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Data-Driven Subgroups in Depression Derived from Directed Functional Connectivity Paths at Rest

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Depressed patients show abnormalities in brain connectivity at rest, including hyperconnectivity within the default mode network (DMN). However, there is well-known heterogeneity in the clinical presentation of depression that is overlooked when averaging connectivity data. We used data-driven parsing of neural connectivity to reveal subgroups among 80 depressed patients completing resting state fMRI. Directed functional connectivity paths (eg, region A influences region B) within a depression-relevant network were characterized using Group Iterative Multiple Model Estimation, a method shown to accurately recover the direction and presence of connectivity paths in individual participants. Individuals were clustered using community detection on neural connectivity estimates. Subgroups were compared on network features and on clinical and biological/demographic characteristics that influence depression prognosis. Two subgroups emerged. Subgroup A, containing 71% of the patients, showed a typical pattern of connectivity across DMN nodes, as previously reported in depressed patients on average. Subgroup B exhibited an atypical connectivity profile lacking DMN connectivity, with increased dorsal anterior cingulate-driven connectivity paths. Subgroup B members had an over-representation of females (87% of Subgroup B *vs* 65% of Subgroup A; $\chi^2 = 3.89$, $p = 0.049$), comorbid anxiety diagnoses (42.9% of Subgroup B *vs* 17.5% of Subgroup A; $\chi^2 = 5.34$, $p = .02$), and highly recurrent depression (63.2% of Subgroup B *vs* 31.8% of Subgroup A; $\chi^2 = 5.38$, $p = .02$). Neural connectivity-based categorization revealed an atypical pattern of connectivity in a depressed patient subset that would be overlooked in group comparisons of depressed and healthy participants, and tracks with clinically relevant phenotypes including anxious depression and episodic recurrence. Data-driven parsing suggests heterogeneous substrates of depression; ideally, future work building on these findings will inform personalized treatment.
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INTRODUCTION

There is substantial heterogeneity in the clinical presentation of depression. In a representative treatment-seeking sample of 3703 depressed patients, over 1000 unique symptom profiles were observed (Fried and Nesse, 2014). Thus, although group comparisons of depressed and healthy samples have revealed numerous biological and neural features that track with depression on the whole, group averages mask considerable heterogeneity and may not accurately represent even a single individual patient (Gates and Molenaar, 2012; Miller *et al*, 2002; Molenaar and Campbell, 2009). We (Price *et al*, 2017) and others (Clementz *et al*, 2016; Drysdale *et al*, 2017; Karalunas *et al*, 2014; Yang *et al*, 2014) have reported that data-driven parsing of biobehavioral heterogeneity within disorder domains (eg, attention deficit, psychosis, and depression) can yield biologically based subgroups that predict external measures of functioning, clinical outcomes, and neurobiology with better precision than traditional diagnostic subgroups.

Depressive heterogeneity notwithstanding, a large literature suggests depression-related alterations in neural connectivity during the resting state (RS). RS connectivity patterns tend to exhibit trait-like stability over time with a high degree of individual specificity (Finn *et al*, 2015) and are posited to represent neural functional architecture that remains consistent across diverse conditions (eg, under anesthesia). Meta-analyses suggest as a group, depressed individuals exhibit elevated RS connectivity within regions of the default mode network (DMN; (Kaiser *et al*, 2015)), a network that deactivates during many tasks and is associated with internal mentation, including self-referential processing (Andrews-Hanna *et al*, 2010) and negative rumination (Zhu *et al*, 2012). Hyperconnectivity across the DMN and regions of the cognitive control network (CCN), as well as hypoconnectivity between DMN and ventral affective network (VAN) regions, were also reported in a meta-analysis (Kaiser *et al*, 2015). However, the direction of RS findings in individual studies of depression (eg, hyper *vs* hypoconnectivity) is sometimes conflicting (Hasler and Northoff, 2011; Kaiser *et al*, 2015), even though RS methods are considered highly translatable and reliable. Conflicting findings are to be

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expected if meaningful neural heterogeneity is present within depressed patients but overlooked in conventional group-based analysis. This may lead to mixed or spurious findings (Gates and Molenaar, 2012; Miller *et al*, 2002; Molenaar and Campbell, 2009), incomplete etiological models, and confusion within the literature, while overlooking biological subgroups that may represent unique etiologies requiring unique treatments. Consistent with this suggestion, a recent data-driven approach suggested as many as four subtypes of RS connectivity patterns within depression may be conflated when depressed patients are averaged together (Drysdale *et al*, 2017).

Clinically, one of the most widely acknowledged forms of heterogeneity within depressed patients is the presence or absence of comorbid anxiety. Roughly half of treatment-seeking depressed patients report clinically significant anxiety, which is associated with poor prognosis and treatment response (Fava *et al*, 2008), greater severity and disability, and higher risk of severe outcomes (eg, suicidality; (Fava *et al*, 2004)). Few studies have examined the neural substrates of this potential dichotomy within depressed patients. During task performance, anxious depression may have unique neural activation substrates spanning VAN, DMN, and CCN regions (Demenescu *et al*, 2011; Etkin and Schatzberg, 2011b; van Tol *et al*, 2012; van Tol *et al*, 2011). However, brain processes may best be characterized as the coordinated activity of disparate brain regions over time (Heller *et al*, 2009; Sporns *et al*, 2004). RS connectivity offers a glimpse at this coordinated activity in the absence of task demands, which may capture idiosyncratic, endogenous, habitual processing patterns akin to the habitual and intrusive anxious thought patterns and affective states reported by patients. In studies comparing RS connectivity patterns for depressed individuals with and without comorbid anxiety (assessed both categorically and continuously), unique patterns between and within the VAN, DMN, and CCN have been reported (Oathes *et al*, 2015; Pannekoek *et al*, 2015). Findings are generally consistent with the view that anxiety and depression have both common and unique neural substrates, informing an important etiological debate by supporting both 'shared diathesis' and 'independent-factor' models of psychopathology (Etkin and Schatzberg, 2011b). However, the patterns detectable within the neural data in previous studies are necessarily constrained by the investigators' selection of independent variables (ie, anxiety measures).

A more novel approach, facilitated by recent advances in data-driven clustering, is to begin by parsing the heterogeneity contained in neural connectivity maps themselves, enabling detection of biologically derived subgroups with unique neural connectivity profiles. This approach allows the optimal number of subgroups (from one—implying homogeneity—to the full sample size—implying no meaningful similarities) to emerge organically from the connectivity data. Subgroups can then be independently characterized with respect to clinically relevant, observable characteristics. We have previously used this approach in conjunction with a connectivity method—Group Iterative Multiple Model Estimation (GIMME (Gates and Molenaar, 2012; Gates *et al*, in press))—shown to reliably recover, for each individual, both the presence and the direction of connectivity among regions (ie, does A predict B after controlling all other network-wide influences (including B's influence on itself?)). GIMME was motivated by a seminal paper that found no methods to date could reliably detect individual-level models in simulated data (Smith *et al*, 2011). These simulations reflected common issues seen in fMRI data such as lower signal-to-noise ratio, nonstationarity, poor region of interest (ROI) selection, and HRF deviation within individuals, and GIMME was found to perform excellently in these cases (Gates and Molenaar, 2012). A recent independent review highlighted GIMME as one of the only approaches that can reliably capture both the presence and direction of functional connectivity paths within heterogeneous individuals (Mumford and Ramsey, 2014). Furthermore, in extensive Monte Carlo simulations, Subgroup-GIMME ('S-GIMME') can arrive at the correct cluster assignments nearly perfectly, even in sample sizes as small as 25, and at rates far higher than clustering based on more traditional connectivity metrics such as correlation matrices (Gates *et al*, in press). When applied to fMRI data collected from depressed and healthy individuals during a positive mood induction, the resulting connectivity-based subgroups predicted presence or absence of depression, as well as numerous clinically relevant indices of affective dysregulation (Price *et al*, 2017).

Here we applied this data-driven, brain-based categorization approach to RS functional connectivity maps obtained from 80 depressed patients (overlapping with those in our previous report; see Supplementary Information), across key nodes of three networks that show well-replicated roles in depressive symptomatology: DMN, CCN, and VAN. Unique goals of the present analysis were to focus explicitly on parsing fine-grained heterogeneity within depressed patients (rather than more broadly across both depressed and healthy individuals) and to focus on the RS, which has been widely studied in depression and shows promise as a method for identifying clinically relevant biotypes (Drysdale *et al*, 2017). S-GIMME produces subgroup-specific network connectivity maps, informing an empirical data-driven model of connectivity subtypes within depression. Based on previous research suggesting anxious depression may represent a unique phenotype with high clinical relevance, we then assessed the external relevance of connectivity-based subgroups in predicting comorbid anxiety diagnoses. In an effort to further understand whether study-specific sample composition parameters may contribute to variable findings when simple depressed *vs* healthy group comparisons are used, we examined several other important clinical (severity and recurrence) and biological (gender and age) features routinely reported as sample characteristics in existing studies. Our data-driven approach has the capacity to reveal heterogeneity within functional neural architecture that is masked by traditional group comparisons and ultimately could inform development of discrete treatments targeting discrete neurobiological etiologies.

MATERIALS AND METHODS

Participants were 80 unmedicated MDD patients with moderate-to-severe depression (BDI mean = 30.73; SD = 9.5) recruited for a larger treatment study (Siegle *et al*, 2012; Supplementary Information and Table 1).

Table 1 Subgroup Clinical and Demographic/Biological Characteristics

	Subgroup A ($n=57$)	Subgroup B ($n=23$)	Statistic testing group differences	Statistical significance (p)	Effect size (95% CI)
≥ 1 Comorbid anxiety disorder	17.5% ($n=10/57$) $n=3$ GAD $n=1$ PTSD $n=1$ Specific phobia $n=7$ Social phobia	42.9% ($n=9/21$) $n=2$ GAD $n=2$ PTSD $n=1$ Specific phobia $n=7$ Social phobia $n=1$ Panic disorder $n=2$ Anxiety NOS	$\chi^2=3.89$	**0.021**	OR = 3.53 (1.17–10.60)
Severe MDD	19.3% ($n=11/57$)	38.1% ($n=8/21$)	$\chi^2=2.94$	*0.086*	OR = 2.57 (0.86–7.73)
Highly recurrent MDD (≥3 episodes)	31.8% ($n=14/44$)	63.2% ($n=12/19$)	$\chi^2=5.38$	**0.020**	OR = 3.67 (1.19–11.34)
Sex (% female)	64.9% ($n=37/57$)	87.0% ($n=20/23$)	$\chi^2=3.89$	**0.049**	OR = 3.60 (1.18–11.00)
Mean age (SD)	35.3 (11.1)	37.3 (11.6)	$t_{78}=.72$	0.473	$d=0.18$ (−0.31 to 0.66]

Abbreviations: GAD, generalized anxiety disorder; MDD, major depressive disorder; NOS, not otherwise specified; PTSD, posttraumatic stress disorder,
Some individuals ($n=2$ Subgroup A; $n=3$ Subgroup B) met criteria for more than one comorbid anxiety diagnosis. Decreased total N's in denominators are due to loss of diagnostic data due to database error (two participants) and insufficient information to confidently determine number of previous episodes and/or duration of current episode (15 participants). Bold = $p<0.05$; italics = $p<0.10$.

fMRI Acquisition and Preprocessing

Data were acquired during a 7 min eyes-open RS block. T2*-weighted images depicting BOLD contrast (TR = 1500; TE = 27; FOV = 24 cm; flip angle = 80°; Twenty-nine 3.2 mm slices; 280 TRs) were acquired on a 3T Siemens Allegra ($n=4$) or a 3T Siemens Trio ($n=76$). Standard preprocessing steps were applied (see (Price *et al*, 2017; Supplementary Information). AFNI's ANATICOR algorithm was applied to remove artifacts (hardware and motion) that may influence connectivity estimates. AFNI's 3dvolreg motion correction algorithm was applied. Timepoints with incremental translational/rotational movement ⩾0.5 mm or 0.5° (1.7% of data) were removed from analysis (marked as missing data).

Fifteen ROIs were selected *a priori* based on prior literature in depression (emphasizing replicated and meta-analytic findings) with the goal of spanning networks relevant to ventral affective processing (VAN), self-referential processing (DMN), and top–down regulation (CCN). See Supplementary Information and Figure 1 for details of ROI definitions.

Directed Connectivity and Community Detection

The full sample of 80 individuals was processed and clustered without regard to clinical/demographic features. Directed paths (ie, establishing which of two ROIs statistically predicts the other after controlling for other candidate regions (including lagged auto-regressions)) between all pairs of ROIs (both contemporaneous and at lag = 1TR) were derived for each individual using S-GIMME (Gates *et al*, in press; Lane *et al*, 2015). Briefly, using a unified structural equation framework (Kim *et al*, 2007) and a Bayes net formulation, S-GIMME first looks across individuals to detect signal from noise and arrive at a map of lagged and contemporaneous directed connections that exist for the majority ('group-level map'). Next, S-GIMME arrives at a similarity matrix using the individual-level estimates of these group-level connections, as well as anticipated estimates for candidate connections. Walktrap (Pons and Latapy, 2006), an 'unsupervised' community detection algorithm found to be robust across many issues common in clustering (eg, unequal cluster sizes; (Orman and Labatut, 2009)), is conducted on this matrix to arrive at an optimal number of subgroups who have shared connectivity patterns (similar strength, sign (positive/negative), temporal pattern (contemporaneous/lagged), and direction (eg, region A → region B) of connectivity paths). S-GIMME then searches for subgroup-level paths. Finally, S-GIMME robustly identifies individual-level connections using group- and subgroup-derived temporal patterns as a starting point. S-GIMME thus generates group-level, subgroup-specific, and individual (per-participant) connectivity maps characterizing the network structure.

External Variables

Subgroups were independently tested and characterized across several clinical and demographic features selected *a priori* based on established clinical relevance and interpretability. Selected variables were those likely to be reported as sample characteristics in previous depressed *vs* healthy group comparison studies (eg, dichotomous diagnostic designations), increasing relevance of present findings to the extant literature. During a structured interview, clinicians established: the presence/absence of at least one comorbid anxiety diagnosis, the presence/absence of 'severe' depression, and the degree of recurrence of depressive episodes. Recurrent depression was dichotomized as <3 episodes *vs* ⩾3 episodes. Although this cut-point diverges from the clinical definition of 'recurrent depression' (2+ episodes), it has been previously linked to clinical outcomes and prognosis (Piet and Hougaard, 2011; Teasdale *et al*, 2000), and preserved an adequate distribution (eg, sufficient individuals in each cell) for analysis, while circumventing problems with distributional skewing. Gender and age were examined as biological/demographic factors with relevance in depression prevalence, presentation, and treatment

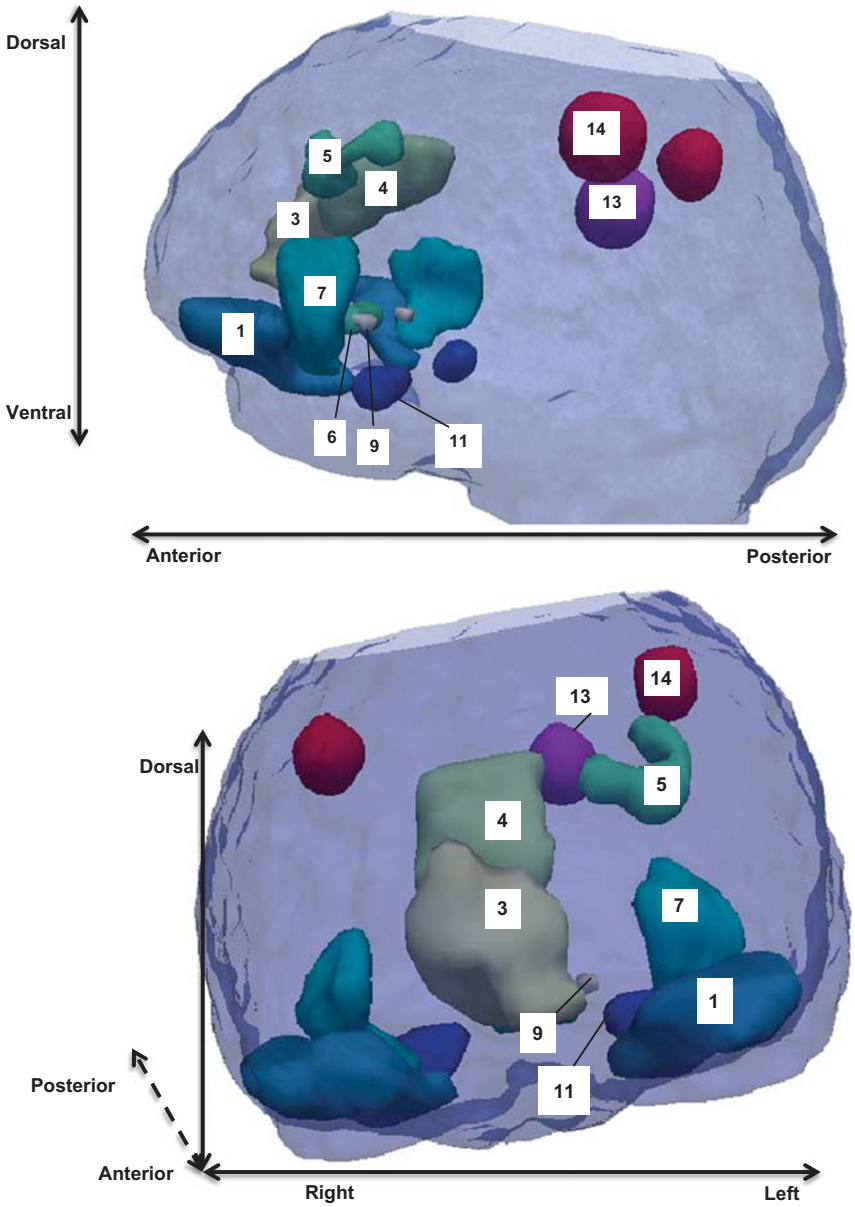

Figure 1 Three-dimensional renderings of region of interest (ROI) locations in template space. For ROIs that are bilateral, a uniform color is used to label both hemispheres and regions are numerically labeled on the left hemisphere only; 1 = left ventrolateral PFC (L VLPFC); 2 = R VLPFC; 3 = perigenual anterior cingulate cortex (pgACC); 4 = dorsal ACC (dACC); 5 = L dorsolateral PFC (L DLPFC); 6 = subgenual ACC (sgACC); 7 = L Insula; 8 = R Insula; 9 = L nucleus accumbens (NucAcc); 10 = R NucAcc; 11 = L Amygdala; 12 = R Amygdala; 13 = posterior cingulate cortex (PCC); 14 = left posterior parietal cortex (L Parietal); 15 = R Parietal. Figure reprinted from *Biological Psychiatry*, Vol 81, Price *et al,* "Parsing Heterogeneity in the Brain Connectivity of Depressed and Healthy Adults During Positive Mood", p. 350, Copyright (2016), with permission from Elsevier.

(Cyranowski *et al*, 2000; Green *et al*, 2005; Szanto *et al*, 2003). Sources of missing data and overlap among external variables are discussed in the Supplementary Information.

RESULTS

Connectivity Maps

Group level. At the group level, connectivity paths depicted in Figure 2 were present, in addition to lagged autoregressive effects at every ROI. ROIs behaved as a strongly interconnected network, including numerous ipsilateral and within-network (VAN → VAN and CCN → CCN) connections, but notably lacked significant connections between the two DMN nodes.

Subgroups. Based on unsupervised search for the optimal number of subgroups, two subgroups emerged (see Supplementary Information for subgroup quality/stability information). Subgroup A contained 71% ($n = 57$) of participants; hence, Subgroup B (29% of participants; $n = 23$) was considered to exhibit 'atypical' connectivity patterns relative to the majority of depressed patients. Subgroup was unrelated to the scanner where data were acquired ($\chi^2 = 0.93$, $p = 0.335$); to motion; and to other data quality measures (Supplementary

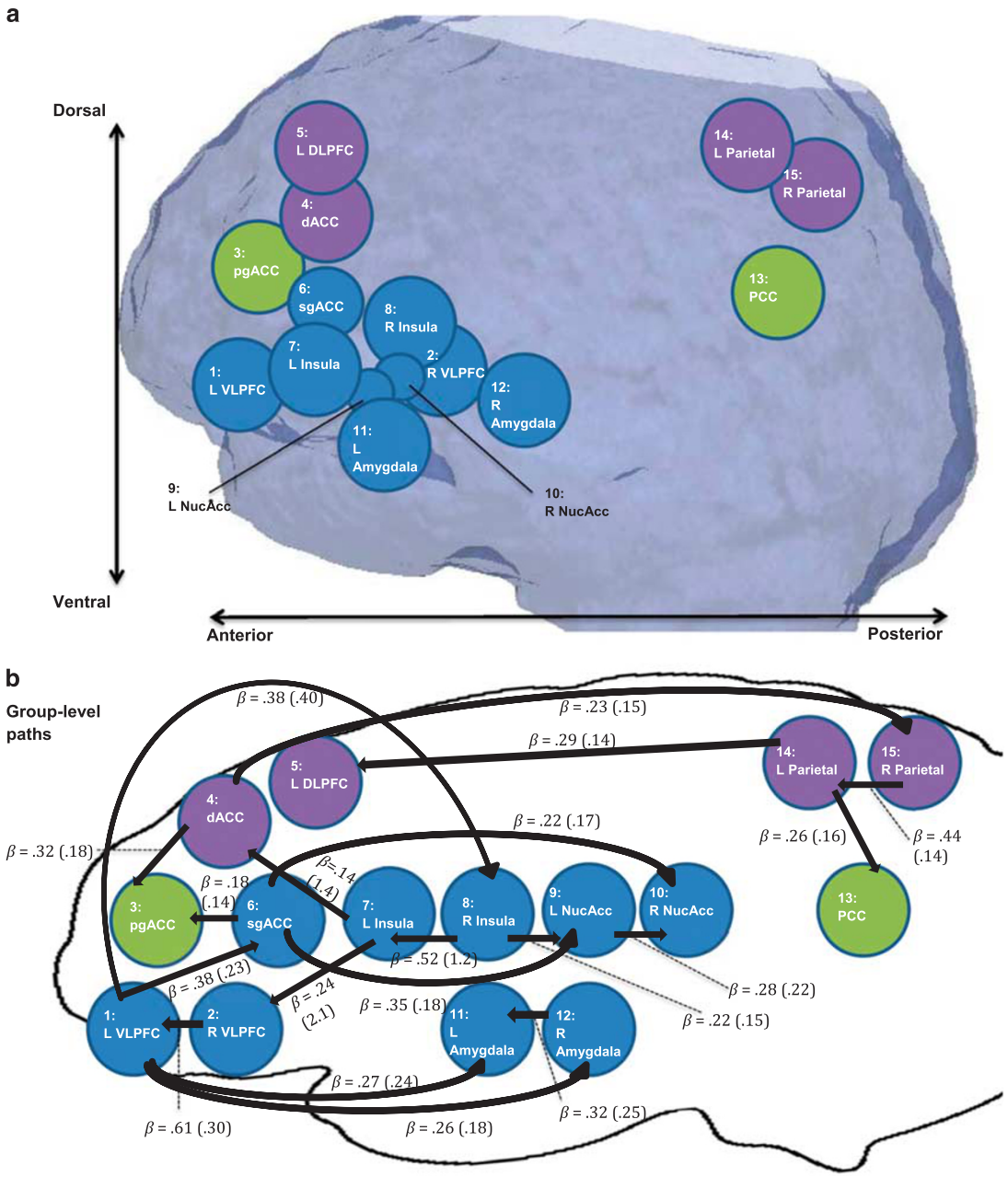

Figure 2 (a) Regions of interest (ROIs) represented as nodes in rough anatomical space. Nodes of the ventral affective network (VAN) are presented in blue; default mode network (DMN) in green; cognitive control network (CCN) in purple. (b) Group-level directed connectivity paths between regions of interest (flattened to two dimensions and stretched in space to facilitate visualization of all significant paths). All identified paths are contemporaneous with positive beta weights; additional lagged autoregressive (positive) paths were found for every ROI (not shown). Superimposed text displays beta-weights as mean (SD) across all individuals with a given path. See Supplementary Information for further discussion.

Information). In paths unique to each subgroup (Figure 3), only Subgroup A showed DMN → DMN connectivity (a pgACC → PCC directed path). Subgroup A further exhibited a unique dACC → R insula path, whereas Subgroup B had a reversed direction of effect for dACC → L DLPFC, as well as an additional dACC → R parietal path and a L insula → R amygdala path.

External Variables

Clinical variables. Subgroup B was associated with multiple markers of poor prognosis in depression (Table 1). Comorbid anxiety was over-represented in Subgroup B (42.9,%), a more than threefold increase in odds ($\chi^2 = 5.34$, $p = 0.021$). Highly recurrent depression was similarly over-represented in Subgroup B (63.2%) relative to Subgroup A (31.8%; $\chi^2 = 5.38$, $p = 0.020$). Subgroup B had a nonsignificant over-representation of 'severe' depression diagnoses (38.1 *vs* 19.3% in Subgroup A; $\chi^2 = 2.94$, $p = 0.086$). See Supplementary Information for analyses of additional clinical variables.

Biological/demographic variables. Subgroup B was almost exclusively comprised of females (87.0%, $n = 20/23$) in

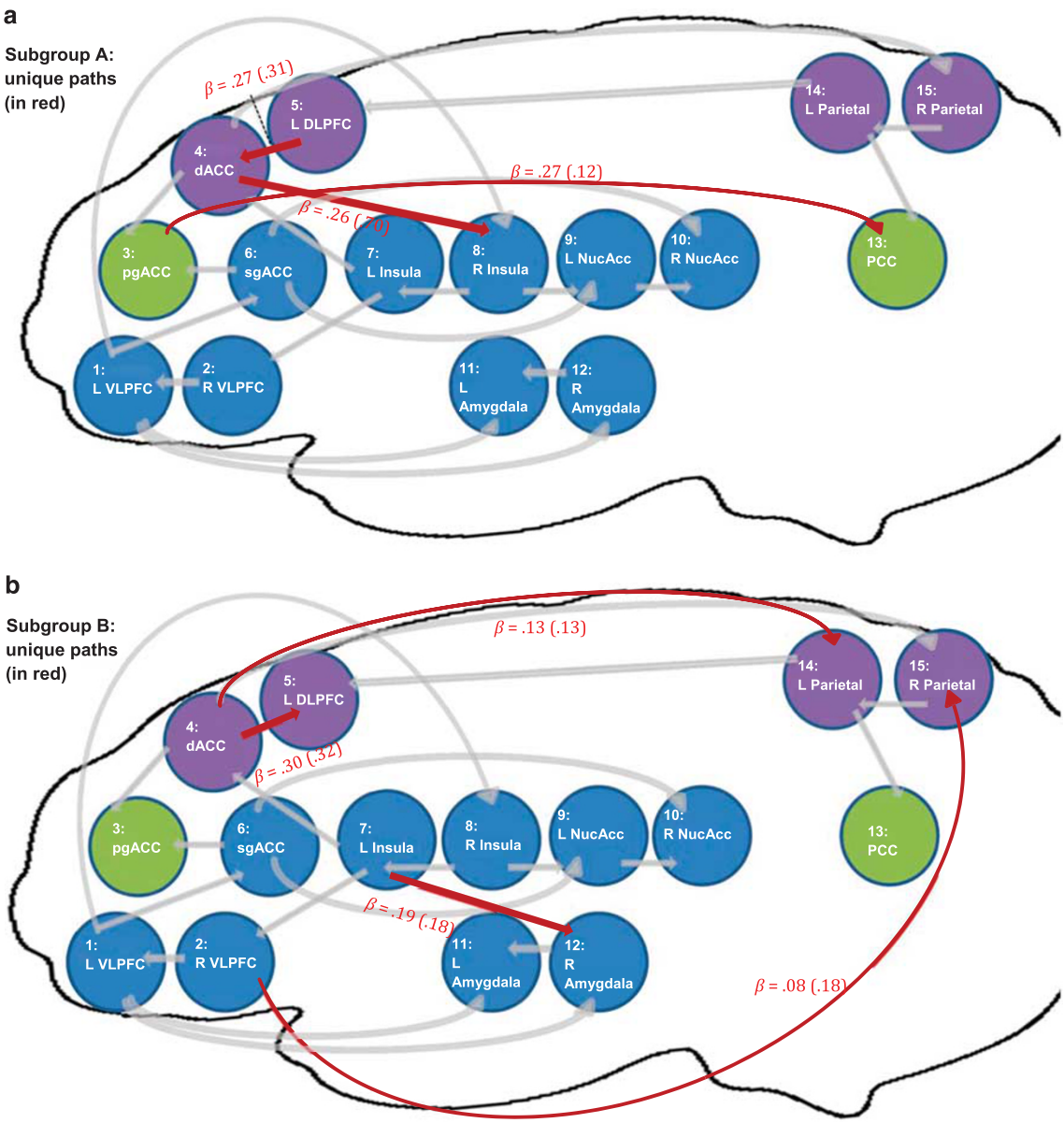

Figure 3 Subgroup-specific connectivity paths. All identified paths are contemporaneous with positive beta weights. Superimposed text displays beta-weights as mean (SD) across all individuals with a given path. (a) Directed connectivity paths unique to subgroup A (in red), superimposed on group-level connectivity map (in gray). (b) Directed connectivity paths unique to subgroup B (in red), superimposed on group-level connectivity map (in gray).

contrast to Subgroup A (64.9%; $\chi^2 = 3.89$, $p = 0.049$). Subgroups did not differ on age ($t_{78} = 0.72$, $p = 0.473$).

Summary. In aggregate, analyses suggested connectivity-based subgroups had external clinical and real-world relevance with regard to key sample composition features.

DISCUSSION

In the present analysis, two data-driven RS connectivity subgroups were identified within a depressed sample using a robust method for recovering functional neural network structure among heterogeneous individuals. The larger group exhibited a directed pathway linking two hubs of the DMN, whereas a smaller subgroup showed an 'atypical' connectivity pattern where within-DMN connectivity was notably lacking and where dACC-driven paths were more prominent. Connectivity-based subgroups tracked with several clinical and demographic features, suggesting clinical relevance. The 'atypical' (smaller) subgroup was associated with markers previously linked to poor prognosis in depression, including comorbid anxiety and high episodic recurrence, and with female gender.

Strength of connectivity within the DMN is posited to reflect perseverative self-focus in the absence of exogenous instruction (Andrews-Hanna *et al*, 2010) and has been tied to negative rumination (Zhu *et al*, 2012), a perseverative form of cognition that increases risk for depression (Nolen-Hoeksema *et al*, 1993). Our findings suggest there is a potent direct influence from the pgACC to the PCC—two prominent nodes of the DMN—in the majority, but not all, of depressed patients. A minority of patients lack this specific

path after controlling for the influence of all other regions included in our network, suggesting the functional influence of these two regions on one another is diminished in this subgroup, with fluctuations in activity over time being more completely explained by other inputs present across the entire sample (depicted in Figure 2b). Meta-analytic findings suggest that, on average, depression is associated with hyperconnectivity between PCC and medial PFC regions (Kaiser *et al*, 2015). Although caution is warranted given the novel directed connectivity method applied here, current findings could suggest such patterns are driven by the majority of depressed patients, whereas a sizable minority do not display strong connectivity within this circuit. Differences in sampling of these two depressive subgroups across studies might therefore yield differential strength of findings which, based on our observations for external variables, may be reflected in clinical and demographic features. Because of our focus on external variables representing widely reported sample characteristics, this hypothesis could be readily tested in future meta-analyses making use of the large number of existing non-directed (correlational) connectivity studies. Within-subject variability in connectivity patterns (ie, coupling dynamics) (Hutchison and Morton, 2015) may also contribute to mixed findings. Quantifying such dynamics, ideally in conjunction with an approach capable of parsing between-subject heterogeneity, represents a promising novel direction for patient classification (Rashid *et al*, 2016).

Our findings were dictated by differential patterns present in the neural connectivity data itself, rather than on *a priori* selection of individual differences variables. Nevertheless, findings are potentially informative regarding the neural substrates of key sources of depressive heterogeneity observed clinically. Anxious depression is a widely-recognized clinical subgroup associated with poor prognosis (Fava *et al*, 2004, 2008), which has distinct neural features including altered activity and connectivity within the networks studied here (Demenescu *et al*, 2011; Etkin and Schatzberg, 2011b; Oathes *et al*, 2015; Pannekoek *et al*, 2015; van Tol *et al*, 2012; van Tol *et al*, 2011). Our findings are potentially consistent with prior studies suggesting that both common (here, group-level) and unique (subgroup-specific) neural substrates underlie depression and anxiety. The data-driven approach reveals that connectivity features track with diagnostic boundaries, yet do not overlap perfectly with these features, suggesting connectivity patterns contain unique information that could be obscured by conventional group comparisons.

One network distinction apparent across the two subgroups included a directed path from left DLPFC → dACC in Subgroup A, which was reversed in Subgroup B. Subgroup B additionally showed a unique dACC → parietal path, whereas Subgroup A showed a unique dACC → insula path. Although the dACC was historically construed as a strictly 'cold' cognitive region (Bush *et al*, 2000), contemporary conceptualizations highlight its additional role in the generation and expression of negative emotion (Etkin *et al*, 2011a), in particular within the context of anxiety research. Thus, its pivotal role in defining depression subgroups that tracked with comorbid anxiety may be significant. For example, greater overall dACC-driven activity might reflect a greater influence of fear-driven processing over cognition. Two additional specific paths present in Subgroup B, right VLPFC → right parietal and left insula → right amygdala, could reflect additional VAN-driven processing at rest. This is potentially consistent with a previous report of increased RS connectivity between VAN and CCN regions that was specific to comorbid anxiety and depression (Pannekoek *et al*, 2015) and also with the prominent roles for exaggerated 'bottom-up' influences of the VAN in neuroanatomical models of both anxiety and depression (Drevets *et al*, 2008; Etkin *et al*, 2009; Mathew *et al*, 2008; Price and Drevets, 2010). Notably, in supplemental analyses, connectivity-based subgroups did not track primarily with any one specific anxiety diagnosis present in the current sample, nor with a continuous measure of anxiety, suggesting the present finding related only to transdiagnostic, clinically diagnosed anxiety. Given that anxiety is itself a highly heterogeneous condition, future studies should include larger and more diverse anxiety presentations, with further attention to heterogeneity across both categorical and continuous measures.

At least 50–60% of depressed patients experience more than one episode (American *et al*, 2001) and it is well-established that risk of future depressive episodes increases with each prior episode (Solomon *et al*, 2000), becoming particularly (⩾90%) likely in individuals who have had three or more episodes (American *et al*, 2001). This pattern could reflect increasingly well-practiced negative cognitions (Beck and Bredemeier, 2016) that become easily re-activated following subsequent stressors through a process termed the 'kindling effect' (Monroe and Harkness, 2005; Post, 1992). Alternatively, there may be two subgroups of patients, each with 'stable-liability'—one prone to high recurrence and another who will experience only a few sporadic (if any) additional episodes (Anderson *et al*, 2016). Few studies in human patients have examined the neural substrates of differential recurrence. In at least some patients with a history of high recurrence, our data suggest involvement of a functional neural architecture with stronger baseline influences stemming from VAN regions and from dACC to other CCN regions, which could be consistent with stronger internally driven affective 'schema' that co-opt cognitive resources. However, findings simultaneously highlight individual differences in connectivity, with a full half of patients with high recurrence showing greater similarity to the 'typical' (larger) depressed subgroup. This could indicate more than one neurocognitive pathway to recurrence, only one of which was characterized here; for example, patients in Subgroup A with high recurrence might differ in non-assessed neural networks or during distinct task states (eg, under emotional provocation). Likewise, 37% of patients in Subgroup B did not report high recurrence at the time of assessment. With prospective follow-up, a highly recurrent pattern might become evident in these specific patients—a testable hypothesis for future work.

One other sample characteristic—gender—tracked with connectivity subgroup, such that the 'atypical' subgroup was almost exclusively female. Females are 2× more likely than males to experience MDD, a gender discrepancy that first appears in adolescence. Posited mechanisms of this gender gap include biological and psychosocial factors (Cyranowski *et al*, 2000; Angold and Costello, 2006; Crone and Dahl, 2012; Hyde *et al*, 2008). Depressed females also show distinct clinical features, including higher rates of comorbid anxiety (Schuch *et al*, 2014). In RS data, healthy females have shown greater connectivity of amygdala subregions to numerous

VAN and CCN regions (Engman *et al*, 2016; Kogler *et al*, 2016; Lopez-Larson *et al*, 2011), possibly consistent with the unique insula → amygdalar pathway found here in Subgroup B. However, group comparisons by gender may set up a false dichotomy, as females were over-represented in Subgroup B, but also well-represented (65%) in Subgroup A. Future analyses should aim to delineate additional biobehavioral features that may distinguish those depressed women in the female-heavy subgroup from those biologically classified together with the vast majority of depressed men.

Using identical S-GIMME methods applied to data collected during a positive mood induction, we previously reported in an overlapping sample (which also included healthy controls) that the majority of depressed patients showed widespread hyperconnectivity across this network of regions, in particular for ventrally driven pathways (Price *et al*, 2017). Here we explicitly searched for and parsed heterogeneity within depressed patients at rest. The subgrouping patterns from the two analyses did not appear highly convergent either in terms of the distinguishing (subgroup-specific) directed pathways or the specific parsing of patients (Supplementary Information), suggesting the two approaches yielded unique information that could be jointly informative for practical goals such as predicting clinical trajectories. Data-driven subgroups derived from correlational (ie, non-directional) patterns within depressed patients' RS data were also divergent anatomically from those identified here, although they likewise tracked with anxious phenotypes of depression (Drysdale *et al*, 2017). Future work should empirically establish combinations of information that are most informative for clinically imperative tasks (eg, matching patients to specific interventions).

Limitations

Although regions analyzed in the network were limited to 15 to increase interpretability and reduce processing time, results may have varied with the inclusion of different or additional regions, as many potentially relevant regions (eg, dorsomedial PFC beyond the ACC boundaries) were omitted in favor of the present set. Alternate forms of data-driven subtype analysis, recently applied to RS connectivity in depression (Drysdale *et al*, 2017), are able to consider a wider network of brain regions, although these methods differ in that they rely on correlational patterns rather than characterizing directional influences—a method that may be less accurate for subgrouping according to simulations (Gates *et al*, in press). Data-driven subgroups are dictated by the individuals in the sample and may have differed with a larger sample or greater representation of certain groups (eg, older participants and more ethnic minorities). Replication is essential to understand the robustness and generalizability of these specific subgroups. In addition, the hard-clustering approach did not allow for multiple subgroup membership, meaning that some individuals could be similar to both subgroups. External clinical variables were assessed cross-sectionally; testing prospective prediction and the stability of subgroups over time are critical next steps. Although assessment tools that accurately parse biobehavioral heterogeneity may one day be informative in clinical decision making, numerous hurdles remain, including: external validation of subgroups in larger, independent samples; developing prediction algorithms with sufficiently high accuracy for specific outcomes; and ideally, translation to clinic-ready assessment methods, given that fMRI is expensive and not widely available to patients. Finally, the directional brain pathways quantified here based on temporal patterns require external validation, eg, using brain stimulation methods to experimentally manipulate one region and observe downstream effects on other regions.

CONCLUSIONS

RS connectivity, an index of coordinated brain activation across a neural network, is posited to reflect stable neural functional architecture impacting the brain's reactive responses to a wide range of stimuli. Although RS connectivity alterations are widely implicated in depression, our findings using a robust data-driven algorithm suggest no one-size-fits-all pattern. In particular, the lack of reliable DMN connectivity within 29% of patients suggests a replicated biomarker of depression, DMN hyperconnectivity, may not apply equivalently to all patients. A clinical implication is that diverse depression treatments that appear to normalize DMN hyperconnectivity (eg, conventional antidepressants, intravenous ketamine, and mindfulness meditation; (Berkovich-Ohana *et al*, 2016; Karim *et al*, 2016; Lv *et al*, 2016), as well as targeted mechanistic treatments (eg, neurofeedback to decrease DMN connectivity (Zhang *et al*, 2013) and transcranial magnetic stimulation (Drysdale *et al*, 2017)), could be ill-matched for a sizable minority of patients. These patients might benefit instead from treatments that reduce dACC- and VAN-driven influences—which could represent fear-driven cognitive processing, particularly given the link observed to comorbid anxiety. Identifying treatments capable of addressing the atypical connectivity pattern may be particularly clinically impactful, as this pattern tracked with markers of poor prognosis.

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