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

Bas, J.M.

Chapter 8

Altered neurobiological processing of unintentional social norm violations: a multiplex, multigenerational fMRI study on social anxiety endophenotypes

Accepted for publication as:

Bas-Hoogendam, J. M., van Steenbergen, H., Tissier, R. L. M., van der Wee, N. J. A., & Westenberg, P. M. (2019). Altered neurobiological processing of unintentional social norm violations: a multiplex, multigenerational fMRI study on social anxiety endophenotypes. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, in press, available online.

Abstract

Background

Patients with social anxiety disorder (SAD) fear negative evaluation in social situations. Specifically, previous work indicated that social anxiety is associated with increased medial prefrontal cortex (mPFC) activation in response to unintentional social norm (SN) transgressions, accompanied by increased embarrassment ratings for such SN violations. Here, we used data from the multiplex, multigenerational Leiden Family Lab study on SAD, which involved two generations of families genetically enriched for SAD, and investigated whether these neurobiological and behavioral correlates of unintentional SN processing are SAD endophenotypes. Of four endophenotype criteria, we examined two: the *co-segregation of these characteristics with social anxiety (SA) within families of SAD-probands* and the *heritability* of the candidate endophenotypes.

Methods

Participants (n = 110, age-range 9.0 - 61.5 years, eight families) performed the revised Social Norm Processing Task; functional magnetic resonance imaging (fMRI) data and behavioral ratings related to this paradigm were used to examine whether brain activation in response to processing unintentional SN violations and ratings of embarrassment were associated with SA-levels. Next, heritability of these measurements was estimated.

Results

As expected, voxelwise fMRI analyses revealed positive associations between SA-levels and brain activation in the mPFC and a cluster encompassing the medial temporal gyrus, superior temporal gyrus and superior temporal sulcus, and these brain activation levels displayed moderate to moderately-high heritability. Furthermore, although SA-levels correlated positively with behavioral ratings of embarrassment for SN transgressions, these behavioral characteristics were not heritable.

Conclusions

These results show, for the first time, that brain responses in the mPFC and medial temporal gyrus, superior temporal gyrus and superior temporal sulcus, related to processing unintentional SN violations, provide a neurobiological candidate endophenotype of SAD.

Introduction

Social anxiety disorder (SAD), a prevalent anxiety disorder, is characterized by an onset during early adolescence, a chronic course and a high risk of comorbid psychopathology (Beesdo-Baum et al., 2015; Blanco et al., 2011; Haller et al., 2015; Kessler et al., 2012; Merikangas et al., 2010; Stein et al., 2017). Furthermore, treatment for SAD is at present often suboptimal (Weisberg, Beard, Moitra, Dyck, & Keller, 2014). Thereby, this psychiatric condition has a large negative impact on the patients' lives (McKnight, Monfort, Kashdan, Blalock, & Calton, 2016; Wittchen et al., 2000) as well as on society (Baxter, Vos, Scott, Ferrari, & Whiteford, 2014). It is therefore essential to gain a better understanding of the vulnerability to develop SAD, in order to improve preventive and therapeutic interventions (Marín, 2016).

A defining feature of SAD-psychopathology is the fear to act in a way that will be embarrassing and humiliating (American Psychiatric Association, 2013). More specifically, it has been postulated that an important fear of SAD patients concerns that they will 'unintentionally generate an embarrassing behavioral blunder in a social situation' (Moscovitch, 2009). The neurobiological and behavioral correlates of this fear of negative evaluation, which is out of proportion to the context and actual threat (Heimberg et al., 2014), can be assessed using the Social Norm Processing Task (SNPT) (Berthoz et al., 2002). In this paradigm, participants read and evaluate three types of stories: stories describing unintentional social norm (SN) violations, stories on intentional SN violations and stories on neutral social situations. This enables examining the effect of intention on processing SN transgressions.

Two previous studies have used the SNPT to investigate SN processing related to social anxiety (SA), indicating that socially-anxious people show increased sensitivity to unintentional SN violations. The first study, an imaging study comparing 16 SAD patients with 16 healthy participants (Blair et al., 2010), revealed increased activation related to unintentional SN violations in the medial prefrontal cortex (mPFC) in SAD patients. Furthermore, patients rated all stories as more inappropriate and more embarrassing, with the most prominent effect for the unintentional SN violations, which SAD patients considered significantly more embarrassing than control subjects did (Blair et al., 2010). This effect of SA on the embarrassment ratings for unintentional SN violations was recently replicated in a community sample (Bas-Hoogendam, van Steenbergen, van der Wee, et al., 2018). Using a revised version of the SNPT (SNPT-R), which enabled investigating SN processing in children, adolescents and adults (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a), we reproduced the general effect of SA on ratings of inappropriateness and embarrassment, and the specific effect of SA on embarrassment ratings for unintentional SN violations: while participants with low-to-intermediate SA-levels rated unintentional SN transgressions as less embarrassing compared to intentional SN transgressions, participants with higher SA-levels rated the unintentional SN violations as equally embarrassing as the

intentional SN violations (Bas-Hoogendam, van Steenbergen, van der Wee, et al., 2018). This distinct experience of embarrassment is a critical factor underlying the development and maintenance of SA, as it could lead to negative self-evaluations and to the increased concerns about the judgments of others which are typical to SAD (Stein, 2015; Wong & Rapee, 2016).

These results suggest that aberrant processing of unintentional SN transgressions, at both the neurobiological and behavioral level, reflects an important component of the SAD phenotype. No study has, however, investigated whether these correlates of SN processing are potential endophenotypes of SAD. Endophenotypes are measurable and heritable traits located on the causal pathway from genotype to phenotype and reflect genetically-based disease mechanisms (Gottesman & Gould, 2003); this definition distinguishes endophenotypes from 'biomarkers', which do not necessarily have a genetic basis, and from the 'intermediate/extended phenotype concept', which is usually used to describe a subclinical form of a serious psychiatric disorder (Lenzenweger, 2013a). As described in more detail elsewhere (Bas-Hoogendam et al., 2016; Miller & Rockstroh, 2013; Puls & Gallinat, 2008), endophenotypes could advance our insight in the pathways leading to serious psychopathology, have potential to identify individuals at risk and can be valuable for improvement of therapeutic interventions. An endophenotype is supposed to be *associated with the disor*der (criterion 1), *state-independent and already present in a preclinical state* (criterion 2), and *heritabl*e (criterion 3). Furthermore, an endophenotype should *co-segregate with the disorder within families of probands, with non-affected family members showing altered levels of the endophenotype in comparison to the general population* (criterion 4) (Glahn et al., 2007; Lenzenweger, 2013a; Puls & Gallinat, 2008). Given that twin- and family studies suggest that genetic factors are involved in the pathogenesis of SAD, by reporting heritability estimates for SAD between 39 - 56% (Bandelow et al., 2016; Isomura et al., 2015; Scaini et al., 2014) as well as a significantly increased risk to develop the disorder in first-degree relatives of SAD patients (Merikangas, Lieb, Wittchen, & Avenevoli, 2003; Stein, Chartier, Hazen, et al., 1998), exploring whether the neurobiological and behavioral correlates of SN processing are candidate endophenotypes will provide more insight into the genetic vulnerability to this impairing disorder (Bas-Hoogendam et al., 2016).

Here, we tested the hypothesis that brain activation related to processing unintentional SN violations, as well as behavioral ratings related to such SN transgressions, are candidate SAD endophenotypes (pre-registration of hypotheses publicly available at osf.io/y5m8q (Bas-Hoogendam et al., 2014c)). We used data from the Leiden Family Lab study on Social Anxiety Disorder (LFLSAD), a unique multiplex, multigenerational family study (Bas-Hoogendam, Harrewijn, et al., 2018). This design is especially suitable to investigate candidate endophenotypes of SAD, as it allows for testing two endophenotype criteria in the same sample: *co-segregation of the candidate endophenotype with social anxiety within families of probands* and the *heritability* of the candidate endophenotype. Based on pre-

vious findings (Bas-Hoogendam, van Steenbergen, van der Wee, et al., 2018; Blair et al., 2010), we predicted a positive correlation with brain activation in the mPFC, specifically related to processing unintentional SN violations; furthermore, we hypothesized to find a positive association between SA-levels and embarrassment ratings on the unintentional SN violations. Next, as genetic influences on brain activation (Blokland et al., 2012; Mattay & Goldberg, 2004; Shan et al., 2016), as well as on personality, temperamental and emotional traits (Nivard et al., 2014; Sallis, Davey Smith, & Munafò, 2018; Stein, Jang, et al., 2002) have been demonstrated, we expected these candidate endophenotypes to be at least moderately ($h^2 \geq 0.20$) heritable.

Methods and materials

Participants

Participants were part of the LFLSAD, including two generations of families genetically enriched for SAD (total sample: $n = 132$, nine families; MRI sample: $n = 110$, eight families; we refer the reader to the *Supplemental Methods* for details about ethics, recruitment and exclusion criteria, as well as an a priori power calculation). More information with respect to the background, aims and methodology of the LFLSAD is provided elsewhere (Bas-Hoogendam, Harrewijn, et al., 2018); a pre-registration is available online (Bas-Hoogendam et al., 2014b). The sample consists of nuclear families who were invited for participation based on the combination of parent with a primary diagnosis of SAD (age 25 - 55 years; 'proband') and a child who met criteria for (sub)clinical SAD (age 8 - 21 years; 'proband's SA-child'). In addition to these two SAD cases, the proband's partner and other children from this nuclear family (age ≥ 8 years), as well as the proband's sibling(s), with their partners and children (age ≥ 8 years), were invited. Thereby, the LFLSAD sample consists of family members of two generations (*Figure 3.1*). Participants took part in several measurements, including a diagnostic interview, self-report questionnaires and an MRI scan (Bas-Hoogendam, Harrewijn, et al., 2018). The LFLSAD was approved by the Medical Ethical Committee of the Leiden University Medical Center and all participants provided informed consent.

Phenotyping

Family members participated in various measurements in order to enable extensive phenotyping (Bas-Hoogendam, Harrewijn, et al., 2018). Here, we focus on the measures of SA (see *Supplemental Methods* and *Supplemental Results* for an extended characterization of the LFLSAD sample). The presence of DSM-IV diagnoses was determined using the Mini-International Neuropsychiatric Interview (M.I.N.I.)-Plus (v5.0.0) (Sheehan et al., 1998; van Vliet & de Beurs, 2007) or the M.I.N.I.-Kid interview (v6.0) (Bauhuis et al., 2013; Sheehan

et al., 2010). Clinical SAD was established using the DSM-IV-TR criteria for the generalized subtype of SAD, but a clinician verified whether the DSM-5 criteria for SAD were also met. A diagnosis of subclinical SAD was established when participants met the DSM-5 criteria for SAD, but did not show impairing limitations in important areas of functioning (American Psychiatric Association, 2013). Furthermore, participants completed age-appropriate questionnaires on the level of SA: the Liebowitz Social Anxiety Scale (Fresco et al., 2001) or the Social Anxiety Scale for Adolescents (La Greca & Lopez, 1998). *Z*-scores were computed (Bas-Hoogendam, Harrewijn, et al., 2018) in order to use these scores over the whole sample,.

MRI experiment

Prior to the MRI scan, participants were informed about the safety procedures, and they were told that they could refrain from continuing the experiment at any time. Children and adolescents were familiarized with the MRI scanner using a mock scanner (Galván, 2010) and all participants received instructions about the task paradigms presented during the scan session. Scanning was performed using a 3.0 T Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands), equipped with a 32-channel Sensitivity Encoding head coil. The MRI experiment (total duration MRI protocol: 54 min 47 s) consisted of several structural scans (Bas-Hoogendam, van Steenbergen, Tissier, et al., 2018b) and functional task paradigms (Bas-Hoogendam, Harrewijn, et al., 2018), including the SNPT-R (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a). Scan parameters are reported in the *Supplemental Methods*.

Revised Social Norm processing Task (SNPT-R)

The SNPT-R (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a) is composed of a story-reading phase, taking place in the MRI scanner, and an unannounced rating phase on completion of the MRI scan (*Figure 8.1*). During the story-reading phase, short stories written in second person are presented. Stories consisted of two sentences: a stem sentence (for example: 'You are baking an apple pie with friends.') followed by an ending sentence which described either a neutral social situation ('…You use the amount of sugar the recipe calls for.'), a situation in which a social norm was unintentionally transgressed ('….You use salt instead of sugar without realizing.') or a situation in which a social norm was intentionally transgressed ('…You use salt instead of sugar as a joke.'). Stories were suitable for a broad audience and age range. However, because of the second-person form of the stories, small adaptations were made in stories describing age- or gender specific elements. Therefore, the SNPT-R has four age- and gender specific versions (boys / girls / men / women). We refer to *Supplemental Table S6.1* for all SNPT-R stories; stories are also available online at the website of the Open Science Framework (osf.io/pt4qt) (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017b).

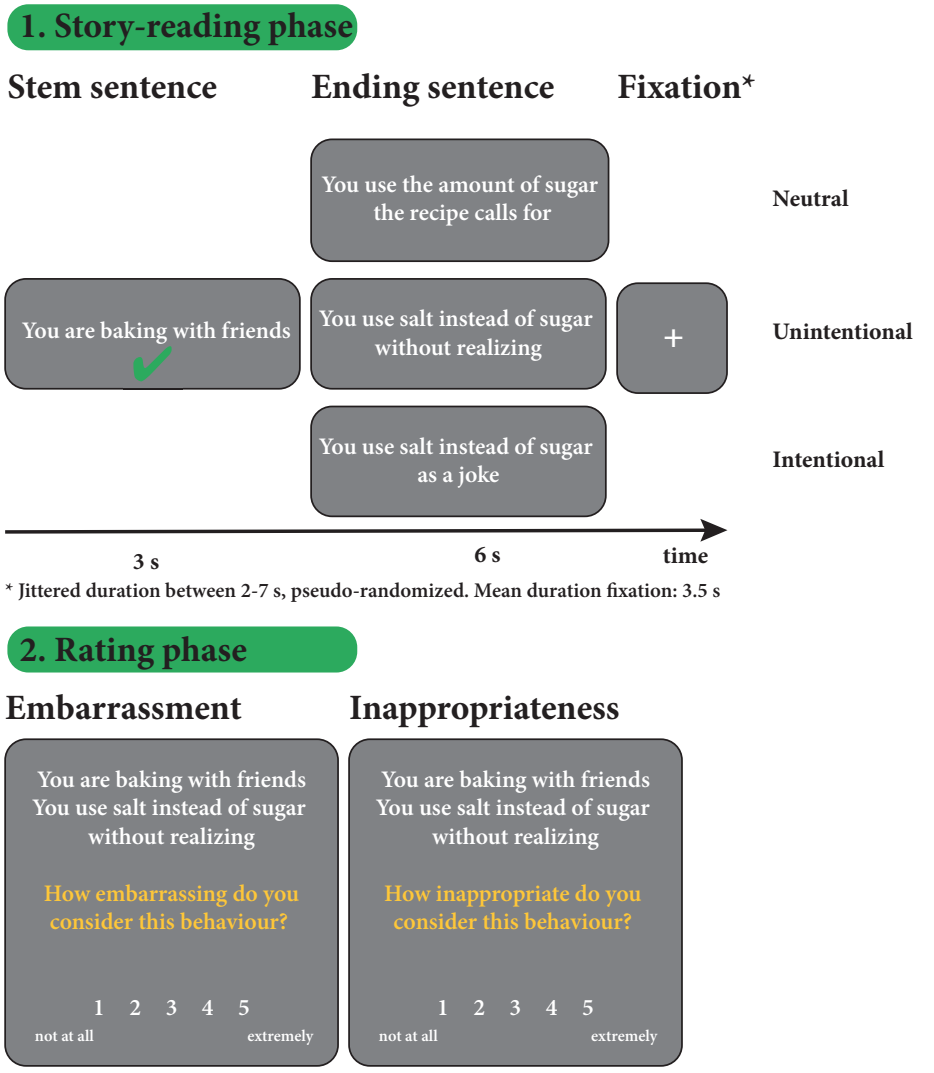


Figure 8.1 Overview of the revised Social Norm Processing Task (SNPT-R).
During the story-reading phase (1), participants read stories consisting of a stem sentence and an ending sentence, describing either a neutral social situation, a situation in which a social norm was unintentionally transgressed or situation in which a social norm was violated intentionally. Participants were instructed to imagine themselves in the situation described. In the rating phase (2), participants rated all stories on embarrassment and inappropriateness.

The SNPT-R consists of 26 stem sentences, each combined with the three different types of endings. These 78 stories were divided into two consecutive blocks of 39 stories. Participants were instructed to imagine themselves in the social situations and to press a

button after reading the stem sentence of each story, in order to verify participants' task engagement.

After the scan, participants rated all stories on a five-point Likert scale on embarrassment and inappropriateness (*Figure 8.1*). Presentation parameters are described in the *Supplemental Methods*.

Data analysis

Sample characteristics

We replaced incidental missing values on the self-report questionnaires by the average value of the completed items. Differences between participants with and without (sub)clinical SAD were examined by fitting regression models in R (R Core Team, 2016),with (sub)clinical SAD as the independent variable and the level of self-reported social anxiety (*z*-score) as dependent variable. Gender and age were included as covariates; genetic correlations between family members were modeled by including random effects.

Imaging data

General processing

Functional (f)MRI data were pre-processed using FMRIB Software Library, version 5.0.9 (Jenkinson et al., 2012); next, event-related statistical analysis was performed (details in *Supplemental Methods*). Briefly, the general linear model included four explanatory variables with their temporal derivatives, representing the presentation of a stem sentence, a neutral ending (EN), an unintentional SN violation ending (EU) and an intentional SN violation ending (EI). Three contrasts were defined: EI > EN, EU > EN and EU > EI. We verified the main effects of the SNPT-R on brain activation by using contrasts EI > EN and EU > EN (*Supplemental Results; Supplemental Table S8.3; Supplemental Figure S8.1*), while the contrast EU > EI was used for the endophenotype analysis, following previous results (Blair et al., 2010).

Neuroimaging candidate endophenotypes

The *co-segregation between SA and brain activation related to processing unintentional SN violations within the families* was investigated using regression models in R (R Core Team, 2016), with self-reported SA (*z*-score, centered) as independent variable and individual activation level related to the contrast EU > EI as dependent variable. Analyses with (sub) clinical SAD as a discrete predictor are included in the *Supplemental Methods* and *Supplemental Results*. Correlations between family members were modeled by including random effects; age (centered) and gender (centered) were included as covariates. Models were ran for each voxel separately, in order to determine the effect of SA on a whole-brain voxelwise basis. Results (*z*-scores) were transformed into a nifti-image with the same dimensions of the MNI T1-template brain. Clusters within this nifti-image, representing the association

between SA and brain activation, were corrected for multiple comparisons at the whole-brain level using the FSL tool easythresh (cluster threshold: $z > 3.1$, cluster extent threshold $p < 0.01$) (Worsley, 2001). Subsequent sensitivity analyses were performed to investigate whether the results of the association analyses were driven by the severity of depressive symptoms or by (comorbid) psychopathology other than SAD (*Supplemental Methods* and *Supplemental Results; Supplemental Tables S8.5-8.7; Supplemental Figures S8.2-8.3*).

Next, we determined the *heritability* of brain activation for voxels in the significant clusters. Voxelwise heritability estimates were obtained with a method which takes the ascertainment process into account and incorporates familial relationships (Tissier et al., 2017). Age and gender (both centered) were included as covariates.

Behavioral Data

Responses during story-reading phase

Analysis of the behavioral responses during the story-reading phase confirmed that participants paid attention to the stories (*Supplemental Results*).

Behavioral candidate endophenotypes

The *co-segregation between SA and the post-MRI SNPT-R ratings within the families* was investigated using linear mixed models in R (package: *coxme*), with self-reported SA (*z*-score, centered) as predictor of interest. Analyses with (sub)clinical SAD (discrete predictor) are described in *Supplemental Methods* and *Supplemental Results*. Separate models were used to investigate the ratings of embarrassment and inappropriateness. Task condition (intentional / unintentional / neutral), age- and gender specific task version (modeled using the dummy variables gender and age group (boys / girls vs men / women)), as well as three interaction terms (condition-by-gender, condition-by-age group, condition-by-SA-level) were added as independent variables and tested for significance. Random effects were included to account for genetic correlations between family members and within-subject correlations between task conditions. Interaction terms lacking significance were removed from the final models. Significance level was set a $p < 0.05$.

Next, we investigated whether the behavioral outcomes were *heritable*, focusing on the ratings displaying a significant association with SA. We estimated heritability by applying an approach that takes the ascertainment process into account and incorporates familial relationships, by jointly modelling the ratings and phenotype on which the family selection was based and by including random effects (Tissier et al., 2017). Age group and gender were included as possible confounders.

Results

Sample characteristics

Characteristics of the sample after quality control (n = 109 for the behavioral analyses; n = 99 for the fMRI analyses; see *Supplemental Results* for a detailed description of data availability) are summarized in *Table 8.1*. Family members with (sub)clinical SAD (n = 22 subclinical SAD; n = 17 clinical SAD) did not differ from family members without SAD (n = 62) with respect to male / female ratio and age. However, as expected, family members with (sub)clinical SAD reported higher levels of social anxiety. A detailed characterization of the sample, including clinical diagnoses other than SAD, is provided in the *Supplemental Results (Supplemental Tables S8.1-S8.2)*.

Table 8.1 Characteristics of participants with and without (sub)clinical SAD.

	Behavioral sample[a]			**fMRI sample[a]**		
	(Sub)clinical SAD (n = 39)	**No SAD (n = 62)**	**Statistical analysis**	**(Sub)clinical SAD (n = 33)**	**No SAD (n = 58)**	**Statistical analysis**
Demographics						
Male / Female (n)	20 / 19	30 /32	$\chi^2(1) = 0.08$, $p = 0.84$	16 / 17	29 / 29	$\chi^2(1) = 0.02$, $p = 1.00$
Generation 1 / Generation 2 (n)	19 / 20	27 / 35	$\chi^2(1) = 0.26$, $p = 0.68$	19 / 14	27 / 31	$\chi^2(1) = 1.02$, $p = 0.39$
Age in years (mean ± SD; range)	30.3 ± 15.5; 9.2 – 59.6	31.3 ± 15.2; 9.0 – 61.5	β (± SE) = -0.9 ± 3.1, $p = 0.76$	33.4 ± 14.9; 13.3 – 59.6	32.7 ± 14.8; 9.6 – 61.5	β (± SE) = 0.7 ± 3.2, $p = 0.83$
Diagnostic information						
Clinical SAD (n)	17	0		15	0	
Self-report measures						
Social anxiety symptoms (z-score; mean ± SD)	3.0 ± 3.3	0.6 ± 1.5	β (± SE) = 2.4 ± 0.5, $p < 0.001$	2.9 ± 3.0	0.7 ± 1.3	β (± SE) = 2.4 ± 0.4, $p < 0.001$

Abbreviations

SAD: social anxiety disorder; SD: standard deviation.

Footnote

[a]: Due to technical reasons, data on the presence of (sub)clinical SAD were lost for eight family members. Data from these participants were, however, included in the endophenotype analyses using SA-level (z-score) as a predictor (behavioral sample: n = 109; fMRI sample: n = 99).

Neuroimaging candidate endophenotypes

Whole-brain voxelwise regression analyses revealed two clusters in which self-reported SA-level was positively associated with brain activation related to the contrast EU > EI (*Table 8.2, Figure 8.2*). The first cluster (6647 voxels, $p = 1.8*10^{-7}$; corrected for multiple comparisons at the whole-brain level) was located in the occipital pole and encompassed the temporal occipital fusiform cortex, lateral occipital cortex, right superior temporal gyrus (STG), right medial temporal gyrus (MTG), superior temporal sulcus (STS) and cuneal cortex. The second cluster (1589 voxels, $p = 0.003$; corrected for multiple comparisons at the whole-brain level) comprised the frontal pole, extending to the paracingulate gyrus and mPFC. There were no clusters displaying negative relationships with SA, while visual inspection of the data confirmed the absence of outliers. Follow-up analyses confirmed the specificity of this positive association for processing unintentional SN violations, while sensitivity analyses, taking the effect of depressive symptoms and (comorbid) psychopathology other than SAD into account, further supported our results *(Supplemental Results)*.

Table 8.2 Effect of self-reported social anxiety on processing unintentional norm violations.

			Peak coordinates (MNI space)			Cluster
Cluster	**Region**	***Z*-score**	**x**	**y**	**z**	**size**
Unintentional norm violations vs intentional norm violations						
1	Temporal occipital fusiform cortex	5.45	32	-60	-18	6647
	Occipital pole	5.29	10	-94	26	
	Superior temporal gyrus, posterior division	4.31	62	-6	-8	
	Medial temporal gyrus, posterior division	3.66	60	-22	-10	
	Cuneal cortex	3.54	20	-76	32	
2	Frontal pole	5.75	-10	56	32	1589
	Frontal pole / frontal medial cortex	3.71	0	58	-4	

Subsequent voxelwise heritability analyses within the two clusters indicated that activation within the right MTG/STG/STS and mPFC, paracingulate cortex and frontal pole was heritable, with 91 voxels (cluster MTG/STG/STS) and 188 voxels (cluster mPFC) showing at least moderate ($h^2 \geq 0.20$) heritability, with some voxels displaying moderately-high ($h^2 \geq 0.40$) heritability (*Figure 8.3*).

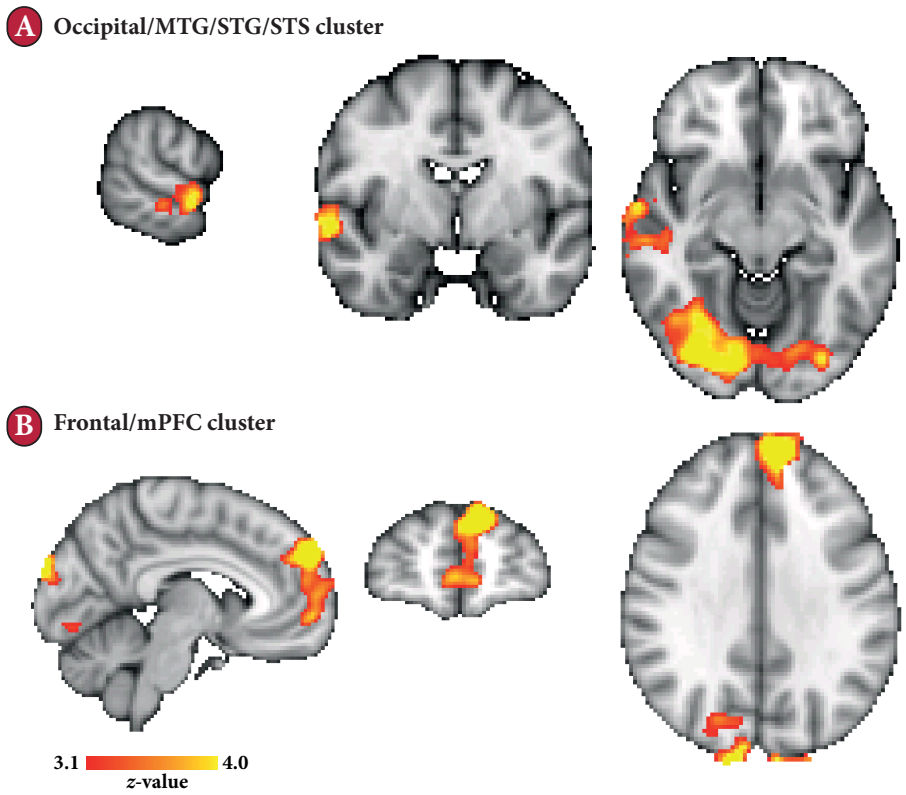


Figure 8.2 Brain activation (related to processing unintentional social norm violations) co-segregates with social anxiety within families.
Significant positive associations between social anxiety (*z*-scores) and activation related to processing stories on unintentional social norm violations versus intentional social norm violations (EU > EI). Coordinates of displayed slices (MNI, xyz): 64, -4, -10 (occipital/MTG/STG/STS cluster) and -6, 56, 32 (frontal/mPFC cluster). Clusters are displayed on the template MNI_T1_152_2mm_brain (partial brain coverage: inferior parts of the frontal medial cortex, superior parts of the postcentral gyrus as well as parts of the cerebellum are not included). Images are displayed according to radiological convention: right in the image is left in the brain.
MTG/STG/STS: medial temporal gyrus/ superior temporal gyrus/ superior temporal sulcus. mPFC: medial prefrontal cortex.

Behavioral candidate endophenotypes

Post-MRI SNPT-R ratings are summarized in *Table 8.3*; detailed results for each task version (boys / girls / men / women) are included in *Supplemental Table S8.8* and illustrated in *Supplemental Figure S8.4*. Analyses revealed significant associations between SA and embarrassment, but no relation with inappropriateness. Follow-up analyses indicated positive relationships between SA and embarrassment in all three conditions (*Figure 8.4*), while sensitivity analyses indicated that these effects were not driven by the clinical SAD cases within the sample (*Supplemental Results*).

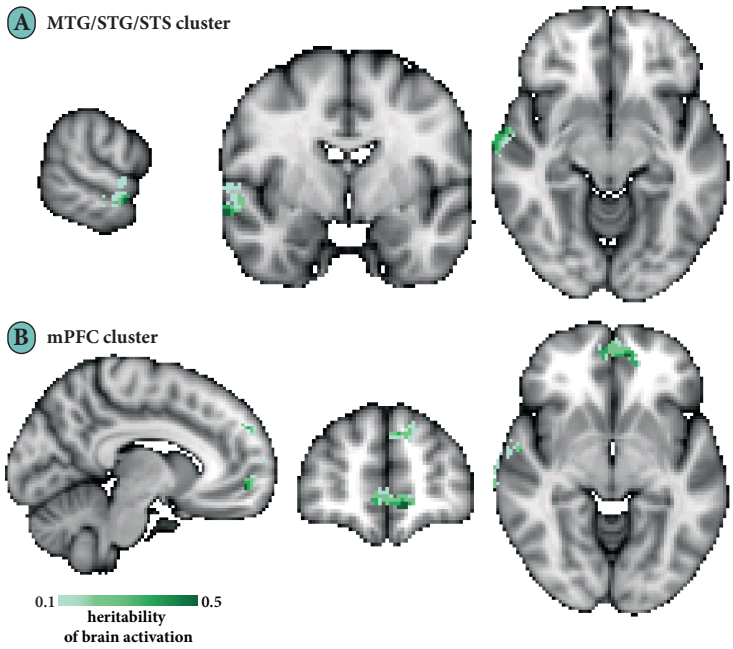


Figure 8.3 Voxelwise heritability estimates.
Coordinates of displayes slices (MNI, xyz): 64, -4, -10 (MTG/STG/STS cluster; 91 voxels with $h^2 \geq 0.20$) and -10, 52, -6 (mPFC cluster; 188 voxels with $h^2 \geq 0.20$).

Table 8.3 Ratings of inappropriateness and embarrassment.

			Effect of social anxiety (*z*-score)		Heritability
	(Sub)clinical SAD	No SAD	β ± SE	*p*	h^2
Inappropriateness			0.002 ± 0.009	0.84	
Intentional stories	4.36 ± 0.40	4.36 ± 0.43	n.i.	n.i.	n.i.
Unintentional stories	2.98 ± 0.73	2.98 ± 0.64	n.i.	n.i.	n.i.
Neutral stories	1.39 ± 0.34	1.31 ± 0.29	n.i.	n.i.	n.i
Embarrassment			0.03 ± 0.01	0.003 *	
Intentional stories	3.92 ± 0.72	3.89 ± 0.58	0.06 ± 0.02	0.010 *	0.17
Unintentional stories	3.45 ± 0.54	3.23 ± 0.51	0.06 ± 0.02	0.003 *	0.01
Neutral stories	1.38 ± 0.38	1.25 ± 0.24	0.03 ± 0.01	0.024 *	0.02

Abbreviations
h^2: heritability estimate; n.i.: not investigated; SAD: social anxiety disorder. SE: standard error.

Footnore
Values represent mean ± standard deviation.

Statistical significance
* : significant at $p < 0.05$.

Heritability analyses demonstrated that embarrassment ratings on the intentional stories had low heritability ($h^2 = 0.17$), while embarrassment scores for unintentional and neutral stories were not heritable (*Table 8.3*).

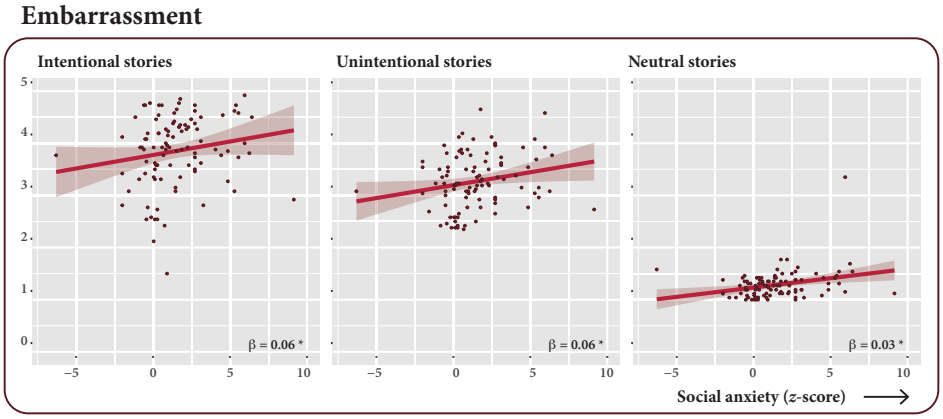


Figure 8.4 Embarrassment ratings co-segregate with social anxiety within families.
Correlation between level of self-reported social anxiety (*z*-score) and ratings of embarrassment. Shaded area represents 95% confidence interval. Asterisks indicate effects of social anxiety at $p < 0.05$.

Discussion

Here, we provide evidence that brain activation related to processing unintentional social norm (SN) violations is a neurobiological candidate endophenotype of social anxiety disorder (SAD), by using data from the Leiden Family Lab study on SAD (LFLSAD) (Bas-Hoogendam, Harrewijn, et al., 2018). This study, with its unique multiplex and multigenerational design, was especially designed to explore SAD endophenotypes (Bas-Hoogendam et al., 2014a), and our data revealed that SAD-related neurobiological alterations in processing unintentional SN violations *co-segregated with social anxiety (SA) within families of probands* ($n = 99$). Next, our data indicated that these aberrant brain activation patterns displayed moderate to moderately-high heritability, providing support for the endophenotype criterion of *heritability*. Thereby, we replicate and extend previous work on the processing of SN violations in SAD, which provided support for the endophenotype criterion of *association with the disorder,* by reporting increased brain activation in the medial prefrontal cortex (mPFC) related to processing unintentional SN violations in SAD patients (Blair et al., 2010). In addition to these neurobiological alterations, we found positive relationships between SA and ratings of embarrassment within the families, but as these behavioral measures were not heritable, our data do not provide support for these ratings as candidate endophenotypes of SAD.

Level of mPFC and MTG/STG/STS activation as a candidate SAD endophenotype

fMRI data revealed a positive relationship between SA-level within the families and brain activation in the frontal cortex, including the mPFC, in response to unintentional SN violations (versus intentional SN violations), as well as an association with activation within the occipital cortex and medial/superior temporal gyrus (MTG/STG), including the superior temporal sulcus (STS) between them (*Table 8.2, Figure 8.2*). Furthermore, activation clusters within the mPFC and MTG/STG/STS displayed moderate to moderately high heritability (maximum $h^2 = 0.47$) (*Figure 8.3*). Thereby, activation within the mPFC and MTG/STG/STS is a promising neurobiological endophenotype of SAD.

The heightened mPFC reactivity in response to unintentional SN violations confirmed our hypothesis, as this finding is in line with previous work reporting on 16 patients with generalized SAD (Blair et al., 2010). The mPFC is engaged during social cognitive processing, including self-referential processing (Amodio & Frith, 2006; Jenkins & Mitchell, 2011) and as such, the exaggerated mPFC activation during processing unintentional SN violations supports the idea that SAD patients consider these transgressions as extremely self-relevant, probably because these unintentional transgressions relate to their strong fear of unintentionally generating an embarrassing behavioral blunder in a social situation (Blair & Blair, 2012; Moscovitch, 2009). The importance of the mPFC in the neurobiological characterization of SAD is further supported by studies indicating increased mPFC activation related to self-referential statements and criticism (Blair et al., 2008; Blair, Geraci, Otero, et al., 2011), as well as in response to performance feedback (Heitmann et al., 2014); see reviews by (Brühl, Delsignore, et al., 2014; Miskovic & Schmidt, 2012).

Although not a priori hypothesized, the increased activation in the posterior STG/MTG/STS could concur with the role of this area in social cognition (Beauchamp, 2015; Deen, Koldewyn, Kanwisher, & Saxe, 2015; Schirmer, Meck, & Penney, 2016), including, but not limited to, understanding intentions from other people's actions (Frith & Frith, 2007; Pelphrey, Morris, & McCarthy, 2004; Saxe, Xiao, Kovacs, Perrett, & Kanwisher, 2004). Interestingly, recent work demonstrated that the posterior STS is involved in experiencing embarrassment with another person's mishaps (Paulus, Müller-Pinzler, Jansen, Gazzola, & Krach, 2015), while work on SAD demonstrated increased bilateral STS activation in response to emotional faces (Gentili et al., 2008; Straube et al., 2004). Furthermore, the STS is functionally connected to the amygdala (Gorka, Torrisi, Shackman, Grillon, & Ernst, 2018; Pitcher, Japee, Rauth, & Ungerleider, 2017). Based on these findings, we cautiously hypothesize that the heightened posterior temporal activation in response to unintentional SN violations could represent the increased affective value that socially-anxious people attribute to making an unintentional slip. Furthermore, as these temporal regions are involved in visual processing and visual imagery (Ganis, Thompson, & Kosslyn, 2004), enhanced activation

within these areas could also represent the increased saliency of the social situations for socially-anxious participants when they imagine themselves in the hypothetical scenarios.

Embarrassment co-segregates with SA within families

Within the LFLSAD sample, family members with higher levels of self-reported SA rated all types of stories as more embarrassing (*Figure 8.4*). These findings are in line with previous work (Bas-Hoogendam, van Steenbergen, van der Wee, et al., 2018; Blair et al., 2010), and confirm the notion that feeling embarrassed is an important characteristic of social anxiety. Our results did not, however, support the specific effect of SA on embarrassment in the unintentional condition, which was reported previously, nor did we replicate the effect of SA on the ratings of inappropriateness (Bas-Hoogendam, van Steenbergen, van der Wee, et al., 2018; Blair et al., 2010), indicating the need for future studies to unravel this complex pattern. Furthermore, heritability of these behavioral endophenotypes was low (intentional condition) or absent (unintentional and neutral condition). So, the present findings reinforce the view that increased reports of embarrassment are associated with SA, but as these embarrassment levels have heritability estimates below our predefined threshold, they do not meet criteria for being a candidate endophenotype.

Limitations and directions for future research

Although the results presented here, from a unique two-generation neuroimaging family study on SAD which was especially designed to explore two endophenotype criteria (Bas-Hoogendam, Harrewijn, et al., 2018), provide support for the *co-segregation of the candidate endophenotypes with social anxiety within families of probands* (criterion 4, first part) and the endophenotype criterion of *heritability* (criterion 3), the cross-sectional nature of the LFLSAD and the lack of control families do not allow for investigation of the *state-independency* (criterion 2) of the candidate endophenotypes, nor do the data enable assessing whether *non-affected family members show altered levels of the endophenotype in comparison to the general population* (criterion 4, second part). Future studies, employing a longitudinal design and including control families from the general population, are needed to investigate these criteria and to replicate the current findings. In addition, given the heterogeneity in the SAD phenotype (Heimberg et al., 2014), future studies could consider using individually-tailored stimuli (cf. (Simon, Kaufmann, Müsch, Kischkel, & Kathmann, 2010)). We hypothesize that such stimuli, representing social situations that are most anxiety-provoking for participants with SAD, might yield even stronger neurobiological and behavioral responses compared to those of the present study. Furthermore, given the fact that the SNPT-R has age- and gender-adjusted task versions, the present design does not allow for determining effects of age and gender on the candidate endophenotypes.

Another interesting avenue for future research would be to link the altered brain activation observed here to changes in brain structure. In a previous study on the same sample, we

found a negative correlation between SA-levels and cortical thickness of the left mPFC and bilateral STG (Bas-Hoogendam, van Steenbergen, Tissier, et al., 2018b). In addition, cortical thickness of the left mPFC and left STG displayed moderately-high and high heritability (Bas-Hoogendam, van Steenbergen, Tissier, et al., 2018b). However, due to the complexity of the present association analyses, in which we had to account for the family structure of the data, we were not able to consider the connection between brain structure and brain function on a voxelwise basis. Moreover, it should be noted that a voxel-based morphometry mega-analysis on the largest sample of SAD patients to date did not reveal gray matter differences in frontal and temporal areas (Bas-Hoogendam, van Steenbergen, Pannekoek, et al., 2017). Furthermore, the alterations in function are specific to processing unintentional SN violations, while the structural changes are independent of any task condition. Therefore, more research is needed to unravel the complex relationship between brain structure and function (Bas-Hoogendam, 2019; Lerch et al., 2017). Besides, longitudinal MRI studies (cf. (Steiger et al., 2017)) could explore the potential of cognitive behavioral therapy enriched with neurofeedback (Haller et al., 2015), to specifically target the altered brain activation patterns in the mPFC. Finally, as we have not yet considered the genetic data collected within the LFLSAD (Bas-Hoogendam, Harrewijn, et al., 2018), we are at present not able to relate the alterations in brain activation to genetic variations, which would be a next step in order to further unravel the genetic susceptibility to SAD.

Conclusions

The findings of this study provide considerable support for increased brain activation in the mPFC and MTG/STG/STS, related to the processing of unintentional SN violations, as a neurobiological candidate SAD endophenotype. Thereby, these results offer novel insights in the neurobiological pathways leading to SAD.

Supplemental Methods

Participants

Exclusion criteria

There was one important exclusion criterion in the LFLSAD, being comorbidity other than internalizing disorders or substance abuse in the proband or proband's SA-child; other family members were included independent from the presence of psychopathology. Insufficient comprehension of the Dutch language was an exclusion criteria for the whole sample, and general MRI contraindications, for example pregnancy, metal implants or dental braces, led to exclusion of the MRI experiment (Bas-Hoogendam, Harrewijn, et al., 2018).

Ethical procedure

Both parents signed the informed consent form for their children, and children between 12 and 18 years of age signed the form themselves as well. Family members received €75 for participation. Confidentiality of the data was maintained by the use of a unique research ID number for each participant.

Recruitment

Families were recruited through media exposure, like interviews in Dutch newspapers, on television and radio; furthermore, the study was brought to the attention of patient organizations, to clinical psychologists, general practitioners and mental health care organizations. Recruitment was targeted at families in which multiple family members experienced 'extreme shyness' and took place between Summer 2013 and Summer 2015. Details about the screening and inclusion flow of the LFLSAD are provided in Bas-Hoogendam et al. (2018).

A priori power calculation and sample size

A priori power calculations were performed to estimate the required sample size of the LFLSAD, as described previously in Bas-Hoogendam, Harrewijn, et al. (2018). Power was computed by simulation, based on an endophenotype with a heritability of 60 % and a correlation of 70 % with SAD; the prevalence of SAD was set at 10 %. Families were generated using linear mixed models and we modeled correlations between family members via normally distributed random effects with a correlation structure of two times the kinship matrix. Only families with at least two affected members in one nuclear family were used for estimation of the power. These power calculations revealed that 12 families with 8 - 12 family members (average: 10 members per family) were required for sufficient power (i.e., minimally 80%) to 1st estimate the association between SAD and neurocognitive putative endophenotypes and 2nd to determine the significance of clustering of these endophenotypes within families (i.e., genetic effects).

Phenotyping

The presence of DSM-IV diagnoses was determined using the Mini-International Neuropsychiatric Interview (M.I.N.I.)-Plus (version 5.0.0) (Sheehan et al., 1998; van Vliet & de Beurs, 2007) or the M.I.N.I.-Kid interview (version 6.0) (Bauhuis et al., 2013; Sheehan et al., 2010); these interviews were administered by experienced clinicians and recorded. Special attention was paid to the presence of (sub)clinical SAD: clinical SAD was established using the DSM-IV-TR criteria for the generalized subtype of SAD, but the clinician verified whether the DSM-5 criteria for SAD were also met. We chose a priori to include patients with generalized SAD, as this is the most prevalent subtype, with a strong familiar pattern and an early age of onset (D'Avanzato & Dalrymple, 2016). A diagnosis of subclinical SAD was established when participants met the criteria for SAD as described in the DSM-5, but did not show impairing limitations in important areas of functioning (criterion G) (American Psychiatric Association, 2013).

In addition to the clinical interviews and the self-report questionnaires on social anxiety (the Liebowitz Social Anxiety Scale (LSAS-SR) (Fresco et al., 2001; Mennin et al., 2002) or the Social Anxiety Scale for adolescents (SAS-A) (La Greca & Lopez, 1998)), participants completed several questionnaires on anxiety-related constructs.

The intensity of fear of negative evaluation was assessed using the revised Brief Fear of Negative Evaluation (BFNE) – II scale (Carleton et al., 2006; Leary, 1983).

Furthermore, the level of self-reported depressive symptoms was evaluated using the Beck Depression Inventory (BDI– II) (Beck et al., 1996; Van der Does, 2002) or the Children's Depression Inventory (CDI) (Kovacs, 1985; Timbremont & Braet, 2002).

The State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) (see (Spielberger & Vagg, 1984) for psychometric properties) was used to determine self-reported trait anxiety, as well as state anxiety before and after the MRI scan.

The sensitivity for the temperamental traits 'behavioral inhibition' and 'behavioral activation' was assessed using the self-report BIS/BAS (Carver & White, 1994; Franken et al., 2005) or the BIS/BAS scales for children (BIS/BAS-C) (Muris et al., 2005).

Two subscales of the Wechsler Adult Intelligence Scale-IV (WAIS-IV) (Wechsler et al., 2008) or Wechsler Intelligence Scale for Children-III (WISC) (Wechsler, 1991), the similarities (verbal comprehension) and block design (perceptual reasoning) subtests, were administered to obtain an estimate of cognitive functioning.

MRI parameters

During the SNPT-R, fMRI scans were acquired using T2*-weighted echo-planar imaging (EPI). Characteristics of these scans with the following characteristics: 38 axial slices, 2.75 mm x 2.75 mm x 2.75 mm + 10 % interslice gap, field of view (FOV) = 220 mm x 115 mm x 220 mm, repetition time (TR) = 2200 ms, echo time (TE) = 30 ms. The first six volumes of

each fMRI scan were dummy volumes; these volumes were removed to allow for equilibration of T1 saturation effects.

In addition, a high-resolution EPI-scan (84 axial slices, 1.964 mm x 1.964 mm x 2 mm, FOV = 220 mm x 168 mm x 220 mm, TR = 2200 ms, TE = 30 ms) and a high-resolution T1-weighted scan (140 slices, resolution 0.875 mm × 0.875 mm × 1.2 mm, FOV = 224 mm × 168 mm × 177.333 mm, TR = 9.8 ms, TE = 4.59 ms, flip angle = 8◦) were acquired. These scans were used for within-subject registration purposes; furthermore, the structural T1-scans were inspected by a neuroradiologist, but no clinically relevant abnormalities were present in any of the participants.

Revised Social Norm Processing Task (SNPT-R)

Story-reading phase

The SNPT-R has 78 stories which were presented in a pseudo-random order using E-Prime software (version 2.0.10, Psychology Software Tools; script available at osf.io/pt4qt (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017b)). Stem sentences were presented for 3 s, while ending sentences had a duration of 6 s. Stories were separated by a fixation cross (jittered duration between 2 - 7 s, determined using Optseq software (https://surfer.nmr.mgh.harvard.edu/optseq/), mean duration fixation: 3.5 s) and the 78 stories were divided into two consecutive blocks of 39 stories (duration each block: 8 min 44 s). Importantly, the stories in the unintentional and intentional condition differed only in the intention of the actor, while the actual outcome of the action (for example, a distasteful pie) was in general the same (see (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a) for a sensitivity analysis).

Rating phase

During the post MRI scan rating phase, participants rated the stories on a 5-point Likert scale on embarrassment (ranging from 1, not embarrassing at all, to 5, extremely embarrassing) and inappropriateness (ranging from 1, not inappropriate at all, to 5, extremely inappropriate), using a laptop. These tasks were presented using E-Prime software (version 2.0.10, Psychology Software Tools) and the scripts are available at osf.io/pt4qt (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017b). Note that the behavioral ratings were performed outside the MRI scanner, for two main reasons. First of all, we aimed to measure brain activation of the participants while they just 'imagined themselves' as being in a certain social situation, without 'priming' or directing participants to think about embarrassment or inappropriateness specifically. Secondly, we had to take the duration of the MRI session into account: the whole MRI session lasted around one hour; having participants rate the stories during the MRI scan would make the session too long and too demanding. As a result, however, we are not able to disentangle which brain areas are activated during

thinking about the inappropriateness of the stories or while considering the amount of embarrassment related to the stories.

fMRI data

General processing steps

FMRI data were denoised using FIX (FMRIB's ICA-based X-noiseifier), a publicly available plugin for FSL (FMRIB Software Library, version 5.0.9) (Jenkinson et al., 2012), which provides an automatic solution for denoising fMRI data via accurate classification of ICA components (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). Next, data underwent several preprocessing steps using FEAT (FMRI Expert Analysis Tool; version 6.00) (Jenkinson et al., 2012; Smith et al., 2004), including motion correction using MCFLIRT (Jenkinson et al., 2002), spatial smoothing using a Gaussian kernel of full-width half-maximum (FWHM) 6.0 mm and grand-mean intensity normalization of the entire 4D dataset by a single scaling factor in order to enable higher-level analyses and registration. Scans were first registered to high-resolution EPI images, which were registered to T1 images, which in turn were registered to the Montreal Neurological Institute (MNI) T1-template brain (resolution 2 mm) using FNIRT nonlinear registration (warp resolution 10 mm) (Andersson et al., 2007; Jenkinson et al., 2002; Jenkinson & Smith, 2001). Next, ICA-AROMA (ICA-based Automatic Removal of Motion Artifacts) was used to remove motion-related artefacts (Pruim, Mennes, van Rooij, et al., 2015; Pruim, Mennes, Buitelaar, & Beckmann, 2015). Data were then submitted to FEAT to perform non-brain removal using BET (Smith, 2002), high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 30.0 s) and registration. Functional scans of each participant were registered to the individual 3D T1-weighted anatomical scan using FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001) and subsequently registered to the MNI T1-template brain (resolution 2 mm) using FNIRT nonlinear registration (warp resolution 10 mm) (Andersson et al., 2007).

Event-related statistical analysis of the time-series was carried out in native space following the method described in (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a). We used FILM with local autocorrelation correction (Woolrich et al., 2001) and included four explanatory variables (EVs) with their temporal derivatives in the general linear model. These EVs were convolved with a canonical double gamma hemodynamic response function and represented the presentation of 1st a stem sentence, 2nd a neutral ending (EN), 3rd an unintentional SN violation ending (EU) and 4th an intentional SN violation ending (EI). The stem EV had a duration of 3 s, ending EVs had a duration of 6 s; onset of the EVs for each individual was determined using custom-written scripts in MATLAB (Mathworks; code available at osf.io/pt4qt (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017b)). Subsequently, three contrasts were defined: 1st EI > EN; 2nd EU > EN; 3rd EU > EI. Contrasts 1 and 2 were used to validate the main effect of the SNPT-R on brain activation (Bas-Hoogendam,

van Steenbergen, Kreuk, et al., 2017a), while contrast 3 (EU > EI) was the contrast of interest for the endophenotype analysis, following previous results (Blair et al., 2010).

We checked whether the individual scans were registered correctly and confirmed that relative motion parameters did not exceed 2.5 mm. The individual contrast images of the two story-reading blocks were combined using a within-subject multi-session fixed-effects analysis. The resulting contrast images were submitted to higher-level mixed-effects group analyses using FMRIB's Local Analysis of Mixed Effects (Beckmann et al., 2003; Woolrich, 2008; Woolrich et al., 2004).

Validation of whole-brain activation patterns

To compare the task-related brain activation patterns to previous findings (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a), whole-brain analyses were used to investigate clusters related to the contrasts EI > EN and EU > EN. To keep the analyses comparable with our previous work, we used a cluster threshold of $z > 2.3$ and a cluster extent threshold $p < 0.05$, but we also used a more stringent threshold (cluster-threshold $z > 3.1$, cluster extent threshold $p < 0.01$).

Endophenotype analyses with (sub)clinical SAD as predictor

For reasons of completeness, we performed voxelwise analyses using (sub)clinical SAD as a discrete predictor in addition to the main analyses using self-reported SA-level (continuous variable) as a predictor. In these analyses, individual activation level related to the contrast EU > EI was used as dependent variable. Correlations between family members were modeled by including random effects; age (centered) and gender (centered) were included as covariates. Models were run for each voxel separately, in order to determine the effect of (sub) clinical SAD on a whole-brain voxelwise basis. Results (z-scores) were transformed into a nifti-image with the same dimensions of the MNI T1-template brain. Clusters within this nifti-image, representing the association between SA and brain activation, were corrected for multiple comparisons at the whole-brain level using the FSL tool easythresh (cluster threshold: $z > 3.1$, cluster extent threshold $p < 0.01$) (Worsley, 2001).

Sensitivity analyses

We performed two sensitivity analyses to examine whether the results of the association analysis (effect of self-reported social anxiety (z-score) on brain activation related to EU > EI) were driven by the severity of depressive symptoms as measured by the BDI-II or the CDI or by (comorbid) psychopathology other than SAD (cf. (Bas-Hoogendam, van Steenbergen, Tissier, et al., 2018b)). To this aim, we added the z-score of the level of depressive symptoms as a covariate in the voxelwise analysis (sensitivity analysis 1) or excluded all family members with past and/ or present psychopathology other than SAD and repeated the association analysis (sensitivity analysis 2). Note however, that this latter analysis may

yield biased and weaker results, as the majority of the probands, on which the selection of the families was based, had comorbid psychopathology and were thus excluded. We used the same statistical threshold as for the main analyses ($z > 3.1$, cluster-threshold $p < 0.01$).

Behavioral data

Endophenotype analyses with (sub)clinical SAD as predictor

For reasons of completeness, we performed analyses using (sub)clinical SAD as a discrete predictor in addition to the main analyses using self-reported SA-level (continuous variable) as a predictor. Separate models were used to investigate the ratings of embarrassment and inappropriateness. Task condition (intentional / unintentional / neutral), age- and gender specific task version (modeled using the dummy variables gender and age group (boys and girls vs men and women)), as well as three interaction terms (condition-by-gender, condition-by-age group, and condition-by-(sub)clinical SAD) were added as independent variables and tested for significance. Random effects were included to account for the genetic correlations between family members and the within-subject correlations between the task conditions. Interaction terms lacking significance were removed from the final models. Significance level was set at $p < 0.05$.

Supplemental Results

Data availability

We acquired MRI data from nine families ($n = 113$) (Bas-Hoogendam, Harrewijn, et al., 2018), but data from one family ($n = 3$ family members) had to be excluded as the proband from this family was not able to participate in the MRI experiment due to an MRI contra-indication. Furthermore, two young participants (aged 18.8 y and 9.4 y) quitted the MRI session before they had completed the two blocks of the SNPT-R, although one of them did perform the rating phase of the SNPT-R after the scan session. As a result, 109 datasets were available for the analyses with respect to the ratings, while 108 fMRI datasets were available for further fMRI pre-processing and quality control. Upon inspection of the relative motion parameters, fMRI data of nine participants had to be excluded, as their motion parameters for at least one of the two blocks of the SNPT-R exceeded 2.5 mm. Thus, the sample available for the fMRI analyses consisted of the data of 99 family members.

Due to technical reasons, data on the presence of subclinical SAD were lost for eight family members and data from these participants were therefore not included in the analysis with respect to the co-segregation of the candidate endophenotypes with (sub)clinical SAD within families.

Sample characteristics

We refer to *Supplemental Table S8.1* and *Supplemental Table S8.2* for detailed information about the sample. Following the design of the study, family members originated from two generations, which differed significantly in age (behavioral sample: $\beta \pm SE = -30.2 \pm 0.7$, $p < 0.001$; fMRI sample: $\beta \pm SE = -29.6 \pm 0.7$, $p < 0.001$), but not in male / female ratio (behavioral sample: $\chi^2(1) = 0.44$, $p = 0.57$; fMRI sample: $\chi^2(1) = 0.50$, $p = 0.55$). Family members with and without (sub)clinical SAD did not differ with respect to male / female ratio, age and estimated IQ. Groups did differ, however, in comorbidity rates: family members with (sub)clinical SAD were more often diagnosed with depression (past), dysthymia (present) and panic disorder. These differences were, however, only significant at an uncorrected significance level. Furthermore, family members with (sub)clinical SAD reported higher levels of fear of negative evaluation, more depressive symptoms, higher levels of trait anxiety and behavioral inhibition (BIS), as well as lower levels of behavioral activation (BAS).

FMRI data

Validation of whole-brain activation patterns

Results of the whole-brain analyses investigating activation related to the two task contrasts EI > EN and EU > EN are summarized in *Supplemental Table S8.3* and *Supplemental Figure S8.1.* In short, the analyses replicated the results reported for a sample of 21 healthy adults (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a), although the current activation clusters were more extended, probably due to the larger sample size of the present study.

***Contrast EI > EN** (Supplemental Figure S8.1A)*

Reading stories on intentional social norm violations (contrasted with reading neutral social stories) was associated with activation in three clusters. The first cluster encompassed the bilateral orbital frontal cortex, the bilateral inferior frontal gyrus, bilateral frontal operculum cortex and bilateral precentral gyrus, extended into subcortical structures like the bilateral amygdala, caudate, putamen, pallidum and thalamus, as well as into occipital areas such as the bilateral lateral occipital cortex, occipital fusiform gyrus and the occipital pole. The second cluster was comprised of the frontal pole, the superior frontal gyrus, the anterior cingulate gyrus and the paracingulate gyrus, while the third cluster included the right precentral and postcentral gyrus.

***Contrast EU > EN** (Supplemental Figure S8.1B)*

Activation related to reading unintentional social norm violations was found in two clusters, again when compared to reading neutral social stories. The first cluster contained the left frontal operculum cortex and inferior frontal gyrus, the left thalamus, the left amygdala, the left superior temporal gyrus and occipital areas like the lingual gyrus, the intracalcarine cortex and the bilateral occipital pole. The second cluster encompassed the superior frontal

gyrus and frontal pole as well as the anterior and posterior cingulate gyrus and the paracingulate gyrus.

Findings were confirmed by analyses using a more stringent threshold (cluster threshold $z > 3.1$, cluster extent threshold $p < 0.01$) – those are reported in *Supplemental Table S8.4*.

Endophenotype analyses with (sub)clinical SAD as predictor

We investigated whether brain activation related to the contrast EU > EI *co-segregated with SA* by performing whole-brain voxelwise regression analyses. The regression analysis using discrete (sub)clinical SAD as a predictor did not yield clusters surviving the predefined threshold. So, it should be noted that we did find a positive association between brain activation and self-reported SA (continuous predictor), but not with (sub)clinical SAD (discrete predictor). We speculate that this lack of a correlation is power-related, as the fMRI sample only contained 33 (sub)clinical SAD cases. This indicates the need for replication of the present findings in a larger sample.

Follow-up analyses

We explored whether the results of the difference contrast EU > EI were driven by a positive relationship between SA-levels and the processing of unintentional SN violations or by a negative association of SA-levels with processing intentional SN violations. We extracted the individual activation levels for the contrasts 'EU > baseline' and 'EI > baseline' within the significant clusters and performed two regression analyses in R (predictor: self-reported SA; dependent variables: activation related to 'EU > baseline' and 'EI > baseline', respectively; models corrected for age and gender; genetic correlations between family members were taken into account). These analyses showed a positive relationship between SA-levels and activation related to processing unintentional SN violations (contrast EU > baseline: $\beta \pm SE = 2.18 \pm 0.80$, $p = 0.006$), but no association between SA-level and activation related to processing intentional SN violations (contrast EI > baseline: $\beta \pm SE = 0.67 \pm 0.76$, $p = 0.36$). We further confirmed the specificity of the main finding for processing unintentional SN violations by repeating the whole-brain voxelwise analyses on the contrasts EI>EN and 'all endings (EU + EI + EN) > baseline'. These analyses did not yield significant clusters at the predefined significance level.

Sensitivity analyses

Results of the first sensitivity analysis, with the level of depressive symptoms as an additional covariate, confirmed the clusters found in the main analysis and revealed even a third cluster showing a significant association between self-reported SA and brain activation related to processing unintentional SN violations in the left temporal pole (*Supplemental Table S8.5, Supplemental Figure S8.2*).

In the second sensitivity analysis, we excluded all participants with past and/or present comorbid psychopathology other than SAD; this resulted in a sample of 64 participants, of which 15 in the (sub)clinical SAD group. Next, we repeated the association analysis with self-reported social anxiety as predictor. The analysis with the standard (stringent) statistical threshold ($z > 3.1$, $p < 0.01$), confirmed the positive association between self-reported social anxiety and brain activation in the occipital pole (*Supplemental Table S8.6; Supplemental Figure S8.3A*), in line with the main analysis. The association between social anxiety and activation in the frontal/mPFC cluster was, however, not significant at this significance level; when we applied a less stringent threshold ($z > 2.3$, $p < 0.05$), we did find a significant positive association (*Supplemental Table S8.7; Supplemental Figure S8.3B*).

Behavioral data

Behavioral responses during story-reading phase

Examination of the behavioral responses during the story-reading phase showed that two participants (female, aged 41 y; male, aged 20 y) forgot to press the button during the first block of the SNPT-R; in between the two blocks, these participants indicated upon request that they had read the stories and after additional instructions, they responded well to the sentences presented in the second block of the task. The other participants ($n = 97$) responded to 95.3 % of the trials (number of missed responses / block of 39 trials (mean ± SD): 1.8 ± 2.5, range 0 - 15), indicating good task compliance.

Effects of gender and age group

Behavioral ratings for each task version (based on age and gender; versions for boys / girls / men/ women) are summarized in *Supplemental Table S8.8* and *Supplemental Figure S8.4*. Detailed statistics are presented in *Supplemental Table S8.9* and *Supplemental Table S8.10*. Females rated the stories are more inappropriate and more embarrassing, while adults (men / women) rated the stories are more inappropriate than the children and adolescents (aged 8 - 18 years) did.

Endophenotype analyses with (sub)clinical SAD as predictor

Analyses examining the *co-segregation of (sub)clinical SAD (discrete variable) with the behavioral candidate endophenotypes within the families* revealed a main effect of (sub) clinical SAD on the ratings of embarrassment, with higher ratings for family members with (sub)clinical SAD, but not on the ratings of inappropriateness (*Supplemental Table S8.9; Supplemental Figure S8.5*). The interaction between (sub)clinical SAD and condition was not significant, while exploratory follow-up analyses on the embarrassment ratings separately for each condition (with age group and gender as covariates) indicated that (sub) clinical SAD was associated with higher embarrassment ratings on the unintentional ($\beta \pm SE = 0.23 \pm 0.10$, $p = 0.025$) and neutral stories ($\beta \pm SE = 0.14 \pm 0.06$, $p = 0.03$) (*Supplemental*

Figure S8.5), but not on the intentional stories (β ± SE = 0.06 ± 0.10, $p = 0.62$). Furthermore, there were main effects of condition and gender, as well as interaction effects for both types of ratings, comparable to previous findings on the SNPT-R (Bas-Hoogendam, van Steenbergen, Kreuk, et al., 2017a; Bas-Hoogendam, van Steenbergen, van der Wee, et al., 2018) (*Supplemental Table S8.9*).

Sensitivity analyses

We performed two additional analyses to investigate whether the effects of social anxiety on the embarrassment ratings were driven by the participants with a diagnosis of clinical SAD.

In the first analysis, we compared the embarrassment ratings for all three task conditions between participants with clinical SAD ($n = 17$) and participants with subclinical SAD ($n = 22$); as in the main analyses, gender and age group were added as covariates and we included random effects to account for the genetic correlations between family members. Results showed no significant differences between the groups in embarrassment ratings for the intentional (β ± SE = 0.03 ± 0.24, $p = 0.89$), unintentional (β ± SE = -0.05 ± 0.16, $p = 0.76$) or neutral condition (β ± SE = -0.01 ± 0.06, $p = 0.80$).

Secondly, we excluded all participants with clinical SAD (remaining $n = 92$) and repeated the analyses with self-reported social anxiety as predictor for the embarrassment ratings. In line with the results of the main analysis, we found a significant main effect of self-reported social anxiety (β ± SE = 0.03 ± 0.01, $p = 0.02$), a main effect of condition (β ± SE = -1.16 ± 0.12, $p < 0.001$), a main effect of gender (β ± SE = 0.56 ± 0.19, $p = 0.003$) and an interaction between condition and gender (β ± SE = -0.17 ± 0.07, $p = 0.01$) (*Supplemental Table S8.11*). Subsequent analyses for the separate conditions revealed a significant positive effect of self-reported social anxiety on the embarrassment ratings for the unintentional condition (β ± SE = 0.05 ± 0.02, $p = 0.03$), while the associations for the other conditions were significant at trend level (intentional: β ± SE = 0.05 ± 0.03, $p = 0.09$; neutral: β ± SE = 0.03 ± 0.02, $p = 0.07$); these non-significant results are most likely due to the loss of statistical power.

Supplemental Tables

Supplemental Table S8.1 Detailed characteristics of participants with and without (sub)clinical SAD: demographics and clinical information.

	Behavioral sample[a]			fMRI sample[a]		
	(Sub)clinical SAD (*n* = 39)	No SAD (*n* = 62)	Statistical analysis	(Sub)clinical SAD (*n* = 33)	No SAD (*n* = 58)	Statistical analysis
Demographics						
Male / Female (n)	20 / 19	30 /32	$\chi^2(1) = 0.08$, $p = 0.84$	16 / 17	29 / 29	$\chi^2(1) = 0.02$, $p = 1.00$
Generation 1/ Generation 2 (n)	19 / 20	27 / 35	$\chi^2(1) = 0.26$, $p = 0.68$	19 / 14	27 / 31	$\chi^2(1) = 1.02$, $p = 0.39$
Age in years (mean ± SD)	30.3 ± 15.5	31.3 ± 15.2	β (± SE)=-0.9 ± 3.1, p= 0.76	33.4 ± 14.9	32.7 ± 14.8	β (± SE) = 0.7 ± 3.2, $p = 0.83$
Estimated IQ (mean ± SD)	104.3 ± 12.2	105.9 ± 10.5	β (± SE)=-2.7±2.2, p= 0.22	103.6 ± 12.6	106.0 ± 10.8	β (± SE) = -2.8 ± 2.4, $p = 0.23$
Diagnostic information (n)						
Clinical SAD	17	0	$\chi^2(1) = 32.5$, $p < 0.001$	15	0	$\chi^2(1) = 31.6$, $p < 0.001$
Depressive episode present	1	1	$\chi^2(1) = 0.15$, $p = 0.70$	1	1	$\chi^2(1) = 0.2$, $p = 0.65$
Depressive episode past	12	9	$\chi^2(1) = 4.8$, $p = 0.03$	12	9	$\chi^2(1) = 6.1$, $p = 0.01$
Dysthymia present	3	0	$\chi^2(1) = 5.3$, $p = 0.02$	3	0	$\chi^2(1) = 5.8$, $p = 0.02$
Dysthymia past	1	1	$\chi^2(1) = 0.2$, $p = 0.65$	0	1	$\chi^2(1) = 0.5$, $p = 0.47$
Panic disorder lifetime	5	2	$\chi^2(1) = 3.9$, $p = 0.05$	5	2	$\chi^2(1) = 4.5$, $p = 0.03$
Agoraphobia present	3	2	$\chi^2(1) = 1.2$, $p = 0.27$	2	2	$\chi^2(1) = 0.4$, $p = 0.52$
Agoraphobia past	0	2	$\chi^2(1) = 1.2$, $p = 0.28$	0	1	$\chi^2(1) = 0.5$, $p = 0.46$
Separation anxiety	0	1	$\chi^2(1) = 0.8$, $p = 0.37$	0	0	n.a.
Specific phobia	2	3	$\chi^2(1) = 0.02$, $p = 0.90$	2	2	$\chi^2(1) = 0.40$, $p = 0.53$
Generalized anxiety disorder	1	0	$\chi^2(1) = 1.7$, $p = 0.19$	1	0	$\chi^2(1) = 1.9$, $p = 0.17$
Obsessive-compulsive disorder	1	0	$\chi^2(1) = 1.7$, $p = 0.19$	1	0	$\chi^2(1) = 1.9$, $p = 0.17$
Attention deficit hyperactivity disorder (ADHD)	3	1	$\chi^2(1) = 2.3$, $p = 0.13$	2	1	$\chi^2(1) = 1.2$, $p = 0.27$
Alcohol dependency present	1	1	$\chi^2(1) = 0.2$, $p = 0.70$	1	1	$\chi^2(1) = 0.2$, $p = 0.65$
Alcohol dependency lifetime	1	3	$\chi^2(1) = 0.3$, $p = 0.62$	1	3	$\chi^2(1) = 0.2$, $p = 0.67$

Supplemental Table S8.1 Detailed characteristics of participants with and without (sub)clinical SAD: demographics and clinical information. (*continued*)

	Behavioral sample[a]			fMRI sample[a]		
	(Sub)clinical SAD (*n* = 39)	No SAD (*n* = 62)	Statistical analysis	(Sub)clinical SAD (*n* = 33)	No SAD (*n* = 58)	Statistical analysis
Present psychotropic medication (n)	4	3	$\chi^2(1) = 1.1$, $p = 0.30$	2	3	$\chi^2(1) = 0.3$, $p = 0.86$
Antidepressants not otherwise specified	3	0		2	0	
ADHD medication not otherwise specified	1	3		0	3	

Footnote

[a]: Due to technical reasons, data on the presence of (sub)clinical SAD were lost for eight family members. Data from these participants were, however, included in the endophenotype analyses using SA-level (*z*-score) as a predictor (behavioral sample: *n* = 109; fMRI sample: *n* = 99).

Supplemental Table S8.2 Detailed characteristics of participants with and without (sub)clinical SAD: scores on self-report questionnaires.

	Behavioral sample			fMRI sample		
	(Sub)clinical SAD (*n* = 39)	No SAD (*n* = 62)	Statistical analysis	(Sub)clinical SAD (*n* = 33)	No SAD (*n* = 58)	Statistical analysis
Self-report measures						
Social anxiety symptoms (z-score; mean ± SD)	3.0 ± 3.3	0.6 ± 1.5	β ± SE = 2.4 ± 0.5, $p < 0.001$	2.9 ± 3.0	0.7 ± 1.3	β ± SE = 2.4 ± 0.4, $p < 0.001$
Fear of negative evaluation (mean ± SD)	23.3 ± 12.3	13.0 ± 8.0	β ± SE = 10.3 ± 2.0, $p < 0.001$	23.4 ± 11.7	13.1 ± 8.0	β ± SE = 10.3 ± 2.1, $p < 0.001$
Depressive symptoms (z-score; mean ± SD)	0.0 ± 0.9	-0.5 ± 0.7	β ± SE = 0.5 ± 0.2, $p < 0.001$	0.1 ± 0.9	-0.5 ± 0.7	β ± SE = 0.6 ± 0.2, $p < 0.001$
STAI – state pre scan (mean ± SD)	n.a	n.a		34.4 ± 7.4	31.5 ± 8.2	β ± SE = 3.1 ± 1.6, $p = 0.06$
STAI – state post scan (mean ± SD)	n.a	n.a		30.5 ± 6.4	28.1 ± 6.2	β ± SE = 2.4 ± 1.4, $p = 0.07$
STAI – trait (mean ± SD)	38.8 ± 9.4	33.0 ± 8.5	β ± SE = 5.5 ± 1.8, $p = 0.002$	39.1 ± 9.4	33.1 ± 8.6	β ± SE = 5.8 ± 1.9, $p = 0.003$
BIS (z-score; mean ± SD)	0.4 ± 1.3	-0.4 ± 0.9	β ± SE = 0.8 ± 0.2, $p < 0.001$	0.3 ± 1.1	-0.4 ± 0.9	β ± SE = 0.7 ± 0.2, $p < 0.001$
BAS (z-score; mean ± SD)	-0.9 ± 1.0	-0.6 ± 1.0	β ± SE = -0.5 ± 0.2, $p = 0.02$	-1.0 ± 0.9	-0.6 ± 1.0	β ± SE = -0.5 ± 0.2, $p = 0.008$

Abbreviations

n.a.: not applicable; SD: standard deviation; SE: standard error.

Supplemental Table S8.3 Brain activity related to reading social stories describing intentional and unintentional social norm violations versus neutral situations ($z > 2.3$, $p < 0.05$).

Cluster	Region	*Z*-score	Peak coordinates (MNI space)			Cluster size
			x	y	z	
Intentional norm violations vs neutral stories (EI > EN)						
1	Left orbital frontal cortex	8.53	-36	22	-14	41548
	Right orbital frontal cortex	6.91	30	20	-16	
	Right occipital pole	5.95	10	-88	40	
	Right operculum cortex	5.62	46	10	0	
	Right operculum cortex / inferior frontal gyrus	5.51	46	26	2	
	Left supramarginal gyrus	5.47	-62	-42	30	
2	Superior frontal gyrus	7.75	-2	54	28	13572
	Paracingulate gyrus	7.31	-6	54	16	
	Anterior cingulate gyrus	7.28	-2	22	22	
	Superior frontal gyrus	6.56	8	12	62	
	Left frontal pole	6.46	-28	46	28	
	Anterior cingulate gyrus	5.95	-2	16	38	
3	Right precentral gyrus	6.25	52	0	48	1429
	Right postcentral gyrus	3.85	64	-8	46	
Unintentional norm violations vs neutral stories (EU > EN)						
1	Lingual gyrus	5.64	-8	-82	-2	27196
	Intracalcarine cortex	5.62	12	-82	2	
	Intracalcarine cortex	5.49	18	-68	2	
	Left orbitofrontal cortex	5.29	-36	22	-14	
	Left frontal operculum cortex	5.09	-44	14	4	
	Right occipital pole	5.06	28	-92	36	
2	Superior frontal gyrus	5.92	0	56	30	6250
	Anterior cingulate gyrus	4.99	-2	18	28	
	Left frontal pole	4.95	-26	48	30	
	Superior frontal gyrus	4.75	0	10	62	
	Posterior cingulate gyrus	4.48	-4	-22	42	
	Paracingulate gyrus	3.90	-6	36	28	

Supplemental Table S8.4 Brain activity related to reading social stories describing intentional and unintentional social norm violations versus neutral situations ($z > 3.1$, $p < 0.01$).

Cluster	Region	*Z*-score	Peak coordinates (MNI space)			Cluster size
			x	y	z	
Intentional norm violations vs neutral stories (EI > EN)						
1	Occipital pole	5.95	10	-88	40	9845
2	Superior frontal gyrus	7.75	-2	54	28	9590
3	Left orbitofrontal cortex	8.53	-36	22	-14	7549
4	Right orbitofrontal cortex	6.91	30	20	-16	3243
5	Right precentral gyrus	6.25	52	0	48	911
6	Left supramarginal gyrus	5.47	-62	-42	30	696
7	Left precentral gyrus	5.14	-44	-14	42	602
8	Right superior temporal gyrus, posterior part	5.12	48	-32	2	601
Unintentional norm violations vs neutral stories (EU > EN)						
1	Lingual gyrus	5.64	-8	-82	-2	9735
2	Superior frontal gyrus	5.92	0	56	30	2572
3	Left orbitofrontal cortex	5.29	-36	22	-14	1766
4	Left thalamus	4.02	-6	-10	6	550

Supplemental Table S8.5 Effect of self-reported social anxiety on processing unintentional social norm violation – with level of depressive symptoms as additional covariate ($z > 3.1$, $p < 0.01$).

Cluster	Region	*Z*-score	Peak coordinates (MNI space)			Cluster size
			x	y	z	
Unintentional norm violations vs intentional norm violations (EU > EI)						
1	Temporal occipital fusiform cortex	6.19	30	-60	-18	12436
	Occipital pole	5.18	10	-94	26	
	Superior temporal gyrus, posterior division	4.33	62	-6	-8	
	Cuneal cortex	4.01	20	-76	32	
	Medial temporal gyrus, posterior division	3.75	60	-22	-10	
2	Frontal pole	6.10	-10	56	32	3520
	Frontal pole / frontal medial cortex	3.30	0	58	-4	
3	Inferior Temporal gyrus	4.79	-54	-18	-22	1464

Supplemental Table S8.6 Effect of self-reported social anxiety on processing unintentional social norm violation – in sample without (comorbid) psychopathology except for SAD ($z > 3.1$, $p < 0.01$).

Cluster	Region	*Z*-score	Peak coordinates (MNI space)			Cluster size
			x	y	z	
Unintentional norm violations vs intentional norm violations (EU > EI)						
1	Occipital pole	5.28	8	-92	26	4850
	Temporal occipital fusiform cortex	5.24	34	-58	-20	
	Occipital fusiform gyrus	4.02	32	-82	-12	

Supplemental Table S8.7 Effect of self-reported social anxiety on processing unintentional social norm violation – in sample without (comorbid) psychopathology except for SAD ($z > 2.3$, $p < 0.05$).

Cluster	Region	*Z*-score	Peak coordinates (MNI space)			Cluster size
			x	y	z	
Unintentional norm violations vs intentional norm violations (EU > EI)						
1	Lateral occipital cortex	5.38	50	-82	8	40324
	Occipital pole	5.28	8	-92	26	
	Frontal pole	4.45	-10	56	30	
	Posterior cingulate gyrus	4.35	-10	-46	30	

Supplemental Table S8.8 Ratings of inappropriateness and embarrassment – summarized for each task version (based on age and gender).

	(Sub)clinical SAD	No SAD		(Sub)clinical SAD	No SAD
Inappropriateness			Embarrassment		
Intentional	4.36 ± 0.40	4.36 ± 0.43	*Intentional*	3.92 ± 0.72	3.89 ± 0.58
Boys	3.99 ± 0.45	4.17 ± 0.69	Boys	3.59 ± 0.67	3.95 ± 0.63
Girls	4.40 ± 0.28	4.29 ± 0.52	Girls	3.84 ± 0.73	3.85 ± 0.48
Men	4.32 ± 0.36	4.35 ± 0.41	Men	3.75 ± 0.62	3.63 ± 0.61
Women	4.63 ± 0.23	4.46 ± 0.32	Women	4.30 ± 0.72	4.13 ± 0.49
Unintentional	2.98 ± 0.73	2.98 ± 0.64	*Unintentional*	3.45 ± 0.54	3.23 ± 0.51
Boys	3.14 ± 0.68	3.19 ± 0.66	Boys	3.14 ± 0.51	3.33 ± 0.49
Girls	2.84 ± 0.78	3.15 ± 0.52	Girls	3.56 ± 0.44	3.43 ± 0.36
Men	3.00 ± 0.62	2.99 ± 0.65	Men	3.32 ± 0.50	3.12 ± 0.52
Women	2.92 ± 0.87	2.83 ± 0.65	Women	3.73 ± 0.52	3.23 ± 0.55
Neutral	1.39 ± 0.34	1.31 ± 0.29	*Neutral*	1.38 ± 0.38	1.25 ± 0.24
Boys	1.57 ± 0.52	1.19 ± 0.15	Boys	1.29 ± 0.27	1.19 ± 0.16
Girls	1.18 ± 0.13	1.44 ± 0.63	Girls	1.28 ± 0.18	1.40 ± 0.52
Men	1.40 ± 0.26	1.30 ± 0.22	Men	1.51 ± 0.64	1.22 ± 0.19
Women	1.33 ± 0.28	1.32 ± 0.19	Women	1.38 ± 0.20	1.25 ± 0.16

Footnote

Values represent mean ± standard deviation.

Supplemental Table S8.9 Effect of (sub)clinical SAD on ratings – detailed statistics.

	Inappropriateness			Embarrassment		
	β	SE	*p*	β	SE	*p*
(Sub)clinical SAD	0.06	0.05	0.24	0.13	0.06	0.02
Condition *Intentional, unintentional, neutral*	-1.15	0.12	< 0.001	-1.07	0.11	< 0.001
Gender *male, female*	0.30	0.12	0.01	0.62	0.17	< 0.001
Age group *boys / girls vs men / women*	0.36	0.13	0.007	0.03	0.06	0.65
Condition-by-gender	-0.09	0.05	0.04	-0.20	0.06	0.001
Condition-by- age group	-0.12	0.05	0.02	*not significant and not included in final model*		
Condition-by-(sub)clinical SAD	*not significant and not included in final model*			*not significant and not included in final model*		

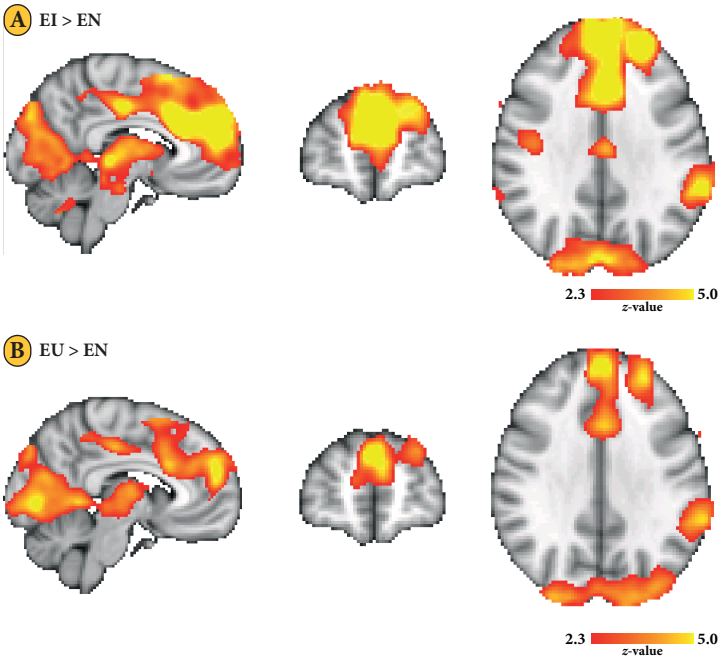
Supplemental Table S8.10 Effect of social anxiety (*z*-score) on ratings – detailed statistics.

	Inappropriateness			Embarrassment		
	β	**SE**	***p***	**β**	**SE**	***p***
Z-score SA	0.002	0.009	0.84	0.03	0.01	0.003
Condition *intentional, unintentional, neutral*	-1.14	0.11	< 0.001	-1.07	0.11	< 0.001
Gender *male, female*	0.33	0.11	0.003	0.69	0.17	< 0.001
Age group *boys / girls vs men / women*	0.32	0.13	0.01	-0.003	0.06	0.96
Condition-by-gender	-0.11	0.05	0.01	-0.22	0.06	p < 0.001
Condition-by- age group	-0.11	0.05	0.02	*not significant and not included in final model*		
Condition-by-*z*-score SA	*not significant and not included in final model*			*not significant and not included in final model*		

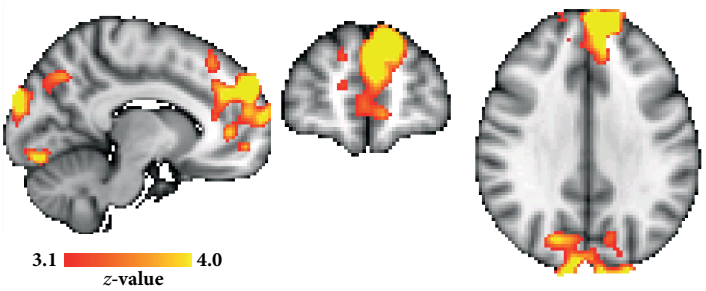
Supplemental Table S8.11 Effect of social anxiety (*z*-score) on embarrassment ratings – detailed statistics for sample without clinical SAD cases.

	Embarrassment		
	β	**SE**	***p***
Z-score SA	0.03	0.01	0.019
Condition *intentional, unintentional, neutral*	-1.16	0.12	< 0.001
Gender *male, female*	0.56	0.19	0.003
Age group *boys / girls vs men / women*	-0.007	0.07	0.92
Condition-by-gender	-0.17	0.07	0.01

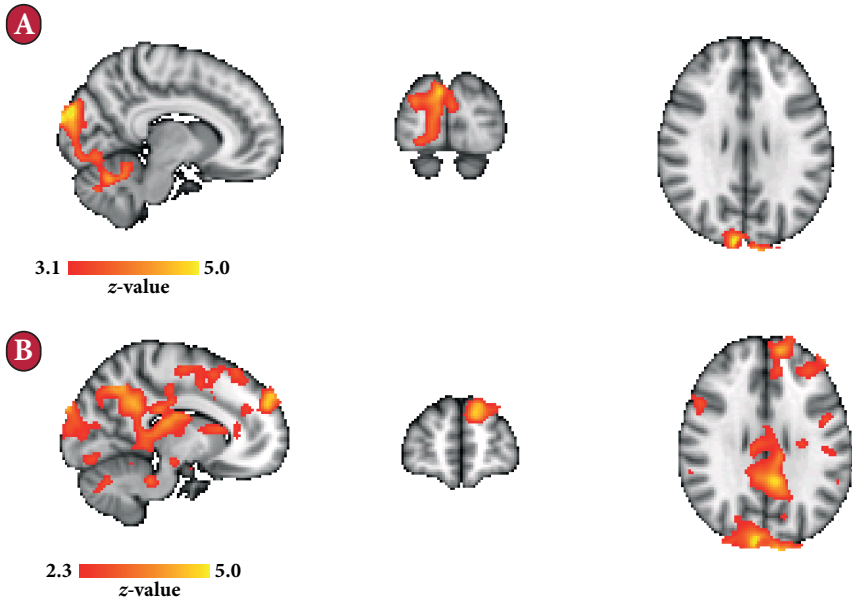
Supplemental Figures



Supplemental Figure S8.1 Significant activation patterns related to processing stories on social norm violations.
Clusters are displayed on the temple MNI_T1_152_2mm_brain (partial brain coverage: inferior parts of the frontal medial cortex, superior parts of the postcentral gyrus as well as parts of the cerebellum are not included). Images are displayed according to radiological convention: right in the image is left in the brain. Coordinates of displayed slices (MNI, xyz): -6, 56, 32. Cluster-threshold $z > 2.3$, cluster extent threshold $p < 0.05$.



Supplemental Figure S8.2 Significant positive associations between social anxiety (*z*-scores) and activation related to processing stories on unintentional social norm violations, corrected for level of depressive symptoms (sensitivity analysis 1).
Clusters are displayed on the temple MNI_T1_152_2mm_brain (partial brain coverage: inferior parts of the frontal medial cortex, superior parts of the postcentral gyrus as well as parts of the cerebellum are not included). Images are displayed according to radiological convention: right in the image is left in the brain. Coordinates of displayed slices (MNI, x, y, z): -10, 56, 32.

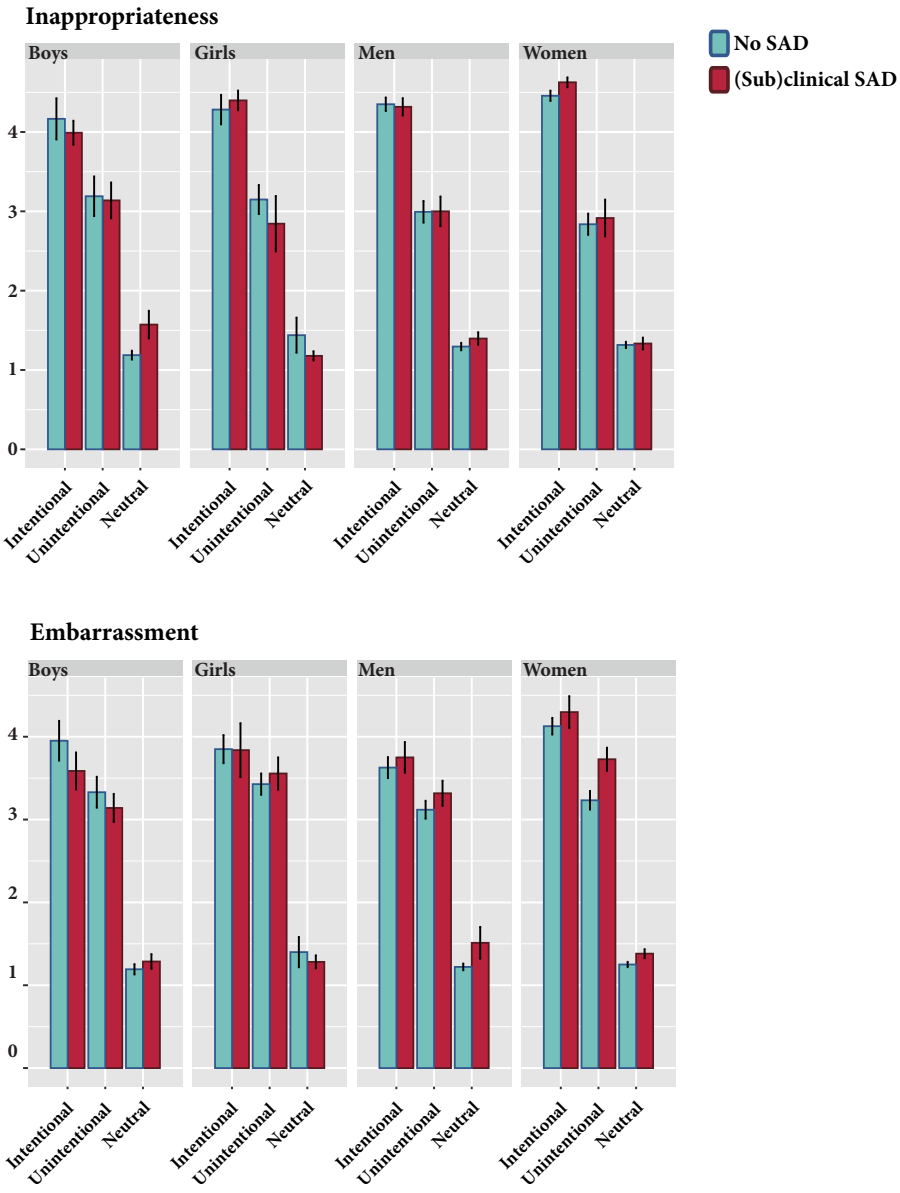


Supplemental Figure S8.3 Significant positive associations between social anxiety (*z*-scores) and activation related to processing stories on unintentional social norm violations, sample without (comorbid) psychopathology other than SAD (sensitivity analysis 2).

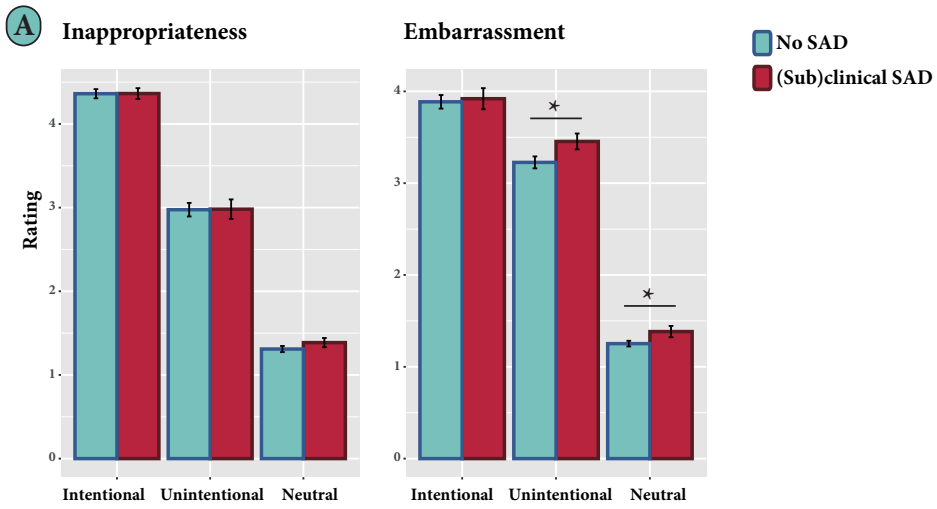
Clusters are displayed on the temple MNI_T1_152_2mm_brain (partial brain coverage: inferior parts of the frontal medial cortex, superior parts of the postcentral gyrus as well as parts of the cerebellum are not included). Images are displayed according to radiological convention: right in the image is left in the brain.

Figure S8.3A Threshold $z > 3.1$, $p < 0.01$. Coordinates of displayed slices (MNI, x, y, z): 10, -90, 28.

Figure S8.3B Threshold $z > 2.3$, $p < 0.05$. Coordinates of displayed slices (MNI, x, y, z): -12, 56, 28.



Supplemental Figure S8.4 Behavioral ratings on the SNPT-R, summarized for each task version (based on age and gender).
Bars represent means ± standard errors of the mean.



Supplemental Figure S8.5 Behavioral ratings on the SNPT-R – effect of (sub)clinical SAD.
Bars represent means ± standard errors of the mean.