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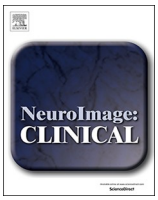

Amygdala hyperreactivity to faces conditioned with a social-evaluative meaning– a multiplex, multigenerational fMRI study on social anxiety endophenotypes

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ABSTRACT

Social anxiety disorder (SAD) runs in families, but the neurobiological pathways underlying the genetic susceptibility towards SAD are largely unknown. Here, we employed an endophenotype approach, and tested the hypothesis that amygdala hyperreactivity to faces conditioned with a social-evaluative meaning is a candidate SAD endophenotype. We used data from the multiplex, multigenerational Leiden Family Lab study on Social Anxiety Disorder (eight families, $n = 105$) and investigated amygdala activation during a social-evaluative conditioning paradigm with high ecological validity in the context of SAD. Three neutral faces were repeatedly presented in combination with socially negative, positive or neutral sentences. We focused on two endophenotype criteria: co-segregation of the candidate endophenotype with the disorder within families, and heritability. Analyses of the fMRI data were restricted to the amygdala as a region of interest, and association analyses revealed that bilateral amygdala hyperreactivity in response to the conditioned faces co-segregated with social anxiety (SA; continuous measure) within the families; we found, however, no relationship between SA and brain activation in response to more specific fMRI contrasts. Furthermore, brain activation in a small subset of voxels within these amygdala clusters was at least moderately heritable. Taken together, these findings show that amygdala engagement in response to conditioned faces with a social-evaluative meaning qualifies as a neurobiological candidate endophenotype of social anxiety. Thereby, these data shed light on the genetic vulnerability to develop SAD.

1. Introduction

Social anxiety disorder (SAD), one of the most prevalent anxiety disorders, has a typical onset during adolescence and runs in families (Haller et al., 2015; Isomura et al., 2015; Miers et al., 2013). Patients with the disorder have an extreme fear of evaluation by others and avoid social situations as much as possible (American Psychiatric Association, 2013; Stein and Stein, 2008). Furthermore, SAD is associated with a chronic course, high rates of comorbid psychopathology, reduced quality of life and far-reaching impairments in school, work and relations (Dams et al., 2017; Fehm et al., 2005). Given the severe consequences of the disorder, for patients and their families as well as for society, insight in the neurobiological functional brain alterations underlying the genetic vulnerability to develop SAD is essential.

One of the key structures in the socially anxious brain is the amygdala (cf. reviews by (Brühl et al., 2014; Etkin and Wager, 2007; Garner et al., 2009)). The amygdala is essential for processing environmental stimuli and learning their predictive value, as demonstrated in both humans and animals (Hariri and Whalen, 2011; Janak and Tye, 2015; Olsson and Phelps, 2007; Paton et al., 2006). More specifically, an elegantly designed neuroimaging study by Davis and colleagues (2010) has provided strong evidence for the role of the amygdala in learning the *social* value of biologically-relevant cues. The authors employed a conditioning paradigm, in which three

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neutral faces (conditioned stimuli, CS) were consistently paired with either a positive endorsement, a negative comment, or a socially-neutral statement (unconditioned stimuli, US; Davis et al., 2010); importantly, as these sentences were directly addressing the participant, the presentation of these face-sentence combinations created a social-evaluative learning context. Behavioral ratings of likeability indicated that healthy participants learned the social value of the faces, and functional magnetic resonance imaging (fMRI) data revealed the involvement of various amygdala subregions during social-evaluative learning (Davis et al., 2010).

At present, and to the best of our knowledge, this social-evaluative conditioning paradigm has not been used in SAD. Nevertheless, given the heightened fear of negative as well as positive evaluation that characterizes socially anxious individuals (Reichenberger et al., 2019; Teale Sapach et al., 2014), investigating the neurobiological underpinnings of social-evaluative learning is of uttermost relevance in SAD (cf. (Pittig et al., 2018)). An electromyography (EMG) study in patients with SAD, using a differential fear conditioning paradigm in which neutral faces (CS) were paired with positive, neutral or negative facial expressions and verbal feedback (US) addressing the participant, reported an elevated fear-potentiated startle reflex in response to faces conditioned with critical facial expressions and insults in SAD patients, while no group differences were present with respect to subjective ratings of the conditioned stimuli, nor during extinction learning (Lissek et al., 2008). Subsequent studies used slightly adapted versions of this differential fear conditioning paradigm. The first, an EMG study on individuals with clinical SAD and participants with high levels of social anxiety (SA) could, however, not replicate fear conditioning in the physiological data, and did not find SA-related differences with respect to self-report measures of anxiety, unpleasantness and arousal due to conditioning (Tinoco-González et al., 2015). The second study (Ahrens et al., 2015), using electroencephalography (EEG), paired neutral faces (CS) with three types of verbal feedback (positive, neutral or negative; US), and demonstrated impaired electrocortical differentiation in students with high levels of SA: while low socially anxious individuals showed differential visuocortical processing in relation to the three conditions, with highest cortical activity to faces paired with insults and lowest activity to faces paired with compliments, this distinction was absent in high socially anxious participants. Again, no group differences were found with respect to ratings of valence (Ahrens et al., 2015). Due to the methodology used, these studies were, however, not able to investigate amygdala reactivity during social conditioning. To the best of our knowledge, only one fMRI study has explored the relation between SA and amygdala activation during social conditioning using disorder relevant stimuli. In that study, Pejic et al. (2013) paired neutral faces (CS) with film-clips of critical comments (US), and showed positive correlations between SA and amygdala activation during social conditioning; at the behavioral level, participants with higher SA-levels reported stronger increases in unpleasantness and fear following social conditioning (Pejic et al., 2013).

Together, these findings suggest that SA is associated with altered physiological and neural responses during social conditioning, although it should be noted that only one study so far directly investigated amygdala activation (Pejic et al., 2013), while the study reporting on an elevated fear-potentiated startle reflex in SAD provides indirect evidence for the involvement of the amygdala (Lissek et al., 2008) – cf. (Pissiota et al., 2003). Furthermore, results on the relation between SA and behavioral indices of social conditioning are mixed. In addition, as Pejic and colleagues (2013) used a sample of healthy students with varying levels of SA and only employed negative unconditioned stimuli, the relation between SA and amygdala function related to social conditioning has until now not been directly investigated in patients with SAD, and the effect of positive and neutral comments as unconditioned stimuli is at present still unknown. Furthermore, it has not been examined whether amygdala activation during social-evaluative learning is a candidate endophenotype of SAD. Such research is however, important, as endophenotypes, which are located on the causal pathway from genotype to phenotype, could shed light on the mechanisms by which genetic risk unfolds (Dick, 2018), and as such, could aid in unravelling the genetic susceptibility to SAD and offer new insights in targets for prevention and intervention (Bas-Hoogendam et al., 2016).

By definition, endophenotypes are quantitative characteristics which are *associated with the disorder* (criterion 1), *state-independent and already present in a preclinical state* (criterion 2), *heritable* (criterion 3), and display *co-segregation 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, 2013). The endophenotype approach has yielded promising results in other psychiatric disorders, for example in depression (Goldstein and Klein, 2014), schizophrenia and psychosis (Blakey et al., 2018; Glahn et al., 2014; Sutcliffe et al., 2016) and obsessive–compulsive disorder (Taylor, 2012) but research on neurobiological endophenotypes of SAD is still scarce.

Here, we present data from the Leiden Family Lab study on Social Anxiety Disorder (LFLSAD), comprising a unique sample of families genetically enriched for SAD (Bas-Hoogendam et al., 2018a). This multiplex (i.e. multiple cases of SAD), multigenerational family-design is eminently suitable to test two important endophenotype criteria within the same sample, being the *heritability* and *co-segregation of a certain candidate endophenotype within families*. Using the social conditioning paradigm developed by Davis and colleagues (2010) for the first time in the context of SAD, we investigated whether amygdala reactivity during social-evaluative learning could serve as a candidate neurobiological endophenotype of SAD. First, we examined evidence for the endophenotype criterion of *co-segregation of the candidate endophenotype with SA within the families* (first element of criterion 4); in case of affirmative results, we established *heritability* (criterion 3). Based on previous research summarized above, we predicted a positive relationship between SA-level and amygdala activation in response to the conditioned stimuli, reflecting the heightened sensitivity to the meaning of the faces; moreover, we expected the most prominent effects for the faces conditioned with the negative and positive (versus neutral) sentences (cf. work on the fear of negative and positive evaluation in SAD (Reichenberger et al., 2019)). Furthermore, on a more exploratory basis, as research on this subject is still scarce, we examined the relation between SA-level and amygdala activation over time, as well as in response to the three particular conditions. Behavioral ratings were used to validate the paradigm; in addition, their relation with SA-level was explored.

2. Materials and methods

2.1. Participants

The sample consisted of participants from the LFLSAD, in which families genetically enriched for SAD are included. Families were invited for participation based on the combination of a primary diagnosis of SAD in a parent (aged 25–55 years old; 'proband') and a child who met criteria for clinical or subclinical SAD ('proband's SA-child'). The proband's SA-child (age 8–21 years) should live at home with the proband; comorbidity other than internalizing disorders or substance abuse was an exclusion criterion for the proband and proband's SA-child. Besides these two SAD-cases, first- and second-degree family-members of two generations were invited to participate, being the proband's partner and other children of the nuclear family (age $\geq$ 8 years), as well as the proband's sibling(s), with their partners and children (age $\geq$ 8 years). These family-members were included independent from the presence of psychopathology. Insufficient comprehension of the Dutch language was an exclusion criterion for all participants, and general MRI contraindications led to exclusion of the MRI experiment.

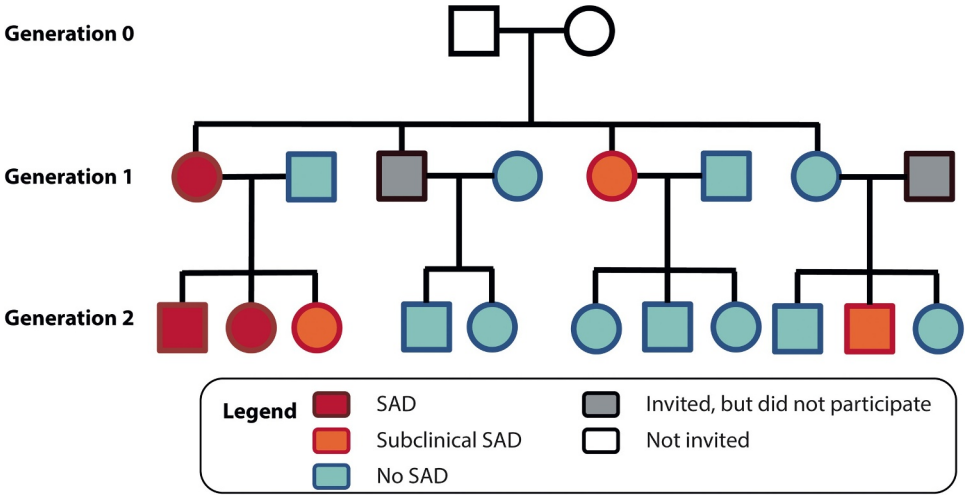

Fig. 1. Family within the LFLSAD. Families were included based on the combination of a parent with SAD ('proband'; depicted in red) and a proband's child with SAD (red) or subclinical SAD (orange). In addition, family-members of two generations were invited, independent from the presence of SAD within these family-members (no SAD: light blue; did not participate: grey). Grandparents (generation 0; white) were not invited for participation. This family is slightly modified to guarantee anonymity; however, the number of family-members and the frequency of (sub) clinical SAD are depicted truthfully. Squares and circles represent men and women, respectively. Reprint of the figure published in (Bas-Hoogendam et al., 2018a). SAD: social anxiety disorder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article) .

Following this inclusion strategy, the LFLSAD sample (total sample: $n = 132$, nine families; MRI sample: $n = 110$, eight families; more information about recruitment is included in the Supplemental Methods) consists of family-members of two generations (Fig. 1). Participants completed a number of measurements, such as a diagnostic interview, self-report questionnaires and an MRI-scan (Bas-Hoogendam et al., 2018a). The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and all participants provided informed consent according to the Declaration of Helsinki.

Detailed information about the LFLSAD and an a priori power-calculation for the study are outlined in a design-paper (Bas-Hoogendam et al., 2018a); furthermore, the study was preregistered online (Bas-Hoogendam et al., 2014b, 2014a).

2.2. Phenotyping

In order to facilitate extensive phenotyping, the LFLSAD protocol consisted of several measurements (Bas-Hoogendam et al., 2018a) (cf. Supplemental Methods). The following assessments are relevant for the present work.

Experienced clinicians determined the presence of DSM-IV diagnoses using the Mini-International Neuropsychiatric Interview (M.I.N.I.)-Plus (Sheehan et al., 1998) or the M.I.N.I.-Kid interview (Sheehan et al., 2010). Given the nature of the LFLSAD sample, 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. 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). The interviews were recorded to enable a considerate evaluation of psychopathology.

Furthermore, participants completed age-appropriate questionnaires on the level of SA-symptoms, being the Liebowitz Social Anxiety Scale for adults (Fresco et al., 2001) and the Social Anxiety Scale for adolescents (La Greca and Lopez, 1998), as well as on the level of depressive symptoms (Beck Depression Inventory (Beck et al., 1996) or the Children's Depression Inventory (Kovacs, 1985). To enable interpreting the scores of the age-appropriate questionnaires over the whole sample, *z*-scores were computed (Bas-Hoogendam et al., 2018a). Incidental missing values were replaced by the average value of the completed items.

2.3. MRI experiment

Scanning was performed using a 3.0T Philips Achieva MRI scanner. The MRI-experiment consisted of several structural scans (Bas-Hoogendam et al., 2018b) and functional task paradigms (Bas-Hoogendam et al., 2019c, 2019b, 2017a); details are provided in the Supplemental Methods.

2.4. Social-evaluative conditioning paradigm

The social-evaluative conditioning paradigm was part of the neutral faces paradigm (NFP), in which we investigated both the initial habituation response to neutral faces as well as brain activation associated with learning their social-evaluative value (Fig. 2). Neutral faces were selected from the FACES database, a set of well-validated images of naturalistic faces of women and men (Ebner et al., 2010). In order to take the so-called 'own gender bias' into account (Wright and Sladden, 2003), participants were presented with faces matching their own sex: we selected photographs of three young males and three young females (see Supplemental Methods for details on these faces). Stimuli were presented using E-Prime software version (2.0.10, Psychology Software Tools).

The NFP consists of two phases, a habituation phase (HP) and the social-evaluative conditioning phase (SCP). Findings on the HP are reported elsewhere (Bas-Hoogendam et al., 2019b); for reasons of completeness, a description of the HP is also included in the Supplemental Methods. During the SCP, which was based on the paradigm by (Davis et al., 2010), three neutral faces, which had been shown to the participants already during the HP, were again presented, but now each face was consistently combined with one type of social-evaluative sentence: positive, negative or neutral. That is, after presentation of the face (duration: 1 s; conditioned stimulus), a social-evaluative sentence was presented (duration: 2 s; unconditioned stimulus) (Figure 2).

One face was always followed by a positive endorsement (for example: 'he/she says you are smart'), the second face was accompanied by a negative comment ('he/she says you are stupid'), while the last face was combined with a socially-neutral statement ('he/she says you are in Leiden'). There were four different sentences within each category (see Supplemental Methods for a list of all sentences), and each face-sentence combination was shown three times. This resulted in 12 trials per condition and a total of 36 trials. The order of the face-sentence combinations was pseudorandomized and the combinations of the faces with the type of self-relevant sentences were counter-balanced across the participants.

Participants were instructed to look at the faces and to read the

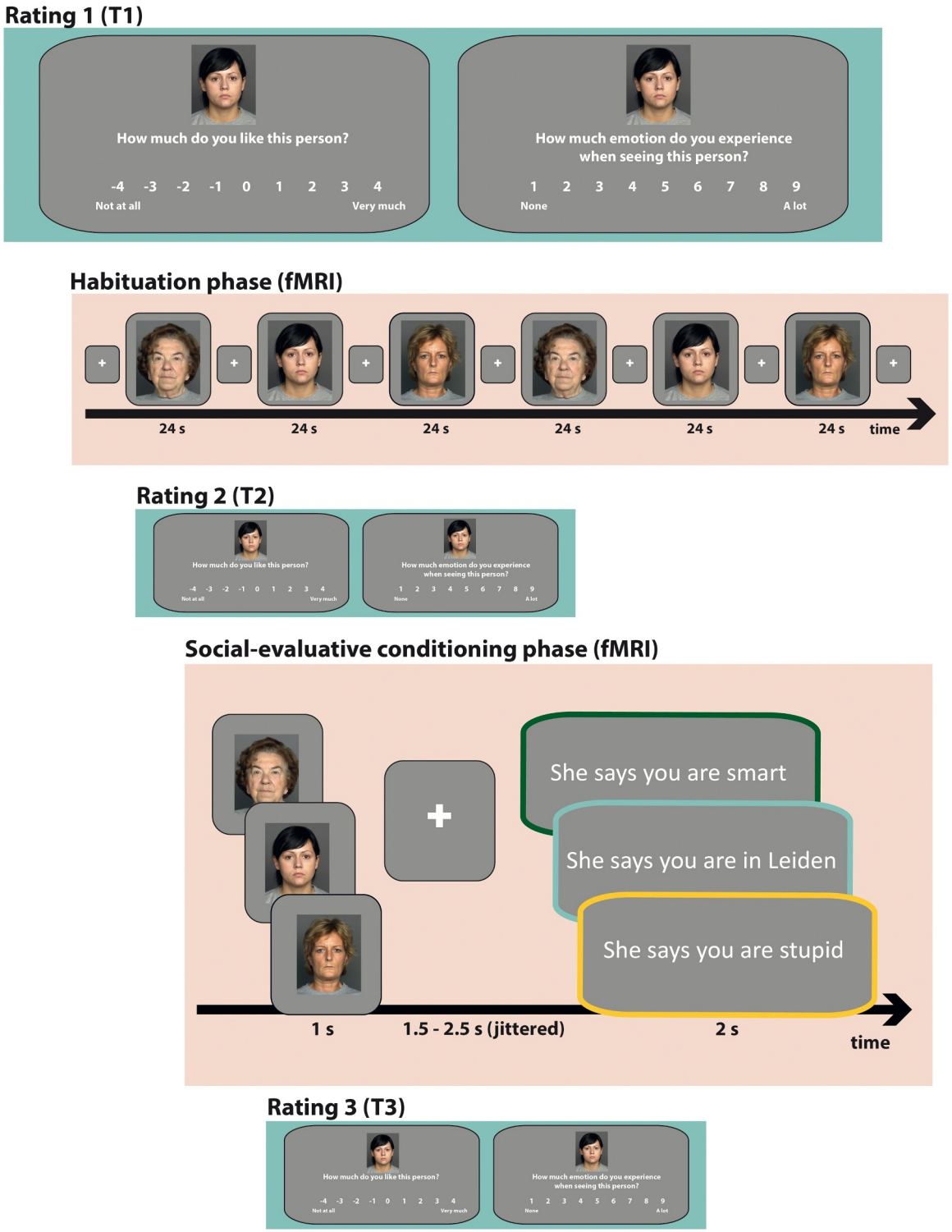

Fig. 2. Overview of the neutral faces paradigm (NFP). Stimuli were neutral faces selected from the FACES database (Ebner et al., 2010) (please note: the selected faces were different from the faces shown in this figure following the FACES Database Release Agreement); the paradigm consists of two fMRI phases, being a habituation phase (described in more detail in Supplemental Information as well as in (Bas-Hoogendam et al., 2019b)) and the social-evaluative conditioning phase (SCP) which is discussed in the present work. During the SCP, three neutral faces were consistently paired with either a positive endorsement, a negative comment or a socially-neutral statement, enabling participants to learn the social value of each face. At different time-points during the neutral faces paradigm (NFP), participants rated the faces on likeability and arousal.

accompanying sentences. As the face presentation always preceded the sentence presentation, participants learned what type of social-evaluative sentence would follow upon presentation of a certain face. The intervals between the presentation of the face and the presentation of the sentence, as well as the intertrial intervals, were jittered in order to optimize the estimation of the blood-oxygen-level-dependent response related to the presentation of the faces and the presentation of the sentences (jitter face-sentence: range 1.5 s–2.5 s, mean 2.0 s; intertrial interval: range 2.0 s–3.5 s, mean 2.7 s; cf. (Davis et al., 2010)). Total duration of the SCP was 5 min 41 s.

At three times during the NFP, participants were asked to rate the faces on likeability and arousal in line with the paradigm described by (Davis et al., 2010); the first measurement was before the HP (T1), the second between the HP and SCP (T2), while the last measurement followed the end of the SCP (T3; Figure 2). These ratings were used to investigate the initial rating of the faces (T1); furthermore, the ratings were used to assess whether participants learned the social-evaluative value of the faces (i.e. to validate the SCP), and to examine the association between this learning process and social anxiety. The three faces were presented sequentially on the screen, accompanied by the question 'How much do you like this person?' (range from -4, 'not at all' to 4, 'very much'), and, on a second screen, the question 'How much emotion do you experience when seeing this person?' (ranging from 1, 'none' to 9,'a lot'). Prior to the start of the MRI-scan, participants were familiarized with these ratings by performing a short version of the task (with faces not used in the fMRI task) on a laptop.

2.5. Data analysis

2.5.1. Sample characteristics

We compared participants with and without (sub)clinical SAD on demographic variables and on the level of self-reported SA, by performing chi-square tests in SPSS (version 25) and by fitting multi-level regression models in R (R Core Team, 2016). Within these regression models, we modelled genetic correlations between family-members by including random effects.

2.5.2. Behavioral data

We examined whether participants learned the social-evaluative value of the faces by performing a repeated measures ANOVA in SPSS, with condition (3 levels: positive, negative, neutral) and time (3 levels; T1, T2 and T3) as within-subjects factors. Significance level was set at $p \leq 0.05$; we applied Greenhouse–Geisser correction when the assumption of sphericity was violated.

Furthermore, we investigated whether the initial behavioral response to the faces, as well as the likeability ratings related to learning their value in the social-evaluative context, were associated with SA. To examine the initial response, we used the average of likeability ratings over the three faces provided at T1 (Likeability_T1); to examine the effect of the social-evaluative context, we calculated the difference in likeability scores between T2 and T3 over the three conditions (ΔLikeability_T3_T2; so irrespective of the content of the evaluation, cf (Reichenberger et al., 2019; Reichenberger and Blechert, 2018)), and for the three conditions separately; furthermore, we calculated difference scores to explore whether SA-level was differentially associated with learning the value of the negative, neutral and positive conditioned faces (ΔLikeability_T3_T2_Neg_vs_Neu; ΔLikeability_T3_T2_Neg_vs_Pos; ΔLikeability_T3_T2_Pos_vs_Neu).

We investigated the association between SA and these likeability ratings using linear mixed models in R (package: coxme) [RRID: SCR_003005], with self-reported SA (continuous variable; *z*-score, centered) as predictor of interest. Separate models were used to investigate the initial response to the faces and the difference scores representing learning the value of the faces in the social-evaluative context. Random effects were included to account for the genetic correlations between family-members; age (centered) and gender (centered) were added as covariates of no interest. Significance level was set at $p < 0.05$. For reasons of completeness, we also performed analyses with (sub)clinical SAD as a discrete predictor (Supplemental Information).

2.5.3. fMRI data

2.5.3.1. General processing steps and statistical analysis. Functional MRI data were pre-preprocessed using standardized procedures in FSL (Jenkinson et al., 2012) [RRID: SCR_002823] – see a detailed description of the processing steps in the Supplemental Methods and (Bas-Hoogendam et al., 2019b, 2019c). Event-related statistical analysis was performed in native space, using FILM with local autocorrelation correction (Woolrich et al., 2001). Following previous analyses (Davis et al., 2010), we included twelve explanatory variables (EVs) as well as their temporal derivatives in the general linear model. The EVs represented the presentation of the faces belonging to the three conditions (negative, neutral, positive) and the presentation of the negative, neutral and positive social-evaluative sentences; separate EVs were created for the stimuli presented during the first half and the last half of the SCP, in order to enable investigating social-evaluative learning over time (cf. (Davis et al., 2010)). As the present work focuses on the processing of the conditioned stimuli (the faces), brain responses to the sentences (unconditioned stimuli) are reported in the Supplemental Information. EVs were convolved with a double gamma hemodynamic response function and onset of the EVs for each individual was determined using custom-written scripts in Matlab. The fixation cross between the face and sentence stimuli and the fixation cross between the trials were not modeled and therefore served as the implicit baseline to which EVs could be compared.

We defined several contrasts of interest. First of all, we examined the contrast 'all faces > baseline', in order to investigate brain activation related to viewing the conditioned stimuli (i.e. faces with a social-evaluative meaning). Furthermore, we examined habituation (cf. (Davis et al., 2010)) by contrasting the faces presented during the first half of the SCP with the faces presented during the last half of the SCP; we refer to this contrast as 'all faces early > all faces late'. Next, we investigated valence-effects by contrasting the conditioned stimuli in the three different conditions ('negative conditioned face > neutral conditioned face'; 'negative conditioned face > positive conditioned face'; 'positive conditioned face > neutral conditioned face').

2.5.3.2. Brain activation at group-level. For all contrasts of interest, we determined brain activation over the whole sample in the amygdala, by using masks of the left and right amygdala (mask description included in the Supplemental Information; cluster threshold: $z > 2.3$, cluster extent threshold $p < 0.05$ within the unilateral regions of interest (ROIs)). Furthermore, for reasons of completeness, we also report explorative whole-brain analyses (cluster threshold: $z > 3.1$, extent threshold $p < 0.05$).

2.5.3.3. Neurobiological candidate endophenotypes. We tested whether altered amygdala activation in response to conditioned faces could serve as a candidate SAD endophenotype, and investigated the '*co-segregation of the candidate endophenotype with the disorder within families*' using regression models in R [RRID: SCR_003005], with self-reported SA-level (*z*-score; centered) as independent variable and individual activation level related to the contrasts of interest as dependent variables. Correlations between family-members were modeled by including random effects; age and gender (both centered) were included as covariates of no interest. Furthermore, analyses were corrected for the level of depressive symptoms (*z*-score; centered). Models were ran for each voxel separately and results (*z*-scores) were transformed into a nifti-image with the dimensions of the MNI T1-template brain.

We examined the relation with SA within the clusters representing significant amygdala activation at group-level; results were corrected for multiple comparisons using the FSL-tool easythresh (cluster threshold: $z > 2.3$, cluster extent threshold $p < 0.05$, minimum of 10 voxels) (Worsley, 2001). For reasons of completeness, we also investigated the association between SA at the level of whole brain activation; furthermore, we performed analyses with (sub)clinical SAD as a discrete predictor (Supplemental Information). A subsequent sensitivity analysis was performed to investigate whether the results of the association analyses were driven by (comorbid) psychopathology other than SAD (Supplemental Methods). Next, we determined the *heritability* of brain activation for voxels in the significant clusters. Heritability

Table 1
Characteristics of participants with and without (sub)clinical SAD

	Behavioral sample[†] (Sub)clinical SAD (n = 39)	No SAD (n = 63)	Statistical analysis	fMRI sample[†] (Sub)clinical SAD (n = 38)	No SAD (n = 60)	Statistical analysis
Demographics						
Male/Female (n)	20/19	31/32	$\chi^2(1) = 0.04, p = 0.84$	19/19	30/30	$\chi^2(1) = 0.0, p = 1.00$
Generation 1 /Generation 2 (n)	19/20	27/36	$\chi^2(1) = 0.33, p = 0.56$	19/19	27/33	$\chi^2(1) = 0.23, p = 0.63$
Age in years (mean ± SD; range)	30.3 ± 15.5 (9.2–59.6)	30.9 ± 15.4 (9.0–61.5)	$\beta \pm SE = -0.6 \pm 3.1, p = 0.85$	30.9 ± 15.3 (9.2–59.6)	31.9 ± 15.0 (9.4–61.5)	$\beta \pm SE = -1.0 \pm 3.1, p = 0.73$
Diagnostic information (n)						
Clinical SAD	17	0		17	0	
Self-report measures						
Social anxiety (z-score; mean ± SD)	3.0 ± 3.3	0.5 ± 1.6	$\beta \pm SE = 2.6 \pm 0.5, p < 0.001$	3.0 ± 3.1	0.6 ± 1.5	$\beta \pm SE = 2.6 \pm 0.5, p < 0.001$
Depression (z-score; mean ± SD)	0.0 ± 0.9	−0.5 ± 0.7	$\beta \pm SE = 0.5 \pm 0.2, p < 0.001$	0.01 ± 0.8	−0.5 ± 0.7	$\beta \pm SE = 0.5 \pm 0.2, p < 0.001$

[†] Due to technical reasons, recordings of the clinical interviews were lost for some family-members; therefore, the presence of subclinical SAD could not be established. Data from these participants were, however, included in the endophenotype analyses using SA-level (z-score) as a predictor, as their questionnaire data were available (behavioral sample: n = 108; fMRI sample: n = 105). SD: standard deviation; SE: standard error.

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.

3. Results

3.1. Sample characteristics

Details on quality checking and data availability are provided in the Supplemental Results. Characteristics of the samples (n = 108 for the behavioral analyses, data on subclinical SAD available for 102 participants; n = 105 for the fMRI analyse, data on subclinical SAD available for 98 participants) are presented in Table 1. Family-members with (sub)clinical SAD did not differ from family-members without SAD with respect to male/female ratio and age, but they reported higher levels of social anxiety and more depressive symptoms. We refer the reader to the Supplemental Tables 1–2 for a detailed characterization of the sample.

3.2. Behavioral data

3.2.1. Validation of the SCP

Likeability ratings for the faces, provided at three timepoints during the NFP, are provided in Table 2 and illustrated in Fig. 3A. As expected, a repeated measures ANOVA with condition and time as within-subject factors indicated a significant interaction between time and condition ($F(3.4, 362.8) = 37.2$, $p < 0.001$, $\eta^2 = 0.18$). Subsequent repeated measures ANOVAs separately for each timepoint, with condition as within-subjects factor, indicated that the faces did not differ with respect to likeability at T1 ($F(2, 214) = 1.0$, $p = 0.38$, $\eta^2 = 0.009$) and T2 ($F(2, 214) = 0.9$, $p = 0.40$, $\eta^2 = 0.009$), which validated the use of these faces for the subsequent SCP. Indeed, after the SCP (T3), a significant effect of condition was present ($F(1.8, 194.5) = 34.5$, $p < 0.001$, $\eta^2 = 0.24$), indicating that participants learned the social-evaluative value of the faces; this finding is in line with the original report on this paradigm (Davis et al., 2010).

Association analyses showed that the initial response to the neutral faces (Likeability_T1) was not significantly related to SA-level within the families (Table 2). SA-level was, however, associated with the change in likeability ratings due to social-evaluative conditioning: there was a significant negative relation between SA-level and ΔLikeability_T3_T2, suggesting that the addition of the social-evaluative sentences (the unconditioned stimuli) was aversive for family-members with higher SA-levels (Table 2; Fig. 3B). This effect was present regardless of the valence of the comments: follow-up analyses indicated that the negative association between SA and ΔLikeability_T3_T2 was present in all three conditions (Table 2), while subsequent regression analyses on the difference scores between the conditions confirmed that the relationship between SA and ΔLikeability_T3_T2 was not different between the conditions (ΔLikeability_T3_T2_Neg_vs_Neu: $\beta \pm SE = -0.04 \pm 0.07$, $p = 0.57$; ΔLikeability_T3_T2_Neg_vs_Pos: $\beta \pm SE = -0.05 \pm 0.09$, $p = 0.60$; ΔLikeability_T3_T2_Pos_vs_Neu: $\beta \pm SE = 0.007 \pm 0.08$, $p = 0.93$). A sensitivity analysis on the difference in likeability between T1 and T2 confirmed that the effect of SA was specific for the SCP of the NFP (Supplemental Results).

In addition to these likeability ratings, we included ratings of arousal in the NFP in line with the task description by Davis et al. (2010). However, it was hard to find a good transcription of the term 'arousal' when translating the question from English to Dutch (cf. (Van Damme, 2013). Indeed, participants indicated during debriefing that they struggled to interpret the question with respect to arousal. Data showed that the changes in the arousal ratings due to conditioning resembled the pattern of the likeability ratings (i.e. increase for the positive condition and decrease for the negative condition), and did not, as expected based on the findings by

Table 2
Behavioral ratings on the neutral faces paradigm

Likeability ratings (mean ± SD)	T1	T2	T3
Average	0.7 ± 1.1	0.8 ± 1.1	0.7 ± 1.1
Negative	0.8 ± 1.6	0.8 ± 1.6	−0.3 ± 1.8
Neutral	0.7 ± 1.6	0.9 ± 1.6	1.0 ± 1.5
Positive	0.6 ± 1.6	0.7 ± 1.6	1.3 ± 1.6
(Effect of social anxiety (z-score)[†]	**β ± SE**	***p***	
Likeability_T1	0.07 ± 0.04	0.07	
ΔLikeability_T3_T2	−0.08 ± 0.03	0.003	
ΔLikeability _T3_T2_positive	−0.06 ± 0.06	0.27	
ΔLikeability_T3_T2_ negative	−0.11 ± 0.06	0.07	
ΔLikeability-T3_T2_ neutral	−0.07 ± 0.05	0.15	

[†] Corrected for age, gender and family structure. SD: standard deviation; SE: standard error.

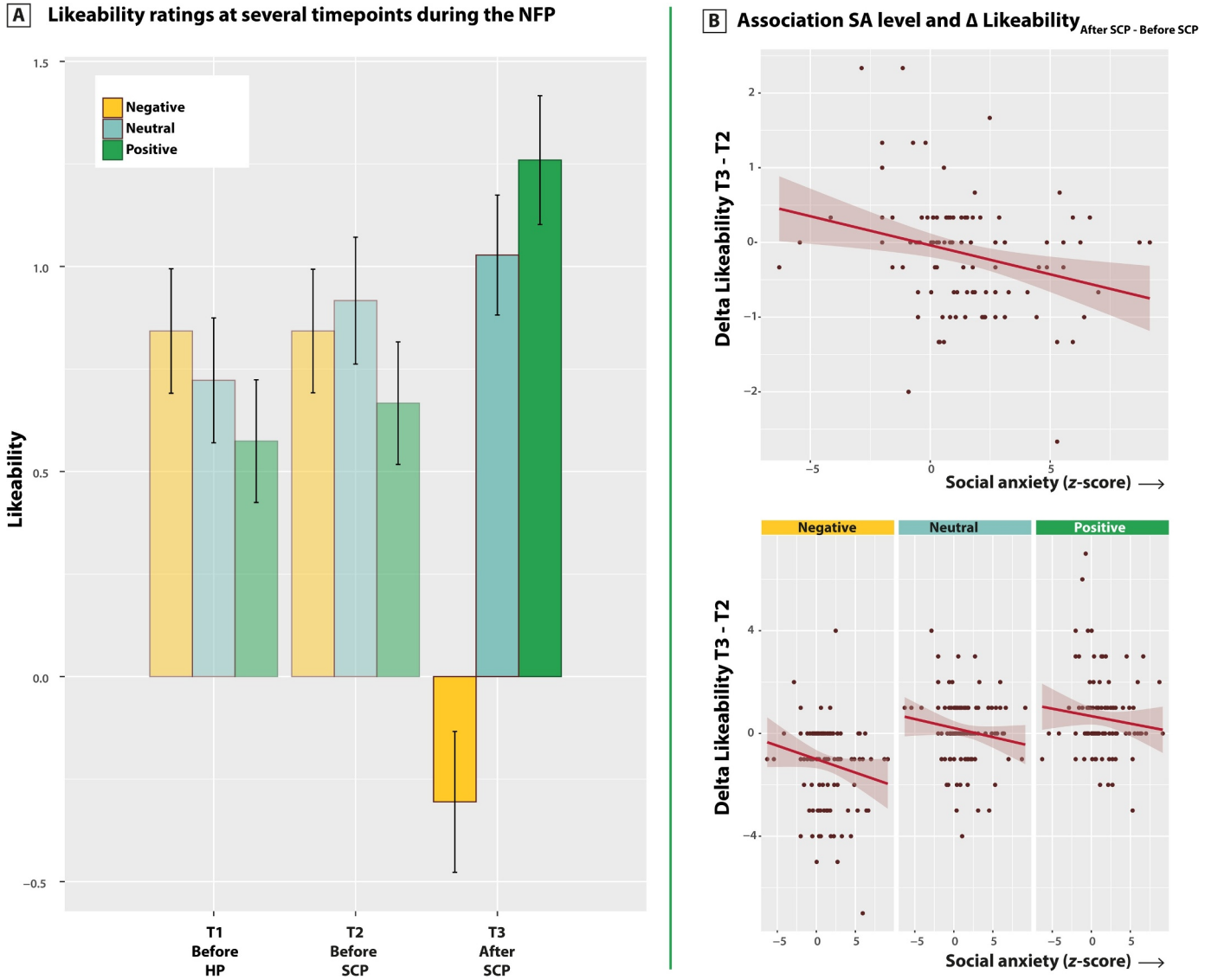

Fig. 3. Behavioral ratings on the NFP. A) Ratings of likeability for the three conditions at the three timepoints. Faded colors at T1 and T2 indicate that the faces were not conditioned yet; at T3, participants had learned the social-evaluative value of the faces, as indicated by a significant interaction between time and condition, as well as an effect of condition at T3. Errorbars represent standard errors of the mean. B) Association between the level of social anxiety and learning the social-evaluative value of the faces (ΔLikeability_T3_T2), depicted over all conditions (upper half) and separate for the three conditions (lower half). HP: habituation phase; NFP: neutral faces paradigm; SA: social anxiety; SCP: social-evaluative conditioning phase.

Table 3
Brain activation independent from level of social anxiety

Cluster	Region	Z-score	Peak coordinates (MNI space)			Cluster size
			x	Y	Z	
All faces > baseline						
	Whole brain					
1	Occipital pole, fusiform gyrus	12.1	14	-94	2	81562
	Middle temporal gyrus	8.3	−62	−46	6	
	Middle frontal gyrus	9.9	−40	0	48	
	Orbitofrontal cortex	7.6	−46	28	−8	
	Amygdala, left	7.47	−20	−6	−14	
	Amygdala, right	6.27	22	−4	−18	
2	Caudate, right	4.93	16	8	8	397
	Amygdala ROI					
1	Amygdala, left	7.47	−20	−6	−14	738
2	Amygdala, right	6.27	22	-4	-18	884
All faces early > all faces late						
	Whole brain					
1	Occipital pole	4.77	16	−92	−4	2335
	Amygdala ROI					
1	Amygdala, right	3.02	16	−4	−12	44
Negative conditioned face > neutral conditioned face						
	Whole brain					
1	Anterior cingulate gyrus	4.35	8	44	8	963
2	Supramarginal gyrus, right	4.33	64	−40	8	513
3	Precentral gyrus, right	4.8	44	8	26	377
4	Cerebellum, left	3.81	−32	−86	−32	367
5	Superior temporal gyrus, right	5.04	44	−26	−2	347
	Amygdala ROI					
1	Amygdala, left	3.87	−16	−8	−10	182
2	Amygdala, right	3.36	16	−12	−10	64
Negative conditioned face > positive conditioned face						
	Whole brain					
1	Inferior frontal gyrus, right	4.36	50	16	20	572
	Amygdala ROI					
1	Amygdala, left	3.37	−16	−10	−12	109
2	Amygdala, right	2.75	18	−4	−14	48
Positive conditioned face > neutral conditioned face						
	Whole brain analysis					
1	Lingual gyrus, right	5.16	20	−66	−12	3876
	Lingual gyrus, left	4.63	−18	−74	−2	
	Lateral occipital cortex	4.59	30	−80	12	
	Amygdala ROI	*No significant clusters*				

Davis et al. (2010), reflect increased levels of arousal for the faces conditioned with the positive and negative social-evaluative sentences when compared to the neutrally-conditioned faces. Therefore, the arousal ratings will not be further considered; for reasons of completeness, they are available in Supplemental Table 3.

3.3. fMRI data

3.3.1. Brain activation at group-level

Significant activation related to the contrasts of interest is summarized in Table 3 and illustrated in Fig. 4 (amygdala ROIs). These results confirmed the role of the amygdala during social-evaluative learning, previously described by Davis et al. (2010). We refer the reader to the Supplemental Results for a more in-depth discussion of these findings; in short, the ROI-analyses on the contrast 'all faces > baseline', 'negative conditioned face > neutral conditioned face', and 'negative conditioned face > positive conditioned face' revealed bilateral amygdala activation, while the contrast 'all faces early > all faces late' showed activation in the right amygdala. No amygdala activation was present for the contrast 'positive conditioned face > neutral conditioned face'. The latter contrast was therefore not further investigated in the endophenotype analysis.

3.3.2. Neurobiological candidate endophenotypes

Voxelwise regression analyses on the association between self-reported SA and amygdala activation related to viewing the conditioned stimuli ('all faces > baseline') revealed significant positive associations within both the left and right amygdala (Table 4; Fig. 5). The amygdala findings were replicated in a sensitivity analysis, in which data from participants with (comorbid) psychopathology other than SAD were excluded (Supplemental Table 5; Supplemental Figure 3). Within the right amygdala cluster, a subset of 22 voxels had at least moderate heritability (range: h^2 = 0.20 (moderate heritability)–0.63 (high heritability); 22 out of 164 voxels = 13 %); in the left amygdala, only one voxel survived the threshold of $h^2 \geq 0.20$ (Table 4) (1 out of 36 voxels = 3 %). Analyses on the association with SA for the three other contrasts of interest ('all faces early > all faces late'; 'negative conditioned face > neutral conditioned face'; 'negative conditioned face > positive conditioned face'; the contrast 'positive conditioned face > neutral conditioned face' was not further investigated because of the lack of amygdala activation at the group-level; cf. Section 3.3.1) did not yield significant results within the amygdala.

4. Discussion

Here, we demonstrated initial evidence for amygdala hyperactivation, in response to faces conditioned with a social-evaluative meaning, as a putative neurobiological social anxiety disorder (SAD)-endophenotype. Using a conditioning paradigm with high ecological validity in the context of SAD, in a unique sample of families genetically enriched for SAD (n = 105) (Bas-Hoogendam et al., 2018a), we showed that amygdala reactivity *co-segregated with social anxiety within families of probands* (endophenotype criterion 4, first element); furthermore, several voxels within these amygdala clusters displayed at least moderate ($h^2 \geq 0.20$) *heritability* (endophenotype criterion 3). Thereby, we extend previous work on the role of the amygdala in SAD (see summary by (Bas-Hoogendam et al., 2016) and the work on the habituation phase of the NFP in this sample, where we reported a relationship between social anxiety and impaired habituation of the amygdala response (Bas-Hoogendam et al., 2019b), and offer novel insights into the genetic vulnerability to SAD.

4.1. Amygdala hyperreactivity during social-evaluative learning

The positive association between SA-level and amygdala activation to social-evaluative conditioned faces (conditioned stimuli, CS) confirmed our a-priori prediction, which was based on a previous neuroimaging study reporting increased SA-related amygdala activation during conditioning of socially threatening stimuli (Pejic et al., 2013). Here, we extend these findings, by using a paradigm which included three types of social evaluation (negative, neutral and positive; unconditioned stimuli, US), and demonstrated amygdala hyperreactivity within SAD patients as well as their family-members.

Interestingly, although the analyses using other contrasts of interest, defined to determine amygdala activation during the course of the social-evaluative conditioning phase (SCP; contrast 'all faces early > all faces late') and related to the three different US conditions ('negative conditioned face > neutral conditioned face'; 'negative conditioned face > positive conditioned face'), revealed amygdala engagement at the group-level, in line with the results of Davis and colleagues (2010), they did not yield significant associations with SA. These results suggest that the SA-related amygdala hyperreactivity seems not to differ between the first and last half of the SCP, nor did these findings support

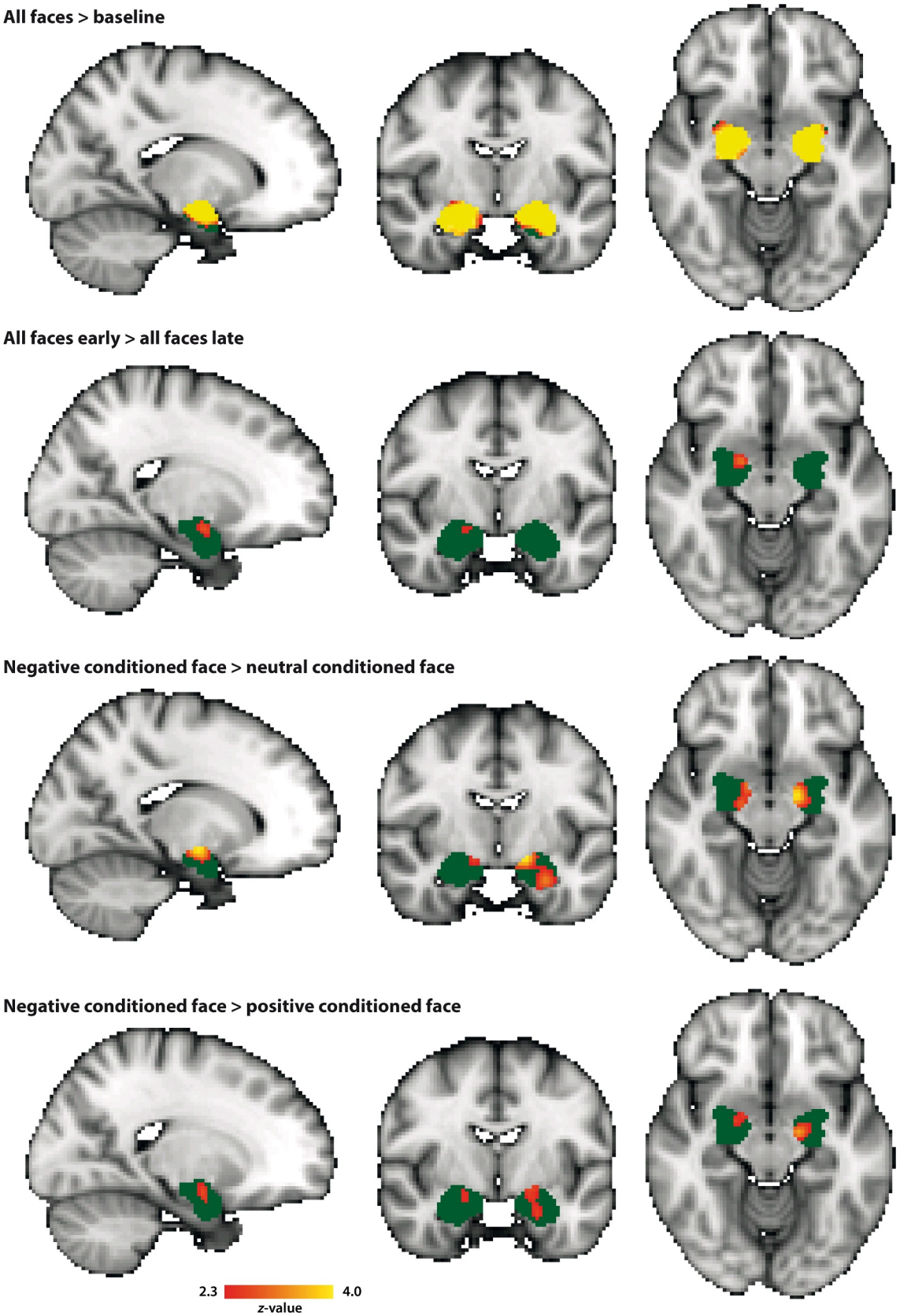

Fig. 4. Amygdala activation (group-level). Activation related to contrasts of interest within the amygdala regions of interest (depicted in green), over the whole sample (n = 105). The contrast 'positive > neutral' did not yield significant amygdala activation. Coordinates displayed slices (MNI space, x,y,z): −16, −8, −12 (contrasts 'all faces > baseline' and 'negative conditioned face > neutral conditioned face') and 20, −6, −12 (contrasts 'all faces early > all faces late' and 'negative conditioned face > positive conditioned face'). Images are displayed according to radiological convention: right in the image is left in the brain.

Table 4
Effect of self-reported social anxiety on neutral face processing

Region	Left/right	*Z*-score	Peak coordinates (MNI space) x	y	z	Cluster size	Number of voxels with $h^2 > 0.20$	Mean h^2, range
All faces > baseline								
Amygdala	Left	2.65	−28	−6	−14	36	1	0.27, n.a.
	Right	3.01	28	−10	−14	164	22	0.31, 0.20–0.63

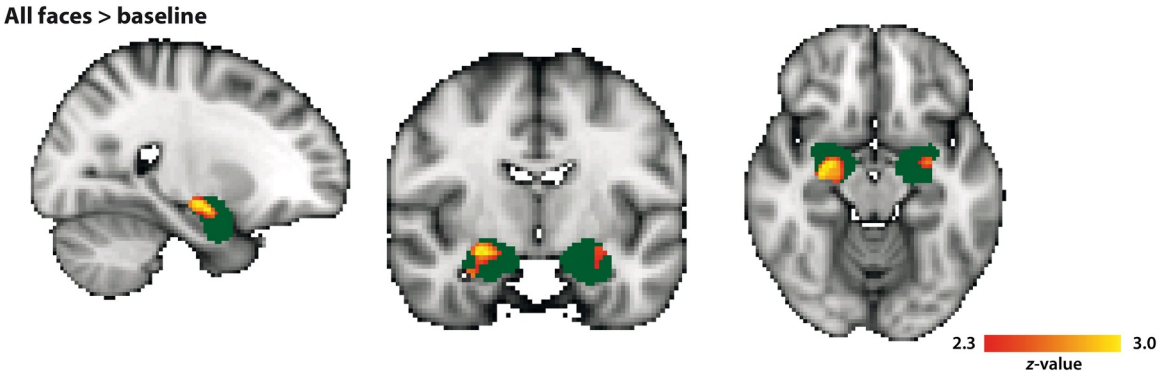

Fig. 5. Association between social anxiety and brain activation in the amygdala. Amygdala activation related to viewing faces conditioned with a social-evaluative meaning (versus baseline) co-segregates with social anxiety within families. Significant positive associations between social anxiety and activation were present in both the left (36 voxels) and right (164 voxels) amygdala. Coordinates displayed slices (MNI space, *x,y,z*): 24, −8, −14. Images are displayed according to radiological convention: right in the image is left in the brain.

our hypothesis that this amygdala hyperreactivity would be most prominent for faces conditioned with negative and positive sentences, although we obviously cannot exclude that the lower statistical power inherent to these difference contrasts (i.e. containing less trials) limited us to detect significant effects of SA. We argue that these results reflect that the amygdala hyperreactivity in family-members with high SA-levels is related to the social-evaluative context of the SCP, in which participants were directly addressed ("He says you are …"), rather than to the valence of the sentences (for example, "He says you are boring" (negative), "He says you are smart" (positive) or "He says you are in Leiden" (neutral)). This idea is supported by the behavioral data, as these showed that family-members with higher SA-levels rated all faces as less likeable after conditioning, independent from the value of the conditioning sentences. Together, these findings underscore the increased saliency of social information, being it negative, positive, or neutrally loaded, in social anxiety, which was present even without a cover story (note that we did not pretend that the faces belonged to 'real people' who did judge the participants in real-life; cf. (Harrewijn et al., 2018)). Complementing this idea is the hypothesis that socially anxious individuals are vigilant and cautious in every condition of the SCP, because due to their tendency to generalize, they fear that all three faces could be followed by negative comments, although, in reality, only one third of the trials was negative (cf. the work by (Everaert et al., 2018) describing inflexible negative interpretations in social anxiety, and the study by (Haller et al., 2016) revealing negative interpretation biases and increased negative internal attributions in adolescents with higher social anxiety). This interpretation coincides with work in SAD patients revealing increased amygdala activation even during the cued anticipation of emotional stimuli without specific social content (Brühl et al., 2011), and the results of Cooney et al. (Cooney et al., 2006) which suggest that neutral faces might be evaluated differently by socially anxious individuals.

The present results concur with contemporary models of social anxiety, acknowledging the multidimensional nature of the disorder (Reichenberger and Blechert, 2018). For example, as illustrated by a recent study, SAD patients displayed elevated scores on fear of negative evaluation as well as on fear of positive evaluation, combined with altered psychophysiological responses to negative as well as to positive social-evaluative videos (Reichenberger et al., 2019). Our findings support the view that social anxiety involves fear and avoidance of all potential social-evaluative interpersonal interactions (Miskovic and Schmidt, 2012), and emphasize that, although the fear of negative evaluation is especially prominent in SAD, the central fear in socially anxious individuals concerns the view that their self-characteristics are deficient or contrary to perceived societal expectations (Moscovitch, 2009). It is of importance to acknowledge this comprehensive fear in cognitive-behavioral therapy for SAD.

Furthermore, our results broaden the knowledge with respect to amygdala overreactivity in SAD. To start, our findings add to those described in a previous paper on the LFLSAD sample, in which we outline a specific form of amygdala hyperreactivity in socially anxious participants, namely an impairment in the adaptive decline of amygdala activation over time (habituation response) (Bas-Hoogendam et al., 2019b). In addition, a recent meta-analysis indicated that SA is associated with increased amygdala responsiveness related to face perception processing (Gentili et al., 2016), and it is commonly hypothesized that amygdala hyperreactivity is reflective of the heightened threat processing that characterizes SAD (Brühl et al., 2014). Indeed, hyperactivation of the amygdala in response to socially-relevant stimuli has been repeatedly reported in SAD patients, as well as in children and adolescents with anxiety disorders (Blair et al., 2011; Ferri et al., 2014; Figel et al., 2019; Kraus et al., 2018; Williams et al., 2015). However, to the best of our knowledge, the present results are the first demonstrating amygdala hyperreactivity in response to conditioned faces with a social-evaluative meaning, and the first to detect amygdala overreactivity within a sample of patients with SAD as well as their family-members of two generations.

4.2. Co-segregation within families

The unique multiplex and multigenerational family-design of the LFLSAD enabled us to investigate two endophenotype criteria within the same sample, namely the *co-segregation within families* and the *heritability* of the candidate endophenotype. In addition to the association between amygdala hyperreactivity and the level of SA within the families, our data revealed that amygdala hyperactivation in a subset of

voxels displayed moderate to even high heritability. Thereby, our results extend previous work reporting genetic influences on amygdala activation (cf. (Bas-Hoogendam et al., 2016)) and indicate that amygdala hyperreactivity is not just a biomarker of SAD (a characteristic associated with the disorder, which is not necessarily positioned on the pathway from genotype to phenotype; cf. (Beauchaine and Constantino, 2017; Lenzenweger, 2013)), but reflective of the genetic vulnerability to SAD, thus providing a starting point for the development of preventive and therapeutic interventions (Beauchaine et al., 2008). Furthermore, the present findings open the way for future imaging studies exploring (epi)genetic variations underlying amygdala responsivity, in line with the work of Furmark et al. (Furmark et al., 2009) and Ziegler and colleagues (Ziegler et al., 2015).

4.3. Amygdala function, structure and connectivity

In the present study, we used a mask of the extended amygdala, based on previous work using this paradigm (Davis et al., 2010), and in line with theories on the role of the extended amygdala in conditioning and treat processing (Fox et al., 2015; Shin and Liberzon, 2010). The amygdala consists of several subnuclei, being the laterobasal, centromedial, and superficial nucleus, with distinct connectivity patterns with other brain regions (Kerestes et al., 2017; Roy et al., 2009); furthermore, these connectivity patterns display different relationships with anxiety-related temperamental traits (Blackford et al., 2014; Roy et al., 2014). According to a probabilistic atlas (Amunts et al., 2005), the hyperreactivity of the amygdala in the present study maps to the bilateral laterobasal nuclei. These nuclei receive information from sensory cortical regions, frontal brain areas and subcortical regions, and play a role in associative processing of environmental cues and the integration of this information with self-relevant cognition (Bzdok et al., 2013). Future studies could explore if there are SA-related changes in connectivity of these nuclei (cf. (Pannekoek et al., 2013; Prater et al., 2013)), and whether such alterations are heritable.

Furthermore, it is interesting to note that, in contrast to the consistent findings with respect to amygdala hyperactivation in SAD, findings on SAD-related alterations in amygdala structure are inconclusive (Brühl et al., 2014). However, both a recent mega-analysis (Bas-Hoogendam et al., 2017b) as well as a recent meta-analysis ((Wang et al., 2018) cf. (Bas-Hoogendam, 2019)) did not report structural alterations in the amygdala in SAD patients, while we, in a previous study on the LFLSAD sample, did not detect SA-related differences in amygdala volume in socially anxious families (Bas-Hoogendam et al., 2018b). Together, these findings suggest that alterations in amygdala function, rather than in its structure, are associated with SAD.

Related to this topic, it is worthwhile to mention that previous work revealed that variability in amygdala volume is moderate-to-highly heritable (den Braber et al., 2013; Satizabal et al., 2019; Swagerman et al., 2014); in a previous report on the LFLSAD sample, we replicated these heritability estimates for the volume of the left (but not the right) amygdala (Bas-Hoogendam et al., 2018b). As the findings of the present study were mainly right-lateralized, and we did not find relationships between SA-level and amygdala volume in our previous work (Bas-Hoogendam et al., 2018b), we hypothesize that the reported hyperreactivity of the amygdala is independent from volumetric amygdala changes.

4.4. No effect of SA on initial likeability ratings

In the present study, we did not find a significant relation between SA-level and the initial response (likeability ratings) to the neutral faces, contradicting previous work reporting that patients with SAD rate neutral faces more negative than healthy controls (Amir et al., 2005; Bell et al., 2011; Peschard and Philippot, 2017), probably because they are inclined to interpret ambiguous social stimuli as more negative due to information-processing biases (Hirsch and Clark, 2004). Based on our data, we are unable to exclude the possibility that the present null findings are due to the fact that the scale used for the likeability was not sensitive enough to capture individual differences in how faces were rated; however, it is important to note that the effects in previous studies were in general small (for example, only present at specific time-points (Amir et al., 2005)), and other studies reported contradictory findings. For example, Stein et al. (2002) did not find significant differences between SAD patients and HC on emotional ratings of facial expressions, while Goldin et al. (2009) reported no differences in ratings of neutral scenes between SAD patients and HC. In addition, Melfsen and Florin found no indication of an enhanced ability to decode negative facial expressions in socially anxious children, nor did these kids have a specific tendency to interpret neutral or positive faces as negative (Melfsen and Florin, 2002). Our findings are in line with these results, and stress the need for future behavioral studies on the relationship between SA and the interpretation of neutral faces.

4.5. Limitations and future research

The LFLSAD was especially designed to investigate the endophenotype criteria of *co-segregation* and *heritability*. Longitudinal studies involving control families from the general population are essential to assess other endophenotype criteria, like the *trait-stability of the candidate endophenotype* (criterion 2) and the *difference between non-affected family-members and participants from the general population* (criterion 4, second element). Furthermore, as the present work focused on the amygdala as an a priori defined, hypothesis-based region of interest, and we only performed an exploratory whole-brain analysis on the association with SA with a stringent statistical threshold, we might have missed functional SA-related alterations in other brain areas. For example, a recent study on reversal learning indicated that trait SA influenced learning rate-related activation of the dorsal anterior cingulate cortex (Piray et al., 2018), while Blair et al. (2016) reported, besides amygdala hyperactivation, increased responsiveness of frontal and parietal cortices during social reference learning in SAD patients. Future studies could explore whether these regions display SA-related functional alterations during social conditioning as well. Moreover, owing to the complexity of the present association analyses, in which we accounted for the family structure of the data, we were at present not able to perform more advanced MRI analyses like physophysiological interaction (PPI) analyses (examining whether the SA-related alterations in amygdala activation were accompanied by differences in functional connectivity specific to the task (cf. (Bas-Hoogendam et al., 2015))) or wavelet-based analyses of the time course of amygdala activation.

In addition, the present study focused on the amygdala response associated with social conditioning, in line with the work of Davis et al. (2010), but did not include an extinction phase which would have allowed to examine the result of the learning process and enables investigating whether amygdala hyperreactivity declines when the neutral faces are presented in absence of the social-evaluative sentences. Given recent work demonstrating altered brain responses during extinction in (social) anxiety (Åhs et al., 2017; Belleau et al., 2018; Marin et al., 2017; Pejic et al., 2013), the important role of extinction learning in exposure therapy (Ball et al., 2017; Pittig et al., 2016), and the possibility to use fear extinction as a translational animal model in psychiatric research (Casey et al., 2011; Erhardt and Spoormaker, 2013; Toth et al., 2012), future studies could employ an extended paradigm in which amygdala responses related to extinction are also measured. Furthermore, as data did not reveal time-dependent effects of SA during the course of the SCP, nor related to the different US conditions, we can't exclude that the amygdala hyperreactivity to the faces (CS) reflects a general higher response to neutral faces in socially anxious individuals, independent from conditioning. This topic is worthy of future investigation. In addition, given the so-called 'own-age' bias which applies to face recognition (Rhodes and

Anastasi, 2012), it would be interesting to repeat the experiment while using age-adjusted neutral face stimuli (same age-group as the participant). Presenting such individualized stimuli might further improve the sensitivity to observe associations between brain activation and SA-level. Finally, it should be noted that, besides genetic influences, environmental factors are important in the development and maintenance of SAD (Bas-Hoogendam et al., 2019a; Scaini et al., 2014; Wong and Rapee, 2016); adverse life events, for example, play a role in the onset of (social) anxiety disorders (Brook and Schmidt, 2008; Miloyan et al., 2018; Norton and Abbott, 2017), and gene-environment interactions with negative life-events, with respect to anxiety in children and adolescents, have been recently reported (Kneer et al., 2019). We did, however, not acquire data on life events, so we are not able to investigate the impact of negative as well as positive life events on SA-level and amygdala activation.

5. Conclusion

In conclusion, the results of the present study provide evidence for bilateral amygdala hyperactivation in response to conditioned faces with a social-evaluative meaning as a candidate neurobiological SAD endophenotype. As such, these findings shed novel light on the genetic susceptibility to SAD.

CRediT authorship contribution statement

Janna Marie Bas-Hoogendam: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Writing - original draft, Visualization. **Henk van Steenbergen:** Conceptualization, Formal analysis, Methodology, Resources, Software, Supervision, Writing - review & editing. **Nic J.A. van der Wee:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing. **P. Michiel Westenberg:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nicl.2020.102247.

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