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

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Chapter 5

Heart rate variability as candidate endophenotype of social anxiety: A two-generation family study

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Abstract

Social anxiety disorder (SAD) is the extreme fear and avoidance of one or more social situations. The goal of the current study was to investigate whether heart rate variability (HRV) during resting state and a social performance task (SPT) is a candidate endophenotype of SAD. In this two-generation family study, patients with SAD with their partner and children, and their siblings with partner and children took part in a SPT (total n = 121, 9 families, 18 patients with SAD). In this task, participants had to watch and evaluate the speech of a female peer, and had to give a similar speech. HRV was measured during two resting state phases, and during the anticipation, speech and recovery phases of the SPT. We tested two criteria for endophenotypes: co-segregation with SAD within families and heritability. HRV did not co-segregate with SAD within families. However, RMSSD during the first resting state phase and recovery, high frequency power during all phases of the task, and LF/HF ratio during anticipation were heritable. HRV during resting state and the SPT is a possible endophenotype, but not of SAD. HRV might reflect a transdiagnostic genetic vulnerability for internalizing disorders, possibly related to reduced flexibility due to impaired inhibition, or generalized unsafety.

Introduction

Social anxiety disorder (SAD) is a common and debilitating psychiatric disorder characterized by extreme fear and avoidance of one or more social situations (APA, 2013). When exposed to socially threatening situations, patients with SAD and individuals with high self-reported levels of social anxiety show extreme physiological reactions, such as increased heart rate (Garcia-Rubio et al., 2017; Gramer, Schild, & Lurz, 2012; Gramer & Sprintschnik, 2008), decreased heart rate variability (HRV) (Garcia-Rubio et al., 2017; Gerlach et al., 2003; Grossman et al., 2001), or increased EEG delta-beta correlation (Harrewijn et al., 2016; Miskovic et al., 2010). Such electrophysiological biomarkers could play a role in the development and maintenance of SAD, and might be helpful in early detection, prevention and treatment of SAD. A promising line of research in psychiatry has focused on delineating endophenotypes, which are heritable (bio)markers of a disorder (Glahn et al., 2007). Endophenotypes are hypothesized to be based on fewer genes than complex psychiatric disorders, and might therefore provide insight in the underlying (genetic) mechanisms of psychiatric disorders (Cannon & Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013). Genetic factors play an important role in SAD, since heritability 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). Therefore, we aim to delineate candidate endophenotypes of SAD.

One such candidate endophenotype of SAD is HRV. According to the neurovisceral integration model (Thayer & Lane, 2000), HRV reflects the interplay between the autonomic nervous system and the central autonomic network of the brain during self-regulation. Higher HRV possibly indicates a general adaptive responsiveness to changes in the internal and external environment, whereas lower HRV indicates less ability to track these environmental changes and respond flexibly. Decreased HRV (and increased heart rate) is supposed to stem from inhibition of the parasympathetic nervous system and disinhibition of the sympathetic nervous system, resulting from decreased activation of the prefrontal cortex which disinhibits the amygdala (Thayer & Lane, 2009). Different measures of HRV have been investigated, but for this study we focused on those that are most often used in SAD: the root mean square of successive differences (RMSSD), high frequency power (usually 0.15-0.4 Hz), and the ratio between low and high frequency power (LF/HF ratio; low frequency power is usually 0.04-0.15 Hz). RMSSD is a measure of parasympathetic activity in the time domain (Chalmers et al., 2014), which is highly correlated high frequency power (Thayer, Ahs, Fredrikson, Sollers,

& Wager, 2012). High frequency power is a measure of parasympathetic (vagal) nervous system (Berntson et al., 1997; Camm et al., 1996), however, this measure might be influenced by respiration (Berntson et al., 1997). LF/HF ratio is interpreted as either reflecting sympathovagal balance or sympathetic control (Berntson et al., 1997; Camm et al., 1996). Decreased HRV is indicated by decreased RMSSD and high frequency power, and increased LF/HF ratio.

A meta-analysis has revealed decreased HRV in anxiety disorders during resting state, presumably reflecting a systemic inflexibility due to poor inhibition (Chalmers et al., 2014). Decreased HRV in anxiety disorders could also be explained by the generalized unsafety theory of stress (Brosschot, Verkuil, & Thayer, 2016), which proposes that patients with anxiety disorders - by default - show chronically low levels of HRV because their ability to recognize safety is compromised (Brosschot et al., 2016). More specifically, the meta-analysis also revealed decreased HRV in patients with SAD during resting state, albeit to a lesser extent than in most other anxiety disorders (Chalmers et al., 2014). Decreased HRV in patients with SAD during resting state was also found by other studies (Alvares et al., 2013; Gaebler, Daniels, Lamke, Fydrich, & Walter, 2013; Garcia-Rubio et al., 2017; Pittig, Arch, Lam, & Craske, 2013; Schmitz, Tuschen-Caffier, Wilhelm, & Blechert, 2013). However, in Schmitz et al. (2013) this was only the case for LF/HF ratio and not for high frequency power. Other studies have found no association between SAD and HRV during resting state (Alkozei, Creswell, Cooper, & Allen, 2015; Alvares et al., 2013; Faucher, Koszycki, Bradwejn, Merali, & Bielajew, 2016; Grossman et al., 2001; Klumbies, Braeuer, Hoyer, & Kirschbaum, 2014).

Furthermore, HRV could also be linked to state anxiety (B. H. Friedman, 2007), which in SAD is often elicited by a social performance task (SPT). In such a task, participants have to give a speech in front of an audience or video camera, to elicit social stress (Davidson et al., 2000; J. F. Van Veen et al., 2009; Westenberg et al., 2009). In general, healthy participants show decreased HRV during negative social interactions (Shahrestani, Stewart, Quintana, Hickie, & Guastella, 2015). Patients with SAD showed decreased HRV compared to healthy controls during anticipation or speech phases in SPTs (Garcia-Rubio et al., 2017; Gerlach et al., 2003; Grossman et al., 2001). However, this was not found in all studies (Alkozei et al., 2015; Klumbies et al., 2014; Schmitz et al., 2013), or only in women (Grossman et al., 2001). Most studies also investigated heart rate besides HRV, but most have found no association between SAD and heart rate during resting state nor SPTs (Alkozei et al., 2015; Gaebler et al., 2013; Gramer et al., 2012; Gramer & Sprintschnik, 2008; Grossman et al., 2001; Hofmann, Moscovitch, & Kim, 2006; Klumbies et al., 2014; Licht, De Geus, Van

Dyck, & Penninx, 2009; Mauss, Wilhelm, & Gross, 2003, 2004; Yoon & Quartana, 2012). Concluding, the findings are mixed, but HRV during resting state and SPTs might be associated with SAD.

The goal of the current study was to investigate whether HRV during resting state and a SPT are candidate endophenotypes of SAD. As candidate endophenotype, HRV might provide additional insight in the underlying (genetic) mechanisms of SAD (Cannon & Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller & Rockstroh, 2013). HRV should meet certain criteria to be seen as an endophenotype: (1) association with SAD; (2) co-segregation with SAD within families; (3) heritability; and (4) increased in unaffected family members compared to the general population (Glahn et al., 2007; Gottesman & Gould, 2003). The first criterion has already been investigated in studies comparing patients with SAD and controls (or high and low socially anxious individuals). In the current study, we employed a two-generation family design to assess two additional endophenotype criteria for HRV: co-segregation within families and heritability. Although different designs have been used, our two-generation family design is particularly suitable because power is increased by including extended families instead of twins or sib-pairs (Gur et al., 2007; Williams & Blangero, 1999), and by selecting families based on two probands with SAD or subclinical SAD (Fears et al., 2014; Glahn et al., 2010). So, patients with SAD and their family members took part in a SPT in which we measured ECG. We tested whether decreased RMSSD and high frequency power, and increased LF/HF ratio during resting state and the SPT are candidate endophenotypes of SAD (Alvares et al., 2013; Chalmers et al., 2014; Gaebler et al., 2013; Garcia-Rubio et al., 2017; Gerlach et al., 2003; Grossman et al., 2001; Pittig et al., 2013).

Methods

Participants

We included 'target participants' with SAD with their partner and children, and the siblings of these target participants with their partner and children. In total, 132 participants divided over nine families took part in this study. However, nine of these participants only filled out questionnaires at home. Data of one participant was excluded because of technical problems, and of one participant because s/he reported heart problems. So, 121 participants (61 females, M_{age} = 30.10, *SD* = 15.65) took part in the first resting state measure and 116 in the SPT (five

participants did not want to take part in any task)[7]. A different number of participants was analyzed for the different phases and measures (Table 1), because not all participants wanted to give a speech, some participants were too tired at the end of the EEG session, and we excluded data with too many ECG artefacts (> 5%) and outliers (> +/- 3 SD).

Table 1

Number of participants included in analysis per phase (first resting state, anticipation, speech, recovery, second resting state) and per measure (RMSSD, high frequency power, LF/HF ratio, heart rate), with the number of participants with SAD displayed between brackets.

	Resting state 1	Anticipation	Speech	Recovery	Resting state 2
RMSSD	117 [17]	103 [16]	76 [11]	100 [16]	108 [17]
High frequency power	120 [17]	105 [16]	78 [11]	101 [16]	110 [17]
LF/HF ratio	117 [16]	104 [16]	74 [9]	100 [15]	107 [16]
Heart rate	118 [17]	105 [16]	78 [11]	101 [16]	109 [17]

Note: Some participants did not want to give a speech (one participant with SAD, eight participants without SAD), so we also excluded the anticipation and recovery phases for these participants. The number of participants is much lower in the speech phase compared to the other phases, because the data contained many artefacts, probably due to movement.

Families were recruited via media exposure and selected based on two probands: an adult with SAD (25-55 years) and his/her child with (sub)clinical SAD. SAD was diagnosed by a psychiatrist using a clinical interview and the Mini-Plus International Neuropsychiatric Interview (MINI Plus version 5.0.0) (Sheehan et al., 1998; Van Vliet & De Beurs, 2007). The MINI interview is based on DSM-IV-TR criteria, but the psychiatrist confirmed that all patients also met DSM-5 criteria. Subclinical SAD was defined as meeting all criteria for SAD, without the criterion 'impairment in important areas of functioning' (criterion G in the DSM-5 (APA, 2013)). In the child of the target, (sub)clinical SAD was diagnosed by a licensed clinician based on a clinical interview and the structured MINI Kid interview (Bauhuis et al., 2013; Sheehan et al., 2010). The MINI interviews are also used to diagnose psychiatric disorders other than SAD. In addition, self-reported symptoms of social anxiety (La Greca & Lopez, 1998; Liebowitz, 1987) and depression (Beck et al., 1996; Kovacs, 1992) were assessed. The inclusion criteria are depicted in Figure 1.

[7] None of the participants with SAD currently underwent psychotherapy. Only one participant with SAD used an SSRI, but the results did not change when we excluded this participant.

A priori power calculations revealed that 12 families with 8 to 12 family members (on average 10 members per family) were required for sufficient power (minimally 80%). This was calculated using simulated data of an endophenotype with heritability of 60% and a correlation of 70% with SAD, based on studies in behavioral inhibition and SAD (Muris et al., 2005; Smoller, Gardner-Schuster, & Covino, 2008). We included fewer families, since the included families were relatively large (on average 14.67 instead of 10 members per family), which results in more power than using smaller families (Dolan et al., 1999; Gur et al., 2007; Rijsdijk et al., 2001; Williams & Blangero, 1999).

Procedure

Figure 1 shows a flow-chart of the inclusion and assessment procedures of the Leiden Family Lab study on SAD. The SPT was part of the EEG session. All adult participants signed an informed consent form, both parents signed the form of their children (children of 12 years and older signed for 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.

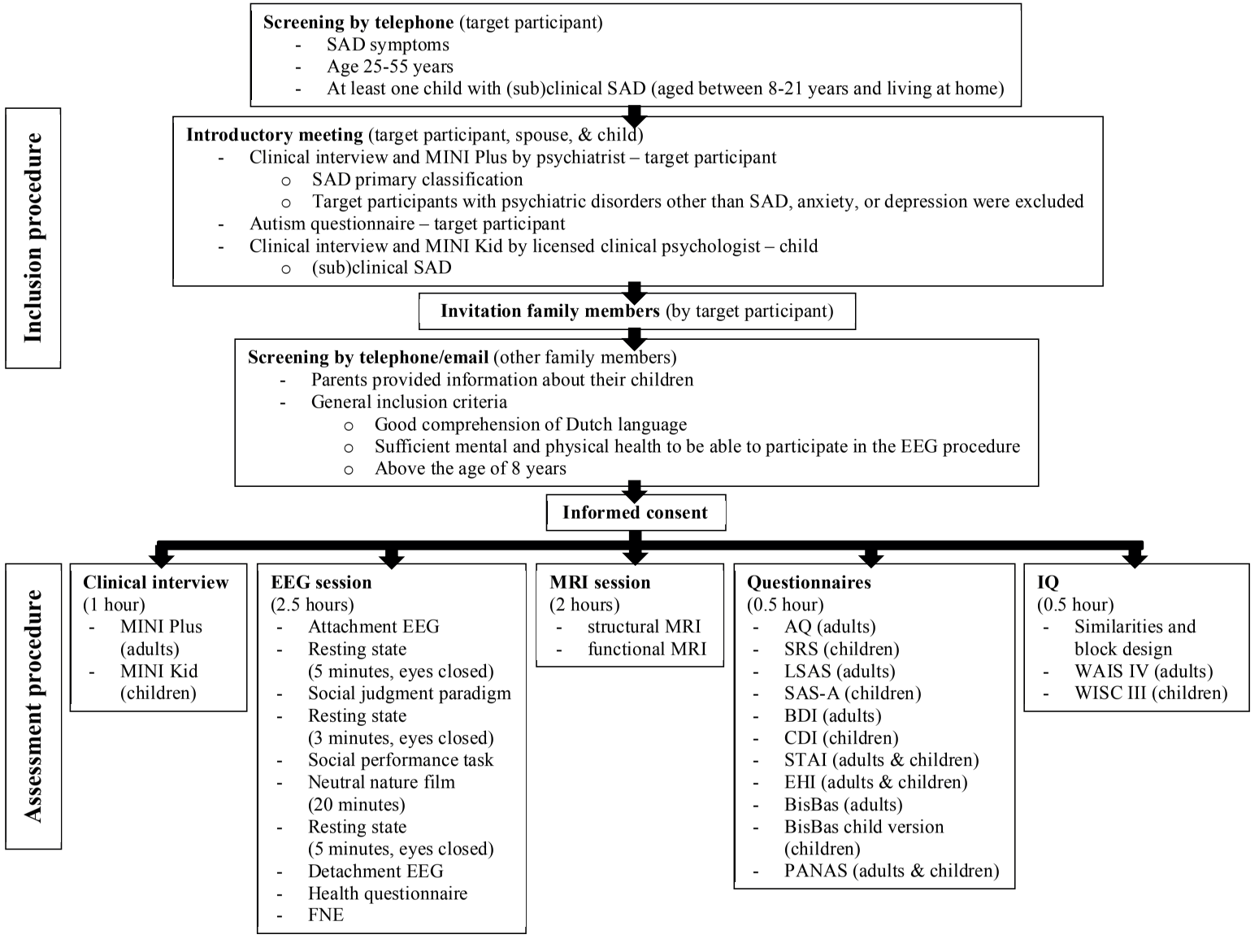

Inclusion procedure
Screening by telephone (target participant)
- SAD symptoms
- Age 25-55 years
- At least one child with (sub)clinical SAD (aged between 8-21 years and living at home)
Introductory meeting (target participant, spouse, & child)
- Clinical interview and MINI Plus by psychiatrist – target 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. Every family member took part in all sessions of the assessment procedure in one or two days. The order of these parts differed between participants, based on their preferences and availability of the labs. Most participants came to the lab with family members.

Note: One target participant scored above the cutoff of the autism questionnaire, but the psychiatrist confirmed that s/he could not be diagnosed with autism spectrum disorder (the high score was probably caused by SAD symptoms). EEG results of the SPT and social judgment paradigm are reported elsewhere (Harrewijn, Van der Molen, Van Vliet, Houwing-Duistermaat, & Westenberg, in press; Harrewijn, Van der Molen, Van Vliet, Tissier, & Westenberg, in press).

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).

Resting state

At the start of the EEG session, we measured ECG (and EEG) for five minutes while participants sat still with their eyes closed. It should be noted that participants were already informed via email about the social judgment paradigm (Harrewijn, Van der Molen, Van Vliet, Tissier, et al., in press; Van der Molen et al., 2014), so this might have influenced this first resting state phase. Therefore, we included a second resting state phase at the end of the EEG session.

Social performance task

The SPT (Harrewijn et al., 2016) was administered to elicit social stress. We also measured EEG during this task, but these data are reported elsewhere (Harrewijn, Van der Molen, Van

Vliet, Houwing-Duistermaat, et al., in press). The SPT consists of five phases presented in a fixed order: instruction, video, anticipation, speech and recovery (Figure 2). We started with an instruction of the entire task, because participants did not know about this task beforehand. Participants then watched a video of a female peer who talked about herself and her positive and negative qualities. After the video, participants were asked to evaluate the person on the video. Next, participants had five minutes to prepare their speech about their own positive and negative qualities (anticipation). They were asked to give this three-minute speech in front of a video camera and were told that their speech would be recorded and shown to a peer. They were led to believe that this peer would evaluate them based on the same criteria as they used to evaluate the person on the video (this was not the case). After the speech, participants had five minutes to relax (recovery). Then, they watched a neutral nature movie (extended recovery). Task-induced mood (nervousness and avoidance) was measured at several time points throughout the SPT. Participants with SAD or (sub)clinical SAD showed more nervousness and avoidance during the SPT than participants without SAD or (sub)clinical SAD (Harrewijn, Van der Molen, Van Vliet, Houwing-Duistermaat, et al., in press). We focused our HRV analyses on the anticipation, speech, and recovery phases of the SPT.

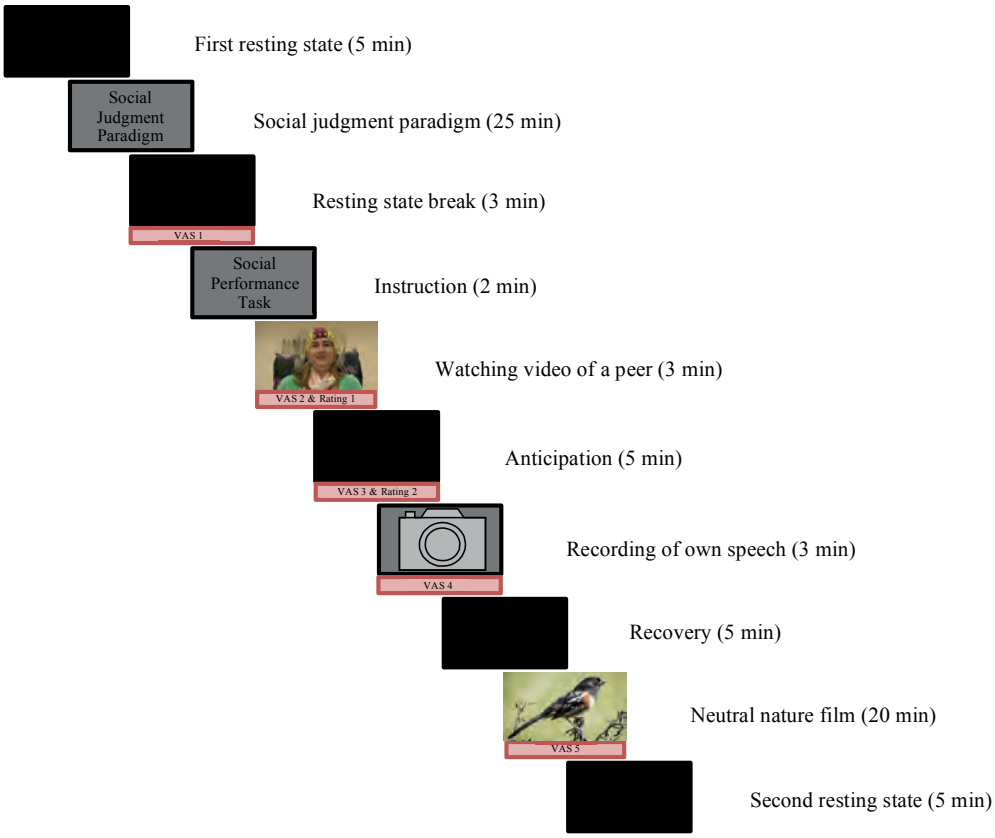


Figure 2. Overview of the social performance task.

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/

ECG recording and signal processing

ECG (and EEG) was recorded during five minutes of resting state (first and second), anticipation, and recovery, and during the first 30 seconds of the speech. The ECG recording of the speech is shorter than is recommended by Camm et al. (1996), because the duration of the speeches varied between participants. Therefore, the results should be interpreted with caution. The phases started when the experimenter was outside the EEG lab. Participants sat upright throughout the entire EEG session, and were asked to move as little as possible. We used a BioSemi Active Two system (Biosemi, Amsterdam, The Netherlands). Two Ag/AgCl

electrodes were placed under the right collarbone and between the ribs on the left side (modified lead-2 placement). The conventional ground electrode was replaced by the common mode sense and driven right leg electrodes in the EEG cap. The sampling rate was 1024 Hz.

HRV was analyzed using Kubios (Kuopio, Finland) (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2014). RR intervals were automatically detected and the ECG data was manually inspected (ectopic beats and artifacts were excluded) by a research assistant who was blind to participant diagnosis. If more than 5% of the data was deleted, the participant was excluded from analysis. See Supplementary table 1 for the percentages of artefacts deleted for participants with and without SAD. We applied the automatic artifact correction as implemented in Kubios, in which artefacts were replaced by interpolated RR values. Then, the smoothness priors detrending method (Lambda = 500) was used to adjust for non-stationarity in the data (Tarvainen, Ranta-aho, & Karjalainen, 2002). We subtracted RMSSD from the data in the time-domain. For the frequency-domain, the fast Fourier transform based on Welch's periodogram method was used to subtract low frequency power (0.04-0.15 Hz) and high frequency power (0.15-0.4 Hz). High frequency power values were log transformed. The ratio between the low and high frequency power was also calculated in Kubios (LF/HF) and log transformed.

Statistical analysis

First, we validated our groups by comparing self-reported symptoms of social anxiety (La Greca & Lopez, 1998; Liebowitz, 1987) and depression (Beck et al., 1996; Kovacs, 1992) between participants with and without SAD. We used different questionnaires for adults and children, so we computed z-scores based on normative samples (Fresco et al., 2001; Inderbitzen-Nolan & Walters, 2000; Miers et al., 2014; Roelofs et al., 2013). Multilevel regression models were fitted in R (R Core Team, Vienna, Austria) with self-report questionnaires as dependent variable, and SAD, age (standardized), age (standardized)^2 and sex as independent variables. Genetic correlations between family members were modeled by including random intercepts.

Second, we used two criteria to test whether HRV during resting state and the SPT is a candidate endophenotype of SAD: co-segregation with SAD within families and heritability. The co-segregation analyses were performed separately for the speech phase, because the duration was much shorter than the duration of the other phases of the task (30 seconds versus five minutes). For the other phases, we fitted one regression model with HRV (RMSSD, high frequency power, or LF/HF ratio) as dependent variable, and time (first resting state,

anticipation, recovery and second resting state as factors), age (standardized), age (standardized)^2, and sex as independent variables. An additional regression model also included the interaction time X SAD. Random intercepts were included to account for genetic correlations between family members and repeated measures within participants. The main effect of SAD across phases was tested using a likelihood ratio test statistic comparing the likelihoods of the regression models with and without SAD. Significance of SAD at a specific time point was assessed using Wald tests. For the speech phase, we fitted multilevel regression models with HRV as dependent variable, and SAD, age (standardized), age (standardized)^2 and sex as independent variables. Genetic correlations between family members were modeled by including random intercepts. We selected families based on a specific criterion (SAD) that is related to the candidate endophenotypes (ascertainment). However, no additional ascertainment-corrections were necessary in co-segregation analyses because we included SAD as independent variable, which is sufficient to correct for ascertainment (Monsees et al., 2009).

SOLAR was used for the heritability analyses (Almasy & Blangero, 1998). In SOLAR, the total variance of the phenotype is decomposed 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). A shared environmental component (e.g. household) was not included to keep the model as simple as possible. Heritability is defined as the ratio of the additive genetic component and the total phenotypic variance (after removal of variance explained by covariates). We used age (standardized), age (standardized)^2 and sex as covariates, but these were removed from the final model if $p > 0.05$. For heritability analyses, it was necessary to correct for ascertainment because we did not include SAD in the analysis. In SOLAR, the likelihood of the probands (target participant with SAD and his/her child with (sub)clinical SAD) is subtracted from the likelihood of the rest of the sample (De Andrade & Amos, 2000; Hopper & Mathews, 1982). For RMSSD and LF/HF ratio (log transformed), the residual kurtosis was not normally distributed, so we applied an inverse normal transformation as implemented in SOLAR (Almasy & Blangero, 1998, 2010). We used a Bonferroni adjusted p-value of 0.0025 to correct for performing multiple [25] tests. We performed additional analysis (co-segregation and heritability) on heart rate, to investigate whether there are differences in heart rate between participants with and without SAD (Camm et al., 1996) (see Supplementary data 1). We also performed additional co-segregation

analyses on HRV and heart rate using (sub)clinical SAD instead of SAD, because more non-target participants were diagnosed with (sub)clinical SAD.

Results

Participant characteristics

Participants with SAD were older than participants without SAD, $\beta = 0.63$, $p = 0.01$. There was no difference in estimated IQ, $\beta = -0.30$, $p = 0.91$. We validated our groups by comparing self-reported symptoms of social anxiety and depression. Participants with SAD reported more symptoms of social anxiety, $\beta = 3.09$, $p < 0.001$, and depression, $\beta = 0.97$, $p < 0.001$, than participants without SAD (Table 2). Psychiatric disorders other than SAD in participants with and without SAD are shown in Table 3.

Table 2

Uncorrected mean (and standard deviation) age, estimated IQ and self-reported symptoms of social anxiety and depression for participants with and without SAD.

	Participants with SAD (12 females, 5 males)	Participants without SAD (49 females, 55 males)
Age	38.88 (13.72)	28.66 (15.53)
Estimated IQ	106.77 (12.34)	105.70 (11.14)
Social anxiety symptoms (z-score)	3.85 (2.13)	0.37 (1.34)
Depressive symptoms (z-score)	0.47 (0.85)	-0.49 (0.66)

Note: Social anxiety symptoms were measured using the Liebowitz Social Anxiety Scale (Liebowitz, 1987) for adults and the Social Anxiety Scale – adolescents (La Greca & Lopez, 1998) for children. Depressive symptoms were measured using the Beck Depression Inventory (Beck et al., 1996) for adults and the Child Depression Inventory (Kovacs, 1992) for children.

Table 3

Number (*n*) and percentage (%) of disorders other than SAD in participants with and without SAD.

		Participants with SAD (12 females, 5 males)		Participants without SAD (49 females, 55 males)	
		n	%	*n*	%
Depression	Current	0	0	1	1.0
	Past	7	41.2	17	16.3
Dysthymia	Past	1	5.9	1	1.0
Bipolair 2	Current	0	0	0	0
	Past	0	0	0	0
Panic disorder	Current	2	11.8	0	0
	Lifetime	3	17.4	4	3.8
Agoraphobia	Current	4	23.5	2	2.0
	Lifetime	0	0	1	1.0
Seperation anxiety disorder	Current	0	0	1	1.0
Specific phobia		1	5.9	4	3.8
Obsessive-compulsive disorder	Current	1	5.9	0	0
Posttraumatic stress disorder	Current	0	0	0	0
Generalized anxiety disorder	Current	2	11.8	0	0

Note: Separation anxiety disorder was only part of the MINI kid interview.

Co-segregation with SAD within families

The first criterion for endophenotypes that we tested was 'co-segregation with SAD within families'. Regression models including SAD did not fit the data better than models without SAD for RMSSD, $X^2(4) = 7.11$, $p = 0.13$, high frequency power, $X^2(4) = 1.40$, $p = 0.84$, and LF/HF ratio, $X^2(4) = 0.41$, $p = 0.98$. These data suggest that HRV across all phases did not co-segregate with SAD within families (Figure 3). The regression models without SAD showed that across phases, RMSSD and high frequency power decreased with age, respectively $\beta = -11.58$, $p < 0.001$ and $\beta = -0.74$, $p < 0.001$. LF/HF ratio increased with age, $\beta = 0.37$, $p < 0.001$. Females showed overall lower LF/HF ratio than males, $\beta = -0.48$, $p < 0.001$.

Co-segregation analyses were performed separately for the speech phase (Figure 3). There was no co-segregation with SAD within families for RMSSD, β = -3.98, p = 0.24, high frequency power, β = -0.61, p = 0.11, and LF/HF ratio, β = -0.12, p = 0.76. RMSSD and high frequency power decreased with age, respectively β = -6.54, p < 0.001 and β = -0.75, p < 0.001.

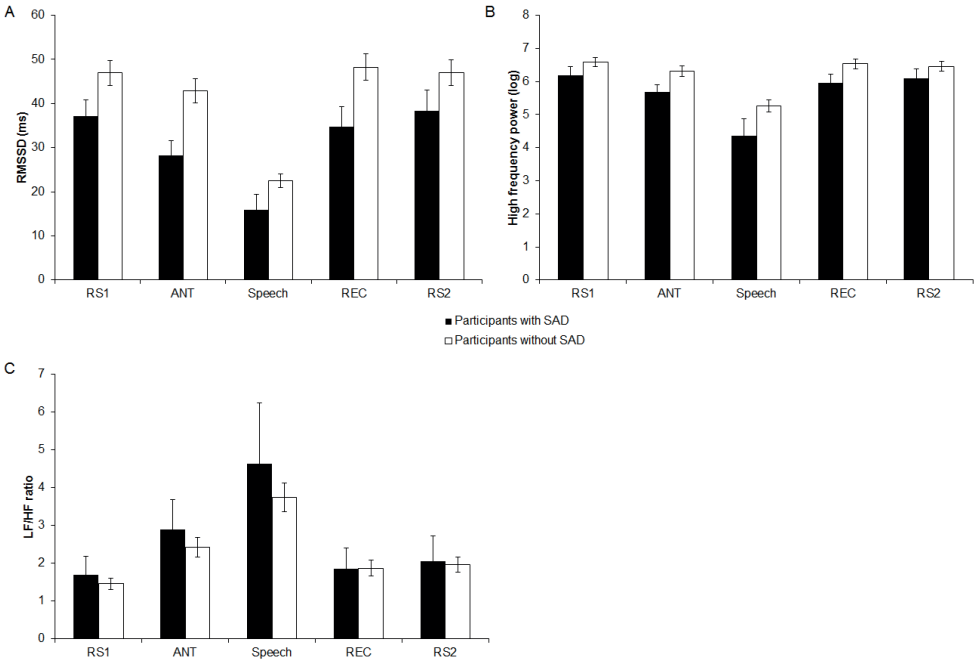


Figure 3. Uncorrected mean RMSSD (A), high frequency power (B), and LF/HF ratio (C) for participants with and without SAD during all five phases of the SPT.

Note: We showed the results of the five phases in one figure, but speech was analyzed separately (due to differences in duration of the phases). RMSSD = root mean square of successive differences; RS1 = first resting state; ANT = anticipation; REC = recovery; RS2 = second resting state; SAD = social anxiety disorder

We repeated all analyses with (sub)clinical SAD instead of SAD, but (sub)clinical SAD did not co-segregate within families with RMSSD, high frequency power, and LF/HF ratio, all ps > 0.27 (for the first resting state, anticipation, recovery, and second resting state) and all ps > 0.10 (for speech).

Heritability

The second criterion for endophenotypes that we tested was 'heritability'. Heritability estimates were significant for RMSSD during the first resting state and recovery, for high frequency power during all phases of the SPT, and for LF/HF ratio during anticipation. Only the heritability estimate for high frequency power during the first resting state remained significant after correction for performing multiple tests. These heritability results are shown in Table 4.

Table 4

Results of the heritability analyses for RMSSD, high frequency power, and LF/HF ratio during all five phases of the SPT.

		Resting state 1	Anticipation	Speech	Recovery	Resting state 2
RMSSD*	h^2	**0.41**	0.25	0.22	**0.25**	0.16
	SE (h^2)	**0.20**	0.21	0.28	**0.19**	0.17
	p (h^2)	**0.003**	0.065	0.20	**0.044**	0.11
	p (age)	**< 0.001**	< 0.001	< 0.001	**< 0.001**	< 0.001
	p (age^2)	**0.71**	0.98	1.00	**0.93**	0.93
	p (sex)	**0.11**	0.72	0.76	**0.15**	0.17
High frequency power	h^2	**0.40**	**0.36**	**0.61**	**0.31**	**0.25**
	SE (h^2)	**0.17**	**0.24**	**0.22**	**0.20**	**0.20**
	p (h^2)	**< 0.001**	**0.01**	**0.002**	**0.02**	**0.04**
	p (age)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	p (age^2)	**0.93**	**0.75**	**0.97**	**0.25**	**0.85**
	p (sex)	**0.03**	**0.26**	**0.77**	**0.12**	**0.04**
LF/HF ratio*	h^2	0.10	**0.27**	0.00	0.00	0.15
	SE (h^2)	0.11	**0.17**	-	-	0.21
	p (h^2)	0.15	**0.03**	0.50	0.50	0.20
	p (age)	< 0.001	**< 0.001**	0.42	0.01	0.11
	p (age^2)	0.05	**0.02**	0.44	0.34	0.50
	p (sex)	< 0.001	**0.001**	0.23	< 0.001	< 0.001

* These variables were inverse normalized in SOLAR. Variables displayed in bold font are heritable.

Discussion

The goal of the current study was to investigate whether HRV during resting state and a SPT is a candidate endophenotype of SAD. We measured HRV in patients with SAD, their partner and children, and their siblings with partner and children during two resting state phases and a SPT. In this SPT, participants had to watch and evaluate a video of a female peer, and then give a similar speech about their own positive and negative qualities in front of a video camera. We tested two criteria for endophenotypes (co-segregation with SAD within families and heritability) for RMSSD, high frequency power, and LF/HF ratio during the first resting state, anticipation, speech, recovery and the second resting state. Co-segregation analyses revealed no effect of SAD or (sub)clinical SAD on HRV across all phases. Heritability analyses revealed that RMSSD during the first resting state and recovery, high frequency power during all phases of the task, and LF/HF ratio during anticipation were heritable.

We found no co-segregation within families between SAD and HRV during resting state and the SPT. Previous studies have revealed mixed results, and our null finding is in line with several other studies in SAD (Alkozei et al., 2015; Alvares et al., 2013; Faucher et al., 2016; Grossman et al., 2001; Klumbies et al., 2014; Schmitz et al., 2013). This might be related to the type of anxiety disorder, since studies comparing different anxiety disorders have shown that the effect of SAD on HRV was smaller than that of other anxiety disorders (Chalmers et al., 2014; B. H. Friedman, 2007; Pittig et al., 2013). This difference between SAD and other anxiety disorders could suggest that cognitive processes and subjective experience of physiological symptoms are more important in SAD, than actual differences in physiological symptoms between patients with SAD and controls (Mauss et al., 2003, 2004). Even though the results were not significant, they were in the expected direction: participants with SAD showed decreased HRV compared to participants without SAD. According to the generalized unsafety theory of stress (Brosschot et al., 2016), chronically reduced levels of HRV are related to not recognizing safety in the environment. In this light, our findings would indicate that the situation was equally (un)safe for participants with and without SAD. There might not have been sufficient variation in feelings of safety to reveal HRV-differences, because the EEG session was very structured, we tried to make the participants feel as comfortable as possible throughout the testing day(s), and the situation was new for most participants (almost none of the participants had participated in a study before). In addition, if feelings of unsafety were too intense, participants could stop the experiment. So, participants

with SAD possibly felt less safe than participants without SAD, but the differences in HRV were not large enough to reach statistical significance.

Age seemed to influence HRV, with older participants showing decreased HRV across resting state and SPT phases (reflected by decreased RMSSD and high frequency power, and increased LF/HF power). This is in line with previous studies showing decreased HRV with age in adolescents (Goto et al., 1997; Hollenstein, McNeely, Eastabrook, Mackey, & Flynn, 2012) and adults (Nunan, Sandercock, & Brodie, 2010). This effect of age complicates our findings, as participants with SAD were older than participants without SAD. Figure 3 seems to suggest an effect of SAD, and this effect was indeed significant for RMSSD when we did not include age. However, we were not able to disentangle the effects of age and SAD, because we included not enough children with SAD. A reason for this might be that children are not often diagnosed with SAD, because they are obligated to go to school and thus cannot avoid social situations. Future studies with more children with SAD should investigate the effects of age and SAD on HRV.

All HRV measures during resting state and/or the SPT were heritable. This corroborates previous studies that have estimated the heritability of HRV during 5-minute resting state between 31-60 % (Golosheykin, Grant, Novak, Heath, & Anokhin, 2017; Uusitalo et al., 2007), and adds that HRV during a SPT is also heritable. However, it should be noted that only high frequency power during the first resting state survived stringent correction for performing multiple tests. This might suggest that high frequency power is most suitable for genetic analyses of HRV. Given the heritability of HRV, it is proposed that HRV is a possible endophenotype related to panic disorder specifically, or to psychopathology more generally (Thayer & Lane, 2009). HRV is probably a more general endophenotype, because it is not only related to several anxiety disorders (Chalmers et al., 2014; B. H. Friedman, 2007; Pittig et al., 2013) but also to depression (Kemp, Quintana, Felmingham, Matthews, & Jelinek, 2012; Kemp et al., 2010). Indeed, others have proposed that HRV is a transdiagnostic factor related to worry (Chalmers, Heathers, Abbott, Kemp, & Quintana, 2016), or to self-regulation and cognitive control (Beauchaine & Thayer, 2015). Persons with this genetic vulnerability might be inflexible to environmental changes due to impaired inhibition (Chalmers et al., 2014; Thayer & Lane, 2000), or their ability to recognize safety is comprised (Brosschot et al., 2016), which might lead to different internalizing disorders. Taken together, HRV might be a possible transdiagnostic endophenotype of internalizing disorders, not specifically of SAD.

A few limitations of the current study should be taken into account. First, the differences in HRV were very small, and the power might have been insufficient to detect these differences. This was because only a small number of non-target participants was diagnosed with SAD. Although, we included extended families and selected families based on two persons with (sub)clinical SAD to enhance the power as much as possible (Fears et al., 2014; Glahn et al., 2010; Gur et al., 2007; Williams & Blangero, 1999). Second, the duration of the speech phase varied between participants, was shorter than the other phases (30 seconds versus five minutes), and was not in line with the recommendations of Camm et al. (1996). In addition, many participants were excluded due to artefacts in the ECG data (probably due to movement). Therefore, we analyzed the speech phase separately and interpreted these findings with caution. Third, participants were informed about the social judgment paradigm before the EEG session (Harrewijn, Van der Molen, Van Vliet, Tissier, et al., in press; Van der Molen et al., 2014), which might have influenced the first resting state phase. However, there were no differences between participants with and without SAD during the first resting state.

To conclude, HRV during resting state and the SPT is a possible endophenotype, but not of SAD. HRV might be a transdiagnostic genetic vulnerability for internalizing disorders, reflecting reduced flexibility due to impaired inhibition (Chalmers et al., 2014; Thayer & Lane, 2000) or generalized unsafety (Brosschot et al., 2016). Future research should investigate which factors influence the development of psychopathology in persons with decreased HRV during resting state or stress.

Supplementary table 1

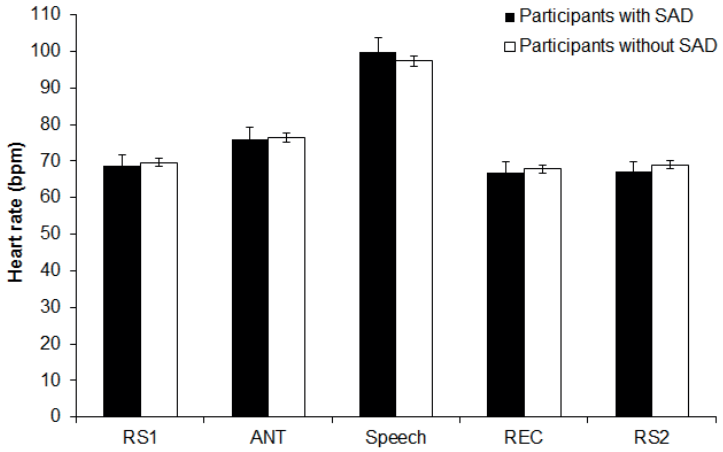
Overview of the percentage of deleted artefacts in HRV data for participants with and without SAD.

	Participants with SAD				Participants without SAD			
	Mean	*SD*	Min	Max	Mean	*SD*	Min	Max
Resting state 1	1.26	0.38	0,773	2.31	1.38	1.23	0.57	13.09
Anticipation	1.17	0.56	0,527	2.90	1.64	3.37	0.47	28.22
Speech	3.77	2.28	0.00	7.31	4.60	3.05	0.00	22.92
Recovery	1.35	0.80	0,535	3.83	1.86	2.44	0.66	16.73
Resting state 2	1.33	0.89	0,286	4.53	1.37	0.57	0.57	4.17

Note: SAD = social anxiety disorder; *SD* = standard deviation; Min = minimum; Max = maximum.

Supplementary data 1

Additional analyses focused on heart rate to investigate whether there are differences in heart rate between participants with and without SAD (Camm et al., 1996). However, most of the previous studies on heart rate have found no effect of SAD during speech or SPTs (Alkozei et al., 2015; Gaebler et al., 2013; Gramer et al., 2012; Gramer & Sprintschnik, 2008; Grossman et al., 2001; Hofmann et al., 2006; Klumbies et al., 2014; Licht et al., 2009; Mauss et al., 2003, 2004; Yoon & Quartana, 2012). During the first resting state, anticipation, recovery and second resting state, heart rate did not co-segregate with SAD within families, $X^2(4) = 5.51$, $p = 0.24$. Overall, heart rate decreased with age, $\beta = -5.43$, $p < 0.001$, and showed a quadratic effect of age, $\beta = 2.73$, $p = 0.01$. During the speech phase, heart rate tended to co-segregate with SAD within families, $\beta = 5.89$, $p = 0.08$. Heart rate also tended to decrease with age, $\beta = -2.32$, $p = 0.054$ (Supplementary figure 1). There was no effect of age^2 nor sex. Heart rate during speech was heritable, $h^2 = 0.84$, $p = 0.01$ (Supplementary table 2).



Supplementary figure 1. Uncorrected mean heart rate for participants with and without SAD during all five phases of the SPT.

Supplementary table 2

Results of the heritability analyses for heart rate during all five phases of the SPT.

	Resting state 1	Anticipation	Speech	Recovery	Resting state 2
h^2	0.11	0.00	**0.84**	0.03	0.34
SE (h^2)	0.21	-	**0.22**	0.19	0.36
p (h^2)	0.27	0.50	**0.008**	0.44	0.14
p (age)	< 0.001	0.02	**0.28**	< 0.001	< 0.001
p (age^2)	0.07	0.25	**0.44**	0.02	0.10
p (sex)	0.05	0.055	**0.18**	0.58	0.21

Note: h^2 = heritability; *SE* = standard error.