# Extremely shy & genetically close : investigating neurobiological endophenotypes of social anxiety disorder

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# Part 2

# Structural brain characteristics as putative SAD endophenotypes

# Chapter 4

## Voxel-based morphometry multi-center mega-analysis of brain structure in Social Anxiety Disorder

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# Abstract

Social anxiety disorder (SAD) is a prevalent and disabling mental disorder, associated with significant psychiatric co-morbidity. Previous research on structural brain alterations associated with SAD has yielded inconsistent results concerning the direction of the changes in gray matter (GM) in various brain regions, as well as on the relationship between brain structure and SAD-symptomatology. These heterogeneous findings are possibly due to limited sample sizes. Multi-site imaging offers new opportunities to investigate SAD-related alterations in brain structure in larger samples.

An international multi-center mega-analysis on the largest database of SAD structural T1-weighted 3T MRI scans to date was performed to compare GM volume of SAD patients ($n$ = 174) and healthy control (HC) participants ($n$ = 213) using voxel-based morphometry. A hypothesis-driven region of interest (ROI) approach was used, focusing on the basal ganglia, the amygdala-hippocampal complex, the prefrontal cortex, and the parietal cortex. SAD patients had larger GM volume in the dorsal striatum when compared to HC participants. This increase correlated positively with the severity of self-reported social anxiety symptoms. No SAD-related differences in GM volume were present in the other ROIs.

Thereby, the results of this mega-analysis suggest a role for the dorsal striatum in SAD, but previously reported SAD-related changes in GM in the amygdala, hippocampus, precuneus, prefrontal cortex and parietal regions were not replicated. Our findings emphasize the importance of large sample imaging studies and the need for meta-analyses like those performed by the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium.

# Introduction

Social anxiety disorder (SAD) is one of the most common anxiety disorders (Stein & Stein, 2008), with an estimated lifetime prevalence between 6 and 13 % (Kessler et al., 2012; Stein et al., 2010). Patients with SAD are characterized by intense fear of, distress in, and avoidance of situations in which they may be scrutinized (American Psychiatric Association, 2013). The disorder is highly disabling, as impairments in social life and work situations are frequently reported (Mack et al., 2015). In addition, the disorder is associated with significant psychiatric co-morbidity, such as depressive disorders and substance abuse (Stein & Stein, 2008). These findings stress the need for improvements in the treatment of SAD. Understanding the neurobiological mechanisms that underlie this disorder has the potential to advance treatment.



Previous magnetic resonance imaging (MRI) studies on brain anatomy differences in SAD have reported heterogeneous findings, implicating regions such as the frontal cortex, the parietal cortex, occipital cortex, temporal regions and subcortical limbic areas, as reviewed by Brühl, Delsignore, et al. (2014); see also Goodkind et al. (2015) reporting on a transdiagnostic meta-analysis of structural neuroimaging studies. Several of these changes were correlated with clinical characteristics, such as the severity of social anxiety symptoms (Brühl, Hänggi, et al., 2014; Frick, Engman, et al., 2014; Irle et al., 2010, 2014; Liao et al., 2011; Syal et al., 2012; Talati, Pantazatos, et al., 2013) or disease duration (Meng et al., 2013). In addition, recent treatment studies in SAD patients have identified structural changes in bilateral caudate and putamen, right thalamus and cerebellum after eight weeks of paroxetine treatment (Talati, Pantazatos, Hirsch, & Schneier, 2015), and alterations in parieto-occipital and prefrontal GM volumes after cognitive behavioral group therapy (Steiger et al., 2017), while a classification study using multi-voxel pattern analysis was able to discriminate SAD patients from healthy control participants based on the pattern of regional gray matter (GM) volume over the whole brain (Frick, Gingnell, et al., 2014). Together, these studies provide evidence for the idea that certain brain regions are clinically associated with SAD.

Functional MRI (fMRI) studies have also identified important candidate brain regions that may be related to structural changes associated with SAD-related psychopathology. These fMRI studies, typically examining brain activity in response to emotional stimuli or in response to cognitive tasks (Brühl, Delsignore, et al., 2014), most consistently point towards an increase of brain activation in SAD in the bilateral amygdala and hippocampus, prefrontal brain regions, bilateral insula, bilateral parietal cortex and bilateral precuneus, while findings on the direction of changes in the basal ganglia are mixed (Brühl, Delsignore, et al., 2014; Cremers & Roelofs, 2016). In addition, studies on functional connectivity, during rest as well as during cognitive tasks (Brühl, Delsignore, et al., 2014), revealed changes in connectivity of, among others, the putamen (Cremers, Veer, Spinhoven, Rombouts, & Roelofs, 2015) and the amygdala (Hahn et al., 2011; Pannekoek et al., 2013; Sladky et al.,

2015), while recent positron emission tomography (PET) studies showed decreased serotonin receptor binding (Lanzenberger et al., 2007) and increased serotonin synthesis and transporter availability in the hippocampus, amygdala, anterior cingulate cortex (ACC) and striatal regions like the putamen and globus pallidus (Frick et al., 2015; Furmark et al., 2016). These results, together with the findings of a treatment study revealing a relationship between changes in amygdala structure and amygdala function in SAD (Månsson et al., 2016), suggest that the brain regions showing functional changes in SAD overlap to a large extent with the regions that have showed differences in brain structure.

However, the available evidence with respect to structural brain alterations in SAD is inconclusive, as both increases as well as decreases in GM volumes in various brain regions have been reported (Brühl, Delsignore, et al., 2014). Furthermore, findings concerning the relationship between brain structure and SAD-symptoms are inconsistent (Brühl, Hänggi, et al., 2014; Frick, Engman, et al., 2014; Irle et al., 2014; Tükel et al., 2015). These heterogeneous results are possibly due to differences in the employed methods, as well as the relatively small sample sizes employed in studies on SAD-related changes in brain structure (ranging from 12 to 67 SAD patients), and variability in clinical parameters between the samples. Recent advances in multi-site imaging offer new opportunities to investigate the structural brain alterations associated with SAD.

In this international multi-center mega-analysis, which is part of the European and South African Research Network in Anxiety Disorders (EURSANAD) program initiated by the Anxiety Disorders Research Network (Baldwin & Stein, 2012), we investigated GM volume in a priori defined regions of interest (ROIs) in a sample of 174 SAD patients and 213 healthy control participants, using an optimized voxel-based morphometry (VBM) protocol (Ashburner & Friston, 2000; Lerch et al., 2017). VBM analyses have the advantage of using unbiased, standardized methods to investigate brain structure, and have been extensively used to investigate alterations in brain morphology across numerous major psychiatric conditions (Ashburner & Friston, 2000; Goodkind et al., 2015). The large sample of the present work provides the best statistical power to date to investigate GM alterations associated with SAD. Data were collected in multiple scan centers located in five countries (Germany, South Africa, Sweden, the Netherlands and the United States of America; *Figure 4.1*). Based on the available evidence reviewed above, our analysis focused on changes in GM volume in four a priori defined ROIs that seem to be most prominently involved in SAD: the basal ganglia, the amygdala-hippocampal complex, the prefrontal cortex and the parietal cortex including the precuneus. Given the mixed findings on SAD-related increases versus decreases in GM in the previous structural MRI studies (Brühl, Delsignore, et al., 2014), we did not make specific predictions about the direction of the changes within these ROIs. Significant results within the ROIs were followed up by regression analyses to investigate the relationship between GM volumes and the severity of social anxiety symptoms within the patient group.

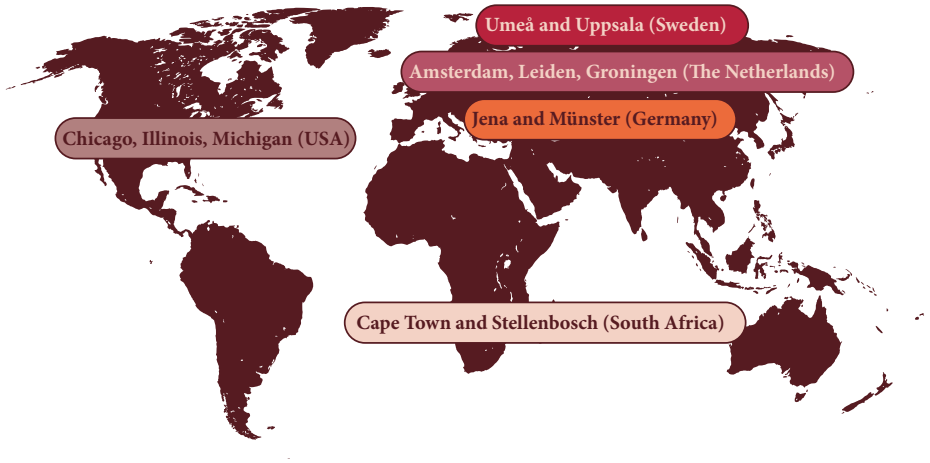


**Figure 4.1 Research sites represented in mega-analysis.**

# Material and methods

## Participants

Structural T1-weighted 3T MRI scans were collected at research centers located in Europe, Africa and North-America, and brought together for quality control and initial analysis in Cape Town, South-Africa. Final analyses took place in Leiden, the Netherlands. The initial sample consisted of 251 SAD patients and 230 healthy control (HC) participants (*Table 4.1*), and results on these datasets have been published previously (Boehme, Ritter, et al., 2014, 2015; Boehme, Mohr, Becker, Miltner, & Straube, 2014; Cremers et al., 2014; Geiger et al., 2016; Howells et al., 2015; Klumpp et al., 2016; Månsson et al., 2013, 2015; Pannekoek et al., 2013; Phan et al., 2013; Syal et al., 2012; van Tol et al., 2010) – see *Supplemental Methods* for more details on the in- and exclusion criteria and recruitment of participants for each sample. At each site, the local ethical committee approved data collection and all participants provided written informed consent after the procedure had been fully explained.

Participants (18 years or older) were recruited through public announcements (online and within the community), consumer advocacy groups, general practitioners and clinical centers, and screened using structured clinical interviews in their native language: the Mini-International Neuropsychiatric Interview (Sheehan et al., 1997), the Composite Interview Diagnostic Instrument version (Kessler & Ustün, 2004) or the Structured Clinical Interview for DSM-IV disorders (First, Spitzer, Gibbon, Williams, & Benjamin, 1998). SAD patients had to meet criteria for a primary diagnosis of SAD, while HC participants had to be free of any psychopathology. General MRI contraindications (ferromagnetic implants, claustrophobia, pregnancy) were a reason for exclusion in both groups.

**Table 4.1 Sample composition and number of scans.**

| | | Initial # scans | | Excluded # scans | | | | Included # scans | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Country** | **Research center** | **SAD** | **HC** | **Comorbidity[a]** | **Insufficient quality[b]** | **Brain pathology** | **Other reason[c]** | **SAD** | **HC** | **Total** |
| Germany | University of Jena; University of Münster | 53 | 22 | 31 | 3 | 0 | 0 | 19 | 22 | 41 |
| The Netherlands | VU Medical Center Amsterdam, NESDA Study | 10 | 27 | 1 | 3 | 0 | 0 | 6 | 27 | 33 |
| | University of Groningen, NESDA Study | 9 | 12 | 1 | 1 | 0 | 0 | 8 | 11 | 19 |
| | Leiden University Medical Center, NESDA Study | 9 | 26 | 1 | 0 | 0 | 0 | 8 | 26 | 34 |
| | Leiden University Medical Center, Social Anxiety Study | 20 | 20 | 0 | 0 | 0 | 0 | 20 | 20 | 40 |
| South-Africa | University of Cape Town; Stellenbosch University | 18 | 17 | 2 | 10 | 0 | 0 | 12 | 11 | 23 |
| Sweden | Umeå University | 26 | 26 | 0 | 3 | 0 | 0 | 26 | 23 | 49 |
| | Uppsala University | 24 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 |
| United States of America | University of Chicago | 27 | 25 | 3 | 4 | 0 | 0 | 24 | 21 | 45 |
| | University of Illinois | 12 | 12 | 1 | 0 | 0 | 0 | 12 | 11 | 23 |
| | University of Michigan | 43 | 43 | 2 | 3 | 1 | 0 | 39 | 41 | 80 |
| Total | | 251 | 230 | 42 | 27 | 1 | 24 | 174 | 213 | 387 |

**Abbreviations and symbols**

SAD: social anxiety disorder patients; HC: healthy control participants. #: number of scans.

**Footnotes**

[a] Other than depression or anxiety (SAD patients only).

[b] Insufficient scan-quality: scans with motion artefacts, scans being unsegmentable or scans for which brain extraction failed after multiple attempts.

[c] No data from HC participants to balance design.

In addition to the T1-weighted 3T MRI scans, demographic (age, gender, handedness) and clinical data were collected at each research center. Furthermore, information about education level, comorbidity, medication use and the scores on several questionnaires (Liebowitz Social Anxiety Scale (LSAS) (Heimberg et al., 1999), Beck Depression Inventory (BDI) (Beck, Steer, & Carbin, 1988) and State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970)) were available for a subset of participants.

## Data acquisition, quality checks and final sample

Parameters of the T1-weighted MRI scans are presented in *Table 4.2*. Scans from SAD patients with comorbid psychopathology other than any other anxiety disorder or major depressive disorder (MDD) were excluded from the analysis ($n$ = 42, see *Supplemental Table S4.1*). Next, scans were extensively checked for pathology and quality, leading to the exclusion of an additional 28 scans (*Table 4.1*). Furthermore, all scans from the research center in Uppsala ($n$ = 24 SAD patients) were excluded due to the lack of scans from HC participants from this center, necessary for our analytic approach. This resulted in a final sample of 174 SAD patients and 213 HC participants. Characteristics of the final sample are presented in *Table 4.3*. Statistical analyses on differences between groups were performed using IBM SPSS Statistics (Version 23), with a significance level of $p < 0.05$.



## Voxel-based morphometry analysis

Voxel-wise GM volumes were investigated using an optimized voxel-based morphometry (VBM) protocol, using the default pipeline as implemented in FSL (version 5.0.7) (Good et al., 2001; Smith et al., 2004). Structural T1-weighted images were first brain-extracted using FSL and FreeSurfer software. Each brain was closely visually inspected and brain-extraction was repeated until all non-brain tissue was properly removed from the image. Subsequently, images were segmented into GM, white matter (WM) and cerebrospinal fluid (CSF) (Zhang, Brady, & Smith, 2001). Next, a study-specific GM template was created, in order to avoid biases during registration that could favour either the SAD or HC-group (Good et al., 2001), by randomly selecting GM images from an equal number of SAD patients and HC participants from each research center ($n$ = 166 SAD patients and $n$ = 166 HC participants). These GM images were non-linearly registered to the Montreal Neurological Institute (MNI) T1-template brain, averaged and flipped along the x-axis to create a left-right symmetric study-specific GM template with a resolution of 2 x 2 x 2 mm. Subsequently, the original GM images from all participants were non-linearly registered to this template (Andersson, Jenkinson, & Smith, 2007), modulated to correct for local expansion or contraction and smoothed using a kernel with an isotropic Gaussian kernel ($\sigma$ = 3 mm).

**Table 4.2 Characteristics of T1-weighted MRI scans.**

| Country | Research Site / Sample | Scanner | Voxels | Dimensions | Reference |
|---|---|---|---|---|---|
| Germany | University of Jena; University of Münster | Siemens/ TrioTim 3T | 192 x 256 x 256 | 1 x 1 x 1 mm | (Boehme, Ritter, et al., 2014, 2015; Boehme, Mohr, et al., 2014) |
| The Netherlands | VU Medical Center Amsterdam, NESDA study | Philips 3 T | 170 x 256 x 256 | 1 x 1 x 1 mm | (Pannekoek et al., 2013, 2015; Penninx et al., 2008; van Tol et al., 2010) |
| | University of Groningen - NESDA study | Philips 3 T | 170 x 256 x 256 | 1 x 1 x 1 mm | |
| | Leiden University Medical Center - NESDA study | Philips 3 T | 170 x 256 x 256 | 1 x 1 x 1 mm | |
| | Leiden University Medical Center - Social Anxiety Study | Philips 3 T | 256 x 256 x 140 | 0.875 x 0.875 x 1.2 mm | (Cremers et al., 2014) |
| South-Africa | University of Cape Town; Stellenbosch University | Siemens Magnetom Allegra 3T | 128 x 256 x 256 | 1.33 x 1 x 1 mm | (Geiger et al., 2016; Howells et al., 2015; Syal et al., 2012) |
| Sweden | Umeå University | General Electric 3T | 512 x 512 x 176 | 0.48 x 0.48 x 1 mm | (Månsson et al., 2013, 2015) |
| | Uppsala University | Philips Achieva 3T | 480 x 480 x 170 | 0.5 x 0.5 x 1 mm | |
| United States of America | University of Chicago | GE Signa System 3T | 256 x 256 x 120 | 0.94 x 0.94 x 1.5 mm | (Klumpp et al., 2016; Phan et al., 2013) |
| | University of Illinois | GE Signa System 3T | 256 x 256 x 182 | 0.86 x 0.86 x 1 mm | |
| | University of Michigan | GE Signa System 3T | 256 x 256 x 124 | 1 x 1 x 1.2 mm | |

## Region of interest (ROI) analysis: differences between groups

In order to maximize the statistical power to detect GM differences between SAD patients and HC participants, we used a region of interest (ROI) approach (Poldrack, 2007), focusing on brain areas in which functional and structural brain changes related to SAD have been reported previously (see *Introduction*). Four ROIs were created in standard space (resolution 2 x 2 x 2 mm) using the Harvard-Oxford Cortical Structural Atlas and Harvard-Oxford Subcortical Structural Atlas implemented in FSLView (version 3.2.0). The *basal ganglia* ROI consisted of voxels with a probability of at least 50 % of belonging to the bilateral accumbens, caudate, pallidum or putamen (total size of ROI: 3224 voxels, 25792 $mm^3$). The second ROI, the *amygdala-hippocampus* ROI, consisted of voxels with a probability of at least 50 % of belonging to the bilateral amygdala, hippocampus and the anterior and posterior para-

hippocampal gyrus (total size of ROI: 3066 voxels, 24528 $mm^3$). The *prefrontal cortex* ROI included voxels with a probability of at least 50 % of belonging to the middle frontal gyrus, the subcallosal cortex, the anterior cingulate gyrus, paracingulate gyrus, frontal medial cortex and frontal orbital cortex (total size of ROI: 20601 voxels, 164808 $mm^3$). Finally, the *parietal* ROI encompassed voxels with a probability of at least 50 % of belonging to the superior parietal lobule, the precuneus cortex and the posterior cingulate gyrus (total size of ROI: 5478 voxels, 43824 $mm^3$).

Within these ROIs, we examined differences in GM volume between SAD patients and HC participants using a general linear model (GLM). In this model, scan center (coded by dummy variables) and gender were added as nuisance regressors, and age and total GM volume were included as covariates. Before we analyzed this GLM, we tested the homogeneity of regression slopes assumption that applies to covariate analysis, by building a separate GLM that included a diagnosis-by-age and a diagnosis-by-total GM regressor in addition to the other regressors. No significant interactions at the whole-brain level were observed, thus justifying the use of the abovementioned GLM that investigated the effect of diagnosis while correcting for the covariates.



Voxelwise statistics were applied using permutation-based non-parametric testing (5000 permutations), correcting for multiple comparisons across space. FSL's default threshold-free cluster enhancement (TFCE) was used to detect significant clusters (Smith & Nichols, 2009) and we used a familywise error (FWE)-corrected threshold of $p < 0.05$ within each ROI. Given the fact that ROIs were a priori defined and are part of a network of brain areas involved in SAD (Brühl, Delsignore, et al., 2014), we report *p*-values uncorrected for the number of ROIs. Significant results within the ROIs were followed up by a multiple regression analysis using IBM SPSS Statistics, in order to examine the relationship between average individual GM volume in the extracted cluster and the severity of total social anxiety symptoms (measured with the LSAS), while controlling for scan center, gender, age and total GM volume. In line with previous work (Frick, Engman, et al., 2014; Irle et al., 2014; Meng et al., 2013; Syal et al., 2012), this analysis was performed in SAD patients only.

For reasons of completeness, we also performed an exploratory whole-brain VBM analysis to examine a main effect of diagnosis and interactions with age and scan center outside the predefined ROIs using the same GLM. Again, we used TFCE-results based on an FWE-corrected threshold of $p < 0.05$.

# Results

## Sample characteristics

Characteristics of SAD patients ($n$ = 174) and HC participants ($n$ = 213) are presented in *Table 4.3*. SAD patients did not differ from HC participants in terms of age, gender distribu-

**Table 4.3 Demographic and clinical characteristics of social anxiety disorder (SAD) patients and healthy control (HC) participants.**

| | SAD (*n* = 174) | HC (*n* = 213) | Statistical analysis |
|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | ***p*** |
| Age (years) | 30.6 ± 10.0 | 32.4 ± 10.5 | 0.13 [g] |
| Age of onset (years)[a] | 14.8 ± 7.1 | | |
| | ***n* (%)** | ***n* (%)** | ***p*** |
| Males | 72 (41.4) | 97 (45.5) | 0.41 [h] |
| Education level[b] | | | 0.10 [h] |
| *Low* | 1 (0.7) | 6 (3.2) | |
| *Intermediate* | 56 (36.8) | 54 (29.0) | |
| *High* | 95 (62.5) | 126 (67.7) | |
| Right-handed | 172 (98.9) | 206 (96.7) | 0.17 [h] |
| Comorbidity | | | |
| *SAD only* | 114 (65.5) | | |
| *SAD + MDD* | 8 (4.6) | | |
| *SAD + MDD + PD* | 2 (1.1) | | |
| *SAD + GAD* | 10 (5.7) | | |
| *SAD + GAD + SP* | 3 (1.7) | | |
| *SAD + GAD + PD* | 2 (1.1) | | |
| *SAD + PD* | 3 (1.7) | | |
| *SAD + SP* | 6 (3.4) | | |
| *Unknown* | 26 (14.9) | | |
| Medication use at time of scan[c] | 24 (14.2) | | |
| *SSRI* | 17 | | |
| *Betablocker* | 2 | | |
| *Antidepressivum NOS* | 4 | | |
| *Unknown medication* | 1 | | |
| | **Mean ± SD** | **Mean ± SD** | ***p*** |
| LSAS[d] | 77.9 ± 17.9 | 14.3 ± 12.6 | <0.001 [g] |
| BDI[e] | 13.8 ± 8.8 | 2.3 ± 3.2 | <0.001 [g] |
| STAI– State [f] | 43.2 ± 10.1 | 20.9 ± 11.0 | <0.001 [i] |
| STAI -Trait [f] | 50.1 ±10.2 | 22.6 ± 11.5 | <0.001 [i] |
| Total GMV (mL) | 519.3 ± 49.9 | 522.3 ± 58.7 | 0.47 [g] |

**Abbreviations**

BDI: Beck Depression Inventory; GAD: generalized anxiety disorder; GMV: Gray Matter Volume; LSAS: Liebowitz Social Anxiety Scale; MDD: Major Depressive Disorder; NOS: not otherwise specified; PD: panic disorder; SD: standard deviation; SP: specific phobia; SSRI: selective serotonin reuptake inhibitor; STAI: State-Trait Anxiety Inventory.

**Footnotes**

[a] Data from 65 SAD patients.
[b] Data from 152 SAD patients and 186 HC participants.
[c] Data from 169 SAD patients.
[d] Data from 148 SAD patients and 140 HC participants.
[e] Data from 113 SAD patients and 111 HC participants.
[f] Data from 75 SAD patients and 73 HC participants.
[g] Independent Samples Mann-Whitney U test.
[h] $\chi^2$ test.
[i] Independent Samples T-Test.

tion, level of education, handedness and total GM volume, but they reported significantly more social anxiety symptoms (measured with the LSAS) and anxiety symptoms (measured with the STAI) in comparison to HC participants. In addition, SAD patients reported significantly more depressive symptoms than HC participants, as measured with the BDI. It should, however, be noted that the degree of reported depression symptoms in the SAD patients indicates only minimal depression (mean ± standard deviation: 13.8 ± 8.8) (Beck et al., 1988), whereas the mean scores on the LSAS for the SAD patients (mean ± standard deviation: 77.9 ± 17.9) are in line with a clinical diagnosis of SAD (Mennin et al., 2002).

## ROI analyses: differences between SAD patients and HC participants



There was an effect of diagnosis in the basal ganglia ROI: SAD patients had larger GM volume in the right putamen, extending into the pallidum (*Figure 4.2A-B*; extent = 78 voxels, peak coordinate in MNI space (x, y, z): 26, -8, 0; $p = 0.022$, small-volume corrected; result did not survive correction when all ROIs were taken together), with a small effect size ($\beta = 0.14$, Cohen's d = 0.20). A subsequent analysis, that regressed social anxiety symptoms within the SAD patients on individual extracted GM volume in this region, revealed a significant positive correlation with a small effect size (zero-order correlation: Spearman's rho = 0.21, $p = 0.010$; multiple regression analysis while controlling for scan center, gender, age and total GM volume: $\beta = 0.13$, $p = 0.048$; see also *Figure 4.2C*).

Given the fact that SAD often co-occurs with major depressive disorder (MDD) (Stein & Stein, 2008), we investigated whether the GM difference in the putamen was influenced by comorbid depression, by performing three subsequent analyses. Firstly, we excluded SAD patients with a diagnosis of comorbid MDD (excluded: $n = 10$ SAD patients; *Table 4.3*) and performed a multiple regression analysis with individual GM volume of the right putamen cluster as dependent variable, and diagnosis as independent variable while controlling for scan center, age, gender and total GM volume (remaining sample: $n = 164$ SAD patients and 213 HC participants). This analysis still showed a significant effect of diagnosis ($\beta = 0.14$, $p = 0.002$). Secondly, we excluded participants with a BDI score ≥ 30, indicating severe depression (Beck et al., 1988), (excluded: $n = 7$ SAD patients; remaining sample: $n = 106$ SAD patients and 111 HC participants). Again, the effect of diagnosis was significant ($\beta = 0.14$, $p = 0.017$). In the third analysis, we examined the relationship between BDI score and GM volume in the SAD group ($n = 113$ SAD patients; regression analysis, controlling for scan center, age, gender, and total GM volume). This analysis revealed a significant effect of BDIscore on GM volume ($\beta = 0.17$, $p = 0.034$). Importantly, when LSAS score and BDI score were both entered in the regression model, the effect of BDI was not significant anymore ($\beta = 0.13$, $p = 0.13$), while LSAS score was still a significant predictor of GM volume ($\beta = 0.16$, $p = 0.049$). These results indicate that variation in BDI scores in the SAD sample did not significantly account for GM variance in the putamen-pallidum over and above effects of LSAS.

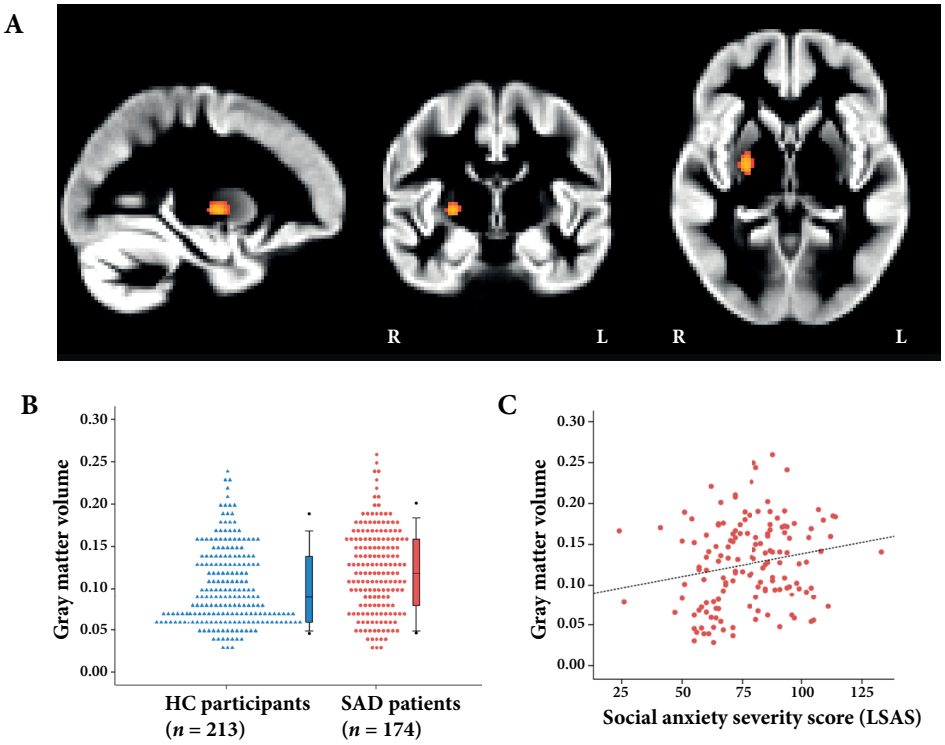


**Figure 4.2 Larger GM volume in social anxiety disorder (SAD) patients relative to healthy control (HC) participants.**
***Figure 4.2A*** Larger gray matter (GM) volume in SAD patients relative to HC participants in the right dorsal striatum ($p < 0.05$, small-volume corrected).
***Figure 4.2B*** Dot density plot illustrating the group difference in GM volume in the dorsal striatum.
***Figure 4.2C*** Scatterplot illustrating the relationship between social anxiety symptoms in a subset of SAD patients ($n = 148$; measured with the Liebowitz Social Anxiety Scale, LSAS) and GM volume in the dorsal striatum (Spearman's rho = 0.21, $p < 0.05$).

However, when we performed two additional sensitivity analyses to investigate the effect of 1st general comorbidity and 2nd medication use on the GM difference in the putamen, using multiple regression analyses with individual GM volume of the right putamen cluster as dependent variable, and diagnosis as independent variable while controlling for scan center, age, gender and total GM volume, the effect of diagnosis lacked significance (sensitivity analysis 1, including only patients without comorbidity: remaining sample: $n = 114$ SAD patients and 213 HC participants; $\beta = 0.06$, $p = 0.28$; sensitivity analysis 2, including only patients without present medication use: remaining sample: $n = 59$ SAD patients and 117 HC participants; $\beta = 0.13$, $p = 0.13$).

There were no clusters in the basal ganglia ROI where HC participants had larger GM volume relative to SAD patients. In addition, we did not find significant group differences in

the other ROIs using the VBM approach. To explore these null findings, we extracted the individual GM volumes from the regions within each of the larger ROIs tested and examined the presence of between-group differences using multiple regression analyses controlled for scan center, age, gender and total GM volume. Because of the exploratory nature of these analyses, we corrected for the number of tests using Bonferroni-correction (13 regions, $p \leq 0.004$). There were no regions in which the effect of diagnosis was significant at this Bonferroni-corrected significance level (*Supplemental Table S4.2*), although two effects were significant at the uncorrected level. Furthermore, we explored the possibility that these null findings were present due to gender differences between patients, by investigating gender-by-diagnosis interactions. Again, no significant interactions were found at the Bonferroni-corrected significance level ($p \leq 0.004$) (*Supplemental Table S4.2*).



### Whole-brain analysis: no group differences

The exploratory whole-brain VBM analysis did not reveal a significant main effect of diagnosis. Significant diagnosis-by-age or diagnosis-by-scan center interactions were also not observed at whole-brain level.

## Discussion

In this study we investigated differences in GM volume between SAD patients and HC participants, in the largest sample of 3T structural MRI scans available for analysis to date ($n$ = 174 SAD patients and 213 HC participants). We used a hypothesis-driven ROI approach and focused on differences in GM volume in the amygdala-hippocampal complex, the basal ganglia, the prefrontal cortex and parietal areas. The results showed larger GM volume in the right putamen in SAD patients in comparison to HC participants (*Figure 4.2A-B*), and this increase in GM was positively correlated with the total score on the Liebowitz Social Anxiety Scale (LSAS) within the patient group (*Figure 4.2C*). This effect remained significant when we performed several sensitivity analyses examining the effect of comorbid depression; however, the effect did not survive in two other sensitivity analyses in which patients with any type of comorbidity and medication use were excluded, possibly due to the fact that the remaining sample size was relatively small.

We did, however, not find diagnosis-related alterations in GM volumes in the amygdala-hippocampal, prefrontal or parietal ROIs. Furthermore, there were no group differences in an exploratory whole-brain analysis. To examine these results, we performed post-hoc analyses to examine group differences in individual structures of these ROIs, but again, no SAD-related GM differences were present (*Supplemental Table S4.2*). Furthermore, we checked whether GM differences between male and female SAD patients might have

confounded the results, but we did not find significant gender-by-diagnosis interactions (*Supplemental Table S4.2*).

## No SAD-related changes in amygdala-hippocampal, prefrontal and parietal ROIs

The null findings in the amygdala-hippocampal, prefrontal, and parietal ROIs were unexpected, because previous studies have reported SAD-related changes in GM in, among others, the amygdala, hippocampus, precuneus, prefrontal cortex, and parietal regions (Brühl, Hänggi, et al., 2014; Irle et al., 2010, 2014; Liao et al., 2011; Machado-de-Sousa et al., 2014; Meng et al., 2013; Syal et al., 2012; Talati, Pantazatos, et al., 2013; Tükel et al., 2015). Although applying the usual caveat when interpreting null effects, our results based on the largest SAD sample to date suggest that GM volume in regions outside the basal ganglia is likely not systematically related to SAD and thus might not underlie the alterations in brain functioning consistently reported and replicated in these regions (Brühl, Delsignore, et al., 2014). This idea is in line with the findings of a recent voxel-wise machine learning study, which suggested that SAD is easier to detect using multivariate analyses that take into account the global relationships between GM volume alterations in different regions, than by applying analyses that only focus on local changes in specific brain regions (Frick, Gingnell, et al., 2014).

With respect to the previous studies reporting SAD-related GM differences, it should be noted that the findings of these studies were often inconsistent, with increases as well as decreases in the same regions having been reported. E.g. for the amygdala, see (Irle et al., 2010; Machado-de-Sousa et al., 2014; Meng et al., 2013); see also (Brühl, Hänggi, et al., 2014; Syal et al., 2012) reporting no volumetric differences between SAD patients and HC participants, and the work of Shang et al. (2014), who did not observe changes in amygdalar GM volumes in a meta-analysis on structural neuroimaging findings across several anxiety disorders. These inconsistencies are most likely due to small sample sizes, which may have increased the probability of obtaining false-positive findings (Blackford, 2017; Button et al., 2013) – see also Cremers & Roelofs (2016) for a critical overview of neuroimaging research findings in SAD. Furthermore, the inconsistencies are likely due to differences in methodology, for example the use of manual vs. automatic segmentation, the choice and size of ROIs, and to differences in clinical characteristics. Thus, the results of this study stress the need for studies with sufficient sample sizes and meta-analyses such as those performed by the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium and its working groups (Bearden & Thompson, 2017; Thompson et al., 2014).

## Larger GM volume in right putamen

We did find GM differences in the right putamen, which, together with the caudate, forms the dorsal striatum (Marchand, 2010). The striatum is the major input structure of the basal ganglia, receiving information from the cortex, amygdala and hippocampus. The dorsal

striatum is part of a network that is important for learning actions based on their predicted outcomes (i.e. reward-related behavior), as well as for regulating cognitive and emotional behavior (Marchand, 2010; Shohamy, 2011; Stathis, Panourias, & Themistocleous, Sakas, 2007); for a recent review on the role of the striatum in anxiety we refer to Lago, Davis, Grillon, & Ernst (2017). Interestingly, our findings converge with earlier research on the structural and functional basis of inhibited temperament, a characteristic that refers to the innate tendency to be shy, quiet and extremely cautious in novel social and non-social situations (Miskovic & Schmidt, 2012). Inhibited temperament substantially increases the risk for developing SAD (Clauss & Blackford, 2012; Fox & Kalin, 2014) and is correlated with larger volumes of both the amygdala and the caudate in young adults, and hyperactivation in, among other areas, putamen, globus pallidus and caudate (Clauss, Seay, et al., 2014; Clauss et al., 2015) – see *Supplemental Table S4.3* for coordinates of these and other findings discussed in this section. Moreover, Clauss and colleagues showed that the GM increase in the caudate was positively related to the level of activation in this area in response to neutral faces (Clauss, Seay, et al., 2014). Because larger GM volume of the caudate was also associated with increased functional connectivity to regions that respond to social stimuli, the authors have proposed that larger caudate volume might facilitate the saliency of social and novel stimuli for individuals with an inhibited temperament, which could predispose them for developing SAD (Clauss, Seay, et al., 2014). Combined with our observation that SAD is associated with larger GM volume in the putamen, it may be hypothesized that structural changes in the dorsal striatum, as an integral part of limbic circuitry (Stathis et al., 2007), might underlie the biased processing of stimuli typically observed in SAD (Miskovic & Schmidt, 2012).

Evidence consistent with this idea comes from recent fMRI studies on SAD-related threat processing (Cremers et al., 2015; Heitmann et al., 2016). Anticipation of social punishment versus reward was associated with increased local activity in the putamen in SAD patients compared to healthy controls. In addition, SAD patients showed increased negative connectivity between the putamen and the ACC during social punishment and reward compared to HC participants (Cremers et al., 2015). Another study indicated that viewing ecologically valid, disorder-related complex visual scenes evoked increased activation in SAD patients in, among others, the putamen and globus pallidus. Here, hyperactivation in the dorsal striatum was accompanied by increased connectivity with the amygdala, medial prefrontal cortex and ACC, regions playing an important role in emotion processing (Heitmann et al., 2016). These findings are supported by another resting-state study indicating hyperconnectivity of the putamen and the globus pallidus in SAD (Arnold Anteraper et al., 2014) and two meta-analyses on task-related activity in SAD, reporting increased activation of the globus pallidus (Gentili et al., 2016; Hattingh et al., 2013).

Additional support for our hypothesis comes from a within-subject longitudinal study on the neuro-anatomical effects of paroxetine in a small sample of fourteen patients with

SAD, showing treatment-related decreases in symptom severity and concomitant reductions in GM in bilateral caudate and putamen (Talati et al., 2015). Furthermore, a 1H-magnetic resonance spectroscopy study demonstrated a relationship between social anxiety symptoms and the concentration of choline metabolites in the left caudate and right putamen (Howells et al., 2015), while single-photon emission computed tomography (SPECT) studies reported on alterations in the striatal dopaminergic system in patients with SAD (Schneier et al., 2000; Tiihonen et al., 1997; van der Wee et al., 2008), which are possibly related to striatal dysfunction (Sareen et al., 2007a). In addition, two recent PET studies indicated enhanced serotonin synthesis capacity in the striatum (Frick et al., 2015; Furmark et al., 2016). Given the role of serotonin in neuroplasticity and brain circuit development (Lesch & Waider, 2012), concomitant brain structure alterations may be expected in this region. Combined with these previous findings, our results support the idea stated before (Brühl, Delsignore, et al., 2014; Gentili et al., 2016), that SAD-related changes in brain function and structure may be found outside the traditional fear circuitry, consisting of the amygdala, insula, prefrontal cortex and anterior cingulate cortex (Etkin & Wager, 2007).

Notwithstanding the results of the present study, it should be noted that, despite the use of the largest database of structural MRI scans of SAD patients available to date, the effect sizes obtained in our study were small (see *Figure 4.3* for an illustration of the relationship between effect size and the power to detect an effect, given the sample size of our study). However, small effect sizes are not uncommon for studies on structural brain abnormalities in mental disorders (Ioannidis, 2011); we refer the reader to the recent viewpoint articles by Blackford (2017) and Reddan and colleagues (Reddan, Lindquist, & Wager, 2017) for important insights on improving the validity and reproducibility of neuroimaging studies in psychiatry. Furthermore, because of the hypothesis-driven ROI approach, we did not correct the *p*-value for the number of ROIs tested. In addition, it should be mentioned that the GM increase was present in a region with a low GM density (mean GM volume ± SD in significant cluster: SAD patients: 0.12 ± 0.05; HC participants 0.10 ± 0.05; see also *Figure 4.2B*). Together with the fact that it is hard to link neuroimaging results showing changes in brain structure directly to underlying cellular and molecular mechanisms like synaptogenesis, neurogenesis and changes in neuronal morphology (Lerch et al., 2017; Zatorre, Fields, & Johansen-Berg, 2012), this finding underscores that more research is needed to understand how the macroscopic SAD-related GM increase relates to effects at the microscopic level. It is also unclear, given the correlational nature of this study, whether and how structural differences in the dorsal striatum might play a causal or compensatory role in the pathogenesis of SAD. This underscores the need for future longitudinal studies on SAD, as well as for experiments that incorporate the dorsal striatum in animal models of social anxiety (compare (Fox & Kalin, 2014)).

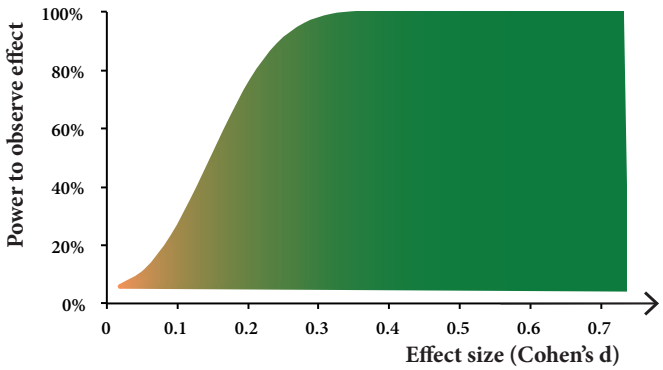


**Figure 4.3 Illustration of relation between effect size and power to detect effect.**
Power to observe an effect as a function of the real effect size given the current sample size ($n$ = 174 SAD patients and $n$ = 213 HC participants), calculated using *https://www.ai-therapy.com/psychology-statistics/power-calculator*.

## Study limitations and future studies

The present study has several limitations. First, data on medication use and comorbidity were not available for all participants (*Table 4.3*). Furthermore, only the current use of medication and present comorbidity were known, so we could not exclude heterogeneity within the sample due to past medication use or past comorbidity. Another possible source of heterogeneity within the sample arises from the fact that we pooled data from multiple research centers located in various countries, which could add confounding effects of, for example, ethnicity and differences in scanner settings. However, we do not believe that these potential confounds have substantially influenced our results, as we corrected for scan center within our statistical model and since we did not find any diagnosis-by-scan center effects.

In the present study, we have exclusively investigated SAD-related differences in GM volumes. Future studies on structural brain alterations should examine changes in other parameters of brain anatomy, like cortical thickness, white matter integrity, and the shape of brain structures. The latter is especially interesting, given the recent insight that the shape of the putamen exhibits moderate-to-high heritability (Ge et al., 2016; Roshchupkin et al., 2016). This, together with the understanding that SAD is familial and moderately heritable (Isomura et al., 2015; Middeldorp et al., 2005; Scaini et al., 2014; Torvik et al., 2016), raises the question whether putamen shape could be considered a candidate endophenotype of SAD (compare (Bas-Hoogendam et al., 2016)) and it will be interesting to investigate this in future studies. In addition, it would be worthwhile to perform multivariate pattern analyses (Adluru et al., 2013; Pereira, Mitchell, & Botvinick, 2009) to examine whether it is possible to discriminate SAD patients from HC participants based on GM volumes – see for example (Frick, Gingnell, et al., 2014). Together with ongoing work on the functional brain altera-

tions, as well as with the results of PET studies on brain metabolism in SAD, these findings may aid in unraveling the neurobiological basis of this serious and disabling disorder.

## Conclusions

In summary, the results of the present mega-analysis of the largest database of SAD brain scans to date showed larger GM volume in the dorsal striatum in SAD, which correlated positively with the severity of self-reported social anxiety symptoms. Combined with previous work on inhibited temperament and imaging studies on SAD, our results suggest that the dorsal striatum may play a role in the biased processing of social stimuli that is characteristic of SAD psychopathology. Importantly, we could not replicate GM alterations in the amygdala, hippocampus, prefrontal cortex and precuneus, regions previously implicated in SAD in imaging studies with smaller sample sizes. We take these null findings as an indication that large sample sizes and investigations such as the meta-analyses performed by the ENIGMA Consortium are necessary for the reliable detection of neuro-anatomical changes in SAD.

# Supplemental Methods

Details on in- and exclusion criteria for each site/study, as described in original research protocols and/or publications.

## Germany

*Full name of research centers* Biological Psychology, University of Jena, Germany; Institute of Medical Psychology and Systems Neurosciences, University of Münster, Germany.

*Data published in* Boehme, Ritter, et al. (2014, 2015); Boehme, Mohr, et al. (2014).

*Exclusion criteria both groups* General fMRI contraindications (e.g. ferromagnetic implants, pregnancy, claustrophobia, …).

*Inclusion criteria SAD* Primary diagnosis of SAD – determined by extensive semi-structured interviews with individual subjects. For SAD, diagnoses were confirmed (and comorbidities were assessed) by clinical psychologists administering the Structured Clinical Interview for DSM-IV Axis I and II disorders (SCID I and II; (Fydrich, Renneberg, Schmitz, & Wittchen, 1997; Wittchen, 1997).

*Exclusion criteria SAD* (i) a diagnosis of obsessive–compulsive disorder, current alcohol or substance abuse, any psychotic disorder or dementia and current primary or secondary major depression; (ii) a history of seizures or head injury with loss of consciousness; (iii) a severe uncontrollable medical condition; or (iv) the use of any psychotropic medication within the preceding 6 months.

*Inclusion criteria HC* Healthy adults, age-, gender-, and education-matched to patients.

*Exclusion criteria HC* Presence of any psychopathology.

*Recruitment of participants* Both groups were recruited via public announcement (flyers distributed at university, in the community, and by online advertisement on department website).

## The Netherlands - The Netherlands Study of Depression and Anxiety (NESDA)

*Full name of research centers* Leiden University Medical Center, Leiden, the Netherlands; VU University Medical Center, Amsterdam, the Netherlands; University Medical Center Groningen, Groningen, the Netherlands.

*Design of NESDA published in* Penninx et al. (2008); *data published in* Pannekoek et al. (2013, 2015; van Tol et al. (2010).

*Exclusion criteria both groups* Presence or history of major internal or neurological disorder, dependence on or recent abuse (past year) of alcohol and/or drugs, hypertension, and general magnetic resonance imaging contraindications.

*Inclusion criteria SAD* Half-year diagnosis of SAD, established using the structured Composite International Diagnostic Interview (lifetime version 2.1) given by a trained interviewer (Robins et al., 1988).

*Exclusion criteria SAD* Known personality disorders; presence of axis-I disorders other than MDD, PD, SAD, or GAD and any use of psychotropic medication other than stable use of SSRIs or infrequent benzodiazepine use (ie, equivalent to 2 doses of 10 mg of oxazepam 3 times per week or use within 48 hours prior to scanning).

*Inclusion criteria HC* Controls were currently free of, and had never met criteria for, depressive or anxiety disorders or any other axis-I disorder and were not taking any psychotropic drugs.

*Exclusion criteria HC* Lifetime DSM-IV diagnosis, established using the structured Composite International Diagnostic Interview (lifetime version 2.1) given by a trained interviewer (Robins et al., 1988).

*Recruitment of participants* (cited from (Penninx et al., 2008)): "NESDA has been designed to be representative of those with depressive and anxiety disorders in different health care settings and stages of the developmental history. Therefore, the sample is stratified for setting (community, primary care and specialized mental health) and set up to include a range of psychopathology: those with no symptoms or disorders ('controls'), those with earlier episodes or at risk because of subthreshold symptoms or family history, and those with a current first or recurrent depressive or anxiety disorder. The focus is on Dysthymia, Major Depressive Disorder, General Anxiety Disorder, Panic Disorder, Social Phobia and Agoraphobia. A general inclusion criterion was an age of 18 through 65 years."

"In order to maintain representativity, only two exclusion criteria existed: (1) a primary clinical diagnosis of a psychiatric disorder not subject of NESDA which will largely affect course trajectory: psychotic disorder, obsessive-compulsive disorder, bipolar disorder, or severe addiction disorder, and (2) not being fluent in Dutch since language problems would harm the validity and reliability of collected data."

"The NESDA community sample builds on two cohorts that were already available through prior studies. The first cohort is from the Netherlands Mental Health Survey and Incidence Study (NEMESIS), a community-based study described in detail elsewhere (Bijl et al., 1998)."

"The second cohort exists of participants of the Adolescents at Risk for Anxiety and Depression (ARIADNE) study (Landman-Peeters et al., 2005), a prospective cohort study among 528 biological children (aged 13– 25 years) of parents who were treated for depressive or anxiety disorder as outpatient at a mental health organization."

"Recruitment from primary care practices: Primary care patients were recruited from 65 general practitioners (GPs) in the vicinity of the field sites (Amsterdam, Groningen, Leiden). In selecting these GPs, attention was paid to the use of an appropriate electronic patient record databases which allows uniform data extraction for research purposes."

"Recruitment from mental health organizations: The specialized mental health patients were recruited from outpatient clinics of regional facilities for mental health care around the three research sites."

## The Netherlands - Social Anxiety Study

*Full name of research center* Leiden University Medical Center, Leiden, the Netherlands.

*Data published in* Cremers et al. (2014, 2015).

*Inclusion criteria SAD* SAD participants had to meet criteria for general SAD according to DSM-IV as a primary diagnosis (1994) based on the Mini-International Neuropsychiatric Interview (MINI; (Sheehan et al., 1997)).

*Exclusion criteria SAD* Other co-morbid anxiety, psychotic or substance abuse disorders.

*Inclusion criteria HC* Matched to SAD with respect to age, gender and years of education; no history of psychiatric diseases or psychotropic medication use.

*Recruitment of participants* SAD participants were recruited through an advertisement ($n$ = 7), local participating treatment centers ($n$ = 8) and, social anxiety web forums ($n$ = 5).

## South Africa

*Full name of research centers* University of Cape Town, Cape Town, South Africa; US/UCT MRC Unit on Anxiety & Stress Disorders, Department of Psychiatry, University of Stellenbosch, Cape Town, South Africa.

*Data published in* Geiger et al.(2016) ; Hattingh et al. (2013); Howells et al. (2015); Syal et al. (2012).

*Inclusion criteria SAD* Primary diagnosis of SAD, established by SCID-I; SCID-OCSD done by clinical psychologist / psychiatrist; right-handed.

*Exclusion criteria SAD* Clinically significant comorbidity; Psychotropic medication; Psychotic disorder.

*Inclusion criteria HC* No psychiatric disorders; Righthanded.

*Exclusion criteria HC* Medication use. Psychotic disorder.

*Recruitment of participants* Advertisements in media; radio talks; Letters to clinicians. Consumer advocacy groups (e.g. SADAG).



## Sweden

*Full name of research center* Uppsala University, Department of Psychology, Uppsala, Sweden

*Data published in* Frick, Engman, et al. (2014).

*Inclusion criteria SAD* Social anxiety disorder (social phobia), according to DSM-IV, must be the main diagnosis as assessed with the structured clinical interview for DSM disorders. Otherwise somatically healthy; age 18 or older but not postmenopausal; willingness to participate in a symptom provocation brain imaging trial.

*Exclusion criteria SAD* Treatment of social anxiety within the three months preceding the study; Current serious or dominant psychiatric disorder other than social anxiety disorder (e.g., psychosis, major depressive disorder, bipolar disorder); Suicidal ideation; Chronic use of prescribed medication that could influence the results; Abuse of alcohol or narcotics; Pregnancy or planned pregnancy during the study period; Menopause; Previous PET examination; Contra-indications for MRI investigations (e.g. implants or other metal objects in the body, brain and heart operations).

*Inclusion criteria HC* Somatically healthy; Age 18 or older but not postmenopausal; Willingness to participate in a symptom provocation brain imaging trial.

*Exclusion criteria HC* History of or current psychiatric disorder. To exclude the presence of psychopathology, Mini International Neuropsychiatric Interview was administered by psychology grad students, (during their final phase of education) trained in the administration of the interviews and under supervision. Suicidal ideation; Chronic use of prescribed medication that could influence the results; Abuse of alcohol or narcotics; Pregnancy or planned pregnancy during the study period; Menopause; Previous PET examination; Contrainidications for MRI investigations (e.g. implants or other metal objects in the body, brain and heart operations).

*Recruitment of participants* SAD patients were recruited through newspaper advertisements and volunteered to participate by signing up at a dedicated website. HC participants were recruited from public bulletin boards at Uppsala University.

## United States of America - University of Michigan, University of Chicago

*Full name of research centers* UIC: University of Illinois at Chicago Mood and Anxiety Disorders Research Program; UofM: University of Michigan; UofC: University of Chicago Brain Imaging and Emotions Laboratory.

*Data published in* Phan et al. (2013).

*Inclusion criteria both groups* Able to give informed consent; Physically healthy; Age 18-55.

*Exclusion criteria both groups* Clinically significant medical or neurologic condition; Life history of bipolar disorder, schizophrenia, presence of an organic mental syndrome, mental retardation, or pervasive developmental disorder; Positive drug screen results; Pregnant or lactating; Left-handed; Presence of ferrous-containing metal in the body; Inability to tolerate small, enclosed spaces; Unwilling/unable to sign informed consent.

*Inclusion criteria SAD* Current social anxiety disorder, generalized subtype; Master's level clinician determined diagnoses by SCID for DSM-IV; LSAS > 60 at screening visit.

*Exclusion criteria SAD* Primary comorbid anxiety disorder; Current Major Depressive Disorder or Major Depression within the past 6 months; HAM-D > 18; Current alcohol/drug abuse or dependence or within the past year; Current suicidal ideation; Diagnosis of any of the following Axis II personality disorders: paranoid, schizoid, schizotypal, antisocial, borderline, histrionic, narcissistic. Concomitant treatments with psychotropic/psychoactive medications within the last 2 weeks (8 weeks for fluoxetine, 4 weeks for MAOIs) before screening, including beta-adrenergic blockers, SSRIs, benzodiazepines, tricyclic/mono-amine oxidase inhibitor antidepressants, lithium, antiepileptic/anticonvulsants, neuroleptic/antipsychotics; Clinically significant medical condition which interferes with the metabolism of sertraline (e.g. severe hepatic or renal insufficiency); Ongoing psychotherapy treatment; History of known or suspected hypersensitivity to sertraline or another SSRI; Prior failure of response to sertraline or another SSRI for social anxiety.

*Inclusion criteria HC* Free of a lifetime diagnosis of any Axis I or Axis II disorder

*Recruitment of participants* Community, internet, clinic.

## United States of America - University of Illinois

*Full name of research centers* University of Illinois at Chicago Mood and Anxiety Disorders Research Program.

*Data published in* Klumpp et al. (2015).

*Inclusion criteria both groups* Age 18-55; Subject is able to give informed consent; Physically healthy.

*Exclusion criteria both groups* Clinically significant and active medical or neurological condition; Primary comorbid anxiety disorder (defined by which disorder was the more debilitating and clinically salient than SAD or MDD); Life history of bipolar disorder, schizophrenia, or presence of an organic mental syndrome, mental retardation, or pervasive developmental disorder; Life history of or current psychotic symptoms; Current alcohol/drug abuse or dependence or in the past 6 months based on the SCI; Current suicidal/homicidal ideation (i.e., an active suicidal plan or history of serious suicide attempt in the last six months); Evidence of chronic self-injurious behavior in the past six months (i.e., cutting, burning, etc.) as determined by self-report and the Primary Investigator; Prior treatment of a clinical dose of cognitive behavioral therapy as determined by the Primary Investigator; Ongoing active psychotherapy (e.g. CBT) treatment of any kind; Current treatment with any psychotropic medication (anti-depressants, anti-obsessionals, anxiolytics, anti-psychotics, mood stabilizers); prior

treatment with a psychotropic medication is not an exclusion criteria as long as the treatment was discontinued at least 2 weeks prior to study entry (4 weeks if potential participant was taking fluoxetine); Presence of ferrous-containing metal in the body; Inability to tolerate small, enclosed spaces.

*Inclusion criteria SAD* Current SAD based on SCID diagnosis; Master's level clinician determined diagnoses by SCID for DSM-IV; LSAS score of $\geq 55$ for SAD.

*Inclusion criteria HC* Free of a lifetime diagnosis of Axis I or Axis II disorder.

*Recruitment of participants* Community, internet, clinic.

# Supplemental Tables

**Supplemental Table S4.1 Scans excluded based on comorbidity other than anxiety and/or MDD.**

| Exclusion comorbidity | Number of scans |
|---|---|
| SAD + OCD | 3 |
| SAD + OCD + panic disorder | 1 |
| SAD + personality disorder | 8 |
| SAD + dysthymia | 5 |
| SAD + dysthymia + personality disorder | 3 |
| SAD + dysthymia + personality disorder + panic disorder | 1 |
| SAD + dysthymia + personality disorder + OCD | 1 |
| SAD + MDD + alcohol dependency | 1 |
| SAD + MDD + personality disorder | 8 |
| SAD + MDD + alcohol dependency + personality disorder | 2 |
| SAD + MDD + eating disorder + personality disorder | 1 |
| SAD + MDD + panic disorder + personality disorder | 1 |
| SAD + self-injury disorder | 1 |
| SAD + cannabis dependency | 2 |
| SAD + dissociative disorder NOS | 1 |
| SAD + sexual arousal disorder | 1 |
| SAD + panic disorder + eating disorder | 1 |
| SAD + somatoform disorder + intermittent explosive disorder | 1 |
| **Total** | 42 |

**Abbreviations**
MDD: major depressive disorder; NOS: not otherwise specified; OCD: obsessive-compulsive disorder.

**Supplemental Table S4.2 Overview results multiple regression analyses individual regions.**

| | | Mean GM[a] | | Effect of diagnosis | | Interaction diagnosis x gender | |
|---|---|---|---|---|---|---|---|
| **Original ROI** | **Individual regions** | **SAD** | **HC** | **β** | ***p* value uncorrected[b]** | **β** | ***p* value uncorrected[b]** |
| Prefrontal cortex | Anterior cingulate gyrus | 0.585 | 0.591 | -0.040 | 0.417 | -0.075 | 0.098 |
| | Frontal medial cortex | 0.566 | 0.584 | -1.020 | 0.015 | 0.012 | 0.763 |
| | Frontal orbital cortex | 0.580 | 0.578 | 0.023 | 0.614 | 0.013 | 0.750 |
| | Middle frontal gyrus | 0.529 | 0.529 | 0.006 | 0.905 | -0.082 | 0.065 |
| | Paracingulate gyrus | 0.614 | 0.632 | -0.102 | 0.029 | -0.016 | 0.710 |
| | Subcallosal cortex | 0.591 | 0.587 | 0.021 | 0.583 | 2.053 | 0.041 |
| Amygdala-hippocampus | Amygdala | 0.649 | 0.639 | 0.033 | 0.318 | 0.330 | 0.275 |
| | Hippocampus | 0.613 | 0.605 | 0.039 | 0.258 | 0.033 | 0.302 |
| | Anterior parahippocampal gyrus | 0.661 | 0.663 | -0.013 | 0.774 | 0.062 | 0.159 |
| | Posterior parahippocampal gyrus | 0.506 | 0.510 | -0.030 | 0.537 | 0.120 | 0.008 |
| Parietal | Precuneus | 0.576 | 0.586 | -0.050 | 0.245 | -0.028 | 0.486 |
| | Superior parietal lobule | 0.473 | 0.481 | -0.050 | 0.304 | 0.010 | 0.832 |
| | Posterior cingulate gyrus | 0.573 | 0.585 | -0.072 | 0.130 | 0.010 | 0.823 |

**Footnotes**

[a] Estimated marginal mean;

[b]: None of these effects survived correction for multiple comparisons (13 regions, Bonferroni-corrected *p*-value: $p \leq 0.004$).

**Supplemental Table S4.3 Coordinates of findings summarized in *Discussion*.**

| | | Coordinates peak voxel | | |
|---|---|---|---|---|
| **Publication** | **Region** | **x** | **y** | **z** |
| Clauss et al., 2014 | Left caudate | -19 | 6 | 17 |
| Clauss et al., 2015 | Left caudate | -14 | 10 | 18 |
| | Right amygdala, parahippocampal gyrus, globus pallidus, putamen | 22 | -6 | -16 |
| Cremers et al., 2015 | Left putamen | -20 | 12 | 4 |
| Heitmann et al., 2016 | Left globus pallidus / putamen | -22 | -1 | 2 |
| Arnold Anteraper et al., 2014 | Caudate seed | *Seeds for functional connectivity analysis defined according to the Wake Forest University Pickatlas* | | |
| | Left and right putamen seeds | | | |
| | Globus pallidus seeds | | | |
| Gentili et al., 2016 | Right globus pallidus | 18 | -2 | -8 |
| Hattingh et al., 2013 | Right globus pallidus | 20 | -2 | -8 |
| Talati et al., 2015 | Right caudate, putamen | 21 | 14 | -3 |
| | Left caudate, putamen | -12 | 14 | -5 |
| Howells et al., 2015 | Left caudate | *Not applicable (MRS voxels)* | | |
| | Right putamen | | | |
| Schneier et al., 2000 | Striatum | *Not further specified* | | |
| Tiihonen et al., 1997 | Striatum | *Not further specified* | | |
| Van der Wee et al., 2008 | Striatum – right putamen (post-hoc) | *Manually drawn VOIs for SPECT analyses* | | |
| Frick et al., 2015 | Caudate nucleus | *ROIs defined according to the Wake Forest University Pickatlas* | | |
| | Putamen | | | |
| Furmark et al., 2016 | Globus pallidus | 24 | 2 | 0 |
| | Putamen | -26 | -14 | 18 |

**Abbreviations**

MRS: magnetic resonance spectroscopy; ROI: region of interest; VOI: volume of interest.