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Functional connectivity of the anterior cingulate cortex predicts treatment outcome for rTMS in treatment-resistant depression at 3-month follow-up

Ruiyang Ge [a], Jonathan Downar [b, c], Daniel M. Blumberger [b, d], Zafiris J. Daskalakis [b, d], Fidel Vila-Rodriguez [a, *]

[a] Non-Invasive Neurostimulation Therapies (NINET) Laboratory, Department of Psychiatry, University of British Columbia, 2255, Wesbrook Mall, Vancouver, BC V6T 2A1, Canada
[b] Department of Psychiatry, University of Toronto, Toronto, ON, Canada
[c] MRI-Guided RTMS Clinic, Toronto Western Hospital, University Health Network, Toronto, ON, Canada
[d] Temerty Centre for Therapeutic Brain Intervention, Campbell Family Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada

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ABSTRACT

Background and objective: Repetitive transcranial magnetic stimulation (rTMS) is a first-line treatment for treatment-resistant depression (TRD). The mechanisms of action of rTMS are not fully understood, and no biomarkers are available to assist in clinical practice to predict response to rTMS. This study aimed to demonstrate that after-rTMS clinical improvement is associated with functional connectivity (FC) changes of the subgenual cingulate cortex (sgACC) and rostral anterior cingulate (rACC), and FC of sgACC and rACC might serve as potential predictors for treatment response.
Methods: Resting-state functional magnetic resonance imaging (rs-fMRI) data were collected within 1 week before rTMS initiation in 50 TRD patients to predict subsequent response to rTMS on the left dorsolateral prefrontal cortex (DLPFC). Follow-up rs-fMRI was obtained 12 weeks after completion of rTMS and neural correlates of rTMS in sgACC- and rACC-related FC patterns were compared to before rTMS data and with rs-fMRI from healthy participants.
Results: Treatment response was associated with lower FC of sgACC to right DLPFC and higher FC of rACC to left lateral parietal cortex (IPL) measured at baseline. Using sgACC-DLPFC and rACC-IPL connectivity as features, responder-nonresponder classification accuracies of 84% and 76% (end-of-treatment), 88% and 81% (3-month follow-up), respectively were achieved. Longitudinal rs-fMRI data analyses revealed that the hyperconnectivity between sgACC and visual cortex was normalized to a level which was comparable to that of healthy participants.
Conclusions: Brain activity patterns in depression are predictive of treatment response to rTMS, and longitudinal change of brain activity in relevant brain circuits after rTMS is associated with treatment response in depression. Target engagement paradigms may offer opportunities to increase the efficacy of rTMS in TRD by optimal selection of patients for treatment.
Trial registration: ClinicalTrials.gov Identifiers: NCT01887782 and NCT02800226.

Introduction

Major depressive disorder (MDD) is the leading cause of disability in the world and is associated with personal, family and social burden [1]. In psychiatry, treatment is prescribed on an empirical basis based on clinical profile. The identification of reliable and robust biologically-based predictors of treatment response and neural correlates of symptom improvement may improve clinical outcomes and decrease risk of side effects by indicating specific interventions based on *a priori* likelihood of improvement.

Neuroimaging and neurophysiological measures have been proposed as potential biomarker candidates [2,3]. Specifically, brain activity patterns within two sub-regions of the anterior cingulate cortex (ACC), namely the subgenual ACC (sgACC [4]) and rostral ACC

* Corresponding author.
E-mail address: fidel.vilarodriguez@ubc.ca (F. Vila-Rodriguez).

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(rACC [5]), show promise in this regard. The sgACC is an extensively connected component of the limbic system that modulates emotional processing [4,6] and its functional connectivity with other brain regions has shown hyperconnectivity in patients with treatment-resistant depression (TRD) [4,7]. Thus, FC and morphology of sgACC have been investigated as potential predictive biomarkers of treatment response in depression across diverse treatment modalities, including antidepressant medications [8,9], evidence-based psychotherapy [9,10], repetitive transcranial magnetic stimulation (rTMS) [11–14], and electroconvulsive therapy [15,16]. Unlike the sgACC, which is located underneath the genu of the corpus callosum, the rACC is located anterior to the genu, and is often considered as a hub region of the default mode network (DMN) [17]. Similar to the sgACC, the activity and morphology of rACC have also been proposed as promising biomarkers of treatment response in depression [18]. However, contrary to the findings of sgACC, rACC activity has consistently been found to be hypoactive in nonresponders, relative to responders (see review by Pizzagalli [5]), treated with antidepressant medication [19], sleep deprivation [20], and rTMS [21,22]. The understanding of the pathophysiology of MDD has shifted to a model based on dysregulation of neural networks, rather than a single neuroanatomical location [23]. In this light, studies looking at the sgACC and the rACC in isolation view these two regions as nodes of separate neural brain networks with different patterns of activity/connectivity and predictive capacities between responders and nonresponders.

The main goal of the present study was to examine whether the FC of sgACC and rACC could serve as short- and long-term predictors of response to rTMS A secondary goal was to identify longitudinal resting-state functional connectivity (FC) correlates of treatment response to rTMS. We hypothesized that rTMS would normalize the abnormal hyperconnectivity of the sgACC and this change in brain activity would be associated with change in symptoms.

Methods

Participants

Participants were outpatients between the ages of 18–65 with a diagnosis of unipolar MDD who did not respond to at least one adequate or two inadequate antidepressant trials during the current episode, as assessed by the Antidepressant Treatment History Form (ATHF) [24]. Detailed inclusion and exclusion criteria have been previously described [25] and are provided in Supplementary Tables S1 and S2. In total, 50 patients were used to investigate baseline predictors; 32 patients and 24 HCs were used to investigate neural correlates of treatment response. The present study employed data from the UBC site of the THREE-D trial [25].

Written informed consent was obtained from all participants. The trial was approved by the Clinical Research Ethics Board of the University of British Columbia (UBC) and Vancouver Coastal Health Authority, and registered as two separate trials with identical inclusion and exclusion criteria and treatment intervention (clinicaltrials.gov identifiers NCT01887782 and NCT02800226).

Clinical outcome measures

The 17-item Hamilton Rating Scale for Depression (HRSD) served as the primary outcome measure. Endpoint outcomes were measured upon completion of the final session of treatment and at follow-up (12 weeks after the final treatment session). Response was defined as $\geq$50% reduction in HRSD from baseline, remission was defined by a total score <8 as in the primary THREE-D analysis [25].

rTMS treatment protocol

Patients completed 20 to 30 sessions of 10 Hz (high-frequency left stimulation, HFL; n = 26) or intermittent theta-burst (iTBS; n = 24) rTMS treatment on a MagPro-X100 stimulator with a Cool-B70 fluid-cooled coil (Magventure, Farum, Denmark) over the left dorsolateral prefrontal cortex (DLPFC), with a neuronavigation system (Visor 2.0, ANT Neuro, Enschede, Netherlands) to target the Montreal Neurological Institute (MNI) coordinate (X-38 Y+44 Z+26) [11]. The details of neuronavigation and treatment procedures are described in the Supplement Material.

Imaging data acquisition and preprocessing

Baseline and follow-up imaging data for each participant were collected within 1 week before rTMS initiation, and 12 weeks after the final rTMS session, respectively. For HCs (they did not receive rTMS), the follow-up data were collected 18 weeks post baseline. Imaging was performed on a single Philips Achieva 3T scanner at UBC MRI Research Centre. Participants were asked to keep still with their eyes open and to try not to think of anything in particular. A total of 300 vol of echo-planar images and high-resolution T1-weighted images were obtained. MRI acquisition and preprocessing details are described in the Supplement Material.

Functional connectivity analysis

Seed-based analyses were conducted to assess whole-brain baseline FC of the sgACC [12] and rACC seeds [17] (Fig. 1A). Cluster-level threshold was set at $p < 0.05$ using family-wise error (FWE) rate correction for multiple comparisons, with voxel-wise threshold $p < 0.001$. Data processing and multiple-comparison correction procedures are described in the Supplement Material.

Subject-level prediction with baseline functional connectivity of sgACC and rACC

Regression analyses were used to identify functional connections at baseline that were significantly correlated with improvement of HRSD scores at both end of rTMS treatment and 12 weeks post rTMS (Supplement Material). We examined the sensitivity and specificity of these connections in distinguishing responders and nonresponders by using receiver operating characteristic (ROC) curves. We employed non-parametric permutation tests to determine whether the discriminative performances occurred by chance [26] (Supplement Material).

Longitudinal rs-fMRI analyses at 3-month follow

A two-way analysis of covariance (ANCOVA) and *post-hoc* analyses were performed to determine the group $\times$ time interaction, main effects of group (responders, nonresponders and HCs) and time (baseline and follow-up) on sgACC- and rACC-based FC, with age, sex, educational level, handedness, treatment protocol and frame-wise displacement (FD) of the head motion as nuisance covariates. As a supplementary analysis, we re-analyzed our data without any nuisance variables added to the statistical model to mitigate the risk that the present results were driven by these nuisance variables. The results of ANCOVA were presented with a threshold at cluster-level $p < 0.05$ using family-wise error rate correction for multiple comparisons, with voxel-wise threshold $p < 0.001$. Next we performed correlational analysis between the changes of the HRSD scores (from baseline to follow-up) and brain measurements (i.e. sgACC- and rACC-related FC patterns) in the areas showing a significant main effect of time, main effect of group

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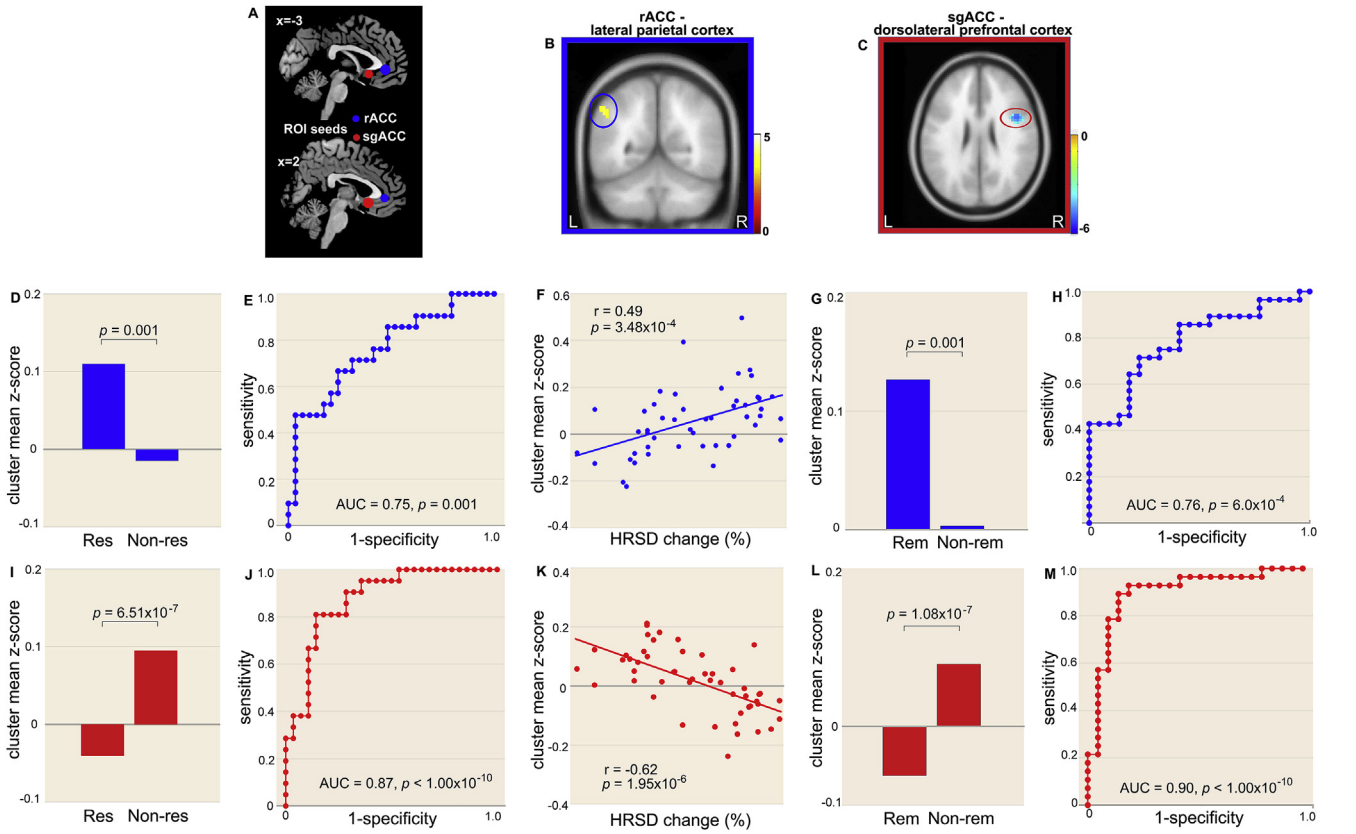

Fig. 1. Neuroimaging predictors of treatment response, with the responders defined with > 50% improvements in HRSD obtained after the final rTMS treatments. (**A**) Seeds used in the present study (sgACC MNI coordinate: (X+2 Y+18 Z-8); rACC MNI coordinate: (X-3 Y+39 Z-2)). Connectivity of the left inferior parietal lobule (IPL, blue circle) with rACC (**B**), and right dorsolateral prefrontal cortex (DLPFC, red circle) with sgACC (**C**) that exhibited significant positive and negative correlation with HRSD improvement. L: left hemisphere, R: right hemisphere. Mean sgACC-DLPFC (**E**) (red bars) and rACC-IPL (**D**) (blue bars) connectivity (Fisher's z) in each group. sgACC-DLPFC (**G**) and rACC-IPL (**F**) connectivity plotted against percent change in depression severity across all samples. Receiver operating characteristic (ROC) curves for classification of response status with sgACC-DLPFC (**I**) and rACC-IPL (**H**) connectivity. AUC: area under curve. Sensitivity (true-positive rate) depicts the proportion of nonresponders who were correctly identified; specificity (true-negative rate) depicts the proportion of responders who were correctly identified. $^{**}p < 0.001$. sgACC: subgenual anterior cingulate cortex; rACC: rostral anterior cingulate cortex; DLPFC: dorsolateral prefrontal cortex; IPL: lateral parietal cortex; HRSD: Hamilton Rating Scale for Depression. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

or group × time interaction. Moreover, to identify functional connections whose changes between baseline and 3-month follow-up were significantly correlated with improvement of HRSD scores, we have conducted regression analysis (in the whole brain) on changes of sgACC-related FC and changes of rACC-related FC with improvement of HRSD scores as regressor.

Mediation analysis was performed to explore the role of baseline sgACC-DLPFC FC (as well as sgACC-DLPFC change) in the association between the FC changes of sgACC-fusiform s and improvement of depression symptoms in patients (Supplement Material).

Results

Participant demographics

Responders and nonresponders did not differ on age, sex, educational level, handedness (Table 1 and Supplement Table S3), head motion, or treatment protocol allocation. Although not significant, these variables were included as covariates of no interest in group-level analyses.

Clinical outcomes

Demographic and clinical characteristics of responders and nonresponders are shown in Table 1. Baseline HRSD scores of responders were very similar to that of nonresponders ($p > 0.50$), and these scores significantly decreased in both groups following treatment ($p < 0.001$). For the immediate post-treatment HRSD scores, there was no significant difference in the number of responders and nonresponders for the iTBS and HFL protocols (16 responders and 8 nonresponders with iTBS; 13 responders and 13 nonresponders with HFL, $\chi^2 = 0.82$, $p = 0.36$ with Yates correction), and number of remitters and nonremitters for the iTBS and HFL protocols (12 remitters and 12 nonremitters with iTBS; 10 remitters and 16 nonremitters with HFL, $\chi^2 = 0.29$, $p = 0.59$ with Yates correction). For 3-month post-treatment HRSD scores, there was no significant difference in the number of responders and nonresponders for the iTBS and HFL protocols (16 responders and 4 nonresponders with iTBS; 11 responders and 10 nonresponders with HFL, $\chi 2 = 2.36$, $p = 0.12$ with Yates correction), and number of remitters and nonremitters for the iTBS and HFL protocols (10 remitters and 11 nonremitters with iTBS; 7 remitters and 14 nonremitters with HFL, $\chi^2 = 0.40$, $p = 0.53$ with Yates correction). There was no difference in baseline HRSD scores ($p = 0.54$), immediate post-treatment HRSD scores ($p = 0.14$), change of HRSD scores immediately after treatment ($p = 0.14$), 3-month post-treatment HRSD scores ($p = 0.10$), change of HRSD scores 3-month after treatment ($p = 0.09$), or treatment course length ($p = 0.45$) between iTBS and HFL protocols. Treatments were well tolerated, with similar rates of side effects and dropout rates in both

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Table 1
Demographics and clinical characteristics of the participants (analyzed sample).

	Baseline patients (n = 50)						Longitudinal patients subsample (n = 32)						HCs (n = 24)
	responders	non-responders	*p*	remitters	non-remitters	*p*	responders	non-responders	*p*	remitters	non-remitters	*p*	
Sex (M/F)	11/18	10/11	0.69[a]	8/14	13/15	0.67[a]	7/14	6/5	0.43[a]	5/9	8/10	0.89[a]	12/12
Age (yrs), mean (SD)	43.58(11.50)	43.76(12.88)	0.96[b]	44.00(11.67)	43.39(12.40)	0.86[b]	43.71(11.97)	45.45(9.50)	0.68[b]	43.71(11.65)	44.78(10.89)	0.79[b]	45.25(12.19)
Educational level (yrs), mean (SD)	15.21(2.44)	15.14(2.10)	0.92[b]	15.64(2.56)	14.82(2.02)	0.21[b]	15.76(2.12)	15.73(2.41)	0.97[b]	16.14(1.92)	15.44(2.38)	0.38[b]	16.50(2.23)
Handedness (L/R/A)	4/25/0	4/16/1	–	4/18/0	4/23/1	–	3/18/0	1/11/0	–	2/12/0	2/16/0	–	2/20/2
HRSD at baseline, mean (SD)	22.03(4.61)	21.71(2.65)	0.78[b]	20.82(4.38)	22.75(3.26)	0.08[b]	21.57(4.77)	21.64(1.75)	0.97[b]	19.71(4.56)	23.06(2.73)	0.02[b]	–
HRSD at the end of treatment, mean (SD)	5.38(4.00)	15.62(3.43)	<0.001[b]	3.50(2.11)	14.54(3.69)	<0.001[b]	4.95(4.12)	17.09(3.59)	<0.001[b]	2.71(1.90)	14.11(5.21)	<0.001[b]	–
HRSD at 3-mo follow-up, mean (SD)	7.46(6.42)	14.72(7.46)	0.002[b]	4.89(4.34)	14.87(7.18)	<0.001[b]	5.52(3.84)	19.45(5.01)	<0.001[b]	3.29(1.98)	15.78(6.26)	<0.001[b]	–
Age at onset (yrs), mean (SD)	25.52(13.38)	24.76(9.61)	0.83[b]	25.95(14.00)	24.61(10.05)	0.71[b]	28.52(14.02)	22.36(8.08)	0.19[b]	26.57(14.19)	26.28(11.47)	0.95[b]	–
Duration of current episode (mo.), mean (SD)	32.00(33.14)	38.79(51.67)	0.58[b]	33.93(45.50)	36.00(36.99)	0.86[b]	37.05(37.55)	48.36(67.62)	0.54[b]	38.14(38.33)	43.11(57.17)	0.78[b]	–
ATHF scores	7.34(3.66)	7.62(2.75)	0.77[b]	6.82(2.87)	7.96(3.53)	0.22[b]	7.33(3.69)	8.27(3.41)	0.49[b]	6.71(2.92)	8.39(3.93)	0.19[b]	–

Abbreviations: M, male; F, female; yrs, years; SD, standard deviation; L, left; R, right; A, ambidextrous; HDRS, 17-item Hamilton Rating Scale for depression; mo, months; ATHF, Antidepressant Treatment History Form. [a] Chi-square test. [b] Two-sample *t*-test for responder/remitter and non-responder/non-remitter groups.

treatment arms. There was one serious adverse event: one participant in the pilot trial completed suicide (NCT02800226).

Baseline neuroimaging predictors of treatment response

The rACC-based FC showed a significant left inferior parietal lobule cluster (IPL, peak voxel (x, y, z) = (−48, −54, 36), $t(49) = 4.05$; 648 mm^3; Fig. 1B). Specifically, the stronger the FC between the rACC and the IPL, the greater the improvement on HRSD (Fig. 1F, $r = 0.49$, $p = 3.48 \times 10^{-4}$); responders and remitters had higher FC between the rACC and IPL relative to nonresponders and nonremitters, respectively (Fig. 1D and G).

On the other hand, sgACC-based FC showed the opposite pattern for a right DLPFC cluster (peak voxel (x, y, z) = (42, 6, 21), $t(49) = 6.08$; 2214 mm^3) (Fig. 1C) with HRSD improvement as the regressor of interest. Specifically, the stronger the FC between sgACC and right DLPFC, the lesser improvement on HRSD (Fig. 1K, $r = -0.62$, $p = 1.95 \times 10^{-6}$); nonresponders and nonremitters had higher FC between sgACC and right DLPFC relative to responders and remitters, respectively (Fig. 1I and L).

We computed correlations between HRSD improvement and the rACC-IPL and sgACC-DLPFC connectivity pairs for the two different intervention types (iTBS and HFL). The results showed that for both interventions, HRSD improvement significantly correlated with the rACC-IPL and sgACC-DLPFC connectivity pairs (Fig. S2), and these correlations did not differ from each other ($p = 0.92$ for rACC-IPL pair of iTBS versus HFL; $p = 0.90$ for sgACC-DLPFC pair of iTBS versus HFL; see Supplement Fig. S2).

ROC curves were employed to characterize the predictive value of the FC measures for sgACC and rACC, respectively. Connectivity within both seed regions exhibited significantly higher discriminative performance than chance level. For the classification of responders and nonresponders, the AUC of the sgACC-DLPFC connectivity was 0.87 (95% confidence interval (CI), 0.76 to 0.98; $p < 0.001$ with permutation test repeated 10,000 times, Fig. 1J) and the AUC of the rACC-IPL connectivity was 0.75 (95% CI, 0.61 to 0.89; $p = 0.001$ with permutation test repeated 10,000 times, Fig. 1E). Classification accuracies were 84% (sensitivity 81%, specificity 86%) and 76% (sensitivity 48%, specificity 97%) for the sgACC-DLPFC connectivity and rACC-IPL measures, respectively. For the classification of remitters and nonremitters, the AUC of the sgACC-DLPFC connectivity was 0.90 (95% confidence interval (CI), 0.81 to 0.99; $p < 0.001$ with permutation test repeated 10,000 times, Fig. 1M) and the AUC of the rACC-IPL connectivity was 0.76 (95% CI, 0.61 to 0.89; $p < 0.001$ with permutation test repeated 10,000 times, Fig. 1H). Classification accuracies were 88% (sensitivity 89%, specificity 86%) and 74% (sensitivity 71%, specificity 77%) for the sgACC-DLPFC connectivity and rACC-IPL measures, respectively. Furthermore, the effect sizes (Cohen's d) of the difference in average connectivity values for responders versus nonresponders in right DLPFC and left IPL were 1.68 and 0.99, and the difference in average connectivity values for remitters versus nonremitters in right DLPFC and left IPL were 1.77 and 0.66 (values conventionally interpreted as constituting medium to large effect sizes) [27]. The discovery-replicate analysis showed that the results of the discovery and replicate dataset replicated the primary findings (Fig. S5 and Fig. S6, see details in Supplement Material).

For all patients (n = 42) who received two MRI scans, the sgACC- and rACC-related FC results replicated that of Fig. 1 (with accuracy of 76% and 67% for sgACC and rACC for classification of responders and nonresponders at the 3-month follow-up visit, accuracy of 79% and 64% for sgACC and rACC for classification of remitters and nonremitters at the 3-month follow-up visit, respectively) (Fig. 2). For the 32 patients who did not change their responsive or remissive status at the 3-month follow-up visit, the sgACC- and

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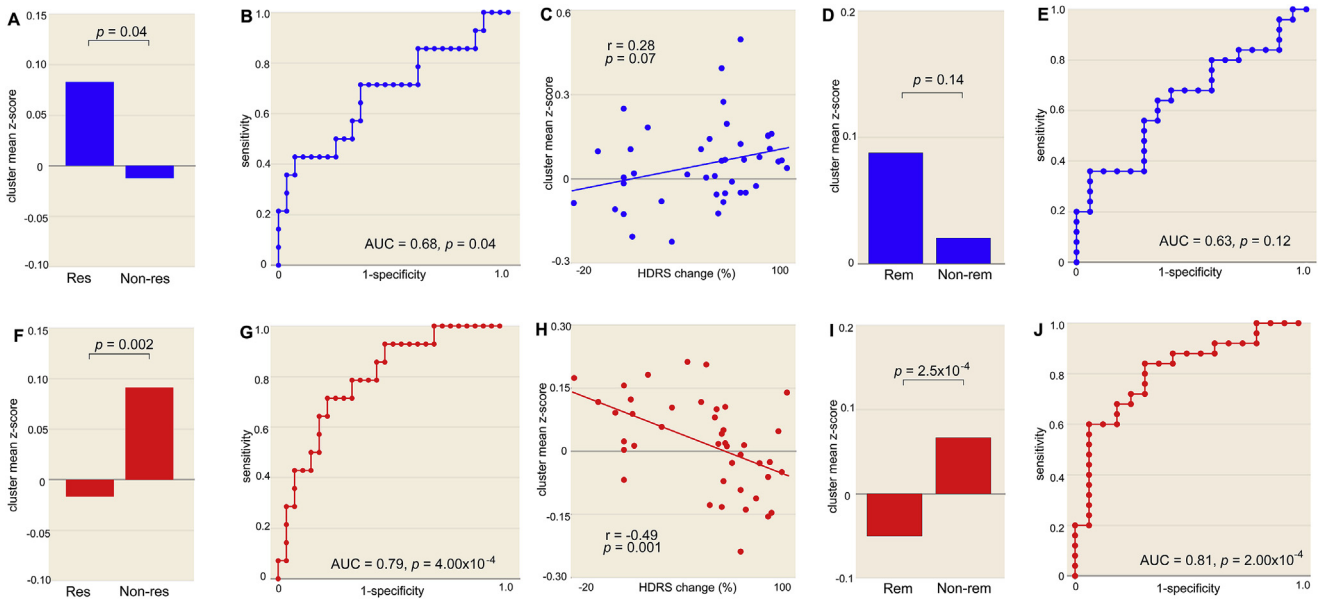

Fig. 2. Neuroimaging predictors of treatment response. Note that the responders were defined with > 50% improvements in HRSD obtained at 3 months after the final rTMS treatments. Mean rACC-LPC (**A**) (blue bars) and sgACC-DLPFC (**B**) (red bars) connectivity (Fisher's *z*) in each group. rACC-LPC (**C**) and sgACC-DLPFC (**D**) connectivity plotted against percent change in depression severity across patients who received two MRI scans. Receiver operating characteristic (ROC) curves for classification of response status with rACC-LPC (**E**) and sgACC-DLPFC (**F**) connectivity. The results of the permutation test (10,000 times) on area-under-curve (AUC) values were presented. sgACC: subgenual anterior cingulate cortex; rACC: rostral anterior cingulate cortex. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rACC-related FC results replicated that of Fig. 1 (with accuracy of 88% and 81% for sgACC and rACC for classification of responders and nonresponders at the 3-month follow-up visit, accuracy of 84% and 66% for sgACC and rACC for classification of remitters and non-remitters at the 3-month follow-up visit, respectively) (Supplement Fig. S3).

Longitudinal rs-fMRI analyses and association to clinical outcomes

For sgACC-related FC patterns, a significant main effect of group was found in the right DLPFC (peak voxel (x, y, z) = (48, 9, 27), $F(1,54) = 9.02$; 171 mm^3, Fig. 3A). A main effect of time was observed in the left occipitotemporal region (appearing to mainly include left fusiform and extending to the left inferior temporal cortex, peak voxel (x, y, z) = (−39, −63, −6), $F(1,54) = 14.93$; 513 mm^3) and in the middle occipital cortex (MOC, peak voxel (x, y, z) = (−45, −87, 18), $F(1, 54) = 19.50$; 972 mm^3) (Fig. 3B). No significant group × time interaction was found. No significant result was found for rACC-related FC patterns. For the comparisons between responders, nonresponders and HCs, our results revealed that responders and nonresponders showed significant decrease in FC of the sgACC-DLPFC, sgACC-fusiform, and sgACC-MOC pairs between baseline and follow-up scans, whereas HCs' FC of these pairs remained stable between baseline and follow-up scans. Nonresponders showed higher FC of the sgACC-fusiform and sgACC-MOC pairs at baseline scan relative to HCs, and this hyper-connectivity was normalized to the HCs' level at the follow-up scan, whereas the sgACC-DLPFC still showed higher connectivity than HCs at the follow-up scan. At baseline, responders showed similar FC of the sgACC-DLPFC, sgACC-fusiform, and sgACC-MOC pairs of the HCs'. Moreover, our results revealed that non-remitters showed significant decrease in FC of the sgACC-DLPFC, sgACC-fusiform, and sgACC-MOC pairs between baseline and follow-up scans, whereas remitters'FC and HCs' FC of these pairs remained stable between baseline and follow-up scans. Non-remitters showed higher FC of the sgACC-DLPFC and sgACC-fusiform pairs at baseline scan relative to HCs, and this hyper-connectivity was normalized to the HCs' level at the follow-up scan. Remitters showed similar FC of the sgACC-DLPFC, sgACC-fusiform, and sgACC-MOC pairs of the HCs' (Fig. 3A and B). When the analyses were repeated without inclusion of any nuisance variables, we found that the observed main effects of group and time largely overlapped with ANCOVA results with nuisance variables (Supplement Fig. S7). This result suggested that the present results were not driven by the nuisance variables.

No significant clusters were identified in the regression analyses on the changes of sgACC and rACC-related FC with HRSD changes as regressor in the whole brain. However, correlational analysis conducted on clusters that show a significant effect of group or time in the ANCOVA model revealed that lesser decrease of sgACC-left-fusiform connectivity was associated with more HRSD improvement ($r = -0.38$, $p = 0.03$) (Fig. 3C). Path analysis showed that lower baseline sgACC-DLPFC connectivity was associated with greater decrease of sgACC-fusiform connectivity, which in turn was associated with greater improvement of depressive symptoms. Analysis indicated that there was a significant indirect association between changes of sgACC-fusiform connectivity and depressive symptoms, mediated through baseline sgACC-DLPFC connectivity (Fig. 3D). Our path analysis did not reveal a significant mediation effect of sgACC-DLPFC FC changes in the association between sgACC-fusiform FC changes and HRSD changes.

Discussion

The etiopathogenesis of depression has an unquestionable biological basis, and abnormalities in brain function must underlie its symptomatic expression; therefore, resolution of symptoms shall be the expression of underlying changes in brain function. The results of this study provide an account of brain function status before a treatment intervention as well as changes in brain function after the intervention using rs-fMRI signal in patients with TRD who receive excitatory rTMS to the left DLPFC; the focus is on ascertaining links between brain function abnormalities and clinical symptoms, and how changes in both dimensions are associated.

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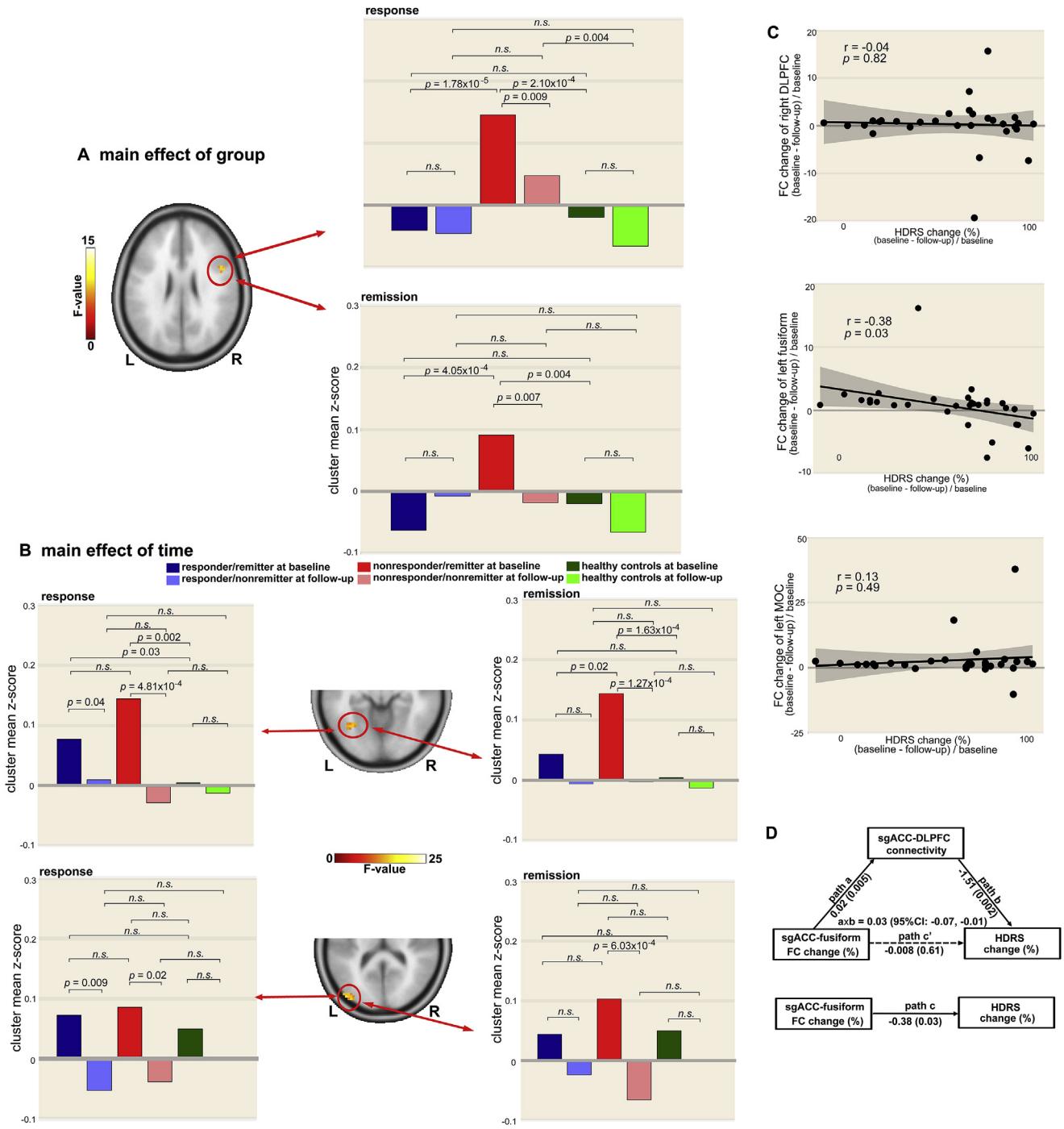

Fig. 3. Significant main effect of group and time. Significant main effect of group (**A**) of sgACC-related functional connectivity patterns was observed in the right DLPFC. Significant main effects of time (**B**) of sgACC-related functional connectivity patterns were observed in the left fusiform and MOC. (**C**) Changes of sgACC-fusiform functional connectivity negatively correlated with changes of HRSD scores. (**D**) Path analysis indicated that changes of sgACC-fusiform functional connectivity were related to changes of depressive symptoms and to the sgACC-DLPFC functional connectivity. In the first linear regression model, we found that changes of sgACC-fusiform were significantly associated with sgACC-DLPFC connectivity (beta = −0.02, $p = 0.002$). In the second linear regression model, we found that while controlling for changes of sgACC-fusiform connectivity, sgACC-DLPFC connectivity was significantly associated with changes of HRSD (beta = −1.41, $p = 0.003$). Lastly, we used bias-corrected bootstrapping to confirm the significance of the indirect effect of changes of sgACC-fusiform connectivity on changes of HRSD in the presence of sgACC-DLPFC connectivity (coefficient = 0.03; 95% CI = 0.01–0.07). n.s.: non-significant. sgACC: subgenual anterior cingulate cortex; DLPFC: dorsolateral prefrontal cortex; MOC: middle occipital cortex; HRSD: Hamilton Rating Scale for Depression.

Our results show that rTMS is associated with changes in brain function three months after completing treatment, and brain function changes are associated with changes in depressive symptoms. To the best of our knowledge this is the first such evidence in the context of a randomized controlled trial of rTMS.

Short and mid-term brain function biomarkers of treatment response

We found that while stronger baseline sgACC FC with right DLPFC was consistently associated with less clinical improvement

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both at the end of rTMS treatment (6 weeks) and at 3-month follow up, stronger rACC FC with left IPL was associated with greater clinical improvement; both were predictive biomarkers of outcome at both time points. In other words, before treatment there was a brain function signature of patients with TRD who eventually responded to or achieved remission with rTMS and sustained that response after 3 months. This brain function profile was characterized by lower levels of FC of sgACC and right DLPFC and higher levels of FC of rACC and leftIPL. The predictive capacity of these two candidate biomarkers remained stable when we re-defined responsiveness and remission based on the longitudinal follow-up data (Fig. 2 and Fig. S3). This result demonstrates the robustness of these two predictors over a longer time frame (i.e., 3 months); to the best of our knowledge, this is a novel finding not reported in the prior literature.

Our results support and extend earlier findings of sgACC activity [8,10] and connectivity patterns [9,11] predicting treatment outcome in MDD, and strengthen the case for the role of sgACC both in the pathophysiology of MDD and as a target for its treatment. The sgACC is a major neural substrate in processing of emotional stimuli and is involved in emotional behavior output [5], and has been used as a probe examining the affective network in depressed patients [6]. The activity of the right DLPFC has repeatedly been linked to emotional modulation. Specifically, it is involved in attentional modulation of emotional judgment in MDD patients [34,35], and its hyperactivity in an emotional task correlates with depression severity [34]. The fact that FC between right DLPFC and sgACC distinguished responders and nonresponders argues for a role of the sgACC in the affective system. The greater baseline connectivity between sgACC and right DLPFC in nonresponders may reflect the availability of this system to be recruited in negative valence emotional bias as well as increased attention to self-judgments which are predominant traits in MDD patients [35]. Additionally, it is possible that higher FC between sgACC and right DLPFC is an index of improper integration within large emotional processing networks and inadequate top-down emotional regulation in nonresponders. A recent study found that rTMS efficacy was predicted by anticorrelation between the left DLPFC and sgACC [13]. Weigand and colleagues used a ROI approach focused only on the left DLPFC, while we opted for a whole brain strategy, and thus it cannot be ruled out they might have found a similar result to the present study regarding the right DLPFC. In our sample, the left DLPFC connectivity with sgACC did not survive correction for multiple comparisons across the entire brain (Supplement Fig. S4), but a more liberal approach would have retained that finding. In addition, it is important to note that we used neuronavigation to target in every rTMS session, while Weigand and colleagues [13] used a rule of 5.5 cm and they likely had a larger variability with regards to target engagement. Instead, our results indicate predictive capability of nonresponsiveness of sgACC to the contralateral site of stimulation (i.e. right DLPFC). This finding is intriguing and poses the question of whether patients with a strong sgACC-right-DLPFC connectivity might be better candidates for inhibitory rTMS to the right DLPFC. Since low-frequency right DLPFC stimulation has been shown to be an effective treatment of TRD [36], it would be worthwhile in future research to investigate predictive values of the FC patterns of sgACC in clinical trials that stimulate right DLPFC. Our findings might have relevant clinical implications as triaging out for excitatory left DLPFC rTMS patients with higher baseline sgACC-right-DLPFC connectivity would be more likely to be associated with increased efficacy rates by decreasing the percentage of patients unlikely to respond to excitatory left DLPFC.

Employing a data-driven multivariate approach, we recently reported that nonresponders displayed hypoconnectivity in rACC/VMPFC (ventromedial prefrontal cortex) within the DMN and that this region exhibited good performance in discriminating the treatment response status of patients [37]. Our current study using an *a priori* seed-driven approach provides converging evidence for the predictive capability of rACC. The rACC, with its adjacent VMPFC, activates across a diverse range of mnemonic, social and emotional tasks that involve personally significant information, and it is a hub region of the DMN [38], which has been shown to be altered in MDD [39]. In light of evidence showing the involvement of the DMN and rACC/VMPFC in self-referential thinking [39,40], our results would support the hypothesis that elevated rACC/VMPFC connectivity with the IPL, a conventional region of the DMN [38], confers better treatment outcome by fostering adaptive self-referential processing and adaptive aspects of rumination [5,41]. The present results also demonstrated that rACC connectivity is opposite to that of sgACC in terms of predictive direction, and this may suggest that the sgACC- and rACC-related neural systems work in concert to oppositely regulate and/or balance treatment effects.

Changes in brain function after rTMS and their association to clinical improvement

The longitudinal analysis showed that rTMS had a normalizing effect on abnormally higher sgACC-related connectivity in the right DLPFC and occipitotemporal region. We observed that the normalization of sgACC-DLPFC connectivity only presented in nonresponders, who had differences relative to HCs at baseline. Our results echoed a recent report [28] that demonstrated the baseline FC of the visual regions and the reduced local FC patterns of the visual regions predicted treatment response of electroconvulsive therapy. Abnormality of fusiform activity in MDD patients has been repeatedly linked to perception of emotion with facial stimuli and declarative memory [29], and the abnormal neural activity of fusiform has been associated with symptoms of depression in both task state and resting state [30,31]. The abnormal activation of this region in emotional processing was normalized after antidepressant treatment [32]. The present study demonstrated higher FC between this region and sgACC in both patient groups relative to HCs at baseline, and this hyperconnectivity decreased to the HCs' level after treatment. The changes of sgACC-fusiform connectivity were associated with improvement of clinical symptoms, and these associations were mediated through sgACC-DLPFC connectivity. These findings suggest that sgACC-fusiform connectivity contributes to hyperconnectivity of sgACC-DLPFC, which, in turn, may decrease the possibility of symptom relief with rTMS. Although path analysis can imply a causal relationship between the variables of interest, our study cannot impute causality since we did not observe any mediation effect of sgACC-DLPFC FC changes in the association between changes of sgACC-fusiform FC and symptoms. Thus, these results should only be interpreted as supportive, or preliminary, in nature. Nevertheless, our findings suggest that rTMS therapeutic substrates include interactions between different brain systems. Specifically, rTMS treatment might have led to changes between systems in charge of emotional social perception, within which fusiform plays an important role [33], as well as changes in emotion regulation systems where sgACC and DLPFC play roles [2]. Informed by the present findings, future studies could test the therapeutic interactions of different systems by employing tasks spanning different emotional and cognitive processes. Although sgACC-MOC connectivity decreased in both responders and nonresponders in the follow-up scans, this connectivity pair *per se* had no difference at baseline relative to HCs. Also, we did not find a correlation between the decrease in sgACC-MOC connectivity and depressive symptoms. Accordingly, the change was unlikely to reflect the therapeutic substrates of rTMS, which indicates a

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possibility that this change was a byproduct rather than the underlying mechanism of rTMS.

Our findings should be considered in light of some limitations. First, type of treatment protocol might have been a source of variability confounding results. We considered this in our analyses by modeling type of protocol as a nuisance variable. In addition, it is important to note that outcomes were not statistically different at any time point. Secondly, drug-naïve patients would have eliminated the potential confounding effect of different antidepressant regimens across patients. This is a limitation intrinsic to the TRD population as patients by definition had previous exposure to antidepressants. On the other hand, this feature of our study population reflects real-world practice in rTMS clinics, where patients typically maintain a stable medication regimen rather than discontinue pharmacotherapy during rTMS treatment, and thus our results may have greater external validity. Thirdly, we did run analyses using treatment type as a between factor and it did not yield any significant differences between different treatment types. Since our sample size is limited though we appreciate this might be false negative. Finally, our results are converging with previous research regarding the ability of sgACC connectivity to predict rTMS response in TRD [11], but our longitudinal results required replication.

In summary, our findings may provide the foundation for a prospective clinical trial using these biomarkers and neural substrates. From a mechanistic perspective, our demonstration of differential effects of baseline and longitudinal sgACC and rACC connectivity between responders and nonresponders may contribute to a better understanding of neural substrates associated with rTMS treatment response at a network level. From a clinical standpoint, the present findings suggest that it may be possible to determine with a significant degree of accuracy individuals who may respond to excitatory left DLPFC rTMS. With further development, a sgACC and rACC connectivity index (especially that of sgACC) may prove useful for outcome prediction prior to engaging in rTMS treatment.

Disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.10.012.

References

[1] Organization WH. Depression and other common mental disorders: global health estimates. World Health Organization; 2017.
[2] Phillips ML, Chase HW, Sheline YI, Etkin A, Almeida JR, Deckersbach T, et al. Identifying predictors, moderators, and mediators of antidepressant response in major depressive disorder: neuroimaging approaches. Am J Psychiatry 2015;172(2):124–38.
[3] Fu CH, Steiner H, Costafreda SG. Predictive neural biomarkers of clinical response in depression: a meta-analysis of functional and structural neuroimaging studies of pharmacological and psychological therapies. Neurobiol Dis 2013;52:75–83.
[4] Drevets WC, Savitz J, Trimble M. The subgenual anterior cingulate cortex in mood disorders. CNS Spectr 2008;13(8):663–81.
[5] Pizzagalli DA. Frontocingulate dysfunction in depression: toward biomarkers of treatment response. Neuropsychopharmacology 2011;36(1):183–206.
[6] Sheline YI, Price JL, Yan Z, Mintun MA. Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. Proc Natl Acad Sci U S A 2010;107(24):11020–5.
[7] Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, et al. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biol Psychiatry 2007;62(5):429–37.
[8] McGrath CL, Kelley ME, Dunlop BW, Holtzheimer III PE, Craighead WE, Mayberg HS. Pretreatment brain states identify likely nonresponse to standard treatments for depression. Biol Psychiatry 2014;76(7):527–35.
[9] Dunlop BW, Rajendra JK, Craighead WE, Kelley ME, McGrath CL, Choi KS, et al. Functional connectivity of the subcallosal cingulate cortex and differential outcomes to treatment with cognitive-behavioral therapy or antidepressant medication for major depressive disorder. Am J Psychiatry 2017:533–45.
[10] Siegle GJ, Thompson WK, Collier A, Berman SR, Feldmiller J, Thase ME, et al. Toward clinically useful neuroimaging in depression treatment: prognostic utility of subgenual cingulate activity for determining depression outcome in cognitive therapy across studies, scanners, and patient characteristics. Arch Gen Psychiatr 2012;69(9):913–24.
[11] Fox MD, Buckner RL, White MP, Greicius MD, Pascual-Leone A. Efficacy of transcranial magnetic stimulation targets for depression is related to intrinsic functional connectivity with the subgenual cingulate. Biol Psychiatry 2012;72(7):595–603.
[12] Liston C, Chen AC, Zebley BD, Drysdale AT, Gordon R, Leuchter B, et al. Default mode network mechanisms of transcranial magnetic stimulation in depression. Biol Psychiatry 2014;76(7):517–26.
[13] Weigand A, Horn A, Caballero R, Cooke D, Stern AP, Taylor SF, et al. Prospective validation that subgenual connectivity predicts antidepressant efficacy of transcranial magnetic stimulation sites. Biol Psychiatry 2018;84(1):28–37.
[14] Cash RF, Zalesky A, Thomson RH, Tian Y, Cocchi L, Fitzgerald PB. Subgenual functional connectivity predicts antidepressant treatment response to transcranial magnetic stimulation: independent validation and evaluation of personalization. Biol Psychiatry 2019;82(2):e5–7. https://doi.org/10.1016/j.biopsych.2018.12.002.
[15] Argyelan M, Lencz T, Kaliora S, Sarpal D, Weissman N, Kingsley P, et al. Subgenual cingulate cortical activity predicts the efficacy of electroconvulsive therapy. Transl Psychiatry 2016;6(4):e789.
[16] Redlich R, Opel N, Grotegerd D, Dohm K, Zaremba D, Bürger C, et al. Prediction of individual response to electroconvulsive therapy via machine learning on structural magnetic resonance imaging data. JAMA Psychiatr 2016;73(6):557–64.
[17] Fair DA, Cohen AL, Power JD, Dosenbach NU, Church JA, Miezin FM, et al. Functional brain networks develop from a "local to distributed" organization. PLoS Comput Biol 2009;5(5):e1000381.
[18] Boes AD, Uitermarkt BD, Albazron FM, Lan MJ, Liston C, Pascual-Leone A, et al. Rostral anterior cingulate cortex is a structural correlate of repetitive TMS treatment response in depression. Brain Stimul 2018;11(3):575–81.
[19] Pizzagalli D, Pascual-Marqui RD, Nitschke JB, Oakes TR, Larson CL, Abercrombie HC, et al. Anterior cingulate activity as a predictor of degree of treatment response in major depression: evidence from brain electrical tomography analysis. Am J Psychiatry 2001;158(3):405–15.
[20] Wu J, Buchsbaum MS, Gillin JC, Tang C, Cadwell S, Wiegand M, et al. Prediction of antidepressant effects of sleep deprivation by metabolic rates in the ventral

Please cite this article as: Ge R et al., Functional connectivity of the anterior cingulate cortex predicts treatment outcome for rTMS in treatment-resistant depression at 3-month follow-up, Brain Stimulation, https://doi.org/10.1016/j.brs.2019.10.012

anterior cingulate and medial prefrontal cortex. Am J Psychiatry 1999;156(8):1149–58.
[21] Kito S, Fujita K, Koga Y. Regional cerebral blood flow changes after low-frequency transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in treatment-resistant depression. Neuropsychobiology 2008;58(1):29–36.
[22] Langguth B, Wiegand R, Kharraz A, Landgrebe M, Marienhagen J, Frick U, et al. Pre-treatment anterior cingulate activity as a predictor of antidepressant response to repetitive transcranial magnetic stimulation (rTMS). Neuroendocrinol Lett 2007;28(5):633–8.
[23] Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA. Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting-state functional connectivity. JAMA Psychiatr. 2015;72(6):603–11.
[24] Sackeim HA, Prudic J, Devanand DP, Decina P, Kerr B, Malitz S. The impact of medication resistance and continuation pharmacotherapy on relapse following response to electroconvulsive therapy in major depression. J Clin Psychopharmacol 1990;10:96–104.
[25] Blumberger DM, Vila-Rodriguez F, KE T, K F, Y N, Giacobbe P, et al. Theta burst versus high frequency repetitive transcranial magnetic stimulation effectiveness evaluation in depression (THREE-D): a randomized non-inferiority trial. The Lancet 2018;391(10131):1683–92.
[26] Ge R, Blumberger DM, Downar J, Daskalakis ZJ, Tham JC, Lam R, et al. A sparse representation-based method for parcellation of the resting brain and its application to treatment-resistant major depressive disorder. J Neurosci Methods 2017;290:57–68.
[27] Cohen J. Statistical power analysis for the behavioral sciences. Routledge; 2013.
[28] Moreno-Ortega M, Prudic J, Rowny S, Patel G, Kangarlu A, Lee S, et al. Resting state functional connectivity predictors of treatment response to electroconvulsive therapy in depression. Sci Rep 2019;9(1):5071.
[29] Schaefer HS, Putnam KM, Benca RM, Davidson RJ. Event-related functional magnetic resonance imaging measures of neural activity to positive social stimuli in pre-and post-treatment depression. Biol Psychiatry 2006;60(9):974–86.
[30] Demenescu L, Renken R, Kortekaas R, Van Tol M-J, Marsman J, Van Buchem M, et al. Neural correlates of perception of emotional facial expressions in outpatients with mild-to-moderate depression and anxiety. A multicenter fMRI study. Psychol Med 2011;41(11):2253–64.
[31] Wu QZ, Li DM, Kuang WH, Zhang TJ, Lui S, Huang XQ, et al. Abnormal regional spontaneous neural activity in treatment-refractory depression revealed by resting-state fMRI. Hum Brain Mapp 2011;32(8):1290–9.
[32] Delaveau P, Jabourian M, Lemogne C, Guionnet S, Bergouignan L, Fossati P. Brain effects of antidepressants in major depression: a meta-analysis of emotional processing studies. J Affect Disord 2011;130(1–2):66–74.
[33] Davidson RJ, Irwin W, Anderle MJ, Kalin NH. The neural substrates of affective processing in depressed patients treated with venlafaxine. Am J Psychiatry 2003;160(1):64–75.
[34] Grimm S, Beck J, Schuepbach D, Hell D, Boesiger P, Bermpohl F, et al. Imbalance between left and right dorsolateral prefrontal cortex in major depression is linked to negative emotional judgment: an fMRI study in severe major depressive disorder. Biol Psychiatry 2008;63(4):369–76.
[35] Bermpohl F, Walter M, Sajonz B, Lücke C, Hägele C, Sterzer P, et al. Attentional modulation of emotional stimulus processing in patients with major depression—alterations in prefrontal cortical regions. Neurosci Lett 2009;463(2):108–13.
[36] Isenberg K, Downs D, Pierce K, Svarakic D, Garcia K, Jarvis M, et al. Low frequency rTMS stimulation of the right frontal cortex is as effective as high frequency rTMS stimulation of the left frontal cortex for antidepressant-free, treatment-resistant depressed patients. Ann Clin Psychiatr 2005;17(3):153–9.
[37] Ge R, Blumberger DM, Downar J, Daskalakis ZJ, Dipinto AA, Tham JC, et al. Abnormal functional connectivity within resting-state networks is related to rTMS-based therapy effects of treatment resistant depression: a pilot study. J Affect Disord 2017;218:75–81.
[38] Andrews-Hanna JR. The brain's default network and its adaptive role in internal mentation. The Neuroscientist 2012;18(3):251–70.
[39] Sheline YI, Barch DM, Price JL, Rundle MM, Vaishnavi SN, Snyder AZ, et al. The default mode network and self-referential processes in depression. Proc Natl Acad Sci U S A 2009;106(6):1942–7.
[40] Gusnard DA, Akbudak E, Shulman GL, Raichle ME. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc Natl Acad Sci U S A 2001;98(7):4259–64.
[41] Hamilton JP, Furman DJ, Chang C, Thomason ME, Dennis E, Gotlib IH. Default-mode and task-positive network activity in major depressive disorder: implications for adaptive and maladaptive rumination. Biol Psychiatry 2011;70(4):327–33.

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