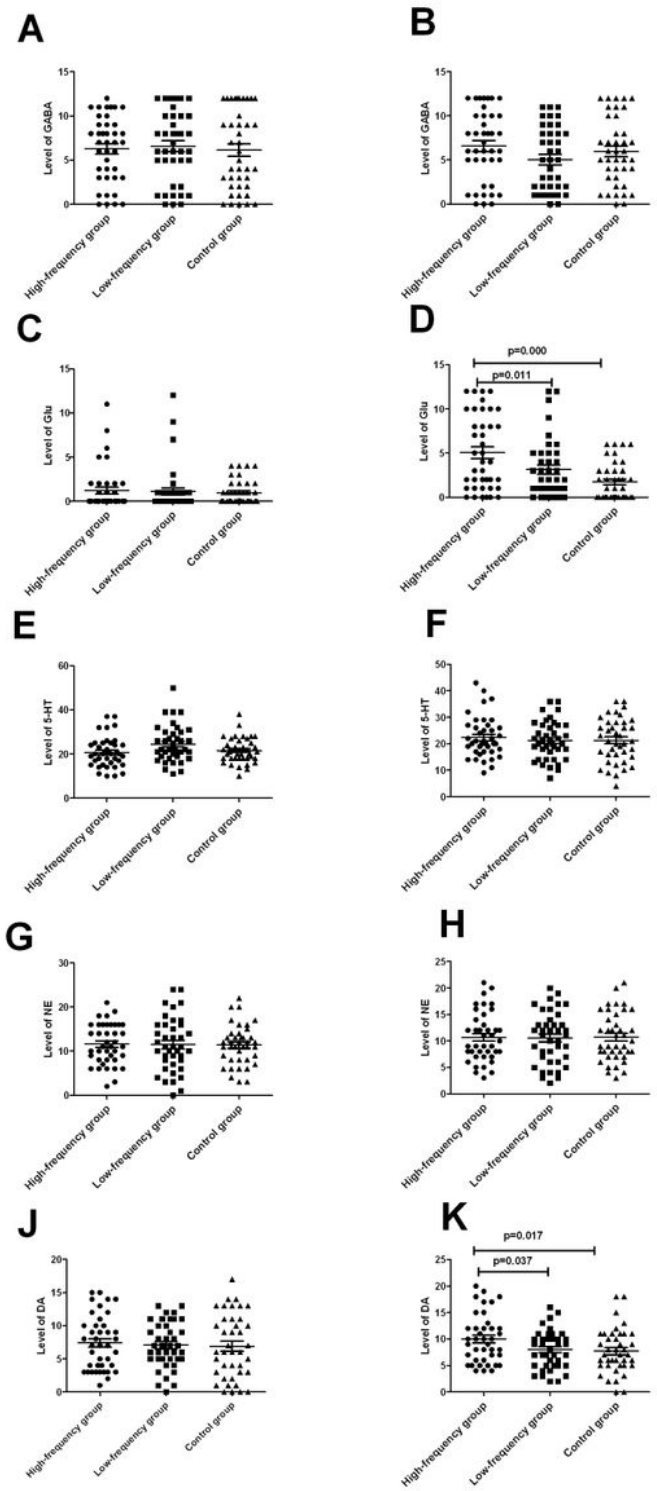

Figure 4

4A Intracranial GABA in three groups before treatment; Figure 4C Intracranial Glu in three groups before treatment; Figure 4E Intracranial 5-HT in three groups before treatment; Figure 4G Intracranial NE in three groups before treatment; Figure 4J Intracranial DA in three groups before treatment; Figure 4B Intracranial GABA in three groups after treatment; Figure 4D Intracranial Glu in three groups after treatment; Figure 4F Intracranial 5-HT in three groups after treatment; Figure 4H Intracranial NE in three groups after treatment;

Figure 4K Intracranial DA in three groups after treatment (Descriptive statistics were performed to determine whether the data were normally distributed, and the results confirmed that the data were indeed normally distributed. Comparisons among the groups were performed with one-way analysis of variance (ANOVA), and a post hoc test was used to determine the differences among the groups.)