Repetitive transcranial magnetic stimulation can improve the fixation of eyes rather than the fixation preference in children with autism spectrum disorder.

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Research Article

Abstract

Background

Transcranial magnetic stimulation (TMS) has been introduced into the intervention of autism spectrum disorders (ASD) as a possible new therapeutic option for modifying pathological neuroplasticity. However, the stimulating protocols of rTMS for ASD have not been approved unanimously, which affects the clinical popularization and application of rTMS. In addition, there is little research on the improvement of social processing of autistic children by rTMS.

Methods

We explored the clinical efficacy of rTMS and improvement of face processing with the protocol of left high-frequency and right low-frequency on bilateral dorsolateral prefrontal cortex (DLPFC), with a sample of 45 ASD participants aged 2–18.

Results

Our results showed that both the score on the Childhood Autism Rating Scale (CARS) and the fixations on the eyes of the human faces improved by two-session rTMS intervention, except for the eye preference. The mediation analysis indicated the item of "Adaptation to Change" of CARS mediated dominantly the improvement of eye-gaze behavior of ASD participants by rTMS.

Conclusions

Our study revealed the mechanism of rTMS in improving the eye-gaze behavior of the autism population, deepened the understanding of the function of rTMS in treating autistic social disorders, and provided a reference for combined treatment for ASD.

Introduction

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder, which occurs in early childhood and is characterized by social disorder, language communication disorder, limited interest range and/or repetitive behaviors (1). The prevalence of autism spectrum disorders continues to rise worldwide (2). For example, according to the Autism and Developmental Disabilities Monitoring (ADDM) Network in the United States, ASD prevalence estimates have increased from 6.7 (one in 150) per 1,000 children aged 8 years at ADDM Network sites in surveillance years 2000 and 2002 to 23.0 (one in 44) in surveillance year 2018 (3). In China, the prevalence rate of autism spectrum disorder is estimated to reach 1% (4). In the ASD population, more than 70% of the individuals need lifelong care and rehabilitation (5). The average lifetime cost of each ASD individual is about 3.6 million US dollars (6), bringing a

substantial economic burden to their families and society. Therefore, finding a more safe and effective intervention method has become a significant problem to be solved urgently in the research field of ASD.

At present, most therapeutic interventions in ASD only provide symptomatic treatment, and the outcomes of the intervention are judged by subjective endpoints (such as behavioral evaluation) which together with the high heterogeneity of ASD account for the wide variability in the effectiveness of treatments (7). Transcranial magnetic stimulation (TMS) is one of the first treatments that targets a putative core pathological feature of autism, specifically the cortical inhibitory imbalance that alters gamma frequency synchronization (8, 9). More and more studies have shown that low-frequency TMS over the dorsolateral prefrontal cortex (DLPFC) of individuals with ASD decreases the power of gamma activity and increases the difference between gamma responses to target and non-target stimuli (10), which improves executive function skills related to self-monitoring behavior and the ability to take corrective measures (7). These improvements are not only reflected in the reduction of stimulus-bound behaviors (7), but also shown as diminished sympathetic arousal (11). Moreover, the improvement also presents a dose-response relationship, i.e., the more number of TMS sessions, the more improvement of ASD symptoms (12). Although TMS has shown some optimistic effects in treating ASD, there are still some key problems that have not been solved, such as the stimulating protocols and the stimulating sites, which affect the clinical popularization and application of rTMS (13). For example, Baruth et al. used low-frequency rTMS to stimulate the DLPFC of ASD patients and found that irritability and repetitive behavior could be improved (14); while Enticott et al. found that they improved the social disorder and anxiety of ASD patients by stimulating bilateral dorsomedial prefrontal cortex with high-frequency rTMS (15). Our previous study has shown that the high-frequency rTMS on left DLPFC and low-frequency on right DLPFC can improve ASD symptoms as well as sleep disturbances (16). Meanwhile, there are also few studies on the improvement of social processing of autistic children by rTMS, such as the improvement of facial processing features of autistic children. ASD individuals' abnormal processing of human faces has always been a concern, and is considered to be the most significant social defect feature (17). On the one hand, ASD individuals are shown to avoid others' eyes contact in the social situations (18, 19); on the other hand, they lack of attentional preference for faces (that is, the attention preference for human faces, relative to non face stimuli, presented by typically developed children at birth (20)) in the environment (21–23). The accumulative evidence has proved that ASD children under the age of 3 show a series of social visual attention deficits, such as decreased fixation on the eyes (24, 25) and face area (26), which have been regarded as biomarkers of early social development abnormalities in ASD individuals (27). Neuroimaging studies also indicated that ASD individuals' defects in face processing may be related to the abnormal activation of DLPFC (28). The exploration of improvement of abnormal face processing by rTMS on DLPFC of ASD subjects will play a positive role in deepening the understanding of neural mechanism of social processing of ASD subjects and promoting the better application of rTMS in the clinical intervention of ASD. Unfortunately, however, studies of face processing among autistic individuals by rTMS on DLPFC have hardly appeared.

Therefore, we explored the clinical efficacy of rTMS and improvement of face processing with the protocol of left high-frequency and right low-frequency on bilateral DLPFC. For the face processing, the

preferential looking paradigm was used, with the area of interest (AOI) of the eyes and the whole face, to check the fixations on eyes before and after the intervention. To further explore the possible mechanism of rTMS on face processing, we also planned to make the mediation analysis, with the score of the Childhood Autism Rating Scale (CARS) as the mediator. We hypothesized that rTMS with above protocol could effectively improve the facial fixation of ASD children, not only on the eyes of faces, but also the attentional preference to eyes (28).

Methodology

Subjects

We mainly carried out this study in Tianjin Anding (psychiatric) Hospital from October 2018 to October 2021, with a convenient sampling method. We released the recruitment information to hospitalized patients or outpatients with ASD and evaluated the subjects who wanted to participate in the development lab of Tianjin Medical University. The eligibility criteria included: (1) it meets the diagnostic criteria of ASD in the fifth edition of the American Diagnostic and Statistical Manual of Mental Disorders (DSM-V); (2) age of 2–18 years old; (3) none no medication during the rTMS intervention; (4) right-handed; (5) the total score of CARS in the baseline $\geq$ 30 (29). The exclusion criteria were (30, 31): (1) contraindications to rTMS, such as metal or electronic instruments near the coil stimulation site; participants with a history of epilepsy (excluding epilepsy according to their electroencephalogram and medical record); participants with a history of brain trauma, brain tumors, and other diseases; participants with severe or recent heart disease; or other major physical illness. (2) Diagnosis of other mental illness (e.g., attention-deficit hyperactivity disorder, schizophrenia and depression). (3) Other neurodevelopmental disorder, genetic metabolic disease, or severe neurological disease. (4) Participants who could not cooperate with the eye movement experiment.

The study was conducted under the Code of Ethics of the World Medical Association (Declaration of Helsinki). Also, the study complied with all relevant national regulations and institutional policies and had been approved by the Medical Ethics Committee of Tianjin Medical University. Participants and their parents (or legal guardians) obtained all information about the research, including the purpose, requirements, responsibilities, compensation, risks, benefits, and alternatives. All questions were answered before asking for the consent signature.

TMS Procedure

A trained electrophysiologist delivered rTMS stimulation over the cortical area controlling the contralateral First Dorsal Interosseous (FDI) using a Magnetic Field Stimulator (CCY-1, YIRUIDE Medical Corporation, Wuhan, China) to detect resting motor threshold (MT). The MT was determined for each hemisphere in all individuals by gradually increasing the output of the machine by 5% until a 5 mV deflection or a visible twitch in the FDI muscle was identified in 2out of 3 trials (31). Electromyographic

(EMG) responses were monitored continuously from the hand contralateral to the stimulated hemisphere using the MEP module in Magnetic Stimulator (YIRUIDE Medical Corporation, Wuhan, China). Subjects were familiarized with the laboratory and procedure before the first TMS session.

In this study, rTMS was selected to stimulate left DLPFC with high frequency (10 Hz) and right DLPFC with low frequency (1 Hz) based on the evidence-based basis proposed by the European Union of Neurological Societies (32), and the electrode positioning cap was used for accurate positioning. Specific parameters are as follows: stimulation frequency of right dorsolateral prefrontal lobe is 1Hz, stimulation time is 32s, stimulation number is 32, intermittent time is 1s, repetition number is 28, the stimulation intensity is 25% MT; stimulation frequency of left dorsolateral prefrontal lobe is 10Hz, stimulation time is 3.2s, stimulation number is 32. Intermittent time is 10s, repetition number is 45. Stimulation intensity is 25% MT. The intervention time of rTMS was 5 times/week, and every 4 weeks was a course of intervention.

Eye Tracking Procedure

The stimuli were selected from the Chinese Affective Picture System (33) and consisted of 48 different pictures (48 emotional pictures and 48 neutral pictures). Each picture included two black-and-white photographs of the same person with varied emotional valence (positive/ negative + neutral), and the two photographs were equal in size and symmetrical in position. When appearing together, the two photographs were located approximately 5° of visual angle away from each other. The size of every picture was 720×480 pixels, subtending a visual angle of 13.78°in height by 7°in width. There were three factors in this study, including the gender of faces (male, female), the left or right visual field where the emotional pictures presented (LVF, RVF), and the picture valence (positive, negative). There was one block for each condition and 6 trials in each block. Thus, 48 trials were included in total during the experiment. Examples of face stimuli are presented in Fig. 1.

We used a Tobii TX300 eye tracker and the Tobii Studio software to present the stimuli, record eye movements, and analyze the gazing behavior of the participants. The fixation was the defined as continuous gazing for more than 80 ms within a 1 degree of visual angle or 30 pixels. The experiment took place in a controlled environment (illumination, temperature, etc.) in the development laboratory of the Department of Maternal, Child & Adolescent Health at the Tianjin Medical University.

Participants were instructed to look at the pictures on the monitor in a relaxed way. After completing 9-point calibration, the test started with instruction text displayed on the screen explaining the procedure in detail. The pictures were presented in randomized order for duration of 5 s at a sampling rate of 120 Hz by using Tobii Studio 3.0 Eye Tracking Software. Between two trials, an image of a cartoon penguin over a white background was presented at the center of the screen for 1 s. While viewing the picture, the subject was not required to give a response. For younger or uncooperative subjects, the caregiver was allowed to accompany the participant, but not to see the screen. A flow chart of the experiment is presented in Fig. 1.

insert Fig. 1

We used the eyes of the left and right faces as the Area of Interest (AOI). The eye movement parameters analyzed in this study included: fixation count (FC), refers to the number of times the participant fixated on an AOI; total fixation duration (TFD): the sum of the duration of the subject's fixation in the AOI. In order to show the eye preference, we calculated the percentage of eyes fixation, i.e., TFD of eyes in one certain face was divided by the TFD of that whole face to derive the proportion of time spent on eyes (i.e., "% eyes").

Clinical Assessments

We evaluated the symptoms of ASD with CARS. The CARS consists of 14 domains assessing behaviors associated with autism, with a 15th domain rating general impressions of autism. Each field has a scale of one to four. Higher scores indicate a higher level of impairment. Total scores can range from 15 to 60. Scores below 30 mean that the individual is in the non-autistic range, a score between 30 and 36.5 indicates mild to moderate autism, and scores between 37 and 60 indicate severe autism (34).

Statistical analysis

We used EpiData to build the database and SPSS 22.0 to make statistical analysis. We used the repeated Measures Analysis of Variance (RMANOVA) to compare the effect of rTMS, with FC, TFD and the number of pictures that ASD participants neglected (no fixation on the whole picture) as dependent measure respectively. As to eye preference, and *location* (left visual field vs. right visual field), *gender* (male face vs. female face) and *emotion* (positive vs. negative face) as within-subject factors, and *time* (before vs. One-session rTMS vs. Two-session rTMS) as the between-subjects factors. For the mediation analysis, the model of Bayesian mediation analysis was created with *time* as the independent variable (Time = 0, 1, 2 as pre, post rTMS), the score of CARS, including the total score and scores of the subscales as the mediator respectively, and the FC or TFD as the dependent variable by using the procedure of MCMC of SAS 9.4 (35).

Community Involvement

A total of 45 autistic children were involved in this study. Their parents also provided help for the smooth implementation of this study. The publicity of The China Disabled Persons Federation (CDPF) of Tianjin also helped the smooth implementation of this study.

Results

The demographics of the participants

In the study, 45 ASD participants completed at least two intervention sessions (4 weeks per session) and completed the assessment. Among them, 36 completed two sessions, 8 completed three sessions, and one subject completed four sessions. There were 37 males (77.8%), 8 females (22.2%), with the average age of 8.80±4.171 years; and the average score of CARS was 36.95±6.82. See Table 1.

insert Table 1

The results of fixation on eyes after rTMS

For the fixation on the facial eyes, the FC, and TFD as the independent variables respectively, the Time was statistically significant (F_{FC}=6.147, P = 0.003; F_{TFD}=10.159, P<0.001). Both FC and TFD were significantly improved comparing to the baseline (before rTMS). However, as to the numbers of the pictures that ASD participants gazed, Time was not statistically significant. For further comparisons of different time, only the comparison between baseline and two-session rTMS was statistically significant for FC; but for TFD, the comparisons were statistically significant except the comparison between one-session rTMS and two-session rTMS, see Table 2, Fig. 2.

insert Fig. 2

insert Table 2

The results of the percentage of eyes fixation after rTMS

For FC, the results of RMANOVA of the percentage of eyes fixation showed that the main effect of *time* (F = 0.563, P = 0.571) was not statistically significant, which indicated that rTMS fail to improve the percentage of eyes fixation. None of the interactions of *gender * time* (F = 0.176, P = 0.838), *emotion * time* (F = 0.563, P = 0.571), and *location * time* (F = 1.005, P = 0.369) was statistically significant. Similarly, for TFD, the main effect of *time* was not statistically significant either, F = 0.022, P = 0.978, neither did the interactions (*gender * time*: F = 0.029, P = 0.997; *emotion * time*: F = 1.301, P = 0.277; *location * time*: F = 0.850, P = 0.430).

The improvement of CARS by rTMS

The total score of CARS showed constant improvement by rTMS, from 36.95 ± 6.82 (baseline) to 33.178 ± 5.921 (after one session), then to 29.756 ± 5.974 (after two sessions). By one-way ANOVA, the change of CARS score was statistically significant (F = 21.203, P<0.001), the further comparisons showed that the improvement of different time was all statistically significant, T_0 vs. T_1: t = 3.765, P = 0.001; T_1 vs. T_2: t = 2.729, P = 0.023; T_0 vs. T_2: t = 6.463, P<0.001.

The Results Of Mediation Analysis

First, we performed a Bayesian mediation analysis with a total score of CARS mediating the relationship between rTMS intervention and the change of TFD (the score of TFD after two sessions minus the baseline). However, the 95% central credibility interval was [-0.731, 0.276], which contained 0 and meant the mediated effect is not statistically significant. Then the score of each item (such as Relating to People, Imitation, Emotional Response, Body Use, Object Use, Adaptation to Change, Visual Response, Listening Response, Taste, Smell, and Touch Response and Use, Fear or Nervousness, Verbal Communication, Nonverbal Communication, Activity Level, Level and Consistency of Intellectual Response and General Impressions) was taken as the mediator respectively, and only Item 6 (Adaptation to Change) was the mediator, the rest was rejected due to the poor convergence of the Markov chain or the containment of 0 in the 95% central credibility interval, which meant the mediated effect is not statistically significant. As shown in Fig. 3, the trace plots indicated the good mixing for parameters, and chains that mix well tend to converge sooner, as well as the kernel density plots of the posterior distribution for the given parameter. The posterior mean of the mediated effect of rTMS intervention through the score of *Adaptation to Change* on change in the TFD was $\alpha\beta$ =-1.735 ± 0.515 with a 95% central credibility interval [-2.950, -1.231]. Given that zero was not between the two credibility limits, the mediated effect of SSP was statistically significant. Further calculation ($\alpha\beta/c\times100\%$) reflected the mediation effect was 83.24%, meaning that 83.24% of the total effect between rTMS intervention and change in the TFD was mediated by the score of *Adaptation to Change* (see Table 3). This indicated that rTMS intervention improved the fixation on eyes mainly by promoting their adaptation to environmental change.

insert Fig. 3

insert Table 3

Discussion

We mainly examined the efficacy of rTMS protocol (high frequency on left + low frequency on right) on bilateral DLPFC for both the clinical symptoms (score of CARS) and facial fixation in ASD participants. In the current study, we found that after rTMS intervention, CARS scores significantly decreased, and the decline of CARS score correlated with the extension of the treating sessions, showing a significant dose-response relationship, which was consistent with Casanova's review (7). The possible therapeutic mechanism of rTMS is related to the improvement of abnormal brain wave activity patterns in the gamma bandwidth in ASD patients (10, 36). Especially, low frequency rTMS over the DLPFC has been proven to normalize gamma oscillation abnormalities (14, 36), executive functions (37–39), and repetitive behaviors (38, 40) in ASD individuals. Also, our results showed that the ASD participants fixated more on the eyes of the human faces after two sessions of rTMS, including FC and TFD. But we failed to find similar results in ASD participants, except one study of rTMS intervention on gazing behaviors in healthy population. Saitovitch et al. (41) once reported that TMS could influence the behavior of orienting toward the eyes in normal participants and confirmed the potential value of this discovery in the treatment of autism. The possibility of rTMS intervention for the improvement of gazing behaviors of

ASD individuals is related to cortical excitation/inhibition (e/I) imbalances and abnormal excitatory-inhibitory ratio in ASD patients. The high-frequency rTMS on left DLPFC and low-frequency rTMS on right DLPFC in the current study worked for ASD symptoms may also result from the cortical excitation/inhibition (E/I) imbalances and abnormal excitatory-inhibitory ratio in ASD patients (42, 43). The high frequency rTMS stimulation of the left DLPFC could cause long-term potentiation (LTP) of synaptic transmission in the stimulation area (44), and LTP could spread to the cortex and sub-cortical neural network (45, 46), which leaded to the enhancement of excitability of mirror neuron system (MNS) system in ASD patients, so as to improve the understanding of social environment in ASD patients, enhance the ability of imitation (47), thus it finally improved their eye-gazing behaviors. Meanwhile, the low frequency rTMS of the right DLPFC could activate inhibitory GABAergic double bouquet interneurons to improve the excitation / inhibition balance of prefrontal cortex in patients with ASD (48).

Although our results showed that rTMS intervention could improve the eye-gazing behavior of ASD subjects, they did not show the eye gaze preference of healthy people. The lack of eye-gaze preference indicates the insufficiency of using eye information in face processing, which is also one of the characteristics of autistic subjects (49, 50). These abnormalities are usually explained as the result of both congenital injury of specialized nervous systems and the secondary result of reduced social interest (51). Face processing is an emergent and developmental skill that is greatly influenced by early experience with faces (52–54). ASD individuals may possess central nervous system irregularities that fail to attribute special status to faces (55), which attenuates the visual input required for the development of neural regions specialized for face processing (51, 56). Even if rTMS treatment could improve the central nervous system abnormalities and the decreased social interest of autistic individuals, it is unlikely to make up for their lack of early face processing experience in a limited period. Therefore, it is necessary to provide other measures, such as social skills training, to promote their social impairments besides rTMS intervention. Meanwhile, our results suggest the value of early application of rTMS intervention in autistic population, especially in the critical period of their social development, so that their social development can be corrected as early as possible, and obtain as many social skills as possible. Further, the results of mediation analysis showed that the improvement of adaptability of ASD children to environmental changes played a critical role in the increment of fixation on the eyes. However, we failed to find any literature on this issue. As we know, changes, especially unexpected changes, can be extremely stressful for children with ASD (57). When change occurs, children with ASD may feel anxious and respond in a variety of ways, including exhibiting withdrawal, repetitive behaviors, tantrums, or even aggression (58). And the rTMS protocol (left high-frequency and right low-frequency on bilateral DLPFC) in our study has been proved effective in treating anxiety (59, 60). Thus, we speculate that relieving anxiety will play a role in increasing the eye-gaze behavior of autistic individuals, because anxiety symptoms are associated with eye gaze avoidance (61, 62). We will further explore whether the anxiety symptoms play a role in the visual avoidance of autism in the future, by using the anxiety scale (such as the self-rating anxiety scale) and neurophysiological indicators (such as pupillary response, heartbeat, skin resistance, etc.) among high-function autism patients, or we can study the eye-gaze behavior of autism patients through the anti-anxiety medications. Our results have deepened the understanding of

the function of rTMS in treating the social disorders of the autism population and provided a reference for combined treatment.

There are some limitations in our study, for example, the utilization of the static facial images with the relatively low ecological validity, and the sample size in the current study was relatively small. Only one rTMS protocol was used without others (such as different frequency on bilateral DLPFC or other stimulation locations) due to the limitation of time and research funds.

Declarations

Availability of data and materials

The datasets presented in this article are not readily available because their containing information in the data probably compromise the privacy of research participants. Requests to access the datasets should be directed to Lei Gao,gaolei98@tmu.edu.cn

Ethics approval and consent to participate

The study had been approved by the Medical Ethics Committee of Tianjin Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Consent for publication

Informed consent for publication was obtained from all participants.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Authors' contributions

LT, SM, and LG contributed to the conception and design of the study and wrote the first draft of the manuscript. LT and SM contributed equally to this work and share first authorship. LT, SM, YL, M-fZ, CX, CW and XZ contributed to the acquisition, analysis, or interpretation of data. YL is the second author. M-fZ is the third author. CX is the fourth author. CW is the fifth author. XZ is the sixth author. LG performed the statistical analysis. LG and ZX obtained funding. All authors contributed to the article and approved the submitted version.

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Acknowledgments

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Tables

Table 1 The demographics of the ASD participants (n=45)

	n(%)	
gender		
	male	37 77.8%
	female	8 22.2%
age		
	2~	12 26.7%
	6~	25 55.5%
	12~18	8 17.8%
CARS		
	30~36	29 64.4%
	≥36	16 35.6%

Table 2 The comparisons of FC and TFD among different time

Parameters	(I) Time	(J) Time	Δ (I-J)	S.E	P	95% CI	
						lower	upper
FC	T_0	T_1	-4.244	2.091	0.131	-9.339	0.851
		T_2	-7.667	2.358	0.005	-13.407	-1.926
	T_1	T_0	4.244	2.091	0.131	-0.851	9.339
		T_2	-3.422	2.113	0.293	-8.573	1.729
	T_2	T_0	7.667	2.358	0.005	1.926	13.407
		T_1	3.422	2.113	0.293	-1.729	8.573
TFD	T_0	T_1	-1.941	0.753	0.037	-3.790	-0.093
		T_2	-4.576	1.041	0.001	-7.145	-2.008
	T_1	T_0	1.941	0.753	0.037	0.093	3.790
		T_2	-2.635	1.210	0.094	-5.588	0.318
	T_2	T_0	4.576	1.041	0.001	2.008	7.145
		T_1	2.635	1.210	0.094	-0.318	5.588

Note: FC=fixation count; TFD=Total fixation duration; T_0=before rTMS intervention; T_1=after one-session rTMS intervention; T_2=after two-session rTMS intervention.

Table 3 Parameter summary of Bayesian mediation analysis with SSP as mediator

Parameter	N	Mean	Stand deviation	95% C I of HPD lower	upper
α	50000	-0.616	0.103	-0.841	-0.458
β	50000	3.066	0.422	2.359	3.635
c	50000	2.295	0.352	1.659	3.436
h	50000	-0.769	0.108	-0.976	-0.566
αβ	50000	-1.735	0.595	-2.950	-1.231

Note: "c" represents the total effect of independent variable (X) on the dependent variable (Y), "αβ" represents the effect of X on Y adjusted for the effect of the mediator M, "β" measures the relation between the mediator M and the dependent variable Y adjusted for the independent variable X, and "α" measures the relation between X and M.
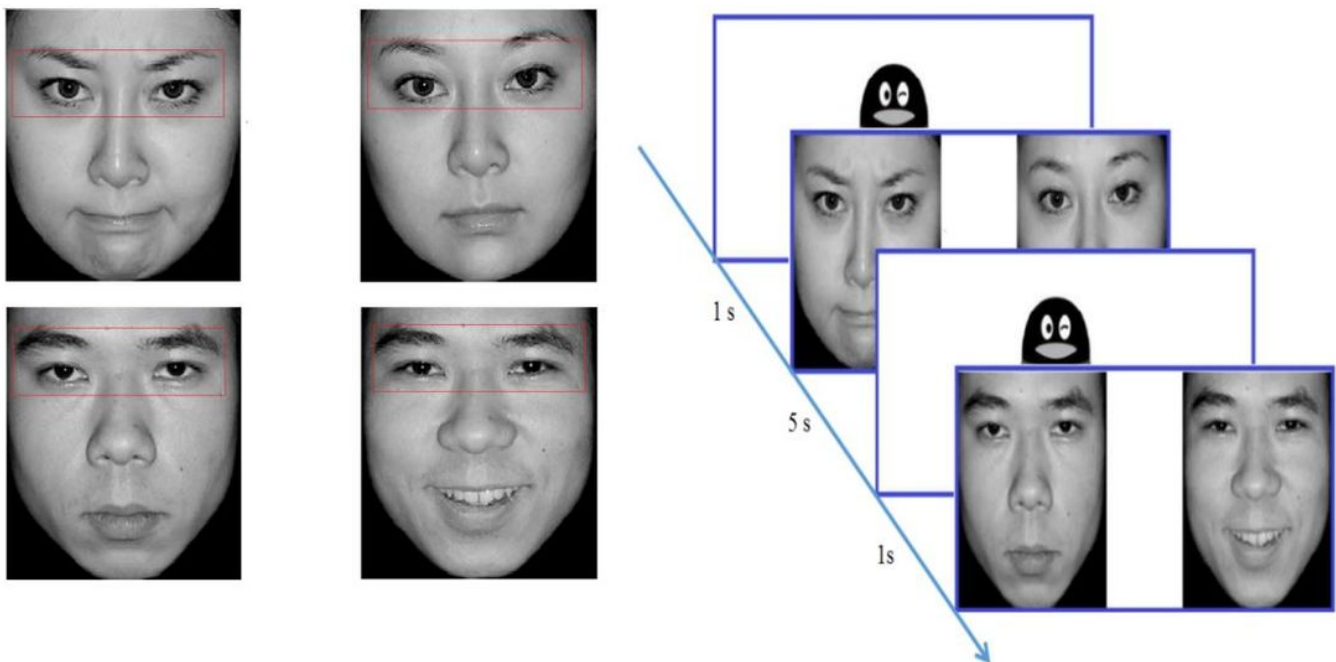
Figures

Figure 1

The examples, definitions of AOI & flowchart in the visual preference experiments
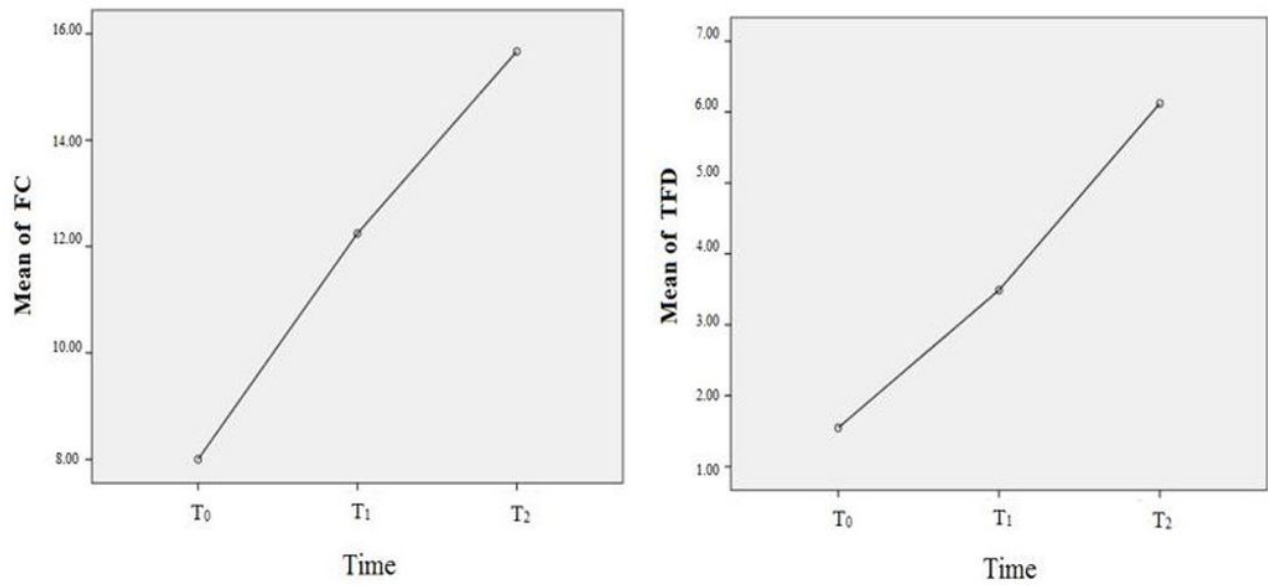

Figure 2

The improvement of fixation on eyes before, after rTMS

Note: T_0=before rTMS intervention; T_1=after one-session rTMS intervention; T_2=after two-session rTMS intervention.
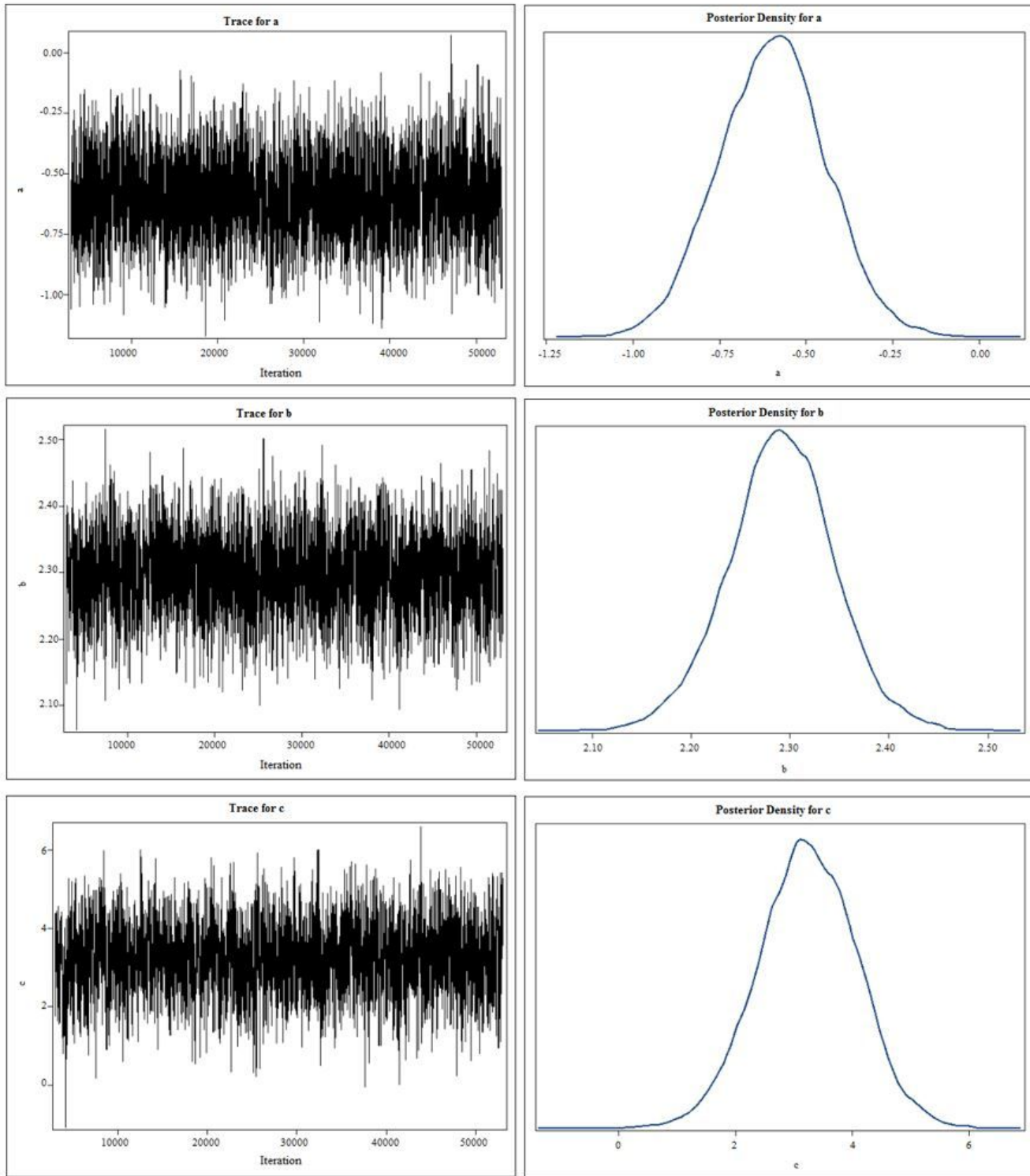
Figure 3

The trace plots & kernel density plots of the posterior distribution for the parameters

Note: "c" represents the total effect of independent variable (X) on the dependent variable (Y), "b" measures the relation between the mediator M and the dependent variable Y adjusted for the independent variable X, and "a" measures the relation between X and M.