Parsing Heterogeneity in the Brain Connectivity of Depressed and Healthy Adults During Positive Mood

Supplemental information

Supplemental Methods & Materials

Participants

Participants were recruited for a larger treatment study; depressed patients were the primary focus of the larger study and were recruited in larger numbers to facilitate tests of treatment outcomes, while a sample of healthy controls approximately one third the size was recruited to provide comparisons of healthy and depressed neurocognitive function. Diagnoses were established by experienced clinicians with the Structured Clinical Interview for DSM-IV Disorders, Patient edition (1). Healthy controls were free of history of a depressive episode and other Axis I disorders. Depressed participants were recruited through the Mood Disorders Treatment and Research Program at the Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine. Controls were recruited from advertisements and lists of participants who consented to being re-contacted during previous research. Inclusion criteria for depressed participants included meeting DSM-IV criteria for current Major Depressive Disorder; depressive severity score greater than 14 on the day of testing measured using the Beck Depression Inventory-II (BDI-II; 2) (four patients did not complete the BDI, but since no other patients were excluded solely on this basis, they were retained); Hamilton Rating Scale for Depression (HAM-D; 3) greater than 14 within two weeks of testing; and free of psychotropic medications for at least two weeks (six weeks for fluoxetine). As this assessment was part of a larger ongoing treatment study, all participants agreed to be treated with their preference of either Cognitive Therapy or medication management using selective-serotonin reuptake inhibitors. Additional exclusions included medical conditions that could cause depressive symptoms or would preclude their participation in the study; psychosis; inability or refusal to consent to the study; and pregnancy. All participants had verbal IQ scores greater than 85 as measured by The North American Adult Reading Test (NART; 4).

The study was approved by the Internal Review Board of the University of Pittsburgh.

Protocol Deviations and Missing Data

Mood induction task deviations occurred for 1 control and 5 depressed subjects (happy music did not play—n=3; mouse malfunction affecting continuous mood ratings—n=3). These subjects were not included in analyses of self-rated mood during the induction; removing them entirely did not alter any other finding in the main text. Symptom severity scores were missing from 12 patients and 4 controls (7.4% of sample) due to failure to collect or store the full battery of questionnaires. Resting state task connectivity data were unusable from 1 control and 3 patients (4.3% of sample) due to technical errors at data acquisition.

An additional 13 individuals (8 depressed, 5 controls) completed the positive mood induction task but were excluded prior to all analyses (i.e., no ROI timeseries extracted or GIMME performed) because quality-checking procedures revealed inadequate acquisition coverage of some regions of interest. In the vast majority of these cases, coverage of one or both amygdalae was inadequate due to MRI technician error.

fMRI Preprocessing

The following standard fMRI preprocessing steps were applied, as described in more detail previously (5): slice time correction, motion correction, linear detrending to correct drift, voxelwise outlier rescaling, conversion to percent change, temporal smoothing [7-point gaussian filter], 32-parameter nonlinear warping to the Montreal Neurological Institute Colin-27 brain data set, spatial smoothing [6-mm full width half maximum]. Prior to the warping step, AFNI's ANATICOR algorithm was applied to remove artifacts (hardware, motion) influencing connectivity data. The GIMME algorithm included further steps to reduce the influence of "micro-movement" on connectivity data (see 'Handling and impact of motion during scanning' below).

Considerations in Connectivity Method Selection

We have shown that abnormalities of negative information processing are associated with abnormalities of temporally varying directed associations representing the unfolding of emotional information processing and its regulation over time (6). As our previous techniques are only appropriate for designs with trials that are a few seconds long and we have shown that the phenomena of interest happen on the time-course of minutes (7), our current approach used a more general framework capable of capturing sustained variation on the time-course of minutes. The GIMME algorithm robustly recovers individual-level directed paths both when stationarity in time is assumed and when it is not (8). As noted in the main text, the approach yields similar results to Dynamic Causal Modeling (DCM), but unlike DCM, it has the added benefits of being applicable to block design and resting state data, and can readily handle a larger number of regions of interest.

Region of Interest Definitions

Network nodes were defined using a combination of anatomical masks based on standardized (MNI and Talairach) atlases [with anterior cingulate cortex (ACC) parcellation boundaries as in (9)], metaanalytic coordinates showing altered resting state connectivity in depression (using 8mm radius spherical ROIs around peak meta-analytic coordinates from (10,11)], and one functional ROI from our previous studies of depression (12). VAN regions included: bilateral amygdala, nucleus accumbens (NA), anterior insula, ventrolateral PFC (VLPFC), and the subgenual ACC (sgACC). DMN regions included: perigenual ACC (pgACC) and posterior cingulate cortex (PCC; coordinates from (11)). CCN regions included: dorsal ACC (dACC), left dorsolateral PFC (DLPFC; functionally defined as in (12)), and bilateral posterior parietal cortex (PPC; meta-analytic coordinates from (10)).

Symptom Severity Scores: Principal Components Analysis (PCA)

For data reduction across questionnaires measuring a broad spectrum of affective dysregulation, a single factor score was calculated across the 13 questionnaire measures listed below. A single factor solution was selected based on examination of the Scree plot, Eigenvalues, and variance explained in an unrestricted PCA, all of which suggested a steep decline in incremental explanatory benefit for additional factors beyond the first (largest) factor. The factor explained 49.4% of variance across questionnaires (eigenvalue=6.41) and strongly differed across patients and controls as expected (t_{74}=16.93, p<.001).

Measure	**Construct**	**Factor Loading**
Beck Depression Inventory (2)	Depressive symptoms	.898
Spielberger State Anxiety Inventory (13)	Acute anxiety	.886
Response Styles Questionnaire (14)	Rumination	.870
Acceptance and Action Questionnaire (15)	Emotional avoidance	.867
Penn State Worry Questionnaire (16)	Uncontrollable worry	.834
Thought Control Questionnaire (17)	Adaptive and maladaptive coping strategies	
Subscales:	Distraction	-.509
	Social Interaction	-.553
	Worry	.631
	(self) Punishment	.638
Emotion Regulation Questionnaire (18)	Emotion regulation strategies	
Subscales:	Reappraisal	-.485
	Suppression	.456
Positive and Negative Affect Schedule (19)	Positive Affect	-.593
	Negative Affect	.673

Handling and Impact of Motion During Scanning

No participant was excluded based on *a priori* definitions of acceptable motion during the scan (<25% of scans with incremental motion >.5mm or degree). Timepoints with incremental translational/rotational movement >=.5 mm or .5 degrees (1.7% of data) were marked as missing data and skipped by the algorithm to further safeguard against spurious (motion-

induced) connectivity. While similar to 'scrubbing' (i.e. deleting timepoints), this approach maintains the temporal ordering of scans.

Although this approach mitigates the influence of motion on connectivity patterns (20), we further verified that motion did not influence findings. Across 12 motion parameters calculated for each participant (maximum absolute change from baseline and maximum incremental movement across each of 6 movement planes: roll, pitch, yaw, right-left, front-back, up-down), no parameter differed as a function of connectivity subgroup (*p*'s>.21). The number of TRs 'scrubbed' for micromovement also did not differ by subgroup (*p*=.93). Furthermore, no external validator finding in the main text was altered after adding additional nuisance covariates representing average degree of motion across the 12 parameters and/or number of TRs scrubbed for micromovement.

Unsupervised Clustering: Distinctions From Alternative Approaches

The S-GIMME approach utilizes Walktrap, an unsupervised classification approach which does not rely on an *a priori* number of subgroups specified by the researcher; instead, it produces an optimal number of subgroups based solely on shared patterns of connectivity across individuals. Specifically, the sign (positive/negative), significance, direction of influence (e.g, region A->region B), and temporal pattern (contemporaneous or lagged) of the connection must be the same in order for that connection to be considered similar between two given individuals. This is distinct from approaches utilizing an *a priori* number of subgroups (e.g., supervised methods, median split on number of connectivity paths, etc.) in a number of ways. First, by using an unsupervised approach, no subgroups will be selected if none truly exist. By contrast, if a specific number of subgroups is predetermined, this may force subgroups to exist in the presence of relatively homogeneous patterns of connectivity; alternatively, it may oversimplify the subgroup structure in the presence of very heterogeneous patterns of connectivity. Additionally, the search procedure integrates information across multiple dimensions, clustering

individuals based on similarities in the overall pattern of connections, rather than along one dimension (e.g., average strength of connections, number of connections). Consequently, diverse patterns in the data can be captured in a data-driven fashion. For instance, subgroups can emerge that have a different number of significant connections; or that have an equivalent number of significant connections, but for which these connections occurred across distinct regions, or had a different sign (positive vs. negative relationship), direction of influence, or temporal pattern (lagged vs. contemporaneous).

Resting State Task

Resting state PCC->pgACC values analyzed as an external neurobiological variable were derived from a group-level path that emerged after applying identical preprocessing and S-GIMME network analyses to an identical network of ROIs. The 7min resting state task was performed with eyes open prior to the positive mood induction task.

Supporting Findings

Discussion of Group-Level Connectivity Findings

The group-level connectivity map (Figure 2, main text) revealed a highly interconnected cortico-limbic-striatal network consistently engaged during the positive mood induction across all participants, suggesting the network's relevance to the task. Notably, no negative or cross-lagged connections were observed at the group or subgroup level, suggesting function within the network is best described by positive, relatively contemporaneous influences at the resolution of many seconds (as afforded by fMRI).

Analyses of Quality of Subgroup Assignment

A series of t-tests were conducted to compare the degree of similarity for individuals within a subgroup ("within-group similarity") to the degree of similarity with those in a different subgroup ("between-group similarity"). For each individual, we computed an average representing the number of shared connections "within-group" and "between-group." A connection was considered shared between any two individuals if it was significant in both individuals and had the same sign (positive/negative), directionality of influence (e.g.., region A->region B), and temporal pattern (contemporaneous or lagged).

Overall quality of subgroups: Across the sample, there was greater within-group than between-group similarity, $t(91) = 13.37$, $p < .001$.

Diagnosis-specific quality: The between/within test for MDD patients was significant ($t(67) = 14.83$, $p < .001$), such that individuals with MDD were more connected to their data-driven subgroup than to members of the opposite group. Importantly, despite there being fewer control individuals, the between/within test for controls was also significant ($t(23) = 3.72$, $p < .001$), such that controls were more connected to members of their data-driven subgroup than to members of the opposite group.

Post Hoc Comparisons of the types of Network Paths Present Across Subgroups

Post-hoc analyses were conducted on individual-level connectivity maps to quantify subgroup differences in the number of connections evident within and between each network. For each pair of networks (e.g., VAN->VAN, VAN->DMN, DMN->DMN, DMN->VAN, etc.), the number of significant connections present in an individual's connectivity map (which could have resulted from either a group-level, subgroup-level, or individual-level path being present) was tallied for each individual, and these tallies were then compared across Subgroups A and B. Subgroup B exhibited a greater number of connections in VAN->DMN paths ($t(90) = -5.60$, $p < .001$), VAN-

>VAN paths (t(90) = -3.83, p < .001), CCN->VAN paths (t(90) = -3.74, p <.001), CCN -> DMN paths (t(90) = -5.29, p < .001), and CCN -> CCN paths (t(90) = -4.04, p < .001). Subgroup A exhibited greater connectivity in DMN->VAN paths (t(90) = 3.31, p < .005) and DMN -> DMN paths (t(90) = 2.29, p = < .05). No other pairs of networks exhibited subgroup differences.

Pathway	**Subgroup 1**		**Subgroup 2**	
	M	SD	M	SD
VAN -> DMN	1.52	0.82	2.76	0.99
VAN -> CCN	2.64	1.87	2.42	2.27
VAN -> VAN	21.96	5.92	26.96	5.42
CCN -> DMN	0.64	0.76	1.58	0.76
CCN -> VAN	1.88	1.88	3.69	2.13
CCN -> CCN	8.84	1.31	10.01	1.21
DMN -> VAN	3.00	1.19	1.69	1.84
DMN -> CCN	1.48	0.82	1.43	0.80
DMN -> DMN	3.16	0.37	3.03	0.17

Post Hoc Analysis of Resting State PCC->pgACC Path: Prediction of Patient Status Based on Two Forms of Connectivity

We further interrogated the role of resting state connectivity in a *post hoc* logistic regression analysis designed to assess the connectivity measures' utility in distinguishing healthy from depressed individuals. Specifically, we sought to explicitly test whether individuals high on network-wide connectivity (during positive mood—i.e., those groups in subgroup B), but *low* on DMN connectivity at rest, were likely to be healthy, while individuals high on both connectivity measures were likely to be depressed. In a logistic regression, connectivity subgroup (from the primary positive mood task) and the independent resting state DMN connectivity index (beta weights for the PCC->pgACC pathway at rest) were entered as predictors of patient status (healthy or depressed).

Both connectivity subgroup (A vs. B; b=-1.73, SE=.58, *p*=.003) and resting state connectivity values (b=3.70, SE=1.85, *p*=.045) explained independent variance in patient status. The model with both predictors correctly classified 76% of participants (80% sensitivity, 64% specificity using an optimal cut value=.67; full model χ^2=11.5, *p*=.003). These confirmatory results are consistent with the conclusion that individuals in the “at-risk” subgroup (subgroup B) are less likely to be depressed when they also exhibit decreased PCC->pgACC connectivity at rest—a potential protective factor. Nevertheless, unexplained variance clearly persists, suggesting these two indices do not fully capture the heterogeneous substrates of depressed and healthy functioning.

Supplemental References

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