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Title: Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression

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Keywords: resting-state functional connectivity; large-scale connectivity networks; biomarkers of treatment response; placebo effects; Major Depression

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Abstract: Background: Recent neuroimaging studies have demonstrated resting-state functional connectivity (rsFC) abnormalities among intrinsic brain networks in Major Depressive Disorder (MDD); however, their role as predictors of treatment response has not yet been explored. Here, we investigate whether network-based rsFC predicts antidepressant and placebo effects in MDD.
Methods: We performed a randomized controlled trial of two weeklong, identical placebos (described as having either "active" fast-acting, antidepressant effects or as "inactive") followed by a ten-week open-label antidepressant medication treatment. Twenty-nine participants underwent a rsFC fMRI scan at the completion of each placebo condition. Networks were isolated from resting-state blood-oxygen-level-dependent signal fluctuations using independent component analysis. Baseline and placebo-induced changes in rsFC within the default-mode, salience, and executive networks were examined for associations with placebo and antidepressant treatment response.
Results: Increased baseline rsFC in the rostral anterior cingulate (rACC) within the salience network, a region classically implicated in the formation of placebo analgesia and the prediction of treatment response in MDD, was associated with greater response to one week of active placebo and ten weeks of antidepressant treatment. Machine learning further demonstrated that increased salience network rsFC, mainly within the rACC, significantly predicts individual responses to placebo administration.
Conclusions: These data demonstrate that baseline rsFC within the salience network is linked to clinical placebo responses. This information could be employed to identify patients who would benefit from lower doses of antidepressant medication or non-pharmacological approaches, or to develop biomarkers of placebo effects in clinical trials.

Trial name: Predictors of antidepressant response.
URL: http://clinicaltrials.gov/ct2/show/NCT02178696?term=zubieta&rank=3.

Trial number: NCT02178696.

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Dr. Carter: September 9th, 2015

Thank you for the opportunity to resubmit a revised version of the manuscript: *"*Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression*"* to the journal *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging.*

Our data demonstrate that in Major Depression, baseline resting-state functional connectivity of the rostral Anterior Cingulate Cortex (rACC) within the salience network predicts placebo and antidepressant responses. Moreover, we found that this predicted effect was confirmed at a single-subject level. By linking inter-individual variation in treatment response with specific neural circuits, these findings could lead to development of simpler treatment-response biomarker, or in the clinical context, the identification of patients who are more likely to response to cognitive interventions.

This study aims to investigate the neurobiological predictors of placebo and antidepressant effects and therefore should not be considered a classical efficacy clinical trial, but a mechanistic study that included placebo and drug administrations. For this reason, and for the purpose of its review, it has not been included within the clinical trial category. However, and for the purposes of regulatory compliance, this study has been registered in the clinicaltrials.gov website, and therefore has a trial number.

The authors attest that the manuscript has been read and approved by all authors and is not under submission to any other journal. The authors have no interests to disclose that are or might be perceived to be in conflict with the work reported in this study.

Thank you again for your consideration of this manuscript.

With best regards,

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Response Letter

Biological Psychiatry

Manuscript: BPS-D-15-01151

Title: "Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression"

We thank the reviewers for their constructive critiques and the editors for the opportunity to resubmit our manuscript to the new journal Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. Each reviewer's comments are addressed point-by-point below. Changes are highlighted in yellow in the separate, revised manuscript.

REVIEWER COMMENTS:

Reviewer #1:

1. Some weaknesses of the paper include the use of a single self-report subjective measure of depression severity (QIDS) as their outcome variable.

Response: The QIDS-16SR was the primary outcome measure of the study and was completed at screening, during the experimental phase and during the 10-week open-label treatment with the SSRI treatment. We chose the QIDS-SR16 as the primary outcome measure based on three main reasons: 1) This questionnaire, together with the Montgomery Asberg Depression Rating Scale (MADRS), are recognized to be more sensitive to change in depressive symptoms than the Hamilton Rating Scale for Depression (HDRS) and is recommended to be used in clinical trials; 2) it is a self-reported questionnaire, which makes it easier to be administered under circumstances where an experienced rater might not be available (e.g. after each fMRI scan), as it was the case for this study; 3) It was also the main outcome measure of the STAR*D study [Control Clin Trials. Feb 2004; 25 (1); 119-42]. However, participants also completed the MADRS and HDRS as secondary measures at screening and during the ten-week open-label trial. These physician-administered questionnaires were chosen to confirm the validity of the self-reported QIDS-16SR, as demonstrated by their high correlation at screening and at each clinical visit. The table below lists MADRS and HDRS correlation with QIDS-16SR.

Table 1: Correlation (r-values reported below) between QIDS-16SR and MADRS and HDRS. *p<0.05; **p<0.01; ***p<0.001

	Screening	Week 0	Week 2	Week 4	Week 8	Week 10
MADRS	0.5*	0.5**	.8***	.9***	.9***	0.8***
HDRS	0.25	0.6***	.7***	.8***	.8***	0.6***

The Methods section now includes: *"This measure was selected as a primary outcome measure for its sensitivity to fluctuations in depression symptoms and its availability as a self-reported measure. Additionally, patients completed the Montgomery Asberg Depression Rating Scale (MADRS) and the Hamilton Rating Scale for Depression (HDRS) at pre-randomization and during each visit within the antidepressant trial."*

2. There was a tendency to overinflate study findings that were not significant throughout the paper.

Response: Initially, we included trending findings due to their interesting nature in the context of MDD treatment literature, but we agree with Reviewer 1 that these are not significant findings and these results have now been removed from the manuscript. However, due to our significant finding that rACC rsFC within the SN predicts placebo responses and the region's consistent role in placebo analgesia neurobiology [Science. Mar 2002; 295 (5560); 1737-40; Pain. Jan 2006; 120(1-2): 8-15] and antidepressant response predictions [reviewed: Neuropsychopharm. Jan 2011; 36 (1); 183-06], we have modified our 'Results' section to implement a small volume correction onto the rACC in subsequent analyses (baseline SN rsFC and antidepressant treatment response; placebo-induced SN rsFC changes and placebo response, and antidepressant treatment response). The 'Results' section now includes: *"Based on this initial finding and the rACC's essential involvement in placebo effects* [Science. Mar 2002; 295 (5560); 1737-40; Pain. Jan 2006; 120(1-2): 8-15] *and the prediction of antidepressant treatment* [reviewed: Neuropsychopharm. Jan 2011; 36 (1); 183-06], *we allowed subsequent activation of rACC rsFC to be significant after small volume correction ($p<0.05$-FWE) using a rACC region-of-interest sphere centered around the MNI coordinates 4, 42, 6 [Biol Psychiatry. Dec 2005; 58(11):843-53] with a 6mm radius* (created using MarsBar *MarsBar[Functional Map Hum Brain. June 2002; 16 (2); abstract 497)]."*

3. Inter-network connectivity was not addressed.

Response: Our main objective for this manuscript was to identify predictors of specific and non-specific antidepressant treatment effects within three major intrinsic connectivity networks. However, as suggested by Reviewer 1, we have now addressed inter-network connectivity to investigate between-network connectivity, which is included as Supplemental Results. The table below shows the r-values between each network. Supplemental Results now includes*: "To examine the relationship between the DMN, SN, and EN, we investigated inter-network connectivity. The table below shows the r-values between the component time series of each network. DMN and SN did not demonstrate a significant difference in their component time series. Classically, the SN is known to associate within a "task-positive network" and the DMN is termed "the task-negative network". These two networks demonstrate an anti-correlated relationship in healthy individuals [PNAS. Jul 2005;102(27);9673-8]; a recent meta-analysis of resting-state functional connectivity in MDD found increased connectivity between the anterior*

portions of the DMN with the SN [Neurosci Biobehav Rev. 2015 Jul 30;56:330-344], consistent with our findings. Additionally, we found that SN and EN connectivity were positively correlated as they are both engaged during the processing of external, salient stimuli [J Neurosci. 2007 Feb 28;27(9):2349-56]. Of interest, the left and right EN demonstrate a dissociation with the DMN: the left EN was not significantly correlated with the DMN, whereas the right EN was significantly positively correlated with the DMN. As the EN is mainly involved in processing goal-directed, cognitive processes and the DMN is mainly involved in introspection, a negative correlation would be expected [PNAS. Jul 2005;102(27);9673-8], whereas the right EN was significantly positively correlated with the DMN. This increase connectivity between the anterior portions of the DMN with the SN, as well as the right EN and the DMN, may represent key features of MDD pathophysiology, which will need to be further investigated in future studies and might help clarify some of our, apparently inconsistent inter-network findings."

Table 2: Correlations between network component time series (r-values are listed below). *p<0.05; **p<0.01; ***p<0.001 Bonferroni-corrected

	SN	***DMN***	***R EN***	***L EN***
SN	*1.00*	*0.07*	*0.11**	*0.24****
DMN		*1.00*	*0.30****	*0.00*
R EN			*1.00*	*0.27****
L EN				*1.00*

4. The rationale as to why rs-fc either group level or individual subject would detect active placebo but not open label antidepressant induced changes was not adequately addressed.

Response: Originally, we hypothesized baseline resting-state functional connectivity would predict response to placebo administration as well as open-label antidepressant response. Because of its nature, responses to the open-label treatment would include the drug effects + the placebo effects + other non-specific effects, and therefore, the same rsFC activity at baseline should predict both, the response to one-week of placebo and ten-weeks of open-label treatment, or at least a portion of it. In the current version of the manuscript, this hypothesis remains the same. However, during the open-label antidepressant treatment, the loss of follow-up data in 6 participants probably resulted in reduced power to detect significant effects during the open-label treatment.

5. In the introduction (p 6) the author's hypothesize that 'increased baseline rsFC within the DMN would be associated with greater depression severity' but do not address the other 2 networks of interest or provide a rational as to why they chose to focus on DMN baseline deficits vs. salience or cognitive networks in relationship to depression severity ratings and then hypothesize that all three networks would 'predict response to both one week of placebo and 10-weeks of antidepressant treatment' without providing a clear rationale, especially since the remainder of the paper focuses primarily on the SN.

Response: We agree with Reviewer 1 that specific details regarding the implication of each network in the neurobiology of MDD and its response to placebo and antidepressant treatment were needed. A revised hypothesis has been incorporated into the manuscript: "*First, among the three networks, only the DMN has previously been related to MDD duration [Biol Psychiatry. Sep 2007; 62 (5); 429-37] and maladaptive rumination [Biol Psychiatry. Aug 2011]; therefore, we hypothesized that increased baseline rsFC within the DMN would be associated with greater depression severity . Second, with respect to the prediction of treatment response, none of the connectivity networks have been specifically related to placebo or antidepressant medication responses in patients with MDD. However, central regions of the DMN, SN, and EN — specifically the rACC, INS, and dlPFC, respectively — have a key role in mechanisms implicated in antidepressant and placebo responses, as described above, as well as the role of these networks in processes necessary for treatment responses: internal monitoring, saliency and higher-level cognition, respectively. Therefore, we hypothesized that increased baseline functional connectivity within central regions of the DMN, SN and EN would predict response to both one-week of placebo and ten-weeks of open-label antidepressant treatment. Finally, we evaluated whether application of multivariate relevance vector regression (RVR) to baseline rsFC maps of the three networks would allow for prediction of placebo and antidepressant responses at an individual level.*"

6. Wording is confusing, e.g."increased baseline functional connectivity within the SN in the rACC and at a trend level in the pINS and dlPFC was significantly associated with placebo response" and again "increased baseline rsFC of the pINS, dlPFC... were also associated with reductions in depression symptoms in response to 10 weeks of open-label antidepressant treatment but did not meet full criteria of significance by extent." This pattern over overinflating study findings continues throughout the results and discussion sections. Non-significant findings are simply non-significant (allowing here even for the multiple analyses conducted and hence uncontrolled multiple comparisons).

Response: The sentence has been removed from the manuscript because all trend results have been removed (see response to Reviewer 1 #2).

7. The explanation as to why the authors expected one-week of placebo administration not to result in significant changes within rs-fc at a group level "given inter-individual variation in response to placebo" needs to be further elaborated. It is unclear how rsfc of the SN was significantly predictive of placebo response on an individual level if placebo administration did not result in significant changes within network functional connectivity on a group level.

Response: As the Reviewer notes, we expected the variability within each subject's placebo response to negate a potential main effect on network rsFC. The response to one-week of placebo administration with expectations of antidepressant effects is a highly inter-individual variable: the variability in this response ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$) ranged from -8 to 11 (mean ± S.D.: 1.8 ± 4.2) which is a likely reason for a null main effect of placebo on network rsFC. While

overall placebo response did not show a significant effect on rsFC within any of the investigated networks, the significant relationship between placebo response and baseline rsFC still exists independently as demonstrated in our results. We included the range of placebo responses within the Results section of the manuscript: *"The total placebo response variable ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$) was highly variable, ranging from -8 to 11 (mean ± S.D.: 1.8 ± 4.2)."*

8. p 19 "the engagement of rACC and dlPFC in the context of this functional connectivity network (e.g. SN) and not their classically associated networks e.g. DMN and EN..." It would be helpful if the authors investigated inter-network connectivity, in order to better address this in their discussion.

Response: Based on the lack of significance of the dlPFC peak, these results are not relevant to be the discussion anymore. We have examined internetwork connectivity (see response to Reviewer 1, #3) in the current manuscript.

9. It is not clear why antidepressant effects were quantitated as wk 10 vs 0 instead of wk 10 vs post-placebo.

Response: In a few cases during the study, participants started the antidepressant treatment a few days (less than three) after the placebo experiment. For this reason, a baseline week 0 was created, which in most cases correspond to the post-placebo measure. This has been clarified in the Supplemental Methods and Materials section by the following sentence: *"In four cases, participants started the antidepressant treatment a less than three days after the placebo experiment. A baseline QIDS-16SR Week 0 measure was created to measure antidepressant treatment response for this reason."*

10. In general, the rationale for ICA analyses is lacking. Often ICA analyses distort an understanding of connectivity since they select for the most intact-looking components, which may be very disrupted in some individuals (which seeded connectivity would pick up). In general I would recommend seeded connectivity, particularly because of the regional hypotheses regarding particular areas previously implicated in antidepressant or placebo effects.

Response: Resting-state functional connectivity is a relatively novel field, especially in the study of the neurobiology inherent to psychiatric patient populations such as MDD; thus, employment of a variety of methods is necessary to fully capitalize on this neuroimaging field. ICA and seed-based connectivity are both valid methods in this endeavor. With the availability of different analytical techniques, we agree with Reviewer 1 that a more sufficient rationale for ICA is needed. Although we hypothesized involvement of a variety of *a priori* regions in the context of large-scale networks, we determined that with our main objective centered on the identification of potential biomarkers of non-specific treatment effects, a data-driven approach was better suited for our analysis than a more regional hypothesis-driven approach. For a biomarker to be reliable and applicable across studies, scanners, and geography, data-driven methodology like

ICA is, in our opinion, a stronger candidate—after following model-order recommendations from large-scale rsfMRI studies [J Cog Neurosci. Dec 2011; 23(12): 4022-37; PNAS Aug 2009; 106(31): 13040-5] in order to isolate major large-scale networks. Moreover, any significant regions within our findings exist in the context of these intrinsic, large-scale networks, better placing our results within the inherent mechanisms of human brain functioning.

While seed-based connectivity is an approach that also provides important insight regarding the specific network associated with a particular seed; in contrast to ICA, any significant peaks would exist only in relation to the extent of their functional connectivity to the selected seed, instead of a cohesive, well-documented large-scale network [PNAS Sep 2006; 103(37): 13848-53]. Furthermore, resultant seed-based connectivity networks are highly dependent on seed location; thus, seed-based results are more subject to outside influence such as seed placement, noise artifacts, subject motion, and ultimately, have a strong probability of excluding potential significant results [Front Syst Neurosci. Apr 2010; 4 (8)]. Taken together, we determined the more data-driven ICA approach to be better suited for our purposes of identifying predictors of treatment effects. In order to better identify subject-specific networks, we implemented 'dual regression' which uses regression of the group-ICA spatial maps against each individual subject's resting-state data in order to generate individualized subject networks [NeuroImage. 2009; 47 (S1); S148; Neuroimage. May 2004; 22(1):252-7].

The following sentence was incorporated into the Introduction section concerning ICA implementation*: "a data-driven approach that produces results within a framework of the brain's intrinsic connectivity networks and allows for identification of reliable, exploratory-based treatment response predictors".* Within the Materials and Methods section, we incorporated the following description to rationalize our use of ICA: *"Among available rsFC analytical methods, in our opinion, ICA, a data-driven approach, is an optimal choice for isolating connectivity networks, to provide an inherent framework for any resultant predictors, versus manual selection of a seed region."*

11. The RVR analyses are a nice complimentary analytical tool, however they are not used to their full potential. Here as well seeded analyses would be helpful in establishing the most predictive classifiers.

Response: RVR was implemented in order to test for potential application of individual-level placebo response predictors to further our group-level results. We agree that seed-based analyses may be interesting predictive classifiers and may augment a comprehensive understanding of the full potential of resting-state functional connectivity in MDD. However, due to limitations in seed-based methodologies (susceptibility to seed location effects, output results in context of a single seed region, see response to Reviewer 1 #10), we decided that examining whole-network activity as individual predictors would provide greater potential for uncovering biomarkers of treatment response. Instead, we believe that data-driven approaches are better suited to predict

network-based resting-state data, which mimics inherent brain functioning, for single-subject level predictors of treatment response.

Reviewer #2:

1. The sample size is relatively modest, although the longitudinal trial design and the repeated scans on all participants necessarily reduces the sample size.

Response: While our sample size was relatively small, this sample size was powered to detect significant effects. Still, this has now been included as a limitation in the new version of the manuscript (see Reviewer 3 #5).

2. Regarding the preprocessing of the resting state data, it is not clear whether movement parameters were included as regressors in analyses, as is usually the case in such analyses (see Satterthwaite, T. D., M. A. Elliott, et al. (2013). "An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data." Neuroimage 64: 240-256.). Can the authors clarify?

Response: Yes, movement parameters were included as regressors in the pre-processing of the resting-state data. For clarification, we have added the following change to this portion of our Materials and Methods section (in the Supplemental): Nuisance signal removal from voxel time series was performed on the preprocessed resting state scans using linear regression in AFNI's 3dTproject. The nuisance signals removed were linear/quadratic trends to account for scanner drift; the six rigid body realignment *(motion)* parameters and their first derivatives; and the top five principal components from both white matter (WM) and cerebral spinal fluid (CSF) BOLD time course."

3. Many of the findings presented appear at trend level. The only significant finding regarding association between resting state connectivity and treatment response appears to be the positive association between baseline resting state connectivity in the salience network and placebo response (ie., placebo administration with expectation of antidepressant effects). The authors should de-emphasize the trend-level findings. This is especially relevant to the main Discussion, where findings are presented as if they were significant.

Response: Originally, we incorporated close to significant findings due to their interesting nature in the context of MDD treatment literature, but we agree with Reviewer 2 and these results have now been removed from the manuscript (see Reviewer 1 #2).

4. In analyses described in p. 15 examining relationships between resting state connectivity in response to placebo and associated reduction in depression symptoms in response to placebo, and to antidepressant medication in the subsequent phase of the trial, I assume that the resting state connectivity measure in these analyses was the change in resting state connectivity for the "active" versus "inactive" placebo conditions. Can the authors clarify?

Response: For each network component, we subtracted rsFC within the "active" condition from the "inactive" placebo conditions. We have revised the sentence and incorporated this in the results: One-week of placebo administration with expectations of antidepressant effects did not result in significant changes within rsFC of the networks—*as measured by the changes in rsFC from "active" to "inactive"*— given the high inter-individual variation in the placebo response measure.

5. On a related note, if there were no significant changes in resting state connectivity in any of the networks to the "active" versus "inactive" placebo conditions (reported at the top of p.15), why would there be an expectation of a relationship between such change in resting connectivity and placebo response? In other words, the range across participants of any change in resting state connectivity would likely be too small to show any significant relationship with either placebo response to subsequent response to antidepressant medication. This may explain the trend-level only relationship between change in resting state connectivity in the salience network and placebo response, and the lack of any association between change in resting state connectivity in any network and antidepressant response.

Response: As pointed by Reviewer 2, we did not find any significant changes in resting-state connectivity within any of the networks between the "active" and "inactive" placebo conditions. This is possibly explained by the fact that placebo responses are inherently, a highly variable measure: in this MDD group, placebo response ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$) ranged from -8 to 11 (mean ± S.D.: 1.8 ± 4.2) which, mostly likely, is the main contributing factor in the resultant null main effect of placebo intervention on network connectivity. However, to account for the placebo response measure's variability, we wanted to explore whether any placebo-induce changes in network connectivity related to placebo or antidepressant responses. These findings highlight which brain regions associate with placebo and/or treatment responses and moreover, the nature of the region's connectivity changes associated with the placebo response. Generally, this type of examination may illustrate more dynamic connectivity characteristics associated with treatment responses. As the Reviewer states, it is most likely that the changes in resting-state connectivity were too small due to the range and individualized nature of placebo responses and were ultimately not strong predictors of treatment responses. However, upon newly incorporated post-hoc rACC small volume correction (see Reviewer 1 #2), we found a significant decrease in rACC connectivity within the SN in response to placebo. The following is now included in the Results section: *"In order to account for the variability inherent in the placebo response measure, statistical component maps of network rsFC in response to one-week of placebo administration were then examined against associated reductions in depression symptoms in response to one-week of placebo administration and in response to ten weeks of open-label antidepressant treatment."*

6. I assume that the resting state connectivity measures used for the RVR analyses were those to the baseline ("inactive" placebo) condition. Can the authors clarify? That said, given the earlier demonstration of a significant relationship, from the regression analyses, between baseline

resting state connectivity in the salience network and placebo response, by using these same resting state connectivity measures in the RVR analyses, the RVR analysis could be criticized for being a "double-dipping" analytic step.

Response: Based on our group-level regression analyses, we input the baseline ("inactive" placebo resting-state scan) SN component for each subject in the RVR analyses. We implemented RVR to determine whether rsFC of the SN as a whole could predict response to placebo at an individual level with significant accuracy. The same analysis was performed to determine whether rsFC of the SN could predict responses to ten weeks of open-label antidepressant treatment. While we used the same SN component in the group-level regression analyses and the RVR analyses, the outcome measures were distinct. The group-level regression analyses are mass univariate analyses that allowed us to determine whether a relationship exists between functional connectivity within certain areas (voxels) of each network and treatment responses. However, the RVR analyses were multivariate analyses that incorporated the SN as a whole to determine what regions within the SN had greatest weight in predicting placebo responses. Importantly, the RVR implemented a leave-one-out cross validation analysis to determine whether SN rsFC could predict placebo response with significant accuracy at an individual level, which the group-level analysis did not allow us to deduce. In essence, the RVR analysis was a separate test to better evaluate SN rsFC as a potential treatment response predictor for potential future clinical use. Again, this will need to be confirmed in substantially larger sample sizes. For clarification, we have now incorporated *"baseline ('inactive')"* into the first sentence of the Methods and Materials: Multivariate RVR section.

7. Together, findings (including those at trend level) indicate relationships between greater baseline resting state connectivity in the salience network and greater placebo response, but greater decreases in resting state connectivity to active versus inactive placebo conditions and placebo response. How do the authors interpret these findings? Is it that greater baseline resting state connectivity confers greater capacity for change in resting state connectivity in the network, that in turn means that both measures are associated with greater placebo response?

Response: As pointed by Reviewer 2, we found that increased baseline functional connectivity of the SN, specifically within the rACC, is associated with greater decreases in depressive symptoms in response to one week of placebo administration. Additionally, and now confirmed with post-hoc rACC small volume correction (see Reviewer 1 #2), greater decreases in rACC rsFC within the SN were also associated with greater placebo response. We speculate that enhanced activity within the rACC within the SN signifies a greater capacity for integrating salient stimuli and related cognitive and affective processes, such as those related to treatment outcome. That this pattern of activity occurs within the context of the SN is apt, as the placebo response requires the processing of salient, emotionally cognitive mechanisms associated with SN functioning. Of interest, this increased rACC metabolism has shown to differentiate antidepressant responders from non-responders, the latter of which displayed hypometabolism in the region, as compared to controls [Neuroreport. Mar 1997; 8(4):1057-61]. The decreases in

rsFC of the region within the SN could possibly suggest normalization to healthy affective states, as those matching healthy controls. In support of this, hyperactivity in the rACC also normalized in responders but not within non-responders to sleep deprivation [Psychiatry Res. Jan 2006; 146 (1):43-51]. This heightened level of activity and ultimate decrease may suggest adaptive and healthier functioning of the rACC that is necessary for a successful treatment response. A more concrete reference to this finding has now been elaborated in the discussion: *"A relevant hypothesis may state that enhanced rACC rsFC within the SN may represent a greater capacity for integrating salient stimuli with adaptive, cognitive-affective functioning, which is conducive to the manifestation of a placebo effect. Normalization of heightened rACC activity has been demonstrated in MDD responders to sleep deprivation, but not in non-responders [Psychiatry Res. Jan 2006; 146 (1):43-51] (a placebo-arm was not incorporated). Thus, malleability within rACC may extend to necessitate a successful antidepressant treatment response or to the placebo mechanisms inherent in these responses."*

Reviewer #3:

1. The statistical power of this study is relatively low - 29 patients but only 23 completers. This limits the analysis of individual prediction incorporated in the paper and highlights the need for independent replication. This needs to be acknowledged directly in the manuscript discussion. Perhaps related to this, the results section details a number of trends which are difficult to fully interpret.

Response: We address this by adding the following sentence in the discussion: *"The potential applicability of SN rsFC as an individual-subject predictor of placebo response will need to be further confirmed in larger samples."* We have additionally included this in the limitation section: "*A limitation of the current study was the small sample size (N = 29 for the placebo phase; N = 23 for the antidepressant phase). This limitation is especially relevant to the RVR analysis, where independent confirmation is needed to establish whether SN rsFC is a predictor of placebo response at an individual level.*" In relation to the comment regarding trend findings, originally, we incorporated our findings which exist at trending levels due to their interesting nature in the context of MDD treatment literature, but we have removed these findings from the manuscript (Reviewer 1# 2).

2. I think the main weight of the paper is evidence suggesting that rACC activity (or cohesion here) is predictive of placebo responses: this challenges a whole body of work using rACC activation to predict therapeutic response to treatment (often in the absence of a placebo control). However, I think this point gets lost in the paper, which could be presented in a more straightforward, briefer and structured way.

Response: We thank Reviewer 3 for the comment and we agree that this point was not adequately addressed and has been highlighted in the current manuscript. To address this we have added the following to the discussion section: *"Our study is an initial attempt in separating predictors for different aspects of treatment effects, such as placebo. Here, we demonstrated that greater baseline rsFC of the rACC within the SN to be a significant predictor of placebo response and separately, open-label antidepressant treatment response. Consistent with these data, a recent RCT found that elevated pretreatment theta current density in the rACC predicted treatment outcome in both the medicated and placebo group [Psychiatry Res. Jun 2011;192(3):188-94]*.

In all, these findings point to the predictive role of rACC within the SN in placebo response and the overall response to antidepressant treatment (antidepressant + placebo + other non-specific effects). Furthermore, we observed the normalization of heightened baseline rACC rsFC after placebo administration to be associated with greater placebo responses; this suggests that rACC modulation is an important element of placebo neurobiology. A relevant hypothesis may state that enhanced rACC rsFC within the SN may represent a greater capacity for integrating salient

stimuli with adaptive, cognitive-affective functioning, which is conducive the manifestation of a placebo effect. Normalization of heightened rACC activity has also been demonstrated in MDD responders to sleep deprivation, but absent in non-responders (a placebo-arm was not incorporated) [Psychiatry Res. Jan 2006; 146 (1):43-51]. Thus, malleability within the rACC may extend to necessitate a successful antidepressant treatment response or to the placebo mechanisms inherent in these responses."

3. The prediction of placebo vs antidepressant drug response was made sequentially - that is that patients received placebo first and then entered into a real treatment phase. The weaker associations with the antidepressant are therefore difficult to interpret. Again I think the main strength of the paper is the initial prediction of placebo response rather than the later response to treatment.

Response: We agree with Reviewer 3's comment that the main strength of the paper lies in placebo response prediction. We have amended the discussion to elaborate on the placebo prediction piece and acknowledge the limitation of the trial design (see response Reviewer 3 #2 and #5).

4. More details on how the associations presented in the figures in the scatterplot were extracted are needed - do these methods avoid the problem of double dipping (i.e. by picking the most significant voxel for plotting?)

Response: The figures in the scatterplot [now only one (Figure 3)] were created by extracting entire resultant significant clusters from the analyses. This modification has been added in the Materials and Methods: "These *significant data clusters* were extracted using MarsBar (66), for quantification of regional functional connectivity at baseline and placebo-induced changes, graphing, determination of correlation coefficients…"

5. The limitations of this study need to be acknowledged in the discussion including the points made above.

Response: We have included the following paragraph addressing the limitations of this study: *"A limitation of the current study was the small sample size (N = 29 for the placebo phase; N = 23 for the antidepressant phase). This limitation is especially relevant to the RVR analysis, where independent confirmation is needed to establish whether SN rsFC is a predictor of placebo response at an individual level. Finally, the subsequent nature of the open-label treatment after the placebo phase was not optimal for dissecting drug and placebo-specific effects. Future RCTs with parallel placebo and drug arms will need to be conducted to better identify and separate predictors of treatment response."*

Title Page

Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression

Short Title: Salience Network Linked to Placebo Effects in MDD

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ABSTRACT

Background: Recent neuroimaging studies have demonstrated resting-state functional connectivity (rsFC) abnormalities among intrinsic brain networks in Major Depressive Disorder (MDD); however, their role as predictors of treatment response has not yet been explored. Here, we investigate whether network-based rsFC predicts antidepressant and placebo effects in MDD.

Methods: We performed a randomized controlled trial of two weeklong, identical placebos (described as having either "active" fast-acting, antidepressant effects or as "inactive") followed by a ten-week open-label antidepressant medication treatment. Twenty-nine participants underwent a rsFC fMRI scan at the completion of each placebo condition. Networks were isolated from resting-state blood-oxygen-level-dependent signal fluctuations using independent component analysis. Baseline and placebo-induced changes in rsFC within the default-mode, salience, and executive networks were examined for associations with placebo and antidepressant treatment response.

Results: Increased baseline rsFC in the rostral anterior cingulate (rACC) within the salience network, a region classically implicated in the formation of placebo analgesia and the prediction of treatment response in MDD, was associated with greater response to one week of active placebo and ten weeks of antidepressant treatment. Machine learning further demonstrated that increased salience network rsFC, mainly within the rACC, significantly predicts individual responses to placebo administration.

Conclusions: These data demonstrate that baseline rsFC within the salience network is linked to clinical placebo responses. This information could be employed to identify patients who would benefit from lower doses of antidepressant medication or non-pharmacological approaches, or to develop biomarkers of placebo effects in clinical trials.

Trial name: Predictors of antidepressant response.

URL: http://clinicaltrials.gov/ct2/show/NCT02178696?term=zubieta&rank=3.

Trial number: NCT02178696.

TEXT

INTRODUCTION

Major depressive disorder (MDD) has recently been conceptualized as a disorder with a network-based pathophysiology (1). Particularly, MDD manifests in cortico-limbic network dysregulation reflected by deficits in cognitive control and increased sensitivity of limbic networks (2-5), which is thought to result in excessive and negatively-skewed focus on introspective processes, difficulty regulating emotions, and persistent difficulties in sustaining attention (6, 7). Regions involved in these networks have emerged as biological markers of response to antidepressant treatments (8-13). Yet, the ability of these biomarkers to selectively distinguish drug-specific effects from other non-specific elements of the treatment response, such as the placebo effect, is still limited, with very few studies specifically addressing biomarkers of non-specific elements of antidepressant treatment response (14, 15). This is not a small concern, as placebo response rates in antidepressant clinical trials average 31-45% compared with response rates to antidepressants of ~50% and have increased at a rate of 7% per decade over the last 30 years (16, 17). Hence, further investigation is warranted in order to dissect the neural predictors of drug-specific and non-specific effects in MDD treatment.

Of the major functionally connected networks identified within the brain's inherent organization (18-20), three have received special attention in MDD and the prediction of treatment response: the default-mode (DMN), salience (SN) and executive (EN) network (21, 22). The DMN, with key regions in the posterior cingulate, medial prefrontal, and bilateral parietal and temporal cortices (23), is associated with introspective cognition (24) and demonstrates heightened connectivity and abnormal down-regulation in MDD, which may

contribute to the disorder's hallmark attributes of excessive self-focus, inattention and rumination (25-28). Elevated pretreatment activity of the rostral anterior cingulate (rACC), a region encompassed within the main anterior DMN node (24) has consistently been identified as a predictive marker of treatment response across imaging and treatment modalities (9, 10, 29, 30). It has been hypothesized that heightened baseline rACC activity fosters better treatment outcome in patients with MDD by implementing adaptive self-reflection through its connectivity within the DMN (31). Moreover, antidepressant medication has been shown to decrease functional connectivity of the DMN (32). The SN, anchored by the anterior insula (aINS) and dorsal anterior cingulate cortex (dACC), is enlisted during the integration of salient stimuli and interoceptive information to guide motivated behavior (33). In particular, the aINS is a hub of meta-awareness and affective processing (34, 35) and has long been associated with MDD pathophysiology (25, 36-39). A recent meta-analysis illustrates its activity as a neural predictive marker of MDD treatment response, where increased baseline insular activity is associated with poor clinical response (40). Finally, MDD is characterized by reduced functional connectivity of the EN (4) and hypo-activation of the network's key node: the dorsolateral prefrontal cortex (dlPFC) (41). The EN, which includes cohesive functional communication between the dlPFC and parietal cortex, is responsible for orienting to and engaging in attentive, goal-directed behavior (33); its dysfunction may contribute to a lack of control over heightened affective responses in MDD (21, 42). The dlPFC is involved in current MDD treatments and successful recovery from the disorder (43-45); while reduced grey matter volume in the region is an indicator of non-response to standard MDD treatments (11).

Nodes within these three networks have also been implicated in the neurobiological mechanisms of non-specific treatment effects in the field of placebo analgesia, where substantial

headway has been made to identify the cognitive, neural, and molecular bases of the neurobiology of placebo effects (46-51). These studies have demonstrated a key role of the rACC in the formation of placebo analgesia (48, 52, 53), potentially through its interactions with additional subcortical brain areas involved in endogenous opioid-mediated analgesic effects, such as the amygdala (54) and the periaqueductal gray (48), but also the anterior and posterior INS (50, 51, 55). A number of neuroimaging studies have also reported placebo-associated changes in dlPFC function, thought to be related to expectancies and anticipatory mechanisms (56-58), consistent with the role of this region in cognitive executive function (59). In this regard, activity in EN-associated regions during pain anticipation was found to be predictive of the magnitude of placebo analgesia (60). Conversely, minimal information has been acquired as to the mechanisms implicated in antidepressant placebo effects, with notable exception of one investigation demonstrating an overlap in regions involved in placebo and medication effects (15) and our recent work describing the role of the opioid system in the formation of placebo responses in MDD (61).

Here, we take a network-based univariate and multivariate approach to the prediction of the response to placebo and antidepressant treatment using resting-state functional connectivity (rsFC) with independent component analysis (ICA) (62), a data-driven approach that produces results within a framework of the brain's intrinsic connectivity networks and allows for identification of reliable, exploratory-based treatment response predictors. We investigated the relationship between baseline rsFC of three networks (DMN, SN, left and right EN) and: 1) depression severity; 2) clinical response to one-week of placebo treatment; and 3) clinical response to ten-weeks of open-label antidepressant treatment. First, among the three networks, only the DMN has been previously related to MDD duration (63) and maladaptive rumination

(25); therefore, we hypothesized that increased baseline DMN rsFC would be associated with greater depression severity. Second, with respect to the prediction of treatment response, none of the connectivity networks have been specifically related to placebo or antidepressant medication responses in patients with MDD. However, central regions of the DMN, SN, and EN — specifically the rACC, INS, and dlPFC, respectively — have a key role in mechanisms implicated in antidepressant and placebo responses, as described above, as well as the role of these networks in processes necessary for treatment responses: internal monitoring, saliency and higher-level cognition, respectively. Therefore, we hypothesized that increased baseline functional connectivity within central regions of the DMN, SN and EN would predict response to both one-week of placebo and ten-weeks of open-label antidepressant treatment. Finally, we applied multivariate relevance vector regression (RVR) to evaluate the hypothesis that baseline rsFC maps of the three networks would allow for prediction of placebo and antidepressant responses at an individual level.

METHODS and MATERIALS

Subjects

Twenty-nine right-handed, un-medicated subjects with a DSM-V diagnosis of MDD were recruited via local advertisements [21 females; age: 18-59 (mean ± S.D.: 32 ± 13)]. See Supplemental Methods and Material for additional subject information and description of informed consent and authorized deception. All of the procedures used were approved by the University of Michigan Investigational Review Board for Human Subject Use Research Committee.

Placebo Randomized Controlled Trial (RCT) and Antidepressant Open-Label Trial

As previously described (61), subjects were randomized into either 1) one-week "active" placebo treatment (two pills/day), with expectations that the pill represented a potentially fast-acting antidepressant agent or 2) one-week "inactive" placebo treatment described as an control condition, without pharmacological effects (two pills/day). After a three-day "washout" period of no pills, subjects crossed over into the alternative condition to which they had not been assigned. After each week of placebo, subjects underwent a resting-state scanning session (Fig. 1).

Depression symptoms were assessed using the 16-item self-rated version of the Quick Inventory of Depressive Symptomatology (QIDS) (64) at the following time points: pre-randomization, baseline (pre)-, and post- *active* and *inactive* conditions. This measure was selected as a primary outcome measure for its sensitivity to fluctuations in depression symptoms and its availability as a self-reported measure. Additionally, patients completed the Montgomery Asberg Depression Rating Scale (MADRS) and the Hamilton Rating Scale for Depression (HDRS) at pre-randomization and during each visit within the antidepressant trial.

The change in QIDS ($\Delta QIDS = QIDS_{BASELINE} - QIDS_{POST}$) was calculated for active and inactive treatment conditions, and the difference between conditions was taken as an index of placebo response ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$). Positive values reflected greater reductions in depressive symptoms as a result of "active" placebo administration.

Following the placebo RCT and the two resting-state fMRI scan sessions, subjects received a ten-week open-label antidepressant trial with citalopram as an initial agent (starting at 20 mg/day and up to 40 mg/day in 45% of cases). When citalopram was not clinically indicated (e.g. prior non-response or side-effects), another antidepressant was given [sertraline (n=1), mirtazapine (n=1), fluoxetine (n=2), duloxetine (n=1), and bupropion (n=1)]. Participants' symptom changes were evaluated at weeks 0, 2, 4, 8, and 10. Antidepressant response was measured by the difference in QIDS between week 0 and 10 (ΔQIDS = Week 0 – Week 10). In four cases, participants began the antidepressant treatment less than three days after the placebo experiment; because of this, a baseline QIDS Week 0 measure was created to measure antidepressant treatment response.

Resting State Functional Connectivity Networks

Image data acquisition, preprocessing, movement analysis, and ICA are detailed in Supplemental Methods. Among available rsFC analytical methods, in our opinion, ICA, a data-driven approach, is an optimal choice for isolating connectivity networks, to provide an inherent framework for any resultant predictors, versus manual selection of a seed region. Briefly, 20 components were output through ICA utilizing the Infomax algorithm within the Group ICA methodology in GIFT software (Medical Image Analysis Lab, University of New Mexico, Albuquerque, New Mexico; http://icatb.sourceforge.net/). Of the resultant components, the networks of interest were selected using templates from the BrainMap

(http://www.brainmap.org/icns) database: a comprehensive resting-state fMRI data source (65). To determine the component with the "best-fit" for each particular network, a linear-template matching procedure was performed on all 20 components, as described elsewhere (63). Briefly, for each network template: all 20 components were scored based on their best-fit with the template by computing the average z-score of voxels falling outside the template in the component subtracted from the average z-score of voxels falling within the template. The component with the greatest value of this measure was identified as the network-of-interest: DMN, SN, right or left EN. For all networks, the component-of-interest had a best-fit score of at least two SD greater than the mean [network: best-fit score (mean ± SD); DMN: 15.7 (1.63 ± 3.58); SN: 5.2 (0.7±1.45); LEN: 12.0 (1.1 ± 2.7); REN: 4.6 (0.7 ±1.4)].

Data Analysis

All data analysis was performed using SPM8 (Welcome Department of Cognitive Neurology, University College, London, England) and Matlab (MathWorks, Natick, Massachusetts) software. One-sample t-tests and paired t-tests were used to analyze within-network functional connectivity within- and between-conditions, respectively (see Supplemental Methods for paired t-tests). For each network, one-sample t-tests were performed: network-of-interest components from all 29 subjects during the "post-inactive" scan (baseline) were entered into the analysis and ICA-assigned z-scores at each voxel were averaged across all subjects and compared to zero (Fig. 2). Significance threshold was set at $p<0.05$ family-wise corrected (FWE).

For each network, a whole brain voxel-by-voxel regression analysis was performed between network functional connectivity (z-score of the weight on the ICA component of interest) at baseline (post-inactive) with the following variables: 1) depression severity (as measured by QIDS score at pre-randomization); 2) reductions in depression symptoms in response to placebo

administration with expectations of antidepressant effects [($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$), see above]; and 3) reductions in depression symptoms in response to ten weeks of antidepressant treatment (as measured by ΔQIDS scores = Week 0 - Week 10). All analyses were restricted to voxels within those defined by the network-of-interest's one-sample t-test composed of corresponding network component maps from all 29 subjects' inactive scan (masked at p<0.05-FWE). Depression severity score and scan order were input as covariates in all analyses.

All results were constrained within network component maps [one-sample t-test (above) masked at p<0.05-FWE]. For all *a priori* regions (rACC, INS, dlPFC), significance was set at a threshold height of p<0.005 uncorrected and 3dClustSim [AFNI (http://afni.nimh.nih.gov)] was used with 1000 Monte Carlo simulations to determine cluster-based, whole-brain correction at p<0.005 to account for testing of three bilateral regions-of-interest and four networks (total of ten). See Supplemental Material for extent cluster information, which is network-size dependent. Significance for all other peaks was set at p<0.05-FWE. These significant data clusters were extracted using MarsBar (66), for quantification of regional functional connectivity at baseline and placebo-induced changes, graphing, determination of correlation coefficients (Pearson/Spearman correlations at p<0.05). Data are expressed as the mean ± one S.D., unless otherwise indicated.

Multivariate Relevance Vector Regression (RVR) Analysis

We also investigated single-subject predictability of baseline ('inactive' scan) rsFC onto measured placebo responses using multivariate RVR as implemented in PRoNTo (http://www.mlnl.cs.ucl.ac.uk/pronto/) running under Matlab (Mathworks, 2012a release). RVR is detailed elsewhere (67). Briefly, it is a sparse kernel learning multivariate regression method formulated in Bayesian framework. The model weights are assigned a zero-mean Gaussian prior

in which each weight is governed by its own hyperparameter. Iterative estimation from the fMRI data identifies the most probable values for these hyperparameters with sparseness achieved due to the posterior distributions of many of the weights peaking around zero. The voxels associated with non-zero weights are marked as relevance vectors, which can then be used to predict the target value (placebo-induced ΔQIDS) for a novel input vector (e.g. baseline SN rsFC). RVR's major strength relative to other multivariate machine learning techniques [i.e. support vector machine (SVM), reviewed (68)] is that it computes quantitative prediction of a variable of interest without a need for discrete categorical estimation (e.g. patients vs. controls) (69). Here, the subjects' SN components were mean centered and input into the RVR analysis. An estimate for the model's predictability was calculated via leave-one-out cross validation, indexed using the Pearson correlation coefficient and mean squared error (MSE) between actual and the predicted placebo response measure. The significance of these metrics was determined through non-parametric permutation tests. In a single iteration, we randomly paired subjects' SN functional connectivity with subjects' placebo response values. Subsequently, we calculated the MSE and correlation for the random pairing. This was completed for 5000 iterations and built a distribution of MSE and correlation values from which p-values were calculated for accuracy of placebo response prediction.

RESULTS

Patient Characteristics

Twenty-nine participants with MDD [QIDS-16SR (mean ± SD): 16.1 ± 4.1; Hamilton Rating Scale for Depression (HRSD-17): 21.6 ± 5.1] were enrolled in the study and completed the two-week randomized placebo trial and entered the ten-week treatment with an open-label antidepressant (Fig. 1). 79% of them (23/29) completed the entire open-label antidepressant treatment portion; those who dropped out before full completion were not significantly different in their depression severity and placebo responsiveness from those who completed the study (see Supplemental Results, also for additional participant data).

Placebo- and Medication-induced Changes in Depression Symptoms

Reductions in depression symptoms after one week of the "active" placebo were significantly greater than after the "inactive" placebo treatment [ΔQIDS: 2.0 ± 3.4 for active; 0.17 ± 2.4 for inactive; $F = 7.2$, $df = 1$, $p = 0.012$]. The total placebo response measure ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$) was highly variable, ranging from -8 to 11 (mean ± S.D.: 1.8 ± 4.2). Ten weeks of open-label antidepressant treatment was also associated with a significant reduction in depression symptoms [QIDS at Week 0: 11.6 ± 4.3; at Week 10: 6.9 ± 3.9; $t = 4.9$, $df = 22$, $p < 0.001$].

Association of Baseline Resting Functional Connectivity, Pre-Randomization Depression Severity, Response to Placebo, and Response to Antidepressant Medication

Statistical component maps for each network were examined against symptoms of depression severity (QIDS-16SR) at time-point of pre-randomization. We did not find a

significant relationship with any of the networks (*a priori*: p>0.005, mm^3 > network-specific mm^3; other: p>0.05-FWE).

Statistical component maps for each network were then examined against reductions in depression symptoms in response to one-week of placebo administration with expectations of antidepressant effects (Fig. 3) and in response to ten weeks of open-label antidepressant treatment. Significance was established at p<0.005 and extent > 928 mm^3 for the SN (Supplemental Material). Increased baseline rsFC of the rACC within the SN was significantly associated with greater placebo response: rACC: 0, 38, 4, 1784 mm^3; Z= 4.35. No other significant effects were observed.

A linear regression analysis that included baseline rsFC of the rACC within the SN predicted 65% of the variance in placebo responsiveness (adjusted R^2=0.65).

Based on this initial finding and the rACC's essential involvement in placebo effects (50-52) and the prediction of antidepressant treatment response (70, 71), we allowed subsequent activation of rACC rsFC to be significant after small volume correction (p<0.05-FWE) using a rACC region-of-interest sphere centered around the MNI coordinates 4, 42, 6 (72) with a 6mm radius [created using MarsBar (66)].

Increase baseline rsFC of the rACC within the SN was significantly associated with reductions in depression symptoms in response to ten weeks of open-label antidepressant treatment (rACC: 0, 40, 8; 64 mm^3; Z = 3.09; p<0.05-SVC:FWE-corr). No other effects were observed.

Placebo-induced Changes in Network Functional Connectivity Predicts Response to Placebo and Antidepressant Medication

As expected, one-week of placebo administration with expectations of antidepressant effects did not result in significant changes within rsFC of the networks—as measured by the changes in rsFC from "active" to "inactive"—given high inter-individual variation in response to placebo (*a priori*: p>0.005; other: p>0.05-FWE-corr, see Supplemental for network-specific cluster size).

In order to account for the variability inherent in the placebo response measure, statistical component maps of rsFC changes in response to one-week of placebo administration were then examined against associated reductions in depression symptoms in response to one-week of placebo administration and ten weeks of open-label antidepressant treatment. Placebo-induced rsFC reduction in the rACC within the SN was significantly associated with greater placebo response (rACC: 0, 38, 4; 192 mm^3; Z = 3.97; p<0.05-SVC:FWE-corr). No other significant effects were observed.

Multivariate Relevance Vector Regression (RVR): Single-Subject prediction of placebo and treatment response

Based on the results described above, we first applied RVR to rsFC of the entire SN, where significant results were observed, in order to enable quantitative prediction of depression symptom reductions in response to placebo administration and in response to ten weeks of antidepressant treatment at the individual level. RsFC of the SN was significantly predictive of placebo responses [correlation = 0.41; p-value = 0.018; mean sum of squares = 14.36; p-value = 0.019; (Fig. 4)], with the greatest weights contributing to placebo response prediction within the rACC. SN rsFC was not a significant predictor of response to antidepressant treatment (correlation = 0.03; p-value = 0.34; mean sum of squares = 21.31; p-value = 0.36). No effects were found in the other networks.

DISCUSSION

In this study, we illustrated the viability of network-based rsFC to identify potential imaging-based predictive markers of treatment response in MDD. We found that more cohesive rsFC of the SN, through connectivity of the rACC, predicted greater reductions in depressive symptoms following one-week of placebo administration with expectations of antidepressant effects and ten weeks of open-label antidepressant treatment. In addition, the clinical response to one-week of placebo treatment was also associated with placebo-induced decreases in rsFC of the rACC within the SN. Finally, multivariate RVR demonstrated that SN rsFC predicted placebo responses with significant accuracy at an individual level.

Elevated baseline rsFC of the rACC within the SN predicted greater reductions in depression symptoms in response to placebo administration with expectations of antidepressant effects. Specifically, the rACC alone predicted 65% of the response variance. This finding potentially advances a more general understanding of the neurobiology of placebo effects across diseases: Both opioidergic and BOLD activity within the rACC have been consistently implicated in placebo analgesia (48, 52, 53) which evinces a hypothesis that corresponding mechanisms of action are involved in the formation of placebo effects across disorders (47), with recent evidence in further support of this notion (61). Our data, to our knowledge, are also the first demonstration of SN involvement in MDD placebo effects and thus, supplement the small existing body of work in the neurobiology of placebo in patients with MDD (15, 61). This is consistent with the role of the SN in saliency attribution: particularly, selecting and integrating biologically relevant stimuli with interoceptive states to guide input of attentional resources and

behavior (73). In this case, the network was involved in attending to and incorporating a set of expectations within a therapeutic environment with internal states resulting in a reduction of depression symptoms. If the former hypothesis remains valid, SN rsFC may be the functional context of prior placebo analgesia-associated rACC findings.

Mounting evidence from studies employing a variety of neuroimaging modalities and treatments indicate that increased pretreatment activity in the rACC is associated with better response to antidepressant treatment, as recently reviewed (31). More specifically, metabolism in the region has been observed to differentiate responders from non-responders to antidepressant medication (70). However, the open-label nature of these and prior studies (10, 70, 74, 75) makes it difficult to dissect drug-specific from non-specific treatment effects. Our study is an initial attempt in isolating predictors for different aspects of treatment effects, such as placebo. Here, we demonstrated that greater baseline rsFC of the rACC within the SN to be a significant predictor of placebo response *and* separately, open-label antidepressant treatment response. Consistent with these data, a recent RCT found that elevated pretreatment theta current density in the rACC predicted treatment outcome in both the medicated and placebo group (14).

In all, these findings point to the predictive role of rACC within the SN in the response to placebo and the overall response to antidepressant treatment (antidepressant + placebo + other non-specific effects). Furthermore, we observed the normalization of heightened baseline rACC rsFC after placebo administration to be associated with greater placebo responses; this illustrates that rACC modulation may be an important element of placebo neurobiology. A relevant hypothesis may state that enhanced rACC rsFC within the SN may represent a greater capacity for integrating salient stimuli with adaptive cognitive-affective functioning, which is conducive for the manifestation of a placebo effect. Normalization of heightened rACC activity has also

been demonstrated in MDD responders to sleep deprivation, but absent in non-responders (a placebo-arm was not incorporated) (76). Thus, malleability within the rACC may extend to necessitate a successful antidepressant treatment response or to placebo mechanisms inherent in the antidepressant response.

Finally, we demonstrated that multivariate RVR application to baseline SN rsFC, with the greatest weight within the rACC, was significantly predictive of placebo responses at an individual-subject level, although this was not the case for antidepressant responses. The utilization of a similar method, SVM, has gained substantial attention in the last years as a predictive classifier in MDD with diagnostic and prognostic qualities (77-79). For example, in a sample of 37 depressed patients and 37 healthy controls, whole-brain structural MRI significantly classified nearly 90% of patients exhibiting clinical remission and nearly 70% of those with an MDD diagnosis (80). Instead, we employed RVR to enable quantitative prediction without need for group classification, as it has been recently described (69). The potential applicability of SN rsFC as an individual-subject predictor of placebo response will need to be further confirmed in larger samples; yet, this information could ultimately augment clinical treatment through identification of patients who possess a greater likelihood in benefiting from lower dosages of antidepressant medication or cognitive interventions. This may also be relevant in the context of clinical trials to better distinguish patients with greater susceptibility to placebo effects, allowing for patient stratification and possibly, more detailed clarification of the effects of treatment components (specific versus non-specific).

A limitation of the current study was the small sample size (N = 29 for the placebo phase; N = 23 for the antidepressant phase). This is especially relevant to the RVR analysis, where independent confirmation is needed to establish whether SN rsFC is a predictor of placebo

response at an individual level. Finally, the subsequent nature of the open-label treatment after the placebo phase was not optimal for dissecting drug and placebo-specific effects. Future RCTs with parallel placebo and drug arms will need to be conducted to better identify and separate predictors of treatment response. However, our data have the potential to inform future designs of antidepressant clinical trials and personalized medicine for MDD patients by identifying individuals with greater likelihood in responding to non-pharmacologically specific interventions and who may ultimately be selected for less intensive treatments, lower dosages of medication, or for enhanced patient-clinician interaction.

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FINANCIAL DISCLOSURES

The authors have no interests to disclose that are or might be perceived to be in conflict with the work reported in this study.

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FIGURES

Figure 1: Experimental Design. A) After pre-randomization (screening), subjects are randomized into one of two conditions each lasting seven days: 1) *Active*: placebo administration with disclosure that it may provide antidepressant-like treatment effects; 2) *Inactive*: placebo administration with disclosure that it is an inactive agent. B) A three day washout occurs during which the patient receives no medication. C) Subjects cross-over to the alternative condition. D) *Resting-state fMRI scans are obtained immediately after each condition*. E) After full completion of the placebo trial, subjects undergo ten weeks of open-label antidepressant treatment. Depression measures are administered (* marks QIDS-16SR administration) at pre-randomization, pre- and post-active, pre- and post-inactive, and week 0, 2, 4, 8, 10 of the antidepressant trial.

Figure 2: Functional Connectivity of Networks. One-sample t-tests including baseline (inactive condition) resting-state fMRI scans for all 29 subjects for each ICA-component corresponding to the network: A) Default-mode, B) Salience, C) Left (left-side) and right (right-side) executive networks. The t-score bars are shown at the right; all images are displayed at a threshold of $p<0.05$ family-wise corrected. Images are shown in standard space of the Montreal Neurological Institute (MNI) template. N=29.

Figure 3: Baseline Functional Connectivity of the Salience Network Predicts Response to Placebo Administration. N=29. (Top left and right): Voxel-by-voxel correlational analysis between baseline functional connectivity of the SN and decreases in depression symptoms in response to placebo administration. Clusters passing significance threshold are labeled. Image display is at $p<0.01$; t-score bar is shown in the bottom right. (Bottom Left): Association between reductions in depression symptoms in response to placebo administration and functional

connectivity of rACC within the SN. Functional connectivity of this region predicted 65% of the variance in placebo responses ($p<0.001$).

Figure 4: Multivariate Relevance Vector Regression. Left: Mean predictor map with an arbitrary threshold of >50% of minimum and maximum voxel weight values. The map shows the relative contribution of each voxel to the regression function in relation to all other voxels. Color bar signifies weight value for all voxels. Right: Scatter plot showing the predicted placebo response value derived from each subject's baseline SN functional connectivity using RVR leave-one-out cross validation versus their actual placebo response *value* ($r = 0.41$; p-value = 0.018).

Title Page

Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression

Short Title: Salience Network Linked to Placebo Effects in MDD

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ABSTRACT

Background: Recent neuroimaging studies have demonstrated resting-state functional connectivity (rsFC) abnormalities among intrinsic brain networks in Major Depressive Disorder (MDD); however, their role as predictors of treatment response has not yet been explored. Here, we investigate whether network-based rsFC predicts antidepressant and placebo effects in MDD.

Methods: We performed a randomized controlled trial of two weeklong, identical placebos (described as having either "active" fast-acting, antidepressant effects or as "inactive") followed by a ten-week open-label antidepressant medication treatment. Twenty-nine participants underwent a rsFC fMRI scan at the completion of each placebo condition. Networks were isolated from resting-state blood-oxygen-level-dependent signal fluctuations using independent component analysis. Baseline and placebo-induced changes in rsFC within the default-mode, salience, and executive networks were examined for associations with placebo and antidepressant treatment response.

Results: Increased baseline rsFC in the rostral anterior cingulate (rACC) within the salience network, a region classically implicated in the formation of placebo analgesia and the prediction of treatment response in MDD, was associated with greater response to one week of active placebo and ten weeks of antidepressant treatment. Machine learning further demonstrated that increased salience network rsFC, mainly within the rACC, significantly predicts individual responses to placebo administration.

Conclusions: These data demonstrate that baseline rsFC within the salience network is linked to clinical placebo responses. This information could be employed to identify patients who would benefit from lower doses of antidepressant medication or non-pharmacological approaches, or to develop biomarkers of placebo effects in clinical trials.

Trial name: Predictors of antidepressant response.

URL: http://clinicaltrials.gov/ct2/show/NCT02178696?term=zubieta&rank=3.

Trial number: NCT02178696.

TEXT

INTRODUCTION

Major depressive disorder (MDD) has recently been conceptualized as a disorder with a network-based pathophysiology (1). Particularly, MDD manifests in cortico-limbic network dysregulation reflected by deficits in cognitive control and increased sensitivity of limbic networks (2-5), which is thought to result in excessive and negatively-skewed focus on introspective processes, difficulty regulating emotions, and persistent difficulties in sustaining attention (6, 7). Regions involved in these networks have emerged as biological markers of response to antidepressant treatments (8-13). Yet, the ability of these biomarkers to selectively distinguish drug-specific effects from other non-specific elements of the treatment response, such as the placebo effect, is still limited, with very few studies specifically addressing biomarkers of non-specific elements of antidepressant treatment response (14, 15). This is not a small concern, as placebo response rates in antidepressant clinical trials average 31-45% compared with response rates to antidepressants of ~50% and have increased at a rate of 7% per decade over the last 30 years (16, 17). Hence, further investigation is warranted in order to dissect the neural predictors of drug-specific and non-specific effects in MDD treatment.

Of the major functionally connected networks identified within the brain's inherent organization (18-20), three have received special attention in MDD and the prediction of treatment response: the default-mode (DMN), salience (SN) and executive (EN) network (21, 22). The DMN, with key regions in the posterior cingulate, medial prefrontal, and bilateral parietal and temporal cortices (23), is associated with introspective cognition (24) and demonstrates heightened connectivity and abnormal down-regulation in MDD, which may

contribute to the disorder's hallmark attributes of excessive self-focus, inattention and rumination (25-28). Elevated pretreatment activity of the rostral anterior cingulate (rACC), a region encompassed within the main anterior DMN node (24) has consistently been identified as a predictive marker of treatment response across imaging and treatment modalities (9, 10, 29, 30). It has been hypothesized that heightened baseline rACC activity fosters better treatment outcome in patients with MDD by implementing adaptive self-reflection through its connectivity within the DMN (31). Moreover, antidepressant medication has been shown to decrease functional connectivity of the DMN (32). The SN, anchored by the anterior insula (aINS) and dorsal anterior cingulate cortex (dACC), is enlisted during the integration of salient stimuli and interoceptive information to guide motivated behavior (33). In particular, the aINS is a hub of meta-awareness and affective processing (34, 35) and has long been associated with MDD pathophysiology (25, 36-39). A recent meta-analysis illustrates its activity as a neural predictive marker of MDD treatment response, where increased baseline insular activity is associated with poor clinical response (40). Finally, MDD is characterized by reduced functional connectivity of the EN (4) and hypo-activation of the network's key node: the dorsolateral prefrontal cortex (dlPFC) (41). The EN, which includes cohesive functional communication between the dlPFC and parietal cortex, is responsible for orienting to and engaging in attentive, goal-directed behavior (33); its dysfunction may contribute to a lack of control over heightened affective responses in MDD (21, 42). The dlPFC is involved in current MDD treatments and successful recovery from the disorder (43-45); while reduced grey matter volume in the region is an indicator of non-response to standard MDD treatments (11).

Nodes within these three networks have also been implicated in the neurobiological mechanisms of non-specific treatment effects in the field of placebo analgesia, where substantial

headway has been made to identify the cognitive, neural, and molecular bases of the neurobiology of placebo effects (46-51). These studies have demonstrated a key role of the rACC in the formation of placebo analgesia (48, 52, 53), potentially through its interactions with additional subcortical brain areas involved in endogenous opioid-mediated analgesic effects, such as the amygdala (54) and the periaqueductal gray (48), but also the anterior and posterior INS (50, 51, 55). A number of neuroimaging studies have also reported placebo-associated changes in dlPFC function, thought to be related to expectancies and anticipatory mechanisms (56-58), consistent with the role of this region in cognitive executive function (59). In this regard, activity in EN-associated regions during pain anticipation was found to be predictive of the magnitude of placebo analgesia (60). Conversely, minimal information has been acquired as to the mechanisms implicated in antidepressant placebo effects, with notable exception of one investigation demonstrating an overlap in regions involved in placebo and medication effects (15) and our recent work describing the role of the opioid system in the formation of placebo responses in MDD (61).

Here, we take a network-based univariate and multivariate approach to the prediction of the response to placebo and antidepressant treatment using resting-state functional connectivity (rsFC) with independent component analysis (ICA) (62), a data-driven approach that produces results within a framework of the brain's intrinsic connectivity networks and allows for identification of reliable, exploratory-based treatment response predictors. We investigated the relationship between baseline rsFC of three networks (DMN, SN, left and right EN) and: 1) depression severity; 2) clinical response to one-week of placebo treatment; and 3) clinical response to ten-weeks of open-label antidepressant treatment. First, among the three networks, only the DMN has been previously related to MDD duration (63) and maladaptive rumination

(25); therefore, we hypothesized that increased baseline DMN rsFC would be associated with greater depression severity. Second, with respect to the prediction of treatment response, none of the connectivity networks have been specifically related to placebo or antidepressant medication responses in patients with MDD. However, central regions of the DMN, SN, and EN — specifically the rACC, INS, and dlPFC, respectively — have a key role in mechanisms implicated in antidepressant and placebo responses, as described above, as well as the role of these networks in processes necessary for treatment responses: internal monitoring, saliency and higher-level cognition, respectively. Therefore, we hypothesized that increased baseline functional connectivity within central regions of the DMN, SN and EN would predict response to both one-week of placebo and ten-weeks of open-label antidepressant treatment. Finally, we applied multivariate relevance vector regression (RVR) to evaluate the hypothesis that baseline rsFC maps of the three networks would allow for prediction of placebo and antidepressant responses at an individual level.

METHODS and MATERIALS

Subjects

Twenty-nine right-handed, un-medicated subjects with a DSM-V diagnosis of MDD were recruited via local advertisements [21 females; age: 18-59 (mean ± S.D.: 32 ± 13)]. See Supplemental Methods and Material for additional subject information and description of informed consent and authorized deception. All of the procedures used were approved by the University of Michigan Investigational Review Board for Human Subject Use Research Committee.

Placebo Randomized Controlled Trial (RCT) and Antidepressant Open-Label Trial

As previously described (61), subjects were randomized into either 1) one-week "active" placebo treatment (two pills/day), with expectations that the pill represented a potentially fast-acting antidepressant agent or 2) one-week "inactive" placebo treatment described as an control condition, without pharmacological effects (two pills/day). After a three-day "washout" period of no pills, subjects crossed over into the alternative condition to which they had not been assigned. After each week of placebo, subjects underwent a resting-state scanning session (Fig. 1).

Depression symptoms were assessed using the 16-item self-rated version of the Quick Inventory of Depressive Symptomatology (QIDS) (64) at the following time points: pre-randomization, baseline (pre)-, and post- *active* and *inactive* conditions. This measure was selected as a primary outcome measure for its sensitivity to fluctuations in depression symptoms and its availability as a self-reported measure. Additionally, patients completed the Montgomery Asberg Depression Rating Scale (MADRS) and the Hamilton Rating Scale for Depression (HDRS) at pre-randomization and during each visit within the antidepressant trial.

The change in QIDS ($\Delta QIDS = QIDS_{BASELINE} - QIDS_{POST}$) was calculated for active and inactive treatment conditions, and the difference between conditions was taken as an index of placebo response ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$). Positive values reflected greater reductions in depressive symptoms as a result of "active" placebo administration.

Following the placebo RCT and the two resting-state fMRI scan sessions, subjects received a ten-week open-label antidepressant trial with citalopram as an initial agent (starting at 20 mg/day and up to 40 mg/day in 45% of cases). When citalopram was not clinically indicated (e.g. prior non-response or side-effects), another antidepressant was given [sertraline (n=1), mirtazapine (n=1), fluoxetine (n=2), duloxetine (n=1), and bupropion (n=1)]. Participants' symptom changes were evaluated at weeks 0, 2, 4, 8, and 10. Antidepressant response was measured by the difference in QIDS between week 0 and 10 ($\Delta QIDS$ = Week 0 – Week 10). In four cases, participants began the antidepressant treatment less than three days after the placebo experiment; because of this, a baseline QIDS Week 0 measure was created to measure antidepressant treatment response.

Resting State Functional Connectivity Networks

Image data acquisition, preprocessing, movement analysis, and ICA are detailed in Supplemental Methods. Among available rsFC analytical methods, in our opinion, ICA, a data-driven approach, is an optimal choice for isolating connectivity networks, to provide an inherent framework for any resultant predictors, versus manual selection of a seed region. Briefly, 20 components were output through ICA utilizing the Infomax algorithm within the Group ICA methodology in GIFT software (Medical Image Analysis Lab, University of New Mexico, Albuquerque, New Mexico; http://icatb.sourceforge.net/). Of the resultant components, the networks of interest were selected using templates from the BrainMap

(http://www.brainmap.org/icns) database: a comprehensive resting-state fMRI data source (65). To determine the component with the "best-fit" for each particular network, a linear-template matching procedure was performed on all 20 components, as described elsewhere (63). Briefly, for each network template: all 20 components were scored based on their best-fit with the template by computing the average z-score of voxels falling outside the template in the component subtracted from the average z-score of voxels falling within the template. The component with the greatest value of this measure was identified as the network-of-interest: DMN, SN, right or left EN. For all networks, the component-of-interest had a best-fit score of at least two SD greater than the mean [network: best-fit score (mean ± SD); DMN: 15.7 (1.63 ± 3.58); SN: 5.2 (0.7±1.45); LEN: 12.0 (1.1 ± 2.7); REN: 4.6 (0.7 ±1.4)].

Data Analysis

All data analysis was performed using SPM8 (Welcome Department of Cognitive Neurology, University College, London, England) and Matlab (MathWorks, Natick, Massachusetts) software. One-sample t-tests and paired t-tests were used to analyze within-network functional connectivity within- and between-conditions, respectively (see Supplemental Methods for paired t-tests). For each network, one-sample t-tests were performed: network-of-interest components from all 29 subjects during the "post-inactive" scan (baseline) were entered into the analysis and ICA-assigned z-scores at each voxel were averaged across all subjects and compared to zero (Fig. 2). Significance threshold was set at $p<0.05$ family-wise corrected (FWE).

For each network, a whole brain voxel-by-voxel regression analysis was performed between network functional connectivity (z-score of the weight on the ICA component of interest) at baseline (post-inactive) with the following variables: 1) depression severity (as measured by QIDS score at pre-randomization); 2) reductions in depression symptoms in response to placebo

administration with expectations of antidepressant effects [($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$), see above]; and 3) reductions in depression symptoms in response to ten weeks of antidepressant treatment (as measured by ΔQIDS scores = Week 0 - Week 10). All analyses were restricted to voxels within those defined by the network-of-interest's one-sample t-test composed of corresponding network component maps from all 29 subjects' inactive scan (masked at $p<0.05$-FWE). Depression severity score and scan order were input as covariates in all analyses.

All results were constrained within network component maps [one-sample t-test (above) masked at $p<0.05$-FWE]. For all *a priori* regions (rACC, INS, dlPFC), significance was set at a threshold height of $p<0.005$ uncorrected and 3dClustSim [AFNI (http://afni.nimh.nih.gov)] was used with 1000 Monte Carlo simulations to determine cluster-based, whole-brain correction at $p<0.005$ to account for testing of three bilateral regions-of-interest and four networks (total of ten). See Supplemental Material for extent cluster information, which is network-size dependent. Significance for all other peaks was set at $p<0.05$-FWE. These significant data clusters were extracted using MarsBar (66), for quantification of regional functional connectivity at baseline and placebo-induced changes, graphing, determination of correlation coefficients (Pearson/Spearman correlations at $p<0.05$). Data are expressed as the mean ± one S.D., unless otherwise indicated.

Multivariate Relevance Vector Regression (RVR) Analysis

We also investigated single-subject predictability of baseline ('inactive' scan) rsFC onto measured placebo responses using multivariate RVR as implemented in PRoNTo (http://www.mlnl.cs.ucl.ac.uk/pronto/) running under Matlab (Mathworks, 2012a release). RVR is detailed elsewhere (67). Briefly, it is a sparse kernel learning multivariate regression method formulated in Bayesian framework. The model weights are assigned a zero-mean Gaussian prior

in which each weight is governed by its own hyperparameter. Iterative estimation from the fMRI data identifies the most probable values for these hyperparameters with sparseness achieved due to the posterior distributions of many of the weights peaking around zero. The voxels associated with non-zero weights are marked as relevance vectors, which can then be used to predict the target value (placebo-induced ΔQIDS) for a novel input vector (e.g. baseline SN rsFC). RVR's major strength relative to other multivariate machine learning techniques [i.e. support vector machine (SVM), reviewed (68)] is that it computes quantitative prediction of a variable of interest without a need for discrete categorical estimation (e.g. patients vs. controls) (69). Here, the subjects' SN components were mean centered and input into the RVR analysis. An estimate for the model's predictability was calculated via leave-one-out cross validation, indexed using the Pearson correlation coefficient and mean squared error (MSE) between actual and the predicted placebo response measure. The significance of these metrics was determined through non-parametric permutation tests. In a single iteration, we randomly paired subjects' SN functional connectivity with subjects' placebo response values. Subsequently, we calculated the MSE and correlation for the random pairing. This was completed for 5000 iterations and built a distribution of MSE and correlation values from which p-values were calculated for accuracy of placebo response prediction.

RESULTS

Patient Characteristics

Twenty-nine participants with MDD [QIDS-16SR (mean ± SD): 16.1 ± 4.1; Hamilton Rating Scale for Depression (HRSD-17): 21.6 ± 5.1] were enrolled in the study and completed the two-week randomized placebo trial and entered the ten-week treatment with an open-label antidepressant (Fig. 1). 79% of them (23/29) completed the entire open-label antidepressant treatment portion; those who dropped out before full completion were not significantly different in their depression severity and placebo responsiveness from those who completed the study (see Supplemental Results, also for additional participant data).

Placebo- and Medication-induced Changes in Depression Symptoms

Reductions in depression symptoms after one week of the "active" placebo were significantly greater than after the "inactive" placebo treatment [ΔQIDS: 2.0 ± 3.4 for active; 0.17 ± 2.4 for inactive; $F = 7.2$, $df = 1$, $p = 0.012$]. The total placebo response measure ($\Delta QIDS_{ACTIVE} - \Delta QIDS_{INACTIVE}$) was highly variable, ranging from -8 to 11 (mean ± S.D.: 1.8 ± 4.2). Ten weeks of open-label antidepressant treatment was also associated with a significant reduction in depression symptoms [QIDS at Week 0: 11.6 ± 4.3; at Week 10: 6.9 ± 3.9; $t = 4.9$, $df = 22$, $p < 0.001$].

Association of Baseline Resting Functional Connectivity, Pre-Randomization Depression Severity, Response to Placebo, and Response to Antidepressant Medication

Statistical component maps for each network were examined against symptoms of depression severity (QIDS-16SR) at time-point of pre-randomization. We did not find a

significant relationship with any of the networks (*a priori*: $p>0.005$, mm^3 > network-specific mm^3; other: $p>0.05$-FWE).

Statistical component maps for each network were then examined against reductions in depression symptoms in response to one-week of placebo administration with expectations of antidepressant effects (Fig. 3) and in response to ten weeks of open-label antidepressant treatment. Significance was established at $p<0.005$ and extent > 928 mm^3 for the SN (Supplemental Material). Increased baseline rsFC of the rACC within the SN was significantly associated with greater placebo response: rACC: 0, 38, 4, 1784 mm^3; Z= 4.35. No other significant effects were observed.

A linear regression analysis that included baseline rsFC of the rACC within the SN predicted 65% of the variance in placebo responsiveness (adjusted $R^2=0.65$).

Based on this initial finding and the rACC's essential involvement in placebo effects (50-52) and the prediction of antidepressant treatment response (70, 71), we allowed subsequent activation of rACC rsFC to be significant after small volume correction ($p<0.05$-FWE) using a rACC region-of-interest sphere centered around the MNI coordinates 4, 42, 6 (72) with a 6mm radius [created using MarsBar (66)].

Increase baseline rsFC of the rACC within the SN was significantly associated with reductions in depression symptoms in response to ten weeks of open-label antidepressant treatment (rACC: 0, 40, 8; 64 mm^3; Z = 3.09; $p<0.05$-SVC:FWE-corr). No other effects were observed.

Placebo-induced Changes in Network Functional Connectivity Predicts Response to Placebo and Antidepressant Medication

As expected, one-week of placebo administration with expectations of antidepressant effects did not result in significant changes within rsFC of the networks—as measured by the changes in rsFC from "active" to "inactive"—given high inter-individual variation in response to placebo (*a priori*: p>0.005; other: p>0.05-FWE-corr, see Supplemental for network-specific cluster size).

In order to account for the variability inherent in the placebo response measure, statistical component maps of rsFC changes in response to one-week of placebo administration were then examined against associated reductions in depression symptoms in response to one-week of placebo administration and ten weeks of open-label antidepressant treatment. Placebo-induced rsFC reduction in the rACC within the SN was significantly associated with greater placebo response (rACC: 0, 38, 4; 192 mm^3; Z = 3.97; p<0.05-SVC:FWE-corr). No other significant effects were observed.

Multivariate Relevance Vector Regression (RVR): Single-Subject prediction of placebo and treatment response

Based on the results described above, we first applied RVR to rsFC of the entire SN, where significant results were observed, in order to enable quantitative prediction of depression symptom reductions in response to placebo administration and in response to ten weeks of antidepressant treatment at the individual level. RsFC of the SN was significantly predictive of placebo responses [correlation = 0.41; p-value = 0.018; mean sum of squares = 14.36; p-value = 0.019; (Fig. 4)], with the greatest weights contributing to placebo response prediction within the rACC. SN rsFC was not a significant predictor of response to antidepressant treatment (correlation = 0.03; p-value = 0.34; mean sum of squares = 21.31; p-value = 0.36). No effects were found in the other networks.

DISCUSSION

In this study, we illustrated the viability of network-based rsFC to identify potential imaging-based predictive markers of treatment response in MDD. We found that more cohesive rsFC of the SN, through connectivity of the rACC, predicted greater reductions in depressive symptoms following one-week of placebo administration with expectations of antidepressant effects and ten weeks of open-label antidepressant treatment. In addition, the clinical response to one-week of placebo treatment was also associated with placebo-induced decreases in rsFC of the rACC within the SN. Finally, multivariate RVR demonstrated that SN rsFC predicted placebo responses with significant accuracy at an individual level.

Elevated baseline rsFC of the rACC within the SN predicted greater reductions in depression symptoms in response to placebo administration with expectations of antidepressant effects. Specifically, the rACC alone predicted 65% of the response variance. This finding potentially advances a more general understanding of the neurobiology of placebo effects across diseases: Both opioidergic and BOLD activity within the rACC have been consistently implicated in placebo analgesia (48, 52, 53) which evinces a hypothesis that corresponding mechanisms of action are involved in the formation of placebo effects across disorders (47), with recent evidence in further support of this notion (61). Our data, to our knowledge, are also the first demonstration of SN involvement in MDD placebo effects and thus, supplement the small existing body of work in the neurobiology of placebo in patients with MDD (15, 61). This is consistent with the role of the SN in saliency attribution: particularly, selecting and integrating biologically relevant stimuli with interoceptive states to guide input of attentional resources and

behavior (73). In this case, the network was involved in attending to and incorporating a set of expectations within a therapeutic environment with internal states resulting in a reduction of depression symptoms. If the former hypothesis remains valid, SN rsFC may be the functional context of prior placebo analgesia-associated rACC findings.

Mounting evidence from studies employing a variety of neuroimaging modalities and treatments indicate that increased pretreatment activity in the rACC is associated with better response to antidepressant treatment, as recently reviewed (31). More specifically, metabolism in the region has been observed to differentiate responders from non-responders to antidepressant medication (70). However, the open-label nature of these and prior studies (10, 70, 74, 75) makes it difficult to dissect drug-specific from non-specific treatment effects. Our study is an initial attempt in isolating predictors for different aspects of treatment effects, such as placebo. Here, we demonstrated that greater baseline rsFC of the rACC within the SN to be a significant predictor of placebo response *and* separately, open-label antidepressant treatment response. Consistent with these data, a recent RCT found that elevated pretreatment theta current density in the rACC predicted treatment outcome in both the medicated and placebo group (14).

In all, these findings point to the predictive role of rACC within the SN in the response to placebo and the overall response to antidepressant treatment (antidepressant + placebo + other non-specific effects). Furthermore, we observed the normalization of heightened baseline rACC rsFC after placebo administration to be associated with greater placebo responses; this illustrates that rACC modulation may be an important element of placebo neurobiology. A relevant hypothesis may state that enhanced rACC rsFC within the SN may represent a greater capacity for integrating salient stimuli with adaptive cognitive-affective functioning, which is conducive for the manifestation of a placebo effect. Normalization of heightened rACC activity has also

been demonstrated in MDD responders to sleep deprivation, but absent in non-responders (a placebo-arm was not incorporated) (76). Thus, malleability within the rACC may extend to necessitate a successful antidepressant treatment response or to placebo mechanisms inherent in the antidepressant response.

Finally, we demonstrated that multivariate RVR application to baseline SN rsFC, with the greatest weight within the rACC, was significantly predictive of placebo responses at an individual-subject level, although this was not the case for antidepressant responses. The utilization of a similar method, SVM, has gained substantial attention in the last years as a predictive classifier in MDD with diagnostic and prognostic qualities (77-79). For example, in a sample of 37 depressed patients and 37 healthy controls, whole-brain structural MRI significantly classified nearly 90% of patients exhibiting clinical remission and nearly 70% of those with an MDD diagnosis (80). Instead, we employed RVR to enable quantitative prediction without need for group classification, as it has been recently described (69). The potential applicability of SN rsFC as an individual-subject predictor of placebo response will need to be further confirmed in larger samples; yet, this information could ultimately augment clinical treatment through identification of patients who possess a greater likelihood in benefiting from lower dosages of antidepressant medication or cognitive interventions. This may also be relevant in the context of clinical trials to better distinguish patients with greater susceptibility to placebo effects, allowing for patient stratification and possibly, more detailed clarification of the effects of treatment components (specific versus non-specific).

A limitation of the current study was the small sample size (N = 29 for the placebo phase; N = 23 for the antidepressant phase). This is especially relevant to the RVR analysis, where independent confirmation is needed to establish whether SN rsFC is a predictor of placebo

response at an individual level. Finally, the subsequent nature of the open-label treatment after the placebo phase was not optimal for dissecting drug and placebo-specific effects. Future RCTs with parallel placebo and drug arms will need to be conducted to better identify and separate predictors of treatment response. However, our data have the potential to inform future designs of antidepressant clinical trials and personalized medicine for MDD patients by identifying individuals with greater likelihood in responding to non-pharmacologically specific interventions and who may ultimately be selected for less intensive treatments, lower dosages of medication, or for enhanced patient-clinician interaction.

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FINANCIAL DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

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FIGURES

Figure 1: Experimental Design. A) After pre-randomization (screening), subjects are randomized into one of two conditions each lasting seven days: 1) *Active*: placebo administration with disclosure that it may provide antidepressant-like treatment effects; 2) *Inactive*: placebo administration with disclosure that it is an inactive agent. B) A three day washout occurs during which the patient receives no medication. C) Subjects cross-over to the alternative condition. D) *Resting-state fMRI scans are obtained immediately after each condition*. E) After full completion of the placebo trial, subjects undergo ten weeks of open-label antidepressant treatment. Depression measures are administered (* marks QIDS-16SR administration) at pre-randomization, pre- and post-active, pre- and post-inactive, and week 0, 2, 4, 8, 10 of the antidepressant trial.

Figure 2: Functional Connectivity of Networks. One-sample t-tests including baseline (inactive condition) resting-state fMRI scans for all 29 subjects for each ICA-component corresponding to the network: A) Default-mode, B) Salience, C) Left (left-side) and right (right-side) executive networks. The t-score bars are shown at the right; all images are displayed at a threshold of $p<0.05$ family-wise corrected. Images are shown in standard space of the Montreal Neurological Institute (MNI) template. N=29.

Figure 3: Baseline Functional Connectivity of the Salience Network Predicts Response to Placebo Administration. N=29. (Top left and right): Voxel-by-voxel correlational analysis between baseline functional connectivity of the SN and decreases in depression symptoms in response to placebo administration. Clusters passing significance threshold are labeled. Image display is at $p<0.01$; t-score bar is shown in the bottom right. (Bottom Left): Association between reductions in depression symptoms in response to placebo administration and functional

connectivity of rACC within the SN. Functional connectivity of this region predicted 65% of the variance in placebo responses ($p<0.001$).

Figure 4: Multivariate Relevance Vector Regression. Left: Mean predictor map with an arbitrary threshold of >50% of minimum and maximum voxel weight values. The map shows the relative contribution of each voxel to the regression function in relation to all other voxels. Color bar signifies weight value for all voxels. Right: Scatter plot showing the predicted placebo response value derived from each subject's baseline SN functional connectivity using RVR leave-one-out cross validation versus their actual placebo response *value* ($r = 0.41$; p-value = 0.018).

Figure1

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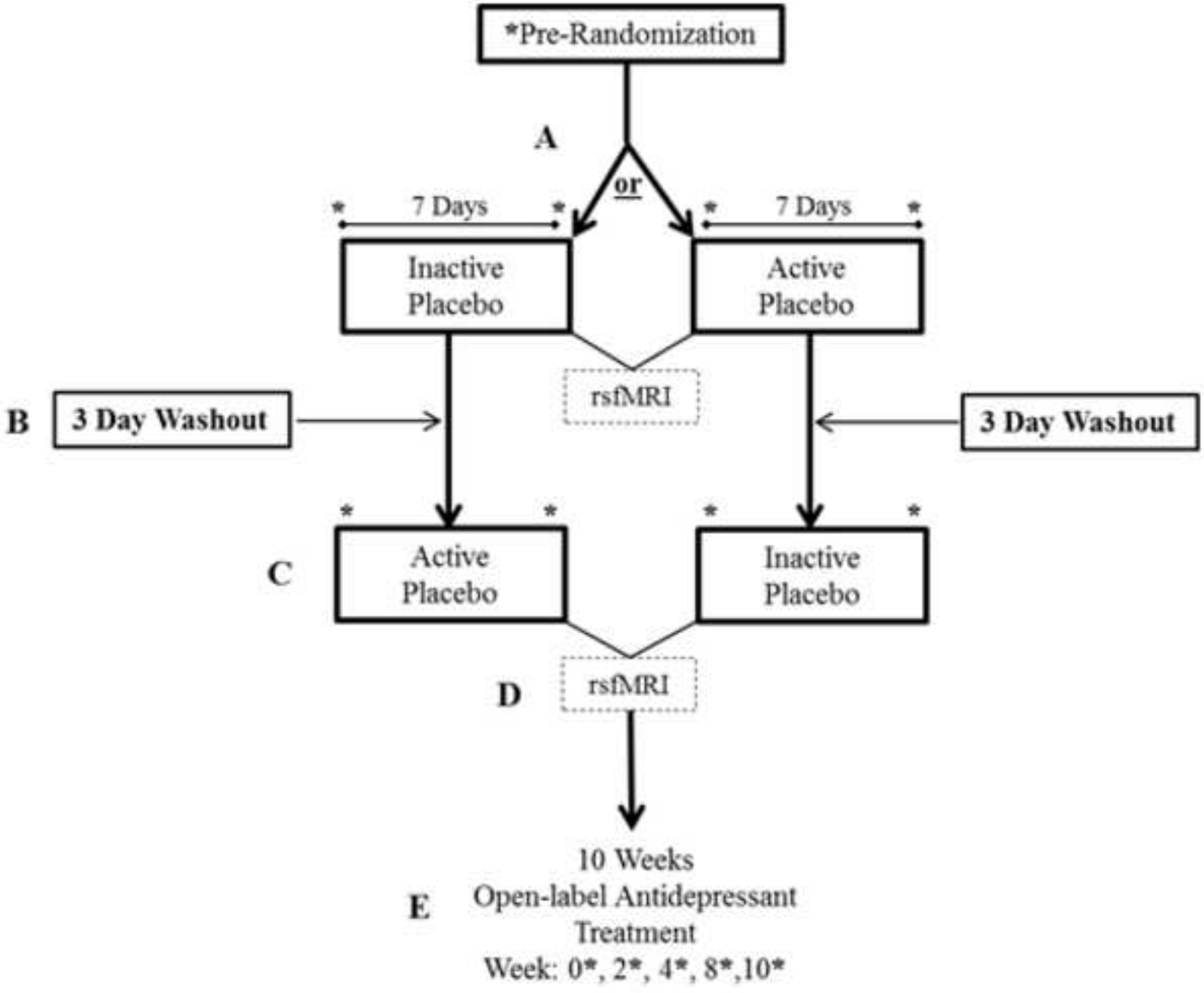

Figure2

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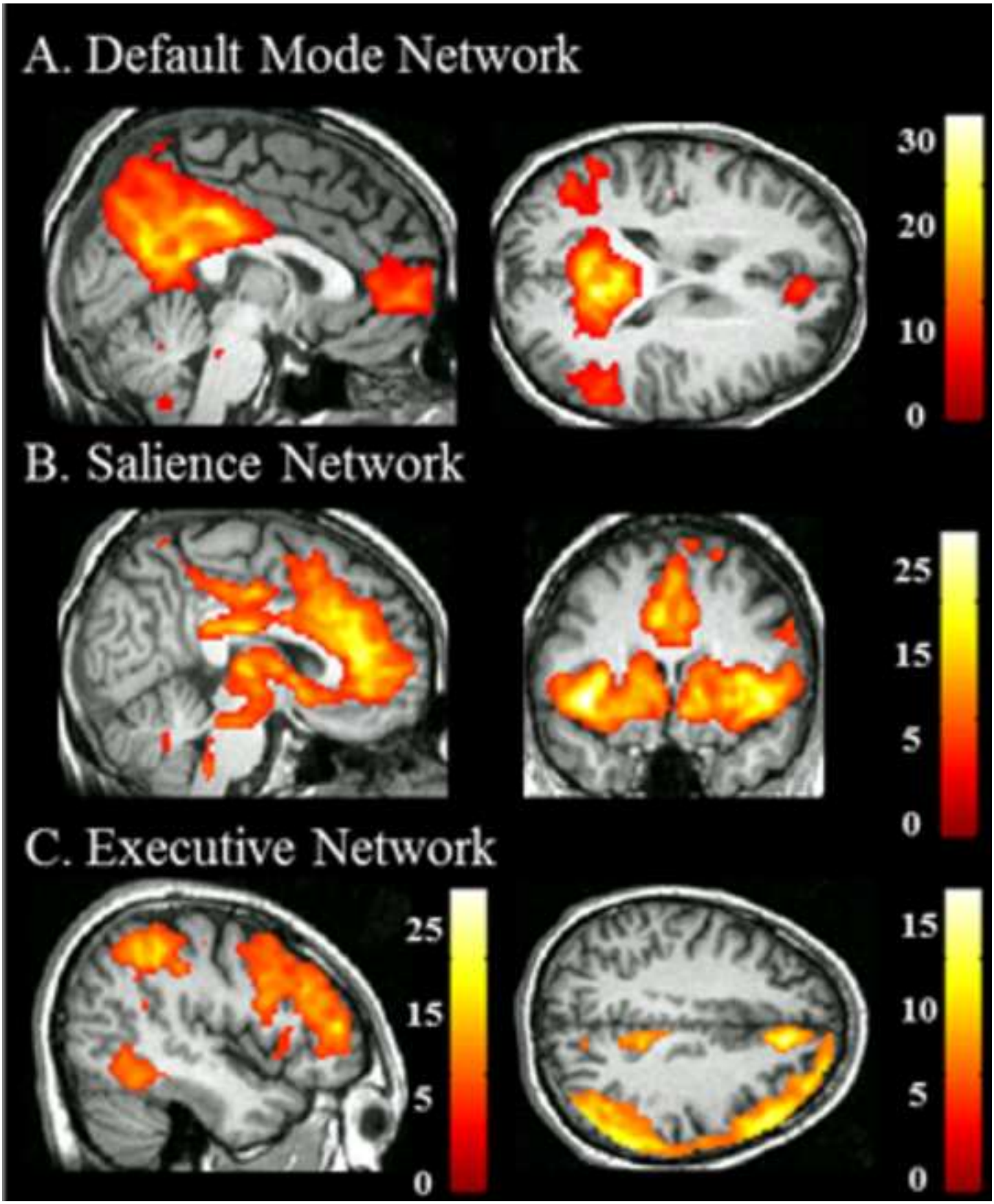

Figure 3

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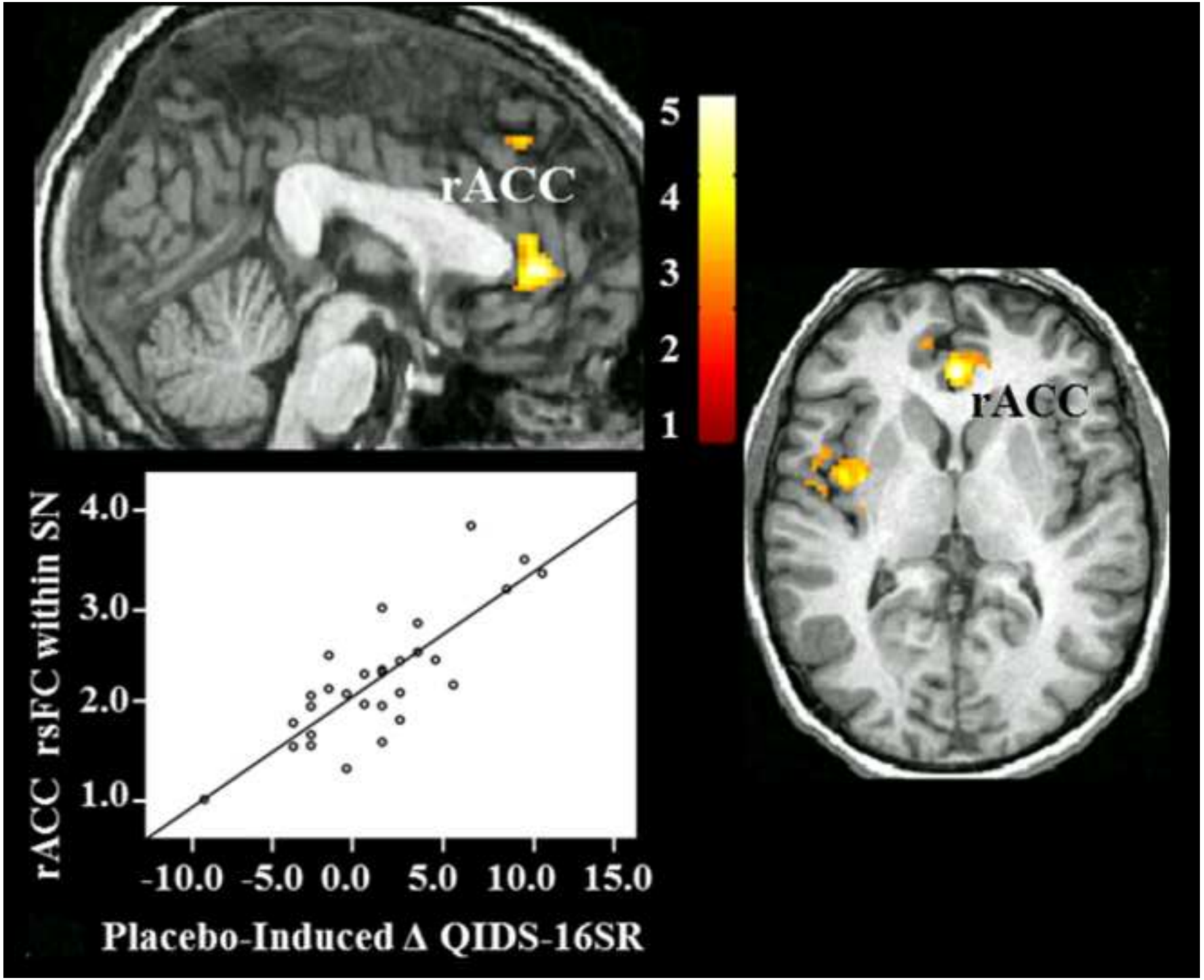

Figure 4

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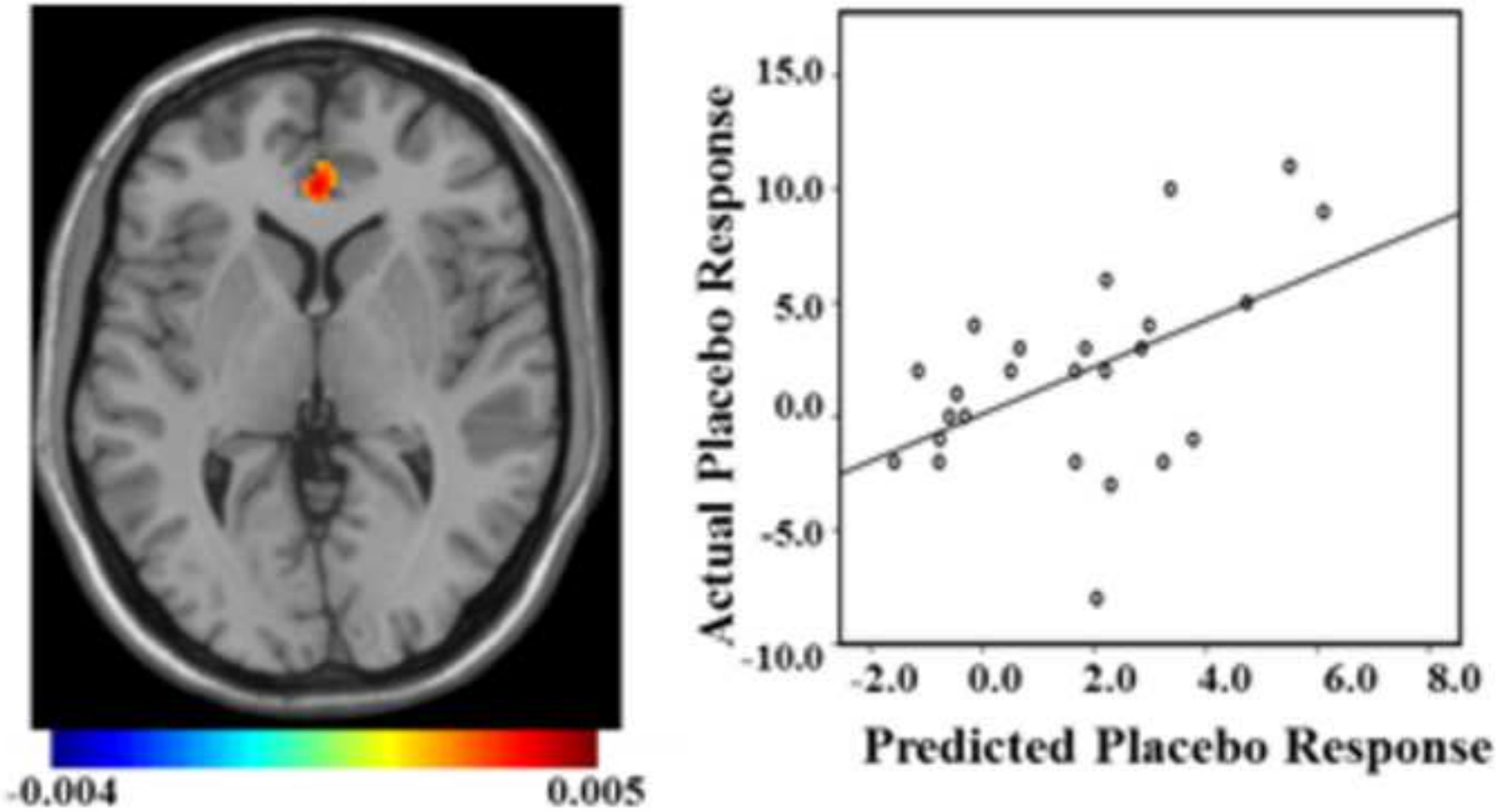

Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression

Supplemental Information

Supplemental Methods and Materials

Subjects

In addition to completing physical and neurological examinations, subjects were screened using the Mini International Neuropsychiatric Interview (1); inclusion criteria included a diagnosis of MDD, 17-item Hamilton Depression Rating Scale scores >12 and excluded suicidal ideation, comorbid conditions (medical, neurological or psychiatric, substance abuse or dependence), the use of psychotropic agents and pregnancy. Patients were either medication-naïve or had not received antidepressant treatment for at least 6 months. Current anxiety disorder diagnoses (generalized anxiety, panic, agoraphobia, social phobia, and specific phobia) were included because of the shared risk factors between MDD and anxiety. Left-handed individuals and subjects who had used any centrally acting medications, nicotine, or recreational drugs within the past two months were excluded. Data was collected and stored using Research Electronic Data Capture (2).

Authorized Deception Procedure

During the consent process, subjects were not informed of the purpose of the study (investigation of placebo mechanisms), nor the manipulations in expectations that took place in the study by the labeling of placebos as active or inactive. To resolve this ethical dilemma, we followed the recommendations of Miller *et al.* (3) and Martin and Katz (4) by incorporating the following

information into the consent form: “You should be aware that the investigators have intentionally withheld certain aspects of the study. This is necessary to obtain valid results. However, an independent research committee has determined that this consent form describes the major risks or benefits of the study. The investigators will explain the withheld aspects of the study to you at the end of your participation.” Upon completion of the study or if the subjects wished to discontinue the study at any point, subjects were debriefed about the study’s purpose and the use of placebos.

Neuroimaging Methods

Resting State Functional MRI Acquisition and Prepreprocessing

After both the active and inactive placebo treatment (Fig.1), all subjects underwent an eight-minute resting state fMRI scan during which they were instructed to hold still in the scanner with eyes open.

Functional images were acquired on a 3-Tesla scanner (Philips Achieva, Best, Netherlands) using a single-shot echo planar imaging sequence (39 slices, slice thickness = 3.5 mm, TR = 2000, TE = 35 ms, FA = 90, FOV = 20 cm, 64 x 64 matrix). A high resolution structural image was obtained for anatomic normalization using a T1-weighted, gradient echo (MPRAGE) sequence (220 slices, slice thickness = 1 mm, echo time = 4.6 msec, repetition time = 9.8 msec, flip angle = 8°, field of view = 240 mm^2).

Each resting-state scan was preprocessed using FSL (http://www.fmrib.ox.ac.uk), SPM8 (www.fil.ion.ucl.ac.uk), and AFNI (afni.nimh.nih.gov/afni) at various stages. Preprocessing included slice time correction (SPM8), motion correction (SPM8), linear registration of resting state scan to the structural image using the boundary based registration algorithm in FSL’s

FLIRT (5-7), and nonlinear registration of structural images to MNI space with FSL's FNIRT (8, 9). The subject's scan was then normalized to MNI space with the field coefficients generated from FNIRT, resampled to 2 x 2 x 2 mm voxels, and smoothed with a 5-mm FWHM Gaussian kernel (SPM8).

Nuisance signal removal from voxel time series was performed on the preprocessed resting state scans using linear regression in AFNI's 3dTproject. The nuisance signals removed were linear/quadratic trends to account for scanner drift; the six rigid body realignment parameters (motion) and their first derivatives; and the top five principal components from both white matter (WM) and cerebral spinal fluid (CSF) BOLD time courses (10). The subject-specific WM and CSF masks used for principal component analysis were generated by segmenting the normalized structural image into tissue probability maps with FSL's FAST (11) (threshold at 0.99). To further reduce partial volume effects, the WM mask was twice eroded and the CSF mask was restricted to the ALVIN mask (12) of the ventricles.

Movement Analysis

To minimize the effect of motion, the instantaneous displacement between all volumes was calculated using the six motion parameters and any subject with a maximum movement greater than 3 mm was excluded. Frame displacement (13) was also calculated for each volume and if a scan had more than 60% volumes greater than 0.3 mm frame displacement, the subject was removed. Eleven subjects were excluded from the analysis for a total of 29 subjects.

Group Independent Component Analysis and Intrinsic Network Selection

Independent component analysis (ICA) was conducted for all subjects' active and inactive scans using the Infomax algorithm within the Group ICA methodology in GIFT software (Medical Image Analysis Lab, University of New Mexico, Albuquerque, New Mexico;

http://icatb.sourceforge.net/). ICA is a data-driven, source separation technique, that when applied to fMRI, separates the BOLD signals into statistically independent spatiotemporal components (14). Principle component analysis (PCA) was first utilized to reduce each subject's resting state fMRI data, after which, subjects' data were concatenated and further reduced with PCA. ICA was then applied to reduce the complete data set into large-scale patterns of functional connectivity; a model order of 20 components was selected in order to isolate these major functional connectivity networks, as has been previously shown (15). The infomax ICA algorithm was repeated 20 times in ICASSO to ensure the analysis converged to stable components. To identify subject-specific spatial maps and associated time courses which correspond to the group ICA components and avoid PCA bias from either scan condition, a spatiotemporal regression algorithm was applied (16, 17). During which, group-level spatial maps from group ICA results were used as spatial regressors in order to find the temporal dynamics associated with each map. In turn, these time courses were employed as a set of temporal regressors to find subject-specific maps (of the multi-subject spatial maps).

In resting state fMRI, the BOLD time series of intrinsic connectivity networks are composed of low frequency fluctuations (18); thus, components in which the power spectrum of its associated time course consisted of 50% high frequency signal (> 0.1 Hz) were considered as noise and removed from further analysis.

Inter-network Connectivity

Inter-network connectivity was investigated between the DMN, SN, and ENs. As described elsewhere (19), inter-network connectivity was calculated by using the network-associated time courses that were output from the ICA. For each subject, a correlation matrix between each pair

of networks was calculated and then converted to z-scores using the Fisher r-to-z transformation. Finally, a one-sample *t*-test across subjects was used to determine the significance of inter-network connectivity. All results were corrected for multiple comparisons using Bonferroni correction at $p < 0.05$.

Data Analysis

All data analysis was performed using SPM8 (Welcome Department of Cognitive Neurology, University College, London, England) and MATLAB (MathWorks, Natick, Massachusetts) software.

A paired *t*-test for each network was conducted: network components from both conditions were entered into the analysis and ICA-determined z-scores at each voxel were averaged within each condition and compared between active and inactive (Active > Inactive; Inactive > Active). Each paired *t*-test was restricted to voxels within the network-of-interest as defined by a one-sample *t*-test including all 29 subject's active and inactive component-of-interest maps (masked at $p < 0.05$ FWE corrected). Subject scan order (active vs. inactive first) was input as a covariate.

To test the relationship between placebo-induced network functional connectivity changes with placebo and antidepressant treatment response, subtracted images (inactive-active network component for each subject) were input into the voxel-based correlation analysis; results were restricted to voxels within one-sample *t*-test composed of corresponding inactive and active network components from all 29 subjects (masked at $p < 0.05$ FWE corrected). Depression severity and scan order were input as covariates.

Significance threshold for *a priori* regions in all correlational analyses was held at a height of $p < 0.005$ uncorrected and extent at $p < 0.005$ (Bonferroni-corrected for number of networks and their corresponding main regions of interest in this investigation). To address differences in smoothness across the multiple correlation analyses performed for each network (depression severity, placebo, and antidepressant response): we selected the most stringent critical cluster size from all analyses within each network (see table below: "baseline" is for all analyses regarding "inactive" scan's network functional connectivity; "placebo-induced Δ" is for all analyses regarding network functional connectivity differences between "inactive" – "active" scans). This value was used for all analyses regarding that specific network. All results were constricted to corresponding network one-sample *t*-test masks as described throughout the main and supplemental text. Significance threshold for all other regions was set at $p < 0.05$ FWE.

Table S1. Critical cluster sizes for each functional connectivity network. Cluster sizes were calculated with 3dClustSim of AFNI and 1000 Monte Carlo Simulations for each network map (analyses examining baseline network functional connectivity and analyses examining placebo-induced changes in functional connectivity used different masks; thus, had different cluster sizes).

	Analysis	Cluster Size Threshold ($mm^{3)}$)
DMN	Baseline	864
	Placebo-Induced Δ (Inactive-Active)	936
SN	Baseline	928
	Placebo-Induced Δ (Inactive-Active)	1024
L EN	Baseline	784
	Placebo-Induced Δ (Inactive-Active)	928
R EN	Baseline	712
	Placebo-Induced Δ (Inactive-Active)	856

Supplemental Results

Subjects

Changes in QIDS [ΔQIDS = (QIDS baseline - post) *active* placebo – (QIDS baseline - post) *inactive* placebo] were not significantly correlated with patient's age (r = -0.13; p = 0.51), nor depression severity at baseline (r = 0.04; p = 0.83); there was no significant sex effect (Females: 2.7 ± 4.1; Males: -0.5 ± 3.8; p = 0.13); there was also no significant effect on scan order (Active First: 3.4 ± 5.0; Inactive First: 0.7 ± 3.1; p = 0.23). However, scan order was input as a covariate in all analyses due to the importance of this measure in our study's experimental design. A sex effect was not seen in depression severity at baseline (Females: 16.1 ± 3.9; Males: 16.1 ± 4.6; p = 0.99) nor antidepressant treatment response (Females: n = 19; 5.1 ± 3.9; Males: n = 4; 2.3 ± 6.6; p = 0.34).

Drop Off Participant Characteristics

Participants who dropped off from the study were not significantly different from completers in their severity scores (QIDS-16SR: Dropped off (n = 6): 15.8 ± 5.4; Completers (n = 23): 16.2 ± 3.8; t = 0.2, p = 0.8) or placebo responsiveness (ΔQIDS-16SR: Dropped off (n = 6): 2.6 ± 3.0; Completers (n = 23): 1.6 ± 4.4; t = -0.5, p = 0.6). In nearly all cases of participant drop off, this occurred within the initial four weeks of the antidepressant administration. Reasons included: fear or reluctance to take medication, long distance to appointments and/or small compensation during the trial period.

Participant Expectations

The level of expectations about the potential effectiveness of the potentially fast-acting antidepressant treatment was collected in 20 out of the 29 participants using the question: “from 0 to 100, how effective do you think the fast-acting antidepressant treatment will be?”. The mean level of expectations was 51.5 (SD = 22.4). Levels of expectations did not correlate with reductions in depression symptoms in response to either the placebo or the antidepressant treatment or any functional connectivity measures.

Inter-network Connectivity

To examine the relationship between the DMN, SN, and EN, we investigated inter-network connectivity. The table below shows the r-values between the component time series of each network. DMN and SN did not demonstrate a significant difference in their component time series. Classically, the SN is known to associate within a “task-positive network” and the DMN is termed “the task-negative network”. These two networks demonstrate an anti-correlated relationship in healthy individuals (20); a recent meta-analysis of resting-state functional connectivity in MDD found increased connectivity between the anterior portions of the DMN with the SN (21), consistent with our finding. Additionally, we found that SN and EN connectivity were positively correlated as they are both engaged during the processing of external, salient stimuli (22). Of interest, the left and right EN demonstrate a dissociation with the DMN: the left EN was not significantly correlated with the DMN, whereas the right EN was significantly positively correlated with the DMN. As the EN is mainly involved in processing goal-directed, cognitive processes and the DMN is mainly involved in introspection, a negative correlation would be expected (20). This increase in connectivity between the anterior portions

of the DMN with the SN, as well as the right EN and the DMN, may represent key features of MDD pathophysiology, which will need to be further investigated in future studies and might help clarify some of our apparently inconsistent, inter-network findings.

Table S2. Correlations between network component time series (r-values are listed below). (*p < 0.05; **p < 0.01; ***p < 0.001 Bonferroni-corrected).

	SN	**DMN**	**R EN**	**L EN**
SN	*1.00*	0.07	0.11*	0.24***
DMN		*1.00*	0.30***	0.00
R EN			*1.00*	0.27***
L EN				*1.00*

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