Section 1: A Protocol for Robot-Assisted Neuronavigated Transcranial Magnetic Stimulation in Bipolar Disorder

The detailed procedures for robot-assisted neuronavigated transcranial magnetic stimulation (rTMS) targeting individualized target have been previously described by our group. Specifically, the full protocol, including individualized target definition, robot-assisted neuronavigation, stimulation setup, and quality control procedures, has been reported in our previous publication (Zhou H, Zhao X, Du W, Chen Q, Zhang P. A Protocol for Robot-assisted Neuronavigated Transcranial Magnetic Stimulation Targeting Individualized Brain Networks in Bipolar Disorder. J Vis Exp. 2025 Sep 30;(223). doi: 10.3791/68987.).

In the present study, we followed a similar robot-assisted neuronavigated rTMS workflow as previously reported. Nevertheless, both the intervention population and the stimulation targets were modified in accordance with the specific research objectives and hypotheses of this study：

1. Participant selection

In this study, patients diagnosed with bipolar disorder (BD) were recruited from the psychiatric outpatient clinic of the First Affiliated Hospital, Zhejiang University School of Medicine. All participants were thoroughly informed about the purpose, procedures, potential risks, and benefits of the study, and written informed consent was obtained prior to participation. The study protocol was reviewed and approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, in accordance with the Declaration of Helsinki, and it has obtained informed consent from each participant. This study has been registered with the Clinical Trial Registry, with the registration number NCT05929183.

The inclusion criteria included: (i) 14–28 years of age; (ii) diagnosis of BD depressive phase established by two professional psychiatrists, in accordance with the Mini-International Neuropsychiatric Interview (MINI) and DSM-5 diagnostic criteria; (iii) the score of the Young Mania Rating Scale (YMRS)$\leqslant$6; (iv) Montgomery–Åsberg Depression Rating Scale score between 12-30; (v) righthandedness; (vi) education $\geqslant$9 years.

The exclusion criteria included: (i) History of severe somatic or brain organic diseases and craniocerebral trauma; (ii) Abnormal brain structure or any MRI contraindications were found by magnetic resonance examination; (iii) Those who do not cooperate or cannot effectively complete

the experiment; (iv) Drug, alcohol or other psychoactive substance abusers; (v) Pregnant, lactating or planned pregnancy; (vi) Severe suicidal ideation and behavior; (vii) ECT or rTMS treatment was performed within six months.

2. Acquisition of magnetic resonance images

Imaging was performed at the First Affiliated Hospital of Zhejiang University School of Medicine using a 3.0 Telsa scanner with a standard full head coil. Participants were asked to lie on the scanner with their eyes closed. Foam pads were positioned on either side of the head to limit head movement, and earplugs were used to minimize noise. High-resolution 3D anatomical images were captured through T1-weighted magnetization prepared rapid acquisition gradientecho (MPRAGE) sequence. The parameters used were: TR = 7.1 ms; TE = 2.9 ms; FOV=260 × 260mm^2; matrix size = 256 × 256; slices = 146; slice thickness = 1 mm; flip angle = 8°. Functional images were acquired by the echo planar imaging (EPI) sequence, with the following parameters: TR= 1800 ms; TE =30 ms; FOV=240 × 240mm^2, gap= 0.8 mm; matrix size = 64 × 64; slices = 28; slice thickness =4 mm; time points = 180.

3. Measurement of motor threshold

On the day each patient began treatment, the operator first measured each patient's motor threshold to help determine the appropriate rTMS stimulation intensity for that patient.

3.1 Each patient was asked to sit comfortably in the treatment chair. The operator then fit the patient with a sensor-equipped headband, ensuring it was securely fastened to the head and that there were no obstructions between the sensors and the tracking cameras.

3.2 Enter the patient ID into the system, verify the patient information, and click to enter the corresponding interface. The patient's head model will be displayed on the screen, with markers at five locations: the center of the forehead, both ear tips, the tip of the nose, and the top of the head. Use the mouse to adjust the marker points to ensure they are in the correct positions.

3.3 The operator then used a neuro-navigation pen to mark specific anatomical landmarks on the head (including the glabella, bilateral ear tips, nasal tip, and vertex). The marked locations

appeared as green dots on the screen, and once completed, the patient's head was precisely aligned with the uploaded MRI model.

3.4 The neuro-navigation pen was placed at the aforementioned reference points on the patients. When the operator pressed the pedal, green markers were displayed in real time on the screen. Once all reference points had been successfully located, the neuro-navigation pen was moved across the scalp, and its movement trajectory was projected onto the screen as green dots to determine the alignment of the patient's head model with its position in space, thereby completing the registration.

3.5 Professional operators used the robot-assisted navigation system equipped with an octagonal coil to control the device to reach the M1 target position recommended by the system and applied a single pulse stimulation. Among 10 consecutive trials, the minimum stimulation intensity at which the maximum motor evoked potential (MEP) was recorded in at least 5 trials was defined as the maximum resting motor threshold (RMT) for the right first dorsal interosseous muscle (FDI).

4. Robotic neuronavigated rTMS intervention

Each study participant received 15 days of robotic neuronavigated rTMS treatment. participants in the Active group received 15 days of effective rTMS treatment, and those in the Sham group received 15 days of pseudo-rTMS treatment. Each train consisted of 50 pulses (5 seconds duration), with inter-train intervals of 15 seconds, totaling 3,000 pulses per session and 45,000 pulses over the entire 3-week treatment course (15 sessions in total), the total duration of each session 20 minutes. For sham treatments, the coils were directed to the same target, simulating scalp sensation and producing the same sound.

4.1 Entered the MNI coordinates corresponding to the stimulation target point in the patient's V1 brain region.

4.2 The stimulation intensity was 100% of the patient's threshold for active rTMS group, the stimulation frequency was 10Hz, and the number of pulses per session was 3000.

4.3 The angle and height of the seat were adjusted to ensure that the position of the patient's head was in the green box on the screen, and the robot brought the coil to the patient's corresponding target position according to the neuronavigation system. Used robot navigation to adjust the target point of the actual stimulus (shown as a red ball) to coincide with the location on the patient's head model on the screen (shown as a green ball). The distance between the two points would be displayed in the lower left corner of the screen. When the distance was less than 1 mm, it indicated that the two points are basically aligned, and rTMS stimulation cuold begin.

4.4 During the whole treatment process, the distance between the coil and the target point was displayed on the screen, which could be calibrated when the patient's head shifted to ensure that the scalp was always close to the coil.

4.5 During treatment, the patient's condition was also monitored. After each daily treatment session, the patient was asked about and any adverse reactions such as dizziness or headaches were recorded.

Section 2: Sensitivity Analysis on Global Signal Regression

A sensitivity analysis was conducted to evaluate the impact of global signal regression (GSR). The results without GSR are detailed in **Additional file 1: Table 1**. In brief, while the analysis without GSR revealed a significant effect in the right cerebellum that was not previously observed, the key finding of altered connectivity in the ACC remained highly stable. It is noteworthy that the ACC cluster was spatially extensive across both hemispheres in both analyses. The shift of the peak coordinate within this stable cluster highlights the methodological influence of GSR on precise localization, without undermining the core finding of ACC involvement.

Additional file 1: Table 1. Significant functional connectivity differences between groups in the absence of GSR.

Anatomical region	Cluster size (voxels)	Peak *F*-values	Peak MNI (X Y Z) coordinates (mm)
Cingulum_Ant_R	43	8.8543	3 33 9
Cerebelum_9_R	60	8.4279	21 -45 -42