Gray Matter Abnormalities in Social Anxiety Disorder: Primary, Replication, and Specificity Studies

Supplemental Information

Corollary Analysis Using a Continuous Measure of Social Anxiety

In addition to examining the differences between the social anxiety disorder (SAD) and control groups based on diagnostic status, we also performed a corollary analysis using a continuous clinician-rated measure of social anxiety, the Liebowitz Social Anxiety Scale (LSAS) total score. [Note that this analysis was only performed in Sample 2, as the measure was not collected in the first sample]. We first examined the association between symptom severity and gray matter (GM) across all subjects, regardless of their diagnostic status. The results, listed in Table S1, show that cerebellar, inferior parietal and precentral GM volumes were positively correlated with social anxiety severity; conversely, temporal pole, as well as superior, middle and inferior frontal cortices, was negatively correlated with severity.

We then explored whether severity could further predict GM variation *within* the SAD group. As listed in Table S1, GM within left medial frontal and right middle occipital gyri, as well as right thalamus and hippocampus, was associated with greater severity among subjects with SAD; conversely, three GM clusters in the (predominantly right hemispheric) dorsal anterior cingulate were inversely correlated with SAD severity.

Table S1. Relationship between Social Anxiety Severity and Gray Matter Volume

		BA	Size	x	y	z	*t*
GM-SAD Severity Correlation, Full Sample							
Positively Associated with LSAS							
1	L,R Cerebellum (Vermis)	-	294	0	-39	-14	4.11
2	Inferior Parietal Lobule	40	145	-38	-44	53	3.82
3	L Middle Temporal, L Inferior Temporal	20,21	92	-59	-44	-15	4.73
4	L Precentral	6	69	-26	-12	63	4.57
5	R Precentral	6	26	30	-11	56	3.58
6	R Cerebellum (Posterior)	-	25	14	-57	-18	3.57
7	R Precentral	6	22	39	-8	56	3.79
Negatively Associated with LSAS							
1	R Superior Temporal, Temporal Pole	38	480	35	15	-30	-4.37
2	R Middle Frontal	11	341	33	47	-11	-5.85
3	L Superior Frontal	11	69	-17	44	-15	-3.97
4	L Inferior Frontal	11	33	-26	29	-15	-3.67
5	L Inferior Frontal	47	23	-35	32	-8	-3.80
6	L Superior Temporal, Temporal Pole	38	19	-42	20	-27	-3.67
7	L Inferior Frontal	10	14	35	35	-8	-4.10
GM-SAD Severity Correlation, within SAD Group							
Positively Associated with LSAS							
1	L Superior / Medial Frontal	6	103	-9	26	57	5.36
2	Thalamus (Pulvinar Nuclei)	-	73	8	-33	2	6.19
3	R Middle Occipital	19	13	33	-80	8	6.83
4	R Hippocampus	-	11	32	-24	-8	4.52
Negatively Associated with LSAS							
1	R Middle Cingulate	23,31	52	11	-36	33	-5.31
2	L Cerebellum (Tonsil)	-	36	-3	-50	-45	-4.49
3	R Middle Cingulate	24	26	9	2	35	-4.70
4	L Middle Temporal	22	20	-51	-45	0	-4.55
5	L Cingulate	24	16	-6	-5	36	-4.74

BA, Brodmann area; GM, gray matter; L, left; LSAS, Liebowitz Social Anxiety Scale; R, right; SAD, social anxiety disorder.

$p < .001$; k = 10.

Analysis includes Sample 2 only as the LSAS measure was not collected in Sample 1.

Full sample: $n = 34$ (17 SAD, 17 Controls); SAD group: $n = 17$ SAD only.

Higher LSAS scores indicate greater anxiety.

All correlations between LSAS severity and GM volume were > .5 within the peak voxel of each cluster.

Testing for Overall Replicability across Samples

In addition to combining the two samples into a single dataset (Table 2 of the manuscript), we also formally tested whether individual findings from one sample were replicated in the other. To do this, we used non-stationary cluster extent correction to identify clusters that were significant at $p < 0.05$ or 0.1, corrected, in either sample alone (clusters indicated by a * or + in Table 2). Contrasts values of *SAD > control* or *control > SAD* contrasts from the 2nd level model of one sample were first averaged over all voxels within the above regions of interest (ROIs), and submitted to an independent 2nd level analysis (using the same group level design as for whole-brain analysis as described in the manuscript). This analysis identified three clusters as detailed below in Table S2. Independent ROI analyses (a single test of the same contrast in the other sample) showed that for left cerebellum/parahippocampal gyrus and right temporal pole, findings were replicated across samples, whereas findings for right precentral/postcentral gyrus were not.

Table S2. Second Level Analysis

Cluster	Sample	Contrast	Region	x	y	z	*t*	*p*
1	1	SAD > Control	L Cerebellum/Parahippocampal	-24	-29	-21	1.92	0.03
2	1	Control > SAD	R Precentral/Post Central	42	-18	38	-1.2	0.88
3	2	Control > SAD	R Temporal Pole	38	17	-29	1.62	0.058

L, left; R, right; SAD, social anxiety disorder.

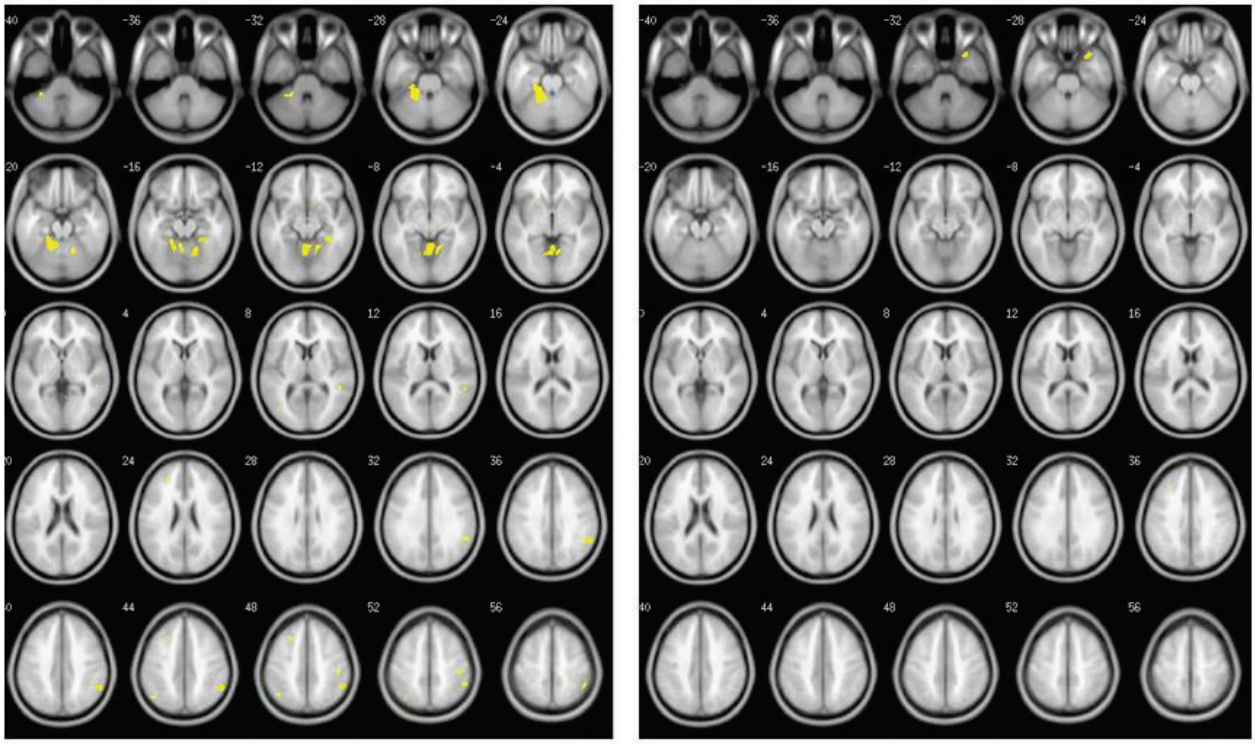
We also explored overall consistency between the two samples by formally quantifying the significance in overlap of the *SAD > control* and *control > SAD* differences across the two samples. To do this, we used the cluster_overlap_npm.m script available from the laboratory of Tor Wager, Ph.D. (http://wagerlab.colorado.edu) in which 2 T-maps (one from each sample for the *cases > controls* comparison) were thresholded at $p < 0.05$ uncorrected, cluster size > 10

and binarized to include only positive T-values (the analysis was repeated for negative T-values, or *controls* > *cases*). The number of overlapping voxels between the two maps was then calculated. The probability of this overlap occurring by chance was calculated by comparing its observed value to a null distribution, which was derived by randomizing the locations of the centers of the clusters of each map 2,000 times.

We observed a significant overlap of voxels for the *SAD* > *control* contrast across both samples (observed: 3096, expected under null: 910, $p = 0.007$; Figure S1, left panel), but not for the *control* > *SAD* contrast (observed: 188, expected: 533 under null, $p = 0.89$, right panel). Thus, the spatial overlap in regions with greater GM volume among the SAD groups (which included cerebellum, parahippocampal gyrus, fusiform and inferior parietal lobe) was relatively extensive and significantly greater than that expected by chance, whereas the spatial overlap across regions with greater GM in the control groups was not extensive and was less than the overlap expected by chance.

Figure S2. Overlap of Spatial Contrast Patterns Across Sample 1 and 2

(a) *SAD > Control* **(b)** *Control > SAD*



Sample 1: N = 16 SAD; 20 Control; Sample 2: N = 17 SAD, 17 Control
T1 -weighted axial images; image left is brain left. Group results are adjusted for differences in age, gender, intracranial volume, and population source.
Clusters represent voxels that survived p < .05, uncorrected, in both samples individually.

ROI Analysis

We conducted an additional exploratory analysis in the combined sample within three ROIs which have been implicated in the functional neurobiology of social anxiety but were not detected in our whole-brain analysis: 1) bilateral amygdala; 2) bilateral insula; and 3) anterior cingulate cortex. For these analyses we employed a looser statistical threshold than that used for the preceding analyses ($p < 0.05$, k = 10) and also applied small-volume, non-stationary cluster extent correction. The results are shown below in Table S3.

Table S3. ROI Analysis.

	BA	**Size**	**x**	**y**	**z**	***t***
SAD > Control						
No significant regions						
Control > SAD						
R Amygdala		87	36	3	-26	2.92
L Anterior Cingulate	32,24	261	-3	36	22	2.41
L Insula		108	-46	12	-8	2.78
R Insula		100	42	-13	-6	2.47
		84	45	8	-3	2.30

BA, Brodmann area; L, left; R, right; SAD, social anxiety disorder.

We found no voxels that survived the above uncorrected threshold for the *SAD > control* contrast within any ROI. However, gray matter in the left anterior cingulate cortex, right amygdala, and bilateral insula were lower in subjects with SAD (Table S1). None of these regional differences survived whole-brain cluster extent correction, and only the amygdala observations survived small-volume and cluster extent correction ($p = 0.03$, corrected); these coordinates should thus be viewed provisionally and probed further in subsequent studies.