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

Short title: Heart rate variability in socially anxious families

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Abstract

Background

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.

Methods

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, 3-30 persons per family, age range: 8-61 years, 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 anticipation, speech and recovery phases of the SPT. We tested two criteria for endophenotypes: co-segregation with SAD within families and heritability.

Results

HRV did not co-segregate with SAD within families. Root mean square of successive differences during the first resting phase and recovery, and high frequency power during all phases of the task were heritable.

Limitations

It should be noted that few participants were diagnosed with SAD. Results during the speech should be interpreted with caution, because the duration was short and there was a lot of movement.

Conclusions

HRV during resting state and the SPT is a possible endophenotype, but not of SAD. As other studies have shown that HRV is related to different internalizing disorders, HRV might reflect

a transdiagnostic genetic vulnerability for internalizing disorders. Future research should investigate which factors influence the development of psychopathology in persons with decreased HRV.

Introduction

SAD[1] is a common and debilitating psychiatric disorder characterized by extreme fear and avoidance of one or more social situations (APA, 2013). Some studies have shown that patients with SAD show enhanced physiological reactions to socially threatening situations, such as increased heart rate (Garcia-Rubio et al., 2017; Gramer et al., 2012; Gramer and Sprintschnik, 2008), decreased HRV (Garcia-Rubio et al., 2017; Gerlach et al., 2003; Grossman et al., 2001), reflecting increased sympathetic nervous system activity and decreased parasympathetic nervous system activity. This pattern of physiological activity could play a role in the development and maintenance of SAD, and assessing it 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 and Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller and 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). Heritability of HRV is estimated around 31-60 % (Golosheykin et al., 2017; Uusitalo et al., 2007), so the goal of the current study is to test HRV as a candidate endophenotype of SAD.

According to the neurovisceral integration model (Thayer and 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

[1] SAD = social anxiety disorder; HRV = heart rate variability; RMSSD = root mean square of successive differences; SPT = social performance task; ECG = electrocardiogram

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 and Lane, 2009). Different measures of HRV have been investigated, but for this study we focused on those that are most often used in SAD: RMSSD, and high frequency power (usually 0.15-0.4 Hz). RMSSD is a measure of parasympathetic activity in the time domain (Chalmers et al., 2014), which is highly correlated high frequency power (Thayer et al., 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).

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 et al., 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 using RMSSD (Alvares et al., 2013; Garcia-Rubio et al., 2017) or high frequency power (Gaebler et al., 2013; Pittig et al., 2013). However, most studies have found no association between SAD and HRV during resting state using RMSSD (Klumbies et al., 2014) or high frequency power (Alkozei et al., 2015; Alvares et al., 2013; Faucher et al., 2016; Grossman et al., 2001; Schmitz et al., 2013).

HRV could also be linked to state anxiety (Friedman, 2007), with healthy participants showing decreased HRV during negative social interactions (Shahrestani et al., 2015). Studies on SAD often elicit state anxiety by using a SPT, in which participants have to give a speech

in front of an audience or video camera (Davidson et al., 2000; Van Veen et al., 2009; Westenberg et al., 2009). Patients with SAD showed decreased HRV compared to healthy controls during anticipation or speech phases in SPTs, measured with RMSSD (Garcia-Rubio et al., 2017) or high frequency power (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). Hence, given that the findings are mixed, the goal of the current study is to gain more insight in the role between SAD and HRV during a SPT. As social anxiety is related to increased state anxiety during SPTs (Davidson et al., 2000; Harrewijn et al., 2016; Miskovic et al., 2010) and HRV is linked to state anxiety (Friedman, 2007), decreased HRV during a SPT is a possible endophenotype of SAD. Furthermore, both HRV and SAD have shown to be heritable (Distel et al., 2008; Golosheykin et al., 2017; Isomura et al., 2015; Kendler et al., 1992; Middeldorp et al., 2005; Nelson et al., 2000; Uusitalo et al., 2007).

Therefore, the goal of the current study was to investigate whether HRV during a SPT is a candidate endophenotype of SAD. As candidate endophenotype, HRV might provide additional insight in the underlying (genetic) mechanisms of SAD (Cannon and Keller, 2006; Glahn et al., 2007; Iacono et al., 2016; Miller and 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 and Gould, 2003). The first criterion has already been investigated in studies comparing patients with SAD and controls (or high versus low socially anxious individuals). We employed a two-generation family design to assess two additional endophenotype criteria for HRV: co-segregation within families and heritability. Although different designs (such as twin or sibling studies) have been used, our two-generation family design is particularly suitable because power is

increased by including extended families with many different types of relationships within one family (Gur et al., 2007; Williams and Blangero, 1999). Furthermore, families were selected 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 hypothesized that decreased RMSSD and high frequency power during the SPT (and not during resting state) are candidate endophenotypes of SAD (Alkozei et al., 2015; Alvares et al., 2013; Faucher et al., 2016; Garcia-Rubio et al., 2017; Gerlach et al., 2003; Grossman et al., 2001; Klumbies et al., 2014; Schmitz et al., 2013).

Methods

Participants

This study was part of the Leiden Family Lab study on Social Anxiety Disorder (Bas-Hoogendam et al., In press). We included 'target participants' with SAD with their partner and children, and the siblings of these target participants with their partner and children. The inclusion criteria are depicted in Figure 1. 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. Supplementary Figure 1 shows the flow of participants from recruitment to inclusion. 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 and 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, and self-reported symptoms of social anxiety (La Greca and Lopez, 1998; Liebowitz, 1987) and depression (Beck et al., 1996; Kovacs, 1992) were assessed.

In total, 132 participants divided over nine families took part in this study. However, nine of these participants only filled out questionnaires at home. ECG data of one participant was excluded because of technical problems, and of one participant because s/he reported heart problems. So, 121 participants (3-30 persons per family, in total 61 females, M_{age} = 30.10, *SD* = 15.65, range 8-61 years), including 16 children aged 8-12 years (2-4 children per family), and 20 children aged 13-17 years (1-7 children per family) took part in the first

resting state measure and 116 in the SPT (5 participants did not want to take part in any task). Of these 121 participants[2], 17 were diagnosed with SAD and an additional 25 were diagnosed with subclinical SAD (so the group with (sub)clinical SAD consisted of 42 participants). A different number of participants was analyzed for the different phases and measures, 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 shows how many participants were excluded for each of these reasons, table 2 shows the number participants (including the number of participants with SAD and subclinical SAD) per phase and per measure.

[Insert Table 1 about here]

[Insert Table 2 about here]

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%). The power was estimated by simulations of endophenotypes within families using a linear mixed model in R (R Core Team, Vienna, Austria). Multivariate normally distributed random effects were sampled to generate correlations between family members. A correlation structure of two times the kinship matrix was used. The outcome variable was generated assuming a heritability of 60% (i.e. 60% of the total variance was caused by the random effects) and a correlation of 70% with SAD. These numbers were based on studies in behavioral inhibition and SAD (Muris et al., 2005; Smoller et al., 2008). From the generated families, only families with two family members with SAD in one nuclear family were stored and used to estimate the power. Since in practice our families appeared to be relatively large (on average 14.67

[2] 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.

instead of 10 members per family), we included less families. Note that larger families have more power than smaller families (Dolan et al., 1999; Gur et al., 2007; Rijsdijk et al., 2001; Williams and 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. The EEG session started with the first resting state phase, then participants performed a social judgment paradigm[3] and the SPT. 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.

[Insert Figure 1 about here]

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 et al., 2018b; Van der Molen et al., 2014), so this might have influenced this first resting state measure. Therefore, we included a second resting state phase at the end of the EEG session.

Social performance task

[3] For the social judgment paradigm, participants had sent in a picture of themselves. During the task, they received feedback from peers indicating whether they liked or disliked the participants. This feedback was generated by a computer and always 50% 'like' and 50% 'dislike' (for the results, see Harrewijn et al., 2018b).

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 et al., 2018a). 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. Five different videos were used, for five different age categories (8-11, 12-17, 18-25, 26-39, 40+ years)[4]. These videos were performed by confederates who had practiced their speech, so it was a good model of giving a speech (to further increase stress). 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 et al., 2018a). We also administered the fear of negative evaluation questionnaire (Carleton et al., 2006). We focused our HRV analyses on the anticipation, speech, and recovery phases of the SPT.

[Insert Figure 2 about here]

[4] The videos were validated in an independent sample of participants (n = 142, age 9-55 years, M = 25.58, SD = 14.69) who rated how emotional the video was (from happy to neutral to angry). There was a main effect of age, F (4, 137) = 3.99, p = 0.004, showing that 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.

Other measures

In a separate session, we administered two subtests (similarities and block design) Wechsler Adult Intelligence Scale IV (Wechsler et al., 2008) or Wechsler Intelligence Scale for Children III (Wechsler, 1991) to calculate an 'estimated IQ'. In another session, we administered a series of questionnaires to measure social anxiety (Liebowitz Social Anxiety Scale (Liebowitz, 1987) for adults and Social Anxiety Scale – adolescents (La Greca and Lopez, 1998) for children), depression (Beck Depression Inventory (Beck et al., 1996) for adults and Child Depression Inventory (Kovacs, 1992) for children), trait anxiety (State-Trait Anxiety Inventory (Spielberger et al., 1983)), handedness (Edinburgh handedness inventory (Oldfield, 1971)), behavioral inhibition and activation (Behavioral Inhibition and Behavioral Activation Scales (Carver and White, 1994) for adults and Behavioral Inhibition and Behavioral Activation Scales, child version (Muris et al., 2005) for children), positive and negative affect (Positive and negative affect scale (Watson et al., 1988)), and autism (Autism-spectrum quotient questionnaire for adults (Baron-Cohen et al., 2001) and Social responsiveness scale (parent-rated) for children (Constantino et al., 2003)).

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 collar bone 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 et al., 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 et al., 2002). We calculated the root mean square of successive differences (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 calculate low frequency power (0.04-0.15 Hz) and high frequency power (0.15-0.4 Hz). High frequency power values were log transformed.

Statistical analysis

We performed all analyses separately for SAD and (sub)clinical SAD, since only few (n = 17) participants were diagnosed with SAD. First, we validated our groups by comparing self-reported symptoms of social anxiety (La Greca and 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 and 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, or high frequency power) as dependent variable, and time (first resting state, anticipation, recovery and second resting state as factors), age (standardized), age (standardized)^2, 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 and Blangero, 1998). In SOLAR, the total variance of the phenotype is decomposed into genetic (σ^2_a) and environmental ($I\sigma^2_e$) components (in formula: $\Omega=2\Phi\sigma^2_a+I\sigma^2_e$) (Almasy and Blangero, 2010). This is estimated using maximum likelihood techniques, based on a kinship matrix for the

genetic component (2Φ) and an identity matrix for the unique environmental component (I; 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 $p > .05$. 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 and Amos, 2000; Hopper and Mathews, 1982). For RMSSD, the residual kurtosis was not normally distributed, so we applied an inverse normal transformation as implemented in SOLAR (Almasy and Blangero, 1998, 2010). We used a Bonferroni adjusted *p*-value of .005 to correct for performing multiple [10] 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).

Results

Participant characteristics

Participants with SAD were older than participants without SAD, β = 9.83, p = .01. There was no difference in estimated IQ, β = -0.30, p = .91. We validated our groups by comparing self-reported symptoms of social anxiety and depression. Participants with SAD reported more symptoms of social anxiety, β = 3.09, p < .001, and depression, β = 0.97, p < .001, than participants without SAD (Table 3). Psychiatric disorders other than SAD in participants with and without SAD are shown in Supplementary table 2.

Participants with (sub)clinical SAD did not differ in age and IQ from participants without (sub)clinical SAD, respectively β = -1.63, p = .58 and β = -1.68, p = .41. Participants with (sub)clinical SAD also reported more symptoms of social anxiety, β = 1.81, p < .001, and depression, β = 0.50, p < .001 (Table 3).

[Insert Table 3 about here]

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 = .13, and high frequency power, $X^2(4) = 1.40$, p = .84[5]. 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 β = -11.58, p < .001 and β = -0.74, p < .001. There were no effects of age[2] and sex, all βs < 1.01 and >-0.58, ps > 0.11.

[5] We also analyzed the high frequency power data while controlling for respiration (using the 'ECG derived respiration' measure from Kubios), and the results did not differ from the results without controlling for respiration.

Co-segregation analyses were performed separately for the speech phase (Figure 3). There was no co-segregation with SAD within families for RMSSD, $\beta = -3.98$, $p = .24$, and high frequency power, $\beta = -0.61$, $p = .11$. RMSSD and high frequency power decreased with age, respectively $\beta = -6.54$, $p < .001$ and $\beta = -0.75$, $p < .001$.

We repeated all analyses with (sub)clinical SAD instead of SAD, but (sub)clinical SAD did not co-segregate within families with RMSSD, and high frequency power, all *p*s > .27 (for the first resting state, anticipation, recovery, and second resting state) and all *p*s > .78 (for speech).

[Insert Figure 3 about here]

Heritability

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

[Insert Table 4 about here]

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 measures 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, and high frequency power 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, and high frequency power during all phases of the task were heritable.

We found no co-segregation within families between SAD or (sub)clinical SAD and HRV during resting state and the SPT. This was expected for resting state (Alkozei et al., 2015; Alvares et al., 2013; Faucher et al., 2016; Grossman et al., 2001; Klumbies et al., 2014; Schmitz et al., 2013), but the findings on SPT were mixed (Alkozei et al., 2015; Garcia-Rubio et al., 2017; Gerlach et al., 2003; 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; 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). 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.

Age seemed to influence HRV, with older participants showing decreased RMSSD and high frequency power across resting state and SPT phases. This is in line with previous studies showing decreased HRV with age in adolescents (Goto et al., 1997; Hollenstein et al., 2012) and adults (Nunan et al., 2010). This effect of age complicates our findings, as participants with SAD were older than participants without SAD in our study. Figure 3 seems to suggest an effect of SAD, and this effect was indeed significant for RMSSD if we did not include age. However, we were not able to disentangle the effects of age and SAD, because only few children were diagnosed with SAD. 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 et al., 2017; Uusitalo et al., 2007), and adds that HRV during a SPT is also heritable. However, it should be noted that only RMSSD during the first resting state and high frequency power during the first resting state and during speech survived stringent correction for performing multiple tests. 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 and Lane, 2009). HRV is probably a more general endophenotype, because it is not only related to several anxiety disorders (Chalmers et al.,

2014; Friedman, 2007; Pittig et al., 2013) but also to depression (Kemp et al., 2012; Kemp et al., 2010). Indeed, others have proposed that HRV is a transdiagnostic factor related to worry (Chalmers et al., 2016), or self-regulation and cognitive control (Beauchaine and Thayer, 2015). Persons with this genetic vulnerability might be inflexible to environmental changes due to impaired inhibition (Chalmers et al., 2014; Thayer and 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 we might not have had sufficient power 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 SAD to enhance the power as much as possible (Fears et al., 2014; Glahn et al., 2010; Gur et al., 2007; Williams and 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). Also, movement and altered respiration patterns might have influenced the measures during speech. 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 et al., 2018b; 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. Fourth, some participants were too anxious to give the speech, and these were mostly participants with SAD (n = 1) or subclinical SAD (n = 5). Fifth, we used the 'ECG derived respiration' measure from

Kubios to control for respiration, but this measure is not as accurate as a more direct measure of respiration.

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, since other studies have shown decreased HRV in other anxiety disorders and depression (Chalmers et al., 2014; Friedman, 2007; Kemp et al., 2012; Kemp et al., 2010; Pittig et al., 2013). Decreased HRV might reflect reduced flexibility due to impaired inhibition (Chalmers et al., 2014; Thayer and 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.

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Table/Figure Legends

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. Reprinted from Journal of Affective Disorders, 227, Harrewijn, A., Van der Molen, M.J.W., Van Vliet, I.M., Houwing-Duistermaat, J.J., & Westenberg, P.M., Delta-beta correlation as a candidate endophenotype of social anxiety: A two generation family study, 398–405, Copyright (2018), with permission from Elsevier.

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 et al., 2018a; Harrewijn et al., 2018b). SAD = social anxiety disorder; MINI Plus = Mini-Plus International Neuropsychiatric Interview (MINI Plus version 5.0.0) (Sheehan et al., 1998; Van Vliet and 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 and 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

and 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).

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/

Figure 3

Uncorrected mean RMSSD (A), and high frequency power (B) for participants with and without SAD during all five phases of the SPT. Error bars represent standard errors.

Note: We show the uncorrected means for clarity, but we used a regression model to test the effect of SAD on HRV. 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

Tables

Table 1

Overview of the reasons for exclusion of participant data per phase of the task.

	Resting state 1	Anticipation	Speech	Recovery	Resting state 2
# ppn starting task	121	116	116	116	116
No speech		-9	-9	-9	
Too tired at the end					-3
Technical failure			-1	-2	-3
> 5% artefacts	-1	-2	-28	-3	
Outliers (+/- 3 SD) in RMSSD	-3	-2	-2	-2	-2
Outliers (+/- 3 SD) in HF				-1	
Outliers (+/- 3 SD) in HR	-2			-1	-1

Note: Of the 9 participants that did not want to give a speech 1 was diagnosed with SAD and 5 were diagnosed with subclinical SAD. Outliers were only excluded for that specific measure, not for the other measures (e.g. data of 117 participants were included for RMSSD, and of 120 participants for high frequency power). The speech data contained many artifacts, probably due to movement.

RMSSD = root mean square of the successive differences, HF = high frequency power, HR = heart rate

Table 2

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

	Resting state 1	Anticipation	Speech	Recovery	Resting state 2
RMSSD	117 [17, 25]	103 [16, 20]	76 [11, 16]	100 [16, 18]	108 [17, 24]
High frequency power	120 [17, 25]	105 [16, 20]	78 [11, 16]	101 [16, 18]	110 [17, 24]
Heart rate	118 [17, 25]	105 [16, 20]	78 [11, 16]	101 [16, 18]	109 [17, 24]

Table 3

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 with subclinical SAD (10 females, 15 males)	Participants without (sub)clinical SAD (35 females, 35 males)
Age	38.88 (13.72)	21.36 (11.54)	29.99 (15.83)
Estimated IQ	106.77 (12.34)	103.00 (11.92)	105.96 (10.61)
Social anxiety symptoms (z-score)	3.85 (2.13)	0.69 (1.85)	0.24 (1.15)
Depressive symptoms (z-score)	0.47 (0.85)	-0.38 (0.64)	-0.55 (0.67)

Note: social anxiety symptoms were measured using the Liebowitz Social Anxiety Scale (Liebowitz, 1987) for adults and the Social Anxiety Scale – adolescents (La Greca and 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. Due to technical problems, data on (sub)clinical SAD is missing from 9 participants, these participants were excluded from analyses on (sub)clinical SAD.

Table 4

Results of the heritability analyses for RMSSD, and high frequency power 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	.20	**0.044**	.11
	p (age)	**< .001**	< .001	< .001	**< .001**	< .001
	p (age^2)	**.71**	.98	1.00	**.93**	.93
	p (sex)	**.11**	.72	.76	**.15**	.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)	**< .001**	**.01**	**.002**	**.02**	**.04**
	p (age)	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**
	p (age^2)	**.93**	**.75**	**.97**	**.25**	**.85**
	p (sex)	**.03**	**.26**	**.77**	**.12**	**.04**

Note: RMSSD was inverse normalized in SOLAR. Variables displayed in bold font are heritable.