Archival Report

Pretreatment Rostral Anterior Cingulate Cortex Connectivity With Salience Network Predicts Depression Recovery: Findings From the EMBARC Randomized Clinical Trial

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ABSTRACT

BACKGROUND: Baseline rostral anterior cingulate cortex (rACC) activity is a well-replicated nonspecific predictor of depression improvement. The rACC is a key hub of the default mode network, which prior studies indicate is hyperactive in major depressive disorder. Because default mode network downregulation is reliant on input from the salience network and frontoparietal network, an important question is whether rACC connectivity with these systems contributes to depression improvement.

METHODS: Our study evaluated this hypothesis in outpatients (N = 238; 151 female) enrolled in the Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) 8-week randomized clinical trial of sertraline versus placebo for major depressive disorder. Depression severity was measured using the Hamilton Rating Scale for Depression, and electroencephalography was recorded at baseline and week 1. Exact low-resolution electromagnetic tomography was used to compute activity from the rACC, and key regions within the default mode network (posterior cingulate cortex), frontoparietal network (left dorsolateral prefrontal cortex), and salience network (right anterior insula [rAI]). Connectivity in the theta band (4.5–7 Hz) and beta band (12.5–21 Hz) was computed using lagged phase synchronization.

RESULTS: Stronger baseline theta-band rACC–rAI (salience network hub) connectivity predicted greater depression improvement across 8 weeks of treatment for both treatment arms (B = −0.57, 95% confidence interval = −1.07, −0.08, p = .03). Early increases in theta-band rACC–rAI connectivity predicted greater likelihood of achieving remission at week 8 (odds ratio = 2.90, p = .03).

CONCLUSIONS: Among patients undergoing treatment, theta-band rACC–rAI connectivity is a prognostic, albeit treatment-nonspecific, indicator of depression improvement, and early connectivity changes may predict clinically meaningful outcomes.

Keywords: Depression, EEG, Functional connectivity, Rostral ACC, Salience network, Sertraline

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Although a variety of interventions exist for major depressive disorder (MDD), fewer than 50% of individuals respond to first-line treatment (1). Consequently, there is an urgent need to better understand which factors predict depression recovery. Abnormal rostral anterior cingulate cortex (rACC) activity is critically implicated in MDD pathophysiology and has emerged as a prognostic (i.e., treatment-nonspecific) predictor of depression improvement (2). First observed by Mayberg et al. (3), heightened pretreatment rACC activity/metabolism predicts greater response to a range of antidepressants, including paroxetine (4), nortriptyline (5), citalopram (6), and fluoxetine (7), but also to placebo (8). Highlighting the robustness of this finding, a meta-analysis showed that depression improvement was linked to higher pretreatment rACC activity in 19 separate studies (2), although a number of nonreplications emerged (9–12). This finding was recently replicated a 20th time (13) in the Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) study, an 8-week clinical trial of sertraline for MDD (14). Importantly, pretreatment rACC theta current density [associated with heightened rACC metabolism (15)] displayed incremental predictive validity in relation to treatment outcome (across both sertraline and placebo conditions) over and above a range of clinical and demographic factors previously associated with better MDD prognosis.

The rACC may influence treatment responsiveness by facilitating adaptive communication among large-scale functional networks (2). It is the main node within the anterior portion of the default mode network (DMN) and shows coordinated activity under task-free conditions with other regions in this network, including the posterior cingulate cortex (PCC) (the main node within the posterior portion of the DMN), angular gyrus, middle and superior frontal gyri, and middle temporal gyrus. The DMN is thought to support self-referential processing and exhibits greater activity under task-free conditions relative to conditions requiring external focus (16). Resting-state functional connectivity studies have revealed hyperconnectivity within the DMN in MDD, which might support persistent negative self-referential thinking (17).

Given its location within the DMN and structural connections with other areas of the prefrontal cortex, the rACC also communicates with the frontoparietal network (FPN) to support emotion regulation and goal-oriented responding (18)—two processes that require a downregulation of DMN activity. The FPN and DMN are typically anticorrelated (19), but meta-analyses indicate that individuals with MDD exhibit weaker anticorrelations between these networks (17), potentially leading to DMN interference in conditions requiring external focus. Similarly, a recent electroencephalography (EEG) source localization study showed that elevated connectivity between the DMN and FPN in the beta frequency band was linked to a more recurrent illness course (20), indicating that aberrant communication between these networks may be associated with MDD trajectory.

Finally, the rACC also has anatomical connections to regions in the salience network (SN), particularly the right anterior insula (rAI) (21,22), which is thought to play a critical role in emotional processing (23). This network supports the detection of emotionally salient stimuli, and the rAI in particular is thought to coordinate anticorrelated activity between the DMN and FPN (24,25). The SN is typically anticorrelated with the DMN (26); however, there is debate as to whether more or less anticorrelated rACC–SN activity may facilitate depression improvement. Weaker anticorrelated rACC and SN activity (particularly AI activity) has been observed in individuals with severe depression (21). Furthermore, greater baseline rACC–SN connectivity has been found to predict depression improvement following 1 week of placebo and 10 weeks of antidepressant treatment (22). It has been suggested that enhanced rACC–SN connectivity may confer a greater capacity for adaptively responding to emotionally salient stimuli, highlighting a potential link between rACC–SN connectivity and the responsiveness of the depressed state to intervention.

Together, these findings suggest that rACC activity may influence depression improvement via connections with other regions within the DMN and also by facilitating DMN connectivity with other networks such as the FPN and SN. Building on recent findings in the EMBARC study showing that baseline rACC theta activity prognostically predicted treatment outcome (13), this study examined whether theta-band synchronization between the rACC and other regions of the DMN, as well as the FPN and SN, predicts depression improvement. Because an independent study showed that elevated beta-band DMN–FPN connectivity was associated with a more

recurrent depressive illness course (20), we also evaluated connectivity within the beta frequency.

In line with prior work (22), we hypothesized that greater depressive symptom reduction would be predicted by increased pretreatment rACC–SN connectivity. In contrast, given prior work linking heightened within-DMN connectivity (17) and DMN–FPN connectivity (20) to greater depression severity, we hypothesized that greater depressive symptom reduction would be predicted by decreased rACC–DMN and rACC–FPN connectivity. In addition, given that the local activity/baseline metabolism of a region has been found to determine that same region's resting-state functional connectivity (27), we also examined whether rACC connectivity moderated or mediated the link between rACC activity and depression improvement. Finally, recent evidence (also based on data from the EMBARC trial) indicates that early changes in rACC cortical thickness following the first week of treatment with sertraline—potentially reflecting increases in cortical serotonin 1A receptor concentrations—predicted greater reduction in depressive symptoms over the course of treatment (28). Because sertraline may also have acute effects on functional connectivity of the rACC with other regions, we also examined whether early changes in rACC connectivity during the first week of treatment were associated with the likelihood of achieving remission.

METHODS AND MATERIALS

The EMBARC study design, recruitment, randomization methods, power calculation, and assessment measures can be found elsewhere (14) and in the Supplement. Methods pertinent to this study are outlined below.

Study Design

Using a double-blind design, participants were randomly assigned to 8 weeks of sertraline or placebo. The primary outcome was depression severity on the 17-item clinician-rated Hamilton Rating Scale for Depression (HRSD-17) (29) administered at baseline and weeks 1, 2, 3, 4, 6, and 8. EEG was recorded at baseline and week 1.

Sample

Outpatients aged 18 to 65 years meeting criteria for MDD based on the Structured Clinical Interview for DSM-IV (30) were recruited at Columbia University College of Physicians and Surgeons, Massachusetts General Hospital, the University of Michigan, and the University of Texas Southwestern Medical Center. A Quick Inventory of Depressive Symptomatology (31) score of ≥14 (moderate depression) was required at screening and randomization visits. Study procedures were approved by the institutional review boards of all sites. Participants provided written informed consent after receiving a complete study description.

From July 2011 to December 2015, 634 individuals were screened and 296 were randomized to sertraline or placebo. Of the latter individuals, 9 dropped out before taking medication, 266 (92.3%) had EEG data collected, and 248 were included in the final model reported by Pizzagalli et al. (13). Of this sample, 10 subjects were excluded from the current study for having <40 seconds of artifact-free segments available for

connectivity analysis (the recommended amount), leaving a final sample of 238 subjects. The study flow diagram is shown in Supplemental Figure S1, with dropout reasons listed in Supplemental Table S1.

EEG Acquisition and Preprocessing

EEG data were recorded in four 2-minute eyes-open and eyes-closed trials. Different EEG acquisition systems were used across sites; therefore, a manual was developed to standardize recording techniques (see Supplemental Methods). Briefly, EEG data from each site were interpolated to a common 72-channel montage and resampled at 256 Hz. Then, a standardized preprocessing pipeline was used to extract 2-second nonoverlapping artifact-free epochs for connectivity analyses (32). In line with prior work (20,33), the first 40 seconds of artifact-free data were analyzed.

Region-of-Interest Selection

To probe FPN connectivity, a left dorsolateral prefrontal cortex seed was defined using coordinates from Dosenbach *et al.* (34). For DMN connectivity analyses, a midline PCC seed was defined using coordinates from Yeo *et al.* (35). For SN analyses, an rAI seed was defined using coordinates from Seeley *et al.* (36) because this right hemisphere region is thought to modulate DMN and FPN activity (24). Finally, an rACC seed was defined using prior work examining predictors of treatment response (5,13). Seed coordinates are shown in Supplemental Table S2. Seeds were used to create regions of interest (Figure 1) consisting of gray matter voxels within a 10-mm radius of the seed. Intracortical current source density at each region of interest was then computed using the linear inverse solution, exact low-resolution electromagnetic tomography (33).

Source-Based Functional Connectivity

Connectivity between sources was computed using lagged phase synchronization, which quantifies the nonlinear non-instantaneous relationship between two signals (33). Instantaneous EEG-based connectivity measures have limited utility given that they are susceptible to volume conduction, which leads to artificially correlated activity at different regions because the electrical signal spreads out laterally when it reaches the skull. However, non-instantaneous or lagged connectivity measures correct for this by computing the connectivity between two regions after any instantaneous contribution has been removed. Lagged phase synchronization was computed in the theta (4.5–7 Hz) and beta (12.5–21 Hz) frequencies (see Supplemental Methods for details).

Statistical Analyses

Linear mixed-effect models (implemented in STATA 13.1; StataCorp, College Station, TX) evaluated whether rACC connectivity predicted HRSD score reductions across 8 weeks. Participants were treated as random effects, with subject-specific estimates for both intercept (estimated week 8 HRSD scores) and slope (weekly change in HRSD scores). Analyses were conducted in two stages. First, we entered
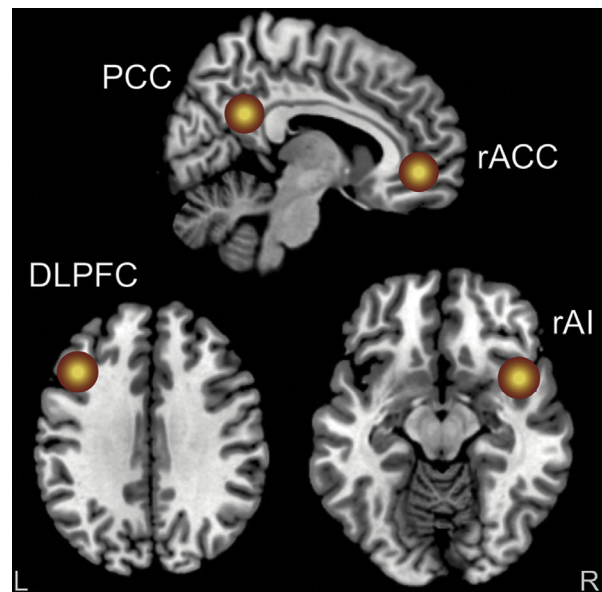
Figure 1. Regions of interest (10-mm radius) that were created in the rostral anterior cingulate cortex (rACC), posterior cingulate cortex (PCC), left dorsolateral prefrontal cortex (DLPFC), and right anterior insula (rAI). Resting-state functional connectivity was then computed (by means of lagged phase synchronization) between the rACC and the PCC (default mode network), left DLPFC (frontoparietal network), and rAI (salience network) in both the theta (4.5–7 Hz) and beta (12.5–21 Hz) frequency bands. For the purposes of visualization, regions of interest shown here are displayed on a 2 × 2 × 2 Montreal Neurological Institute template brain (5 mm resolution is used for analyses in exact low-resolution electromagnetic tomography). L, left; R, right.

demographic/clinical covariates linked to treatment response in MDD (Supplemental Table S3) as well as the baseline rACC theta activity terms that were included in the final model reported in Table 2 of the earlier study published by Pizzagalli *et al.* (13). Second, connectivity and connectivity × time (weeks 0, 1, 2, 3, 4, 6, and 8 centered at week 8) terms were added to the model. We applied a conservative criterion (13) whereby connectivity terms needed to be associated with both the intercept (connectivity effect) and slope (connectivity × time interaction) at $p < .05$ to be considered significant. For models containing significant connectivity terms, we used a likelihood ratio test to evaluate the goodness of fit of this extended model relative to the model containing only the covariates and baseline rACC theta activity terms.

For connectivity terms that were associated with both the intercept and slope, and that yielded a significantly improved model fit, we tested whether rACC connectivity moderated the relationship between baseline rACC theta activity and depression improvement by adding a connectivity × rACC theta term and a connectivity × rACC theta × time term. A significant interaction term was taken as evidence of moderation.

For mediation analyses, we evaluated a model in which baseline rACC connectivity mediated the relationship between baseline rACC theta activity and HRSD score improvement (baseline to week 8). Because prior work has shown that rACC connectivity changes after 1 week of placebo are correlated

Table 1. Demographic and Clinical Characteristics of the Analyzed Sample

	Whole Sample (N = 238)	CU Site (n = 75)	MG Site (n = 36)	TX Site (n = 83)	UM Site (n = 44)	p Value
Age in Years, Mean (SD)	36.9 (13.2)	33.5 (11.0)[a]	33.2 (13.1)[a]	43.5 (12.4)[b]	33.4 (14.0)[a]	<.001
Female, n (%)	151 (63.4)	49 (65.3)	18 (50.0)	52 (62.7)	32 (72.7)	.21
Years of Education, Mean (SD)	15.1 (2.4)	15.6 (2.1)	15.0 (2.5)	14.6 (2.7)	15.1 (2.3)	.09
Caucasian, n (%)	163 (68.5)	45 (60.0)	26 (72.2)	57 (68.7)	5 (11.4)	.16
Hispanic, n (%)	42 (17.6)	19 (25.3)[a]	2 (5.6)[b]	18 (21.7)[a]	3 (6.8)[b]	.01
Married, n (%)	49 (20.6)	9 (12.0)	7 (19.4)	22 (26.5)	11 (25.0)	.13
Employed, n (%)	135 (56.7)	41 (54.7)	26 (72.2)	40 (48.2)	28 (63.6)	.07
Age of MDD Onset, Mean (SD)	16.3 (5.7)	17.1 (5.9)[a]	16.2 (4.3)[a,b]	16.8 (6.4)[a,b]	14.2 (4.5)[b]	.04
Current MDE Length in Months, Median	15.5	20.0	8.5	30.0	6.0	.09
Number of Prior MDEs, Median	4	3	5	5	6	.19
QIDS, Mean (SD)	18.2 (2.8)	18.8 (2.8)[a]	17.5 (2.8)[a,b]	17.5 (2.5)[b]	18.7 (3.1)[a,b]	.01
17-Item HRSD, Mean (SD)	18.5 (4.5)	17.9 (4.4)	19.9 (4.0)	18.6 (4.5)	18.0 (4.8)	.11

The p values indicate the significance value associated with the main effect of site. Where the main effect of site was significant at $p < .05$, superscript letters are used to denote the results of Bonferroni-adjusted pairwise comparisons between sites. Sites with the same superscript letter did not differ significantly from each other.

CU, Columbia University College of Physicians and Surgeons; HRSD, Hamilton Rating Scale for Depression; MDD, major depressive disorder; MDE, major depressive episode; MG, Massachusetts General Hospital; QIDS, Quick Inventory of Depressive Symptoms; TX, University of Texas Southwestern Medical Center; UM, University of Michigan.

with depressive symptom improvement (22), we tested a second mediation model in which early change (baseline to week 1) in rACC connectivity was the mediator.

Finally, we examined whether connectivity was associated with clinically meaningful outcomes: 1) treatment response, defined as >50% reduction in HRSD scores by week 8, and 2) depression remission, defined as an HRSD score ≤7 at week 8.

RESULTS

Sample characteristics of the 238 subjects included in this analysis are shown in Table 1, with further details shown in Supplemental Table S4.

Effects of Baseline rACC Connectivity on Depression Improvement

A main effect of connectivity ($B = -3.01$, 95% confidence interval [CI] = -5.65, -0.37, $p = .03$) and a connectivity × time interaction ($B = -0.59$, 95% CI = -1.07, -0.10, $p = .02$) emerged for rACC–rAI (SN hub) connectivity in the theta band. Specifically, across the entire sample (placebo and sertraline groups), elevated theta-band rACC–rAI connectivity predicted lower week 8 HRSD scores and greater symptom improvement over 8 weeks, controlling for demographic/clinical covariates and baseline rACC theta activity. A likelihood ratio test showed that a model containing these two connectivity terms (Table 2) provided improved fit relative to a covariates + rACC theta activity–only model (likelihood ratio = 6.69, $p = .04$). Notably, when connectivity terms were entered into the model, both rACC theta activity terms remained significant predictors of symptom improvement (rACC theta term: $B = -3.82$, 95% CI = -6.50, -1.15, $p = .01$; rACC theta × time term: $B = -0.57$, 95% CI = -1.07, -0.08, $p = .02$). Furthermore, baseline theta-band rACC–rAI connectivity was uncorrelated with rACC theta activity ($r = .06$, $p = .39$), indicating that these

two metrics were independent predictors of depression improvement. Aligning with rACC theta activity findings reported by Pizzagalli et al. (13), connectivity terms did not interact with treatment condition in predicting symptom change (both $ps > .05$), suggesting that they are treatment-nonspecific (i.e., prognostic) predictors of symptom improvement.

In contrast, neither theta-band rACC–PCC (the key posterior DMN region) connectivity nor theta-band rACC–left dorsolateral prefrontal cortex (the key FPN region) connectivity emerged as a predictor of depression improvement (all $ps > .05$). Furthermore, when considering beta-band connectivity, no models showed both a significant effect of connectivity and a connectivity × time interaction (all $ps > .05$) (see Supplemental Results). Taken together, these results specifically highlight theta-band rACC–rAI connectivity as a predictor of depression improvement.

rACC Connectivity as a Moderator or Mediator of the Effect of Baseline rACC Activity on Depression Improvement

For theta-band rACC–rAI connectivity, neither the connectivity × rACC theta interaction ($B = 3.30$, 95% CI = -8.47, 15.06, $p = .58$) nor the connectivity × rACC theta × time interaction ($B = 0.61$, 95% CI = -1.54, 2.75, $p = .58$) was significant, indicating no moderation. We also found no evidence for theta-band rACC–rAI connectivity acting as a mediator. The two mediation models tested are described in the Supplemental Results and shown in Supplemental Figure S2.

rACC Connectivity as a Predictor of Depression Remission

Theta-band rACC–rAI connectivity changes from baseline to week 1 predicted remission status after controlling for baseline HRSD scores (odds ratio = 2.90, 95% CI = 1.11, 7.58, $p = .03$). Specifically, as theta rACC–rAI connectivity changes from

Table 2. Linear Mixed Model Showing Theta-Band rACC–rAI Connectivity as a Predictor of HRSD Score Improvement Across 8 Weeks

Model Term	Coefficient	SE	Z	p
Time	−3.19	0.94	−3.41	<.001
Treatment	5.86	2.68	2.19	.03
Time × Treatment	−0.19	0.25	−0.74	.46
Site	1.52	0.37	4.15	<.001
Time × Site	0.17	0.07	2.50	.01
Treatment × Site	−0.17	0.53	−0.33	.74
Time × Treatment × Site	−0.02	0.10	−0.25	.80
Depression Severity[a]	0.48	0.09	5.55	<.001
Time × Depression Severity	−0.07	0.01	−5.39	<.001
Treatment × Depression Severity	−0.27	0.11	−2.54	.01
Anxiety Severity[b]	0.10	0.05	2.21	.03
Age	0.14	0.03	4.67	<.001
Time × Age	0.01	0.00	1.41	.16
Treatment × Age	−0.08	0.04	−2.14	.03
Gender	−0.53	0.51	−1.03	.31
Race	0.33	0.34	0.99	.32
Time × Race	0.06	0.06	0.99	.32
Marital Status	−0.96	0.31	−3.10	<.001
Employment Status	−0.08	0.35	−0.23	.82
Treatment × Employment Status	0.49	0.53	0.93	.35
rACC Theta	−3.82	1.37	−2.80	.01
Time × rACC Theta	−0.57	0.25	−2.28	.02
Theta-Band rACC–rAI Connectivity	−3.01	1.35	−2.23	.03
Time × Theta-Band rACC–rAI Connectivity	−0.59	0.25	−2.37	.02

rACC, rostral anterior cingulate cortex; rAI, right anterior insula.

[a]Depression Severity from baseline Hamilton Rating Scale for Depression (HRSD) total score.

[b]Anxiety Severity from Anxious Arousal subscale of the Mood and Anxiety Symptom Questionnaire.

baseline to week 1 increased by 1 unit, a participant was 2.9 times more likely to achieve symptom remission by week 8 (connectivity change in remitters [n = 73]: mean = 0.44, SD = 0.34; change in nonremitters [n = 122]: mean = 0.32, SD = 0.31). Theta-band rACC–rAI connectivity changes in remitters and nonremitters are shown in Figure 2, with tests of potential confounds reported in the Supplemental Results.

DISCUSSION

Baseline theta rACC activity has emerged as an important indicator of clinical response to a range of depression interventions, including antidepressants, electroconvulsive therapy, and sleep deprivation as well as placebo (13), and—in combination with known clinical/demographic predictors of depression prognosis—could be used to identify patients who require careful monitoring and more intensive intervention. Because the rACC has rich anatomical connections with large-scale functional networks involved in attention, emotion regulation, and cognitive control (2), we hypothesized that rACC connectivity with other brain systems may play a mechanistic role in depression recovery. Several key findings emerged. First, greater theta-band connectivity between the
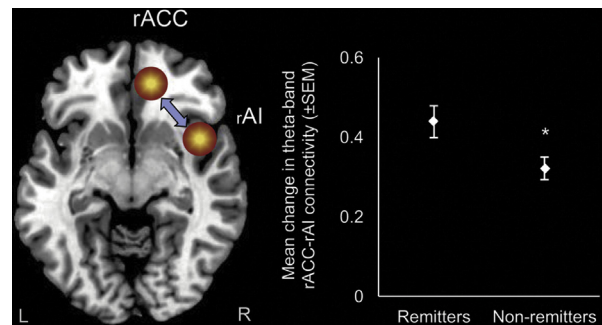
Figure 2. Early changes (baseline to week 1) in theta-band connectivity between the rostral anterior cingulate cortex (rACC) and the right anterior insula (rAI)—a major region in the salience network—as a function of depression remission status. Remission was defined as a Hamilton Rating Scale for Depression score of ≤7 at week 8. *p = .02. L, left; R, right.

rACC and rAI—a key region within the SN—predicted greater reduction in depression severity across treatment conditions, controlling for demographic/clinical covariates and baseline rACC activity. Second, adding theta-band rACC–rAI connectivity as a predictor provided an improved model fit compared with a model containing only the demographic/clinical covariates and rACC activity. Importantly, in this final model, rACC activity remained a significant predictor of depression improvement. Combined with the lack of evidence for rACC connectivity moderating or mediating the link between baseline rACC activity and symptom improvement, this suggests that rACC activity and rACC connectivity are independent predictors of depression improvement. Third, baseline theta-band rACC–rAI connectivity did not interact with treatment group, indicating that it represents a nonspecific prognostic predictor of depression improvement [as previously found for baseline rACC activity (13)]. Fourth, increases in theta-band rACC–rAI connectivity from baseline to week 1 predicted a greater likelihood of achieving remission by week 8, indicating that early connectivity changes may be a useful marker of clinically meaningful outcomes.

Prior work has shown that rACC activity increases under conditions involving emotional conflict (37) or inhibiting attention to irrelevant emotional information (38). Consequently, elevated rACC activity may reflect a greater ability to modulate emotional responding using top-down control (2), and this may in turn promote better outcomes. Our findings extend this by showing that communication between the rACC and a region that is involved in the detection of personally salient events, and that regulates communication between the DMN and FPN (25), may be another important predictor of future symptom improvement. One explanation is that rACC–SN synchronization may aid in DMN downregulation in response to emotionally salient events, and this may be a mechanism that facilitates depression recovery. Support for this comes from a study in healthy individuals, which showed that ignoring task-irrelevant unpleasant words was associated with task-evoked increases in rACC–SN functional connectivity (39). Furthermore, disruption of this functional coupling via brain injury–related damage to the white matter tract linking the rAI to the ACC results in difficulty in deactivating the DMN under conditions requiring external task focus (40).

Communication between the rACC and rAI may also be implicated in monitoring the salience of one's emotions and interoceptive states, and this may partially explain the link between rACC–rAI connectivity and clinical response to placebo observed in our study and in other work (22). For example, rAI and ACC coactivation has been observed when subjects view pictures of their bodies (41), and connectivity between these regions has been found to be negatively correlated with impairments in social awareness and self-awareness in healthy adults (42). This hints at the role of rACC–rAI connectivity in adaptive self-related processes, which may play an important role in both antidepressant and placebo effects. Furthermore, our observations that rACC connectivity with the DMN (the PCC region) or the FPN (the left dorsolateral prefrontal cortex region) was not a predictor of depression improvement suggests that the integrity of systems that coordinate DMN–FPN switching (i.e., the SN), rather than the integrity of the DMN or FPN per se, may be more closely associated with the responsiveness of the depressed state to intervention. Moreover, the specificity of our findings to the theta band may reflect the putative role that the ACC (including the rACC) has in generating frontal midline theta frequency synchronization [e.g., see (15)].

Our finding that early changes (i.e., after 1 week of treatment) in theta-band rACC–rAI connectivity predicted depressive symptom improvement aligns with prior findings showing that changes in rACC cortical thickness after 1 week of sertraline treatment [potentially reflecting increased serotonin 1A receptor concentrations (28)] predicted greater depressive symptom improvement. Furthermore, involvement of the rAI is consistent with prior studies showing that changes in activity among a set of brain regions (including the insula) following 1 week of treatment with a selective serotonin reuptake inhibitor were predictive of greater therapeutic response (22). However, in the current study, early changes in theta-band rACC–rAI connectivity (and the relationship between these early changes and better depression improvement) cannot be entirely attributed to the effects of sertraline given that theta-band rACC–rAI connectivity predicted better response to both sertraline and placebo. Future research is needed to determine what neuromodulatory processes may influence early changes in functional connectivity in individuals undergoing treatment with placebo. In the context of our findings, enhanced baseline theta-band rACC–rAI connectivity and early changes in this connectivity may be an indicator of the degree to which an individual's depressive symptoms are responsive to intervention more generally. An important avenue for future studies will be to examine whether this reflects 1) a unique subtype of depression characterized by early response to treatment or 2) a marker that is indicative of remission that is currently/already in progress. Examining changes in theta-band rACC–rAI connectivity over a longer time course during treatment (e.g., from baseline to week 8) would allow for these competing interpretations to be tested. Furthermore, it will be important to link this marker to previously reported depression endophenotypes (43).

We initially hypothesized that rACC–outcome associations observed in prior work [e.g., (32)] may be driven by rACC connectivity; however, we found no evidence for rACC connectivity acting as a moderator or mediator. Although we cannot infer directionality from our analysis, the link between rACC–rAI connectivity and depression improvement may be driven by inputs coming from the rAI. Support for this comes from dynamic causal modeling research showing that the rAI acts as a "causal outflow hub" within the SN that triggers FPN modulation of the DMN in accordance with salient events (24). Another dynamic causal modeling study points to the relevance of excitatory rAI signaling in depression, showing weaker excitatory input from the rAI to the middle frontal gyrus in MDD patients compared with control subjects (44). In the context of our study, coordinated input from the rAI to the DMN (via the rACC) may facilitate adaptive processing of emotionally salient events, which may in turn promote treatment responsiveness.

An important next step is to determine whether malleability of theta-band rACC–rAI connectivity identifies patients whose depression is likely to spontaneously remit or whether it indicates patients who show greater susceptibility to placebo effects. Although these two processes are likely to be closely related, links between rACC–rAI connectivity and greater placebo response will have important implications for clinical trials. For example, if the mechanism by which elevated baseline theta-band rACC–rAI connectivity facilitates greater symptom improvement is via greater susceptibility to placebo effects, then this may be used to identify individuals for whom treatment-nonspecific factors are likely to play a larger role in determining treatment outcome. This in turn might allow for a better estimation of treatment-specific effects.

Some limitations must be emphasized. First, although EEG source functional connectivity has high temporal resolution for examining connectivity at discrete frequencies, lagged phase synchronization quantifies only synchronization strength (ranging from 0 to 1) and does not indicate synchronization direction. Studies using metrics that assess both connectivity strength and direction are needed to confirm whether greater positive or greater anticorrelated theta-band rACC–rAI connectivity predicts depression improvement. Causal links between rACC–rAI connectivity and depression improvement should also be probed using neurostimulation techniques that modulate fronto–insula connectivity [e.g., prefrontal theta-burst stimulation (45)]. Second, source localization techniques cannot estimate connectivity involving subcortical regions. Subcortical dysfunction is critical to MDD pathophysiology; therefore functional magnetic resonance imaging–based connectivity studies must examine relationships between rACC–subcortical connectivity and depression improvement. Third, in addition to showing significant main effects and interactions involving theta-band rACC–rAI connectivity, the final model also revealed a number of unanticipated significant effects that warrant further investigation. These include a main effect of site and a site $\times$ time interaction, both of which were unanticipated owing to standardization of treatment across study sites. The significant treatment $\times$ age interaction was also unanticipated because there is little evidence to suggest that the effects of sertraline (relative to placebo) are moderated by patient age in adults aged 18 to 65 years. Finally, because our sample was composed of individuals with chronic or recurrent MDD with onset before 30 years of age, further research is needed to

assess the generalizability of our findings to individuals with milder or later onset depression.

In sum, our findings suggest that in patients with MDD undergoing treatment with sertraline or placebo, elevated baseline theta-band connectivity between the rACC and rAI—a key region of the SN—is an important prognostic treatment-nonspecific indicator of depression improvement, and early changes in this connectivity may be useful for identifying patients likely to achieve remission. In conjunction with recent findings (13), our results indicate that lower pretreatment rACC activity and reduced rACC–rAI connectivity at baseline may be useful markers for identifying patients with MDD who would benefit from more careful monitoring or intensive intervention.

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ARTICLE INFORMATION

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**Pretreatment Rostral Anterior Cingulate Cortex Connectivity
With Salience Network Predicts Depression Recovery:
Findings from the EMBARC Randomized Clinical Trial**

Supplementary Information

Contents:

Supplementary Methods and Materials

Sample size and power analyses for the clinical trial

The sample size of 300 was chosen to allow at least 80% power (α=0.05, two-tailed) to detect interaction effects of multiple (~40) potential moderators of the treatment on depressive symptom improvement, after adjusting for multiple comparisons. Based on prior work, the effect sizes of the moderators were hypothesized to be between 0.15 and 0.2.

Methods used to generate the random allocation sequence

Randomization was conducted according to site, depression severity and depression chronicity. Within each of these levels, block randomization with a random block size of 2 or 4 was applied using the commercial clinical trial data management software StudyTrax. For each potential participant, a site coordinator would input information regarding all inclusion/exclusion criteria, after which the software crosschecked this information for eligibility. If the participant was deemed to be eligible, the software provided a random assignment, which was communicated directly to the site pharmacist.

Participant inclusion/exclusion criteria

All patients reported MDD onset before 30, and had either a chronic (episode duration > 2 years) or recurrent ($\geq$ 2 recurrences including the current episode) illness course. Participants were excluded from the study if they were currently pregnant, breastfeeding or were planning to become pregnant in the near future; had a lifetime history of bipolar disorder or psychotic disorder; met criteria for substance dependence in the past six months or substance abuse in the past two months; displayed evidence of unstable medical or psychiatric symptoms that required hospitalization; had any study medication contraindications; had clinically significant laboratory abnormalities; had a

history of epilepsy or any condition requiring anticonvulsant medication; had received transcranial magnetic stimulation, vagal nerve stimulation or electroconvulsive therapy during the current depressive episode; were currently taking psychotropic medications; were currently receiving psychotherapy; displayed evidence of significant suicide risk; failed to respond to any antidepressant at adequate dose and duration in the current episode.

Participant compensation

Compensation for the study components relevant to the current analyses was as follows:

- Completion of the detailed interview and questionnaires administered at screening – $150
- Completion of the two EEG recordings – $68

Compensation for other study components that are not presented in this study, was as follows:

- Completion of two MRI scans – up to $200
- Completion of a behavioral task – up to $32
- Completion of blood samples for research purposes – $25 per sample, up to $175 total
- Completion of genetic blood sampling – $50
- Completion of the final clinical rating session of the study – $50

The total possible compensation for the study was $725.

Participants lost to follow-up

Of the 143 participants who received sertraline, 117 completed all 8 weeks of the intervention, whereas 26 discontinued (7 of whom were lost to follow-up). Of the 144 participants who received placebo, 125 completed all 8 weeks of the intervention, whereas 19 discontinued (5 of whom were lost to follow-up). A summary of the reasons why participants dropped out is provided in Table S1.

EEG systems used across the four recording sites

Columbia University. 72-channel EEG was recorded using a 24-bit BioSemi system with a Lycra stretch electrode cap (Electro-Cap International Inc., Ohio), sampled at 256 Hz (bandpass: DC-

251.3 Hz). An active reference (ActiveTwo EEG system) at electrode locations PPO1 (common mode sense) and PPO2 (driven right leg) were used.

McLean Hospital. 129-channel EEG was recorded using a Geodesic Sensor Net system (Electrical Geodesics, Inc., Eugene, Oregon), sampled at 250 Hz (bandpass: 0.01-100 Hz). Data were referenced to the vertex (Cz) at acquisition.

University of Michigan. 60-channel EEG was recorded using a 32-bit NeuroScan Synamp system (Compumedics, TX) using a Lycra stretch electrode cap (Electro-Cap International Inc., Ohio), sampled at 250 Hz (bandpass: 0.5-100 Hz). A nose reference was used during acquisition.

University of Texas. 62-channel EEG was recorded using a 32-bit Neuroscan Synamp system (Compumedics, TX) using a Lycra stretch electrode cap (Electro-Cap International Inc., Ohio), sampled at 250 Hz (bandpass: DC-100 Hz). A nose reference was used during acquisition.

EEG preprocessing

A standardized analysis pipeline was developed and implemented by researchers at the Columbia site to minimize cross-site differences (1). First, data were interpolated to a common, 72-channel montage using spherical spline (2) and resampled at 256 Hz. Second, electrodes with poor signal were interpolated using a spherical spline interpolation (recordings with less than 80% of usable data were discarded). Third, a spatial principal component analysis was used to correct for blink artifacts (3). Fourth, artifact-free data were segmented into 2 second, non-overlapping epochs, and bandpass filtered at 1-60 Hz (24-dB/octave). Fifth, residual artifacts were identified on an individual channel basis within each epoch using a semiautomated reference-free approach (4). Finally, flagged channels were interpolated using spherical spline from data of all valid channels for a given epoch if less than 25% of channels were flagged for this epoch.

Evidence for the validity of the LORETA algorithm

The eLORETA solution space consists of 6239 cortical gray matter voxels in a realistic head model using the Montreal Neurological Institute 152 template. Validation for the LORETA algorithm comes from studies using simultaneous EEG and fMRI (5) as well as in an EEG localization study for epilepsy (6). The algorithm has also received validation from studies examining LORETA and fMRI data (7-9), or LORETA and PET data (10-12) in the same samples. In a review of independent source localization techniques, sLORETA – the algorithm upon which the eLORETA algorithm used in the current study was based – was found to perform best in terms of localization error (13). In the context of functional connectivity, eLORETA has been found to minimize the detection of false positive connections significantly more so compared to other EEG source localization methods (14).

Additional information about computation of lagged phase synchronization

Lagged phase synchronization is a metric that refers to the nonlinear dependence between the phases of pairs of intracortical EEG source estimates. It is a measure of phase synchrony between intracortical signals in the frequency domain (calculated using normalized Fourier transforms). The strength of this method is its ability to minimize the impact of volume conduction on EEG source-based connectivity estimates. Specifically, volume conduction refers to the tendency for intracortical signals to spread laterally upon contact with the skull, and this causes spurious correlations in activity detected at neighboring scalp-level electrodes. To minimize the effects of volume conduction, the instantaneous "zero-lag" contribution is excluded from the total phase synchronization, leaving only non-instantaneous synchronization.

Total phase synchronization (which is susceptible to volume conduction effects) is typically computed using the following formula:

$$\varphi^2{}_{x,y}(\omega) = |f_{x,y}(\omega)|^2 = \{Re[f_{x,y}(\omega)]\}^2 + \{Im[f_{x,y}(\omega)]\}^2 \qquad (1)$$

where: $\qquad f_{x,y}(\omega) = \frac{1}{N_R}\sum_{k=1}^{N_R}\left|\frac{x_k(\omega)}{|x_k(\omega)|}\right|\left|\frac{y_k^*(\omega)}{|y_k(\omega)|}\right| \qquad\qquad (2)$

In this algorithm, "ω" refers to the frequency band, and "x" and "y" are the intracortical sources (i.e., two ROIs in each connectivity pair). "Re" and "Im" indicate the real and the imaginary parts of a complex element C, respectively; x (ω) and y (ω) denote the Fourier transforms of the two signals x and y, respectively, at frequency "ω".

The second part of the formula (2) explains the cycle of C and "*" denotes a complex conjugate (this is where the sign of the imaginary part of a complex number is flipped but the real part is left unchanged). The instantaneous connectivity component is closely related to the real ("Re") part of the phase synchronization.

Lagged phase synchronization partials out the instantaneous component of total connectivity, and is defined as:

$$\varphi_{x,y}^2(\omega) = \frac{\{Im[f_{x,y}(\omega)]\}^2}{1-\{Re[f_{x,y}(\omega)]\}^2} \qquad\qquad (3)$$

This measures the similarity of two time series according to the phases of the signal, after the instantaneous similarity has been removed. A value of 0 indicates no synchronization and 1 indicates perfect synchronization. This measure is thought to capture only physiological connectivity. Additional details on the eLORETA connectivity algorithm can be found in Pascual-Marqui et al (15). In the current study, lagged phase synchronization was computed in the theta (4.5-7 Hz) and beta (12.5-21 Hz) frequency bands using a normalized Fourier transform.

Supplementary Results

Models showing significant connectivity effects at only the intercept or the slope, but not both

Two of the connectivity variables under consideration were found to have significant effects at either the intercept or the slope, but not both, specifically:

In the beta band, there was a significant *Connectivity* x *Time* interaction (effect on the slope) for rACC-PCC (the DMN hub) connectivity (B=-0.54, 95% CI=-1.00, -0.09, *p*=0.02) but the main effect of *Connectivity* (effect at intercept) was at trend (B=-2.12, 95% CI=-4.59, 0.36, *p*=0.09). Exploratory analyses confirmed that adding beta-band rACC-PCC connectivity terms did not provide a significantly better model fit compared to the reduced model that contained the covariates and rACC theta activity (LR=5.62, *p*=0.06).

Also in the beta band, there was a main effect of *Connectivity* (effect at intercept) for rACC-rAI (the SN hub) connectivity (B=2.75, 95% CI=0.15, 5.35, *p*=0.04) however the *Connectivity* x *Time* interaction term was not significant (B=0.10, 95% CI=-0.38, 0.58, *p*=0.68). The addition of beta-band rACC-rAI connectivity terms did not provide a better model fit than the reduced model containing covariates and rACC theta activity model (LR=5.20, *p*=0.07).

Results of mediation models

For illustration purposes, the results of the two mediation models tested are shown in Figure S2. The indirect effect of baseline rACC theta activity on HRSD improvement through baseline theta-band rACC-rAI connectivity was -0.17 (SE=0.30; 95% CI=-0.88, 0.34). In the second mediation model, where change in theta-band rACC-rAI connectivity from baseline to week 1 was evaluated as the potential mediator, the indirect effect was 0.02 (SE=0.03; 95% CI= -0.49, 0.49). The inclusion of

zero within the CIs for both models indicated that neither baseline theta-band rACC-rAI connectivity nor early change (baseline to week 1) in this connectivity was a mediator.

Control analyses examining potential confounds in the link between theta-band rACC-rAI connectivity and remission status

The between-subjects variability in theta-band rACC-rAI connectivity at baseline and week 1, between the placebo and sertraline groups is shown in Fig. S3. As is evident, there were no differences in connectivity between the groups at either time point.

Theta-band rACC-rAI connectivity changes from baseline to week 1 predicted remission status after controlling for baseline HRSD scores (odds ratio=2.90, 95% CI=1.11, 7.58, p=0.03). Aligning with the absence of moderation or mediation effects, we confirmed that theta-band rACC-rAI connectivity changes predicted remission status even when rACC theta activity change was entered into the model (odds ratio=2.94, 95% CI=1.12, 7.71, p=0.03) indicating that the relationship between early theta-band rACC-rAI connectivity changes and symptom remission was not related to early rACC theta activity changes. Theta-band rACC-rAI connectivity also remained a significant predictor when recruitment site was entered into the model as a covariate (p=0.04).

Link between rACC connectivity and depression chronicity

Relative to those with non-chronic MDD at baseline (n=122), those with chronic (episode duration longer than 2 years) MDD (n=116) had lower baseline theta-band rACC-rAI connectivity, $t(236)$=2.83, p=0.005, Cohen's d=0.37 [chronic M=-1.12, SD=0.22; non-chronic M=-1.04, SD=0.21]. This was not driven by differences in symptom severity, as chronic and non-chronic MDD patients did not differ in baseline HRSD scores, $t(236)$=-0.62, p=0.53, and connectivity differences remained significant when controlling for baseline HRSD scores, $F(1, 235)$=7.93, p=0.005, η_P^2=0.03.

Tests of whether early changes in theta-band rACC-rAI connectivity reflect a marker of depression remission that is already in progress during the first week of treatment

As requested by an anonymous Reviewer, we examined whether remitters who were predicted by early changes in theta-band rACC-rAI connectivity were those who showed a decline in HRSD scores from baseline to week 1. If this were the case, then this may indicate that early changes in theta-band rACC-rAI connectivity represents a potential marker of depression remission that is already in progress during the first week of treatment.

To do this, we generated the predicted group membership (remitter vs. non-remitter) from the binary logistic regression model examining the degree to which early changes in theta-band rACC-rAI connectivity from baseline to week 1 predict depression remission status at week 8. The model accurately classified 109 of the 122 individuals who did not remit (89.3% accuracy) but only 12 of the 73 individuals who did remit (16.4% accuracy). Next, we ran a *Remitter* (predicted remitter, predicted non-remitter) x *Week* (baseline, week 1) repeated measures ANOVA to determine whether predicted remitters showed greater depressive symptom reductions from baseline to week 1 relative to predicted non-remitters. Of the 195 individuals with remission status data available, 186 had HRSD data at both baseline and week 1. The *Remitter* x *Week* interaction was not significant, $F(1,184)=0.09$, $p=0.73$, $\eta_p^2<0.001$, indicating that the predicted remitters and predicted non-remitters did not differ in their overall change in HRSD scores from baseline to week 1. There was a main effect of *Week*, $F(1,184)=25.06$, $p<0.001$, $\eta_p^2=0.12$, where across both groups, HRSD scores decreased significantly from baseline to week 1. Furthermore, there was a main effect of *Remitter*, $F(1,184)=23.75$, $p<0.001$, $\eta_p^2=0.11$, where averaged across baseline and week 1, the HRSD scores of the predicted remitters

was significantly lower than the predicted non-remitters (predicted remitters: *M*=13.60, *SE*=0.76; predicted non-remitters: *M*=17.58, *SE*=0.29).

These results suggest that remitters, as predicted by early changes in theta-band rACC-rAI connectivity, were more likely to have lower HRSD scores at the beginning of treatment. This is consistent with the widely-replicated link between lower baseline depression severity and greater responses to treatment.

Symptom trajectories in first and second-stage treatment remitters

At the suggestion of an anonymous Reviewer, we also conducted an exploratory analysis that sought to compare the symptom trajectories of individuals who remitted at the first stage of treatment (i.e., at week 8 and who were predicted by early changes in theta-band rACC-rAI connectivity) to individuals who remitted after a second stage of treatment (i.e., at week 16 and who were not predicted by early changes in theta-band rACC-rAI connectivity) to individuals who never remitted.

The second stage of treatment was conducted from weeks 9 to 16, where some individuals who were randomized to placebo at the first stage of treatment received either placebo or sertraline at the second stage, and some individuals who received sertraline at the first stage were randomized to sertraline again, or to bupropion or placebo at the second stage. To inspect the rate of symptom improvement, we first divided the sample into those who remitted at the first stage of treatment (i.e., those who had a HRSD score ≤7 at week 8), those who remitted at the second stage of treatment (i.e., those who had a HRSD score ≤7 at week 16), and those who never remitted (i.e., those who had a HRSD score >7 at week 16) and plotted the raw mean HRSD (±SEM) scores over time (Fig. S4). Pairwise comparisons focused on differences in HRSD scores at week 8, since early changes in theta-band rACC-rAI connectivity predicted remission status at week 8.

Results showed that week 8 HRSD scores in second stage remitters (*M*=12.87, *SD*=3.42) were significantly higher than first stage remitters (*M*=3.96, *SD*=2.25), $t(119)=17.27$, $p<0.001$, but were

significantly lower than non-remitters (M=16.65, SD=5.25), t(129)=18.84, p<0.001. These findings suggest that second stage remitters fall intermediate between remitters who were predicted by early changes in theta-band rACC-rAI connectivity (i.e., first-stage remitters) and non-remitters in terms of symptom severity. It is possible that second stage remitters may be captured by changes in theta-band rACC-rAI connectivity over a longer time course (e.g., from baseline to week 8). Although EEG data were only obtained at baseline and week 1 in the current study, future studies examining changes in theta-band rACC-rAI connectivity over a longer time course would assist in determining whether this connectivity marker reflects remission that is "in progress" or whether it is a marker that indicates a person's likelihood of achieving remission/early response.

Enrollment

Assessed for eligibility (n = 634)

Excluded (n = 338)
☐ Did not meet inclusion/exclusion criteria (n = 325)
☐ Randomized but did not meet criteria (n = 3)
☐ Treated with different medication (n = 10)

Randomized (n = 296)

Allocation

Allocated to SERTRALINE (n = 146)
   -   Dropped out before 1st dose (n = 3)
☐ Received SERTRALINE (n = 143)

Allocated to PLACEBO (n = 150)
   -   Dropped out before 1st dose (n = 6)
☐ Received PLACEBO (n = 144)

**Analysis
(n = 238)**

Analyzed (n = 117)
☐ Missing baseline EEG (n = 12)
☐ Excluded from analysis because poor EEG data quality (n = 10) or insufficient amount of artifact-free data (n = 4)

Analyzed (n = 121)
☐ Missing baseline EEG (n = 9)
☐ Excluded from analysis because poor EEG data quality (n = 8) or insufficient amount of artifact-free data (n = 6)

Discontinued (n = 22)[a]
☐ Lost to follow-up (n = 8)
☐ Non-adherent (n = 4)
☐ Found Study too Burdensome (n = 3)
☐ Wanted to Discontinue Meds (n = 3)
☐ Believed Treatment not Working (n = 1)
☐ Side Effects Unacceptable (n = 6)
☐ Developed Medical Condition (n = 1)
☐ Became Danger to Self (n = 1)
☐ Hospitalized for Worsening Dep (n = 1)
☐ Hospitalized for Suicidal Id. (n = 1)
☐ Other (n = 3)

Discontinued (n = 10)
☐ Moved from area (n = 1)
☐ Lost to Follow-up (n = 2)
☐ Non-adherent (n = 5)
☐ Wanted to Discontinue Meds (n = 2)
☐ Believed Treatment not Working (n = 1)
☐ Other (n = 6)

Completed 8 week Intervention (n = 95)

Completed 8 week Intervention (n = 111)

[a]Note that there are more reasons for exclusion than there are total discontinued participants as some participants discontinued for more than one reason.

Figure S1. CONSORT flow diagram showing numbers of participants who were randomized to treatment, who received treatment, who had valid EEG data available for the current analyses, and who completed 8 weeks of treatment.
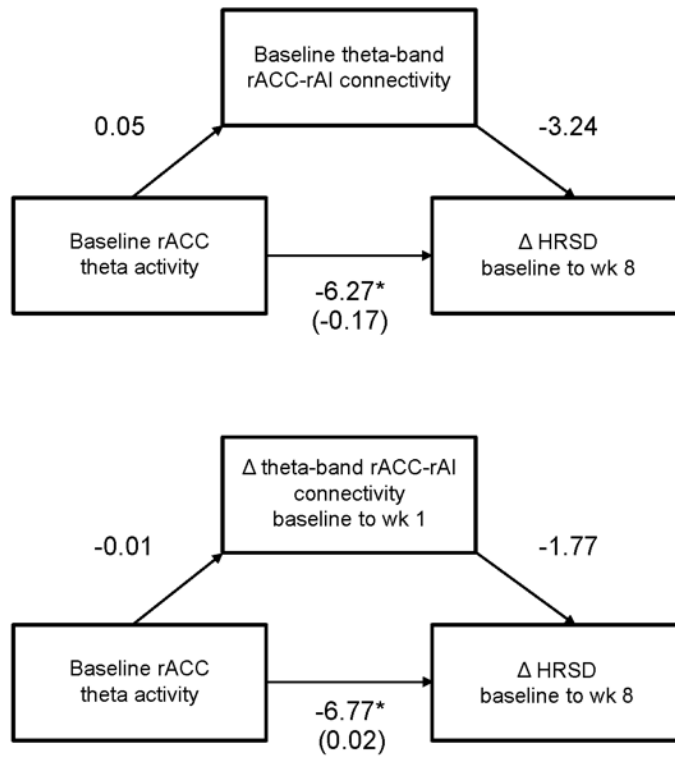
Figure S2. Figure shows mediation models examining the indirect (mediating) effect of baseline theta-band rACC-rAI connectivity (top model) and changes in theta-band rACC-rAI connectivity from baseline to week 1 (bottom model) as potential mediators of the link between elevated baseline rACC theta activity and greater reduction in HRSD scores from baseline to week 8. Neither model shows evidence of theta-band rACC-rAI connectivity acting as a mediator. rACC=rostral anterior cingulate cortex; rAI=right anterior insula, ΔHRSD=change in Hamilton Rating Scale for Depression scores from baseline to week 8; *=significant at p<0.05.
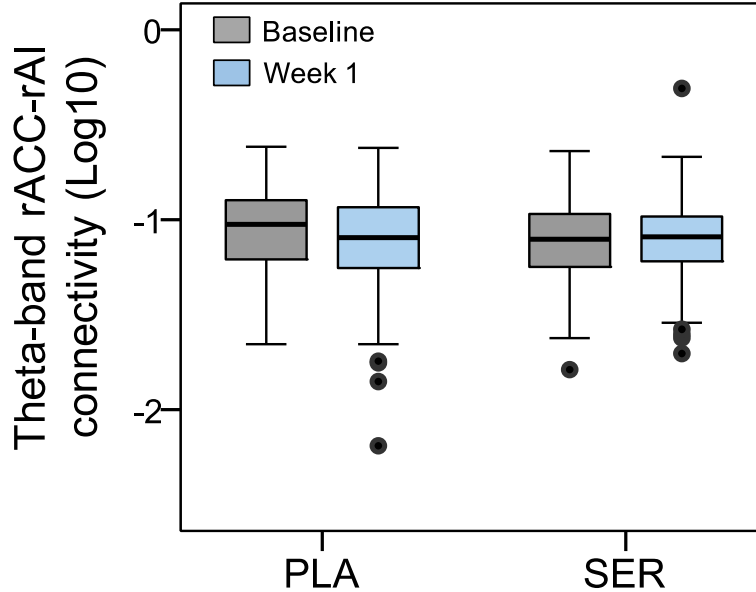
Figure S3. Box plots showing the between-subject variability in theta-band rACC-rAI connectivity between the placebo (PLA) and sertraline (SER) groups at baseline (grey bars) and week 1 (blue bars). Cases represented by black dots are greater than ±2SD from the mean but less than ±3SD, and are not considered outliers. The figure shows that there were no differences in theta-band rACC-rAI connectivity between the two treatment arms either at baseline or at week 1. This suggests that early changes in theta-band rACC-rAI connectivity and their relationship with depression remission at week 8 cannot be solely attributable to the acute effects of sertraline (since the same effects were observed for the placebo group). This further highlights theta-band rACC-rAI connectivity as a prognostic, yet treatment non-specific indicator of depression improvement.
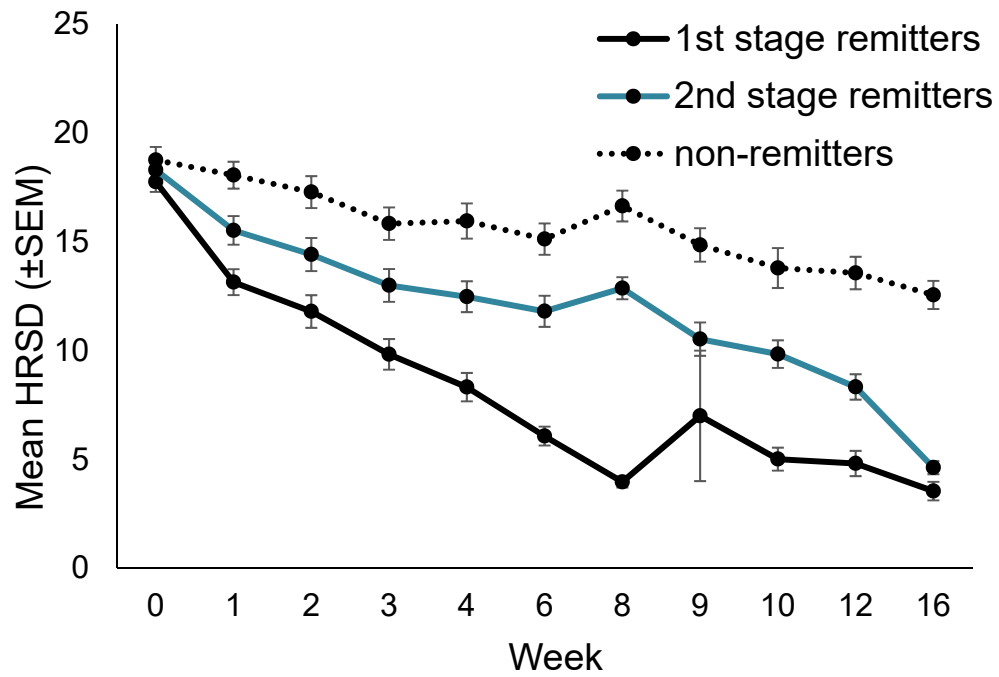
Figure S4. Mean (±SEM) HRSD scores across the first stage (weeks 0-8) and second stage (weeks 9-16) of treatment in individuals who were classified as: 1st stage remitters (HRSD ≤7 by week 8); 2nd stage remitters (HRSD ≤7 by week 16); non-remitters (HRSD >7 at weeks 8 and 16).

Table S1. Reasons for participant dropout across the sertraline and placebo groups

Discontinued Sertraline (n=26)	**Discontinued Placebo (n=19)**
Lost to follow-up (n=7)	Lost to follow-up (n=5)
Non-adherent (n=6)	Non-adherent (n=6)
Wanted to discontinue medication (n=3)	Wanted to discontinue medication (n=4)
Believed treatment was not working (n=1)	Believed treatment was not working (n=2)
Side effects unacceptable (n=9)	Side effects unacceptable (n=1)
Found study too burdensome (n=3)	Moved from area (n=1)
Developed medical condition (n=1)	Became pregnant (n=1)
Became danger to self (n=1)	Other (n=6)
Hospitalized for worsening depression (n=1)	
Hospitalized for suicidal ideation (n=1)	
Other (n=4)	

Note. Numbers add up to more than the totals because participants discontinued for more than one reason.

Table S2. Seed regions used for connectivity analyses

Region	X	Y	Z	Reference
Rostral anterior cingulate cortex	11	45	-6	Pizzagalli et al. (2001), Fig. 1
Posterior cingulate cortex	0	-52	26	Yeo et al. (2011), Table 5
Left dorsolateral prefrontal cortex	-43	22	34	Dosenbach et al. (2007), Table 1
Right anterior insula	42	10	-12	Seeley et al. (2007) Supp. Table 2

Note. X=left(-) to right(+); Y=posterior(-) to anterior(+); Z=inferior(-) to superior(+). Note that regions-of-interest were not registered to subject space from the MNI template, but rather, were retained in MNI space.

Table S3. Demographic and clinical factors that have been identified as predictors of poor outcome in prior studies of depression. Variables capturing each of these factors were used as covariates in our final model and the model reported in Table 2 of Pizzagalli, Webb, et al. (2018)

Covariate	Reference
Greater baseline depression severity (QIDS-SR, HRSD)	Trivedi *et al.* (2006)
Anxious depression (anxiety factor score on the HRSD)	Fava *et al.* (2008)
Anhedonia (CIDI)	Spijker *et al.* (2001)
Male gender	Trivedi *et al.* (2006)
Older age	Fournier *et al.* (2009)
Lower socioeconomic status	Jakubovski *et al.* (2014)
Being non-Caucasian	Trivedi *et al.* (2006)
Being unmarried	Fournier *et al.* (2009)

Note. QIDS-SR=Quick Inventory of Depressive Symptoms, Self-Report; HRSD=Hamilton Rating Scale for Depression; CIDI=Composite International Diagnostic Interview.

Table S4. Demographic and clinical characteristics of the sertraline and placebo groups for the subsample included in the current analysis (n=238)

	Whole sample (n=238)	Sertraline (n=117)	Placebo (*n*=121)	*P* Value
Age in years, M (SD)	36.9 (13.2)	36.6 (13.5)	37.3 (13.0)	0.68
Female, No. (%)	151 (63.4)	79 (67.5)	72 (59.5)	0.20
Years of education, M (SD)	15.1 (2.4)	14.9 (2.4)	15.3 (2.4)	0.21
Caucasian, No. (%)	163 (68.5)	78 (66.7)	85 (70.2)	0.55
Hispanic, No. (%)	42 (17.6)	20 (17.1)	21 (17.4)	0.90
Married, No. (%)	49 (20.6)	22 (26.5)	29 (24.0)	0.19
Employed, No. (%)	135 (56.7)	64 (54.7)	71 (58.7)	0.54
Age of MDD onset, M (SD)	16.3 (5.7)	16.5 (5.8)	16.1 (5.6)	0.63
Current MDE length (months), median	15.5	13.0	18.0	0.49
Number of prior MDEs, median	4	4	5	0.42
QIDS, M (SD)	18.2 (2.8)	18.6 (2.8)	17.7 (2.8)	0.02
HRSD 17-item, M (SD)	18.5 (4.5)	18.4 (4.5)	18.5 (4.4)	0.89

Note. MDD=Major Depressive Disorder; MDE=Major Depressive Episode; QIDS=Quick Inventory of Depressive Symptoms; HRSD=Hamilton Rating Scale for Depression; *P* Values indicate the significance value for tests of differences between the sertraline and placebo group.

Supplementary References

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