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Individualized rTMS Treatment for Depression Using an fMRI-based Targeting Method

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TITLE:

Individualized rTMS Treatment for Depression Using an fMRI-based Targeting Method

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SUMMARY:

The present protocol describes the application of repetitive transcranial magnetic stimulation (rTMS), where a subregion of the dorsolateral prefrontal cortex (DLPFC) with the strongest functional anticorrelation with the subgenual anterior cingulate cortex (sgACC) was located as the stimulation target under the assistance of a fMRI-based neuronavigation system.

ABSTRACT:

To achieve greater clinical efficacy, a revolution in treatment for major depressive disorder (MDD) is highly anticipated. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive and safe neuromodulation technique that immediately changes brain activity. Despite its wide application in the treatment for MDD, the treatment response remains different among individuals, which may be attributable to the inaccurate positioning of the stimulation target. Our study aims to examine whether the functional magnetic resonance imaging (fMRI)-assisted positioning improves the efficacy of rTMS in treating depression. We intend to identify and stimulate the subregion of dorsolateral prefrontal cortex (DLPFC) in MDD with strongest anti-correlation with the subgenual anterior cingulate cortex (sgACC), and to conduct a comparative investigation of this novel method and the traditional 5-cm rule. To achieve more precise stimulation, both methods were applied under the guidance of neuronavigation system. We expected that the TMS treatment with individualized positioning based on resting state functional connectivity may show better clinical efficacy than the 5-cm method.

INTRODUCTION:

Major depressive disorder (MDD) is characterized by significant and persistent depression, and in more severe cases, patients can encounter hallucinations and/or delusions[1,2]. Compared with the general population, the risk of suicide among MDD patients is approximately 20 times higher[3].

While medication is currently the most used treatment for MDD, 30% - 50% of the patients lack adequate response to antidepressants[4]. For the responders, the symptom improvement tends to appear after a relatively long latent period and is accompanied by side effects. Psychotherapy, although effective for some patients, is costly and time-consuming. A safer and more effective treatment for MDD is therefore urgently required.

Repetitive transcranial magnetic stimulation (rTMS)is a non-invasive and safe technique and has been approved for the treatment of various mental disorders[5-7]. Although its therapeutic mechanism remains unclear, rTMS was speculated to work by regulating the activity of the stimulated brain regions and the neural plasticity[8-10], thus normalizing specific functional networks[10-12]. rTMS also causes network effect, which evokes changes in remote brain areas through connection pathways, leading to an amplified therapeutic effect[13]. Although rTMS changes brain activity immediately and robustly, its response rate in the treatment of MDD is only about 18%[14]. The main reason may be the inaccurate location of stimulation targets[15].

The subgenual anterior cingulate cortex (sgACC) is mainly responsible for emotional processing and plays a role in regulating the response to stressful events, emotional response to internal and external stimuli, and emotional expression[16-18]. This subregion of ACC shares substantial structural and functional connectivity with the cerebral cortex and the limbic system[19,20]. Interestingly, studies have shown that the post-stimulation activity of this area is closely related to the clinical efficacy of TMS. For instance, the blood flow of sgACC decreased after a course of TMS targeted on the right dorsolateral prefrontal cortex (DLPFC), which was associated with the alleviation of depressive symptoms[21]. Vink et al.[8] found that stimulation targeted on DLPFC was propagated to sgACC, and suggested that sgACC activity can be a biomarker of the treatment response of TMS. According to previous researches, Fox and colleagues[22] proposed that targeting on a subregion of DLPFC that shows strongest functional anti-connectivity with sgACC (MNI coordinate: 6, 16, -10) enhances the antidepressant effect. Here, we demonstrate a study protocol aimed to examine this hypothesis.

PROTOCOL:

Inform all participants about the study and ask them to sign the informed consent form prior to the start of the study. The present study was approved by the Research Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University.

NOTE: In this double-blind study, patients with depression were randomly divided into two groups. In the experimental group, stimulation targets were located by the DLPFC-sgACC-based individualized location method (Please see 3.3 for detailed description). The targets of the control group were obtained by the average 5-cm method (i.e. (-41, 16, 54))[22].

1. Participants' selection

1.1. Recruit patients with a diagnosis of MDD as confirmed by an expert psychiatrist.

NOTE: Confirm the diagnosis with the standardized MINI-International Neuropsychiatric

Interview (M.I.N.I.)[23] . The total score of Montgomery-Asberg Depression Rating Scale (MADRS)[24] should be no less than 22.

1.2. Exclude patients who meet the exclusion criteria: (1) serious physical diseases such as malignant tumor, acute heart failure, multiple organ failure, or severe neurological conditions including but not limited to epilepsy, stroke, encephalitis, brain trauma; (2) comorbidity of other mental illness, or a history of substance use disorder; (3) having metallic implants, especially in brain or heart; (4) women during pregnancy or lactation; (5) had suicidal behaviors or attempted suicide in the past six months; and (6) a diagnosis of bipolar depression or psychotic depression.

NOTE: Recruit at least 36 subjects for each group to ensure statistical power. It is recommended that there is no significant difference in sex and age between the two groups.

2. Preparation of Magnetic Resonance Imaging (MRI) and TMS

2.1. Obtain fMRI images by a 3T MRI scanner before performing TMS.

2.1.1. Reconfirm that the patient has no contraindications before an MRI scanning. Instruct the patient to try to lie still and think of nothing during the scan.

2.1.2. Conduct a resting-state fMRI (rs-fMRI) scan using the FE-EPI sequence with the following parameters: TR/TE = 2000/30 ms, FA = 90°, field-of-view = 220 x 220 x 150 mm^3, matrix = 64 x 64, voxel size = 3.44 x 3.44 x 4 mm^3, gap = 0.6 mm, number of signal averages = 1, volumes = 240, number of slices = 33, scanning time = 8m 43s.

2.1.3. Conduct a structural MRI scan using the sagittal T1 weighted 3D turbo field echo (T1W 3D TFE) sequence with the following parameters: field of view = 256 x 256 mm^2, TR/TE = 8.2/3.8 ms, view matrix= 256 x 256, slice thickness= 1 mm.

2.2. Set TMS parameters.

NOTE: The protocol of TMS in our study is the intermittent theta-burst stimulation (iTBS). A daily treatment session includes 60 cycles of 10 bursts of 3 pulses at 50 Hz delivered at 100% RMT in 2-s trains, with an interval of 8 s. The whole treatment consisted of 10 sessions carried out on weekdays of two consecutive weeks.

3. Treatment (Figure 1)

3.1. Conduct MRI scans and clinical assessments of symptoms and cognitive performance one day before the treatment.

3.2. Assign the patient randomly to one of the two groups, after scanning.

3.3. For the experimental group, identify the subregion of DLPFC that shows strongest

functional anti-connectivity with sgACC. For the control group, simply locate the target in the standard-space using the average 5-cm method, then convert it to the individual space coordinates.

3.3.1. rs-fMRI data preprocessing

3.3.1.1. Preprocess the rs-fMRI data using an MRI analysis software: (a) remove the first 10 volumes; (b) correct the slice timing correction; (c) correct the head motion; (d) co-register EPI images to T1 images; (e) perform segmentation; (f) perform normalization using T1 images; (g) smooth with a 6-mm Gaussian kernel of full-width half maximum (FWHM); (h) band-pass filter (0.009 – 0.08 Hz); and (i) perform nuisance regression (head motion effects, linear trends, white matter, cerebrospinal fluid, and global mean time course).

3.3.2. Functional connectivity (FC) of the sgACC

3.3.2.1. Select the sgACC (MNI coordinate: 6, 16, −10; Fox et al.) as the region of interest (ROI)[25] with a 10-mm radius.

3.3.2.2. Remove the white matter and cerebrospinal fluid in the ROI based on the Harvard-Oxford cortical atlas (http://www.cma.mgh.harvard.edu/), using a gray matter probability threshold of 0.25.

3.3.2.3. Extract the average time course of the ROI.

3.3.2.4. To generate FC map, compute Pearson's correlation coefficients between the ROI (sgACC) and DLPFC in a voxel-wise manner. Normalize each correlation coefficient using the Fisher's r-to-z Transformation.

NOTE: The DLPFC mask is a combination of 20mm radius spheres centered along the left hemisphere at BA9 (x=-36, y=39, z=43), BA46 (x=-44, y=40, y=29), the 5-cm approach site (x=-41, y=16, z=54), and the F3 Beam group-average stimulation site (x=-39, y=26, z=49)[26].

3.3.2.5. According to the FC map, identify the peak coordinate in DLPFC that has the largest Pearson’s anti-correlation coefficient with sgACC. This is the subregion of DLPFC with the strongest negative FC with sgACC, which will be later targeted in the TMS treatment for the experimental group.

3.4. Determine the resting motor threshold (RMT) for each subject and record the hotspot.

3.4.1. Instruct the patient to sit back and relax, then put two recording electrodes on the thenar of the right hand and a reference electrode on the bony part of the wrist.

3.4.2. Stimulate the motor hotspot with 10 consecutive stimulations with different intensities; in the meanwhile, record the times of thenar muscle contraction.

3.4.3. Identify the minimum TMS intensity at which a motor evoked potential (MEP) ≥ 50 μV is recorded at least 5 times. Define it as the patient's RMT.

3.5. Assess the severity of depression using clinical scales as described in *Clinical Data Collection*.

3.6. Perform TMS treatment twice a day for 10 days.

NOTE: For a subject who did not receive treatments as planned, perform additional stimulations after the end of treatment course as needed. However, any subject who missed the treatment for more than four consecutive days should be excluded.

3.6.1. Create a new patient entry.

3.6.1.1. Select the option **Create New Patient**. Input the patient's ID number or name in the textbox.

3.6.2. Overlay the structural MRI images onto the navigation system.

3.6.2.1. Select **Import patient MRI**, and then import the structural image of the patient and select the image type.

3.6.3. Create individual head model and define the stimulation target.

3.6.3.1. Press the button **Specify MRI Fiducials**.

3.6.3.2. Place the crosshair on these spots in the MRI image: (1) Fiducial Markers: nasion, both left and right tragi; (2) AC-PC Markers for Talairach: anterior commissure, posterior commissure, inter-hemispheric point; (3) Talairach Markers: anterior point, posterior point, superior point, inferior point, left point, and right point.

NOTE: The "Talairach Markers" mark the borders of the brain.

3.6.3.3. Press **Create Head Model**. Select **Manual Brain Segmentation** and adjust the threshold of the scalp, the lower brain, and the upper brain.

3.6.3.4. Click **Define Target** to proceed.

3.6.3.5. Select **Target Marker** page. Click **…** to input the coordinate of the treatment target as identified in step 3.3, and then press **Go to**. Press **Add Marker** to name the point.

NOTE: The coordinates of the control group were (-41, 16, 54).

3.6.4. Coil calibration

3.6.4.1. Click **Proceed to Neuronavigation**. In the textbox, select the right type of tools to be used in the treatment. Ensure all the tools of reference are in the view of the infrared camera.

3.6.4.2. Press **Validate Coil**. Put the tip of pointer on the marked coil point. Press Validate (or the green button on the remote control) when the indicator of each tool turns green.

3.6.5. Select patient and target.

3.6.5.1. Select patient's name or ID on the **Select Patient** page. Click **Select Targets** on the next page.

3.6.5.2. Choose **Read target markers** to browse the file of targets. Import the file and select the target as in step 3.6.3.5.

3.6.6. Define the coordinate system.

3.6.6.1. Click **Define Coordinate System**. Put a headband with a reference tool on the patient. Make sure the coil tracker and the reference tool are in the vision of the navigation system.

3.6.6.2. Place pointer's tip on the nasion and both tragi in turn. Press the green button on the remote control each time when the indicator of a marker turns green.

3.6.7. Head shape generation

3.6.7.1. Continuously move the pointer's tip over the top of the head. Press the button on the remote control (or **Fit**) to continue.

NOTE: One can press **Pause** to stop the process, and resume by pressing the **Start** button again, once the pointer has been placed correctly.

3.6.8. Neuronavigation and stimulation

3.6.8.1. Press **Neuronavigation**. On the **Active Coil** page, set the stimulation intensity to 100% RMT. Choose **Stimulate at targets** to see the target on the head model online.

3.6.8.2. When the coil matches the target crosshair, that is, when the indicator text turns green, stimulate.

3.6.9. Prepare and start the treatment from step 3.6.4 directly if the patient's entry had been created before.

3.7. Conduct follow-up assessments on Day 1, Day 28, and Day 56 after the whole treatment course.

4. Clinic data collection (Figure 1b)

4.1. Perform clinical assessments using MADRS[24], Hamilton Depression Rating Scale (HAMD)[27], Beck Depression Inventory–II (BDI-II)[28], Hamilton Anxiety Scale (HAMA)[29], Clinical Global Impression (CGI)[30] and MATRICS Consensus Cognitive Battery (MCCB)[31,32].

NOTE: MINI and MADRS are used for the screening. All the above scales are applied for pre- and post-treatment clinical assessment.

REPRESENTATIVE RESULTS:

ROI-wise FC analysis should show that sgACC is significantly anti-correlated with DLPFC, in which the strongest negative correlation is the stimulus target to be chosen. Significant anti-correlation between the sgACC-DLPFC functional connectivity and the treatment response should be found in the correlation analysis[33].

The current protocol is based on an innovative TMS targeting method that no previous studies have applied. Here we present results from an fMRI-guided TMS trial that applied the traditional 5-cm method. This study[34] proposed a new treatment protocol, the Stanford Accelerated Intelligent Neuromodulation Therapy (SAINT), a high-dose iTBS regimen with fMRI-guided targeting. The response rate (a MADRS score was 50% lower from the baseline) among 23 MDD patients was 90.48%. 19 of 22 participants (86.4%) met the remission criteria in the intent-to-treat analysis[34]. Two participants dropped out due to therapeutic intolerances and high motor threshold. **Table 1** presents the scores of clinical assessments post-TMS treatment. Therefore, we conjecture that the TMS treatment base on the FC can produce remarkable effectiveness.

FIGURE AND TABLE LEGENDS:

Figure 1. Treatment diagram. (**a**) Process of acquiring stimulation targets and the treatment. See 3.3 for the detailed description on obtaining the target coordinates for the experiment group. The target coordinate for the control group is defined as (-41, 16, 54). (**b**) Time points of MRI scan and clinical evaluation. Clinical data were collected on the screening, the baseline (i.e., before treatment), as well as Day 1, Day 28, and Day 56 after the treatment. The MRI scan was only performed on the baseline.

*Evaluate patients with M.I.N.I.[23] and MADRS[24].

**Evaluate patients with all the scales mentioned in Step 4.

Table 1. Clinical assessment scores immediately after and 1 month after the Stanford Accelerated Intelligent Neuromodulation Therapy (SAINT) for treatment-resistant depression[a] [34]

[a] Treatment response was defined as a reduction on score by ≥ 50%; remission was defined as a score of < 8 for the 17-item HAM-D, < 5 for the 6-item HAM-D, < 11 for the MADRS, < 13 for the BDI-II, and zero for the C-SSRS.

[b] Suicidal Ideation subscale.

DISCUSSION:

The sgACC is responsible for emotional processing and plays an important role in stress regulation[16-18]. A study suggests that targeting on a subregion of DLPFC that shows strongest functional anti-connectivity with sgACC (6, 16, -10) may enhance the antidepressant effect[25]. Therefore, precisely locating this target is the critical step of this protocol. Before the stimulation, the borders of the brain should be accurately marked out with the assistance of neuronavigation, and the head should be carefully registered to ensure the accuracy of a head model. Also, note that the 5-cm rule generally stimulates very posterior regions of the frontal brain, while our sgACC-DLPFC targeting protocol usually leads to a very anterior region[35,36]. Thus, the differential clinical efficacy among targeting methods may be associated with the orientation. Our method should be carefully evaluated by comparison with other approaches that define the stimulate target based on other functional connectivities.

Our protocol has some limitations. First of all, sgACC is located near the sphenoidal sinus, which causes severe signal loss due to the non-uniformity of the magnetic fields[37]. Besides, the accuracy of the neuronavigation largely depends on the quality of MRI images, which may lead to inaccurate stimulation targets. Improvement of the signal-to-noise ratio of MRI or a better replacement for sgACC may help address this problem. Another limitation is the time-consuming procedures that potentially affect patients' compliance for the treatment, since preparation such as establishing a head model takes a long time, not to mention the whole treatment course that lasts for about two weeks.

Despite these limitations, this method has its strength. Despite the fact that the 5-cm rule has been widely applied in clinical settings, it overlooks the individual differences on the anatomical features, which is considered an important reason for the heterogenous efficacy of TMS[38]. The neuronavigation system models the head individually by referring to structural MRI images, thus improving the accuracy of coil positioning. Research has proven that a neuronavigated TMS therapy is more effective than a traditional treatment using the 5-cm targeting method[38], Furthermore, an operator can adjust the coil in real time under the guidance of the system[39,40].

Traditional TMS therapy targets at DLPFC as a whole. In this protocol, the subregion of DLPFC with the strongest negative connectivity with sgACC was selected as the target. Baeken et al.[41] found that sgACC is related to suicidal ideation and hopelessness. Patients with treatment-resistance depression show a stronger FC between sgACC and the right lateral frontotemporal cortex, which may be related to the refractory state of the patient[42]. In addition, strong connectivity between sgACC and DLPFC was found in MDD patients[43], and the negative FC between sgACC and default mode network (DMN) was correlated with the clinical improvement. Therefore, we speculate that the connectivity of sgACC is closely related to the therapeutic effect of TMS, and that stimulating a specific region of DLPFC may change its FC with sgACC, which can improve the effectiveness of the treatment[25,44].

In summary, the present TMS protocol is operated under the MRI-assisted neuronavigation

system and targets the subregion of DLPFC that shows the strongest negative functional connectivity with sgACC. Although no previous studies have applied this targeting method, it may help enhance the accuracy of positioning and possibly improve the treatment response.

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DISCLOSURES:

Authors have no disclosures to report.

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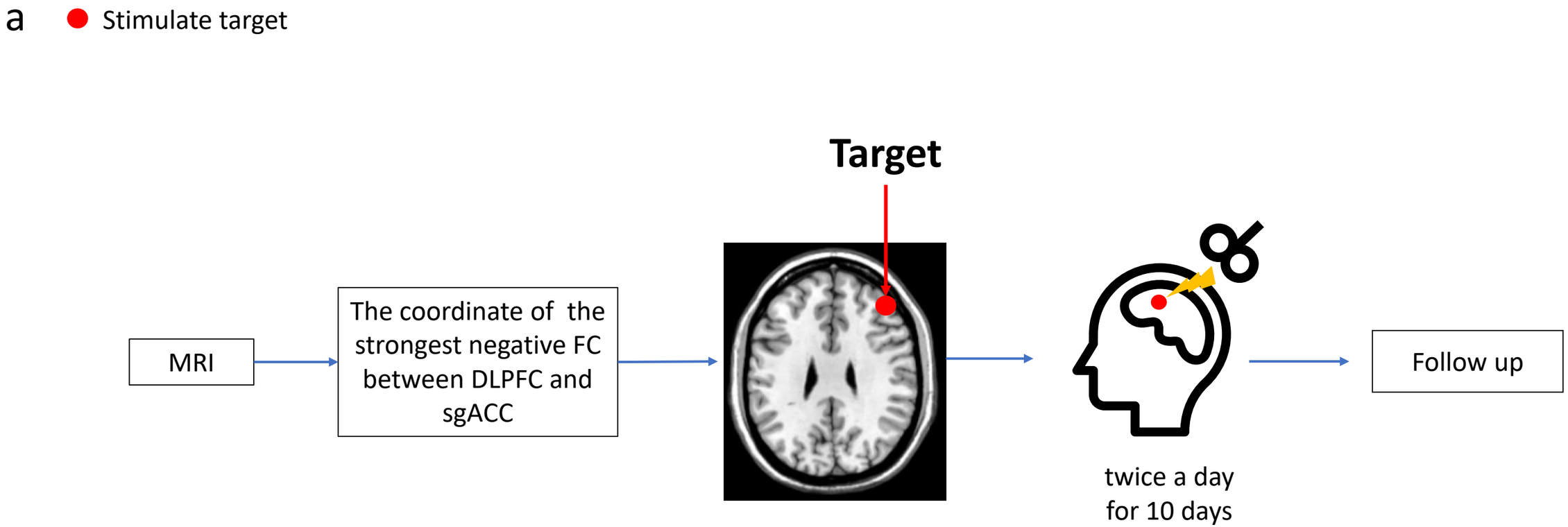
a



b

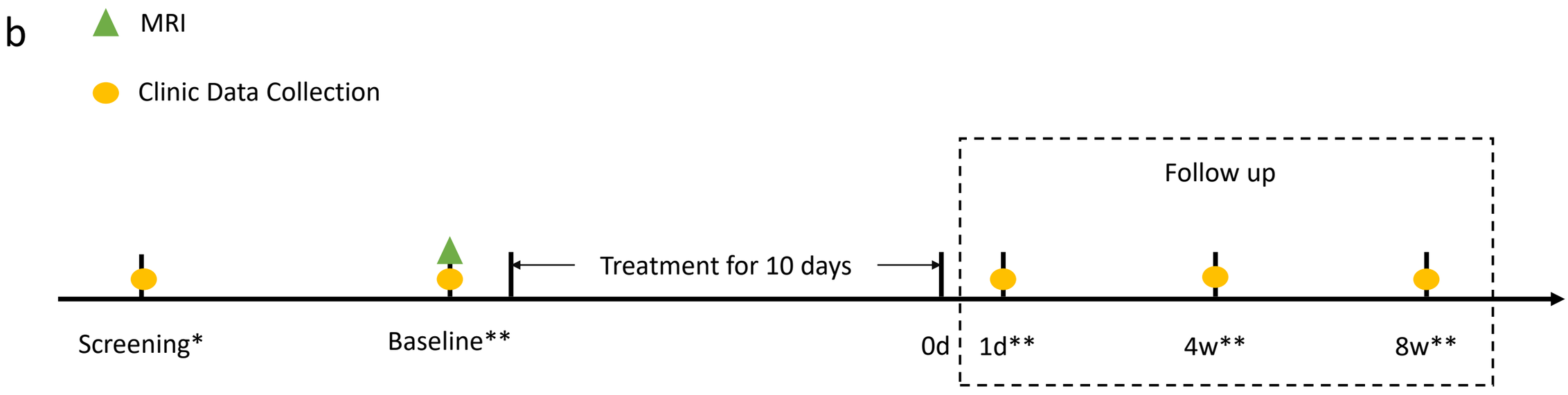


Table1 Click here to access/download;Table;Table1.xlsx

Measure	Mean	SD	N	Post-SAINT Response(%)	N	Remission (%)
MADRS	5	6.37	21	90.48	21	90.48
HAM-D, 17-item	4.29	4.43	21	90.48	21	80.95
HAM-D, 6-item	2.24	3.1	21	85.71	21	85.71
BDI-II (N=18)	4.47	5.76	15	100	12	93.33
Suicidal ideation						
C-SSRS[b]	0	0	18	100	14	100
HAM-D, item 3	0.05	0.22	21	100	19	95.24
MADRS, item 10	0.1	0.44	21	95.24	21	95.24

			One Month Post-SAINT				
N	Mean	SD	N	Response(%)	N	Remission (%)	N
21	10.95	11.76	20	70	20	60	20
21	8.05	8.31	20	75	20	65	20
21	4.4	4.72	20	75	20	70	20
15	12.25	13.06	16	57.14	14	62.5	16
18	0	0	19	100	14	100	19
21	0.1	0.31	20	100	18	90	20
21	0.35	0.75	20	90	20	80	20

Dear Editor and Reviewers,

Thank you for giving us the opportunity to revise our manuscript. The comments have been carefully considered. The responses are as follows.

To Editors:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

RESPONSE: Thanks for your suggestion. We asked one native speaker to polish our manuscript.

2. Please define all abbreviations during the first-time use.

RESPONSE: We have added the definition of abbreviations during the first-time use as follows: repetitive transcranial magnetic stimulation (rTMS), dorsolateral prefrontal cortex (DLPFC), default mode network (DMN), magnetic resonance imaging (MRI), functional connectivity (FC).

3. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”

RESPONSE: We have revised the text to address your concerns and hope that it is now more appropriate.

4. Please revise the following lines to avoid overlap with previously published work: Step 3.3.1, step 3.3.2 and paragraphs after 3.3.2 and before 3.4.

RESPONSE: Thank you for underlining the deficiency, we have modified the text from

step 3.3.1 to 3.3.2 (page 3-4).

5. The Protocol should contain only action items that direct the reader to do something.

RESPONSE: We are sorry for our negligence. We have modified it throughout the text according to the comment.

6. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?

RESPONSE: According to the comment, we have added some descriptions to complete our expression of the protocol. Please see step 2.1.1, step 3.3.2.5, and notes of step 3.3.2.4, step 3.7.3.9, and step 4. The figure 1 and its legend also were modified to clarify how the course performed.

7. Please include a citation for MINI questionnaire.

RESPONSE: We have carefully checked the manuscript and added the citation for the questionnaire in 1.1, 1.2, and 4.

8. Is there any age and sex bias for the participants? How many patients and controls included in the study?

RESPONSE: Thank you for raising these questions. To clarify the issues the comment mentioned, we have added NOTE in the last paragraph of 1 as follows:

“We suggest that recruit at least 36 subjects for each group to ensure statistical power. It is recommended that there is no significant difference in sex and age between the two groups.”

9. Please use complete sentences to describe the actions being discussed in a step.

RESPONSE: Thank you for your suggestion, and we have modified the incomplete

sentences throughout the text.

10. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: Philips Achieva MRI, Statistical Parametric Mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm), etc.

RESPONSE: Thank you for providing the necessary information above. The commercial information was removed from our manuscript and written in the Table of Materials. The template mentioned in 3.3.2.2 (Harvard-Oxford cortical atlas) an open assessment tool, is noncommercial.

11. Please ensure that the protocol section is a cohesive story. After recruitment of the patients what do you do before taking the MRI? Do you check their vitals? Etc.

RESPONSE: Thanks for your comment. According to the comment, we have added a brief description in 2.1.1 as follows:

“Reconfirm that the patient has no contraindications before an MRI scanning. Instruct the patient to try to lie still and think of nothing during the scan”.

12. How is the MRI performed?

RESPONSE: We have added a paragraph in 2.1.1 as follows:

“Reconfirm that the patient has no contraindications before an MRI scanning. Instruct the patient to try to lie still and think of nothing during the scan”.

The parameters used in MRI were written in 2.1.2 and 2.1.3.

13. We cannot have paragraphs of text in the protocol section. All text should be numbered action steps. Each step can have no more than 2-3 action sentences.

RESPONSE: We have modified throughout text according to the comment.

14. 3.3: How do you determine the negative functional connectivity point?

RESPONSE: We have added a descript in 3.3.2.5 as follows:

“According to the FC map, identify the peak coordinate in DLPFC that has the largest Pearson’s anti-correlation coefficient with sgACC. This is the subregion of DLPFC with the strongest negative FC with sgACC, which will be later targeted in the TMS treatment for the experimental group.”

15. 3.3.2: How is this done?

RESPONSE: Thank you for the question. We have added some details in 3.3.2.4 and 3.3.2.5 as follows:

“The DLPFC mask is a combination of 20mm radius spheres centered along the left hemisphere at BA9 (x=-36, y=39, z=43), BA46 (x=-44, y=40, y=29), the 5-cm approach site (x=-41, y=16, z=54), and the F3 Beam group-average stimulation site (x=-39, y=26, z=49)” (Page 4, step 3.3.2.4)

“According to the FC map, identify the peak coordinate in DLPFC that has the largest Pearson’s anti-correlation coefficient with sgACC. This is the subregion of DLPFC with the strongest negative FC with sgACC, which will be later targeted in the TMS treatment for the experimental group.” (Page 4, step 3.3.2.7)

16. 4: Citations for all these? How are these used?

RESPONSE: We feel sorry for our carelessness. We have added the citation for the scales in 4. When they are used has been illustrated in figure 1b and NOTE as follows:

“Time points of MRI scan and clinical evaluation. Clinical data were collected on the screening, the baseline (i.e., before treatment), as well as Day 1, Day 28, and Day 56 after the treatment. The MRI scan was only performed on the baseline.” (Page 7, Legend of Figure 1b)

“MINI and MADRS are used for the screening. All the above scales are applied for pre-

and post-treatment clinical assessment." (Page 6, NOTE in 4)

17. There is a 10-page limit for the Protocol, but there is a 3-page limit for filmable content. Please highlight 3 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

RESPONSE: Thank you for point this out. We have highlighted the essential steps of the protocol. (Step 3.1 – 3.8)

18. Please include a figure or a table in the Representative Results showing the effectiveness of your technique backed up with data.

RESPONSE: Thank you for your suggestion. However, there are no studies that performed TMS in our method so that we have no backup data for our experiment to offer. But the studies we cited in Representative Results show their support for the effectiveness to our technique.

19. Please discuss all figures/Tables in the Representative Results.

RESPONSE: Thank you for your careful reading. We have added a paragraph of page 6 as follows:

" Therefore, we conjecture that the TMS treatment base on the FC can produce remarkable effectiveness." (Line 16, page 6)

20. Please describe the result with respect to your experiment, you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title.

RESPONSE: According to the comment, we have added a paragraph of page 6 as follows:

" The current protocol is based on an innovative TMS targeting method that no previous

studies have applied. Here we present results from an fMRI-guided TMS trial that applied the traditional 5-cm method." (Line 9, page 6)

" Therefore, we conjecture that the TMS treatment base on the FC can produce remarkable effectiveness." (Line 16, page 6)

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RESPONSE: Thank you for the comment. The figures used in the article are original. Only table 1 has been cited from other articles.

22. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols.

RESPONSE: We have added a brief description of our figure on page 7 as follows:

"a. Process of acquiring stimulation targets and the treatment. See 3.3 for the detailed description on obtaining the target coordinates for the experiment group. The target coordinate for the control group is defined as (-41, 16, 54).

b. Time points of MRI scan and clinical evaluation. Clinical data were collected on the screening, the baseline (i.e., before treatment), as well as Day 1, Day 28, and Day 56 after the treatment. The MRI scan was only performed on the baseline.

*Evaluate patients with M.I.N.I.[23] and MADRS[24].

**Evaluate patients with all the scales mentioned in Step 4."

23. As we are a methods journal, please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

RESPONSE: Thank you for your comment. We have ensured the discussion included the detail mentioned above.

a) Critical steps within the protocol:

> "Therefore, precisely locating this target is the critical step of this protocol." (Line 43, page 6)

b) Any modifications and troubleshooting of the technique:

"Thus the differential clinical efficacy among targeting methods may be associated with the orientation. Our method should be carefully evaluated by comparison with other approaches that define the stimulate target based on other functional connectivities". (Line 2, page 7)

c) Any limitations of the technique:

> "Our protocol has some limitations." (Line 8, page7)

d) The significance with respect to existing methods:

> "Despite these limitations, this technique has its strength." (line 17, page 7)

e) Any future applications of the technique:

> "Although no previous studies have applied this targeting method, it may help enhance the accuracy of positioning and possibly improve the treatment response. "(Line 38, page 7)

To Reviewer 1:

1. You claim to have recruited 23 subjects to this experiment in the result part, but in

the following table 1, table 2 and figure legends, there are only 21 subjects written. Would it be possible to explain it?

RESPONSE: Thanks for your careful reading. In this study, we do the experiment instead of ourselves by quoting the experimental results of our predecessors. The previous cohort recruited 23 patients. Two of the patients were excluded during treatment. We have added the explanation of their dropout as follows:

“Two participants dropped out due to therapeutic intolerances and high motor threshold.” (Line 15, page 6)

2. There are some spelling or symbol mistakes, such as the abbreviation for Mini Mental State Examination should be MMSE instead of MINI (in page 3, 1.1);

RESPONSE: We were really sorry for our careless mistake. The Mini Mental State Examination has been modified to the MINI-International Neuropsychiatric Interview (M.I.N.I.) throughout the text.

3. Since you have another table for the scales measured at the timepoint of 1 month post treatment, it might be better to describe your prognosis schedule in the part "clinic data collection".

RESPONSE: We think this is a valuable suggestion. The time point for scales measured has been illustrated in Figure 1 and NOTE in 4.

4. In figure 1, it might be misleading to see the red dot and the word "target" labeled on the sgACC region, for the word target also means the TMS localization, but actually the target region for TMS should be the DLPFC most anticorrelated with sgACC. Would it be possible to replace this figure with a 3-dimention image or illustrate in more details in figure legends?

RESPONSE: Thank you for underline this deficiency. Figure 1 has been replaced the target localization.

To Reviewer 2:

Major Concerns:

While connectivity analyses are easy to follow (basically identical to Fox approach), the authors did not describe how to select the maximally anticorrelated cluster/voxel in the DLPFC. This is a critical point since usually the resulting maps feature more anterior as well as posterior clusters in the DLPFC. Please clarify.

RESPONSE: Thank you for the suggestion. We have added the information in 3.3.2.

Minor Concerns:

1. Please mention the neuronavigation system used.

RESPONSE: Thank you for the comment. The manuscript of JoVE cannot contain commercial information. The neuronavigation system we use is the Visor 2 system which is mentioned in the Table of Materials.

2. One limitation is that the resulting differences in clinical efficacy could be due to the fact that the 5cm rule in principle stimulates very posterior regions of the frontal brain while sgACC anticorrelation targets usually lead to a very anterior region. To evaluate the superiority of individualization one might need to compare this approach to a group-MNI target based on e.g. HCP sgACC anticorrelation. This is beyond this paper but should be mentioned in the limitations.

RESPONSE: Your suggestion really means a lot to us. A brief discussion has been added as follows:

“Also, note that the 5-cm rule generally stimulates very posterior regions of the frontal brain, while our sgACC-DLPFC targeting protocol usually leads to a very anterior region35,36. Thus the differential clinical efficacy among targeting methods may be associated with the orientation. “ (Line 2, page 7)

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PL17995

June 23, 2021

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Eleanor J. Cole, et al.

American Journal of Psychiatry 2020 177:8, 716-726

1. Table 2, page 7

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