Neurobiological Signatures of Anxiety and Depression in Resting-State Functional Magnetic Resonance Imaging

Supplemental Information

Supplemental Results

Low Frequency Signal Amplitudes: Patients Only

As a secondary analysis, we examined whether the findings across all participants also largely held true when restricted to the patients alone. In the repeated measures categorical only model, we found a significant main effect of major depressive disorder (MDD) diagnosis ($F_{1,49}$ = 4.8, p = .034), and again a significant interaction between principle component and generalized anxiety disorder (GAD) diagnosis ($F_{2,98}$ = 3.2, p = .047). Participants with GAD had greater limbic/paralimbic low frequency (LF) signal amplitudes than those without GAD (GAD vs. MDD [patient non-GAD]; t_{50} = 2.1, p = .045; other ps > 0.28) (Figure S2A). In a stepwise model, only the GAD diagnosis was a significant predictor of limbic/paralimbic signal (sβ = .279, p = .045). Consistent with the pattern in the full group analysis, an MDD diagnosis was associated with lower overall LF signal amplitude (t_{50} = 2.3, p = .028; only significant stepwise predictor, sβ = -.305, p = .028; Figure S2B).

The dimensional only analysis repeated the full group principle component by general distress interaction ($F_{2,96}$ = 5.9, p = .002). This interaction was driven by a positive trend relationship between general distress and limbic/paralimbic LF signal amplitude (single factor forced entry model, sβ = .250, p = .073; with other dimensional factors, stepwise, sβ = .412, p = .055; see Figure S2C) and a negative relationship

between general distress and dorsolateral prefrontal cortex (DLPFC) / medial prefrontal cortex (MPFC) LF signal amplitudes (stepwise, sβ = -.339, p = .014; other ps > 0.07; Figures S1 and S2D).

Finally, combining categorical and dimensional predictors revealed a persistent main effect of MDD ($F_{1,46}$ = 6.9, p = .011) and again the interaction between component and general distress ($F_{2,92}$ = 5.4, p = .006). Covarying other factors, an MDD diagnosis was associated with lower LF signal amplitudes ($F_{1,46}$ = 6.9, p = .011; only stepwise significant predictor, sβ = -.305, p = .028; see Figure S3A), as in the full group. The positive relationship between general distress and limbic/paralimbic signal was at the trend level in the combined model (forced entry, sβ = .250, p = .073; see Figure S3B) and there was also a significant negative relationship between distress and DLPFC/MPFC signal (only stepwise significant predictor, sβ = -.339, p = .014; see Figure S3C).

Functional Connectivity: Patients Only

In the patient only categorical model on functional connectivity data, there were again no significant findings (ps > 0.15). In the dimensional only model, the same pattern was observed as in the full participant model: a significant interaction between factor and anxious arousal ($F_{5,240}$ = 3.1, p = .010) driven by a positive relationship between anxiety and subgenual anterior cingulate cortex (sgACC) / ventral striatum (VS) connectivity (only stepwise significant predictor, sβ = .275, p = .049) (see Figure S4A). There was also a significant general distress by component interaction ($F_{5,240}$ = 2.4, p = .040) driven by combinations of subthreshold significance level positive and

negative correlations (*p*s > 0.22) similar to the finding with anhedonia in the full group.

Combining categorical and dimensional data to determine the strongest predictors in patients, we found no main effects (*p*s > .39) but several significant interactions between factor and anxious arousal ($F_{5,230}$ = 2.8, *p* = .017), general distress ($F_{5,230}$ = 2.6, *p* = .028), an MDD diagnosis ($F_{5,230}$ = 2.4, *p* = .038), and marginally with anhedonia ($F_{5,230}$ = 1.9, *p* = .097). Follow up stepwise regressions suggested that sgACC/VS connectivity was predicted positively by anxious arousal (stepwise, sβ = .301, *p* = .027; see Figure S4B) and negatively by an MDD diagnosis (stepwise, sβ = -.283, *p* = .037; see Figure S3C) to partially drive the combined model. As in the full group, an MDD diagnosis pushes down sgACC/VS connectivity whereas anxious arousal was associated with greater connectivity in this circuit.

Global Signal Analysis: Full Group

There were no effects from the categorical, dimensional, or combined models that approached significance for global signal betas used to denoise resting fMRI data (*p*s > 0.36) indicating that this step did not account for our findings.

Interrogating Small MDD Only Group

Since we may have been underpowered to detect effects related to a pure MDD diagnosis (non-GAD) abnormality given our small cell that did not also have a GAD diagnosis (*n* = 12), we ran independent samples *t*-tests between MDD only and control participants for average LF signal amplitude and average functional connectivity across the 3 and 6 factors, respectively. We then calculated effect sizes for each metric and

then an *a priori* power analyses (alpha .05; power .80) to determine a required sample size to reach significance (1). For functional connectivity, 128 subjects per group would have been necessary to establish this effect and for signal amplitude, more than 12,000 subjects per group would have been required. Thus, for our particular measures of interest, the small MDD only cell did not likely prevent us from finding robust effects were we to have reasonably increased our non-GAD MDD only participant pool.

Table S1. Demographic and symptom information across groups.

Group (Sample Size)	Age, Mean (SD)	% Female	Education, Mean (SD)
Patients (52)*	31.29 (9.17)	69.2	16.12 (2.15)
GAD only (17)	30.35 (7.74)	76.5	16.35 (2.00)
GAD/MDD (23)	33.48 (10.77)	65.2	16.17 (2.35)
MDD only (12)	28.42 (7.09)	66.7	15.67 (2.06)
Healthy Controls (38)	34.00 (10.04)	71.1	16.92 (1.87)
GAD + (40)	32.15 (9.61)	70.0	16.25 (2.18)
GAD - (50)	32.66 (9.65)	70.0	16.26 (1.97)
MDD + (35)	31.74 (9.86)	65.7	16.00 (2.24)
MDD - (55)	32.87 (9.47)	72.7	16.74 (1.91)

MASQ Subscales**	General Distress, Mean (SD)	Anxious Arousal, Mean (SD)	Anhedonic Depression, Mean (SD)
Patients	34.20 (7.80)	28.19 (9.34)	83.60 (12.24)
GAD only	28.65 (6.90)	26.94 (8.06)	73.06 (12.86)
GAD/MDD	39.20 (5.48)	31.83 (10.86)	87.70 (8.04)
MDD only	32.50 (7.03)	23.00 (3.84)	90.67 (7.94)
Healthy Controls	15.61 (3.14)	18.50 (2.39)	47.26 (9.26)
GAD +	34.71 (8.02)	29.75 (9.96)	81.48 (12.57)
GAD -	19.66 (8.47)	19.58 (3.36)	57.68 (20.73)
MDD +	36.90 (6.77)	28.80 (9.96)	88.71 (8.02)
MDD -	19.64 (7.61)	21.83 (7.03)	56.38 (16.43)

*Comorbidities: Primary analyses tested models of GAD+ vs. GAD- (GAD only and GAD/MDD vs. MDD only and Controls) as well as MDD+ vs. MDD- (MDD only and GAD/MDD vs. GAD only and Controls). In the patient only analyses, the models were the same but with controls dropped from the analyses. Of 52 patients, 15 had other comorbid diagnoses besides GAD and MDD. Among GAD/MDD patients, 6/23 had other diagnoses (4 generalized social phobia; 1 panic disorder; 1 obsessive-compulsive disorder and bulimia). Among "GAD only" patients (no MDD), 8/17 had other diagnoses (2 generalized social phobia only; 1 non-generalized social phobia only; 2 dysthymia only; 1 generalized social phobia and obsessive-compulsive disorder; 1 non-generalized social phobia and panic disorder; 1 generalized social phobia, agoraphobia, and panic disorder). Among "MDD only" patients (no GAD), only 1/12 had another diagnosis for bulimia.

**Across all participants, consistent with the conceptual formulation driving creation of the Mood and Anxiety Symptom Questionnaire (MASQ) scale, the anxious arousal and anhedonia

subscales shared 24% of their variance (r = .49, p < .001), but shared a greater degree of variance with the general distress subscale (r > .68, p < .001). Amongst patients alone, shared variance between these subscales was near-zero (r = .01, p = .96), with a greater degree of variance shared with the general distress subscale (r > .5, p < .001).

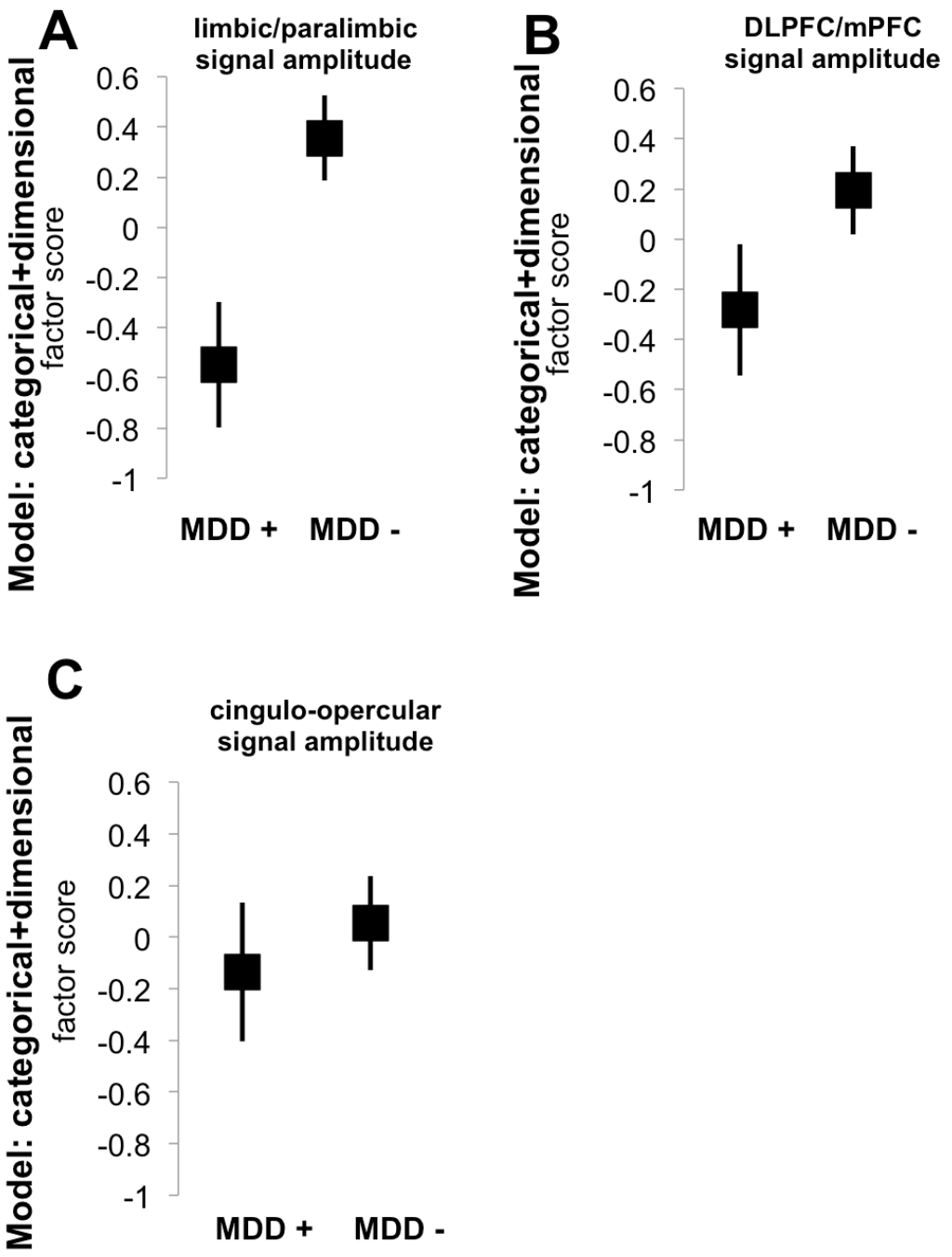

Figure S1. To illustrate individual factor contributions to the primary analysis MDD main effect (Figure 3D) for the combined dimensional and categorical model, each of the three signal amplitude factors is individually displayed in **A**-**C**. As shown, MDD patients compared to the non-MDD group (GAD only or Healthy Control) consistently had lower signal amplitudes that was especially pronounced for the limbic/paralimbic as well as DLPFC/mPFC factors.

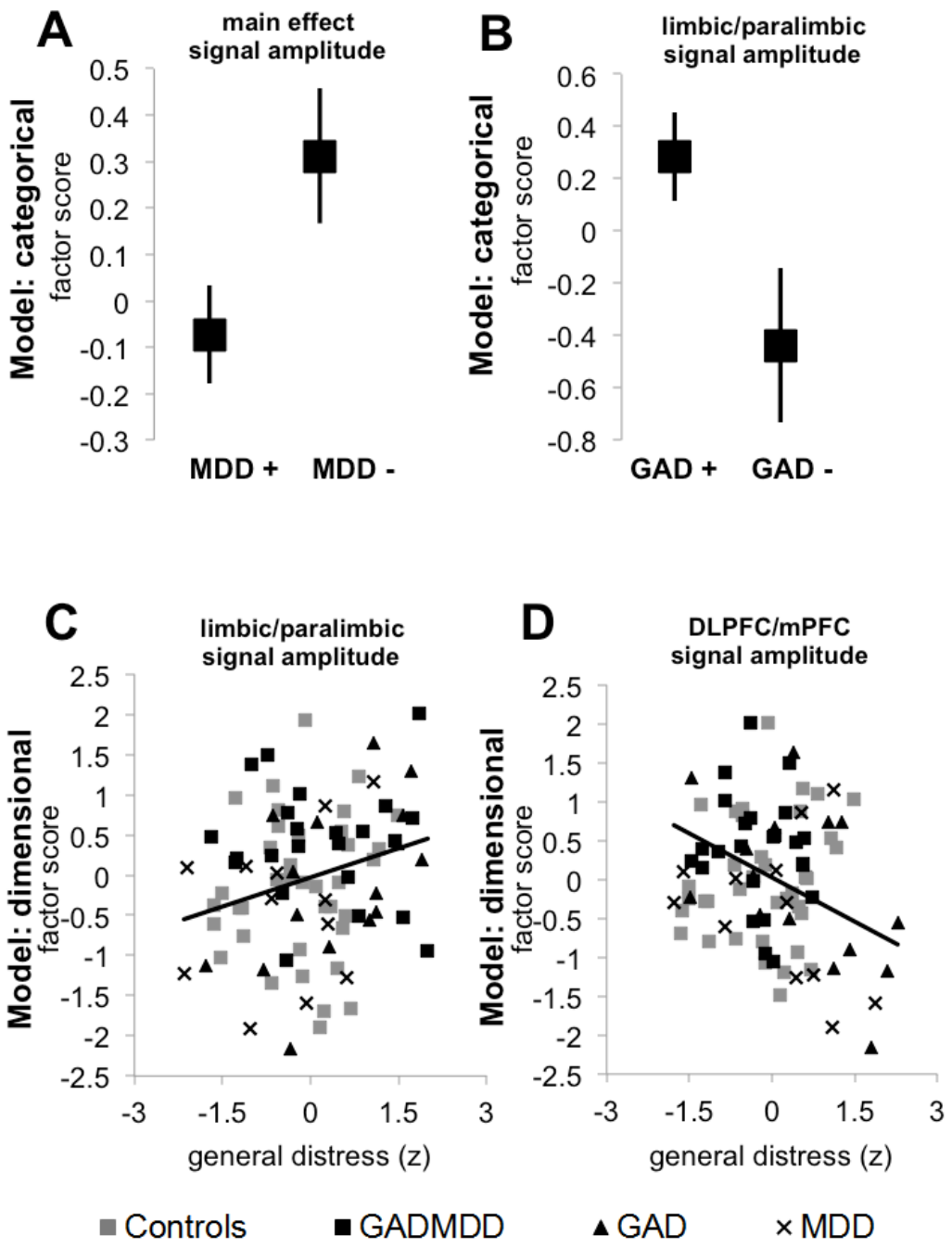

Figure S2. Signal amplitude results from models of only categorical or dimensional definitions of anxiety and depression for the patient only subsample, plotting estimated marginal means and their standard errors. For the categorical model, (**A**) a main effect of MDD was found, as well as (**B**) a specific effect of GAD on limbic/paralimbic signal amplitude. For the dimensional only model, effects of general distress on (**C**) limbic/paralimbic, and (**D**) DLPFC/MPFC signal were found. Control subject data are shown for visual comparison in the scatterplots but were not used in the linear trend line calculation.

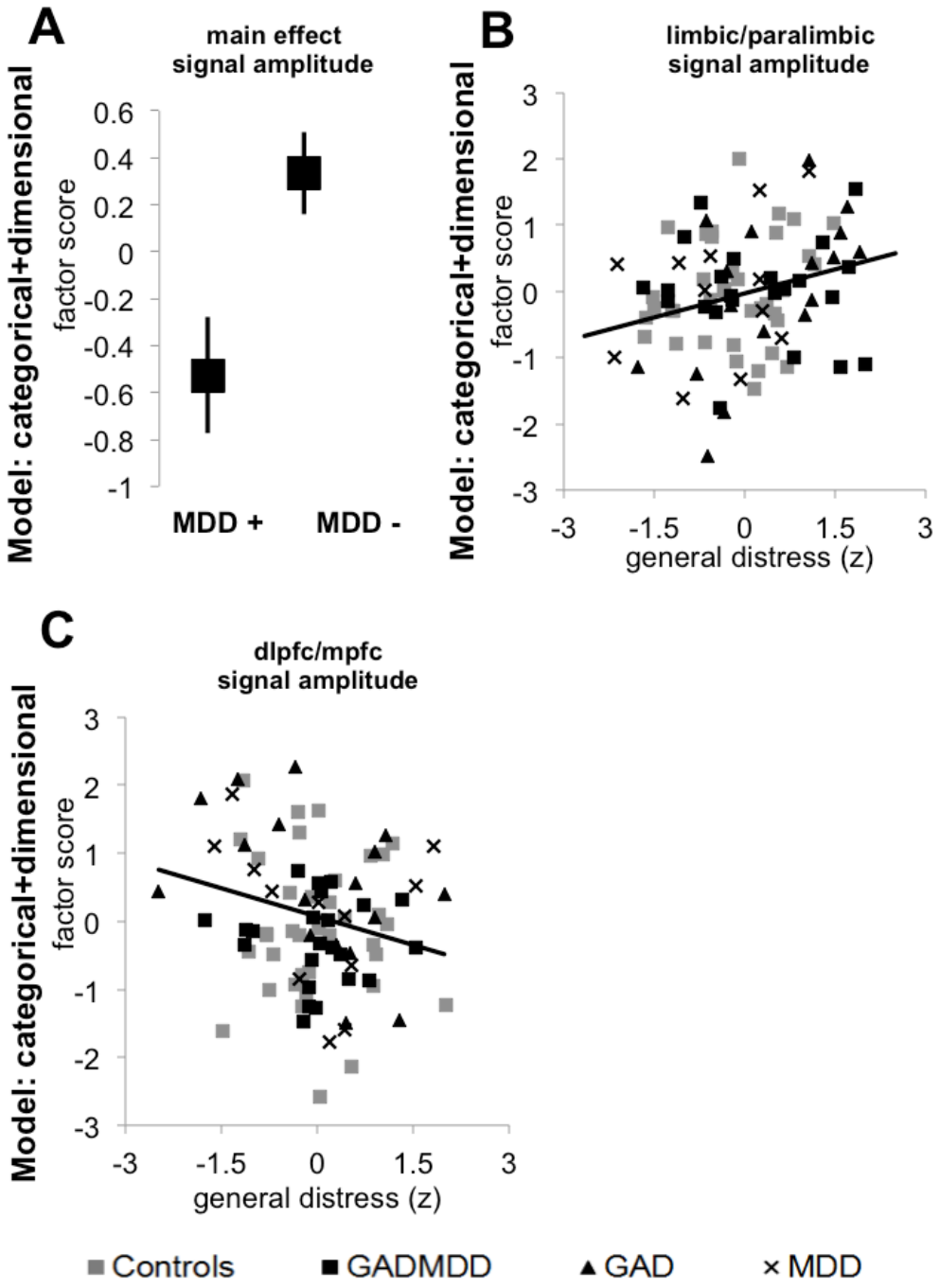

Figure S3. Signal amplitude results from the model combining categorical and dimensional definitions of anxiety and depression for the patient only subsample, plotting estimated marginal means and their standard errors. (**A**) A main effect of MDD was found, as well specific effects of general distress were found on (**B**) limbic/paralimbic, and (**C**) DLPFC/MPFC signal. Control subject data are shown for visual comparison in the scatterplots but were not used in the linear trend line calculation.

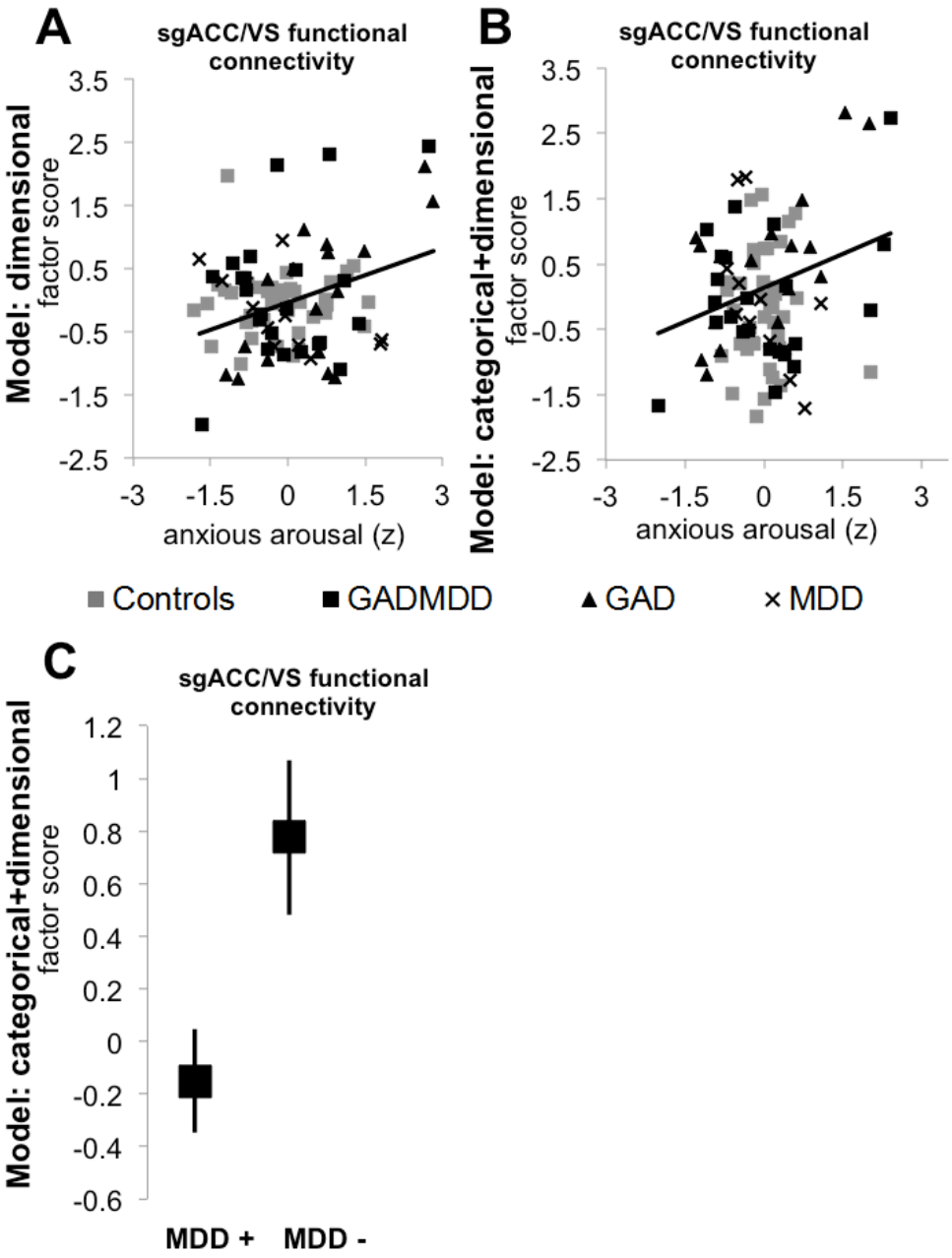

Figure S4. Functional connectivity results from dimensional only or models combining dimensional and categorical definitions of anxiety and depression for the patient only subsample, plotting estimated marginal means and their standard errors. For the dimensional only model, a positive relationship was found between anxious arousal and sgACC/VS connectivity (**A**) which also remained significant in the combined dimensional and categorical model (**B**). In the combined model, an MDD diagnosis was associated with lower sgACC/VS connectivity (**C**). Control subject data are shown for visual comparison in the scatterplots but were not used in the linear trend line calculation.

Supplemental Reference

1. Faul F, Erdfelder E, Lang AG, Buchner A (2007): G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 39:175-191.