Shy parent, shy child ? : delineating psychophysiological endophenotypes of social anxiety disorder

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Chapter 4

Delta-beta correlation as a candidate endophenotype of social anxiety: A two-generation family study

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Abstract

Social anxiety disorder (SAD) is characterized by an extreme and intense fear and avoidance of social situations. In this two-generation family study we examined delta-beta correlation during a social performance task as candidate endophenotype. Nine families with a target participant (diagnosed with SAD), their spouse and children, as well as target's siblings with spouse and children performed a social performance task in which they gave a speech in front of a camera. EEG was measured during resting state, anticipation, and recovery. Our analyses focused on two criteria for endophenotypes: co-segregation within families and heritability. Co-segregation analyses revealed increased negative delta-low beta correlation during anticipation in participants with (sub)clinical SAD compared to participants without (sub)clinical SAD. Heritability analyses revealed that delta-low beta and delta-high beta correlations during anticipation were heritable. Delta-beta correlation did not differ between participants with and without (sub)clinical SAD during resting state or recovery, nor between participants with and without SAD during all phases of the task. Delta-low beta correlation during anticipation of giving a speech might be a candidate endophenotype of SAD, possibly reflecting increased crosstalk between cortical and subcortical regions. If validated as endophenotype, delta-beta correlation during anticipation could be useful in studying the genetic basis, as well as improving treatment and early detection of persons at risk for developing SAD.

Introduction

Patients with social anxiety disorder (SAD) show extreme fear and avoidance in one or more social situations in which they could experience scrutiny by others (APA, 2013). SAD is a common, debilitating anxiety disorder with a life-time prevalence between 7 and 13% in Western societies (Furmark, 2002; Rapee & Spence, 2004) and severe personal, relational, professional, and economic consequences (Acarturk et al., 2008; Dingemans et al., 2001; Lampe et al., 2003; Wittchen et al., 1999). Previous studies have shown that, besides environmental factors, genetic factors play an important role in the patho-etiology of SAD. That is, family members of patients with SAD have a higher risk of developing SAD than family members of controls (Isomura et al., 2015; Lieb et al., 2000). Heritability of SAD is estimated around 20-56 % (Distel et al., 2008; Isomura et al., 2015; Kendler et al., 1992; Middeldorp et al., 2005; Nelson et al., 2000). A useful method for studying the genetic basis of psychiatric disorders in more detail is by focusing on endophenotypes (Gottesman & Gould, 2003). Studying endophenotypes has advanced understanding of psychiatric disorders such as depression (Goldstein & Klein, 2014) and schizophrenia (Bramon et al., 2005; Glahn et al., 2007; Gottesman & Gould, 2003). Therefore, the goal of the current study is to delineate candidate electrocortical endophenotypes of SAD.

Endophenotypes are genetic trait markers of a disorder, between the genotype and phenotype. To be considered an endophenotype, a trait should be a) associated with the disorder, b) heritable, c) primarily state-independent, d) co-segregate with the disorder within families, and e) increased in non-affected family members compared to the general population (Glahn et al., 2007; Gottesman & Gould, 2003). Endophenotypes could be useful in unraveling genetic factors influencing the development of SAD, because the genetic basis is proposed to be simpler than the genetic basis of complex psychiatric disorders (Cannon & Keller, 2006; Glahn et al., 2007). Endophenotypes could also yield better understanding of the biological mechanisms underlying SAD (Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013), that could help in interpreting genetic findings (Flint et al., 2014). Finally, endophenotypes could be used to identify individuals at risk for developing SAD. Electrocortical endophenotypes are specifically useful because they are presumably more closely related to genes than cognitive-behavioral endophenotypes (Cannon & Keller, 2006).

A putative electrocortical endophenotype of SAD is delta-beta cross-frequency correlation (further referred to as ‘delta-beta correlation’) during socially stressful situations (Harrewijn, Schmidt, Westenberg, Tang, & Van der Molen, 2017). Delta-beta correlation has

been hypothesized to reflect the crosstalk between cortical (as reflected in beta power [14-30 Hz]) and subcortical brain regions (as reflected in delta power [1-4 Hz]) (Miskovic, Moscovitch, et al., 2011; Putman et al., 2012; Schutter & Knyazev, 2012; Schutter et al., 2006; Schutter & Van Honk, 2005; Velikova et al., 2010), which is increased at elevated levels of anxiety (Knyazev, 2011; Knyazev et al., 2006; Schutter & Knyazev, 2012). Source-localization analyses have revealed that delta-beta correlation was associated with a neural network that comprised the orbitofrontal cortex and the anterior cingulate cortex (Knyazev, 2011), key neural structures playing an important role in affective control processes (Bechara, Damasio, & Damasio, 2000; Devinsky, Morrell, & Vogt, 1995). The endophenotype criterion 'association' has already been confirmed in previous studies: social anxiety is associated with stronger delta-beta correlation during anticipation of (Harrewijn et al., 2016; Miskovic et al., 2010; Miskovic, Moscovitch, et al., 2011) and recovery from giving a speech (Harrewijn et al., 2016). Results during resting state appear to be mixed (Harrewijn et al., 2016; Miskovic et al., 2010; Miskovic, Moscovitch, et al., 2011).

The present study was designed to investigate whether delta-beta correlation during anticipation and recovery meets the endophenotype criteria 'co-segregation within families' and 'heritability'. We used a two-generation family design, because examining extended families is better to identify genetic variability and therefore heritability than examining twins or sib-pairs (Gur et al., 2007; Williams & Blangero, 1999). In addition, we selected families based on two probands (adult with SAD and child with (sub)clinical SAD; ascertainment), to ensure we did not focus on a spurious or nongenetic form of SAD and to increase the chance that endophenotypes were related to the genetic factors that influence SAD (Fears et al., 2014; Glahn et al., 2010). To our knowledge, no studies exist that have used a two-generation family design to examine electrocortical endophenotypes of SAD. Adults with SAD and their family members participated in a social performance task (SPT) to elicit social stress (J. F. Van Veen et al., 2009; Westenberg et al., 2009). We measured EEG in all participants during resting state, anticipation and recovery from this socially stressful situation. We expected that delta-beta correlation would be an endophenotype of SAD during anticipation and recovery, but not during resting state (Harrewijn et al., 2017).

Methods

Participants

This was the first study to intensively investigate patients with SAD and their family members – their spouse and children, and the target's siblings with spouse and children. We investigated extended pedigrees instead of nuclear families since larger families result in more power than smaller families (Dolan, Boomsma, & Neale, 1999; Gur et al., 2007; Rijsdijk, Hewitt, & Sham, 2001; Williams & Blangero, 1999). In total, 9 families (total n = 132, on average 14.67 members per family, range 4-35) participated in the Leiden Family Lab study on SAD. Families were recruited via media exposure (newspapers, TV, radio) calling for participation of entire families in a study on 'extreme shyness'.

We selected families based on two probands: one 'target participant' with SAD and one child of the 'target participant' with clinical or subclinical SAD (further referred to as '(sub)clinical SAD'). SAD was diagnosed based on the Mini-Plus structured interview (Sheehan et al., 1998; Van Vliet & De Beurs, 2007), using the DSM-IV-R criteria for SAD generalized subtype. In addition, the psychiatrist made sure that these patients also satisfied DSM-5 criteria. Subclinical SAD was defined as meeting the criteria for SAD, without showing impairment in important areas of functioning (criterion G in the DSM-5 (APA, 2013)).

Nine participants did not participate in the EEG session, and data of 10 participants were excluded due to technical problems. Of the 113 participants taking part in the EEG session, several participants did not finish because of different reasons (e.g. some participants only wanted to participate in resting state measures, others did not want to give a speech, a few children were too tired). Supplementary table 1 displays the number of participants per measure. Of these 113 participants 18 were diagnosed with SAD (15.9%), and 25 were diagnosed with subclinical SAD (22.1%), thus, 43 participants were diagnosed with (sub)clinical SAD.

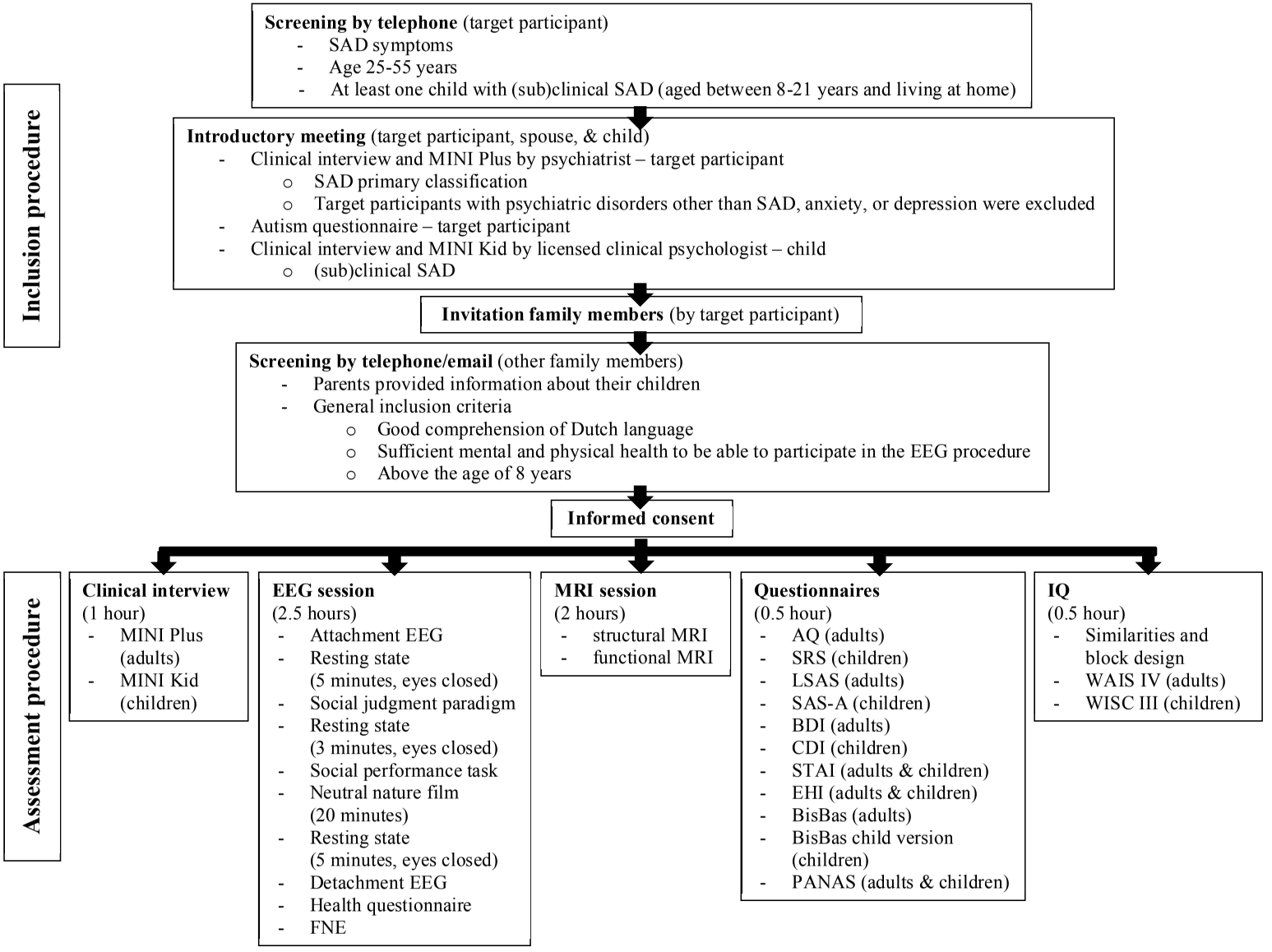

Inclusion procedure
Screening by telephone (target participant)
- SAD symptoms
- Age 25-55 years
- At least one child with (sub)clinical SAD (aged between 8-21 years and living at home)
Introductory meeting (target participant, spouse, & child)
- Clinical interview and MINI Plus by psychiatrist – target participant
o SAD primary classification
o Target participants with psychiatric disorders other than SAD, anxiety, or depression were excluded
- Autism questionnaire – target participant
- Clinical interview and MINI Kid by licensed clinical psychologist – child
o (sub)clinical SAD
Invitation family members (by target participant)
Screening by telephone/email (other family members)
- Parents provided information about their children
- General inclusion criteria
o Good comprehension of Dutch language
o Sufficient mental and physical health to be able to participate in the EEG procedure
o Above the age of 8 years
Informed consent
Assessment procedure
Clinical interview
(1 hour)
- MINI Plus (adults)
- MINI Kid (children)
EEG session
(2.5 hours)
- Attachment EEG
- Resting state (5 minutes, eyes closed)
- Social judgment paradigm
- Resting state (3 minutes, eyes closed)
- Social performance task
- Neutral nature film (20 minutes)
- Resting state (5 minutes, eyes closed)
- Detachment EEG
- Health questionnaire
- FNE
MRI session
(2 hours)
- structural MRI
- functional MRI
Questionnaires
(0.5 hour)
- AQ (adults)
- SRS (children)
- LSAS (adults)
- SAS-A (children)
- BDI (adults)
- CDI (children)
- STAI (adults & children)
- EHI (adults & children)
- BisBas (adults)
- BisBas child version (children)
- PANAS (adults & children)
IQ
(0.5 hour)
- Similarities and block design
- WAIS IV (adults)
- WISC III (children)

Figure 1. Flow-chart of the inclusion and assessment procedures of the Leiden Family Lab study on SAD. All family members performed the same parts of the family study (as depicted in assessment procedure), but the order of the parts differed between family members, dependent on their preferences and availability of the labs. Mostly, family members came together to the lab.

Note: One target participant scored high on the autism questionnaire, but a psychiatrist confirmed that SAD was the correct diagnosis. Results of the social judgment paradigm (Van der Molen et al., 2014) will be reported elsewhere. SAD = social anxiety disorder; MINI Plus = Mini-Plus International Neuropsychiatric Interview (MINI Plus version 5.0.0) (Sheehan et al., 1998; Van Vliet & De Beurs, 2007); MINI Kid = MINI Kid interview (Bauhuis et al., 2013; Sheehan et al., 2010); FNE = Fear of Negative Evaluation (Carleton et al., 2006); AQ = Autism-Spectrum Quotient Questionnaire (Baron-Cohen et al., 2001); SRS = Social Responsiveness Scale
(parent-rated) (Constantino et al., 2003); LSAS = Liebowitz Social Anxiety Scale (Liebowitz, 1987); SAS-A = Social Anxiety Scale – adolescents (La Greca & Lopez, 1998); BDI = Beck Depression Inventory (Beck et al., 1996); CDI = Child Depression Inventory (Kovacs, 1992); STAI = State-Trait Anxiety Inventory (Spielberger et al., 1983); EHI = Edinburgh Handedness Inventory (Oldfield, 1971); BisBas = Behavioral Inhibition and Behavioral Activation Scales (Carver & White, 1994); BisBas child version = Behavioral Inhibition and Behavioral Activation Scales, child version (Muris et al., 2005); PANAS = Positive and Negative Affect Scale (Watson et al., 1988); WAIS IV = Wechsler Adult Intelligence Scale IV (Wechsler et al., 2008); WISC III = Wechsler Intelligence Scale for Children III (Wechsler, 1991).

Procedure

Figure 1 depicts a flow-chart of the inclusion and assessment procedures of our Leiden Family Lab study on SAD, and lists the inclusion criteria. All participants provided informed consent, according to the Declaration of Helsinki (1991). Both parents signed the informed consent form for their children, and children between 12 and 18 years signed themselves as well. Every participant received €75 for their participation and we reimbursed travel expenses. The procedure was approved by the medical ethics committee of the Leiden University Medical Center.

Social performance task

The SPT (Harrewijn et al., 2016) comprised five phases in a fixed order: instruction, video, anticipation, speech, and recovery (Figure 2). We added an extended recovery phase to allow for cortisol measures (the results will be reported elsewhere). Participants did not know

beforehand about this task, so we started with an instruction. Participants then viewed a video of a peer, who talked about her positive and negative qualities (see Supplementary data 1 for validation of the videos in an independent sample). Thereafter, participants were asked to evaluate this peer (Supplementary figure 1). During the anticipation phase, participants prepared a speech about their own positive and negative qualities. Then, participants indicated on a VAS how they expected that their speech would be evaluated by a peer (Supplementary figure 1). Participants gave a three-minute speech in front of a video camera, and were told that their speech would be evaluated by a peer at a later moment. However, this was a cover story to induce social evaluative stress. The SPT ended with the recovery phase in which participants had five minutes to relax, and a neutral nature film that the participants watched for 20 minutes. After the EEG procedure, participants were debriefed and asked not to tell their family members about the SPT, and all but one participant reported that they did not know beforehand about the SPT.

Task-induced mood. To validate whether the SPT indeed elicited more social stress in participants with SAD or (sub)clinical SAD, we asked participants to report on a visual analogue scale (VAS) from 0 (‘not at all’) to 100 (‘very much’) how nervous they felt at six time points and how much they felt like doing the next part of the experiment at five time points (Figure 2). This latter question was used to indirectly measure avoidance, because in our view it was not ethical to ask participants five times if they wanted to avoid the situation and do nothing about it.

Figure 2. Overview of the social performance task.

Adapted from Cognitive, Affective & Behavioral Neuroscience, Harrewijn, A., Van der Molen, M.J.W., & Westenberg, P.M., Putative EEG measures of social anxiety: Comparing frontal alpha asymmetry and delta-beta cross-frequency correlation, Copyright (2016), with permission. Photo indicating neutral nature film from Matsubara, B. (Photographer) (2017, April 27). *Spotted Towhee* [digital image]. Retrieved from https://www.flickr.com/photos/130819719@N05/33925138900/

EEG recording and signal processing

We used the same procedure for EEG recording and signal processing as in Harrewijn et al. (2016). EEG was recorded from 64 Ag-AgCl electrodes mounted in an electrode cap (10/20 placement) using the BioSemi Active Two system (Biosemi, Amsterdam, The Netherlands). Sampling rate was set at 1024 Hz. The common mode sense and driven right leg replaced the conventional ground electrode, and common mode sense was used as online reference. Two electrodes above and below the left eye measured vertical eye movements, and two electrodes at left and right canthus measured horizontal eye movements. Two electrodes were placed at left and right mastoid for offline re-referencing. Two electrodes (under the right collar bone

and between the ribs on the left side) measured heart rate via the modified lead-2 placement (data will be reported elsewhere).

EEG data was offline analyzed with BrainVision Analyzer (BVA, Brain Products GmbH, Gilching, Germany). EEG channels were re-referenced to the average of all EEG electrodes, and filtered between 0.1-50 Hz (24 dB/oct), with a 50 Hz notch filter. We created epochs of 4 sec (4096 samples) with 1 sec (1024 samples) overlap, and manually inspected for artifacts. Noisy channels were interpolated, and eye movements were subtracted from the data with the ocular independent component analysis as implemented in BrainVision Analyzer Epochs were automatically excluded based on the following criteria: maximal allowed voltage step: 50 μV/ms; minimum/maximum amplitude: -200/200 μV; lowest allowed activity in 100 ms intervals: 0.5 μV. If an artifact was found in one channel, the entire epoch was removed during both manual and automatic artifact rejection. Participants with and without (sub)clinical SAD did not differ in their number of clean epochs per phase of the task, all *p*s > 0.19 (Supplementary table 2)[6]. Finally, we ran a fast Fourier transform analysis with a 50% Hanning window to extract relative power (μV^2) from the delta (1-4 Hz), total beta (14-30 Hz), low beta (14-20 Hz), and high beta (20-30 Hz) frequency bands per epoch. Power values for electrodes F3, Fz, F4 were averaged into composite frontal delta and frontal beta power values (Harrewijn et al., 2016; Putman, 2011; Putman et al., 2012). For each participant separately, we calculated the correlation between log-transformed delta power and log-transformed total, low, or high beta power across all epochs per phase of the SPT.

Statistical analysis

We performed all analyses separately for SAD and (sub)clinical SAD, because only few people (*n* = 18) were diagnosed with SAD, which might influence power. First, we verified the differences between participants with and without SAD or (sub)clinical SAD by modeling the relation between SAD or (sub)clinical SAD and self-reported symptoms of social anxiety and depression. Z-scores based on means and standard deviations of normative samples (Fresco et al., 2001; Inderbitzen-Nolan & Walters, 2000; Miers et al., 2014; Roelofs et al., 2013) were calculated to enable comparisons between adult and child questionnaires. Regression models were fitted in R (R Core Team, Vienna, Austria) with self-report questionnaires as dependent variable and SAD, age, age^2, and sex as independent variables.

[6] The number of clean epochs during the second resting state was related to delta-high beta correlation during the second resting state, there were no other correlations between the number of clean epochs and personal characteristics, task-induced mood or EEG measures.

Because the participants in this study were not independent, we modeled genetic correlations between family members by including random effects.

Second, we validated whether the SPT elicited more social stress in participants with SAD or (sub)clinical SAD by modeling the relation between SAD or (sub)clinical SAD and task-induced mood across several time points during the SPT. One regression model was fitted with task-induced mood as dependent variable and time (as a factor), age, age^2 and sex as independent variables. An additional regression model also included the interaction time X SAD or (sub)clinical SAD. We included random effects for taking into account genetic correlations between family members and existing correlations between measurements at various time points within a person. The effect of SAD or (sub)clinical SAD was tested using a likelihood ratio test statistic comparing the likelihoods of the regression models with and without SAD or (sub)clinical SAD. Significance of SAD or (sub)clinical SAD at a specific time point was assessed by using Wald tests.

Third, we tested whether delta-beta correlation during the SPT was a candidate endophenotype of SAD, using the two criteria 'co-segregation within families' and 'heritability' (Glahn et al., 2007). For the co-segregation analysis, one regression model was fitted with delta-beta correlation as dependent variable, and time (as a factor), age, age^2, sex as independent variables. An additional regression model also included the interaction time X SAD or (sub)clinical SAD. We included random effects for taking into account genetic correlations between family members and existing correlations between measurements at various time points within a person. The effect of SAD or (sub)clinical SAD was tested using a likelihood ratio test statistic comparing the likelihoods of the regression models with and without SAD or (sub)clinical SAD. This was performed separately for task data (anticipation and recovery – eyes open), and resting state data (first and second – eyes closed). Individual delta-beta correlations were transformed using the Fisher transformation (0.5*ln(1+r/1-r) and then standardized to zero mean and unit variance variables. Note that to assess the relationship between SAD or (sub)clinical SAD and the self-report questionnaires, task-induced mood, or delta-beta correlation no additional ascertainment-corrections were needed because SAD was included as an independent variable which is sufficient to correct for ascertainment (Monsees, Tamimi, & Kraft, 2009).

Heritability analyses were performed using SOLAR (Almasy & Blangero, 1998). Briefly, SOLAR decomposes the total variance of the phenotype into genetic and environmental components. This is estimated using maximum likelihood techniques, based on a kinship matrix for the genetic component and an identity matrix for the unique

environmental component (with ones on the diagonal and zeros everywhere else, implying that the environment is unique to every person). We did not include a shared environmental component (household) in the final analysis, because this did not influence the effects. Heritability is defined as the ratio of the additive genetic component and the total phenotypic variance (after removal of variance explained by covariates) (Almasy & Blangero, 2010). Age, age^2 and sex were used as covariates, and removed from the final model if $p > 0.05$. Correction for ascertainment was necessary because we selected families based on specific criteria (SAD) that are related to the candidate endophenotypes and SAD was not included in the heritability analyses. In SOLAR this is implemented as subtracting the likelihood for the probands (target participant with SAD and child with (sub)clinical SAD) from the likelihood of the rest of the sample (De Andrade & Amos, 2000; Hopper & Mathews, 1982). Since the assumptions for SOLAR (trait standard deviation higher than 0.5, residual kurtosis normally distributed) were not met for most variables, we applied an inverse normal transformation to all EEG variables in this step, as implemented in SOLAR (Almasy & Blangero, 1998, 2010). For candidate endophenotypes that showed significant heritability, we also performed a bivariate analysis in SOLAR to estimate the genetic correlation between the candidate endophenotype and SAD or (sub)clinical SAD, including only the significant covariates. A Bonferroni correction was applied to correct for performing multiple (12) tests (i.e. $\alpha = 0.004$ as threshold for declaring statistical significance). We did not exclude the few outliers, since these were mostly participants with (sub)clinical SAD, of whom we expected extreme scores.

Results

Participant characteristics

First, we verified the differences between participants with and without SAD or (sub)clinical SAD. Table 1 shows the characteristics of the participants with SAD, subclinical SAD and participants without (sub)clinical SAD. The analyses focusing on SAD revealed that participants with SAD were older than participants without SAD, $\beta = 10.75$, $p = 0.01$. There was no difference in estimated IQ, $\beta = -0.52$, $p = 0.85$. Participants with SAD showed more social anxiety and depressive symptoms than participants without SAD, respectively $\beta = 3.08$, $p < 0.001$ and $\beta = 0.95$, $p < 0.001$. The analyses focusing on (sub)clinical SAD (clinical and subclinical together) revealed no differences in age, $\beta = -1.01$, $p = 0.74$, and estimated IQ, $\beta = -1.74$, $p = 0.39$. Furthermore, participants with (sub)clinical SAD showed more social anxiety and depressive symptoms than participants without (sub)clinical SAD, respectively $\beta = 1.83$, $p < 0.001$ and $\beta = 0.51$, $p < 0.001$.

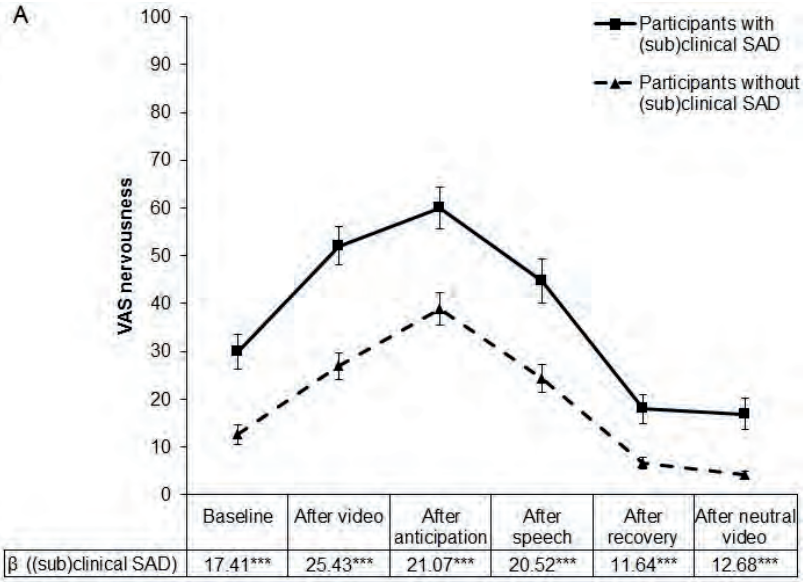
Table 1

Uncorrected means (and standard deviations) of participants with SAD, subclinical SAD, and without (sub)clinical SAD.

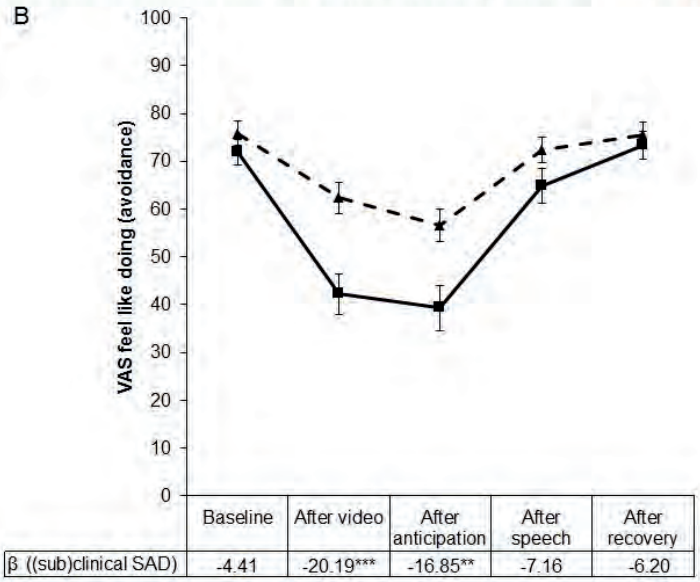
	Participants with SAD (12 females, 6 males)	Participants with subclinical SAD (10 females, 15 males)	Participants without (sub)clinical SAD (35 females, 35 males)
Age	39.67 (13.72)	21.36 (11.54)	29.99 (15.83)
Estimated IQ	106.67 (11.97)	103.00 (11.92)	105.96 (10.61)
Social anxiety (z-score)	3.83 (2.07)	0.69 (1.85)	0.24 (1.16)
Depression (z-score)	0.44 (0.83)	-0.38 (0.64)	-0.55 (0.67)

Task-induced mood

Second, we analyzed task-induced mood to validate whether the SPT elicited more social stress in participants with SAD or (sub)clinical SAD. Indeed, both SAD and (sub)clinical SAD were related to nervousness during the task, respectively X^2 (6) = 49.33, $p < 0.001$ and X^2 (6) = 34.17, $p < 0.001$ (Figure 3). Nervousness was not influenced by age, age^2 or sex, all *p*s > 0.11. Furthermore, both SAD and (sub)clinical SAD were related to avoidance, respectively X^2 (5) = 25.97, $p < 0.001$ and X^2 (5) = 16.98, $p = 0.005$. Avoidance was not influenced by age and age^2, all *p*s > 0.63, but females felt less like doing the SPT than males in models with SAD and (sub)clinical SAD, respectively $\beta = -12.88$, $p < 0.001$, and $\beta = -12.78$, $p < 0.001$. Figure 3 shows the time points on which participants with and without (sub)clinical SAD differ significantly.



	Baseline	After video	After anticipation	After speech	After recovery	After neutral video
β ((sub)clinical SAD)	17.41***	25.43***	21.07***	20.52***	11.64***	12.68***



	Baseline	After video	After anticipation	After speech	After recovery
β ((sub)clinical SAD)	-4.41	-20.19***	-16.85**	-7.16	-6.20

Figure 3. Task-induced nervousness (A) and avoidance (B) for participants with and without (sub)clinical SAD (since analyses of delta-beta correlation also focused on (sub)clinical SAD). Error bars represent standard error of the mean, means are uncorrected.

** $p < 0.01$; *** $p < 0.001$

Delta-beta correlation

Third, we tested whether delta-beta correlation during the SPT was a candidate endophenotype of SAD by focusing on co-segregation within families and heritability. Since we found no co-segregation within families between SAD and delta-beta correlation, we only reported the findings of (sub)clinical SAD (Figure 4). See Supplementary data 2 for results of frontal alpha asymmetry.

Social performance task. Co-segregation analyses showed that (sub)clinical SAD was related to delta-low beta correlation during anticipation and recovery, X^2 (2) = 6.04, p = 0.049. Age, age^2, and sex also influenced delta-low beta correlation during the SPT. Females show more negative delta-beta correlation than males, β = -0.38, p = 0.01. Age is positively related to delta-low beta correlation, β = 0.07, p = 0.01, and also in a non-linear way, β = -0.001, p = 0.001, revealing more negative delta-beta correlation in the youngest and oldest participants. Individual betas indicated that participants with (sub)clinical SAD showed significantly more negative delta-low beta correlation during anticipation, β = -0.47, p = 0.01, but not during recovery, β = -0.09, p = 0.63. Delta-total beta and delta-high beta correlation showed the same pattern, but did not significantly co-segregate with (sub)clinical SAD within families, respectively X^2 (2) = 2.33, p = 0.31, and X^2 (2) = 0.97, p = 0.62.

Heritability analysis showed that delta-low beta and delta-high beta correlations during anticipation were heritable (Table 2). However, if we corrected for performing multiple tests, these results did not remain significant. Bivariate analyses showed that the genetic correlation between delta-low beta correlation during anticipation and (sub)clinical SAD was not significantly different from zero, r = -0.77, SE = 0.46, p = 0.24.

Resting state. Co-segregation analysis showed that (sub)clinical SAD did not co-segregate with delta-total beta, delta-low beta, nor delta-high beta correlation within families during the two resting state phases, all X^2s < 1.53 and ps > 0.46. Heritability analysis showed that only delta-total beta correlation during the second resting state was heritable (Table 2). However, this did not remain significant after correction for performing multiple tests.

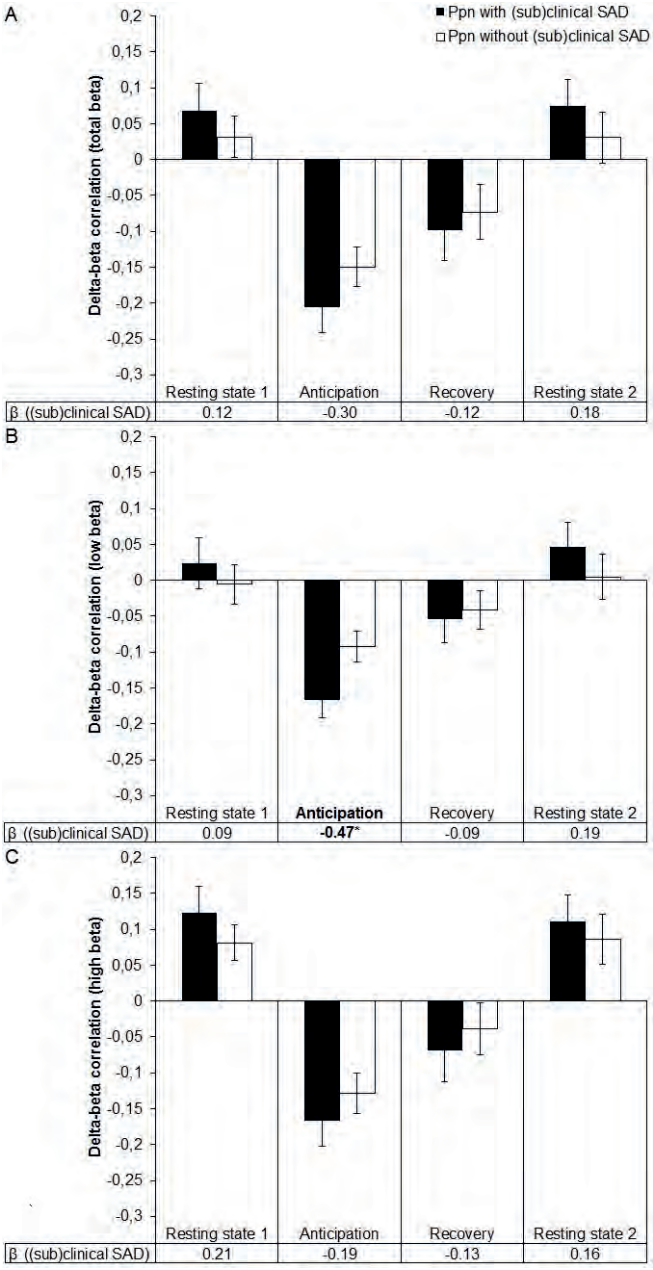


Figure 4. Correlation between delta and total (A), low (B), and high (C) beta power in participants with and without (sub)clinical SAD during the social performance task.

Note: Analyses were done with transformed data, but non-transformed, uncorrected data are shown for clarity. Error bars represent standard error of the mean. * $p < 0.05$

Table 2

Heritability estimates for the correlation between delta and total, low, and high beta during the social performance task.

		Resting state 1	Anticipation	Recovery	Resting state 2
Delta - total beta	h^2	0.00	0.30	0.06	**0.35**
	p (h^2)	0.50	0.07	0.32	**0.04**
	p (age)	0.01	0.02	0.02	0.01
	p (age^2)	< 0.001	0.02	0.05	0.03
	p (sex)	0.18	0.36	0.26	0.27
Delta - low beta	h^2	0.02	**0.37**	0.04	0.24
	p (h^2)	0.43	**0.04**	0.38	0.07
	p (age)	0.12	0.01	0.01	0.13
	p (age^2)	< 0.001	0.13	0.04	0.01
	p (sex)	0.17	0.31	0.12	0.44
Delta - high beta	h^2	0.03	**0.33**	0.14	0.23
	p (h^2)	0.38	**0.04**	0.16	0.11
	p (age)	< 0.001	0.06	0.11	0.001
	p (age^2)	0.04	0.03	0.30	0.36
	p (sex)	0.28	0.36	0.54	0.34

Note: h^2 = heritability.

Discussion

The goal of the current study was to investigate whether delta-beta correlation during anticipation of and recovery from a socially stressful situation is a candidate electrocortical endophenotype of SAD. We used a unique two-generation family design to investigate the endophenotype criteria 'co-segregation within families' and 'heritability' for SAD. Target participants with SAD and their family members participated in a SPT to elicit social stress. We validated our groups and SPT by showing that participants with SAD or (sub)clinical SAD showed increased symptoms of SAD, and increased task-related nervousness and avoidance. Co-segregation analyses for SAD or resting state did not reveal significant effects on delta-beta correlation. Co-segregation analyses revealed that participants with (sub)clinical SAD showed stronger negative delta-beta correlation during anticipation than participants without (sub)clinical SAD. Heritability analyses showed that delta-low beta and delta-high

beta correlations during anticipation were heritable, suggesting that delta-low beta correlation might be a candidate endophenotype of SAD.

Delta-beta correlation is often interpreted as the crosstalk between slow delta waves from subcortical regions and fast beta waves from cortical regions (Miskovic, Moscovitch, et al., 2011; Putman et al., 2012; Schutter & Knyazev, 2012; Schutter et al., 2006; Schutter & Van Honk, 2005; Velikova et al., 2010). The current study showed stronger *negative* delta-beta correlation in (sub)clinical SAD, similar to our previous research (Harrewijn et al., 2016), whereas some other studies showed stronger *positive* delta-beta correlation (Miskovic et al., 2010; Miskovic, Moscovitch, et al., 2011). This might be explained by the use of relative power in this study, whereas other studies have not specified whether they have used absolute or relative power). Or, this might suggest that the relation between delta-beta correlation and stress is not linear but U-shaped, and our SPT is possibly be more stressful than other tasks (indeed, low socially anxious participants also showed increased nervousness during this SPT). These two explanations are described in more detail in Harrewijn et al. (2016). Previously, we argued that negative delta-beta correlation could still be interpreted as increased crosstalk, only in a different direction (Harrewijn et al., 2016). That is, a negative correlation corroborates studies showing an imbalance between cortical and subcortical brain regions in general anxiety (Bishop, 2007) and SAD (Bruhl et al., 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012). This imbalance might be related to increased worrying or rumination, as is often found in cognitive-behavioral studies in SAD (Clark & McManus, 2002; Heinrichs & Hofmann, 2001; Hirsch & Clark, 2004). Delta-beta correlation was not related to (sub)clinical SAD during resting state, like in previous studies with high and low socially anxious participants (Harrewijn et al., 2016; Miskovic et al., 2010) and patients with SAD and controls (Miskovic, Moscovitch, et al., 2011). This might illustrate that a certain social threat is needed to induce worrying or rumination to measure delta-beta correlation as an endophenotype of SAD.

The current study provided an important first step in investigating candidate endophenotypes of SAD. This unique two-generation family design allowed us to investigate two important endophenotype criteria: co-segregation within families and heritability. Although our results suggest that delta-beta correlation is a candidate electrocortical endophenotype of SAD, some caution is warranted with this interpretation. Namely, we did not find this effect for delta-high beta or delta-total beta correlation. Although, other studies focused only on delta-low beta correlation (not on delta-high beta or delta-total beta) and found an effect of social anxiety (Miskovic et al., 2010; Miskovic, Moscovitch, et al., 2011).

In our previous study in high and low socially anxious participants, we did not find an effect on delta-low beta correlation, only on delta-total beta correlation (Harrewijn et al., 2016). However, this sample was not comparable to the current study in terms of age and gender. We also need to be careful because the results were not significant for SAD, nor after correction for performing multiple tests. This might be a power issue, since only few non-target participants were diagnosed with SAD and participants with subclinical SAD varied in their severity of symptoms. Future studies should replicate our finding and investigate the remaining endophenotype criteria, for example by comparing results of families with SAD with the general population. Also, it should be studied whether this candidate endophenotype is specific to SAD, or also present in comorbid disorders (such as depression and other anxiety disorders).

If future research would confirm that delta-beta correlation during anticipation is an endophenotype of SAD, this might guide research into delineating the genetic basis of SAD. It is hypothesized that endophenotypes have a simpler genetic basis than complex psychiatric disorders (Cannon & Keller, 2006; Glahn et al., 2007). So, genes involved in the biological processes implicated in delta-beta correlation during anticipation might be easier to find and might be related to genes involved in SAD. In addition, the biological mechanisms underlying delta-beta correlation in SAD might be targeted in treatment, and might be used to identify people at risk for developing SAD. For example, future studies should investigate which factors influence the development of SAD in persons with increased negative delta-beta correlation during anticipation.

A few limitations of the present study should be taken into account. First, participants were seen once, so future research should investigate whether this candidate endophenotype is stable over time. Second, all participants performed the EEG tasks in the same order, so their experiences in the social judgment paradigm could have influenced the results in the SPT. Third, some participants were too anxious to do the speech, and these might be the people with the most extreme delta-beta correlations. Possibly, if these participants had participated, delta-beta correlation effects would have been stronger.

To conclude, delta-low beta correlation during anticipation of a stressful social situation might be a candidate endophenotype of SAD. Stronger negative delta-beta correlation in participants with (sub)clinical SAD could reflect the alleged imbalance between cortical and subcortical brain regions (Bruhl et al., 2014; Cremers, Veer, Spinhoven, Rombouts, Yarkoni, et al., 2015; Miskovic & Schmidt, 2012). Although more studies are needed to confirm the current findings and examine the specificity of delta-beta correlation for SAD, this candidate

endophenotype during anticipation of a stressful event might be useful in studying the genetic basis of SAD, as well as improving treatment and early detection of persons at risk for developing SAD.

Supplementary data 1

Participants of all ages performed the same task, but we had five different videos for different age categories (8-11, 12-17, 18-25, 26-39, 40+ years), so participants always evaluated a female peer. We have validated these videos in an independent sample of participants (n = 142, age 9-55 years, M = 25.58, SD = 14.69). Age had an effect on how emotional the video was rated (from happy to neutral to angry), F (4, 137) = 3.99, p = 0.004. The person in the third category was rated as more neutral than the persons in the second and fourth category, all Bonferroni adjusted ps < 0.05.

Supplementary data 2

We also analyzed frontal alpha asymmetry during resting state 1, anticipation, recovery and resting state 2, using the same signal processing method as in Harrewijn et al. (2016). Frontal alpha asymmetry did not co-segregate with SAD and (sub)clinical SAD within families during the task and resting state, all X^2s < 2.19, all *p*s > 0.34. Frontal alpha asymmetry during anticipation was heritable, $h^2 = 0.27$, $p = 0.02$, frontal alpha asymmetry during the other phases was not heritable, all h^2s < 0.07 and *p*s > 0.33.

Supplementary table 1

Overview of number of participants per measure, the number of participants with (sub)clinical SAD is in squared brackets.

Measure		Total	Probands	Siblings/Children	Nieces/Nephews	Spouses
EEG	RS1	113 [43]	18 [18]	34 [13]	40 [8]	21 [4]
	ANT	99 [36]	15 [15]	30 [13]	35 [5]	19 [3]
	REC	99 [36]	15 [15]	30 [13]	35 [5]	19 [3]
	RS2	104 [42]	18 [18]	31 [13]	35 [7]	20 [4]
Task-induced	T1	109 [43]	18 [18]	33 [13]	38 [8]	20 [4]
mood	T2	108 [43]	18 [18]	32 [13]	38 [8]	20 [4]
	T3	99 [36]	15 [15]	30 [13]	35 [5]	19 [3]
	T4	99 [36]	15 [15]	30 [13]	35 [5]	19 [3]
	T5	105 [41]	17 [17]	32 [13]	36 [7]	20 [4]
	T6	105 [42]	18 [18]	32 [13]	35 [7]	20 [4]

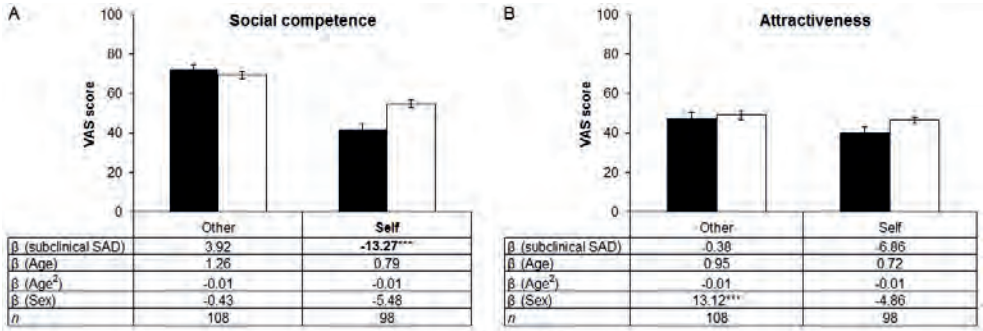
Note: RS1 = resting state 1; ANT = anticipation; REC = recovery; RS2 = resting state 2; T1 = time point 1.

Supplementary table 2

Number of clean epochs per phase of the social performance task for participants with and without (sub)clinical SAD.

		Mean	Standard deviation	Minimum	Maximum
Resting state 1	Participants with (sub)clinical SAD	96.65	3.79	86	99
	Participants without (sub)clinical SAD	96.64	6.45	52	99
Anticipation	Participants with (sub)clinical SAD	91.53	11.83	39	99
	Participants without (sub)clinical SAD	94.14	7.74	56	99
Recovery	Participants with (sub)clinical SAD	94.42	5.04	79	99
	Participants without (sub)clinical SAD	95.60	5.88	65	99
Resting state 2	Participants with (sub)clinical SAD	96.00	5.57	72	99
	Participants without (sub)clinical SAD	95.71	7.48	59	99

Supplementary figure 1



	A: Other	A: Self	B: Other	B: Self
β (subclinical SAD)	3.92	**-13.27****	-0.38	-6.86
β (Age)	1.26	0.79	0.95	0.72
β (Age^2)	-0.01	-0.01	-0.01	-0.01
β (Sex)	-0.43	-5.48	13.12***	-4.86
n	108	98	108	98

	C: Other	C: Self	D: Other	D: Self
β (subclinical SAD)	-2.00	**13.44****	-0.52	-8.28
β (Age)	-2.43**	0.95	0.93	0.44
β (Age^2)	0.03	-0.02	-0.01	0.01
β (Sex)	1.18	9.76	10.15	-1.63
n	108	98	108	98

Supplementary figure 1. Results of how participants evaluated the person on the video (other) and indicated how they expected to evaluated by a peer (self). Ratings of own social competence and nervousness were associated with (sub)clinical SAD.Means are uncorrected, error bars represent standard error of the mean.

Note: * $p < 0.006$; ** $p < 0.0013$; *** $p < 0.00013$ (Bonferroni corrected $p < 0.05$; $p < 0.01$; $p < 0.001$ [8 tests])